International Journal of Fauna and Biological Studies
Available online at www.faunajournal.com

IJFBS International Journal of Fauna And Biological Studies

E-ISSN 2347-2677
P-ISSN 2394-0522
www.faunajournal.com
IJFBS 2021; 8(3): 43-49
Received: 24-03-2021
Accepted: 26-04-2021

Kashif Abbas
Department of Zoology, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Mudassir Alam
Department of Zoology, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Shakir Kamal
Department of Biotechnology, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Corresponding Author:
Mudassir Alam
Department of Zoology, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Heavy metals contamination in water bodies and its impact on fish health and fish nutritional value: A review

Kashif Abbas, Mudassir Alam and Shakir Kamal

DOI: https://doi.org/10.22271/23940522.2021.v8.i3a.833

Abstract
This review attempts to provide some sagacity of our recent knowledge of water body's contamination and exposure of heavy metals with aquatic life. Heavy metals are basically known to be naturally occurring compounds that have a relatively high density compared to water. These metallic are considered systemic toxicants that are directly or indirectly linked to induce multiple organ damage and several other severe consequences even at lower levels of exposure. Due to the immense anthropogenic actions these metals bring in large quantities in different environmental compartment and water bodies that cause water quality deterioration ultimately leading to bioaccumulation of hazardous substances. Toxicity depends on various factors *viz*. dose, route of exposure, chemical species as well as the age, gender, genetics, and nutritional status of exposed organism. Arsenic, cadmium, chromium, lead, and mercury rank among the high-grade toxicants. It has been reported that the aquatic life is greatly affected with these heavy metals accumulation. Several conducted studies suggest that Fishes dwelling in such contaminated water bodies significantly affect their health and further reduce their nutritive value and flesh quality due to the incorporation of non-essential metals which are not required. Such toxicant potentially hampered the normal physiology and functioning of an organism.

Keywords: Heavy metals, hazardous, anthropogenic, bioaccumulation, toxicant, accumulation, nutrition

1. Introduction
Water pollution is one of the major concerns in 21st century. The most significant pollutants are heavy metals in aquatic networks due to their toxicity, accumulation, and bio-magnification by aquatic organisms. Domestic, industrial, and anthropogenic activities may all be sources of heavy metal contamination in natural aquatic systems [1]. Metal concentrations have both beneficial and detrimental effects on aquatic life. As a result, these can be classified as essential, non-essential, or toxic. Iron (Fe), copper (Cu), zinc (Zn), and selenium (Se) are essential elements that play a specific role in body metabolism. Non-essential elements are those that have no known specific function in the body but are not toxic in any significant amount. Finally, toxic elements such as chromium (Cr), nickel (Ni), cadmium (Cd), mercury (Hg), and lead (Pb) are generally associated with pollution. Few metals are extremely toxic in trace amounts, whereas some biologically significant metals are toxic at high concentrations. Intakes of heavy metals in excess react with bio-elements (these elements are essential for life to function) present in the body, disrupting the structure of chief molecules that participate in many metabolic reactions [2]. Heavy metals in fish are primarily ingested through their diet, and levels of bioaccumulation of contaminants are higher in fish leading to increased level of contamination in the food chain [3] (Figure 1). The trend of metal accumulation in fishes varies depending on a variety of factors, including developmental and psychological factors as well as the age of the fish. Fish consumption has increased rapidly in recent years, owing to increased awareness of its nutritional and therapeutic benefits. Fish is rich in essential minerals, vitamins, and unsaturated fatty acids, in addition to being a good source of protein [4] (Figure 2). Fish and shellfish provide 25% of protein to humans worldwide [5]. The American Heart Association suggested intake of fish at least twice a week to meet the recommended daily requirement of omega-3 fatty acids [6]. The presence of toxic metals and metalloids such as lead, cadmium, arsenic, and mercury may jeopardize the numerous health benefits provided by fish consumption. Metal concentrations in fish meat must therefore be monitored in order to ensure compliance with food safety regulations and consumer protection.

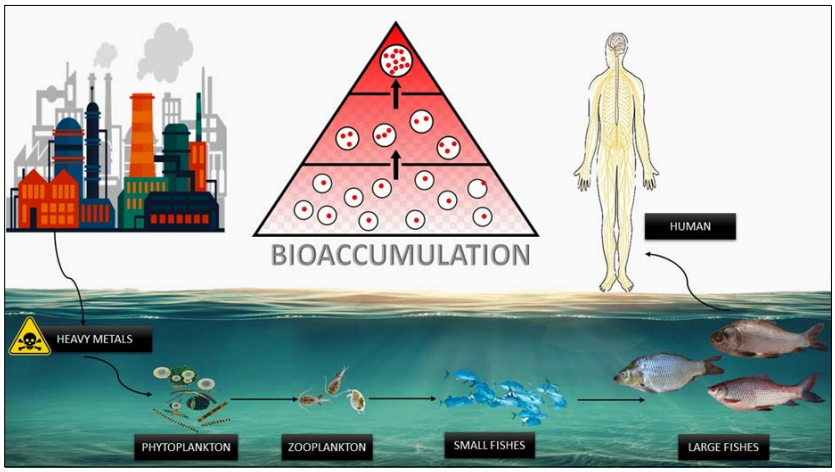

Fig 1: Contamination of water bodies with heavy metals due to effluents from industries leading to bioaccumulation

