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A Comprehensive Review on the Deleterious Effects of Heavy Metal Bioaccumulation on the Gills and Other Tissues of Freshwater Fishes

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Heavy metals can be harmful to aquatic organisms when exposed for a short (acute) or long (chronic) period. They have made a tremendous contribution to human welfare, but they also have considerable negative impacts on organisms that are not their targets. Runoff and groundwater leaching from a range of hazardous metals have a significant risk of contaminating aquatic habitats that pass through industrial or agricultural areas, which could directly threaten freshwater life, especially delicate animals like fish. Fish are the most well-known model for determining the extent of aquatic pollution. Since fish play a significant part in the food chain, the investigation into how toxic metals affect fish might help determine whether or not metals have harmful impacts on human health. This review attempted to consolidate all available scientific findings on the accumulation and uptake of various heavy metals (As, Hg, Cd, Cu, Cr, and Pb) as well as the overall histopathological changes caused by long-term exposure to sublethal doses of these heavy metals on the gills and other tissues of the freshwater fishes. Keeping in mind the above facts, in this review, an effort has been made to elucidate the deleterious impact of metals on the gills of freshwater fishes.

Keywords: Freshwater Fishes; Gills; heavy metals; histopathological changes; sublethal doses; Toxicity.

The health of an aquatic system can be measured/assessed by the quality and quantity of flora and fauna present in it. The direct and indirect health standards of the human community are greatly influenced by the quality of water used. Hence, the aquatic system all around, or in other words, the health of humans and the earth's aquatic system go hand in glove with each other. Fish serve as an excellent model to understand the health of mechanical aspects of chemical toxicity and oxidative stress in the aquatic ecosystem. The organism present in the aquatic environment accumulates toxic chemicals which ultimately affect not only the growth and reproductive capability of the organisms but also the health of human beings. In the aquatic food chains where fish act as top consumers are known to accommodate in their bodies a huge amount of heavy metals[1]. Heavy metals are metallic elements that occur as a natural element in aquatic ecosystems and have

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a permissible limit that has elevated in recent days because of an increase in agricultural and industrial activities[2].

The indiscriminate release of heavy metals from domestic, industrial, and other anthropogenic activities has become a cause for concern due to their ability to contaminate natural aquatic ecosystems[3]. These heavy metals are released directly or indirectly into the aquatic system and all together constitute a serious threat to our aquatic flora and fauna [4,5]. Heavy metals accumulate in higher concentrations in different organs of the fish and thereby, entering the food chain and human metabolism through fish consumption and finally posing a serious threat to human health[6,7]. These heavy metals once entered the blood are transported either to the other organs of storage such as bones, liver, or carried forward to fat, kidney, or gills[8].

Non-biodegradable nature of heavy metals results in their bioconcentration in different tissues of fish through different biosorption and metallic processes[10,11]. According to Specie and Hemelink, 1985 [12] when the rate of elimination of heavy metals exceeds the rate of metal uptake by living organisms, this results in the bioaccumulation of metals in the body of the living organisms. Thus, monitoring the levels of bioaccumulation of heavy metals helps in examining the potential threats that they impose on human health and acts as an indicator of the spatial and temporal extent of the accumulation of metals[13].

Heavy metals are directly or indirectly involved in inducing toxicity of other agents thereby enhancing the genotoxicity[14]. Direct exposure to heavy metals results in pathogenic changes in fish and also causes a decrease in androgenic or estrogenic secretions [15].

Effect of heavy metals on fishes

Aquatic organisms can accumulate heavy metals up to certain limits only. Fishes and shellfish are known to store a higher concentration of heavy metals than that present in sediments or water [16,17,18]. Govind and Madhuri, 2014 [19] while studying the effects of heavy metals on fish found that no doubt, these heavy metals are important for life, but are still known to have adverse effects on living organisms which include a decrease in fitness, effect on reproductive health and eventually leading to Carcinoma and finally death.

