American Journal of Toxicology
http://www.ivyunion.org/index.php/ajt/

Review Article

Fish Liver Biomarkers for Heavy Metal Pollution: A Review Article

Rashid Alijani Ardeshir[1], Abdolali Movahedinia[1,2*], and Sara Rastgar[1]

[1] Department of Marine Biology, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran
[2] Department of Marine Biology, University of Mazandaran, Babolsar, Iran

Abstract
Heavy metals as dangerous and long lasting pollutants in environment have been widely studied and monitored. Liver is the most important organ in storage and detoxification of these pollutants. Assessment of indicators, including changes in intermediate active components between the liver and pollution effect, provide sensitive liver biomarkers and can be a suitable index for health condition of fish. In addition, fish liver is a favorite model to study interaction between environmental factors and liver structure and performance. This review studies variety of liver biomarkers in molecular- cellular (damage to chromosome, DNA and lysosome; increasing metallothionein and ferritin levels), biochemical-physiological (transaminase enzymes and oxidative enzyme parameters) and morphological-histopathological levels, and investigates advantages and disadvantages of these levels. Moreover, restrictions and landscape in the future is discussed for these biomarkers exposed to heavy metals pollution. There are many liver biomarkers that can be used for identification of initial heavy metal pollution, assessment of health condition of fish and ability of fish to defense against pollution challenges. Some of these biomarkers are special for heavy metal pollution while the others are non-special.

Keywords: biomarkers; heavy metals; fish liver; fish; pollution
Received: April 21, 2017; **Accepted**: May 25, 2017; **Published:** June 20, 2017
Competing Interests: The authors have declared that no competing interests exist.
Consent: We confirm that the patients have given the informed consents for the case reports to be published.

***Correspondence to:** Abdol-Ali Movahedinia, Department of Marine Biology, Faculty of Marine Science, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran
Email: amovahedinia@yahoo.com

Introduction

Growth in the human population and the development of industries has led to high levels of water pollution that are a serious threat to various aquatic ecosystems. Heavy metal pollution has long-term toxic effects and unlike many of the organic pollutants, they are not degraded over time. Even though some heavy metals (e.g., Cu, Zn and Mn) [1] are needed for the growth of organisms, they are toxic if their concentration exceeds an allowable limit. Other heavy metals do not have any biologic role (e.g., Cd, Ag, Hg, Pb and Cr), and are described as xenobiotic [2]. The heavy metals are easily dissolved in water, and are therefore absorbed by fish and other aquatic organisms. These pollutants are able to be bioconcentrated even at very low concentrations, most specifically in the liver [3], and, therefore, their concentrations in organisms is higher than that in the environment. Fishes have particular sensitivity to environmental changes, and respond more specifically to pollution than mammals [4]. Thus, measurement of biomarkers, either as individual or as population responses to toxic stress resulting from pollution, could be used to easily and rapidly monitor pollution.

Fish liver is the most important organ for detoxification and the main site of many important metabolic reactions involving carbohydrate, lipids and protein [5]. It also is important for the storage of glucose and vitamins A, D, E, K, and B12 [6]. Numerous environmental stressors affect the liver and cause metabolic disturbances and structural damage, possibly leading to death [4]. At all the different cellular levels (i.e., molecular, cellular, biochemical, physiological, morphological, and histopathological), exposure, effective and susceptibility biomarkers can be used to monitor initiative signals of pollution, liver health and ability of this organ to defense against pollution challenges, respectively.

Fish livers have been a favorite model to study the interactions between environmental factors and the functions and structures of the liver [4]. This article reviews liver biomarkers at the different biological levels when exposed to heavy metal pollution, and evaluates their advantages and disadvantages.

Main text

Cellular-molecular biomarkers in the liver

The liver functions to store and detoxify heavy metals. Thus, heavy metals cause changes in the proteins, DNA, and lysosomes of hepatocytes.

Stored metabolites

Fishes are able to absorb heavy metals through the skin mucosa, gill and alimentary tract [7]. They are transferred through ion channels or specialized transporter systems in the plasma membrane into the body [8] and, finally, into the liver and kidney (detoxification organs) where they are absorbed independent of their concentration [9]. They are then stored intracellularly as metallothionein-bound metals, ferritin-bound complex, and bound within vesicle-granules [8]. While considered the most important organ in detoxification, some studies show that liver is the major organ in storage of heavy metals [10]. However, a few heavy metals (e.g. Cr) are preferentially stored in other organs such as the gill [11, 12], and the extent of storage may differ between males and females [13].

