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Effects of heavy metals on fish physiology – A review

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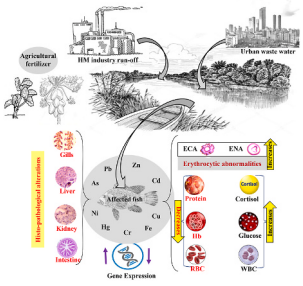
HIGHLIGHTS

- Heavy metal in water cause toxicity to fish is a global concern.
- Describe mode of actions of heavy metals in living tissues.
- Provides a general view on the effects of heavy metals on fish physiology.

GRAPHICAL ABSTRACT



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ABSTRACT

The pollution by heavy metals poses a serious threat to the aquatic environment and to the organisms if the concentration of heavy metals in the environment exceeds the safe limits. Due to their non-biodegradable and long persistence nature in the environment, heavy metals cause toxicity in fish by producing oxygen reactive species through oxidizing radical production. In this review, we investigated the effects of heavy metals on fish physiology with special emphasis on hemato-biochemical properties, immunological parameters especially hormones and enzymes, histopathology of different major organs and underlying molecular mechanisms. All those parameters are significantly affected by heavy metal exposure and are found to be important bio-monitoring tools to assess heavy metal toxicity. Hematological and biochemical alterations have been documented including cellular and nuclear abnormalities in different fish species exposed to different concentrations of heavy metals. Major fish organs (gills, liver, kidneys) including intestine, muscles showed different types of pathology specific to organs in acute and chronic exposure to different heavy metals. This study also revealed the expression of different genes involved in oxidative stress and detoxification of heavy metals. In a nutshell, this article shades light on the manipulation of fish physiology by the heavy metals and sought attention in the prevention and maintenance of aquatic environments particularly from heavy metals contaminations.

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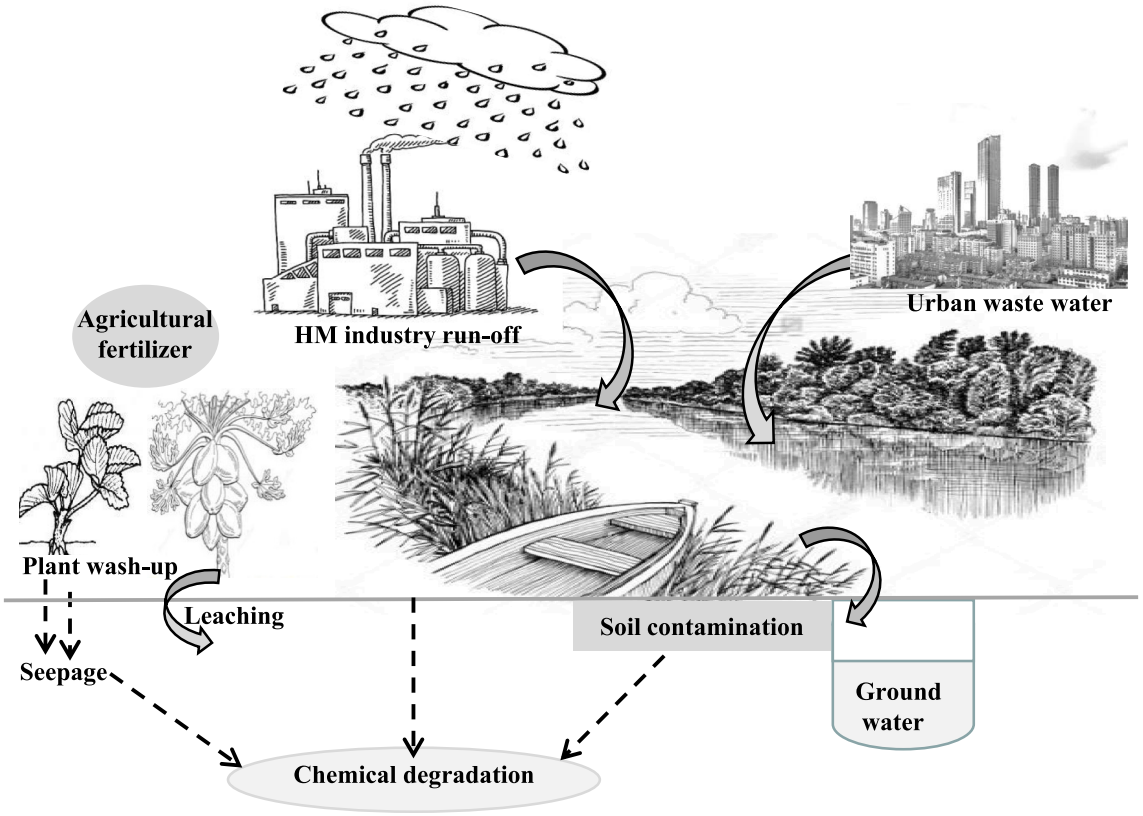

Fig. 1. The possible ways of heavy metals get into water body.

1. Introduction

In the era of industrial revolution, keeping the environment sustainable and sound is a big concern and the rapid expansion of industries in the last couple of decades by added extra fuel to the fire which eventually sinking the environment in the threshold limits of tolerance (Kavitha et al., 2010). Out of 70 metals and metalloids present in the environment 23 are categorized as heavy metals/trace metals, some of which are considered as strong biological poisons (Wood et al., 2011a). Heavy metals are the core group of contaminants found in the environment and divided into essential (Fe, Cu, Zn etc.) and non-essential (Pb, Cd, Cr etc.) metals. They can neither be created nor destroyed and has direct relationship with the environmental pollution concerning their potentiality to cause toxicity in the biota (Duffus et al., 2007; Rahman et al., 2020; Sarkar et al., 2022). The heavy metals are produced from natural and anthropogenic activities including industrial discharge, as contaminants in agrochemicals, traffic, domestic etc. and their indiscriminate discharge without proper or no treatment into the environment possess a great threat to the survival of living organisms (Fig. 1). Heavy metals are stable compounds, non-biodegradable, shows tendency to accumulate in the sediments and long half-life period in the environment making them hard to manage. The properties like bio-accumulation and bio-magnifications in the living tissues, and inability to remove through oxidation, precipitation or bioremediation like organic pollutants making elements of special concern. Aquatic environments, especially rivers and sea are the ultimate receiver of such contaminants/heavy metals and slight alterations in the quality of the environment including physicochemical properties can have a negative impact on the normal physiology of the aquatic organisms especially fish which are very sensitive to such changes (Lakra and Nagpure, 2009; Akter et al., 2021). They affect the aquatic ecosystems either directly by entering into the living forms causing toxicity or indirectly through breaking the food web (Capillo et al., 2018; Merola et al., 2021; Shiry et al., 2021). Bioavailability and the uptake of heavy metals depend on many factors such as concentration of heavy metals, its exposure period, interaction with other metal, age and size of the fish, detoxifying mechanisms, metabolic processes of fish, feeding behavior, physico-chemical parameters of the environment etc. (Delahaut et al., 2020; Kovendan and Vincent, 2013).

Fish is the top consumer in the aquatic food web and are considered as ideal organisms for toxicology and toxicogenomics studies (Aliko et al., 2018, 2019; Burgos-Aceves et al., 2019; Das et al., 2012; Sula et al., 2020b). Fish are highly sensitive to any kind of environmental alterations that make them suitable bioindicators for aquatic ecosystem monitoring because they readily metabolize, detoxify and accumulate heavy metals within the body (Abdel-Baki et al., 2011). Heavy metals can enter into the fish body through feed, water uptake for respiration or ion exchange through semi-permeable membrane followed by accumulating in various tissues within the body (Ahmed et al., 2010; Haque et al., 2005; Islam et al., 2015). Fish are considered as good source of protein and fatty acids for human and as heavy metals can be accumulated within the fish body, they can easily be transmitted to the human body and can cause deleterious effects. Like higher vertebrates, fish responds in a similar way to toxicants and can be used to test heavy metal toxicity that are potentially mutagenic, carcinogenic and teratogenic to human beings (Yilmaz et al., 2004).

Acute and chronic toxicity tests have been performed for a wide range of fish species to evaluate the effect of heavy metals on the life forms such as fish in the aquatic environment. Heavy metal intoxication in fish is highly variable and fish responds to heavy metals through physiological, biochemical, cellular and molecular changes within the body (Khalesi et al., 2017; Mohamed et al., 2020; Phoonaploy et al., 2019). Such changes can be used as potential biomarkers to monitor existence of harmful chemicals such as heavy metals in the aquatic

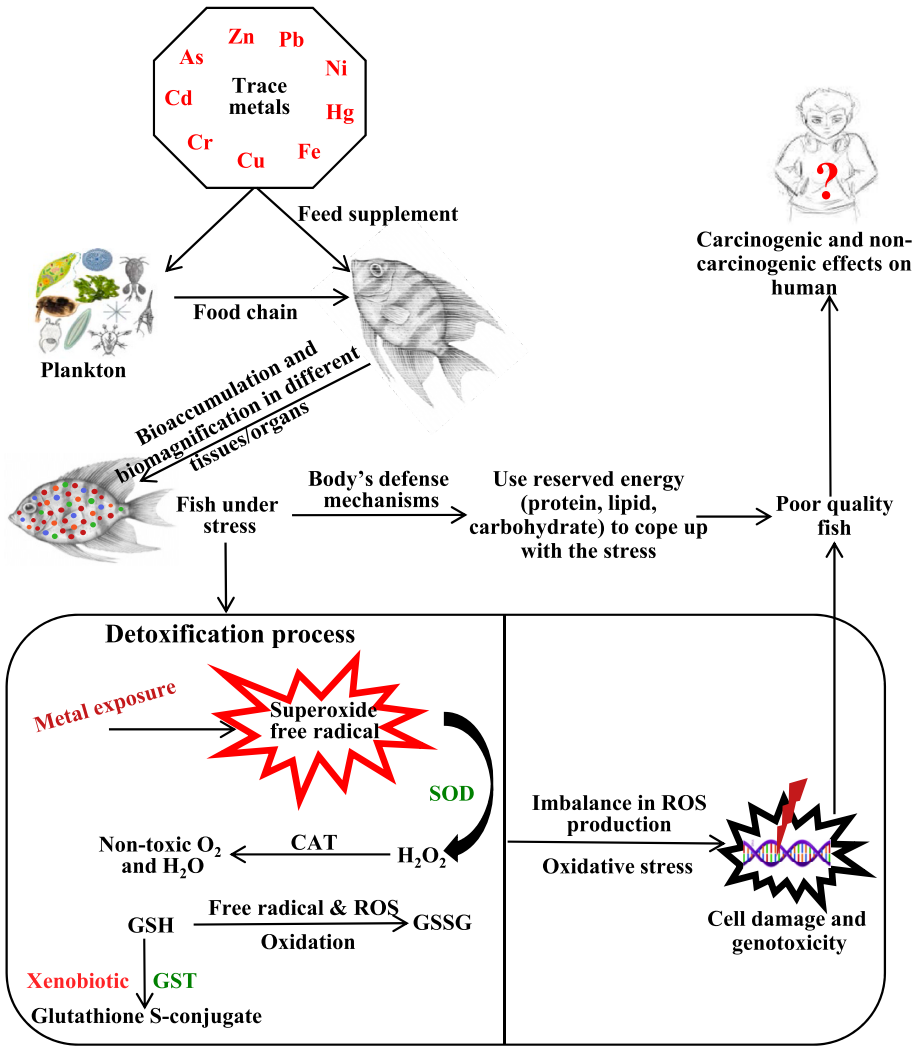

Fig. 2. Routs of heavy metal accumulation in fish and human; and the underlying detoxification mechanism. SOD, superoxide dismutase; CAT, catalase; GST, glutathione S transferase; GSH, glutathione; GSSG, glutathione disulphide; ROS, reactive oxygen species.