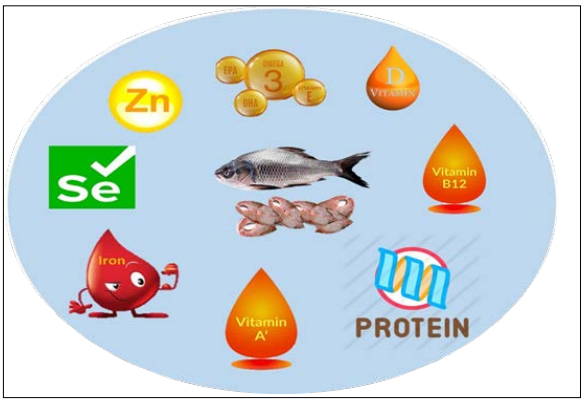

Fig 2: Nutritional content of fish

2. Effect of cadmium toxicity on fish

Cadmium is regarded as one of the most dangerous heavy metals. There are neither any known beneficial properties of cadmium which support life nor any evident fact for its biological necessity [7]. Cadmium-related contamination of aquatic habitats has significantly increased in recent decades, resulting in an increase in cadmium deposits in aquatic organism tissues throughout all food chain systems. Its accumulation in living organisms is a major ecological concern, especially given its ability to accumulate quickly. Cadmium excretion from living organisms, on the other hand, is a slow process and it can cause structural and pathomorphological changes in a variety of organs in fish [8]. The gills (respiratory tract), the intestine (ingestive intake), and the skin are all potential sites for dissolved metal absorption in fish (transcutaneous uptake). Cadmium ions are typically absorbed via passive diffusion or carrier-mediated transport over the gills. It has been reported that cadmium ions may enter the chloride cells of the gills via calcium channels [9].

2.1 Cadmium-induced neurotoxicity

Several studies have shown that cadmium toxicity can damage neuronal network and nervous system in fishes. An experiment done on zebrafish showed that exposure to cadmium significantly reduced its swimming speed. It has also been reported from the study that cadmium exposure disrupted the levels of neurotransmitters such as dopamine, serotonin and acetylcholine. Furthermore, it was found that the expression of gene which governs neuronal development and neurotransmitter metabolism was also altered [10].

2.2 Cadmium-induced nephrotoxicity

Cadmium is a heavy metal that is highly toxic even at low levels of exposure and has both acute and chronic effects on aquatic animal health and the environment. It affects the osmoregulatory function of the kidneys. Studies has shown that acute exposure to cadmium causes low functionality of certain enzymes which disturbs the fish's ability to regulate the calcium level in the blood finally leading to hypocalcemic condition [11].

2.3 Cadmium-induced immunotoxicity

Several studies have suggested that cadmium exposure can induce immunosuppressive effect in fishes. Cadmium exposure causes oxidative stress and disruption of miR-216a-PI3K/AKT axis which promotes apoptosis and necrosis in fish lymphocytes [12]. Furthermore, cadmium can induce immunotoxicity via transcriptional regulation of Nrf2 and NF-κB and gene modifications at transcriptional, translational, post-translational levels [13].

2.4 Cadmium-induced hepatotoxicity

Cadmium accumulates in fish liver at higher concentration. The cadmium accumulation in fish liver causes several pathological changes such as engorgement of blood vessels, congestion, vacuolar degeneration of hepatocytes, necrosis of pancreatic cells and fatty changes in the peripancreatic hepatocytes [14]. These alterations further lead to the hepatomegaly in fishes which causes retarded growth and disruption of metabolic mechanisms.

3. Effect of chromium toxicity on fish

Chromium is regarded as one of the most common and pervasive pollutants in the aquatic ecosystem. It enters the waterbodies through effluents discharged from various industries such as textiles, tanneries, electroplating workshops, ore mining, dyeing, printing-photographic, and medical. The bioaccumulation of the chromium in aquatic organism varies with size and organ. Its concentration has been found to be highest in fish gills, kidneys, and liver, with little tendency for chromium accumulation in muscular tissues [15].

3.1 Chromium-induced neurotoxicity

Chromium can induce neurotoxic effect in fishes. Studies have revealed that exposure to chromium leads to alteration in activity of acetylcholine esterase (AChe) and MT gene

expression [16]. Furthermore, it was observed that exposure to chromium leads to erratic swimming, loss of balance and tendency of convulsion [17]. Very few literature articles are available regarding chromium induced neurotoxicity. More research is required for deep comprehension of induction of neurotoxicity and its mechanism due to chromium in fishes.

3.2 Chromium-induced nephrotoxicity
The osmoregulatory function of various fish species is greatly harmed by trivalent chromium. It was reported that the trivalent as well hexavalent metal has the potential to cause deep fluctuations in the osmoregulatory functions of fish [18]. Chromium toxicity is directly associated with kidney enzyme ATPase activity due to which function of osmoregulation is hampered ultimately leading to the severe consequences and kidney failure.