A lot of research has been carried out on the heavy metal contamination in fish muscle, but due to poor accumulation potential, the whole body of the fish is not enough to determine the contamination of heavy metals. So, it is equally important to study other tissues such as the gills and liver of the fish. Gills which act as an important organ of gaseous exchange in fishes tend to accumulate a large quantity of water-soluble heavy metals through the process of ion exchange. Various physiological and ecological attributes such as species, feeding behavior, habitat, and growth rate determine the distribution and accumulation of different heavy metals in the aquatic ecosystem[21].

Accumulation of metal in fishes

Copper and Zinc

In a toxicology study, Kamaruzzaman *et al.*, 2010 [22] investigated the accumulation of Zn, Cu, and Pb in the gills of Tilapia fingerlings (*Oreochromis niloticus*). Fish were exposed to copper, zinc, and lead at a concentration of 96h LC50, which tends to collect less heavy metal in the gills. On day 30, when the concentration has increased threefold to 142 g/kg, they see an enhanced increase in metal uptake.

Kumar and Ram (2015) [23] investigated the impact of copper and zinc on the oxygen consumption of the gills of the freshwater fish *Clarias batrachus*. For 15 days, they exposed juvenile fish to concentrations of 0.3, 0.4, and 0.5 ppm. They observed histological alterations in the gill that led to necrosis, a fusion of secondary lamella, and separation of epithelial secondary gill lamellae. They found that when exposure time and concentration increased, mortality tended to rise for copper and zinc concentrations. The strong effect of copper resulting from copper uptake by gills induces gill disorder more than zinc.

Further, an investigation by Loro *et al.,* 2012 [24] on the effect of Zinc on the liver, kidney, gills, and white muscle of *Fundulus heteroclitus* (Killifish) exposed to sublethal level (500ug/L) of waterborne zinc for 96h in 0% (freshwater), 10% (3.5ppt), 30% (10.5ppt), 100% seawater (35 ppt). They observed a significant increase in the activities of glutathione-S-transferase (GST) and superoxide dismutase (SOD). Total oxidative scavenger capacity (TOSC) is depleted in Zn-exposed fish which leads to decreased SOD, GST, and GSH, catalase activity.

The effect of dietary Zinc oxide nanoparticles (nano-ZnO) and $ZnCl_2$ on adult fathead minnows (*Pimephales promelas*) were studied by Gagné F *et al.,* 2016 [25]. They exposed fish to 5, 10, and 20% v/v of Zinc oxide for 21 days. They observed decreased leukocyte viability with increasing effluent concentration. They noticed decreased phagocytosis activity.

Copper and Other Metal

The effect of copper on the survival, growth, and gill morphology of *Danio rerio* in the Juvenile stage was studied by Campagna *et al.,* 2008 [26]. They exposed fish at concentrations of 20.0, 60.0, 120, and 360.0 µg/l. They noticed that growth and gill morphology were most suitable parameters than survival to evaluate the toxicity of copper as it affects both survival and growth rate. The negative effect on the survival and growth of fish was observed for the intensity of gill lesions.

Similarly, Chakpram and Gupta, 2014 [27] studied the effect of cadmium and copper on the gill surface ultrastructure of *Anabas testudineus* (Bloch). They exposed fish for 96 hours. They noticed a change in the gill surface, a fusion of the adjacent secondary lamella, edema, and disruption of gill epithelium. The gill emerged as a sensitive indicator of cadmium and copper because of their direct contact with water and the responses of their surface epithelial cells from the effect of cadmium and copper.

Mahboob *et al*., 2016 [28] examined the effects of Cu, Fe, Pb, and Cr on the gills, kidney, liver, and muscle of *Wallago attu* and *Cyprinus carpio*, two fishes that reside in the Indus. They observed the subsequent pattern, i.e Fe>Cu>Cr>Pb in the kidney and muscle of Wallago, and Fe>Cr>Pb>Cu in the gills and muscle of *Cyprinus carpio*. *Cyprinus carpio* and *Wallago attu* have a rise in body weight that is correlated with an increase in the levels of Cu, Cr, Fe, and Pb.