environment (Woo et al., 2009). These metals can bind with the biological particles containing nitrogen, sulfur, oxygen etc. thus affecting/altering the structure and function of proteins, enzymes, hormones etc. which ultimately damage different organs of fish (Banday et al., 2019). Blood is an important biological component and comparatively easy to detect any changes/alterations in the hematological parameters (RBC, WBC, Hb, Glu etc.), enzyme and hormone levels (ALT, AST, GST, Cortisol etc.) within the fish body due to heavy metals exposure. Bunch of literatures have documented how the blood chemistry and biochemical parameters respond in the different groups of fishes reared in different concentrations of heavy metals (Banday et al., 2019; Javed et al., 2017; Javed and Usmani, 2019; Phoonaploy et al., 2019; Soundararajan and Veeraiyan, 2014; Suchana et al., 2021). Non-essential heavy metals usually detoxified or accumulated in the body and the essential metal exceeding the permissible limits also accumulate in the different organs of the body such as gills, liver, kidney, muscle, intestine, skin, bones etc. Due to the failure in detoxification, accumulation of heavy metal in various organs of fish in various degrees causes pathological changes in the tissues such as gills, liver, kidney etc. (Ahmed et al., 2013a; Al-Ghanim et al., 2019; Benjamin and Kutty, 2019; Guardiola et al., 2013; Kawade, 2020; Naz et al., 2021; Rajeshkumar et al., 2017). Heavy metals can cause serious problems in body by producing oxygen reactive species (ROS) which cause oxidative stress and damage the DNA responsible for altering expression pattern of important proteins, different hormones, enzymes etc. (Benhamed et al., 2016; Chen et al., 2015; Gárriz et al., 2019; Javed et al., 2017; Kwong et al., 2011; Morcillo et al., 2016).

There is plenty of information about the effect of heavy metals on fish physiology of different fish species and each literature is basically based on particular heavy metal impact on few physiological parameters for particular fish species. Taking into account the adverse effect of heavy metals in fish and eventually in human, a full-scale investigation is required to ensure healthy ecosystem for the food security and food safety. Therefore, this review has been designed to accumulate information about the heavy metal impacts on fish physiology particularly on blood chemistry, histopathology and molecular changes at the DNA level.

2. Mode of actions of heavy metals in living tissues

Heavy metals have the atomic density greater than 4 g/cm^3 and have the chemical properties to attract and accept electrons, which cause toxicity to aquatic organisms (Javed and Usmani, 2019). Heavy metals can persist in the environment for so long resulting a continuous exposure of fish to the heavy metals. The accumulation process of heavy metals and their mode of action in fish depend on the type of heavy metals, fish species, duration of exposure etc. Bioaccumulation and biomagnification are two very important indicators used for monitoring

Table 1
Lethal concentration of heavy metals for fish.

Species	96 h lethal concentrations (LC50)	References
As		
Labeo rohita	40.20 mg/L	Kousar and Javed (2014)
Cirrhinus mrigala	32.10 mg/L	
Catla catla	14.10 mg/L	
Ctenophanyngodon idella	29.67 mg/L	
Cyprinus carpio	32.00 mg/L	Kovendan and Vincent (2013)
Oreochromis mossambicus	28.22 mg/l	Ahmed et al. (2013a)
Channa punctatus	42.00 mg/l	Das et al. (2012)
Catla catla	20.41 mg/l	Lavanya et al. (2011)
Catla catla	43.78 mg/l	Kavitha et al. (2010)
Clarias gariepenus	89.00 mg/l	Hamdi et al. (2009)
Anabas testudineus	18.21 mg/l	Akter et al. (2008)
Danio rerio	43.00 mg/l	Liu et al. (2008)
Labeo rohita	28.30 mg/l	Vutukuru et al. (2007)
Clarias batrachus	84.00 mg/l	Bhattacharya and Bhattacharya (2007)
Oreochromis mossambicus	28.68 mg/L	Liao et al. (2003)
Cd		
Cyprinus carpio	0.20 μM	Delahaut et al. (2020)
Oreochromis sp.	0.70	Aldoghachi et al. (2016)
Poecilia reticulata	30.40 mg/l	Yilmaz et al. (2004)
Sparus aurata	15.30 mg/l	Guardiola et al. (2013)
Rasbora sumatrana	0.10 mg/l	Shuhaimi-Othman et al. (2015)
Poecilia reticulata	1.06 mg/l	
Danio rario	6.50 mg/l	Wang et al. (2013)
Ctenopharyngodon idella	18.47 mg/l	
Rare minnow	5.36 mg/l	
Leporinus macrocephalus	7.42 mg/l	Gomes et al. (2009)
Prochilodus vimboides	3.16 mg/l	
Pagrus major	5.60 mg/l	Cao et al. (2009)
Catla catla	4.53	Sobha et al. (2007)
Lates calcarifer	20.12 mg/l	Thophon et al. (2003)
Salvelinus confluentus	0.83 mg/l	Hansen et al. (2002)
Oncorhynchus mykiss	0.53 mg/l	
Cu		
Cyprinus carpio	0.77 μM	Delahaut et al. (2020)
Oreochromis niloticus	7.94 mg/l	Alkobaby and El Wahed (2017)
Oreochromis sp.	0.45	Aldoghachi et al. (2016)
Sarotherodon mossambica	58 mg/l	Reddy et al. (2016)
Tilapia nilotica	25.00	Naseem et al. (2015)
Danio rario	0.17 mg/l	Wang et al. (2013)
Ctenopharyngodon idella	0.09 mg/l	
Rare minnow	0.12 mg/l	
Rasbora sumatrana	0.006 mg/l	Shuhaimi-Othman et al. (2015)
Poecilia reticulata	0.038 mg/l	
Rutilus kutum	2.31 mg/l	Gharedaashi (2013)
Rutilus frisii kutum	2.31 mg/l	Gharedaashi et al. (2013)
Channa gachua	1.42 mg/l	Deore and Wagh (2012)
Leporinus macrocephalus	0.09 mg/l	Gomes et al. (2009)
Prochilodus vimboides	0.05 mg/l	
Pomatoschistus microps	568.00 μg/L	Vieira et al. (2009)
Carassius auratus	0.30 mg/l	James et al. (2008)
Xiphophorus helleri	0.36 mg/l	
Cr		
Pangasianodon hypophthalmus	32.47 mg/l	Islam et al. (2020)
Danio rario	105.28 mg/l	Nisha et al. (2016)
Danio rario	26.03 mg/l	
Catla catla	31.41 mg/L	Sanyal et al. (2017)
Oryzias melastigma	83.50 mg/L	Chen et al. (2016)
Labeo rohita	30.36 mg/L	Bakshi (2016)
Heteropneustes fossilis	33.39 mg/L	Bakshi (2016)
Clarius batrachus	36.65 mg/L	Johnson and Radhakrishnan (2015)
Oryzias latipes	57.30 mg/L	Li et al. (2015)

Table 1 (*continued*)

Species	96 h lethal concentrations (LC50)	References
Oreochromis nilitica	164.36 mg/L	Shaukat and Javed (2013)
Cirrhinus mrigala	34.00 mg/L	Virk and Sharma (2003)
Cyprinus carpio	128.89 mg/L	Shaukat and Javed (2013)
Heteropneustes fossilis	35.724 mg/l	Ahmed et al. (2013b)
Cirrhinus mrigala	18.20 mg/l	Palaniappan and Karthikeyan (2009)
Ctenopharyngodon idella	87.01 mg/l	
Carassius auratus	85.70 mg/L	Velma et al. (2009)
Salmo gardnerii	69.00 mg/L	
Pimephales promelas	48.00 mg/L	
Channa punctatus	50.00 mg/L	
Salvelinus fontinalis	59.00 mg/L	
Catla catla	100.00 mg/L	
Labeo rohita	39.40 mg/L	
Channa punctatus	41.75 mg/l	Mishra and Mohanty (2008)
Rainboe trout	28.5 mg/l	Svecevičius (2006)
Three spined stickled back	38.3 mg/l	
Roach	49.3 mg/l	
Perch	33.1 mg/l	
Dace	71.7 mg/l	
Labeo rohita	39.40 mg/l	Vutukuru (2003)
Cirrhinus mrigala	34.00 mg/L	Virk and Sharma (2003)
Cyprinus carpio	128.89 mg/L	Shaukat and Javed (2013)
Fe		
Rasbora sumatrana	1.71 mg/l	Shuhaimi-Othman et al. (2015)
Poecilia reticulata	1.46 mg/l	
Hg		
Oreochromis niloticus	0.30 mg/l	Jasim et al. (2016)
Danio rario	0.14 mg/l	Wang et al. (2013)
Ctenopharyngodon idella	0.23 mg/l	
Rare minnow	0.10 mg/l	
Channa gachua	1.0625 mg/l	Deore and Wagh (2012)
Anabas testudineus	0.606 mg/l	Akter et al. (2008)
Channa punctatus	1.21 mg/l	Pandey et al. (2005)
Ni		
Channa gachua	150 mg/l	Kawade (2020)
Rasbora sumatrana	0.83 mg/l	Shuhaimi-Othman et al. (2015)
Poecilia reticulata	15.62 mg/l	
Catla catla	43.78 mg/l	Kavitha et al. (2010)
Cirrhinus mrigala	10.83 mg/l	Palaniappan and Karthikeyan (2009)
Clarias gariepinus	8.87 mg/l	Ololade and Oginni (2010)
Poecilia reticulata	173.10 mg/l	Selvi (2009)
Catla catla	6.00 mg/l	Javed and Abdullah (2006)
Cirrhina mrigala	23.47 mg/l	
Labeo rohita	20.20 mg/l	
Oncorhynchus mykiss	20.8 mg/l	Brix et al. (2004)
Oncorhynchus mykiss	15.3 mg/l	Pane et al. (2003)
Pb		
Danio rerio	18.62 mg/L	Kim et al. (2020)
Clarias gariepinus	284.189 mg/l	Samuel et al. (2018)
Oncorhynchus mykiss	487 μg/l	Alsop et al. (2016)
Rasbora sumatrana	0.63 mg/l	Shuhaimi-Othman et al. (2015)
Poecilia reticulata	1.99 mg/l	
Rutilus frisii kutum	268.07 mg/l	Gharedaashi (2013)
Chanos chanos	426.49 mg/l	Hesni et al. (2011)
Clarias batrachus	378.00 mg/l	Pandit et al. (2018)
Prochilodus lineatus	95.00 mg/l	Martinez et al. (2004)
Oncorhynchus mykiss	1.04 mg/l	Rogers et al. (2003)
Zn		
Cyprinus carpio	29.89 μM	Delahaut et al. (2020)
Oreochromis sp.	2.10	Aldoghachi et al. (2016)
Labeo rohita	212.90 mg/l	Kousar and Javed (2014)
Cirrhinus mrigala	140.70 mg/l	
Catla catla	57.30 mg/l	
Ctenophanyngodon idella	129.22 mg/l	
Rasbora sumatrana	0.46 mg/l	Shuhaimi-Othman et al. (2015)
Poecilia reticulata	0.17 mg/l	
Danio rario	44.48 mg/l	Wang et al. (2013)

