Biological Trace Element Research (2025) 203:4326–4346
https://doi.org/10.1007/s12011-024-04478-w

REVIEW

Fate and Effects of Heavy Metals in Fishes: Antioxidant Defense System, miRNA/Gene Expression Response, and Histopathological Reproductive Manifestations

Rayees Ahmad Bhat[1] · Absar Alam[1] · Dharm Nath Jha[1] · Vikas Kumar[1] · Jeetendra Kumar[1] · Venkatesh Ramrao Thakur[1] · Basanta Kumar Das[2]

Received: 29 July 2024 / Accepted: 3 December 2024 / Published online: 9 December 2024

Abstract
Heavy metal pollution is a major environmental concern and in particular for aquatic ecosystems. With heavy metals exceeding safe and recommended limits, they pose significant threats to the environment and its inhabitants, including fish. Heavy metals, when accumulated in the different organs of the fish, result in toxicity in fish by producing reactive oxygen species (ROS) through the generation of oxidizing radicals. This oxidative stress mechanism is a key factor in the detrimental effects of heavy metal pollution on aquatic life. Heavy metal exposure profoundly affects fish behavior and physiology. In this review, an attempt was made to report the effects of heavy metals on fish physiology, focusing on toxicological effects on antioxidant enzymes, microRNAs (miRNAs) and molecular genetic responses, histopathology of organs, and underlying molecular mechanisms. This review also highlighted the heavy metal impact on fish gonads (testes and ovaries) and the hormones associated with it. The detection methods and the incorporation of latest developments in AI-based technology for the detection of heavy metals are also included in this review. Understanding the above effects is important for assessing the ecological impact of heavy metal pollution and developing strategies to mitigate its adverse effects on aquatic life. Understanding the consequences listed above is important for analyzing the ecological impact of heavy metal pollution and devising measures to reduce its negative effects on aquatic life and human health.

Keywords Heavy metals · Toxicity · Fish · Biochemical parameters · Quantification techniques

Introduction

Water pollution due to various pollutants like heavy metals, pesticides, microplastics, etc. causes a severe threat to aquatic life and human health [1]. Fishes are more vulnerable to water pollution because of their direct contact with exposed contaminants. Heavy metals are metals with high density (usually more than 5 g/cm^3) and show toxicity even at parts per billion (ppb) levels [2]. These metals offer substantial environmental and health risks due to their persistence, bioaccumulation, and biomagnification in ecosystems. Heavy metals pose a serious threat to aquatic ecosystems, including fish. These metals accumulate in water bodies and sediments, ultimately affecting fish physiology and reproductive performance [3]. The heavy metal toxicity impacts fish by damaging their nervous systems, movement, feeding, and breeding behaviors [4]. The accumulation of heavy metals is concerning because most heavy metals do not degrade into less harmful forms. Their accumulation in aquatic ecosystems leads to long-term detrimental effects not only on fish but also on humans who consume contaminated fish. The uncontrolled release of heavy metals into water bodies remains a critical environmental and public health challenge. In recent times, pollutants like heavy metals have become a global concern due to their intensified emission resulting from anthropocentric activities [5–8]. Increasing population growth, industrialization, and intensive agriculture activities contribute to an array of pollutants which causes a serious impact on aquatic flora and fauna [9, 10]. Anthropogenic and industrial activities like mining, manufacturing, metal

✉ Basanta Kumar Das
basantakumard@gmail.com

[1] ICAR-Central Inland Fisheries Research Institute, Regional Centre, Prayagraj 211 002, Uttar Pradesh, India

[2] ICAR-Central Inland Fisheries Research Institute, Barrackpore, Kolkata 700 120, West Bengal, India

working, and the leather industry release effluents containing a variety of toxic heavy elements viz. chromium (Cr), nickel (Ni), cobalt (Co), aluminium (AI), cadmium (Cd), mercury (Hg), lead (Pb), copper (Cu), arsenic (As), and tin (Sn) (Table 1). Higher levels of iron lead to oxidative stress, while excessive concentrations of copper are involved in neurological disorders and embryonic and larval development [11, 12]. Due to their high toxicity, Cd, As, Cr, Pb, and Hg are classified as priority heavy metals of significant public health concern. These elements are systemic toxicants capable of causing damage to multiple organs, even at low levels of exposure [13].

Exposure to heavy metals during larval and embryonic stages leads to a range of negative effects on fish development and health. Metals entered into the body via direct contact with gills, skin, and dietary intake of metal-contaminated food [14]. Metals can interfere with skeletal development in fish embryos and larvae, leading to abnormalities in the vertebral column and spinal structure [15]. These may disrupt cardiovascular function in developing fish, leading to alterations in heart rate and rhythm and also induce morphological abnormalities in fish embryos and larvae, resulting in deformed body shapes and impaired growth and development [15]. Metal toxicity during early life stages can increase mortality rates in fish populations.

Heavy metal intoxication in fish leads to a wide range of biochemical, physiological, histological, and molecular alteration. Exposure to acute as well as chronic levels of heavy metals leads to significant health impacts, which are reflected in altered enzyme activities, gene expression patterns, histopathological changes, and overall physiological dysfunction [16, 17]. Heavy metals endorse the production of reactive oxygen species, which results in oxidative stress and DNA damage. This oxidative stress alters the expression patterns of important proteins, hormones, and enzymes, affecting numerous physiological and cellular functions [18], Gárriz et al. [19]. Heavy metal alters the hemato-biochemical setup in fishes and results in various cellular and nuclear deformities in blood cells [20]. Various studies have confirmed that heavy metals are involved in different genetic damages [21]. Additionally, documented reports revealed that heavy metal contamination impairs the reproductive performance of fish, viz. reduced gonadosomatic index (GSI), fecundity, gonadotropins, and abnormal shape of reproductive organs [22–24]. Despite the well-documented impacts of heavy metals on aquatic animals, comprehensive patterns detailing these effects across various fish species, especially those crucial to aquaculture, are sparse.

The detection of heavy metals from environmental and human samples is crucial for monitoring and managing contamination. The development and implementation of simplified and precise methodologies for the quantification of heavy metals in environmental matrices can significantly enhance monitoring and management efforts [25]. To address the need for highly simplified and precise methodologies for the quantification of heavy metals in various environmental matrices (water, soil, biological tissues), several key approaches can be considered viz. sample preparation protocol, calibration, and application of internal standards. In recent years, significant advancements have been made in the development of novel, highly robust, sensitive, and selective approaches for the quantification of heavy metals. Two prominent techniques for this purpose are atomic absorption spectrometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS) [26]. However, monitoring pollution levels typically involves direct measurement methods can be labor-intensive, require specialized equipment, and consume significant time and resources. Effective management of heavy metal pollution necessitates comprehensive definition, continuous monitoring, and accurate modeling [27]. Artificial intelligence (AI) and machine learning (ML) offer significant importance in the field of heavy metal detection. Using a machine learning approach, an AI-based methodology has been used to identify and genetically optimize the color and texture features for the classification of fish exposed to heavy metals [28].

This review aims to consolidate current knowledge on how heavy metals influence antioxidant enzymes, histopathology, molecular changes, miRNA, detection techniques, and reproductive impairments with the latest insights.

Table 1 Source of heavy metals

Heavy metals	Sources
Arsenic (As)	Pesticides, fungicides
Cadmium (Cd)	Pesticides, fertilizers, nuclear plants, batteries
Chromium (Cr)	Mining, electroplating, textile
Nickel (Ni)	Industrial dust and electroplating
Cobalt (Co)	Battery production, industrial waste, alloy, mining and fossil combustion
Mercury (Hg)	Pesticides, batteries, paper industries
Zinc (Zn)	Refineries, brass manufacture, metal painting
Lead (Pb)	Paint, pesticides, mining, batteries

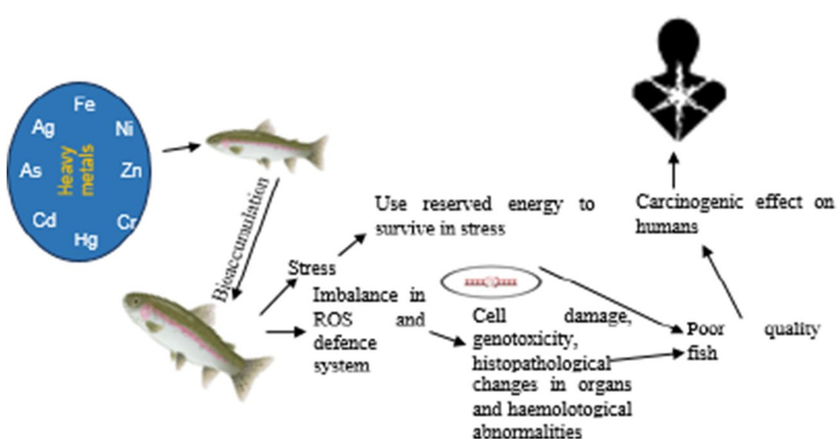
Toxicological Response of Heavy Metals on Antioxidant Enzymes

Reactive oxygen species (ROS) have a role in disrupting biological systems. It can directly damage DNA by causing modifications such as base oxidation and strand breaks; it also initiates lipid peroxidation. Superoxide dismutase (SOD) is an enzyme that catalyzes the dismutation of superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), and catalase (CAT) catalyzes the decomposition of H_2O_2 into O_2 and H_2O. By converting superoxide radicals, SOD and catalase help prevent oxidative stress and cellular damage (Fig. 1). Glutathione S-transferase (GST) is an essential phase II detoxification enzyme, facilitates the removal of toxicants from fish tissues, and plays a critical role in protecting fish from harmful chemicals and maintaining cellular homeostasis [29, 30]. These enzymes together minimize ROS and their adverse effects on cellular components [31]. The presence of ROS, also referred as oxyradicals, triggers the upregulation of antioxidant enzymes as part of the cellular defense mechanism against oxidative stress [31, 32]. Exposure to heavy metals can lead to alterations in the activity levels of antioxidant enzymes such as CAT, SOD, glutathione peroxidase (GPx), and other enzymes [33, 34]. Heavy metals viz. Cu and Cd have been reported to reduce the activity of CAT in tissues of African catfish and other fish species. These metals induce oxidative stress by promoting the generation of ROS, which can overcome the defense mechanism of the body, resulting in harm to cellular components [35]. The reduction of CAT activity by Cd and Cu in different tissues reflects the negative effects of metal-induced oxidative stress on aquatic organisms. Exposure to Cr has been associated with decreased catalase activity in various tissues of *Clarias batrachus*, viz. kidneys and gills. Lower concentrations of CAT in various tissues of exposed fish may result from the accumulation of H_2O_2 within cells [36]. Khalil et al. [37] upon working on lipid peroxidation and antioxidant enzymes in *Oreochromis niloticus* (Nile tilapia) during different seasonal variations reported during winter when water pollution levels were higher compared to summer, the activities of antioxidant enzymes such as SOD, CAT, GPx, and glutathione S-transferase (GST) significantly increased in *O. niloticus* depicting an adaptive mechanism of the fish to cope with increased oxidative stress induced by elevated pollution levels during the winter season. Cd is a significant environmental pollutant known for its toxicity to aquatic animals and fish. Previous studies have reported that exposure to Cd can lead to various adverse effects, including oxidative stress, impaired antioxidant capacity, reduced growth performance, and alterations in the composition of the microbiota in fish ([38].

Vaseem and Banerjee [39] have studied the impact of heavy metal pollution resulting from uncontrolled anthropogenic activities on fish health, particularly in terms of oxidative stress. An elevated activity of enzymes viz. CAT, SOD, and MDA in fish collected from the heavily polluted river was reported. The increase in enzyme activities depicts that the fish were going through oxidative stress caused by the pollutants present in the water. Bhat et al. [40] recorded a reduction in the levels of antioxidant enzymes like SOD, CAT, and GPx, and an increase in MDA levels in *Oncorhynchus mykiss* ovary with increased heavy metal concentration. The reduction suggests that the antioxidant defense mechanisms are overwhelmed by the increased production of ROS induced by heavy metal stress. An increase in MDA levels were noticed in *O. mykiss* ovary collected from metal contaminated water depicted elevated lipid peroxidation. Atli [41] observed a decrease in CAT activity in both the liver and kidney of *O. niloticus* when fishes exposed to Cd but an increase in thiobarbituric acid reactive substances (TBARS) level in the kidney when treated with higher doses of Cu and Zn. Hossain et al. [42] recorded a significant change in antioxidant enzyme activities and GST activity, as well as changes in lipid peroxidation levels, in response to exposure to heavy metal-contaminated river water in *O. niloticus*. An increase in GST activity in blood was observed on exposure to heavy metals compared to the control group. Also, after 10 days of exposure to polluted water, a significant increase in the activities of antioxidant enzymes such as GPx, SOD, and CAT were noticed.