Once stored in the fishes, these will become part of the food of other animals and humans higher in the food chain.

Biomarkers of damage to chromosomes, DNA and lysosomes

The presence of heavy metals in liver tissue often causes oxidative stress and increases of reactive oxygen species (ROS) through induction of O_2 and transformation into $O_2^{\cdot}$- (superoxide), H_2O_2 (hydrogen peroxide) and $OH^{\cdot}$ (hydroxyl radical). The hydroxyl radical is able to react with many compounds. Therefore, these radicals could transfer energy to other molecules including vital molecules. This may cause damage such as DNA single (SSB) and double strand breaks (DSB), micronuclei (MN XX), chromosome aberrations (CA) and sister chromatid exchanges (SCE) in hepatocytes and other cells [14-18].

Among other cell injuries caused by free radicals from heavy metals in hepatocytes is lysosomal damages. Fish hepatocytes often have a small number of lysosomes, and during heavy metal pollution the number and size of the lysosmes increase to store pollutant and lipids [19]. In addition, lysosomal membrane stability decreases due to damage to the lysosomal detoxification system, and enzymes of the lysosomes are released into the cytoplasm and nucleoplasm. This results in more damage to hepatocytes [19, 20]. Although lysosome stability quickly monitors damage and the capacity of hepatocyte in detoxification, observations need to be confirmed with liver histopathologic and other physiologic biomarkers. In addition, the number, size and lysosomal alterations could be affected by sex and season of vitellogenesis in female fish [21] and, therefore, can cause error in pollution monitoring .

Protein biomarkers

After entering heavy metals into the body, some proteins, such as metalothionin, chelate heavy metals via their sulfhydryl groups, and suppress heavy metal activity [22]. These low molecular weight proteins (2-16 kDa) have cysteine amino acids in binding site enriched in thiol ions [23]. Since the liver is the major site accumulating heavy metals, the most metalothionin is found in the liver. Its increase in the liver is a suitable biomarker for heavy metal pollution [24]. P-glycoprotein [25], heat shock proteins [26] and multixenobiotic resistance protein (MXR) [27] are the other known protein biomarkers for heavy metal increases in the liver, although they may also respond to some other xenobiotic compounds.

Biochemical and physiological biomarkers

Reactions in the liver to heavy metal oxidative stress might be enzymatic or non-enzymatic (Figure 1) and seem to be specific for monitoring heavy metals pollution.

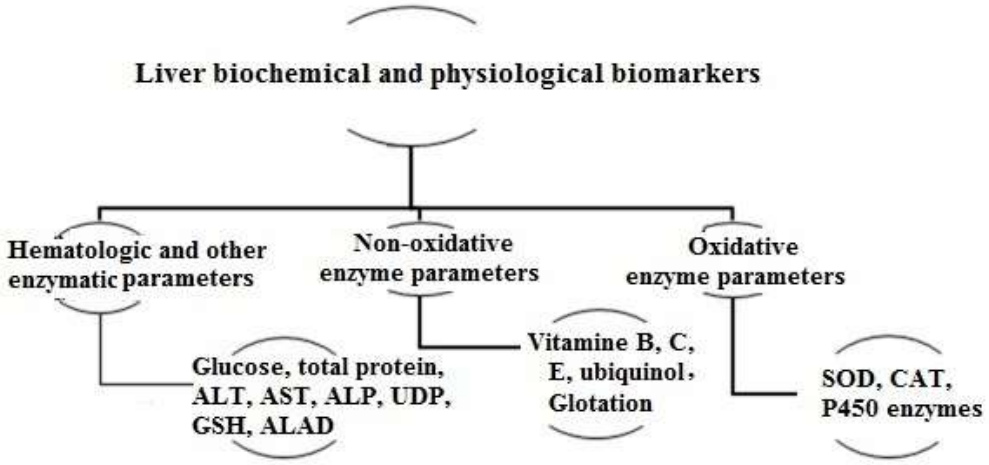

Figure 1 Biochemical and physiological biomarkers in the liver

Hematologic parameters

In fishes, proteins and lipids are used as energy sources [28]. Muscle glycogen obtained from glucose, which in turn is obtained from glycogenesis of exogenous and endogenous amino acids and lactate from anaerobic glycolysis [29]. The liver has the major role in the synthesis of the plasma proteins and the glucose. Some studies showed that pollution, especially heavy metals pollution, disrupted hepatocytes, and, therefore, decreased the removal of plasma glucose and proteins [30].Other studies showed similar results [31, 32].