3.3 Chromium-induced immunotoxicity
Prabakaran *et al*. have conducted an experiment in order to understand the immune system response and non-specific immunity in the tilapia fish (*Oreochromis mossambicus*). Fish was exposed to the sublethal concentrations of tannery effluent having chromium (88.2 mg/l) [19]. It was evaluated that the exposure of fish to such sublethal concentrations of tannery effluent significantly suppressed antibody response as well nonspecific serum lysozyme activity. These types of experimental studies can serve as an essential factor for discussing the role of immunological parameters in monitoring and assessing fish health and risk.

3.4 Chromium-induced hepatotoxicity
Though the liver plays an important role in metabolic processes and xenobiotic neutralization and detoxification, acute exposure to metals such as chromium may leads to its accumulation in the liver and cause pathological changes [20]. Alanine aminotransferase (ALT) and Aspartate amino transferase (AST) are used biomarker for analysis of hepatotoxicity [21]. Fishes exposed to chromium exhibit increase in transferases (ALT and AST) which could be attributed to enzyme leakage across damaged plasma membranes and/or increased enzyme synthesis by the liver [22].

4. Effect of mercury toxicity on fish
The people have become increasingly aware of the negative health effects caused by mercury pollution in the waterbodies since the Minamata disease incident in Japan. Mercury in waterbodies is mainly found in the form of elemental mercury, divalent mercury and methylmercury. Bacteria and phytoplankton serve as gateway for entry of mercury in food chain as they can actively accumulate mercury in their cells [23, 24]. As mercury enters the fish body through food chain, it forms stable and strong bond with protein sulfhydryl groups which makes it excretion and elimination very slow and is retained in the fish's body for longer duration [25]. The highest concentration of mercury was observed in the muscle, followed by liver or kidney.

4.1 Mercury-induced neurotoxicity
Mercury has potency to cross blood brain barrier and accumulate in brain and therefore can cause serious damage. Accumulation of mercury in brain can induce neurotoxicity via oxidative stress, cytoskeletal assembly dysfunction and metabolic disorders [26]. It is established through the proteomic analysis of Atlantic cod brain tissue that on exposure to methylmercury, the level of 71 protein changed by 20% or more [27]. Furthermore, in another study on brain of juvenile beluga authorized the fact that methylmercury has potency to induce neurotoxicity via oxidative stress and apoptosis concluding that it can cause metabolic damage [28]. Several other studies shown that mercury may also induce morphological changes such as total count and volume of neurons and glial cells with changes in swimming behavior [29].

4.2 Mercury-induced nephrotoxicity
The kidney's role in mercury elimination is dependent on the mercurial form, preferably inorganic form, by urine [30]. Earlier studies have shown that after a duration of 4 weeks exposure to methylmercury, the sturgeon fish species kidney exhibit severe degeneration of renal tubules [31]. Another study provided fact that exposure to methyl mercury culminated to necrosis areas, increased number of melano-macrophages center, intercellular spaces among the parenquimal cell and phagocytic areas in head kidney of the fish [32].

4.3 Mercury-induced immunotoxicity
Although the immunotoxic effect of mercury is well studied in context of mammals, its adverse effect in case of fishes is still not well known. Research articles have chalked out the hindrances in assessment of immunotoxicological effects in fish and challenges to choose valid and appropriate parameters out of many immune parameters [33]. Study on snakehead (*Channa punctatus)* exposed to 0.3 mg/L $HgCl_2$ shown up-regulation of pro-inflammatory cytokines like tumor necrosis factor-α (*tnfα*) and interleukin-6 (*il6*). *In vitro* study on gilthead seabream *(Sparus aurata* L.*)* shown down regulation of pro-inflammatory *il1b* gene expression in head kidney leucocytes [34]. Furthermore, European sea bass (*Dicentrarchus labrax*) *in vitro* exposure to mercuric chloride potentially induced apoptosis in head-kidney macrophages [35]. Also, increase in transcription of apoptotic gene was observed after exposure to methylmercury in gilthead seabream [36] leading to apoptosis and necrosis.

4.4 Mercury-induced hepatoxicity
Accumulation of mercury in liver tissues may lead to histopathological damage such as vacuolization, parenchyma disorganization and pyknotic nucleus and thus leads to impairment of liver ultrastructure [37]. Furthermore, using quantitative proteomic analysis, it was proposed that hepatotoxicity induced by mercury may involve oxidative stress, change in rate of energy metabolism and cytoskeleton damage. Above mentioned alteration indicates that mitochondria may be the prime target for mercury attack in cells [38] which further leads to hepatotoxicity.

5. Effect of lead toxicity on fish
Lead has no physiologically relevant role in the body, and its harmful effects are myriad. Lead (from the soil and atmosphere pollutes water bodies and poses detrimental effect on aquatic life. Leads being one of the most toxic elements holds 2nd place in Priority List of Hazardous Substances [39]. The accumulation rate of lead in fishes depends on several factors such as exposure duration, salinity, temperature, fish species and metabolic activity of fish [40]. Exposure to lead

poses adverse effect on physiological functions of fish including pituitary function, gonadosomatic index, chromosomal aberrations, oocyte growth, DNA damage, neurological disorders and scoliosis [41, 42]. Pb exposure causes a large range of toxic effects on fishes and greatly affects their life.