Glutathione (GSH) and its oxidized form glutathione disulfide (GSSG) has an important role in non-catalytic antioxidant defense mechanisms within cells. Glutathione helps to neutralize ROS and protect cellular components from oxidative damage [43]. Changes in GSH levels with

Fig. 1 Heavy metal accumulation, formation of ROS and histopathological changes in fish and human health risk



response to toxicological response have been observed in different fish species [44]. Tanhan et al. [45] studied the relationship between liver GSH levels and lead accumulation in different fish species, namely, *Netuma thalassina, Scatophagus argus*, *Mugil cephalus*, and *Netuma thalassina*, and observed that liver GSH levels were not significantly different among the species studied. A positive correlation between liver GSH levels and Pb concentration specifically in *N. thalassina* was revealed suggesting this species may have a unique response to Pb exposure compared to the other species studied. Table 2 depicts the detailed effects of heavy metals on fish antioxidant enzymes with different concentrations.

miRNAs and their Toxicological Response

ROS are reactive molecules causing damage to various cellular components, including nucleic acids like RNA. It also induces oxidative modifications in RNA molecules, including ribosomal RNA (rRNA), messenger RNA (mRNA), and microRNAs (miRNAs) [58]. miRNAs are small non-coding RNA molecules typically consisting of around 22 nucleotides and play an essential role in post-transcriptional regulation of gene expression by linking to complementary sequences on target mRNA molecules [59, 60]. miRNA expression changes in response to exposure to heavy metals in humans and fish is an emerging field with limited research conducted so far [61, 62]. miR-181a and miR-155 are miRNAs had a role in regulating immune responses, inflammation, and oxidative stress. The increased elevation of miR-155 has been associated with the upregulation of inflammatory cytokines, including interleukin-6 (IL-6), (IL-8) and interleukin-1β (IL-1β) [63].

Cadmium and miRNAs

Liu et al. [38] reported that exposure to Cd at a concentration of 0.275 mg/L for 30 days resulted in the downregulation of novel-miR6, miR-193a-3p, and miR-122 in the kidney or other tissues of *C. carpio*, these all play a role in apoptosis, cell proliferation, and metabolism. Li et al. [64] have studied the effects of Cd exposure on the kidney of *C. carpio* and reported that exposure to 0.5 mg/L Cd^{2+} significantly alters the function of key enzymes and the expression of various immune-related genes and upregulation of miR-181a and miR-155 in the kidneys. Cd exposure hindrances miRNA expression and can induce specific signalling pathways, such as the miR-216a-PI3K/AKT axis in *C. carpio* lymphocytes [65]. miR-122 has been implicated in the regulation of metallothionein (MT) levels, and this regulatory mechanism can influence Cd exposure and the resistance response in fish [66].

Several reports have documented that miRNA viz. miR-9, miR-21, miR-132, miR-210, miR-146, miR-155, miR-181a, and miR-223 play crucial roles in inflammatory processes [67, 68]. miRNAs have been reported to play a significant role in the response of fish to Cd exposure, some miRNAs

Table 2 Effects of heavy metals on antioxidant enzymes

Fish species	Heavy metal and dose	Effect on fish	References
Carassius auratus gibelio	Cd (20 mg/L)	Stress, proliferation mucus cells, and oxidative injury	Zikic et al. [46]
Carassius auratus	Cr^{6+} and Cr^{3} (10 mg/L)	Change in the activity of CAT, and SOD in the brain, liver, gills and kidney	Kubrak et al. [47]
Phoxinus lagowskii	Cr^{6+} (5 and 10 mg/L)	Increase in SOD, CAT, GPx, GST, ROS	Hu et al. [48]
Channa punctatus	7.89 mg/L (Cr^{6+})	Increase in SOD-CAT, GR, increase in ROS and decrease in GSH level	Awasthi et al. [49]
Oryzias latipes	16 mg/L Cr(VI)	Increase in MDA levels in testes	Li et al. [50]
Oreochromis niloticus	Pb (9.12 mg/L), Cd (0.84 mg/L), Hg (0.025 mg/L)	Elevated activity of CAT, SOD, GPX, and MDA	Rahman et al. [51]
Pseudosciaena crocea	MeHg (4.0 μg/L)	Decrease in SOD and increase in CAT, GSH, and GPx levels	Wu et al. [52]
Salmo salar	5 and 10 mg MeHg/kg	Dose dependent activity of SOD, first increase then got decreased in 10 mg dose	Berntssen et al. [53]
Anguilla Anguilla	MeHg (50 ng/L)	Over expression of *sod–cat* system resulting in elevated activity of SOD and CAT	Claveau et al. [54]
Hoplias malabaricus	$HgCl_2$ (0.15 mg/L)	Increase in GST activity in liver	Monteiro et al. [55]
Micropterus salmoides	Cd (0.05 mg/L; 0.15 mg/L; 0.25 mg/L)	SOD, CAT, and GPx first increased then got decreased in 0.25 mg/L in gill as well as in liver	Liu et al. [56]
Oreochromis niloticus	Zn and Ag (25 – 100 μg/L)	Inhibition of CAT	Atli et al. [57]

are involved in modulating various cellular processes and pathways associated with Cd toxicity and stress response [38, 66, 69].

In *C. carpio* treated with Cd, miR-375–4-3p was upregulated and miR-9–6-5p and miR-31–12-5p were downregulated [69]. miRNAs mediate inflammation-immunosuppressive damage by Cd toxicity in the spleens of *C. carpio* through targeted regulation of multiple signalling pathways. Specifically, the targeted regulation of key pathways, including the Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathway, mitogen-activated protein kinase (MAPK) signaling pathway, nuclear factor kappa-light-chain-enhancer of activated B cell (NF-κB) signaling pathway, and Toll-like receptor (TLR) signalling pathway, suggests a comprehensive response to Cd-induced stress and inflammation in the spleens of *C. carpio* [69].

Arsenic and miRNAs

Shaw et al. [70] reported that arsenic exposure on killifish (*Fundulus heteroclitus*) elevates miRNA expression viz. miR-146a, novel-miR6, miR-22a-1, miR-30e-2, miR-203a-1, miR-203a-2, miR-22a-2, miR-21–2, and miR-30e-1. These miRNAs are known to be involved in regulating various cellular processes, including inflammation, oxidative stress, and apoptosis, which were affected by arsenic exposure. miR-10,012 has been documented as the most highly expressed miRNA, followed by miR-10,003 and miR-10,016, in the gills of Atlantic killifish upon exposure to arsenic toxicity [71]. An elevated expression of miR-135b in the gills of killifish following arsenic exposure has also been reported [71].

Copper and miRNAs

Copper exposure has an impact on the normal function of the olfactory system in zebrafish, as well as a direct relation of differentially expressed miRNAs in mediating the toxic effects. miRNAs such as miRNA-7a, miRNA-128, let-7, and miRNA-138 were regulated on Cu exposure, which is known to be involved in neurogenesis, suggesting that Cu exposure interferes with the process of neurogenesis in the olfactory system of zebrafish, while downregulation of miRNAs such as miRNA-16a, miRNA-16C, miRNA-203a, miRNA-199, and miRNA-25 leads to lower mRNA levels of their host genes through post-transcriptional mechanisms [72]. Figure 2 depicting the dysregulation of some important miRNAs upon heavy metal resulting in decreased neurogenesis, molecular changes, inflammation, oxidative stress, and apoptosis.

Toxicological Responses at the Molecular Levels

The genes and their expression involved in oxidative stress response and heavy metal detoxification in fish are valuable indicators of cellular and physiological responses to heavy metal exposure. These genes play crucial roles in defending against the toxic effects of heavy metals by scavenging ROS, chelating metal ions, and facilitating their excretion from cells. Various molecular techniques have been employed to study the profiling/expression of the genes in response to heavy metal exposure. Quantitative polymerase chain reaction (qPCR) allows for the precise measurement of gene

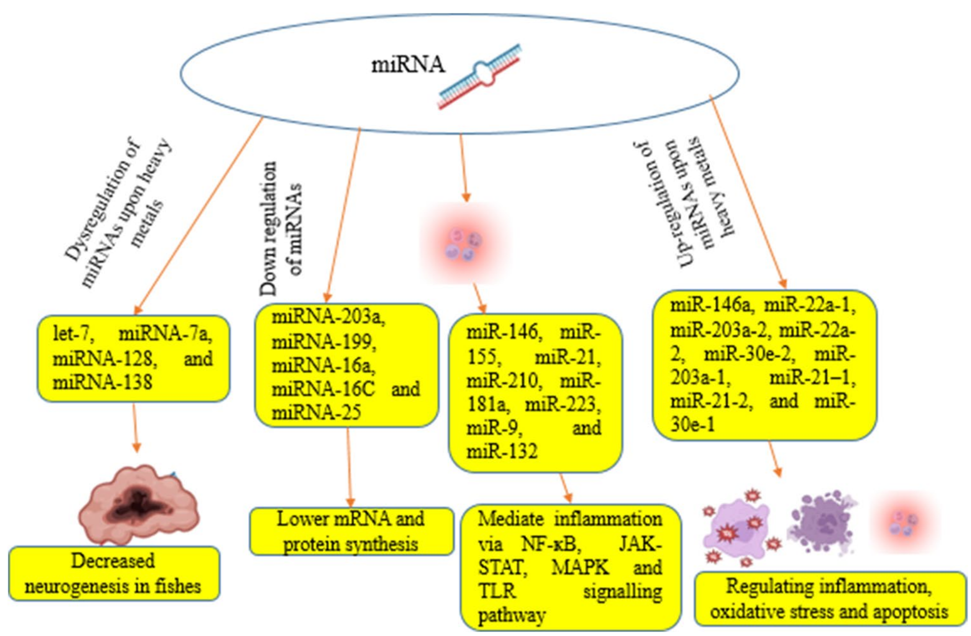


Fig. 2 Role of miRNAs and their regulation in fish physiology

expression levels for specific target genes. It provides high sensitivity and specificity, making it suitable for validating the expression of candidate genes linked to stress and metal detoxification [73, 74]. Microarrays enable the simultaneous amount of gene expression levels for thousands of genes in a single experiment. Transcriptomic techniques, such as RNA sequencing (RNA-seq), offer a comprehensive view of gene expression by sequencing and quantifying the entire transcriptome of an organism [73, 74]. The molecular approaches enable the identification of key pathways and mechanisms that can be targeted for the development of strategies to mitigate the adverse effects of heavy metal pollution on fish and their habitats [73, 74].

A potent method for comprehending the molecular reaction to metal stress is the use of RNA sequencing to examine genes that are expressed differently in the hepato-pancreas of *C. carpio* subjected to Cd [73]. The down-regulation of mRNA expression of SOD and CAT genes in brain tissue of *Tilapia zillii* and *O. niloticus* due to Zn has been reported [75]. Jiang et al. [76] highlighted the impact of Cu exposure on caspase-3 activity and apoptotic processes in the muscles of fish, an increase in *caspase-3* in fish administered to Cu exposure, suggests induction of apoptotic pathways and DNA fragmentation, which are indicative of cellular damage and programmed cell death. *Caspase-3* is an important enzyme in the executing phase of apoptosis, a highly regulated process of programmed cell death for tissue homeostasis and the elimination of damaged cells. Dai et al. [77] reported the molecular mechanisms underlying Cd-induced toxicity, particularly focusing on the role of autophagy-related genes as potential markers of autophagy toxicity. They reported upregulation of genes such as *ulk1, beclin1, atg5, atg4b, atg9a*, and *p62* in response to chronic Cd exposure suggesting the activation of autophagy pathways in an attempt to remove damaged cellular components and mitigate Cd-induced toxicity. The findings of Giri et al. [78] highlight the detrimental effects of Cd exposure on the immune system of *L. rohita*, the downregulation of important immune genes viz. *TNF-α, IL-1β, IFN-γ*, and *IL-10* in the head kidney, suggest immune response compromised, which can lead to increased vulnerability to pathogens and reduced ability to cope with environmental stressors. In another study on zebrafish embryos, upregulation of *TNF-α* and *IL-1β* treated with lower concentrations of Cd was reported [79].