Chromium

The effect of chromium on the histology of the liver and gill of freshwater fish *Labeo rohita* in its fingerling stage was observed by Muthukumaravel and Rajaraman, 2013 [29]. They exposed fish to 10% sub-lethal concentration for 30 days. They observed the fusion of gill lamella, hypertrophy, and degeneration of the epithelium. The gill exposed to chromium showed mild histological alteration.

Afshan *et al.,* 2014 [30] studied the effect of metals like cadmium, zinc, chromium, and lead in polluted fish on the gills of *Cyprinus carpio*. They exposed fish to chromium concentration at 1

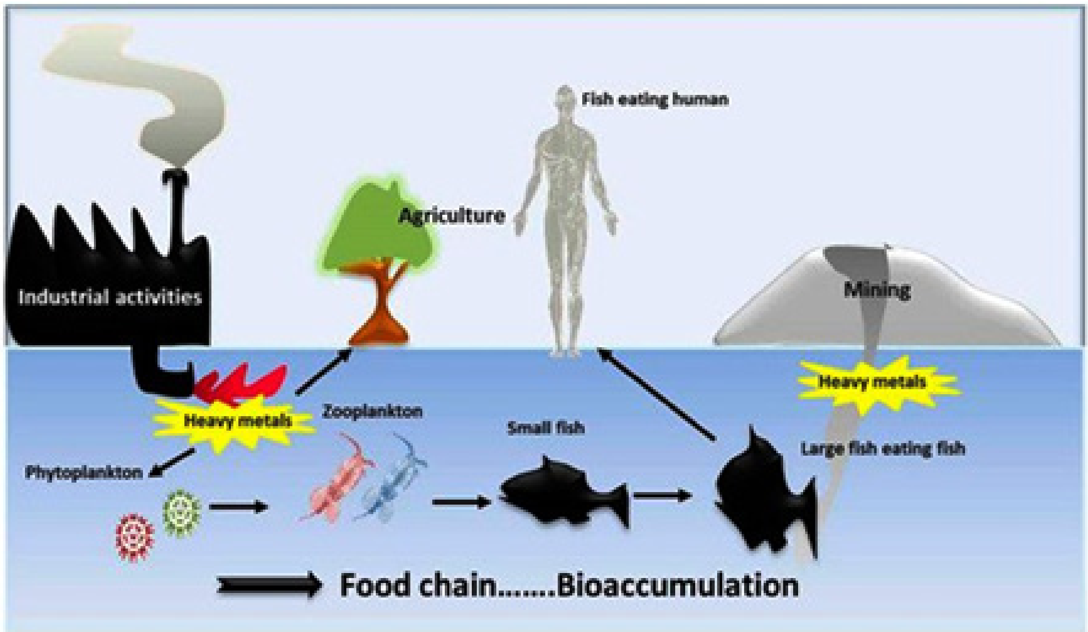

Fig. 1. Heavy metal bioaccumulation in fishes and its diverse impact on the food chain and the aquatic ecosystem. (Source: Mehana et al., 2020 [9])

to 10 µg/l. They observed that metal accumulates through freshwater fishes are badly affected. They reported the following order of accumulation in the liver i.e Pb>Cd>Ni>Cr and in the gills i.e Cd>Pb>Ni>Cr and conclude that the concentration of cadmium and lead was raised in the gills.

Rahmani *et al.*, 2016 [31] studied histopathological alterations in the gill of Zebrafish (*Danio rerio*) exposed to 10, 32 & 100 mg/l of chromium and barium by TiO_2 nanoparticles. They noticed gill alteration like an aneurism, dilated, clubbed tips, hyperplasia, oedema, curvature, an increase in the mucous secretion, and proliferation in the erythrocytes of the cartilaginous core. They observed increased tissue damage to the concentration level of NPs.