5.1 Lead-induced neurotoxicity

Lead Pb is known as neuro-toxicant which causes neurodegenerative disorders, cell signaling deregulation, and neurotransmission impairment. It greatly impacts brain and cognitive function morphological changes in the brain are mostly observed in case of Pb-induced toxicity [43]. It has been reported that oxidative damage by lead Pb exposure is closely associated with neurotoxicity in fishes; also chronic Pb exposure is much toxic to the central nervous system in fish, resulting in to behavioral and cognitive dysfunctions [44]. Pb affects by disrupting the calcium flux and calcium-regulatory functions, it competes with calcium ion which is a critical for neurotransmitter release and regulation. Just by mimicking the calcium cation it binds specifically to the calcium transport system of the nervous system. Thereby inducing changes in calcium homeostasis, that hampered neurotransmission [45]. Pb exposure causes synaptic damage and neurotransmitter changes in fish, resulting in neurological and behavioural consequences. Furthermore, exposure of pb also reduces the activity of ecto-nucleotidase a key enzyme regulating the purinergic signalling. As a result, structural or functional changes in proteins, induces gene expression, and disruptions in the transduction and DNA repair processes [46].

5.2 Lead-induced nephrotoxicity

Lead exposure poses lethal effects on kidney tissues. Chronic lead exposure causes high accumulation in kidney. Higher concentration of lead is found in kidney as compared to other organs as they perform function of detoxification and removal of xenobiotics [47]. Several studies reported degeneration of kidney due to increased exposure to lead [48]. Furthermore, degeneration of kidney leads to loss of essential ions such as calcium due to decrease in reabsorption activity of the fish [49]. Increased peroxidase activity was also observed in kidney cells which indicates higher production of reactive oxygen species [50].

5.3 Lead-induced immunotoxicity

Lead exposure alters the immune response in the fishes. Studies have shown that lead exposure in fish induces decreased hematopoietic activity in the spleen, phagocytic activity and decreased antibody production [51]. Furthermore, it was observed that exposure to lead for short duration result in increase of lymphocytes count while the number decreases when exposed for longer duration due to immune injuries [52]. Also, cortisol secretion was observed due to stress reaction and promotion of lymphocytes apoptosis which led to decrease in white blood cell and lymphocyte count [53]. Lead is known to influence immune responses by regulating the expression of cytokines [54]. Cytokines such as interleukins (ILs) and tumor necrosis factors (TNFs) regulate signals between different cells that cause an immune response and plays an important role in regulating an immune mechanism [55]. It was reported in crucian carp that mRNA expression of IL-10 and TNF-α was increased due to exposure to lead which signifies serious damage done by lead on fish immune system [56]. So, it is well established fact from several studies that lead poses serious negative effect on immune responses in fish, including lymphocytes, leukocytes, inflammation, and apoptosis. Apart from inhibiting the activity of vital biomolecules, it also acts as an immunotoxin by interfering with intracellular signal transduction.

5.4 Lead-induced hepatoxicity

There have been various experimental studies revealed that lead Pb exerts some histological effects on hepatic tissue such as proliferation of connective tissue, hepatic necrosis, vacuolation of hepatic cells These alterations became more pronounced in liver of fishes when tested at increasing concentrations of Pb [57]. Pandi Prabha *et al*. concluded in their experimental study that Pb accumulation is highly toxic to fish liver as its toxicity is directly associated with increased expressions of some liver enzymes such as CYP1A, CYP3A, Hsp60, Hsp70, and metallothionein in the liver tissue. The lead induced toxicity enhances and increased the release of alanine transaminase, aspartate transaminase in serum due to which activity of ALAD in the blood decreased and hepatic damage take place ultimately inhibiting the synthesis of red blood cells in fish. It has also been reported that Pb toxicity causes severe liver injury leading cellular leakage of several hepatic marker enzymes into blood stream finally loss of the actual functional integrity of hepatic membrane architecture [58].

Table 1: Shows maximum permissible limit for heavy metals according to FAO

Heavy metal	Maximum permissible limit in parts per million (ppm)
Cadmium	0.05
Chromium	1
Mercury	0.5
Lead	0.5

6. Effect of heavy metal toxicity on protein content of fish flesh

Protein is essential macromolecule which act as regulatory elements for various vital body functions such as growth, immunity and development. Major population of the world suffers from protein deficiency. Survey conducted by Indian Council of Medical Research in 2017 shows that 73% of Indian have protein deficient in their diet. Fish being nutritionally rich serve as cheapest and best form of protein [59]. Fish flesh have relatively more protein content than the flesh of most terrestrial animals. Furthermore, fish protein is highly digestible and rich in peptides and essential amino acids that are lacking in terrestrial meat proteins, such as methionine and lysine [60]. Proteins are extremely sensitive to heavy metal toxicity. Studies have shown that there has been significant decrease in muscle protein content of the fishes when exposed to heavy metal toxicity [61]. The decrease in muscle protein content is due to the channelization of protein for tissue damage repair and detoxification of the heavy metal stress [62].