The abnormal expression of stress response protein genes, including heat shock proteins (*HSPs*) and metallothioneins (*MTs*), in the liver of *C. carpio* and *L. rohita* exposed to Cd, was reported [80]. The differential expression patterns of anti-stress genes, particularly *HSPs*, in various tissues of *C. carpio* following 96 h of Cd exposure highlight the tissue-specific responses to Cd-induced stress [80]. The expression level of *HSP60* in the liver was significantly induced after Cd exposure, while the expression levels of *HSP70* and *HSP90* in the liver were not significantly changed or inhibited after Cd exposure. The expression level of *HSP70* in the gills first significantly increased and then decreased after Cd exposure, while the expression level of *HSP90* in the gills was significantly inhibited at all times after Cd exposure. *HSP90* is involved in protein folding, signal transduction, and cellular homeostasis [81, 82].

The transcriptome analysis in a study was aimed to find the molecular mechanisms behind brain injury in *C. carpio* due to lead exposure. Upon lead exposure, results identified 2141 differential genes, and these differential genes were significantly enriched in several pathways viz. GnRH signaling pathway, ion channels, Wnt signaling pathway, and calcium signaling pathway, all of which are likely involved in lead-induced brain injury [83]. Studies have also demonstrated that lead can accumulate in the brain tissues of fish, leading to serious neurological injuries [84]. In rainbow trout exposed to Cd, differential expression patterns of *HSP70a* and *HSP70b* genes were noticed. Both these genes showed increased mRNA expression in the intestine while *HSP70a* gene expression was observed only in the kidney in response to Cd exposure [85]. Heavy metal exposure disrupts the function of the brain-pituitary–gonadal BPG axis, leading to alterations in gene expression and reproductive physiology. In a study on *Odontesthes bonariensis*, exposure to Cd, Cu, and Cr has been shown to hinder the expression of key genes involved in the BPG axis. Cd increased *GnRH* mRNA transcripts (1, 2, 3), while a decrease in *cyp19a1b* and *FSHβ* mRNA expression was observed in the pituitary gland in the brain. The alterations in gonadotropin gene expression may disrupt the normal balance of reproductive hormones and impair reproductive function in *O. bonariensis* exposed to heavy metals Gárriz et al. [19]. Figure 3 demonstrating heavy metal exposure resulting in ROS formation, organ damage, DNA damage, apoptosis, and enzyme hindrance in fish.

Toxicological and Genetic Damage Response

Heavy metal pollution poses a threat to the stability and health of freshwater ecosystems and is among the pollutants that commonly accumulate in these environments. Heavy metal pollution affects freshwater ecosystems and induces genotoxicity in fishes [86, 87]. Heavy metals directly damage DNA by causing DNA strand breaks, cross-linking, and oxidative damage [88]. Javed et al. [89] studied the potential for genotoxicity and other biochemical changes in *Channa punctatus* in response to long-term exposure to different metals. Long-term exposure to metals induces genotoxic effects in *C. punctatus*, leading to DNA damage, chromosomal aberrations, and other genetic alterations. Ratn et al.

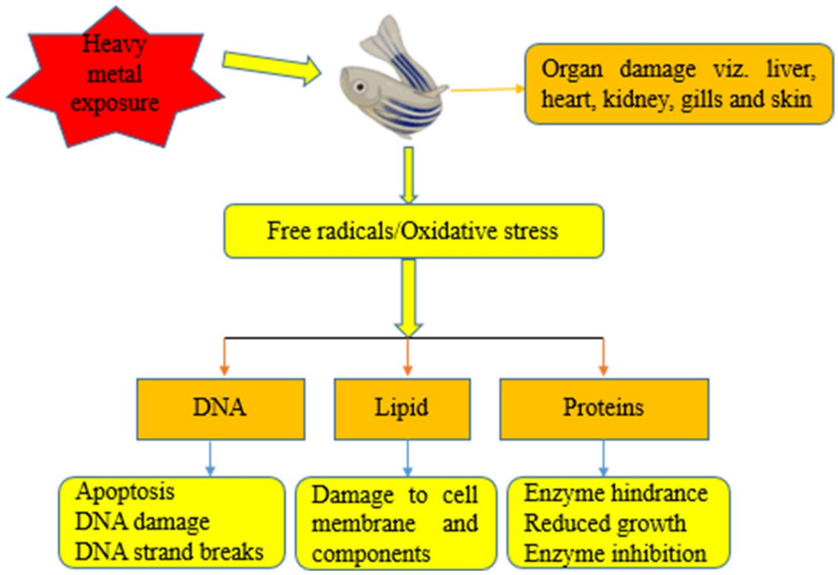


Fig. 3 Impact of heavy metals on fish DNA, lipids, and proteins

[90] reported that higher levels of zinc accumulation in *C. punctatus* are associated with DNA damage. Like other heavy metals, Zn causes direct DNA damage through DNA strand breaks, cross-linking, and oxidative damage. Moussa et al. [91] have observed a direct relation between the accumulation of metals in tissues and the degree of DNA damage as observed in *O. niloticus* and *C. gariepinus* collected from contaminated water. A higher frequency of DNA fragmentation observed in fish exposed to metals indicated that metals impaired the expression of enzymes involved in DNA proofreading and repair mechanisms.

González-Mille et al. [92] observed a higher frequency of DNA damage in the muscle tissues of fish from the Coatzacoalcos River. Higher DNA damage was attributed to the presence of high concentrations of genotoxins in the water, which are substances capable of causing genetic mutations and DNA alterations. Exposure to heavy metals has detrimental effects on organisms, even in relatively short periods. In *Venus verrucosa*, exposure to Pb for six days results in significant histopathological changes in the gills and digestive gland [93]. Exposure to Cr^{6+} at concentrations ranging from 5 to 10 mg/L induced DNA damage in *Ctenopharyngodon idellus*. Even the lowest concentration 5 mg/L of Cr^{6+} induced DNA damage in *C. idellus* [94]. Chaudhary et al. [95] reported that *L. rohita* experiences higher DNA damage when exposed to Cr as compared to the control group. In one study when *L. rohita* were exposed to different non-lethal doses of copper oxide nanoparticles (CuO NPs) showed a clear dose–response relationship regarding genetic damage. According to Aziz and Abdullah [96], *L. rohita* exposed to CuO NPs showed a higher percentage of nuclei damaged and higher concentrations of CuO NPs lead to more pronounced genetic damage. Exposure to inorganic mercury in concentrations ranging from 5 to 20 μg/L has been reported to have adverse effects on *O. mykiss* such as the generation of free radicals, DNA damage, and oxidative stress [97]. Numerous studies have demonstrated that exposure to heavy metals can result in significant DNA damage across various fish species such as *C. punctatus*, *C. gariepinus*, and *O. niloticus* Jamil Emon et al. [98]. The study by Chatha et al. [99] examined the effects of metal exposure on cell DNA in four fish species. Among the four fish species studied, silver carp exhibited the highest percentage of DNA-damaged cells in response to Pb exposure, followed by *L. rohita*, *C. idella*, and *L. catla*. The study of Barjhoux et al. [100] reported that exposure to Cd and Cu induced significant DNA damage in 2-day post-hatch (dph) larvae of Japanese medaka. Table 3 shows a detailed overview related to molecular response in fishes upon different doses of heavy metal administration.

Toxicology and Histopathological Alterations

Fish are extremely susceptible to heavy metal-induced toxicity because they are constantly exposed to heavy metal-contaminated aquatic environments. Among organs affected by heavy metal toxicity in fish, the kidney, liver, and gills are particularly susceptible and commonly studied for histopathological alterations [108]. A study conducted by Awasthi et al. [49] highlights the importance of histological manifestations as end-point biomarkers for evaluating toxicity induced by Cr^{6+} in *C. punctatus*. The observations of gills reveal many structural alterations such as epithelial hyperplasia, necrosis, lamellar fusion, and inflammatory responses while tissues hepatocellular damage, necrosis, vacuolization, necrosis, and inflammation in liver tissue induced by Cr^{6+} exposure. Javed et al. [18] report many

Table 3 Impact of heavy metals on molecular response

Fish species	Heavy metal and dose	Effect on fish	References
Gobiocypris rarus	Cd (0.5 and 2.5 mg/L)	Significant change in genes related to hypothalamic-pituitary-thyroid (HPT) axis and decrease in thyroid hormone	Li et al. [101]
Danio rerio	As (2 or 10 ppb)	Increase in the expression of *IL-6*	Nayak et al. [102]
Pangasianodon hypophthalmus	Cr (0.8, 1.6, and 3.2 mg/L)	DNA damage in liver, kidney and brain	Suchana et al. [21]
Oreochromis niloticus	Cr (43.7 mg/L)	DNA damage and polymorphic bands	Abbas et al. [103]
Oryzias melastigma	Cd (10 µg/L), Pb (50 µg/L), and Zn (100 µg/L)	Upregulation of *FSHβ, B-ER-α, LAR-α, VTG2, LHR, FSHR, G-ER-α, G-ER-β, G-AR-α,* and *3β-HSD* in females and upregulation of *B-AR-α, B-ER-β, L-ER-α, VTG1, LHR, G-ER-β, GAR-α,* and *Star* genes and down regulation of *LHβ* in the testis	Yan et al. [104]
Channa punctatus	7.89 mg/L (Cr^{6+})	Upregulation of *cat, p53, bax, apaf-1*, and down regulation of *bcl-2* gene in liver	Awasthi et al. [49]
Odontesthes bonariensis	Cd (0.25 µg/L), Cr (4 µg/L), Cu (22 µg/L), and Zn (211 µg/L)	Increase in *gnrh1, 2 and 3* in brain, decrease in *cyp19a1b, fshb,* and decrease of fshr in testis	Gárriz et al. [19]
Oreochromis niloticus	Pb (9.12 mg/L, Cd (0.84 mg/L), Hg (0.025 mg/L)	Upregulation of *MT gene* of hepatic	Rahman et al. [51]
Myoxocephalus Scorpius	Pb (0.73 ± 0.35 µg/L)	Upregulation of hepatic *mt* and *igm* mRNA	Jantawongsri et al. [105]
Pseudosciaena crocea	MeHg (0.25, 1, 4.0 µg/L)	Upregulation of *GST3, Hsp70, Hsp27*, and *TCTP* in juveniles	Wu et al. [52]
Anguilla Anguilla	MeHg (50 ng/L)	Upregulation of *12 s* and *coxI* gene in migrant *Anguilla anguilla*	Claveau et al. [54]
Gadus morhua	MeHg (2 mg/kg)	Upregulation of many genes involved in lipid and amino acid metabolism	Yadetie et al. [106]
Danio rerio	MeHg (13 mg/kg)	Decrease in mitochondrial respiration and cytochrome c oxidase (*cox*) activity	Cambier et al. [107]
Labeo rohita	Cd (0.65 mg/L)	Upregulation of *HSP70* in the liver	Giri et al. [78]
Micropterus salmoides	Cd (0.05 mg/L, 0.15 mg/L, 0.25 mg/L)	Upregulation of *HSP70, HSP90, Cyp1a*, and *MT*	Liu et al. [56]
Clarius magur	Zn (50, 200, and 300 mg/kg)	Increase in FSH, LH, and decrease in GTH	Gupta et al. [24]
Oncorhynchus mykiss	Cd (35.9 µg/kg)	Increase in hepatic transferrin, MTs, and HSP70	Kwong et al. [85]

histopathological changes induced by environmental stressors in fish viz. necrosis, leucocyte infiltration, and vacuolization in the liver while in gills, swelled and fused lamellae, gill bridging, and granuloma formation. These histopathological changes observed in the liver and gills of fish provide valuable information about the effects of environmental stressors on tissue morphology and function. Puntoriero et al. [109] observed histological changes in *Odontesthes bonariensis* due to heavy metal toxicity effects, viz. cellular degeneration, apoptosis, and congestion in hepatic sinusoidal and focal necrosis areas.

Cd exposure leads to nephrotoxicity, damage or dysfunction of the kidneys and mesangial hypercellularity, cellular damage with cloudy appearance of tubules, and vacuolation of the renal tubules due to exposure to toxic substances. These histopathological changes collectively reflect the toxic effects of Cd on renal tissues and the pathophysiological processes underlying Cd-induced nephrotoxicity [110]. Li et al. [111] reported that *C. carpio* exposed with Cd leads to a range of histological changes in the kidneys including renal tubular interstitial fibrosis and renal tubular inflammatory cellular infiltration.