Transaminase enzymes, including alanine transferase (ALT), aspartate transferase (AST), and alkaline phosphatase (ALP) are important for amino acid and protein metabolism in fish tissues, and are key metabolic enzymes released into the blood from damaged hepatocytes [33] and also have increased synthesis due to induction [34]. Thus, alterations in plasma levels of transaminase enzymes could be a sign of hepatocytes damages.

With heavy metal pollution, decreases or increases of some other easily measured liver detoxification enzymes, such as glucuronosyltransfrase (UDP-GT) [35] and δ-aminolevulinic acid dehydratase (ALAD) [36] can also be used as suitable biomarkers, although they may not have been heavy metal specific.

Oxidative enzyme parameters

Production of antioxidant compounds is part of an organism's response to free radicals, by trying to neutralize them to slow or prevent oxidative damage. Antioxidants generally stop oxidative chain reactions by giving a hydrogen atom to free radicals. The yield and performance of an antioxidant is dependent on how its hydrogen is released [37].

In the liver, antioxidant defenses occur both enzymatically and non-enzymatically. Antioxidant enzymes of the mitochondria and cytoplasm are able to decompose hydrogen peroxide (H_2O_2) [37, 38]. Thus, measurements of these enzymes are a valid biomarker for monitoring free radicals. Among the most important antioxidant enzymes whose activity changes with pollution are catalase (CAT), glutathione peroxidase (GPx) [39], and superoxide dismutase (SOD) [40]. Although some P450 enzymes also respond to heavy metals [41], their alterations with other organic pollutions make these enzymes more non-specific biomarkers.

Histopathological-morphological biomarkers

Histopathological alterations have special advantages [42]. Differences in biochemical processes in different organelles lead to different responses that can be determined histocytologically. Long-term exposure to heavy metals causes more changes in detoxification organs such as the kidney [43] and the liver. Studies of the livers of fish and birds exposed to heavy metals show degenerative, destructive and inflammatory alterations [43-46]. Again, however, they are non-specific biomarkers.

Bile as a biomarker in fish

The bile secreted by the liver contains salts and bile acids, which have role in emulsification and absorption of fatty acids from the gastrointestinal tract. It also has an important role in the excretion of many xeno- and endobiotics that the kidney is not able to remove them from the liver and blood [47]. Several studies have shown that many heavy metals enter into the gall bladder, and then are excreted from the fish [47-50]. The bile liquid can be more easily analyzed and is not age or sex dependent [50]. With cannulation, bile can be obtained from the bile duct without killing the fish [47]. Thus, monitoring bile and establishing correlations of heavy metals in bile and its storage in the liver can improve the value of

data obtained using the bile.

Problems and future of study on liver biomarkers in response to heavy metals

Some of the limitations mentioned require further research to allow fish to be more successfully used as biomarkers for heavy meat pollution:

Fishes are the most diverse group among vertebrates. Species diversity and factors such as sex and reproduction season may affect some liver biomarkers. So, these need to be better documented.

To better understand the changes in liver biomarkers with heavy metal pollution, it is necessary to understand which non-biological factors (e.g., temperature, salinity, and pH) and biological factors (e.g., fish age and size, liver structure, and fish genotype) affect these biomarkers so that measurement conditions can both be standardized and optimized.

Using cell culture, one can more easily study the effects of heavy metals on hepatocytes. These can be used to determine dose effects and the impact of other pollutants in the same water sample. These then need to be correlated with results in the field.

So are liver biomarkers appropriate and valid as compared to other biomarkers for use in real ecosystems? Additional questions that need to be answered include understanding the time course of liver changes. It may also be important to have a better understanding of the molecular mechanisms by which heavy metals change liver transporters, genes and proteins under different pollution scenarios.