7. Effect of heavy metal toxicity on carbohydrate content of fish flesh

Fish meat contains higher amount of omega 3 long chain polyunsaturated fatty acids (n-3 LC PUFA) and other essential fatty acids as compared to terrestrial animals [63]. Many researchers have found strong links between fish and

seafood consumption and positive health effects, particularly a lower risk of coronary heart and cardiovascular disease, a lower risk of inflammatory disease such as arthritis, and cancer prevention. The European Food Safety Authority (EFSA) has authorized numerous health benefits related to fish consumption or EPA and DHA, such as the maintenance of normal blood triacylglycerol levels, normal brain function, vision, cardiac function and blood pressure [64]. Composition of fatty acid depend on peroxidation in fishes. Study conducted by (Ramirez and Gimenez, 2002) showed that lipid content of the fish muscle significantly decreased when exposed to heavy metals [65]. Also decrease in unsaturated fatty acid was also observed [66]. Furthermore, fishes exposed to cadmium showed decrease in palmitoleic acid, oleic acid, linoleic acid, arachidonic acid, eicosapentaenoic acid, docosapentaenoic acid and docosahexanoic acid content [67]. So, we can be summarized that heavy metals toxicity leads to degradation of fat content of fish flesh.

8. Conclusion

Anthropogenic activities have led to degradation of both terrestrial and aquatic ecosystem. With exponential population growth and shoot in number of industrial setups, the ecosystem health is jeopardized. In recent years, aquatic ecosystem is deteriorating at an alarming rate due to effluents from sewage and industries. It leads to contamination of waterbodies with toxic substances which poses deleterious effect on aquatic ecosystem. Most lethal of those contaminants are heavy metals which have serious effect on both biotic and abiotic components of the water body. These heavy metal toxicants get ingested into the aquatic animal's body and their accumulation (bioaccumulation) affects whole food chain. Fishes being the chief residents of the aquatic ecosystem are badly affected by heavy metal toxicity. It is well known fact that fish serves as one the richest and cheapest form of protein. Bioaccumulation and toxification of fishes with these heavy metals leads to degradation of its nutritive quality and makes it unfit for human consumption as its consumption has serious effect on human health. The accumulation of these heavy metals also leads to increase in mortality in fishes. Apart from the ecological and nutritional aspect, it also has economical aspect as it serves as source of income for the people living in coastal areas. So, we can say that heavy metal contamination of waterbodies poses serious detrimental effect on ecological, nutritional, biological and economical aspect of life. Contamination of waterbodies with heavy metals disturbs whole food chain and leads to bioaccumulation. When these metals are ingested by the fish through food chain, it accumulates in their body and leads to detrimental effects such as neurotoxicity, nephrotoxicity, immunotoxicity, hepatotoxicity, degradation of nutritional value and sometimes increase in mortality rate. The accumulation of these metals makes the fish unfit for human consumption and if consumed causes serious health issues in humans. While on the other hand, increase in mortality rate puts the diversity of the fishes of that particular water body in jeopardy. So, we conclude that strict measures should be taken to keep a check on effluents from sewage and industries. They must be treated before being released into the waterbodies as they have toxic pollutants. There is need of sustainable practice to make fruitful use of the available resources we got while trying to minimize the ill effects simultaneously. Fishes are not only a source of food but an important component of aquatic ecosystem. Decline in their diversity might disturb the aquatic ecological balance. Henceforth, there is needed to keep a check on heavy metal contamination of waterbodies for healthy ecosystem, healthy human and healthy food stuffs.

9. Acknowledgement

We sincerely thank Professor Mukhtar Ahmad Khan, Head of Fishery Science and Aquaculture section (Department of Zoology, Aligarh Muslim University) for his kind support. The authors are also thankful to Department of Zoology, Aligarh Muslim University and Department of Biotechnology, Aligarh Muslim University for providing necessary facilities and aid.

10. Conflict of interest

Authors declare no conflict of interest.

11. References

1. Afshan Sehar, Ali Shafaqat, Ameen Uzma, Farid Mujahid, Bharwana Saima, Hannan Fakhir, *et al*. Effect of Different Heavy Metal Pollution on Fish. Research Journal of Chemical and Environmental Sciences 2014;2:74-79.
2. Naeem S, Ashraf M, Babar ME *et al*. The effects of some heavy metals on some fish species. Environ Sci Pollut Res 2021;28:25566-25578. https://doi.org/10.1007/s11356-021-12385-z.
3. EFSA. Opinion of the Scientific Panel on contaminants in the food chain [CONTAM] related to the safety assessment of wild & farmed fish 2005. http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_ 11786 20762697.html
4. El-Moselhy KM. "Accumulation of copper, cadmium and lead in some fish from the Guif of suez," Egyptian Journal of Aquatic Biology and Fisheries 2000;3(1).
5. Bahnasawy M, Khidr AZ, Dheina N. Seasonal variations of heavy metals concentrations in mullet, Mugil cephalus and Liza ramada (Mugilidae) from Lake Manzala, Eygpt. Journal of Applied Sciences Research 2009;5(7):845-852.
6. Kris-Etherton PM, Harris WS, Appel LJ. "Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease," Circulation 2002;106(21):2747-2757.
7. Nordberg Gunnar, Fowler BA, Nordberg Monica, Friberg L. Handbook on the Toxicology of Metals. Reproductive and Developmental Toxicity of Metals 2007;1:213-249. 10.1016/B978-0-12-369413-3.X5052-6.
8. Thophon S, Kruatrachue M, Upatham ES, Pokethitiyook P, Sahaphong S, Jaritkhuan S. Histopathological alterations of white seabass, *Lates calcarifer*, in acute and subchronic cadmium exposure. Environmental Pollution 2003;121:307-320.
9. Olsson PE. Disorders associated with heavy metal pollution. In: Fish Diseases and Disorders (Non-infectious Disorders). (Eds. Leatherland, J.E. and Woo, P.T.K.), CABI International, U.K 1998;2:105-131.
10. Tian J, Hu J, Liu D, Yin J, Chen M, Zhou L, Yin H. Cadmium chloride-induced trans-generational neurotoxicity in zebrafish development. Environ Toxicol Pharmacol 2021;81:103-545. Doi: 10.1016/j.etap.2020.103545. Epub 2020 Nov 7.