Short-term exposure to Pb has been reported to be linked with histopathological alterations in the gills and digestive gland of *Venus verrucosa*. The morphological changes include structural abnormalities, cellular damage, inflammation, and other morphological disruptions. Pb exposure also leads to DNA damage in *V. verrucosa* [93].

The accumulation of Hg in fish leads to toxicity and tissue damage, particularly in organs such as the gills, kidney, and liver. Histopathological changes include epithelial damage, lamellar fusion, and disruption of gill architecture [112].

The histopathological changes recorded in *C. punctatus* exposed to mercuric chloride highlight the detrimental effects of Hg toxicity on liver tissue. In control, the liver showed normal hepatocytes while the fish exposed to mercuric chloride exhibit degeneration of both the nucleus and cytoplasm of hepatocytes. Other degenerations reported were pyknotic nuclei, vacuolization, and necrosis [113]. Pervaiz et al. [114] investigated the effects of Hg exposure on the liver of *O. niloticus* and observed hepatocyte destruction, sinusoidal abnormalities, pyknotic nuclei, bile duct degeneration, vacuolization, and necrosis. The histological examination of *L. catla* liver showed degeneration of hepatocytes, vacuolar degeneration, and necrotic changes, and the kidney reveals scattered inflammatory cells in the interstitium, focal degeneration of glomeruli, and eosinophilic hyaline casts in tubules upon heavy metal exposure [115]. Elwasify et al. [116] and Sahid et al. [117] have reported various histopathological changes in the kidney and liver induced by the accumulation of potentially toxic metals in *T. zillii* viz. vacuolated hepatocytes, vasculitis in hepatoportal blood vessels, congestion in interstitial blood vessels, and necrosis in renal tubules.

The accumulation of heavy metals leads to histopathological abnormalities in the kidney of *O. niloticus*. Cd has been observed to induce various histopathological changes in the kidney of *O. niloticus* viz. accumulation of fluid in the renal interstitium, resulting in reduction in cell size, edema, and degeneration of renal tubules [118]. Rana et al. [119] demonstrated histopathological changes in the *C. carpio* kidney following exposure to Cr, and the changes observed were accumulation of inflammatory cells and enlargement of the capillaries [119]. The histological analysis of *Boops boops* revealed several histopathological changes in the kidney, indicative of potential renal injury or dysfunction. The histopathological changes noted were renal tubule atrophy; inflammatory cell aggregation; such as lymphocytes, macrophages, or neutrophils; loss of cellular integrity; and renal tubule degeneration. Gill histopathology showed potential damage or dysfunction in the gill tissues such as cartilaginous core and lamellar damage [120]. Exposure to metal-contaminated waters has been reported to various abnormalities in the renal structures of fish. These abnormalities include a decrease in the production of blood cells within the kidney, causing cellular injury, degeneration, necrosis, lesions in the glomeruli, proliferation of connective tissue, and structural changes in the glomeruli [121, 122]. Gills play an important role in ion exchange during osmoregulation and contact with toxicants (heavy metals) have a drastic effect on the gill lamellae. Exposure to heavy metals has been recorded to stimulate abnormal growth or proliferation of epithelial cells in the gill filaments as well as lamellar fusion and necrosis in the epithelial cells [123].

Kumar et al. [124] recorded significant histopathological changes induced by mercuric chloride in multiple organs in *C. punctatus*. Mercuric chloride exposure led to the fusion of adjacent gill lamellae, damage of branchial arches, and necrosis in gills, while in liver degenerative changes in the cytoplasm of hepatic cells, the presence of vacuoles was observed. In kidneys, constriction of the renal tubules and hypertrophy while in muscle abnormalities and necrotic changes in muscle tissues were recorded. Table 4 provides a detailed demonstration related to the changes in different fish organs upon heavy metal administered.

Toxicology and Fish Reproduction

Reproduction is fundamental to the survival and perpetuation of all animal species. Successful reproduction ensures the continuation of a species over time, maintaining biodiversity and ecological balance [133, 134]. Water pollution from various sources, including heavy metals, industrial wastes, pesticides, and agricultural runoff, can all have detrimental effects on fish reproduction [135]. Heavy metals accumulate in the fish leading to a range of adverse effects, including disruptions to reproductive organs, disruption in the production of hormones, and impaired gamete production [136]. Heavy metal pollution, viz. arsenic, has detrimental effects on reproductive cell and organ development in various organisms, including fish. Arsenic is known for its toxic effects on reproductive systems, disrupting processes like spermatogenesis and oogenesis. In fish, exposure to arsenic has been reported to reduce egg and sperm quality and quantity [22, 137]. Several studies have recorded adverse effects on the reproductive performance of fish due to heavy metal pollution viz. abnormal oocyte structure, empty follicles, condensation of the cytoplasm, and a decrease in GSI [104]. Cu and Hg are known to exert toxic effects on the reproductive health of fish, leading to various adverse outcomes such as reduced GSI, decrease in fecundity, structural abnormalities in gonads, and reduced hatching and fertilization rate [23, 138]. Increased heavy metal concentrations have been reported to affect the reproductive health of *T. zillii*, higher concentrations of heavy metals, as observed in the El-Nasria station at the river Nile delta area, induce atresia in the ovaries of *T. zillii* [139]. Bhat et al. [40] observed a significant impact of heavy metal pollution on the ovarian histology of *O. mykiss*. The observed histological changes in the ovaries reported were disorganization of the nucleus and cytoplasm, and abnormal structural changes in the oocytes, retracted cytoplasm, and post-ovulatory follicles.

Table 4 Effect of heavy metals on histopathology of different fish organs

Fish species	Heavy metal/contaminants and dose	Effect on fish	References
Cirrhinus mrigala	Mercuric chloride (0.0206 ppm, 0.0402 ppm); lead acetate (28.2 ppm, 14.1 ppm)	Loss of cellular architecture in the liver, necrosis in hepatocytes, and lamellar degeneration in gills	Chavan and Muley [125]
Labeo catla	Cu (1.25 ppm), Cd (4.5 ppm)	Hepatic cell degeneration, lamellar atrophy, necrosis of lamellar epithelial cells and atrophy of glomeruli, necrosis of renal tubular cells	Naz et al. [126]
Cyprinus carpio	$K_2Cr_2O_7$ (150 µg/L), $CdCl_2$ (100 µg/L) and Pb $(NO_3)_2$ (250 µg/L)	Fusion of secondary lamellae, damage of central vein in hepatocytes, and increase in vacuolization	Rajeshkumar et al. [127]
Cyprinus carpio	Cd (8.4 mg/L) and Pb (6.2 mg/L)	Fusion of gill lamellae, vessel dilatation, hyperemia	Khalesi et al. [128]
Pangasianodon hypophthalmus	Cr (0.8, 1.6, and 3.2 mg/L)	Hypertrophy of cells, lamellar fusion, necrotic lamellae in gills and hemorrhage, pyknotic nucleus hepatocyte hypertrophy in the liver, shrinkage of renal corpuscle, pyknotic nuclei, dilation of Bowman's space, vacuolization, and necrosis in kidneys	Suchana et al. [21]
Phoxinus lagowskii	Cr^{6+} (5 and 10 mg/L)	Diffuse vacuolations of hepatocytes, cytoplasmic vacuolizations, bile stasis and focal necrosis and increase of sinusoidal space in the liver; lamellar curling fusion, necrosis and lamellar fusion in gills	Hu et al. [48]
Channa punctatus	7.89 mg/L (Cr^{6+})	Necrosis and hypertrophy and hepatocytes with large vacuolation in the liver	Awasthi et al. [49]
Anabas testudineus	$K_2Cr_2O_7$ (6 and 12 mg/L)	Hyperplasia in lamellar epithelium, lamellar fusion in gills; hepatic haemorrhage, necrosis, degeneration in hepatocytes in liver; renal haemorrhage and degeneration in renal tubules in kidney	Benjamin and Kutty [129]
Channa gachua	$NiSO_4$ (150 ppm)	Rupture of sinusoids, necrosis and degeneration in hepatocytes, shrinkage of central vein and connective tissue degeneration	Kawade [130]
Gambusia affinis	$PbCl_2$ (20 and 25 mg/L)	Histological lesions, hemorrhage, vacuolar degeneration, necrosis, congestion, and edema in brain	Alkshab and Taha [131]
Oreochromis niloticus	Pb (9.12 mg/L, Cd (0.84 mg/L), Hg (0.025 mg/L)	Necrosis of hepatopancreatic cells, vacuolar degeneration of hepatocytes in liver, and congestion of gill lamellae	Rahman et al. [51]
Anguilla anguilla	MeHg (50 ng/L)	Empty mitochondria, destruction of cristae in mitochondria and increase in space between fiber bundles of muscles	Claveau et al. [54]
Ctenopharyngodon idella	Cu (1.5, 1.4, 1.2, and 1 mg/L) Ch (25.5, 22.5, 20, and 18 mg/L) Pb (250, 235, 225, and 216 mg/L)	Necrosis and degeneration of muscle fibres, lesions in muscles, and gills showed curling filaments, gill bridging, irregular cells, and interlamellar spaces	Shah et al. [132]
Micropterus salmoides	Cd (0.05 mg/L, 0.15 mg/L, 0.25 mg/L)	Necrosis and nuclear shrinkage in liver and swollen lamellae, vacuolization, cell detachment and columnar cell disorder in gills	Liu et al. [56]

Exposure to pollutants can lead to follicular atresia. Follicular atresia refers to the degeneration and eventual apoptosis (cell death) of ovarian follicles, which are structures containing developing oocytes [140]. The findings of Senarat et al. [141] suggest that the decrease in the activities of gonadotropin hormone (GTH) and other estrogen hormones, likely induced by pollutant exposure, contributes to the observed increase in follicular atresia. Jangu et al. [142] study the effects of pollutants on the ovaries of *L. rohita* within the Harike wetland ecosystem. Their findings reported that the ovaries of these fish were subjected to significant damage and disruption. The study by Du et al. [143] also observed changes and improper responses in gonadal development and alterations in tissue histology that occurred because of heavy metal intoxication. A study conducted by Hayati et al. [144] reported that exposure to pollutants significantly impacted spermatogenic development and induced metabolic disorders in fish. Pollutants like heavy metals were found to interfere with the mitotic process, leading to a reduction in the number of spermatocytes compared to the control group.

Heavy metals like Cd have been reported to cause testicular damage to various fish species. One common effect observed in male fish exposed to Cd is a reduction in the total number of spermatocytes and spermatids [145]. Cd toxicity has also been reported to cause a wide range of adverse effects on the male reproductive system in fish, including histological changes in the testis and impaired sperm function, which can ultimately reduce fertilization success [19, 146].

Exposure to heavy metals such as Cd, Cu, As, Hg, and Pb can disrupt various stages of spermatogenesis, leading to impaired sperm production and function [147]. The histological examination of the gonads of *C. gariepinus* has revealed that heavy metals interfere with gonad development in this species [148]. Chronic exposure to Cr has been associated with decreased spawning success in fish populations. Cr exposure led to structural deformities in the testes of male fish and reduced sperm motility [149]. Bhat et al. [150] reported different structural anomalies of testicular tissues in *O. mykiss* viz. the presence of large empty spaces, reduction in germ cells, and disorganization of the epithelium. These structural anomalies observed in the testicular tissues of *O. mykiss* are consistent with the effects of environmental stressors on reproductive health. Heavy metals like Cd, Cr, and Cu are reported to be impacting negatively sperm quality primarily by reducing sperm motility and velocity in pejerrey fish [151] and damage the plasma membrane as well as axoneme of sperm thus leading to a loss of fertility Cousins et al. [152]. A detailed information is provided in below (Table 5) regarding the effects of heavy metals on fish gonads viz. ovary and testes.

Quantification Techniques of Heavy Metals

Quantifying heavy metals is important because biomarker responses of fish to heavy metal exposure and the quantification of heavy metals are interconnected as they provide a comprehensive understanding of environmental contaminations and their biological effects [171]. Additionally, researchers can pinpoint particular dose–response correlations by quantifying the concentrations of heavy metals in organs with biomarker responses [172]. The bioaccumulation potential of metals in different organs in fish or other organisms in response to metal exposure is assessed through quantification of metals in between the contaminant environment and the exposed animals. Therefore, quantification of metals is important for assessing their biomarker responses at specific doses, bioaccumulation potential, environmental impact, and associated health risks. Various analytical techniques are being used for accurate and reliable quantification of metals. This review introduces various heavy metal detection instruments that are important for monitoring heavy metals in aquatic compartments. The key instruments include atomic absorption spectrometry (AAS), inductively coupled plasma mass spectrometry (ICP-MS), and inductively coupled plasma–optical emission spectroscopy (ICP-OES). The procedures for heavy metal detections in fish tissues include sample collection, tissue digestion, filtration, and analysis viz. AAS, ICP-Ms, ICP-AES, AFS, etc. which are demonstrated in Fig. 4 [173–175].