(*continued on next page*)

Table 1 (*continued*)

Species	96 h lethal concentrations (LC50)	References
Ctenopharyngodon idella	31.37 mg/l	
Rare minnow	12.74 mg/l	
Salvelinus confluentus	80.00 mg/l	Hansen et al. (2002)
Salvelinus confluentus	53.30 mg/l	

the geochemical cycling of heavy metals in the aquatic ecosystem. Bioaccumulation is the process where accumulation occur within a single trophic level of the food web, whereas in biomagnification accumulation of heavy metals occur in multiple tropic level of the aquatic food web as a result of consumption of metal contaminated foods by consumers of higher tropic level. The rate of biomagnification depends on the feeding habits and the ability of the aquatic organisms to digest different forms of heavy metals, availability of and concentration of the metals, their accumulation rate in a single tropic level of the aquatic food web etc. The bioaccumulation and biomagnification of heavy metals in aquatic ecosystem can be depicted as shown in Fig. 2. Heavy metals exist in several oxidation states depending on the metal types and not all forms cause toxicity in fish. For example, chromium (Cr) is present in six oxidation states (+1 to +6), and trivalent and hexavalent Cr are more stable. Trivalent Cr acts as a cofactor for insulin, which has a significant role in glucose metabolism. On the other hand, hexavalent Cr cause toxicity in fish and has potential mutagenic and teratogenic activities in living cells (Velma et al., 2009). Fish is under stress when exposed to sub-lethal to lethal concentrations of heavy metals which eventually accumulate in different tissues/organs of the body such as gills, kidney, liver, skin, muscle etc. Fish has its own defense mechanism and when subjected to heavy metal exposure, they try to cope up with this stressful condition by utilizing more energy from reserved carbohydrate, protein and lipid in the fish body. Heavy metals such as As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Zn are redox active components and drive the redox reaction to form reactive oxygen species (ROS) which is important for certain physiological function in the fish body. The excess of ROS indicates imbalance between the productions and scavenging of ROS causes oxidative stress, which eventually interfere with the cellular function by damaging lipid, protein and DNA. Enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione S transferase (GST) and non-enzymatic compounds such as reduced glutathione (GSH) are essential to maintain the dynamic balance of ROS through detoxification. SOD works by converting superoxide free radical to hydrogen peroxide, which is further broken down to non-toxic oxygen and water by CAT enzyme (Vutukuru et al., 2007). GST participate in the detoxification process by catalyzing the conjugation of electrophile to GSH. However, the GSH is also oxidized non-enzymatically by electrophilic substances (free radicals and ROS) to glutathione disulfide (Javed et al., 2017). Hampering in the enzymatic reaction could generate surplus ROS, which accumulate in the fish tissue and cause oxidative stress. Later on, ROS degenerate the cell membrane through lipid peroxidation, which in turn cause genotoxicity through DNA damage.

3. Lethal concentrations of heavy metals for fish

Heavy metals when crossed the tolerance limits are proved to be a potential threat to organisms due to high toxicity and tissue damage within the body. Toxicity tests are performed in a dose-dependent manner to investigate the effects of heavy metals in the physiology of aquatic organisms, which in further help determining the discharge limit of the chemicals to the natural environment (Javed and Usmani, 2013). Determining the lethal concentration is a widely used assay to detect the level of toxicity in the aquatic environment and usually the concentration of the toxicant that kill 50% of the exposed individuals in a given period of time (called LC50) is used to test the toxicity of a chemical. Lethal concentration of different essential and non-essential heavy metals exposed to different fish species are enlisted in Table 1. Degree of toxicity of heavy metals depends on the fish species, metal speciation, age and size of the organisms, environmental condition and water chemistry such as pH, temperature, alkalinity, hardness etc. influence the bioavailability and bioaccumulation of metals in organisms (Das and Das, 2005; Ebrahimpour et al., 2010). Generally, the larger fish is less susceptible to heavy metals than the smaller ones. For example, LC50 values for Cu during a exposure of 96 h for common carp weighed 60 g adult and 2.6 g juvenile fish are 10.4 μM and 0.77 μM respectively, which explains the higher tolerance of larger fish to the toxicants than the smaller fish (De Boeck et al., 2004; Delahaut et al., 2020). On the contrary, in juvenile grass carp (*Ctenopharyngodon idellus*, 1.84 g weight), 96 h LC50 for Cu was found to be 0.09 mg/L which was higher than adult Cyprinid *Rasbora sumatrana* (0.006 mg/L; 3–5 g weight) and for Zn the 96 h LC50 was more than 30 folds (31.37 mg/L) in grass carp than that of adult *R. sumatrana* (0.46 mg/L; (Wang et al., 2013). Different sizes of zebra fish showed variable toxicity level when exposed to Pb. For example, larger size fishes (3.0 cm length) showed less toxicity than the smaller group fish (1.5 cm) and the LC50 value for 96 h exposure increased significantly with increasing the size of the fish (LC50 value was 18.62 mg/L for large and 9.7 mg/L was found in smaller fishes (Kim et al., 2020). Evidence suggests that the presence of calcium and sodium ions in the water decrease the toxicity of metals in fish, for example 6 folds higher concentration of Ca^{2+} ion in the exposure tank increases 5 times tolerance level (LC50 was 149 μM) of fish exposed to Zn (Hattink et al., 2006). Metal uptake significantly decreased in hard water than in soft water. A 96 h Pb exposure experiment in zebra fish showed that the LC50 value in hard water was (28.62 mg/L) higher compared to soft water (18.62 mg/L; Kim et al., 2020). Water temperature has also direct relationship with the metal toxicity; such as higher temperature significantly increased the Zn toxicity level of bull trout and inverse relationship (not significant) was observed in case of rainbow trout (Hansen et al., 2002).

Some of the heavy metals and/or trace elements such as Cu, Zn, Fe, Cr are essential for animals including fish for maintaining the basic metabolic processes, particularly their role as component or co-factor of different enzymes (Wood et al., 2011b). However, when such elements cross the threshold concentration, can lead to the detrimental effects on a wide range of biological pathways. On the other hand, non-essential trace/heavy metals such as Pb, Hg, Cd, As, Ni (Wood et al., 2011a) can cause toxicity even at very low concentration. Generally, the greater the concentration and exposure time of heavy metals, the higher the toxicity level within the organisms. A study reported the metal (Cu, Cd, Zn, Pb, Ni, Fe) toxicity trend for *Rasbora sumatrana* (Cyprinidae) and *Poecilia reticulata* (Poecilidae); Cu has been found to be more toxic followed by Cd and Zn for both species (Shuhaimi-Othman et al., 2012). The LC50 values (96 h exposure) were less for all the heavy metals in case of *R. sumatrana* except for Fe, where the LC50 value was slightly higher for *P. reticulate,* which suggests that *R. sumatrana* was more susceptible to heavy metal toxicity than *P. reticulata*. On the other hand, Cd was found to be more toxic than Cu in freshwater prawn *Macrobrachium lanchesteri* (Shuhaimi-Othman et al., 2012)), juvenile crayfish *Cherax destructor* (Khan and Nugegoda, 2007) and juvenile clams *Mercenaria mercenaria* (Keppler and Ringwood, 2002), which implies that biological variations including the genetic make-up of the species is responsible for the variations in heavy metal toxicity. Two different studies have found almost similar 96 h LC50 value (28 mg/L) for *Oreochromis mossambicus* exposed to As, whereas lower LC50 value (18 mg/L) has been found in the same order (Perciformes) of another species such as *Anabas testudineus* (Ahmed et al., 2013a; Akter et al., 2008; Liao et al., 2003). Exposure of heavy metals at different concentrations in fishes showed behavioral abnormalities such as irregular body movement, continuous gill opercular movement, lateral swimming, loss of equilibrium, jumping out from the media etc. along with different pathological changes (Akter et al., 2008; Mishra and Mohanty, 2008; van Dyk et al., 2007). Freshwater snakehead *Channa punctatus* exposed

Table 2
Effects of heavy metals on hemato-biochemical parameters of fish.