PMID: 33171223.
11. Silva AO, Martinez CB. Acute effects of cadmium on osmoregulation of the freshwater teleost *Prochilodus lineatus*: enzymes activity and plasma ions. Aquat Toxicol 2014;156:161-8. Doi: 10.1016/j.aquatox.2014.08.009. Epub 2014 Aug 30. PMID: 25203423.
12. Zhang J, Zheng S, Wang S, Liu Q, Xu S. Cadmium-induced oxidative stress promotes apoptosis and necrosis through the regulation of the miR-216a-PI3K/AKT axis in common carp lymphocytes and antagonized by selenium. Chemosphere 2020;258:127341. Doi: 10.1016/j.chemosphere.2020.127341. Epub 2020 Jun 11. PMID: 32563067.
13. Zheng JL, Yuan SS, Wu CW, Lv ZM. Acute exposure to waterborne cadmium induced oxidative stress and immunotoxicity in the brain, ovary and liver of zebrafish (*Danio rerio*). Aquat Toxicol 2016;180:36-44. Doi: 10.1016/j.aquatox.2016.09.012. Epub 2016 Sep 14. PMID: 27642707.
14. Dangre AJ, Manning S, Brouwer. Effects of cadmium on hypoxia-induced expression of hemoglobin and erythropoietin in larval sheepshead minnow, Cyprinodon variegates. Aquatic Toxicol 2010;99(2):168-175.
15. Sadiq M. Chromium in Marine Environment, Toxic Metal Chemistry in Marine Environments, Marcel Dekker, Inc. New York 1992;6:154-197.
16. Kim JH, Kang JC. Oxidative stress, neurotoxicity, and metallothionein (MT) gene expression in juvenile rock fish *Sebastes schlegelii* under the different levels of dietary chromium (Cr(6+)) exposure. Ecotoxicol Environ Saf 2016;125:78-84. Doi: 10.1016/j.ecoenv.2015.12.001. Epub 2015 Dec 8. PMID: 26680530.
17. Mishra AK, Mohanty B. Acute toxicity impacts of hexavalent chromium on behavior and histopathology of gill, kidney and liver of the fresh water fish, *Channa punctatus* (Bloch) Environ. Toxicol. Pharmacol 2008;26:136-141.
18. Subashini P, Manavalaramanujam R, Ramesh M, Geetha N. Changes in selected biomarkers in freshwater teleost fish, *Cyprinus carpio* var. communis exposed to sublethal concentrations of chromiumsulphate toxicity. Journal of Environmental Science and Engineering 2005.
19. Prabakaran M, Binuramesh C, Steinhagen D, Dinakaran Michael R. Immune response in the tilapia, *Oreochromis mossambicus* on exposure to tannery effluent. Ecotoxicol. Environ. Saf 2007;68(3):372-378. (PubMed: 17261332).
20. Braunbeck T. Sublethal, and chronic effects of pollutants on freshwater fish. Oxford, UK: Blackwell 1994.
21. Markovich D, James KM. Heavy metals mercury, cadmium and chromium inhibit the activity of the mammalian liver and kidney sulphate transporter sat1. Toxicol. Appl. Pharmacol 1999;154:181-187.
22. Yang, Jen-Lee, Chen, Hon-Cheng. Serum Metabolic Enzyme Activities and Hepatocyte Ultrastructure of Common Carp after Gallium Exposure, Zoological Studies 2003;42(3):455-461.
23. Schaefer JK, Szczuka A, Morel FMM. Effect of divalent metals on Hg(II) uptake and methylation by bacteria. Environ Sci Technol 2014;48:3007-3013.
24. Pickhardt PC, Fisher NS. Accumulation of inorganic and methylmercury by freshwater phytoplankton in two contrasting water bodies. Environ Sci Technol 2007;41:125-131.