Atomic Absorption Spectroscopy (AAS)

Atomic absorption spectrometry (AAS) is one of the most popular and widely used methods for the qualitative and quantitative analysis of heavy metals. AAS is highly valued for its ability to directly measure the metal content in various samples, making it an essential tool in environmental monitoring, industrial quality control, and biomedical research [176]. AAS is a highly advantageous technique for the qualitative and quantitative analysis of heavy metals. Its high selectivity, accuracy, sensitivity, and ability to minimize interferences make it an essential tool in various fields, including environmental science and biomedical research. The ease of operation, fast analysis times, and versatility further contribute to its widespread use and reliability in detecting and quantifying heavy metals in diverse samples [177]. While AAS is a powerful tool for heavy metal analysis, it has its limitations, particularly the narrow range of standard curves and potential interference from complex sample matrices. Its efficacy can also be influenced by the extraction technique used and the

Table 5 Effect of heavy metals on histopathology of fish gonads

Fish species	Heavy metal and dose	Effect on fish	References
Odontesthes bonariensis	0.25 and 2.5 µg/L (Cd), 4 and 40 µg/L (Cr), 220 µg/L	Decrease in sperm motility	Gárriz and Miranda [151]
Odontesthes bonariensis	Cd (0.25 µg/L), Cr (4 µg/L), Cu (22 µg/L), and Zn (211 µg/L)	Shrinkage of spermatic lobules and pyknotic nuclei	Gárriz et al. [19]
Astyanax aff. bimaculatus	Zn (3 and 20 mg/L)	Swollen and broken walls of their spermatic cysts, pyknotic cells in spermatic cells	Santos et al. [153]
Danio rerio	Cu (20 and 40 g L^{-1})	Decrease in GSI of both male and female fish and reduced gonadal maturation	Cao et al. [154]
Poecilia reticulata	Hg (30 to 50 g L^{-1})	Delayed spermatogenesis; decrease in GSI	Hayati et al. [146]
Poecilia reticulate *Oryzias latipes*	Hg exposure (–-)	Testicular degeneration, inhibition in spermatogenesis	Liao et al. [155]
Clarias gariepinus	Pb (6.1, 12.2, 24.4 ppm)	Reduced sperm motility	Alkahemal-Balawi et al. [156]
Colossoma macropomum	Cu (0, 0.4, 0.8, and 1.2 ppm)	Reduced motility and fertilization rate	Pinto et al. [157]
Clarias batrachus	Pb (5 ppm)	Deformities in the testes as well as in the ovary	Authman et al. [158]
Channa punctata	Mercuric chloride (0.081 mg/L)	Disruption of the ovarian follicle, reduction in the mature ovum, oocyte swelling, reduction in GSI of ovary as well as testes	Rai et al. [159]
Colisa fasciatus	As (14.0 mg/L)	Follicular spaces, underdeveloped ovum, atretic follicles	Shukla and Pandy [160]
Oreochromis niloticus	Fe (134.863 ppm), Cu (10.386), Pb (2.659), Cd (1.400)	Loss of shape of seminiferous tubule; severe congestion in testes	Elgaml et al. [161]
Oryzias melastigma	Cd (10 µg/L), Pb (50 µg/L), and Zn (100 µg/L)	Irregular oocytes, empty follicles and increased follicular atresia	Yan et al. [104]
Oryzias latipes	Cr(VI) (1.4 mg/L)	Lower GSI in the ovary as well as testes, follicular atresia, follicular wall degeneration	Chen et al. [162]
Channa punctatus	Cr(VI) (14.0 mg/L) Cr(VI) and Cr(VI) (1.4 mg/L)	Increase in follicular atresia and decrease in the 17-estradiol in the serum	Mishra and Mohanty [163]
Gasterosteus aculeatus	As (1 µg/L)	Decrease in GSI	Hani et al. [164]
Oncorhynchus tshawytscha	Cr (11 to 266 µg/L)	No effect on fish ovary/eggs	Farag et al. [165]
Pangasianodon hypophthalmus	As h 10^{-4} M	Germ cell necrosis, hypertrophy and interstitial tissue enlarged	Yamaguchi et al. [166]
Anguilla japonica	Pb (10^{-7} M), Mo ($10-5$ and 10^{-4} M), As (10^{-5} M)	Inhibition of spermatogenesis and necrosis	Yamaguchi et al. [166]
Anguilla japonica	As (100 mM)	Increase in dead germ cells	Celino et al. [137]
Lepomis macrochirus	Se (10 mg/L)	Reproductive failure	Lemly [167]
Amblypharyngodon mola	Mercuric chloride (0.04583 ppm.), arsenic trioxide (0.2911 ppm.), and Cadmium chloride (0.6279 ppm.)	Size reduction on gonads, damage in germinal elements, increase in follicular atresia and secondary spermatocytes were pycnotic	Shelke [168]
Oncorhynchus mykiss	Pb (10 µg/L)	Increase in spermatogonia cysts and decrease in spermatocytes	Ruby et al. [169]
Carassius gibelio	Pb (8, 13, 24, and 49 mg/kg)	Decrease in GSI, adverse long-term impact on steroidogenesis, gametogenesis, and ovulation	Łuszczek-Trojnar et al. [170]

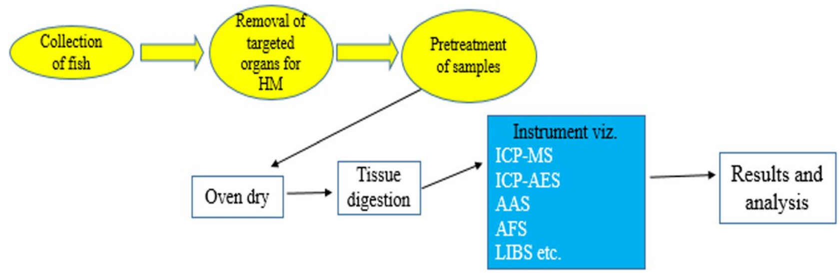


Fig. 4 Procedure of heavy metal detection using different techniques

specific elements being analyzed [178]. While using AAS for detecting heavy metals, Vinodhini and Narayanan [179] examined the bioaccumulation of Cd in *C. carpio* upon exposure to 5 mg/L for 32 days. The results revealed the bioaccumulation rates as follows: gills (6.23–6.94 µg/g), liver (4.82–5.64 µg/g), and kidney (4.31–5.32 µg/g). Similarly, Ali et al. [180] investigated the bioaccumulation of Mn and Cr in *C. carpio* upon exposure to doses of $MnSO_4$ (1.12 mg/L) and $CrCl_3$ (3.41 mg/L). Gills exhibited highest accumulation of Mn and Cr, viz. Mn (0.54 ± 0.04 µg/g) and Cr (0.78 ± 0.01 µg/g). They also reported hematological indices of *C. carpio* following exposure to Mn and Cr and recorded elevated levels of hematocrit, hemoglobin, RBCS, mean corpuscular hemoglobin, and mean corpuscular volume. Similarly, biochemical parameters viz. serum glutamic pyruvic transaminase (SGPT), serum cholesterol, and low-density lipid were recorded significantly high. Szebedinszky et al. [181] have also studied the effects of Cd exposure on *O. mykiss* upon dose of 1.5 mg/kg for a duration of 36 days recorded bioaccumulation rate of 0.20 to 0.30 µg/g in the gills and 0.29 to 0.37 µg/g in the liver.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

In heavy metal analysis, AAS has been a widely used technology due to its reliability, cost-effectiveness, and ability to measure metal concentrations in various environmental samples [182]. However, in recent years, ICP-MS has emerged as the preferred technique due to its greater efficiency, sensitivity, and versatility. ICP-MS is a premier technology in elemental analysis, excelling in the detection of ultra-trace levels of metals and metalloids. Its high sensitivity, wide dynamic range, and ability to perform multi-element and isotopic analysis make it an invaluable tool across various scientific and industrial fields [183, 184]. ICP-MS is the preferred method for detecting heavy metal due to its exceptional accuracy, sensitivity, and low detection limits. Additionally, ICP-MS is often used in combination with other analytical techniques like LA-ICP-MS, SP-ICP-MS, and LC-ICP-MS to enhance its capabilities. Despite its powerful capabilities in ultra-trace elemental analysis, ICP-MS has limitations including high costs, the necessity of pre-transforming samples into solutions, issues with salt tolerance, and interference from certain elements viz. S, Ca, Fe, K, and Se [185]. Pei et al. [186] upon quantifying heavy metals with ICP-MS investigated the bioaccumulation of arsenic in *O. niloticus* when exposed to doses of 806.5 and 772.1 µg/g for 10 days recorded bioaccumulation rates as follows: gills (5.12 ± 0.61 µg/g), liver (9.51 ± 1.68 µg/g), and muscle (3.40 ± 0.24 µg/g).

Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP-OES)

ICP-OES is a standard technique used for heavy metal detection. It is widely used for determining both major and trace elements in various matrices, viz. environmental samples and biological samples. ICP-OES is worth noting for its stability, low detecting limits, high accuracy levels, vast linear ranges of concentrations, and multi-element detection ability [187, 188]. ICP-OES versatility and robustness are demonstrated by its popularity and broad acceptance over the past 50 years. ICP-OES has developed steadily since the first commercial instrument was introduced, establishing itself as one of the most dependable analytical methods for identifying major and trace elements in a variety of sample matrices [187]. Aldoghachi et al. [189] upon determining the bioaccumulation in different tissues of *Oreochromis* sp. exposed to varying concentrations of Cu (0.0, 0.50, 1.0, 3.0, and 5.0 mg/L) by ICP-OES recorded accumulation rates viz. gill (6.3 to 38.4 mg/kg), liver (19.4 to 136 mg/kg), and muscle (1.4 to 4.0 mg/kg).

Artificial Intelligence (AI)

Artificial intelligence (AI) is described as "computers with modern intelligent behavior with minimal human intervention." Its applications in medicine, image processing, and biology are revolutionizing these fields, leading to more accurate diagnostics, enhanced image analysis, and deeper insights into biological processes [190, 191]. In recent years, AI-based technologies have become increasingly valuable in the detection and removal of heavy metals. These technologies leverage advanced algorithms and computational power to enhance the efficiency and accuracy of monitoring and remediation processes [28]. Ucun Ozel et al. [192] conducted a study in which they analyzed concentrations of Cu, Fe, Zn, Mn, Ni, and Pb in samples taken from three stations along the Bartin River. The results of these analyses were then modeled using artificial neural networks (ANN) and adaptive neuro-fuzzy inference system (ANFIS) techniques. The results underscore the potential of artificial intelligence models, such as ANN and ANFIS, to effectively estimate heavy metal concentrations in river systems. The use of these models with easy-to-measure parameters offers a quick, economical, and reliable method for monitoring and managing heavy metal pollution, thereby enhancing the viability of rivers and protecting both environmental and public health. Various neural network models such as multilayer perceptron (MLP), backpropagation neural network (BPNN), general regression neural network (GRNN), and multiple linear regression (MLR) models have also been used in heavy metal prediction. It has been established that the ANN model is an efficient tool for heavy metal forecasting, with many researchers reporting that the results are suitable for predicting heavy metals quickly and cost-effectively [193], Altunkaynak and Basakin [194]. ANN have proven to be a very important tools in the management of water resources, monitoring of water quality, and analysis of hydrologic time series. Their ability to model complex environmental systems accurately and efficiently makes them indispensable for defining, monitoring, and managing river pollution [195].

AI particularly ML algorithms are highly effective in addressing the challenges of interference and large data processing in the detection of heavy metals like Hg, As, and Pb in samples. The ML algorithms significantly enhance the accuracy, speed, and efficiency of data analysis in spectrometric techniques like AAS, ICP-MS, and ICP-OES. Genetic algorithm back-propagation neural network (GA-BPNN), BPNN, successive projections algorithm (SPA), and ANFIS are advanced computational techniques employed in the detection and analysis of heavy metals especially in complex environmental samples [196]. GA-BPNN a hybrid technique that integrates genetic algorithms (GA) and BPNN study method was used for Hg detection using an Avafield spectrometer in samples, and the recorded results were found significant [197]. BPNN in detecting Cu and Cd with the AAS has been studied and recorded accurate levels of concentrations of the metals compared to traditional statistical models [198].