Species	Doses (mg/L)	Exposure time (days)	Hemato-biochemical alterations		References
			Increase	Decrease	
As					
Clarias batrachus	84	4	–	TP	Pichhode and Gaherwal (2020)
Tilapia mossambica	Sub-lethal	21	WBC, MCH, MCHC	Hb, RBC, PCV	Soundararajan and Veeraiyan (2014)
Cd					
Clarias gariepinus	0.316	–	AST, ALP, ALT, Cort, Glu, MCH	CK, TLC, MCV	Banday et al. (2019)
Channa striata	0.001	-	HDL, LDL, TP, AST and ALT	Glu	Phoonaploy et al. (2019)
Colisa fasciatus	1.86	4	–	Protein, gly, nucleic acid	Tripathi et al. (2012)
Labeo rohita	0.826	–	WBC	RBC, Hct,	Chandanshive et al. (2012)
Oreochromis niloticus	1.0	7 and 14	ALT, Cort., Glu and TP	cholesterol levels	Firat and Kargin (2010)
Catla catla	1 to 8	4–7	Glu	Glycogen, TP	Sobha et al. (2007)
Cyprinus carpio	0.05–1.0	10	Glu	Glycogen	Cicik and Engin (2005)
Cyprinus carpio	10	4	Hb, Hct, neutrophil	WBC, leukocyte	Witeska (2005)
Oncorhynchus mykiss	0.00018	28	–	RBC, Hb, Hct, WBC	Vosyliene et al. (2003)
Cr					
Pangasianodon hypophthalmus	0.8, 1.6, and 3.2	30	WBCs	Hb, RBC, and Glu	Suchana et al. (2021)
Pangasianodon hypophthalmus	10, 20, 30, 40, 50 and 60	4	WBC, MCV, MCH, Glu, ECA, ENA	RBC, Hb, PCV	Islam et al. (2020)
Clarias gariepinus	0.203	–	AST, ALP, ALT, Cort, Glu, MCH	CK, TLC, MCV	Banday et al. (2019)
Channa striata	0.001	-	HDL, LDL, TP, AST and ALT	Glu	Phoonaploy et al. (2019)
Channa punctatus	0.08	–	LDL, TP, lipid and chol., SOD, CAT, GST and LPO	Albumin, triglyceride, HDL and VLDL	Javed et al. (2017)
Channa punctatus	0.10		WBC	RBC, Hb,	Javed and Usmani (2014)
Mastacembelus armatus	0.10	–	Glu	–	Javed and Usmani (2013)
Cyprinus carpio,	2	28	Glu	Hct, Hb, RBC, WBC, MCH, and MCHC	Abedi et al. (2013)
Cyprinus carpio	25–150	180	WBC, MCV, AST, ALT, ALP, ESR, AP	RBC, Hb, PCV, MCH, MCHC, Glu, protein, chol.	Shaheen and Akhtar (2012)
Labeo rohita	39.40	4	–	RBC, Hb, MCH, lipids, TP	Vutukuru (2005)
Oncorhynchus mykiss	0.367	4	–	RBC	Vosylienė and Jankaitė (2006)
Oncorhynchus mykiss	0.0028	28	–	RBC, Hb, Hct, WBC	Vosyliene et al. (2003)
Cu					
Clarias gariepinus	100	4	TP, Albumin, globulin	–	Javed and Usmani (2019)
Clarias gariepinus	0.073	–	AST, ALP, ALT, Cort, Glu, MCH	CK, TLC, MCV	Banday et al. (2019)
Channa punctatus	0.08	–	LDL, TP, lipid and chol., SOD, CAT, GST and LPO	Albumin, triglyceride, HDL and VLDL	Javed et al. (2017)
Channa punctatus	0.86		WBC	RBC, Hb,	Javed and Usmani (2014)
Mastacembelus armatus	0.86		Glu	–	Javed and Usmani (2013)
Channa punctatus	0.36	15, 30, 45	WBC, MCV, MCHC, MCH	Hb, RBC, PCV	Singh et al. (2008)
Oncorhynchus mykiss	0.87	4	–	RBC	Vosylienė and Jankaitė (2006)
Cyprinus carpio	5	4	Hb, Hct, neutrophil	WBC, leukocyte	Witeska (2005)
Oncorhynchus mykiss	0.0075	28	–	RBC, Hb, Hct, WBC	Vosyliene et al. (2003)
Prochilodus scrofa	0.029	4	Hct, RBC, Hb, WBC, neutrophils, plasma K+	Lymphocytes, plasma Na^+ and Cl^-	Mazon et al. (2002)
Fe					
Channa punctatus	9.00	–	LDL, TP, chol, SOD, CAT, GST and LPO	Albumin, triglyceride, HDL and VLDL	Javed et al. (2017)
Channa punctatus	8.71		WBC	RBC, Hb,	Javed and Usmani (2014)
Mastacembelus armatus	8.71		Glu	–	Javed and Usmani (2013)
Hg					
Oreochromis niloticus	0.025	30	ALT, AST, SOD	–	Mahboub et al. (2021)
Labeo rohita	0.0987		WBC	RBC, Hct	Chandanshive et al. (2012)
Acanthopagrus lates	0.01, 0.02, 0.04, 0.08	21	Hb, Hct, monocyte	WBC, lympho, eosinophil	Safahieh et al. (2010)
Clarias batrachus	0.6	35	WBC	RBC, Hb	Maheswaran et al. (2008)
Ni					
Clarias gariepinus	0.302	–	AST, ALP, ALT, Cort, Glu, MCH	CK, TLC, MCV	Banday et al. (2019)
Channa punctatus	0.10	–	LDL, TP, chol, SOD, CAT, GST and LPO	Albumin, triglyceride, HDL and VLDL	Javed et al. (2017)
Channa punctatus	0.12		WBC	RBC, Hb,	Javed and Usmani (2014)
Mastacembelus armatus	0.12		Glu	–	Javed and Usmani (2013)
Cirrhinus mrigala		7, 14, 21, 28	MCHC	RBC, WBC, Hb, Ht, MCV	Parthipan and Muniyan (2013)

(*continued on next page*)

Table 2 (*continued*)

Species	Doses (mg/L)	Exposure time (days)	Hemato-biochemical alterations		References
			Increase	Decrease	
Cyprinus carpio	6,9,12,15, 18	4	–	RBC, WBC, Hct and Hb, MCV, MCH, MCHC	Al-Ghanim (2011)
Clarias gariepinus	4, 6, 8, 10, 12	4	–	RBC, WBC, Hct, Hb, MCV, MCH and MCHC	Ololade and Oginni (2010)
Oncorhynchus mykiss	0.47	4	–	RBC	Vosylienė and Jankaitė (2006)
Oncorhynchus mykiss	0.0021	28	–	RBC, Hb, Hct, WBC	Vosyliene et al. (2003)
Pb					
Mugil cephalus	0.0015, 0.0025	4	Glu, MDA	–	Hajirezaee et al. (2021)
Channa striata	0.005	-	HDL, LDL, TP, AST and ALT	Glu	Phoonaploy et al. (2019)
Labeo rohita	0.756	–	WBC	RBC, Hct,	Chandanshive et al. (2012)
Oncorhynchus mykiss	3.6	4	–	RBC	Vosylienė and Jankaitė (2006)
Cyprinus carpio	10	4	Hb, Hct, neutrophil	WBC, leukocyte	Witeska (2005)
Prochilodus lineatus	95	4	Glu	Lipid, plasma Na^+, TP, Cholesterol	Martinez et al. (2004)
Oncorhynchus mykiss	0.0142	28	–	RBC, Hb, Hct, WBC	Vosyliene et al. (2003)
Zn					
Channa punctatus	0.54	–	LDL, TP, lipid and chol., SOD, CAT, GST and LPO	Albumin, triglyceride, HDL and VLDL	Javed et al. (2017)
Channa punctatus	0.30	–	WBC	RBC, Hb,	Javed and Usmani (2014)
Mastacembelus armatus	0.30	–	Glu	–	Javed and Usmani (2013)
Tilapia mossambicus	1.0, 2.5, 5.0	14	Hb, MCV, MCH, MCHC, Hct, lysozyme & MPO	RBC, lymphocyte, WBC	Çelik et al. (2013)
Labeo rohita	1.46	–	WBC	RBC, Hct,	Chandanshive et al. (2012)
Oreochromis niloticus	5.0	7 and 14	ALT, Cort., Glu and TP	cholesterol levels	Firat and Kargin (2010)
Oncorhynchus mykiss	0.7	4	–	RBC	Vosylienė and Jankaitė (2006)
Cyprinus carpio	20	4	Hb, Hct, neutrophil	WBC, leukocyte	Witeska (2005)
Oncorhynchus mykiss	0.064	28	–	RBC, Hb, Hct, WBC	Vosyliene et al. (2003)
Co					
Channa punctatus	0.26	–	LDL, TP, lipid and chol., SOD, CAT, GST and LPO	Albumin, triglyceride, HDL and VLDL	Javed et al. (2017)
Channa punctatus	0.11	–	WBC	RBC, Hb,	Javed and Usmani (2014)
Mastacembelus armatus	0.11	–	Glu	–	Javed and Usmani (2013)
Mn					
Channa punctatus	2.50	–	LDL, TP, lipid and chol., SOD, CAT, GST and LPO	Albumin, triglyceride, HDL and VLDL	Javed et al. (2017)
Channa punctatus	0.21	–	WBC	RBC, Hb,	Javed and Usmani (2014)
Mastacembelus armatus	0.21	–	Glu	–	Javed and Usmani (2013)
Oncorhynchus mykiss	14.0	4	–	RBC	Vosylienė and Jankaitė (2006)
Oncorhynchus mykiss	0.0099	28	–	RBC, Hb, Hct, WBC	Vosyliene et al. (2003)

ALP; alkaline phosphatase, ALT; alanine transaminase, AST; aspartate transaminase, GST; glutathione S transferase, Glu; glucose, Hb; hemoglobin, Hct; hematocrit, HDL; high density lipoprotein, LPO; lipid peroxidation, MDA; malondialdehyde, RBC; red blood cell, TP; total proteins, WBC; white blood cell, and VLDL; very low density lipoprotein.

to Cr at concentration ranged from 20 to 40 mg/L exhibited abnormal behaviour such as erratic swimming and migration to the surface, loss of equilibrium and become lethargic etc. (Mishra and Mohanty, 2008).

4. Heavy metals effect on hemato-biochemical parameters of fish

Fish blood is an important diagnostic tool to detect any stressful or pathological condition in any place within the body to various biotic and abiotic stresses (Ashaf-Ud-Doulah et al., 2020, 2019; Islam et al., 2019; Salam et al., 2015; Shahjahan et al., 2021, 2020, 2019; Sharmin et al., 2016; Van Doan et al., 2018). The exposure of fishes to heavy metal is manifested by numerous changes in hematological and biochemical parameters (Islam et al., 2020; Suchana et al., 2021). How heavy metal exposure to fish changes the hematological and biochemical parameters of fish blood are summarized in Table 2. Hematological parameters such as red blood cell (RBC), hemoglobin (Hb), leucocytes, and lymphocytes decreased significantly irrespective of the heavy metals exposed to different fish species (Çelik et al., 2013; Soundararajan and Veeraiyan, 2014; Suchana et al., 2021; Witeska, 2005). (Mazon et al., 2002) reported that Cu induction at the dose of 25 and 29 μg/L in *Prochilodus scrofa* caused a significant increase in RBC. Anemic condition manifested by heavy metal exposure may trigger the early release of immature RBCs resulting in an increase of RBC. The number of Hb and RBC has been declined due to iron deficiency, structural alteration of heme, decrease utilization of iron for erythropoiesis, hemolysis or suppression of Hb synthesizing enzymes (Vinodhini and Narayanan, 2009). RBCs are very sensitive blood component, which respond immediately to the alterations of environmental parameters and causes morphological aberrations. Different cellular and nuclear (genotoxicity) abnormalities of RBCs have been recorded due to various heavy metals exposure; damage of cell membrane and metal-permeability of the cell are reported to be the main causes of such abnormalities (Sadiqul et al., 2016).