Lead

The toxic effect of lead nitrate on the gill of air-breathing catfish (*Heteropneustes fossilis*) Bloch was studied by Parashai and Banerjee, 2002

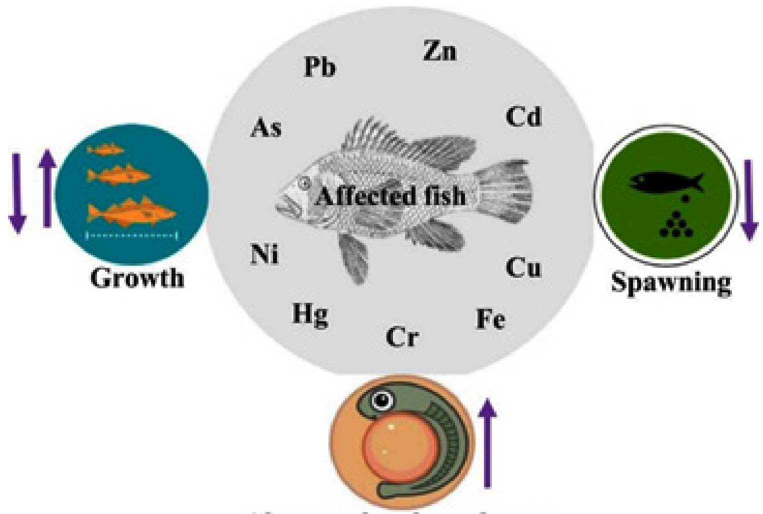

Fig. 2. Depicts the effects of the accumulation of different heavy metals in fish affecting the growth, spawning, and embryonic development in fishes. (Source: Taslima *et al.,* 2022 [20])

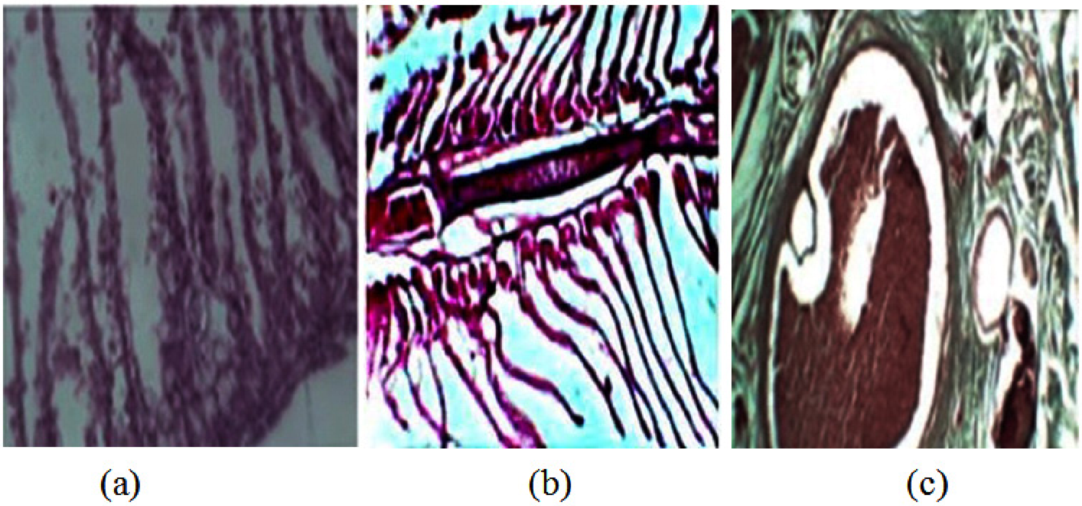

Fig. 3. Exposure of arsenic (As) to the gills of Odontesthes bonariensis and their microscopic view show (A) Hyperplasia of mucous cells (40×). (B) Curling of secondary lamellae (10×) (C) Congestion and telangiectasia in blood vessels in gill filaments (40×) (Source: Puntoriero et al., 2018 [32])

[33]. They exposed fish to a concentration, i.e 96h LC50. They noticed that the fish exhibited rapid alteration that includes detachment and the lifting of epithelial lining from the surface of the gill filament and respiratory lamella.

Aldoghachi *et al.,* 2015 [34] studied the ultrastructural effect on gill tissue induced in red tilapia (*Oreochromis species*) by lead exposure. They exposed fish for 96 hours and observed a change in the epithelial cell, a fusion of secondary lamella, hypertrophy, and coagulation necrosis in the pavement cell. The structural design of gill tissue was disrupted when lead accumulates on it.