Maurya et al. [28] developed a ML-based methodology to classify fish exposed to heavy metals versus non-exposed fish by leveraging color and texture features. These features were identified and genetically optimized, and the performance of the proposed method was further tested using a transfer learning-based approach. This innovative approach underscores the potential of machine learning in addressing environmental challenges. Continuous advancements in detection technology are driving the evolution of AI towards enhanced integration, efficiency, and adaptability in the detection of heavy metals. As detection technologies improve and become more sophisticated, AI methodologies are increasingly capable of integrating diverse data sources, optimizing detection processes for higher efficiency, and adapting to varying environmental conditions and contaminant scenarios.

Conclusion

The aquatic environment in general and the water quality in particular have been severely weakened by the unregulated discharge of sewage and industrial pollutants, which has an impact on the biochemical and physiological condition of aquatic animals, including fish. Toxic heavy metals have bioaccumulated in fish organs thus impairing normal fish functions. Fish physiology has been negatively impacted by the bioaccumulation of these hazardous metals, which has hindered fish growth and reduced fish reproduction. Therefore, it is advised that industrialists not dispose of their waste until it is properly treated in order to preserve the ecological balance. In the long run, fish will have lower levels of heavy metals when the level of pollution in their environment declines. Therefore, in order to prevent pollution of aquatic ecosystems worldwide and to avoid harmful effects on fish and humans, it is imperative that action be taken immediately to make sure that future emissions and releases of heavy metals in aquatic ecosystems be minimized to the greatest extent possible.

AI offers a powerful tool to complement direct measurements in monitoring heavy metal pollution levels over traditional techniques like AAS. By leveraging computational techniques and data-driven approaches, modeling enhances cost-effectiveness, efficiency, and spatial–temporal coverage in heavy metal assessment. With a minimal margin of error and a very high degree of reliability, predictions can be made using AI. Therefore, it is possible to monitor water contamination with a significant reduction in cost, time, and labor force. Many studies have concluded that AI-based approaches for detecting heavy metals are more effective than any other traditional methods. But before using AI techniques, the best model for that area or resource needs to be chosen, preparatory research needs to be done, and the model's degree of reliability needs to be determined.

Acknowledgements The authors are thankful to the Director, ICAR-Central Inland Fisheries Research Institute (CIFRI) for the support to carry out this study. The authors are also thankful to NMCG, Ministry of Jal Shakti, Department of Water Resources, River Development and Ganga Rejuvenation, Government of India, for financing the study without which it would not have been possible.

Author contribution RAB-Writing – writing review & editing; AA-Writing – review & editing, Validation; DNH- review & editing; VK-writing review & editing, Conceptualization, Methodology, proofreading; JK- Writing – review & editing; VRT-Proofreading and editing; BKD- review & editing, Project administration, Funding acquisition, Conceptualization.

Funding The authors are also thankful to NMCG, Ministry of Jal Shakti, Department of Water Resources, River Development and Ganga Rejuvenation, Government of India, for financing the study without which it would not have been possible.

Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

References

1. El Agawany N, Kaamoush M, El-Zeiny A, Ahmed M (2021) Effect of heavy metals on protein content of marine unicellular green alga Dunaliella tertiolecta. Environ Monit Assess 193(9):584
2. Duruibe JO, Ogwuegbu MOC, Egwurugwu JN (2007) Heavy metal pollution and human biotoxic effects. Int J Phys Sci 2(5):112–118
3. Malik DS, Maurya PK (2014) Heavy metal concentration in water, sediment, and tissues of fish species (Heteropneustis fossilis and Puntius ticto) from Kali River, India. Toxicol Environ Chem 96(8):1195–1206
4. Baatrup E (1991) Structural and functional effects of heavy metals on the nervous system, including sense organs, of fish. Comp Biochem Physiol Part C: Comp Pharmacol 100(1–2):253–257. https://doi.org/10.1016/0742-8413(91)90163-N
5. Das BK, Kumar V, Chakraborty L, Swain HS, Ramteke MH, Saha A, Das A, Bhor M, Upadhyay A, Jana C, Manna RK (2023) Receptor model-based source apportionment and ecological risk assessment of metals in sediment of river Ganga, India. Mar Pollut Bull 195:115477
6. Kumar V, Kumar J, Alam A, Thakur VR, Kumar V, Srivastava SK, Kayal T, Jha DN, Das BK (2024) Ecological and human health risk from exposure to metal contaminated sediments in a subtropical river affected by anthropogenic activities: a case study from river Yamuna. Mar Pollut Bull 203:116498
7. Kumar V, Swain HS, Upadhyay A, Ramteke MH, Sarkar DJ, Roy S, Das BK (2024) Bioaccumulation of potentially toxic elements in commercially important food fish species from lower Gangetic stretch: food security and human health risk assessment. Biol Trace Elem Res 202(3):1235–1248
8. Sarkar M, Islam JB, Akter S (2016) Pollution and ecological risk assessment for the environmentally impacted Turag River. Bangladesh. J Mater Environ Sci 7(7):2295–2304
9. Sarkar MM, Rohani MF, Hossain MAR, Shahjahan M (2022) Evaluation of heavy metal contamination in some selected commercial fish feeds used in Bangladesh. Biol Trace Elem Res, pp 1–11
10. Shahjahan M, Islam SM, Bablee AL, Siddik MA, Fotedar R (2021) Sumithion usage in aquaculture: benefit or forfeit? Rev Aquac 13(4):2092–2111
11. Ghazi S, Diab AM, Khalafalla MM, Mohamed RA (2021) Synergistic effects of selenium and zinc oxide nanoparticles on growth performance, hemato-biochemical profile, immune and oxidative stress responses, and intestinal morphometry of Nile tilapia (Oreochromis niloticus). Biol Trace Elem Res 200:364–374
12. Wang RF, Zhu LM, Zhang J, An XP, Yang YP, Song M, Zhang L (2020) Developmental toxicity of copper in marine medaka (Oryzias melastigma) embryos and larvae. Chemosphere 247:125923
13. Chang LW, Magos L, Suzuki T (eds) (2023) Toxicology of metals. CRC Press, Boca Raton. https://doi.org/10.1201/9781003418917
14. Tunçsoy M, Duran S, Ay Ö, Cicik B, Erdem C (2017) Effects of copper oxide nanoparticles on antioxidant enzyme activities and on tissue accumulation of Oreochromis niloticus. Bull Environ Contam Toxicol 99(3):360–364
15. Jagdale SC, Hude RU, Chabukswar AR (2020) Zebrafish: a laboratory model to evaluate nanoparticle toxicity. Model organisms to study biological activities and toxicity of nanoparticles, pp 371–399
16. Mohamed AAR, El-Houseiny W, Abd Elhakeem EM, Ebraheim LL, Ahmed AI, Abd El-Hakim YM (2020) Effect of hexavalent chromium exposure on the liver and kidney tissues related to the expression of CYP450 and GST genes of Oreochromis niloticus fish: Role of curcumin supplemented diet. Ecotoxicol Environ Saf 188:109890
17. Phoonaploy U, Tengjaroenkul B, Neeratanaphan L (2019) Effects of electronic waste on cytogenetic and physiological changes in snakehead fish (Channa striata). Environ Monit Assess 191:1–11
18. Javed M, Ahmad MI, Usmani N, Ahmad M (2017) Multiple biomarker responses (serum biochemistry, oxidative stress, genotoxicity and histopathology) in Channa punctatus exposed to heavy metal loaded waste water. Sci Rep 7(1):1675
19. Gárriz Á, Del Fresno PS, Carriquiriborde P, Miranda LA (2019) Effects of heavy metals identified in Chascomús shallow lake on the endocrine-reproductive axis of pejerrey fish (Odontesthes bonariensis). Gen Comp Endocrinol 273:152–162
20. Islam SM, Rohani MF, Zabed SA, Islam MT, Jannat R, Akter Y, Shahjahan M (2020) Acute effects of chromium on hemato-biochemical parameters and morphology of erythrocytes in striped catfish Pangasianodon hypophthalmus. Toxicol Rep 7:664–670
21. Suchana SA, Ahmed MS, Islam SM, Rahman ML, Rohani MF, Ferdusi T, Ahmmad AS, Fatema MK, Badruzzaman M, Shahjahan M (2021) Chromium exposure causes structural aberrations of erythrocytes, gills, liver, kidney, and genetic damage in striped catfish Pangasianodon hypophthalmus. Biol Trace Elem Res 199:3869–3885
22. Boyle D, Brix KV, Amlund H, Lundebye AK, Hogstrand C, Bury NR (2008) Natural arsenic contaminated diets perturb reproduction in fish. Environ Sci Technol 42(14):5354–5360
23. Forouhar VM, Mohamadi Yalsuyi A, Sattari M, Prokić MD, Faggio C (2020) Effects of copper oxide nanoparticles (CuO-NPs) on parturition time, survival rate and reproductive success of guppy fish, Poecilia reticulata. J Cluster Sci 31:499–506
24. Gupta G, Srivastava PP, Kumar M, Varghese T, Chanu TI, Gupta S, Ande MP, Jana P (2021) The modulation effects of dietary zinc on reproductive performance and gonadotropins' (FSH and LH) expression in threatened Asian catfish, Clarias magur (Hamilton, 1822) broodfish. Aquac Res 52(5):2254–2265
25. Helaluddin ABM, Khalid RS, Alaama M, Abbas SA (2016) Main analytical techniques used for elemental analysis in various matrices. Trop J Pharm Res 15(2):427–434
26. Yadav N, Maurya BM, Chettri D, Pulwani C, Jajula M, Elangovan A, Velusamy P, Iyer M, Vellingiri B (2023) Artificial intelligence in heavy metals detection: methodological and ethical challenges. Hyg Environ Health Adv 7:100071