Other hematological parameters such as neutrophil has increased significantly (Witeska, 2005) but in case of hematocrit, white blood cell (WBC) differential response to various heavy metals exposures have been noticed (Table 2). For example, the number of WBC is increased in striped catfish (*Pangasianodon hypophthalmus*) exposed to Cr (Islam

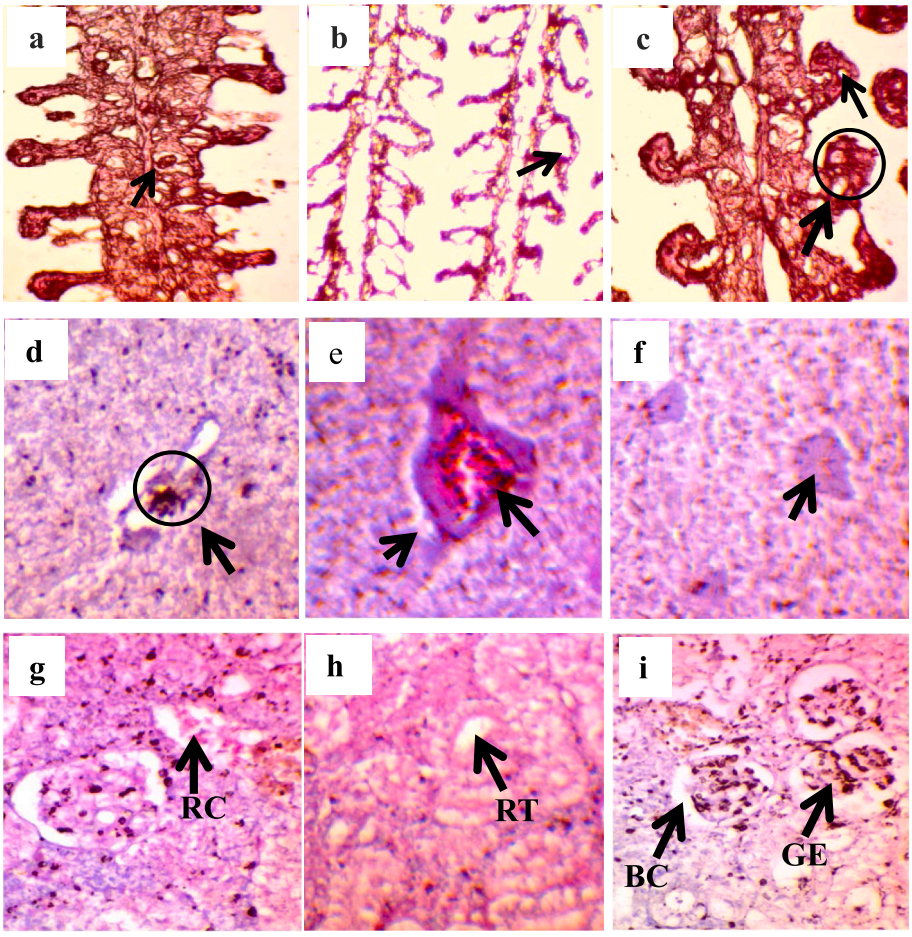

Fig. 3. Histo-pathological changes in gills (a, b & c), liver (d, e & f) and kidney (g, h & i) of striped catfish exposed to sub-lethal concentrations of chromium for 30 days; a. hypertrophy of chloride cells, b. lamellar fusion, c. clubbed lamella, necrotic lamellae, d. melano-macrophage centers, e. blood congestion, f. hemorrhage, g. shrinkage of renal corpuscle; h. increasing in the diameter of renal tubules, and i. glomerular expansion, dilation of bowman's space.

et al., 2020). Those increment of WBC could be due to the normal defence mechanism of the body which causes stimulation of lymphoiesis and enhance the production of lymphocytes from the lymphoid organs. On the contrary, Ni exposure to common carp (*Cyprinus carpio*) induced the reduction of blood WBC content (Al-Ghanim, 2011). Heavy metals accumulate in the kidney and liver, which are the main organs of hematopoiesis and eventually inhibit the process of granulopoiesis or lymphpoiesis causing a reduction in WBC. Hematological indices such as packed cell volume (PCV) always decreased significantly with heavy metal exposure (Soundararajan and Veeraiyan, 2014), whereas mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) showed both increasing and decreasing trend when exposed to heavy metals (Banday et al., 2019; Shaheen and Akhtar, 2012; Singh et al., 2008).

Blood biochemical indices such as glucose, glycogen, cholesterol, proteins (albumin, transferrin, ceruloplasmin), high density lipoprotein (HDL), low density lipoprotein (LDL) etc. have also been deviated from the normal range due to heavy metal exposure. Sometimes glucose level has been reported to decrease when exposed to heavy metal (Zn and Cd exposure in Nile tilapia, Cr toxicity in Common carp). This might be due to the enhanced energy requirement to cope up with oxidative stress or inhibition of the glycolysis process (Firat and Kargin, 2010; Shaheen and Akhtar, 2012). On the contrary, Cd, Cu, Fe exposure in *Catla catla*, *Clarias gariepinus*, *Mastacembelus armatus* etc. caused a significant increase in blood glucose level due to the production of glucose from glycogen through glycogenolysis (Banday et al., 2019; Javed and Usmani, 2013; Sobha et al., 2007). Both glycogenolysis and inhibition of hormone for glycogen synthesis are responsible for the reduction of glycogen level in the blood due to heavy metals exposure (Cicik and Engin, 2005; Sobha et al., 2007; Tripathi et al., 2012). Serum proteins possess metal binding sites, which eventually bind to the specific metal and bioaccumulate in the target organ and cause toxicity (Abedi et al., 2013). Javed et al. (2017) reported that heavy metals (Fe, Mn, Zn, Co, Ni) exposed to *Channa punctatus* showed significant increase in total protein, lipid and cholesterol and LDL.

Cortisol is known as a stress hormone and released from hypothalamic-pituitary-adrenal axis upon exposure to heavy metals in fish. Cortisol level has a direct relation with the level of glucose in blood. The increased level of cortisol in blood activates the process of glycogenolysis and gluconeogenesis which increases the glucose level in blood (Banday et al., 2019; Firat and Kargin, 2010). Serum enzymes such as aspartate aminotransferase (AST); alanine aminotransferase (ALT) and alkaline phosphatase (ALP) are important hepato-toxic biomarkers; and these enzyme levels have been increased in various fish species due to different heavy metals exposure (Banday et al., 2019; Phoonaploy et al., 2019; Shaheen and Akhtar, 2012). The increase in serum enzymes could be due to the hepato-cellular damage triggering the release of these enzymes into the bloodstream. Enzymatic and non-enzymatic oxidative stress markers such as superoxide dismutase (SOD), catalase (CAT), glutathione S transferase (GST) and lipid peroxidation (LPO) were found to be increased due to heavy metal exposure which indicates increasing level of free radicals in different organs or tissues (Javed et al., 2017). These free radicals damage the cell membrane and causes genotoxicity or DNA damage. For example, Cr

Table 3
Effects of heavy metals on histo-pathology of different organs in fish.

Species	Doses	Exposure time (days)	Organs	Changes	References
As					
Heteropneustes fossilis	7.0 and 20.0 ppm	60	muscle	Intramuscular edema, atrophy and edema of muscle bundles, splitting of muscle fibers	Begum et al. (2014)
			intestine	Partial intactness of serosa, disorganized villi, slightly swollen and shorten of villi,	
			liver	Necrosis, hemorrhage and hemolysis	
Oreochromis mossambicus	3.0, 28.0, 56.0 mg/L	8	gills	Epithelial hyperplasia, epithelial lifting and edema, lamellar fusion, aneurism, desquamation and necrosis	Ahmed et al. (2013a)
			liver	Macrophage infiltration, congestion, vacuolization and shrinkage of hepatocytes, cloudy swelling, vacuolar degeneration and nuclear hypertrophy	
Channa punctatus	3.8 and 7.6 mg/l	14	liver	Tissue disorientation and vacuolization accompanied by karyolysis, apoptosis, and necrosis of hepatocytes	Roy and Bhattacharya (2006)
			kidney	Shrinkage of the glomerulus and increase in the Bowman's space	
Clarias batrachus	0.50 μM	30	kidney	Vacuole, depletion in leukocyte number and melano-macrophage population, increased hemosiderin deposition	Datta et al. (2009)
Coregonus clupeaformis	0, 10 & 100 μg/g	64	liver	Nuclear alterations, inflammation, and focal necrosis	Pedlar et al. (2002)
			gall bladder	Sloughing of epithelium, dilation of vascular elements, inflammation, edema, fibrosis	
Cd					
Catla catla	4.5 mg/l	30	gills	Lamellar atrophy, telangiectasia, and necrosis of lamellar epithelial cells	Naz et al. (2021)
			liver	Karyorrhexis, hepatic cells degeneration, congestion, and hemorrhages	
			kidney	Atrophy of glomeruli, necrosis of renal tubular cells, degeneration of renal tubules, and melanomacrophage aggregates	
			intestine	Atrophy of villi, sloughing of epithelial villi, and congestion	
Cyprinus carpio	0.10 mg/L	30	gills	Spiking and fusion of secondary lamellae, formation of club-shaped filaments epithelium in the inter-lamellar regions	Rajeshkumar et al. (2017)
			liver	Hepatocytes showed damage of central vein and rupture of irregular hepatic plate with more number of vacuoles	
Cyprinus carpio	8.4 mg/L	15	gills	Fusion of gill lamellae, vessel dilatation, hyperemia, and hyperplasia of gill epithelial cells	Khalesi et al. (2017)
Sparus aurata	1 mg L−1	30	liver	Vacuolated cytoplasm and displaced the nuclei, hepatocytes showed quite large vacuoles and a high presence of blood cells in the lumen	Guardiola et al. (2013)
Puntius gonionotus	0.06 mg/L	60	gills	Hypertrophy and hyperplasia of primary and secondary gill lamellae	Wangsongsak et al. (2007)
			liver	Hepatic MT levels remained high	
			kidney	Vacuolization in hepatocytes, and prominent tubular and glomerular damage in the kidney	
Dicentrarchus labrax	4.47, 5.63, 7.08 and 8.91 mg/l	2	gills	Telangectasia, diffuse edema and detachment of the lamellar epithelium, secondary lamellar fusion and thickening of primary lamellae	Giari et al. (2007)
			liver	Vacuolization, formation of myelinoid-bodies, lipid droplets accumulated in many hepatocytes (steatosis), diffuse degenerative vacuolation (cellular edema or acute cell swelling) and cytoplasm rarefaction	
			kidney	Cytoplasmic vacuoles (swelling) and apical expansion of cells ('blebbing'), damage in epithelial tubular cells, tubules showed myelinoid bodies, nuclei were swollen, with abnormal shape, dilated nuclear envelope	
Lates calcarifer	10.0 and 0.8 mg/L	90	gills	Edema of the epithelial cells with the breakdown of pillar cell system, aneurisms with some ruptures, hypertrophy and hyperplasia of epithelial and chloride cells	Thophon et al. (2003)
			liver	Blood congestion in sinusoids and hydropic swelling of hepatocytes, vacuolation and dark granule accumulation	
			kidney	Hydropic swelling of tubular cell, tubular degeneration and necrosis	
Cr					
Pangasianodon hypophthalmus	0.8, 1.6 & 3.2 mg/L	30	gills	Lifting of lamellae epithelium, telangiectasia at the tips of secondary lamellae, hypertrophy of chloride cells, lamellar fusion, clubbed lamella, and necrotic lamellae	Suchana et al. (2021)
			liver	Blood congestion, hemorrhage, pyknotic nucleus, melano-macrophage centers, and hepatocyte hypertrophy	
			kidney	Shrinkage of renal corpuscle, pyknotic nuclei, increases in the diameter of renal tubules, dilation of Bowman's space, glomerular expansion, necrosis and vacuolation	
Oryzias melastigma	10.44 & 20.88 mg/L	4	liver	Nuclear migration, cell vacuolization, nuclear pyknosis in some hepatocytes,	Ni and Shen (2021)
Anabas testudineus	6 & 12 mg/L	15	gills	Abnormalities in secondary gill lamellae, lamellar fusion, epithelial lifting and hemorrhage	Benjamin and Kutty (2019)
			liver	Dilated sinusoids, a modest collection of blood in the liver parenchyma, partial necrosis	
			kidney	Renal tissue edema, interstitial hemorrhage, and degeneration of renal tubules	
Labeo rohita	1/10th LC50	60	liver	Hyperplasia, Necrosis of hepatic cells, Cellular disorganization	Velma et al. (2009)