Conclusion

This study showed that there are many liver biomarkers at all different cellular levels that can be used for identification of initial heavy metal pollution (exposure biomarkers), assessment of health condition of fish (effect biomarkers) and ability of fish to defense against pollution challenges (susceptibility biomarkers). Moreover, these biomarkers are useful for environmental monitoring and assessment; some of these biomarkers, such as metallothionein and ferritin, are special for heavy metal pollution while the others are non-special.

Acknowledgment

The author thanks Professor Joe M. Regenstein of Cornell University for his extensive editing of the manuscript to improve both the science and the English.

Reference

1. Singh BP and Tandon PK. Effect of river water pollution on hematological parameters of fish, Wallagoattu. ***Res Environ Life Sci.*** 2009, 2(4):211-214
2. Sadeghi P, Savari A, Movahedinia A, Safahieh A, Azhdari D. An assessment of hematological and biochemical responses in the tropical fish Epinephelus stoliczkae of Chabahar Bay and Gulf of Oman under chromium exposure: ecological and experimental tests. ***Environ Sci Pollut Res.*** 2014, 21(9):6076-6088
3. Agah H, Leermakers M, Elskens M, Fatemi SMR, Baeyens W. Accumulation of trace metals in the muscle and liver tissues of five fish species from the Persian Gulf. ***Environ Monit Assess.*** 2009, 157(1-4):499-514
4. Brusle J, Anadon GG. (1996). The structure and function of fish liver. In: Munshi JSD and Dutta HM, editors. Fish morphology. Boston: Massachusetts. 1996,77-93

5. Mitra V and Metcalf J. Metabolic functions of the liver. ***Anaesth Intensive Care Med.*** 2009, 10(7):334-335
6. Postic C, Dentin R, Girard J. Role of the liver in the control of carbohydrate and lipid homeostasis. ***Diabet Metab.*** 2004, 30(5):398-408
7. Jovanović B, Mihaljev Ž, Maletin S, Palić D. Assessment of heavy metal load in chub liver (Cyprinidae–Leuciscus cephalus) from the Nišava River (Serbia). ***Biologica Nyssana.*** 2011, 2(1)
8. Deb SC and Fukushima T. Metals in aquatic ecosystems: mechanisms of uptake, accumulation and release: Ecotoxicological perspectives. ***Intern J Environ Studies.*** 1999, 56(3):385-417
9. Blanco MV, Cattoni DI, Carriquiriborde P, Grigera JR, Chara O. Kinetics of bioaccumulation of heavy metals in Odontesthes bonariensis is explained by a single and common mechanism. ***Ecol Model.*** 2014, 274:50-56
10. Malik N, Biswas AK, Qureshi TA, Borana K, Virha R. Bioaccumulation of heavy metals in fish tissues of a freshwater lake of Bhopal. ***Environ Monit Assess.*** 2010, 160(1-4):267-276
11. Demirak A, Yilmaz F, Tuna AL, Ozdemir N. Heavy metals in water, sediment and tissues of Leuciscus cephalus from a stream in southwestern Turkey. Chemosphere 2006; 63(9): 1451-1458.
12. Wong CK, Wong PPK, Chu LM. Heavy metal concentrations in marine fishes collected from fish culture sites in Hong Kong. ***Arch Environ Contam Toxicol.*** 2001, 40(1): 60-69
13. Khan AT and Weis JS. Bioaccumulation of heavy metals in two populations of mummichog (Fundulus heteroclitus). ***Bull Environ Contam Toxicol.*** 1993, 51(1):1-5
14. Frenzilli G, Nigro M, Lyons BP. The Comet assay for the evaluation of genotoxic impact in aquatic environments. ***Mutat Res/Rev Mutat Res.*** 2009, 681(1): 80-92
15. Mourón SA, Grillo CA, Dulout F N, Golijow CD. A comparative investigation of DNA strand breaks, sister chromatid exchanges and K-ras gene mutations induced by cadmium salts in cultured human cells. ***Mutat Res /Fundam Mol Mech Mutagen.*** 2004, 568(2):221-231
16. Obe G, Pfeiffer P, Savage JRK, Johannes C, Goedecke W, Jeppesen P, et al. Chromosomal aberrations: formation, identification and distribution. ***Mutat Res/Fundam Mol Mech Mutagen.*** 2002, 504(1):17-36
17. Russo C, Rocco L, Morescalchi MA, Stingo V. Assessment of environmental stress by the micronucleus test and the Comet assay on the genome of teleost populations from two natural environments. ***Ecotoxicol Environ Saf.*** 2004; 57(2):168-174
18. Shugart L and Theodorakis C. Environmental genotoxicity: probing the underlying mechanisms. ***Environ Health Perspect.*** 1994, 102(12):13-17
19. Köhler A. Lysosomal perturbations in fish liver as indicators for toxic effects of environmental pollution. ***Comp Biochem Physiol Part C: Compa Pharmacol.*** 1991, 100(1):123-127
20. Myers MS, Rhodes LD, McCain BB. Pathologic anatomy and patterns of occurrence of hepatic neoplasms, putative preneoplastic lesions, and other idiopathic hepatic conditions in English sole (Parophrys vetulus) from Puget Sound, Washington. ***J Natl Cancer Inst.*** 1987, 78(2):333-363
21. Petersen IM and Emmersen BK. Changes in serum glucose and lipids, and liver glycogen and phosphorylase during vitellogenesis in nature in the flounder (Platichtys flesus). ***Comp Biochem Physiol Part C: Comp Pharmacol.*** 1977, 58(2):167-171
22. Ryan JA and Hightower LE. Stress proteins as molecular biomarkers for environmental toxicology. ***In Stress-Inducible Cell Respon.*** 1996, 77:411-424
23. Hamilton SJ, Mehrle PM. Metallothionein in fish: review of its importance in assessing stress from metal contaminants. ***Trans Am Fish Soc.*** 1986, 115(4):596-609