27. Nhantumbo C, Carvalho F, Uvo C, Larsson R, Larson M (2018) Applicability of a processes-based model and artificial neural networks to estimate the concentration of major ions in rivers. J Geochem Explor 193:32–40
28. Maurya R, Srivastava A, Srivastava A, Pathak VK, Dutta MK (2023) Computer aided detection of mercury heavy metal intoxicated fish: an application of machine vision and artificial intelligence technique. Multimed Tools Appl 82(13):20517–20536
29. Kumar V, Swain HS, Das BK, Roy S, Upadhyay A, Ramteke MH, Kole RK, Banerjee H (2022) Assessment of the effect of sub-lethal acute toxicity of Emamectin benzoate in Labeo rohita using multiple biomarker approach. Toxicol Rep 9:102–110
30. Kumar V, Swain HS, Roy S, Das BK, Upadhyay A, Ramteke MH, Kumar V, Kole RK, Banerjee H (2022) Integrated biomarker approach strongly explaining in vivo sub-lethal acute toxicity of butachlor on Labeo rohita. Comp Biochem Physiol C: Toxicol Pharmacol 261:109427
31. Pinto E, Sigaud-kutner TC, Leitao MA, Okamoto OK, Morse D, Colepicolo P (2003) Heavy metal–induced oxidative stress in algae 1. J Phycol 39(6):1008–1018
32. Barata C, Varo I, Navarro JC, Arun S, Porte C (2005) Antioxidant enzyme activities and lipid peroxidation in the freshwater cladoceran Daphnia magna exposed to redox cycling compounds. Comp Biochem Physiol C: Toxicol Pharmacol 140(2):175–186
33. Atli G, Grosell M (2016) Characterization and response of antioxidant systems in the tissues of the freshwater pond snail (Lymnaea stagnalis) during acute copper exposure. Aquat Toxicol 176:38–44
34. Eroglu A, Dogan Z, Kanak EG, Atli GÜLİ, Canli MUSTAFA (2015) Effects of heavy metals (Cd, Cu, Cr, Pb, Zn) on fish glutathione metabolism. Environ Sci Pollut Res 22:3229–3237
35. Fatima M, Usmani N (2013) Histopathology and bioaccumulation of heavy metals (Cr, Ni and Pb) in fish (Channa striatus and Heteropneustes fossilis) tissue: a study for toxicity and ecological impacts. Pak J Biol Sci 16(9):412–420
36. Arshad R, Abdullah S, Naz H, Abbas K (2018) Catalase activity as a bio-indicator of lead+ nickel toxicity in carnivorous fish, Channa striata: response of CAT activity in fish. Proc Pakistan Acade Sci: B. Life Environ Sci 55(2):37–43
37. Khalil MT, Gad NS, Ahmed NA, Mostafa SSED (2017) Antioxidant defense system alternations in fish as a bio-indicator of environmental pollution. Egypt J Aquat Biol Fish 21(3):11–28
38. Liu Q, Yang J, Gong Y, Cai J, Zheng Y, Zhang Y, Yu D, Zhang Z (2020) MicroRNA profiling identifies biomarkers in head kidneys of common carp exposed to cadmium. Chemosphere 247:125901
39. Vaseem H, Banerjee TK (2016) Evaluation of pollution of Ganga River water using fish as bioindicator. Environ Monit Assess 188:1–9
40. Bhat RA, Bakhshalizadeh S, Guerrera MC, Kesbic OS, Fazio F (2023) Toxic effect of heavy metals on ovarian deformities, apoptotic changes, oxidative stress, and steroid hormones in rainbow trout. J Trace Elem Med Biol 75:127106
41. Atli G (2020) How metals directly affect the antioxidant status in the liver and kidney of Oreochromis niloticus? An in vitro study. J Trace Elem Med Biol 62:126567
42. Hossain Z, Hossain MS, Ema NS, Omri A (2021) Heavy metal toxicity in Buriganga river alters the immunology of Nile tilapia (Oreochromis niloticus L). Heliyon 7(11):e08285
43. Kelly KA, Havrilla CM, Brady TC, Abramo KH, Levin ED (1998) Oxidative stress in toxicology: established mammalian and emerging piscine model systems. Environ Health Perspect 106(7):375–384
44. Garai P, Banerjee P, Mondal P, Saha NC (2021) Effect of heavy metals on fishes: toxicity and bioaccumulation. J Clin Toxicol. S 18:001
45. Tanhan P, Imsilp K, Lansubsakul N, Thong-asa W (2023) Oxidative response to Cd and Pb accumulation in coastal fishes of Pattani Bay. Ital J Anim Sci 22(1):148–156
46. Zikic RV, Stajn A, Saicic ZS, Spasic MB, Ziemnicki K, Petrovic VM (1996) The activities of superoxide dismutase, catalase and ascorbic acid content in the liver of goldfish (Carassius auratus gibelio Bloch.) exposed to cadmium. Physiol. Res 45(6):479–481
47. Kubrak OI, Lushchak V, Lushchak JV, Torous IM, Storey JM, Storey KB, Lushchak VI (2010) Chromium effects on free radical processes in goldfish tissues: comparison of Cr (III) and Cr (VI) exposures on oxidative stress markers, glutathione status and antioxidant enzymes. Comp Biochem Physiol C: Toxicol Pharmacol 152(3):360–370
48. Hu T, Ye C, Ning Z, Liu T, Mu W (2024) Effect of toxicity of chromium (VI) stressors alone and combined to high temperature on the histopathological, antioxidation, immunity, and energy metabolism in fish Phoxinus lagowskii. Fishes 9(5):168
49. Awasthi Y, Ratn A, Prasad R, Kumar M, Trivedi A, Shukla JP, Trivedi SP (2019) A protective study of curcumin associated with Cr6+ induced oxidative stress, genetic damage, transcription of genes related to apoptosis and histopathology of fish, Channa punctatus (Bloch, 1793). Environ Toxicol Pharmacol 71:103209
50. Li L, Chen H, Bi R, Xie L (2015) Bioaccumulation, subcellular distribution, and acute effects of chromium in Japanese medaka (Oryzias latipes). Environ Toxicol Chem 34(11):2611–2617
51. Rahman ANA, ElHady M, Hassanin ME, Mohamed AAR (2019) Alleviative effects of dietary Indian lotus leaves on heavy metals-induced hepato-renal toxicity, oxidative stress, and histopathological alterations in Nile tilapia, Oreochromis niloticus (L.). Aquaculture 509:198–208
52. Wu F, Huang W, Liu Q, Xu X, Zeng J, Cao L, Hu J, Xu X, Gao Y, Jia S (2018) Responses of antioxidant defense and immune gene expression in early life stages of large yellow croaker (Pseudosciaena crocea) under methyl mercury exposure. Front Physiol 10(9):1436
53. Berntssen MH, Aatland A, Handy RD (2003) Chronic dietary mercury exposure causes oxidative stress, brain lesions, and altered behaviour in Atlantic salmon (Salmo salar) parr. Aquat Toxicol 65(1):55–72
54. Claveau J, Monperrus M, Jarry M, Baudrimont M, Gonzalez P, Cavalheiro J, Mesmer-Dudons N, Bolliet V (2015) Methylmercury effects on migratory behaviour in glass eels (Anguilla anguilla): an experimental study using isotopic tracers. Comp Biochem Physiol C: Toxicol Pharmacol 171:15–27
55. Monteiro DA, Rantin FT, Kalinin AL (2013) Dietary intake of inorganic mercury: bioaccumulation and oxidative stress parameters in the neotropical fish Hoplias malabaricus. Ecotoxicology 22:446–456
56. Liu H, Pei Z, Fan K, Liu Y, Wang Y, Liu Y, Huang Y (2023) Effects of cadmium exposure on tissue structure, antioxidant enzyme activity, serum biochemical indices and gene expression in the liver of juvenile largemouth bass (Micropterus salmoides)
57. Atli G, Alptekin Ö, Tükel S, Canli M (2006) Response of catalase activity to Ag+, Cd2+, Cr6+, Cu2+ and Zn2+ in five tissues of freshwater fish Oreochromis niloticus. Comp Biochem Physiol C: Toxicol Pharmacol 143(2):218–224
58. Kong Q, Lin CLG (2010) Oxidative damage to RNA: mechanisms, consequences, and diseases. Cell Mol Life Sci 67:1817–1829
59. Bhat RA, Priyam M, Foysal MJ, Gupta SK, Sundaray JK (2021) Role of sex-biased miRNAs in teleosts–a review. Rev Aquac 13(1):269–281
60. Wang W, Hang C, Zhang Y, Chen M, Meng X, Cao Q, Song N, Itkow J, Shen F, Yu D (2017) Dietary miR-451 protects erythroid

cells from oxidative stress via increasing the activity of Foxo3 pathway. Oncotarget 8(63):107109
61. Tilghman SL, Bratton MR, Segar HC, Martin EC, Rhodes LV, Li M, McLachlan JA, Wiese TE, Nephew KP, Burow ME (2012) Endocrine disruptor regulation of microRNA expression in breast carcinoma cells. PLoS One 7(3):e32754
62. Veiga-Lopez A, Luense LJ, Christenson LK et al (2013) Developmental programming gestational bisphenol-a treatment alters trajectory of fetal ovarian gene expression. Endocrinology 54(5):1873–1884
63. Lyu Y, Qian Y, Fu L (2015) Prediction of regulating network of innate immune signaling molecule hsa-miR-181a in stroke development based on bioinformatics analysis. Xi bao yu fen zi Mian yi xue za zhi= Chin J Cell Mol Immunol 31(8):1042–1047
64. Li H, Di G, Zhang Y, Xue R, Zhang J, Liang J (2019) MicroRNA-155 and microRNA-181a, via HO-1, participate in regulating the immunotoxicity of cadmium in the kidneys of exposed Cyprinus carpio. Fish Shellfish Immunol 95:473–480
65. Zhang J, Zheng S, Wang S, Liu Q, Xu S (2020) Cadmium-induced oxidative stress promotes apoptosis and necrosis through the regulation of the miR-216a-PI3K/AKT axis in common carp lymphocytes and antagonized by selenium. Chemosphere 258:127341
66. Qiang J, Tao YF, He J, Xu P, Bao JW, Sun YL (2017) miR-122 promotes hepatic antioxidant defense of genetically improved farmed tilapia (GIFT, Oreochromis niloticus) exposed to cadmium by directly targeting a metallothionein gene. Aquat Toxicol 182:39–48
67. He X, Jing Z, Cheng G (2014) MicroRNAs: new regulators of Toll-like receptor signalling pathways. BioMed Res Int 2014:1–14
68. Zeng Q, Liu W, Luo R, Lu G (2019) MicroRNA-181a and microRNA-155 are involved in the regulation of the differentiation and function of regulatory T cells in allergic rhinitis children. Pediatr Allergy Immunol 30(4):434–442
69. Chen J, Zhang S, Tong J, Teng X, Zhang Z, Li S, Teng X (2020) Whole transcriptome-based miRNA-mRNA network analysis revealed the mechanism of inflammation-immunosuppressive damage caused by cadmium in common carp spleens. Sci Total Environ 717:137081
70. Shaw JR, Gabor K, Hand E, Lankowski A, Durant L, Thibodeau R, Stanton CR, Barnaby R, Coutermarsh B, Karlson KH, Sato JD (2007) Role of glucocorticoid receptor in acclimation of killifish (Fundulus heteroclitus) to seawater and effects of arsenic. Am J Physiol-Regul Integr Comp Physiol 292(2):R1052–R1060
71. Goodale BC, Hampton TH, Ford EN, Jackson CE, Shaw JR, Stanton BA, King BL (2019) Profiling microRNA expression in Atlantic killifish (Fundulus heteroclitus) gill and responses to arsenic and hyperosmotic stress. Aquat Toxicol 206:142–153
72. Wang L, Bammler TK, Beyer RP, Gallagher EP (2013) Copper-induced deregulation of microRNA expression in the zebrafish olfactory system. Environ Sci Technol 47(13):7466–7474
73. Fu J, Wang M, Chaudhry MT, Tian Y, Liu C (2020) Combined RNA-Seq with small RNA revealed ribosome biogenesis and oxidative stress associated with cadmium response in carp (Cyprinus carpio L.) Hepato-pancreas. Aquaculture 518:734817
74. Hook SE, Skillman AD, Small JA, Schultz IR (2006) Gene expression patterns in rainbow trout, Oncorhynchus mykiss, exposed to a suite of model toxicants. Aquat Toxicol 77(4):372–385
75. Saddick S, Afifi M, Zinada OAA (2017) Effect of Zinc nanoparticles on oxidative stress-related genes and antioxidant enzymes activity in the brain of Oreochromis niloticus and Tilapia zillii. Saudi J Biol Sci 24(7):1672–1678
76. Jiang WD, Liu Y, Jiang J, Wu P, Feng L, Zhou XQ (2015) Copper exposure induces toxicity to the antioxidant system via the destruction of Nrf2/ARE signaling and caspase-3-regulated DNA damage in fish muscle: amelioration by myo-inositol. Aquat Toxicol 159:245–255
77. Dai Z, Cheng J, Bao L, Zhu X, Li H, Chen X, Zhang Y, Zhang J, Chu W, Pan Y, Huang H (2020) Exposure to waterborne cadmium induce oxidative stress, autophagy and mitochondrial dysfunction in the liver of Procypris merus. Ecotoxicol Environ Saf 204:111051
78. Giri SS, Sen SS, Jun JW, Sukumaran V, Park SC (2016) Immunotoxicological effects of cadmium on Labeo rohita, with emphasis on the expression of HSP genes. Fish Shellfish Immunol 54:164–171
79. Cobbina SJ, Xu H, Zhao T, Mao G, Zhou Z, Wu X, Liu H, Zou Y, Wu X, Yang L (2015) A multivariate assessment of innate immune-related gene expressions due to exposure to low concentration individual and mixtures of four kinds of heavy metals on zebrafish (Danio rerio) embryos. Fish Shellfish Immunol 47(2):1032–1042
80. Jiang X, Guan X, Yao L, Zhang H, Jin X, Han Y (2016) Effects of single and joint subacute exposure of copper and cadmium on heat shock proteins in common carp (Cyprinus carpio). Biol Trace Elem Res 169:374–381
81. Gökalp FD, Doğanlar O, Doğanlar ZB, Güner U (2022) The genotoxic effects of mixture of aluminum, arsenic, cadmium, cobalt, and chromium on the gill tissue of adult zebrafish (Danio rerio, Hamilton 1822). Drug Chem Toxicol 45(3):1158–1167
82. Wang H, Feng Y, Ming M, Song J, Chen Z, Xiao Z (2022) Amelioration of Cd-induced bioaccumulation, hematological parameters, and heat shock protein-related genes by vitamin C on common carp. Comp Biochem Physiol C: Toxicol Pharmacol 258:109362
83. Zhang Y, Zhang P, Yu P, Shang X, Lu Y, Li Y (2020) Transcriptome analysis reveals the mechanism of common carp brain injury after exposure to lead. Sci Total Environ 743:140796
84. Shahsavani D, Baghshani H, Alishahi E (2011) Efficacy of allicin in decreasing lead (Pb) accumulation in selected tissues of lead-exposed common carp (Cyprinus carpio). Biol Trace Elem Res 142:572–580
85. Kwong RW, Andrés JA, Niyogi S (2011) Effects of dietary cadmium exposure on tissue-specific cadmium accumulation, iron status and expression of iron-handling and stress-inducible genes in rainbow trout: influence of elevated dietary iron. Aquat Toxicol 102(1–2):1–9. https://doi.org/10.1016/j.aquatox.2010.12.010
86. Kontaş S, Bostancı D (2020) Genotoxic effects of environmental pollutant heavy metals on Alburnus chalcoides (Pisces: Cyprinidae) inhabiting lower Melet River (Ordu, Turkey). Bull Environ Contam Toxicol 104(6):763–769
87. Omar WA, Zaghloul KH, Abdel-Khalek AA, Abo-Hegab S (2012) Genotoxic effects of metal pollution in two fish species, Oreochromis niloticus and Mugil cephalus, from highly degraded aquatic habitats. Mutat Res/Genet Toxicol Environ Mutagen 746(1):7–14
88. Hussain B, Sultana T, Sultana S, AlGhanim KA, Mahboob S (2016) Study on effect of pollution on genotoxic damage in Cirrhinus mrigala and Catla catla from River Chenab. Fresenius Environ Bull 25(7):2500–2508
89. Javed M, Ahmad I, Usmani N, Ahmad M (2016) Studies on biomarkers of oxidative stress and associated genotoxicity and histopathology in Channa punctatus from heavy metal polluted canal. Chemosphere 151:210–219
90. Ratn A, Prasad R, Awasthi Y, Kumar M, Misra A, Trivedi SP (2018) Zn2+ induced molecular responses associated with oxidative stress, DNA damage and histopathological lesions in liver

and kidney of the fish, Channa punctatus (Bloch, 1793). Ecotoxicol Environ Saf 151:10–20