25. Maulvault AL, Custodio A, Anacleto P, Reploho T, Pousao P, Nunes ML *et al*. Bioaccumulation and elimination of mercury in juvenile seabass (*Dicentrarchus labrax*) in a warmer environment. Environ Res 2016;149:77-85.
26. Barboza LGA, Vieira LR, Branco V, Figueiredo N, Carvalho F, Carvalho C *et al*. Microplastics cause neurotoxicity, oxidative damage and energy-related changes and interact with the bioaccumulation of mercury in the European seabass, *Dicentrarchus labrax* (Linnaeus, 1758). Aquat Toxicol 2018a;195:49-57.
27. Berg K, Puntervoll P, Valdersnes S, Goksøyr A. Responses in the brain proteome of Atlantic cod (*Gadus morhua*) exposed to methylmercury. Aquat Toxicol 2010;100:51-65.
28. Keyvanshokooh S, Vaziri B, Gharaei A, Mahboudi F, Esmaili-Sari A, Shahriari-Moghadam M. Proteome modifications of juvenile beluga (*Huso huso*) brain as an effect of dietary methylmercury. Comp Biochem Physiol D-Genomics Proteomics 2009;4(4):243-248.
29. Pereira P, Puga S, Cardoso V, Pinto-Ribeiro F, Raimundo J, Barata M *et al*. Inorganic mercury accumulation in brain following waterborne exposure elicits a deficit on the number of brain cells and impairs swimming behavior in fish (White seabream-*Diplodus sargus*). Aquat Toxicol 2016;170:400-412.
30. Yamamoto Y, Almeida R, Regina S *et al*. Mercury distribution in target organs and biochemical responses after subchronic and trophic exposure to Neotropical fish *Hoplias malabaricus*. Fish Physiol Biochem 2014;40:245-256.
31. Lee JW, Kim JW, De Riu N *et al*. Histopathological alterations of juvenile green (*Acipenser medirostris*) and white sturgeon (*Acipenser transmontanus*) exposed to graded levels of dietary methylmercury. Aquat Toxicol 2012;109:90-99.
32. Mela M, Randi MA, Ventura DF, Carvalho CE, Pelletier E, Oliveira Ribeiro CA. Effects of dietary methylmercury on liver and kidney histology in the neotropical fish *Hoplias malabaricus*. Ecotoxicol Environ Saf 2007;68(3):426-35. Doi: 10.1016/j.ecoenv.2006.11.013. Epub 2007 Feb 12. PMID: 17296226.
33. Segner H, Wenger M, Möller AM. Immunotoxic effects of environmental toxicants in fish-how to assess them? Environ Sci Pollut Res 2012;19:2465-2476.
34. Morcillo P, Cordero H, Meseguer J *et al*. Toxicological *in vitro* effects of heavy metals on gilthead seabream (*Sparus aurata* L.) head-kidney leucocytes. Toxicol *In vitro* 2015;30:412-420.
35. Sarmento A, Guilhermino L, Afonso A. Mercury chloride effects on the function and cellular integrity of sea bass (*Dicentrarchus labrax*) head kidney macrophages. Fish Shellfish Immunol 2004;17:489-498.
36. Morcillo P, Meseguer J, Esteban MA *et al*. *In vitro* effects of metals on isolated head-kidney and blood leucocytes of the teleost fish *Sparus aurata* L. and *Dicentrarchus labrax* L. head-kidney leucocytes. Fish Shellfish Immunol 2016;54:77-85.
37. Chen QL, Sun YL, Liu ZH, Li YW. Sex-dependent effects of subacute mercuric chloride exposure on histology, antioxidant status and immune-related gene expression in the liver of adult zebrafish *(Danio rerio*). Chemosphere 2017;188:1-9.