Lead, Mercury, and Other Metals

The effect of lead, mercury, and arsenic on the gill, muscle, and liver of *Otolithes rubber, Pampus argenteus, parastromateus niger, onnchorynchus mykiss, scomberomorous commerson* were studied by Sobhanardakani *et al.,* 2011 [35]. They observed that the mercury concentration reaches a maximum in the gill of *niger, and rubber*. Lead content reaches a maximum in the gill of *niger.* They noticed that the concentration of lead, mercury, and arsenic in the gill, muscle, and liver was lower than the permissible level.

Brraich and kaur, 2017 [36] studied the effect of the concentration of lead nitrate in the gill, scales, kidney, and liver of *Labeo rohita* at the fingerling stage. They exposed fish from 1mg/l to 50mg/l for 96 hours. They noticed that the median lethal concentration (LC_{50}) of lead nitrate in *Labeo rohita* is 34.20 mg/l. They observed that the effect of lead nitrate was dependent on duration as well as concentration.

Chavan and Muley, 2014 [37] studied the effect of mercury chloride on the gill and liver of *Cirrhinus mrigala*. They exposed $HgCl_2$ at a concentration (of 0.0206- 0.0402) ppm and lead nitrate at (28.2-14.1) ppm. They observed that gill lamella shows lamellar degeneration, epithelial lifting, and necrotic changes in the intercellular cell. They observed a significant increase in glutamate pyruvate transaminase and glutamate oxaloacetate transaminase in the liver.

Accumulation of fluoride in the gill tissue of *Notopterus* under fluoride stress was studied by Aziz *et al.,* 2015 [38]. They exposed fish at 1.5 and 3g/70l concentration for 24, 48,72 hours, and 45 days. They noticed a decreased permeability of gill epithelium which significantly affects the scale loss due to fluoride mobility with an increase in metal accumulation in the gill. They observed that fluoride decreases the absorption of oxygen in the gill filament which results in the death of fish at long time exposure.

Ribeiro, 2015 [39] studied the effect of cerium dioxide nanoparticles in the gill of (Rainbow trout) *Oncorhynchus mykiss.* He exposed juvenile-stage fish to 0.25, 2.50, or 25.00mg/l cerium dioxide for 96 hours and noticed that CuO_2 nanoparticles caused genetic damage and tissue alteration in the

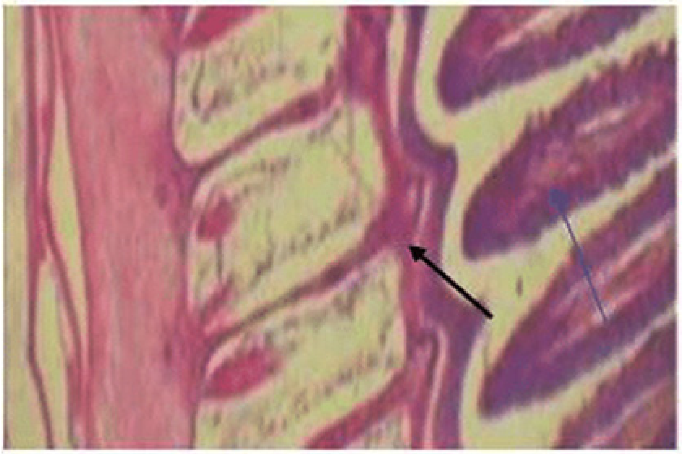

Fig. 4. Represents the gills of *Clarius batrachus* showing the fusion of secondary gill filaments (blue row) and detachment in the gills (black row) on exposure to mercury under the microscope. (Source: Selvanathan *et al.,* 2013 [40])

gill. He noticed the increased activity of catalase in gill exposed to 2.50 and 25.00mg/l cerium dioxide and decreased activity at 0.25 mg/l cerium dioxide in gill.