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Table 3 (*continued*)

Species	Doses	Exposure time (days)	Organs	Changes	References
			kidney	Highly fenestrated Bowman's capsule, Constricted lumen of Renal tube,	
			intestine	Glomerular disorganization	
			gills	Inner epithelial layers highly degraded High Lamellar degradation. Necrosis in epithelial cells. Thickening of blood vessels, Atrophied central axis	
Channa punctatus	20.0, 40 mg/L		gills	Epithelial hyperplasia, lamellar fusion, epithelial lifting, epithelial necrosis	Mishra and Mohanty (2008)
			kidney	Hypertrophy of epithelial cells of renal tubules with reduced lumens, atrophy of the renal tubules, glomeruli contraction in the Bowman's capsules and necrosis of hematopoietic tissues	
			liver	Cytoplasmic vacuolization with the lateral nuclei arrangement. Hepatocytes atrophy and increase in sinusoidal space	
Cyprinus carpio	0.15 mg/L	30	gills	Spiking and fusion of secondary lamellae, formation of club-shaped filaments epithelium in the interlamellar regions	Rajeshkumar et al. (2017)
			liver	Hepatocytes showed damage of central vein and rupture of irregular hepatic plate with more number of vacuoles	
Gambusia affinis	77.62 mg/L	28	gills	Hypertrophy and hyperplasia in secondary lamellae followed by detached epithelium with severe necrosis	Begum et al. (2006)
Cu					
Channa gachua	1.4202 mg/l	4	liver	Vacuolation in the cytoplasm and stroma, degeneration of nuclei	Kawade (2020)
Catla catla	1.25 ppm	30	gills	Microscopic analysis of gills' sections revealed lamellar atrophy, telangiectasia, and necrosis of lamellar epithelial cells.	Naz et al. (2021)
			liver	Karyorrhexis, hepatic cells degeneration, congestion, and hemorrhages	
			kidney	Atrophy of glomeruli, necrosis of renal tubular cells, increased urinary spaces, degeneration of renal tubules, and melanomacrophage aggregates	
			intestine	Atrophy of villi, sloughing of epithelial villi, and congestion	
Oreochromis niloticus	0.5, 1.0 and 2.5 mg/l	21	gills	Edema, lifting of lamellar epithelia and an intense vasodilatation of the lamellar vascular axis	Figueiredo-Fernandes et al. (2007)
			liver	Vacuolation and necrosis	
Hg					
Channa gachua	0.1062, 0.0531 mg/l	4	liver	Cloudy swellings of the cells with large vacuoles, degeneration of nuclei, vacuolation in stroma, pycnotic nuclei	Kawade (2020)
Oreochromis niloticus	500,750, and 1000 mg/kg	60	gills	Epithelial lifting and hypertrophy. Fusion of secondary lamellae	Al-Ghanim et al. (2019)
			liver	Necrosis of hepatocytes and portal veins	
			muscles	Irregular muscle bundles and irregularly bigger gaps between the muscle bundles	
Cirrhinus mrigala	0.0206 and 0.0402 ppm	30	gills	Lamellar degeneration, epithelial lifting and necrotic changes in intercellular epithelial cells	Chavan and Muley (2014)
			liver	Necrosis in hepatocytes, blood vessels dilated, haemolysis, inflammation of hepatic cells, congesion in blood sinusoids	
Channa gachua	1.0625 mg/l	4	liver	Vacuolation in the cytoplasm and stoma, degeneration of nuclei	Deore and Wagh (2012)
Pb					
Myoxocephalus scorpius	4.01 μg/L	28	gills	Telangiectasia, hyperplasia of the epithelial cells, lamellar fusion, and synechia	Jantawongsri et al. (2021)
			liver	Megalocytic hepatosis, necrosis, granuloma, and hepatic neoplasm	
Cyprinus carpio	0.25 mg/L	30	gills	Spiking and fusion of secondary lamellae, formation of club-shaped filaments epithelium in the interlamellar regions	Rajeshkumar et al. (2017)
			liver	Hepatocytes showed damage of central vein and rupture of irregular hepatic plate with more number of vacuoles	
Cirrhinus mrigala	28.2 and 14.1 ppm	30	gills	Dilation and congestion in blood vessel of primary gill filament. Hyperplasia of epithelial cells between secondary lamellae led to fusion. Vacuolation and necrosis of lamellar epithelial cells. Congestion of central lamellar vein and hyperplasia of lamellar epithelial cells	Chavan and Muley (2014)
			liver	Cytoplasmic vacuolation, intravascular haemolysis in blood vessels, dilation and congestion in sinusoids and venules and cellular degeneration, focal necrosis	
Poecilia latipinna	0.0 and0.8 mg/L	4	gills	Hyperplasia, hypertrophy and destruction of the lamellar architecture, fusion of lamellae and lamellar clubbing	Mobarak and Sharaf (2011)
			liver	Disarrangement of hepatic cords, shrinkage of hepatocytes and dilatation of liver sinusoids and extravasation of blood	
Oreochromis niloticus)	0, 100, 400, and 800 μg/g	60	liver	Degeneration of hepatic tissue, irregularly arranged hepatocytes, cell hypertrophy, ambiguous cell outline, and obvious vacuolation in cytoplasm	Dai et al. (2009)
Clarias gariepinus	0.006 and 0.008 mg/l	21	gills	Cyto-architectural distortion of the lamella with primary and secondary lamella overlapping. Thus there was occlusion of inter lamella spaces.	Olojo et al. (2005)
			liver	Density of fibrous connective tissue within and around the hepatic parenchyma.	
Clarias gariepinus	0.0, 6.1, 12.2 and 24.4 mg/l	42	gills	Epithelial hypertrophy and lifting, degeneration of cytoplasm and secondary lamellae	Al-Balawi et al. (2013)
			liver	Necrosis of hepatocytes, glomerular expansion and gaps between the muscular bundles	

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Table 3 (*continued*)

Species	Doses	Exposure time (days)	Organs	Changes	References
			kidney	Glomerular expansion, reduction of Bowman's space, tubular necrosis and blood congestion	
Cyprinus carpio	6.2 mg/L	15	gills	Fusion of gill lamellae, vessel dilatation, hyperemia, and hyperplasia of gill epithelial cells.	Khalesi et al. (2017)
Oreochromis niloticus	0, 100, 400, and 800 μg/g	60	liver	Degeneration of hepatic tissue, irregularly arranged hepatocytes, cell hypertrophy, ambiguous cell outline, and obvious vacuolation in cytoplasm	Dai et al. (2009)
Ni					
Channa gachua	150 mg/l	4	liver	Shrinkage of central vein, accumulation of blood cells in the central vein, rupture of sinusoids, degeneration and necrosis in the hepatocytes and connective tissue	Kawade (2020)
Hypophthalmichthys molitrix	5.7 mg/l	30	gills	Mucus proliferation, fusion of the gill lamellae and hypertrophy of gill tissues were observed.	Athikesavan et al. (2006)
			liver	Necrosis in hepatocytes. Degeneration of blood vessels, vacuolation, hypertrophy, pyknotic nuclei and lesion	
			kidney	Degeneration of tubular cells, hyperplasia was observed in kidney tissues.	

exposure to Striped Catfish (*Pangasianodon hypophthalmus*) caused a significant decrease in amount of DNA in the vital organs such as gill, liver and kidney (Suchana et al., 2021).

5. Heavy metals effect on histopathology of different organs of fish

Major fish organs like gills, liver and kidney are very sensitive to heavy metal toxicants and are extensively studied for different fish species exposed to different heavy metals. These organs respond differently to various heavy metals and are considered to be an important bio-monitoring tool in evaluating their toxic effects in various fish species (Naz et al., 2021). Prolonged exposure of heavy metals induces cytotoxicity and causes degenerative changes in the vital organs of the fish. Histopathological changes in different organs triggered by the heavy metals intoxication have been extensively studied in different fish species (Fig. 3, Table 3).

Fish gills are in direct contact with the external environment for gaseous exchange and act as a main route for heavy metal uptake from the environment through delicate epithelium followed by storage and transfer of the metal to the internal organs (Thophon et al., 2003). Histopathological symptoms in gills differ with heavy metal uptake, exposure time and fish species etc. Generally, edema of lamellar epithelial cells is considered to be the first pathological sign in gill exposed to any heavy metal. This could be due to the lifting away of the epithelium, a covering layer of the secondary lamellae, from the pilaster cell system, which makes the diffusion distance/blood-water diffusion distance larger, thus separating the blood vessel in the lamellae from the external medium. Similarly capillary congestion or aneurysm, lamellar fusion, hypertrophy and hyperplasia of epithelial, chloride and mucous cells etc. have also been noticed in different fishes exposed to heavy metals. These signs are considered to be the body's defense mechanisms that reduce the chances of the gill surface to have a direct contact to the external water (Al-Ghanim et al., 2019; Khalesi et al., 2017; Rajeshkumar et al., 2017; Suchana et al., 2021). Aneurysm was found to be resulted from the breakdown of the pilaster and vascular cell systems due to the release of large volume of blood that pushes the epithelium layer outward (Thophon et al., 2003). Calcium channel along with enzyme act as a carrier for heavy metals such as Cd to transport to the chloride cells of the gill and in response to that, chloride cells try to eject the heavy metals which causes hypertrophy or hyperplasia of the cells (Giari et al., 2007).