38. Olsvik PA, Brattas M, Lie KK, Goksoyr A. Transcriptional responses in juvenile Atlantic cod (*Gadus morhua*) after exposure to mercury-contaminated sediments obtained near the wreck of the German WW2 submarine U-864, and from Bergen Harbor, Western Norway. Chemosphere 2011;83:552-563.
39. Kirillova AV, Danilushkina AA, Irisov DS, Bruslik NL, Fakhrullin RF, Zakharov YA *et al*. Assessment of resistance and bioremediation ability of Lactobacillus strains to lead and cadmium. Int J Microbiol 2017, 7. Doi:10.1155/2017/9869145.
40. Kim JH, Kang JC. Toxic effects on bioaccumulation and hematological parameters of juvenile rockfish *Sebastes schlegelii* exposed to dietary lead (Pb) and ascorbic acid. Chemosphere 2017;176:131-40. Doi:10.1016/j.chemosphere.2017.02.097.
41. Ramsdorf WA, Ferraro MV, Oliveira-Ribeiro CA, Costa JR, Cestari MM. Genotoxic evaluation of different doses of inorganic lead (Pb(II)) in *Hoplias malabaricus*. Environ Monit Assess 2009;158:77-85. doi:10.1007/s10661-008-0566-1.
42. Paul N, Chakraborty S, Sengupta M. Lead toxicity on non-specific immune mechanisms of freshwater fish *Channa punctatus*. Aquat Toxicol 2014;152:105-12. doi:10.1016/j.aquatox.2014.03.017.
43. Verstraeten SV, Aimo L, Oteiza PI. Aluminium and lead: molecular mechanisms of brain toxicity. Arch Toxicol 2008;82:789-802. https://doi.org/10.1007/s00204-008-0345-3.
44. Hsu P. Antioxidant nutrients and lead toxicity. Toxicology 2002;180(1):33-44. Doi:10.1016/s0300-483x(02)00380-3
45. Westerink RH, Vijverberg HP. Ca(2+)-independent vesicular catecholamine release in PC12 cells by nanomolar concentrations of Pb(2+). J Neurochem 2002;80(5):861-73. Doi: 10.1046/j.0022-3042.2001.00751.x. PMID: 11948250.
46. Richetti SK, Rosemberg DB, Ventura-Lima J, Monserrat JM, Bogo MR, Bonan CD. Acetylcholinesterase activity and antioxidant capacity of zebrafish brain is altered by heavy metal exposure. Neuro Toxicology 2011;32(1):116-122. Doi:10.1016/j.neuro.2010.11.001.
47. Zhai Q, Wang H, Tian F, Zhao J, Zhang H, Chen W. Dietary *Lactobacillus plantarum* supplementation decreases tissue lead accumulation and alleviates lead toxicity in Nile tilapia (*Oreochromis niloticus*). Aquac Res 2011;48:5094-5103. https://doi.org/10.1111/are.13326.
48. Rabitto IS, Alves Costa JR, Silve de Assis HC, Pelletier EF, Akaishi FM, Anjos A *et al*. Effects of dietary Pb(II) and tributyltin on neotropical fish, *Hoplias malabaricus*: histopathological and biochemical findings. Ecotoxicol Environ Safety 2005;60:147-156. 10.1016/j.ecoenv.2004.03.002.
49. Patel M, Rogers JT, Pane EF, Wood CM. Renal responses to acute lead waterborne exposure in the freshwater rainbow trout (*Oncorhynchus mykiss*). Aquat Toxicol 2006;30:362-371.
50. Raza Bakhtawar, Javed Muhammad, Ambreen Faiza, Latif Fariha. Toxic effect of lead chloride on antioxidant enzyme in the liver and kidney of fish 2016. 10.13140/RG.2.2.11352.90889.
51. Paul N, Chakraborty S, Sengupta M. Lead toxicity on non-specific immune mechanisms of freshwater fish *Channa punctatus*. Aquat Toxicol 2014;152:105-12. Doi: 10.1016/j.aquatox.2014.03.017. Epub 2014 Mar 28. PMID: 24747082.
52. Adeyemo Olanike, Adedeji Olufemi, Offor CC. Blood lead level as biomarker of environmental lead pollution in feral and cultured African catfish (*Clarias gariepinus*). Nigerian Veterinary Journal 2011, 31. 10.4314/nvj.v31i2.68957.
53. Witeska Malgorzata. Stress in fish-hematological and immunological effects of heavy metals. Electronic Journal of Ichthyology 2005, 1.
54. Yin Y, Zhang P, Yue X, Du X, Li W, Yin Y *et al*. Effect of sub-chronic exposure to lead (Pb) and *Bacillus subtilis* on *Carassius auratus* gibelio: Bioaccumulation, antioxidant responses and immune responses. Ecotoxicol Environ Saf 2018;161:755-762. Doi: 10.1016/j.ecoenv.2018.06.056. Epub 2018 Jun 26. PMID: 29957583.
55. Savan R, Sakai M. Genomics of fish cytokines. Comparative Biochemistry and physiology. Part D, Genomics & Proteomics 2006;1(1):89-101. Doi: 10.1016/j.cbd.2005.08.005.
56. Dai J, Zhang L, Du X *et al*. Effect of Lead on Antioxidant Ability and Immune Responses of Crucian Carp. Biol Trace Elem Res 2018;186:546-553. https://doi.org/10.1007/s12011-018-1316-z.
57. Bothaina Khidr M, Imam Mekkawy AA, Ahmed Harabawy SA, Abdel Salam Ohaida MI. Effect of Lead Nitrate on the Liver of the Cichlid Fish (*Oreochromis niloticus*): A Light Microscope Study. Pakistan Journal of Biological Sciences 2012;15:854-862.
58. Offor SJ, Mbagwu HOC, Orisakwe OE. Lack of beneficial effect of activated charcoal in lead induced testicular toxicity in male albino rats. Middle East Fertility Society Journal 2017;22(3):189-192.
59. Sargent JR. Fish oils and human diet. Br. J Nutr 1997;78:S5-S13.
60. Tacon AGJ, Metian M. Fish matters: importance of aquatic foods in human nutrition and global food supply. Rev. Fisher. Sci 2013;21:22-38.
61. Siddiqui AA, Chang S. Cadmium chloride intoxication and evaluation of protein changes in *Clarias batrachus* (Linn). International Journal of Current Microbiology and Applied Sciences 2014;3(2):787-794.
62. Bedii CiCi K, Kenan Engin. The effect of Cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissue of *Cyprinus Carpio* 2005.
63. Lund EK. Health benefits of seafood; Is it just the fatty acids? Food Chem 2013;140:413-420.
64. EFSA Panel on Dietetic Products. Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA J 2010;8:1461-1568.
65. Ramirez DC, Gimenez MS. Lipid modification in mouse peritoneal macrophages after chronic cadmium exposure. Toxicology 2002;172:1-12.
66. Kawamoto S, Kawamura T, Miyazaki Y, Hosoya T. [Effects of atorvastatin on hyperlipidemia in kidney disease patients]. Jpn. J Nephrol 2007;49:41-48. (In Japanese).
67. Konar V, Aydogmus C, Orun I, Kandemir S. The effects of cadmium on fatty acid composition in the muscle and skin of Juvenile rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792). J Anim. Vet. Adv 2010;9:1191-1196.