Diwakar Ram Tripathi, 2014 [41] studied the effect of 250mg/l, 500mg/l, 750mg/l, and 1000mg/l cadmium in gill, liver, and kidney of *Channa punctatus* (Bloch) and *Mystus tengara* (Hamiltan). They noticed that an increased concentration of cadmium chloride resulted in respiratory problems. Further, the mortality rate was observed to be more in *Mystus tengara* than in *Channa punctatus*.

Toxicology of Other Heavy Metal

Mensoor and Said, 2018 [42] studied the concentration of heavy metals and their toxicity in the freshwater fish species belonging to the genus *Barbus* from the Tigris river, Baghdad. The results of the study showed that the concentration of Chromium (0.002ppm) and Cadmium (0.002ppm) was the highest among all the metals studied and exceeds the acceptable limits for consumption by humans as recommended by WHO and the Food and Agricultural Organization of UN.

Jia *et al.,* 2017 [43] experimented to analyze the concentration of different heavy metals such as Cd, Fe, Cu, Mn, and As in the gills, muscles, and liver of three different fish species collected from Xiang River, Southern China. The results of the study depicted that the liver accumulates Cd and Cu which can be attributed to metallothionein protein while the gills accumulate high concentrations of Pb and Mn indicating that water is the main pathway for metal uptakes. Further investigation revealed that omnivorous species accumulate a high percentage of nutrient elements such as Fe, Zn, Mn, and Cu while the carnivorous species such as *P. fulvidraco* possess a higher concentration of toxic elements like As, Pb, and Cd in their gills, liver, and muscle.

Shah *et al.,* 2019 [44] studied the toxicological impact of LC_{15} of metals like Cr, Cu, and Pb on the cell morphology and RBC nucleus, hematological indices, and gill and muscle tissues of Grass Carp (*Ctenopharyngodon Idella*) for 24, 48, 72 and 96 hrs. The results of the study depicted that Pb was absorbed in maximum concentration by gill and muscle tissues followed by Cu and Cr. Various histological variations like Swelling and fusion of cells, destruction of epithelial cells, epithelial lifting, club gill filaments, irregular cells, gill bridging, inflammatory cells, and cellular necrosis were also seen in the gill tissues when the fish was exposed to different concentrations of heavy metals depicting incorporation of heavy metals to the aquatic fauna and food chain ultimately impacting the human beings.

The accumulation of heavy metals in various fishes and their parts was found to be non-linear. This trend in fisheries has been observed by many different authors. Aghoghovurwia *et al.,* 2016 [45] studied the trend of heavy metals in various fishes based on their different body parts like gills, liver, kidney, and muscles and found the following trend in gills i.e Fe Ã Zn > Pb > Ni > Cr > Cu > Cd and Fe > Zn > Pb > Ni > Cu > Cr > Cd in the liver, muscle, and kidney.

Similarly, Ekeanyanwa *et al.,* 2011 [46] found the trend i.e Cd > Mn > Ni > Cr > Pb in the gills, Mn > Cd > Ni > Cr > Pb in the muscles, and Mn > Cd > Cr > Ni > Pb in the liver of Catfish and Tilapia fish while the trend Mn > Cd > Ni > Cr > Pb was reported in the bones of the catfish and the order Mn > Ni > Cr > Cd > Pb in the bones of only Tilapia fish.

MHuseen and Mohammad, 2019 [47] studied the toxic effect of Pb, Cu, and Zn on the gills, liver, and muscle of *Cyprinus carpio*, *Liza Abu*, and Grass carp during the four seasons of the year. The results of the study showed that Pb and Zn were present in the highest concentrations in the gills of *C. carpio* during the autumn. Similarly, Cu was present in maximum concentrations in the gills of *C. carpio* and Grass carp during the summer season. *Liza abu* contains maximum concentrations of Pb and Zn in the muscle tissue during the autumn and Zn during the summer season. Grass carp on the other hand contain maximum concentrations of Cu in the gills during summer and Pb in the liver during autumn.