Liver is considered to be the main organ for metabolism and detoxification of any toxicants. When the toxicity level reaches to a threshold point, structural and biochemical alterations occur in the liver by changing the hepatic enzyme levels that leads to the degenerative changes in the liver (Hossain et al., 2016; Paris-Palacios et al., 2000; Sharmin et al., 2015). Most of the heavy metals accumulate at higher level in the liver makes it more sensitive to heavy metals than other organs. Histopathological alterations such as hydropic swelling of hepatocytes, degeneration and necrosis of hepatocytes, blood congestion, hepatocyte lysis, cirrhosis, pyknotic nuclei, vacuolation, lipid droplets etc. have been found in the liver of different fish species exposed to heavy metals in an exposure time dependent manner (Benjamin and Kutty, 2019; Chavan and Muley, 2014; Kawade, 2020). Nuclei of hepatocyte have metal binding proteins, which might be the cause of degeneration of the nucleus due to the exposure of contaminants (Al-Balawi et al., 2013). The increased accumulation of heavy metals in the liver might be responsible for the necrosis and degeneration of the hepatic cells. Degeneration of the gills and disruption of the blood vessels are responsible for the insufficient supply of oxygen to the liver, which may cause the damage of the hepatic cells in the liver (Hossain et al., 2016; Sharmin et al., 2015).

Kidney plays an important role in maintaining the osmotic balance and detoxification of xenobiotics including heavy metals. Several studies reported that the histopathological changes in kidney are more severe than the gills and liver and is more susceptible to even minor toxic injury due to its large proportion of post-branchial blood supply to the renal tissue (Liew et al., 2020; Moyson et al., 2016; Sula et al., 2020a; Thophon et al., 2003; Wangsongsak et al., 2007). Therefore, lesion in nephron and hematopoietic tissue indicates the ionic imbalance due to heavy metal exposure and this could be a good indicator of environmental pollution (Ortiz et al., 2003). Like liver, kidney also has metal binding protein such as metallothionein (MT) and the increased level of MT in kidney indicates the increased amount of heavy metal deposition like Cd which causes degenerative changes in the kidney (Wangsongsak et al., 2007). Apart from that, heavy metals exposure to different fish species also causes histopathological changes in the kidney such as shrinkage of renal corpuscle, pyknotic nuclei, atrophy of glomeruli, necrosis of renal tubular cells, increased urinary spaces, degeneration of renal tubules, increased diameter of proximal convoluted tubules, hypertrophy of the epithelial cells etc. (Al-Balawi et al., 2013; Benjamin and Kutty, 2019; Datta et al., 2009).

Unlike gill, liver and kidney, other body parts such as intestine, gallbladder, muscles have got least importance to explore the histopathological changes occurred due to expose to heavy metals in fish species. Mainly feeds contaminated with heavy metal causes degenerative changes in the intestine including atrophy of villi, sloughing of epithelial villi, necrotic changes in the intestinal mucosa, and submucosa with degenerative cells in the intestinal lumen (Al-Balawi et al., 2013; Kaoud et al., 2011; Padrilah et al., 2018) Fish muscle constitutes a large proportion compared to any other organs in fish.

Table 4
Molecular responses of fish to heavy metals exposure.

Species	Doses	Exposure time (days)	Genes expression		References
			Increase	Decrease	
As					
Sparus aurata	0.001, 0.005, 0.01, 0.05, 0.1, 0.5 & 1.0 mM	1	sod	mta, hsp70, prx1 and prx2, f bcl2	Morcillo et al. (2016)
Danio rerio	Arsenic Contaminated Diets	68	–	vitellogenin	Boyle et al. (2008)
Cd					
Odontesthes bonariensis	0.25 μg/L	14	gnrh1, 2 and 3	–	Gárriz et al. (2019)
Monopterus albus	1.10, 10.26, 6.70 & 22.88 mg/kg	–	MT	–	Wahid et al. (2017)
Sparus aurata	0.5 mg/kg	15	Cytochrome P450	HSP90	Benhamed et al. (2016)
Danio rerio		7	ABCC5	–	Sabri et al. (2013)
Oncorhynchus mykiss	35.9 μg/kg	28	hepatic transferrin, MTs and HSP70	–	Kwong et al. (2011)
Fundulus heteroclitus	1.2 μg/mg	7	MT	–	Roesijadi et al. (2009)
Oryzias javanicus	10 & 100 ppb	1	MT, G6PD and HSP70	–	Woo et al. (2009)
Oryzias javanicus	1, 10 & 100 ppb	1	MT	–	Woo et al. (2006)
Oncorhynchus mykiss	0, 1 & 5 μg/l	3	sGnRH1, sGnRH2	–	Vetillard and Bailhache (2005)
Cr					
Oreochromis niloticus	4.57 mg/L	60	caspase-3	Bcl2, CYP450 and GST	Mohamed et al. (2020)
Odontesthes bonariensis	4 μg/L	14	–	fshb	Gárriz et al. (2019)
Sebastes schlegelii	0, 30, 60, 120 and 200 mg/kg diet	28	SOD and GST, MT	GSH	Kim and Kang (2016)
Oryzias javanicus	10, 100 & 1000 ppb	1	–	MT	Woo et al. (2006)
Fundulus heteroclitus	32 mg/L	7	EST – bb93	ALDH4, GSTs, Cyp2X and EST	Roling and Baldwin (2006)
Cu					
Megalobrama amblycephala	1.43, 5.21 and 9.13 mg/kg	70	Nrf2, Hsp70, HO-1, IL-10	Keap1	Linag et al. (2020)
Odontesthes bonariensis	22 μg/L	14	lhb	cyp19a1b	Gárriz et al. (2019)
Pelteobagrus fulvidraco	0.76, 4.18 & 92.45 mg/kg diet	56	6PGD, FAS, ACCα, PPARγ, LXR, HSL, PPARα	–	Chen et al. (2015)
Danio rerio		7	ABCC5	–	Sabri et al. (2013)
Oryzias javanicus	1, 10 & 100 ppb	1	MT	–	Woo et al. (2006)
Hg					
Pseudosciaena crocea	0.25, 1.0, 4.0 μg/l	30	CAT, GSH, GPx, TCTP, GST3, Hsp70, Hsp27	SOD	Wu et al. (2018)
Osteochilus hasseltii	0.025, 0.05 & 0.1 mg/L	60	–	GtH1α, GtH2α and GtHβ	Siregar and Prayogo (2017)
Sparus aurata	0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05 & 0.1 mM	1	mta, hsp70, prx1 and prx2), sod and gr, bax	–	Morcillo et al. (2016)
Danio rerio	15 & 30 μg/l	30	cat1, sod1 and gpx1a,	lhβ, gnrh2, gnrh3, lhr and erα	Zhang et al. (2016)
Channa punctatus	0.3 mg/L	7	TNF-α and IL-6	–	Begam and Sengupta (2015)
Gobiocypris Rarus	0.1 & 0.3 mg/L	4	cr, atpase, gh, hsp70, and mt	prl and igf1	Li et al. (2014)
Danio rerio	0.08, 5.0 & 13.5 μg/g	63	mt2 and rad51	–	Gonzalez et al. (2006)
Ni					
Oryzias javanicus	10, 100 & 1000 ppb	1	MT	–	Woo et al. (2006)
Pb					
Oreochromis. niloticus	0.56, 0.70 & 1.09 ppm	–	–	GST	Hassan et al. (2020)
Sparus aurata	1, 2, 3, 4 or 5 mM	1	mta, hsp70, sod, cat, prx1, prx2, bax	bcl2	Morcillo et al. (2016)
Zn					
Clarias magur	50, 200, 300 mg/kg	60	FSH, LH	GTH	Gupta et al. (2021)
Odontesthes bonariensis	211 μg/L	14	–	fshr, flhcgr	Gárriz et al. (2019)
Oreochromis niloticus	500 & 2000 μg/l	15	GSH, SOD, CAT, GR, GPx, GST	–	Saddick et al. (2017)
Sparus aurata	0.4 μgl^{-1}	15	–	MT	Benhamed et al. (2016)
Oryzias javanicus	10, 100 & 1000 ppb	1	MT	–	Woo et al. (2006)
Oncorhynchus mykiss	3.5 mM	6	GST	–	Hogstrand et al. (2002)

ABCC5; ABC transporters, subfamily C (ATP-binding cassette transporters), GtH; gonadotropin hormone, G6PD; glucose-6-phosphate 1-dehydrogenase, HSP; heat-shock protein, MT; metallothionein, GST; Glutathione-S-transferase; metallothionein-A gene (mta), gr; glutathione reductase.

Although the fish muscle is considered to be a least susceptible to heavy metal exposure but still some pathological changes have been observed in fish muscle in a dose-dependent manner like atrophy and edema of muscle bundles, irregular muscle bundles and bigger gaps between the muscle bundles etc. (Al-Ghanim et al., 2019; Begum et al., 2014). For example, smaller gaps between the muscle bundles have been noticed at the low dose (500 mg/kg) of Hg exposure while the higher doses (1000 mg/kg) create larger gaps between the muscle bundles. Prolonged exposure to heavy metals causes hemorrhage and sloughing of skin due to the depleted production of the mucus (Al-Ghanim et al., 2019). If the fish exposed to mixtures of heavy metals, they may act either as additive, synergistic or antagonistic agent and showed degenerative changes in the exposed fish (Jezierska et al., 2000). The mixture of Cr and Ni exposed to *Cirrhinus mrigala* showed synergistic interactions between the two metals and higher accumulating rate was observed than those of the individual metal (Palaniappan and Karthikeyan, 2009).

6. Molecular responses in fish to heavy metals exposure

Changing the expression profile of different genes involved in oxidative stress response and the detoxification of heavy metals in fishes are being used to reveal the cellular and physiological responses of different heavy metal exposure and considered as potential biomarkers to detect hazardous chemicals in the environment. The standard qPCR analysis, microarray and the most recent genomics technology such as transcriptomics, RNAseq have been used to study genome-wide and simultaneous expression of thousands of genes in cells or tissues of fishes exposed to multiple heavy metals (Fu et al., 2020; Hook et al., 2006). For example, RNAseq has been used to explore differentially expressed genes in hepato-pancreas of carp (*Cyprinus carpio*) exposed to Cd and 15 genes have been identified that regulate cell growth and oxidative function under metal stress (Fu et al., 2020). The up-regulation and down-regulation of genes related to antioxidant enzymes and proteins due to exposure of different heavy metals with varying doses and exposure times in fishes are summarized in Table 4.