24. Hauser-Davis RA, Bastos FF, Tuton B, Rocha RC, Saint'Pierre T, Ziolli RL, Arruda MA. Bile and liver metallothionein behavior in copper-exposed fish. ***J Trace Elem Med Biol.*** 2014, 28(1):70-74
25. Abu-Qare A, Elmasry E, Abou-Donia M. A role for P-glycoprotein in environmental toxicology. ***J Toxicol Environ Health Part B: Crit Rev.*** 2003, 6(3):279-288
26. Rajeshkummar S and Munuswamy N. Impact of metals on histopathology and expression of HSP 70 in different tissues of Milk fish (Chanos chanos) of Kaattuppalli Island, South East Coast, India. ***Chemosphere.*** 2011, 83(4):415-421
27. Archard M, Baudrimont M, Boudou A, Bourdineaud JP. Induction of a multixenobiotic resistance protein (MXR) in the Asiatic clam Corbicula fluminea after heavy metals exposure. ***Aquat Toxicol.*** 2004, 67(4): 347-357
28. Walton MJ, Cowey CB. Gluconeogenesis by isolated hepatocytes from rainbow trout Salmo gairdneri. ***Comp Biochem Physiol Part B: Comp Biochem.*** 1979, 62(1):75-79
29. Moon TW, Walsh PJ, Mommsen TP. Fish hepatocytes: a model metabolic system. ***Canadian j fish aquat sci.*** 1985, 42(11):1772-1782
30. Savari A, Hedayati A, Safahieh A, Movahedinia A. Changes in some serum biochemical values of Yellowfin Sea Bream (Acanthopagrus latus) in Mahshahr creeks (Persian Gulf). ***Glob Vet.*** 2010, 5(4):233-238
31. Vaboonian A, Movahedinia A, Safahieh A, Hedayati A. Serum biochemical changes of yellowfin sea bream (Acanthopagrus latus) in response to sublethal cadmium toxicity. ***Comp Clin Pathol.*** 2015, 24(1):35-39
32. Hedayati A, Safahieh A, Savari A, Movahedinia A, Zare P, Bagheri T. Serum biochemical change induced by in vitro sub chronic mercury chloride in yellowfin sea bream (Acanthopagrus latus). ***Iranica J Energ Environ.*** 2011, 2(3):208-214
33. Savari A, Hedayati A, Safahieh A, Movahedinia A. Determination of some enzymatic indices of yellowfin sea bream (Acanthopagrus latus) in Mahshahr Creeks (North West of Persian Gulf). ***World.*** 2010b, 2(6):475-480
34. Vutukuru SS, Prabhath NA, Raghavender M, Yerramilli A. Effect of arsenic and chromium on the serum amino-transferases activity in Indian major carp, Labeo rohita. ***Intern J Environ Res Public Health.*** 2007, 4(3):224-227
35. Castrén M, Oikari A. Changes of the liver UDP-glucuronosyltransferase activity in trout (Salmo gairdneri Rich.) acutely exposed to selected aquatic toxicants. ***Comp Biochem Physiol Part C: Compar Pharmacol.*** 1987, 86(2):357-360
36. El-Murr A, Ali HA, Elgaml SA, Hashish EA. The β-1, 3-glucan alleviated the hepatotoxicity induced by combination of fipronil and lead in common carp (Cyprinus carpio). ***Comp Clin Pathol.*** 1-9
37. Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods of their quantification. ***Toxicol Pathol.*** 2002, 30 (6):620-50
38. Vieira LR, Gravato C, Soares AMVM, Morgado F, Guilhermino L. Acute effects of copper and mercury on the estuarine fish Pomatoschistus microps: linking biomarkers to behaviour. ***Chemosphere.*** 2009, 76(10):1416-1427
39. Zheng JL, Zhu QL, Wu CW, Zhu AY, Shen B, Zeng L. Zinc acclimation mitigated high zinc induced oxidative stress by enhancing antioxidant defenses in large yellow croaker Pseudosciaena crocea. ***Aquat Toxicol.*** 2016, 172:21-29
40. Doherty VF, Ogunkuade OO, Kanife UC. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in some selected fishes in Lagos, Nigeria. ***American-Eurasian J Agric Environ Sci.*** 2010, 7(3):359-365