Garai P *et al.,* 2021 [48] discussed various bioaccumulation and toxic effects of heavy metals on fish health and focus on enforcing various laws and legislation for the protection of aquatic ecosystems from the toxic effects of heavy metals and suggest various important steps to lessen the deleterious effects of heavy metals on the environment and human health. They also put forward certain recommendations which include regular monitoring of levels of heavy metals in sediments, water, and soil, decontamination of industrial, and agricultural waste and awareness among masses, and encouraging more scientific research.

Wei *et al.,* 2014 [49] studied the concentration of different heavy metals like Cr, Cu, Cd, As, Pb, Zn, and Ni in the muscle tissue of eleven different fish species collected from Poyand Lake in China. During the study, they reported higher concentrations of heavy metals in the benthic fish as compared to the pelagic fish while the concentration of metals in the eleven fish species was found lower than the recommended limits. Pb and Ni were reported to be in higher concentrations in the gills, Cu in the livers while Zn and Cd were present in maximum concentrations in the kidneys.

Lima *et al.,* 2022 [50] studied the Potentially Toxic Elements (PTE) bioaccumulation in fish species collected from the Southeastern Mineral province of Brazil and focus on the associated environmental and human health risks. According to them, various anthropogenic activities are the main reason behind the drastic increase in the concentration of Potentially Toxic Elements in the fish species. The results of the study depicted a higher concentration of PTE in the water, sediments, and fish species of Parauapebas river and the Gelado stream. Moreover, the concentration of Mn (0.2 mg/L) and Fe (0.3 mg/L) in the water and Pb (> 2 mg/kg) and Cr (o.1 mg/kg) in the fish species were found to be higher than the recommended Brazilian Legislature Threshold. The presence of Pb in all the Fish species and Mo in *L. trifasciatus* is associated with risks to human health. The results of the study focus on the implementation of suitable techniques for controlling and monitoring contamination by various environmental agencies.

The concentration of heavy metals (Zn, Hg, and Cu) was detected by £uczyñska *et al.,* 2018 [51] in the gills, gonads, liver, and muscles of perch and roach collected from the lake Pluszne, Poland. Zn was detected in higher concentrations in the gonads of perch and gills of roaches. The concentration of Zn in the gills of perch shows a positive correlation with their weight while in the gills of roach, the concentration of Zn decreased with weight. Moreover, a range from very low to low concentration was seen in all the tissues, and also a very low value of THQ and HI (<1) was seen which indicates that fish is fit for consumption by the consumers.

Velusamy *et al.,* 2014 [52] determined the concentration of heavy metals in the seventeen commercially important fish species from Mumbai Harbor and found that the concentration of toxic elements like Cd, Cr, and Hg was lower than the recommended limits fit for human consumption. All the fish species possess a lower concentration of heavy metals except *C. dussumieri* and *J. elongatus* which contain the highest concentration of all the metals studied. Thus, the results of the study depicted that all the sampled fish species contain heavy metals but within the limits recommended by the FAO, WHO, and the European Union.

The bioaccumulation of heavy metals like Pb, Zn, Cu, and Cr in common fish species like *L. fulviflamma*, *C. chanos*, *Arius sp*., and *T. jarbua* from the Pulicat lake, Chennai was studied by Akila *et al.,* 2022 [53]. The heavy metals showed the following order of accumulation in the fish samples: Cu> Cr> Zn> Pb. The results of the study showed that Pb and Zn were present within the acceptable limits while the concentrations of Cu and Cr exceeds the limit fit for human consumption. The gills of *C. chanos* and *L. fulviflamma* showed higher bioaccumulation of Zn and Cu. Moreover, *C. chanos* also showed the highest concentration of Cr in the gills while Pb showed maximum bioaccumulation in the liver and gills as compared to the muscles of the fish species. Thus, the results of the study depicted that a maximum accumulation of heavy metals was reported in the gills and liver of the fish.