When the ROS level has been override the antioxidant molecules and free radical scavengers, SOD-CAT system provides the first line of defense against oxidative damage. The SOD has direct involvement in the removal of reactive oxygen species by converting super-oxide free radical to hydrogen peroxide and CAT works by breaking down hydrogen peroxide to water and free oxygen. The mRNA of SOD and CAT genes has been down-regulated significantly in the brain tissue of *Oreochromis niloticus* and *Tilapia zillii* due to Zn exposure (Saddick et al., 2017). Another study found that CAT transcript increased significantly in Javanese medaka (*Oryzias javanicus*) exposed to Cd, Cu, Zn and decreased by exposure to Cr and Ni (Woo et al., 2009). The expression differences could be due to different signaling pathways for detoxification of different heavy metals. Glutathione peroxidase (GPx) has a direct role in body defense mechanism against the formation of hydrogen peroxide and GPx transcript expression has been influenced by the heavy metal exposure. Cd, Cu and Zn exposure in Javanese medaka fish has induced the GPx gene expression in liver tissue, whereas inhibited by Cr and Ni (Woo et al., 2009). Glutathione reductase (GR) and Glutathione S-transferase (GST) are also known to play an important role in detoxification and are used to determine the oxidative stress in the cells due to heavy metal exposure. The GR mRNA expression was found to significantly high in the gills and kidney of brown trout but not in the liver collected from a river contaminated with Cd, Cu and Zn (Hansen et al., 2006). On the other hand, significantly higher GST transcript has been expressed in the liver tissue of Japanese medaka fish exposed to five heavy metals such as Cd, Cu, Zn, Cr and Ni in a dose-dependent manner (Woo et al., 2009).

Glucose-6-phosphate dehydrogenase (G6PD), heat shock proteins and metallothionein have been highly expressed in liver of marine medaka fish exposed to Cd (Woo et al., 2009). The G6PD is an important biomarker and has proved to have role in detoxification of reactive oxygen species. The G6PD mRNA has been over expressed in rainbow trout gill cells and in Japanese medaka liver tissue exposed to Cu and Zn (Chung et al., 2005; Woo et al., 2009). Similarly heat-shock proteins (HSP47, HSP60, HSP70, HSP78 and HSP90) are stress defense proteins, which are highly conserved among the taxa and are responsive to the external stimuli. For instances HSP70 and HSP90 genes were found to be down-regulated in the skin of gilthead sea bream exposed to Cd (Benhamed et al., 2016). Tissue-specific expression of HSP70 genes (HSP70a and HSP70b) has been noticed upon Cd exposure. For example, HSP70a is found to be more sensitive to Cd exposure in rainbow trout than HSP70b and increased mRNA expression of both genes (HSP70a and HSP70b) have found in the intestine due to Cd induction, whereas kidney showed only HSP70a gene expression (Kwong et al., 2011). Metallothioneins are low-molecular weight, heat-stable and cysteine-rich peptides which plays an important role in homeostasis of essential metals such as Cu and Zn, and detoxification of non-essential metals such as Cd, Pb and Hg (Cheung et al., 2004). The MT has been widely used as a molecular biomarker for evaluating the heavy metal toxicity and bioaccumulation mechanism in the living organisms. The MT mRNA transcripts were found to express in almost all tissues with the highest expressions in liver may be due to its role as a major detoxifying organ (Yang et al., 2014). Two types of MT genes (MT-A and MT-B) have been identified in a number of teleost fish and MT-A has shown to have a significant role in Cd detoxification in fish than MT-B (Wu et al., 2018). For example, MT-A mRNA gene has been expressed in the liver with the dietary Fe supplementation and MT-B has not been affected. On the other hand, Fe exposure has suppressed the mRNA expression of both MT-A and MT-B genes in the kidney. Likewise, Cd exposure increased the up-regulation of MT-A gene in the intestine and liver whereas kidney was very sensitive to Cd exposure by expressing the MT-B gene (Vergani et al., 2007). Transferrin (Tf), an important iron binding protein synthesized from liver and transported to the blood. Cd is also known to bind Tf, which further reduce the binding capacity of Fe to Tf and has up-regulate the expression of Tf mRNA in the liver. It has been noted that in fish fed with high Fe and Cd rich diet, the binding of Cd-Tf is inhibited by the presence of high amount of Fe thereby Tf mRNA gene expression will not be affected (Kwong et al., 2011). Another protein, ATP-binding cassette transporters subfamily C (ABCC) is known as multidrug-resistance-associated proteins which has role in detoxification and also in protecting body from heavy metal intoxication by pumping out the toxin from cells back into blood (Gómez-Pinedo et al., 2010). The mRNA transcripts of ABCC5 has been found to show a high level of expression in brain, eyes, ovaries and intestine in adult zebrafish treated with Cu, Cd, Pb and As whereas gill, liver, heart and muscle showed low level of expression (Long et al., 2011; Sabri et al., 2013).

Brain-pituitary-gonadal axis genes have been found to be influenced by heavy metal exposure and are shown to play an important role as indicators of environmental pollution. The GnRH mRNA transcripts (1, 2, 3) have been found to increase in the brain of pejerrey fish (*Odontesthes bonariensis*) exposed to Cd and a decrease in cyp19a1b gene expression due to Cu exposure in the same fish. The FSHβ mRNA expression has been found to be down-regulated in the pituitary of pejerrey exposed to Cd (0.25 μg/L) and Cr (4 μg/L); and a significant up-regulation of LHβ mRNA has been noticed in Cu exposure (22 μg/L (Gárriz et al., 2019), in the same species. Cd exposure could inhibit the expression of estrogen receptor (rtER) in the liver of rainbow trout, which has been found to suppress the vitellogenin (Vg) gene expression. If the estrogen level is very high in the blood then Cd exposure cannot inhibit the estrogen receptor expression, which in turn will not be able to inhibit vitellogenin production in the liver in trout (Vetillard and Bailhache, 2005).

Estimation of chromosomal abnormality has long been regarded as a genotoxicity test to evaluate the impacts of heavy metals at molecular level. Four types of chromosomal breakages such as single chromatid gap, isochromatid gap, single chromatid breaks and isochromatid breaks have been found in the kidney tissue of a snakehead fish (*Channa striata*)

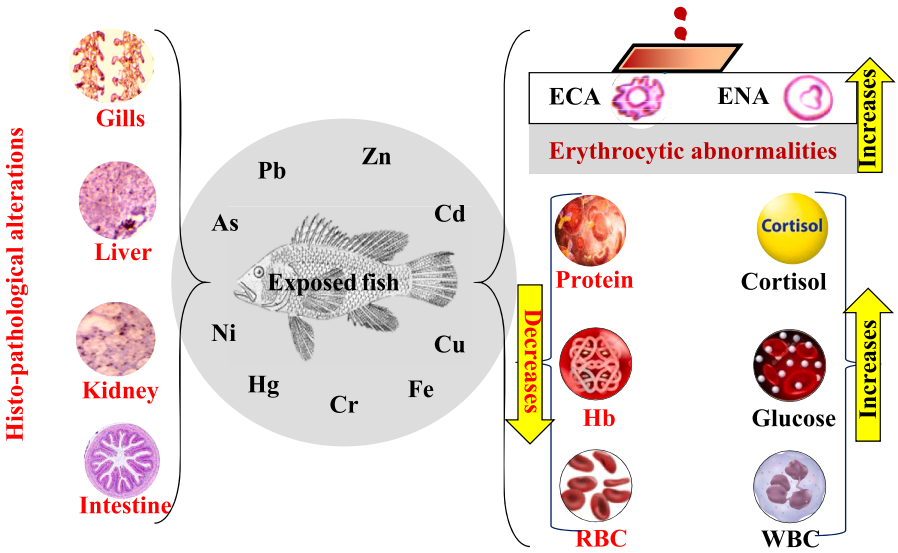

Fig. 4. Schematic illustrations of the impacts of different heavy metals on fish physiology.

collected from natural reservoir contaminated with Hg (Promsid et al., 2015). The Hg is known to cause teratogenic, mutagenic and cytogenetic damage in fish, and interfere with the thiol metabolism leading to disruption of mitotic cell division, which causes chromosomal abnormalities in fish. Another study reported 4.6011% chromosome aberrations in the kidney of *C. striata* collected from a reservoir near an electronic waste dumping area which was found to be contaminated with Cd, Cr and Pb (Phoonaploy et al., 2019). Arsenic has been considered as carcinogenic metal and has found to cause genotoxicity in fish. Arsenic has the ability to inhibit DNA repair system and telomerase activity, and it has been reported that it has been significantly delayed mitotic division, inhibited assembly of the mitotic spindle, caused endoreduplication and chromosomal breaks, chromatid and chromosome gaps (Yadav and Trivedi, 2009).

Most of the heavy metal studies on fish have been conducted *in vivo* either in the laboratory or in the natural environment. But there are very few studies where fish cell lines have been used *in vitro* to explore the biochemical changes and the molecular mechanisms involved due to the heavy metal exposure. For example, fibroblast cell line (SAF1) of sea bream (*Sparus aurata*) has been used to study Cu homeostasis and to identify the expression pattern of oxidative stress genes after exposure to heavy metals such as Cu, Zn and Cd (Minghetti et al., 2011). Three metals have been found to increase the mRNA levels of MT and GR genes whereas ATP7A mRNA levels increased only due to Cu exposure, which may be due to the presence of specific intracellular Cu sensor, which mediates ATP7A transcription.

7. Conclusions

Anthropogenic inputs and industries are the major sources of heavy metal contamination in the aquatic environments. Establishing industries are essential for country's development and when their effluents are discharged directly into the environment without proper wastewater treatment cause deleterious effects in the aquatic biota. Fish is an important and chief source of animal protein and are in direct contact with heavy metals due to aquatic pollution. Unfortunately, nowadays fish are becoming a major source of dietary heavy metals, which is responsible for carcinogenic and non-carcinogenic effects on human. Heavy metal causes toxicity; multiple organs of fish are affected due to oxidative damages and finally accumulate in the tissues. This review documented information about the heavy metal impacts on fish biochemical parameters, histopathology of different major organs and molecular changes at the DNA levels (Fig. 4) and those parameters are proved to be important and effective bio-monitoring tools to evaluate the health status of aquatic organisms and to detect environmental pollutants.

Most of all, further investigations are required to elucidate the underlying specific molecular mechanisms of how a single heavy metal or a combination of those interact with organisms that can cause DNA damage in different fish species including fresh and marine water using modern genomics and transcriptomics technologies. There is a need to evaluate the effect of a single heavy metal covering the physiological, immunological and molecular mechanism in a single species in a single experiment for better understanding of specific heavy metal toxicity with its mode of action. It also creates a room to develop bio-chemical sensors for immediate monitoring of the presence of heavy metals in the fish body as well as in the environment. It also warrants to explore the bioremediation of heavy metals toxicity and accumulation in the fish body as evidenced in African catfish, *Clarias gariepinus* (El-Bouhy et al., 2021a,b). Lastly this review points out the need for formulation or legislation of the laws for establishing an industry with proper discharging protocols considering their devastating effects on the fish and other aquatic biota and will also help to ascertain the safe levels of heavy metals for the aquatic environment.

Data availability statement

Sharing of data is not permissible for this article. The data that support the outcomes of this study are available on request from the corresponding author [M Shahjahan].

Authors' contributions

Md Shahjahan: conceptualization and edited the manuscript. Khanam Taslima: preparation of the first draft of the manuscript. Mohammad Shadiqur Rahman, Md Al-Emran and Shanon Iffat Alam: data collection and preparation of the Tables. Caterina Faggio edited the manuscript. All authors have read the final version and approved the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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