41. Brüschweiler BJ, Würgler FE, Fent K. Inhibitory effects of heavy metals on cytochrome P4501A induction in permanent fish hepatoma cells. ***Arch Environ Contam Toxicol.*** 1996, 31(4):475-482
42. Segner H and Braunbeck T. Cellular response profile to chemical stress. ***Ecotoxicol.*** 1998, 3:521-569
43. Safahieh A, Hedayati A, Savari A, Movahedinia A. Effect of sublethal dose of mercury toxicity on liver cells and tissue of yellowfin seabream. ***Toxicol Industrial Health.*** 2011, 28(7):583-593
44. Chavan VR, Muley DV. Effect of heavy metals on liver and gill of fish Cirrhinus mrigala. Int. J. Curr. Microbiol. ***App Sci.*** 2014, 3(5):277-288
45. Hermenean A, Damache G, Albu P, Ardelean A, Ardelean G, Ardelean DP, et al. Histopatological alterations and oxidative stress in liver and kidney of Leuciscus cephalus following exposure to heavy metals in the Tur River, North Western Romania. ***Ecotoxicol Environ Saf.*** 2015, 119:198-205
46. Salamat N, Etemadi-Deylami E, Movahedinia A, Mohammadi Y. Heavy metals in selected tissues and histopathological changes in liver and kidney of common moorhen (Gallinula chloropus) from Anzali Wetland, the south Caspian Sea, Iran. ***Ecotoxicol Environ Saf.*** 2014, 110:298-307
47. Grosell M, O'Donnell MJ, Wood C M. Hepatic versus gallbladder bile composition: in vivo transport physiology of the gallbladder in rainbow trout. Am J Physiol-Regul, Integ Comp Physiol. 2000, 278(6):R1674-R1684
48. Bunton TE, Frazier JM. Extrahepatic tissue copper concentrations in white perch with hepatic copper storage. ***J Fish Biol.*** 1994, 45(4):627-640
49. Dijkstra M, Havinga R, Vonk RJ, Kuipers F. Bile secretion of cadmium, silver, zinc and copper in the rat. Involvement of various transport systems. ***Life Sci.*** 1996, 59(15):1237-1246
50. Hauser-Davis RA, Bastos FF, de Oliveira TF, Ziolli RL, de Campos RC. Fish bile as a biomarker for metal exposure. ***Marine Pollut Bull.*** 2012, 64(8):1589-1595