Mohiuddin *et al.,* 2022 [54] investigated the concentration of heavy metals in finfish and shellfish species to find out the extent of pollution in the tropical estuary and reported the following trend in the bioaccumulation of heavy metals in the edible tissues of the fishes i.e *A. bato* > *C. cirrhosis* > *A. grammepomus* > *M. dobsoni* > *P. paradiseus* > *O. panna* > *M. rosenbergii* > *N. smithi* > *S. phasa* and the metal concentrations showed the following trend: Zn > Fe > Cr > Ni > Pb. Maximum concentrations of Zn and Pb were exhibited by *C. cirrhosis* whereas maximum Ni concentration was seen in *N. smithi*, Zn in *A. bato,* and Fe in *S. phasa*. In all the shellfish and finfish species except *S. phasa, C. cirrhosis, N. smithi,* and *A. bato*, the mean chromium concentration was much higher than the recommended value of 1mg/kg. However, the concentration of Fe does not exceed the acceptable limit in the fish and the shellfish samples except *S. phasa* while the concentration of Zn and Cr exceeds the standard recommended values of FAO.

El-Moselhy *et al.,* 2014 [55] determined the concentration of heavy metals in the gills, muscles, and liver of pelagic and benthic fish species found in the Egyptian Red Sea. Maximum concentrations of Pb and Mn were reported in the gills, while Zn, Fe, and Cu were found in the highest concentrations in the liver. Minimum concentrations of heavy metals were seen in the muscles. All the metals showed concentrations within the permissible limits set by International legislation. The results of the study showed that the accumulation of metals varied from species to species and may depend on various factors like swimming patterns, feeding behavior and genetic tendency, and other factors like geographical distribution and age.

Mahapatra *et al.,* 2022 [56] examined the histopathological changes occurring in the gills of *H. fossilis* when exposed to Lead nitrate (0.4 and 4 mg/L) for a period of 15 days. The results of the study showed various histopathological changes occurring in the gill tissues of *H. fossilis* which include fusion of epithelium of the gill filaments and secondary lamellae, alteration in the structure of secondary lamellae and hypertrophy and swelling of the epithelial cells. There was a decrease in the activity of enzymes like Peroxidase and Catalase while the anti-oxidant enzymes showed an increase in their activity depicting oxidative stress. Moreover, a decrease in the hematological parameters was also seen with exposure to lead nitrate depicting metal toxicity. Thus, it can be concluded that gills are the vital organ that represents the physiological conditions of the fish and the degree of contamination in the nearby aquatic environment.

Histopathological effects of lead nitrate on the gills of *Puntius ticto* were studied by Choudhary *et al.,* 2019 [57]. The results of the study showed various changes in the gill architecture of *P. ticto* which include deposition of lead, Lamella shrinkage, damage in the formation of tissue vacuole, swelling in the gills epithelial layer, and the fusion of gill lamellae tips

CONCLUSION

It is well known that metals in aquatic environments can have a variety of effects on aquatic animals. Today metals pose a serious concern because they alter the Physicochemical characteristics of water, disrupt the environment's delicate equilibrium, enter food chains, and physically harm aquatic fauna's critical tissues. Numerous abnormalities are brought on by continuous exposure to these chemicals, which also shortens organisms' lives. The most important route of heavy metal incorporation in the fish is the gills and the digestive tract. Metals like nickel, arsenic, cadmium, chromium, copper, zinc, and lead are some of the major toxins that cause deleterious effects in fish. Thus, in this paper, an extensive review of the literature was done and the hazardous effects of various heavy metals on the gills and other tissues have been documented. It can be concluded that Metals cause an instant reaction in fish, as illustrated by various functional and structural changes seen in various organs, including genetic and enzymatic effects, that ultimately increase the susceptibility of the exposed fish to various diseases by alteration in the innate immunity of the fish. Humans are highly dependent on fish for their protein and other nutrient requirements. Therefore, any heavy metal contamination can be equally dangerous for humans. Fish can be used as important bio-indictors, playing an important role in monitoring heavy metals pollution. Moreover, Regular monitoring of heavy metal levels in the aquatic system and the enforcement of law and legislation is highly essential for dealing with such serious environmental threats.

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Conflict of Interest

The authors declare no conflict of interest.

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