91. Moussa MA, Mohamed HRH, Abdel-Khalek AA (2022) Metal accumulation and DNA damage in Oreochromis niloticus and Clarias gariepinus after chronic exposure to discharges of the batts drain: potential risk to human health. Bull Environ Contam Toxicol 108(6):1064–1073. https://doi.org/10.1007/s00128-022-03512-8
92. González-Mille DJ, Ilizaliturri-Hernández CA, Espinosa-Reyes G, Costilla-Salazar R, Díaz-Barriga F, Ize-Lema I, Mejía-Saavedra J (2010) Exposure to persistent organic pollutants (POPs) and DNA damage as an indicator of environmental stress in fish of different feeding habits of Coatzacoalcos, Veracruz, Mexico. Ecotoxicology 19:1238–1248
93. Bejaoui S, Telahigue K, Chetoui I, Trabelsi W, Rabeh I, Nechi S, Chalbi E, Chalghaf M, Cafsi MHE, Soudani N (2020) Effects of lead exposure on redox status, DNA and histological structures in Venus verrucosa gills and digestive gland. Chem Ecol 36(5):434–457
94. Handa K, Jindal R (2020) Genotoxicity induced by hexavalent chromium leading to eryptosis in Ctenopharyngodon idellus. Chemosphere 247:125967
95. Chaudhary A, Gul Javaid K, Bughio E, Faisal N (2023) Study on histo-biochemical biomarkers of chromium induced toxicity in Labeo rohita. Emerg Contam 9(1):100204
96. Aziz S, Abdullah S (2023) Evaluation of toxicity induced by engineered CuO nanoparticles in freshwater fish, Labeo rohita. Turk J Fish Aquat Sci 23(8). https://doi.org/10.4194/TRJFAS18762
97. Nedzvetsky VS, Gasso VY, Novitskyi RO, Hasso IA (2021) Indices of the DNA repair system in the brain of fish as a biomarker of inorganic mercury burden. Ecol Noospherology 32(1):9–16
98. Jamil Emon F, Rohani MF, Sumaiya N, Tuj Jannat MF, Akter Y, Shahjahan M, Abdul Kari Z, Tahiluddin AB, Goh KW (2023) Bioaccumulation and bioremediation of heavy metals in fishes—a review. Toxics 11(6):510
99. Chatha AMM, Naz S, Iqbal SS, Kiran A, Lateef M, Zahra U, Yasmin F, Amjad N, Javaid A (2024) Detection of DNA damage in fish using comet assay. Curr Trends OMICS 4(1):01–16
100. Barjhoux I, Baudrimont M, Morin B, Landi L, Gonzalez P, Cachot J (2012) Effects of copper and cadmium spiked-sediments on embryonic development of Japanese medaka (Oryzias latipes). Ecotoxicol Environ Saf 79:272–282
101. Li ZH, Chen L, Wu YH, Li P, Li YF, Ni ZH (2014) Effects of waterborne cadmium on thyroid hormone levels and related gene expression in Chinese rare minnow larvae. Comp Biochem Physiol C: Toxicol Pharmacol 161:53–57
102. Nayak AS, Lage CR, Kim CH (2007) Effects of low concentrations of arsenic on the innate immune system of the zebrafish (Danio rerio). Toxicol Sci 98(1):118–124
103. Abbas HHH, Mahmoud HM, Miller JD (2007) Vitamin C and cadmium toxicity in fish Oreochromis niloticus. J Vet Res 11:54–74
104. Yan W, Hamid N, Deng S, Jia PP, Pei DS (2020) Individual and combined toxicogenetic effects of microplastics and heavy metals (Cd, Pb, and Zn) perturb gut microbiota homeostasis and gonadal development in marine medaka (Oryzias melastigma). J Hazard Mater 397:122795
105. Jantawongsri K, Nørregaard RD, Bach L, Dietz R, Sonne C, Jørgensen K, Lierhagen S, Ciesielski TM, Jenssen BM, Waugh CA, Eriksen R (2022) Effects of exposure to environmentally relevant concentrations of lead (Pb) on expression of stress and immune-related genes, and microRNAs in shorthorn sculpins (Myoxocephalus scorpius). Ecotoxicology 31(7):1068–1077
106. Yadetie F, Karlsen OA, Lanzén A, Berg K, Olsvik P, Hogstrand C, Goksøyr A (2013) Global transcriptome analysis of Atlantic cod (Gadus morhua) liver after in vivo methylmercury exposure suggests effects on energy metabolism pathways. Aquat Toxicol 126:314–325
107. Cambier S, Benard G, Mesmer-Dudons N, Gonzalez P, Rossignol R, Brethes D, Bourdineaud JP (2009) At environmental doses, dietary methylmercury inhibits mitochondrial energy metabolism in skeletal muscles of the zebra fish (Danio rerio). Int J Biochem Cell Biol 41(4):791–799
108. Bibi S, Naz S, Saeed S, Chatha AM (2021) A review on histopathological alterations induced by heavy metals (Cd, Ni, Cr, Hg) in different fish species. Punjab Univ J Zool 36(1):81–89
109. Puntoriero ML, Fernández Cirelli A, Volpedo AV (2018) Histopathological changes in liver and gills of Odontesthes bonariensis inhabiting a lake with high concentrations of arsenic and fluoride (Chasicó Lake, Buenos Aires province). Rev Int Contam Ambient 34(1):69–77
110. Joardar S, Dewanjee S, Bhowmick S, Dua TK, Das S, Saha A, De Feo V (2019) Rosmarinic acid attenuates cadmium-induced nephrotoxicity via inhibition of oxidative stress, apoptosis, inflammation and fibrosis. Int J Mol Sci 20(8):2027
111. Li H, Di G, Zhang Y, Liang J, Wang X, Xu Z, Kong X (2021) miR-217 through SIRT1 regulates the immunotoxicity of cadmium in Cyprinus carpio. Comp Biochem Physiol C: Toxicol Pharmacol 248:109086
112. Yusnaini Y, Emiyarti E, Nur I, Astuti O, Patadjai RS (2020) Sublethal toxicity test of mercury (Hg) in the flesh and tissue of tilapia (Oreochromis niloticus). Omni-Akuatika 16(2):99–107
113. Trivedi SP, Singh S, Trivedi A, Kumar M (2022) Mercuric chloride-induced oxidative stress, genotoxicity, haematological changes and histopathological alterations in fish Channa punctatus (B loch, 1793). J Fish Biol 100(4):868–883
114. Pervaiz A, Afridi R, Pervaiz Z, Masood R, Pervaiz H (2019) Examination of morphological, behavioral and histopathological effects on Oreochromis niloticus after acute exposure to methylmercury. J Innov Sci 5(1):16–24
115. Khalique N, Khan AM, Ahmed A, Badar R, Abassi NA, Shafique MA (2023) Biochemical and histological effects of heavy metals on fish from River Jehlum, Pakistan. J Xi'an Shiyou Univ Nat Sci Ed 19(2):508–525
116. Elwasify AYH, Ghanem HM, El-Bamby MMM, Ali AFF (2021) Impact of bioaccumulation and biosedimentation of some heavy metals on some biochemical responses in the sole fish, Solea solea inhabiting Lake Qarun, Egypt. Egypt J Aquat Biol Fish 25(1):75–89
117. Sahid S, Sultana T, Sultana S (2021) Histopathological alterations in gills, liver, kidney and muscles of Ictalurus punctatus collected from pollutes areas of River. Braz J Biol l81(3):814–821
118. Younis E, Abdel-Warith AW, Al-Asgah N, Ebaid H (2015) Histopathological alterations in the liver and intestine of Nile tilapia Oreochromis niloticus exposed to long-term sublethal concentrations of cadmium chloride. Chin J Oceanol Limnol 33(4):846–852
119. Rana MA, Jabeen F, Shabbir S, Naureen A, Sultana K, Ahmad I, Shabnam M (2015) Histopathological study of liver and kidney in common carp (Cyprinus Carpio) exposed to different doses of potassium dichromate. Int J Biosci 6(12):108–116
120. Alesci A, Cicero N, Fumia A, Petrarca C, Mangifesta R, Nava V, Lo Cascio P, Gangemi S, Di Gioacchino M, Lauriano ER (2022) Histological and chemical analysis of heavy metals in kidney and gills of Boops boops: melanomacrophages centers and rodlet cells as environmental biomarkers. Toxics 10(5):218
121. Kaur S, Khera KS, Kondal JK (2018) Heavy metal induced histopathological alterations in liver, muscle and kidney of freshwater cyprinid, Labeo rohita (Hamilton). J Entomol Zool Stud 6(2):2137–2144
122. Khan MS, Javed M, Rehman MT, Urooj M, Ahmad MI (2020) Heavy metal pollution and risk assessment by the battery of toxicity tests. Sci Rep 10(1):16593

123. Onita B, Albu P, Herman H, Balta C, Lazar V, Fulop A, Baranyai E, Harangi S, Keki S, Nagy L, Nagy T (2021) Correlation between heavy metal-induced histopathological changes and trophic interactions between different fish species. Appl Sci 11(9):3760
124. Kumar M, Zaveria A, Jain A, Singh S, Tiwari V, Trivedi SP (2023) Toxicological manifestations in gills, liver, kidney and muscles of Channa punctatus exposed to mercuric chloride. J Appl Nat Sci 15(2):498–504
125. Chavan VR, Muley DV (2014) Effect of heavy metals on liver and gill of fish Cirrhinus mrigala. Int J Curr Microbiol App Sci 3:277–288
126. Naz S, Hussain R, Ullah Q, Chatha AMM, Shaheen A, Khan RU (2021) Toxic effect of some heavy metals on hematology and histopathology of major carp (Catla catla). Environ Sci Pollut Res 28:6533–6539
127. Rajeshkumar S, Liu Y, Ma J, Duan HY, Li X (2017) Effects of exposure to multiple heavy metals on biochemical and histopathological alterations in common carp, Cyprinus carpio L. Fish Shellfish Immunol 70:461–472
128. Khalesi MK, Abedi Z, Behrouzi S, Eskandari SK (2017) Haematological, blood biochemical and histopathological effects of sublethal cadmium and lead concentrations in common carp. Bulg J Vet Med 20(2):141–150
129. Benjamin LV, Kutty R (2019) Sub-lethal effects of potassium dichromate on hematological and histological parameters in climbing perch, Anabas testudineus (Anabantidae). Int J Aquat Biol 7(3):140–145
130. Kawade S (2020) Histopathological alterations in the liver of freshwater fish, Channa gachua (Ham.) on acute exposure to nickel. J Emerg Technol Innov Res 7:1025–1030
131. Alkshab AA, Taha AM (2021) Histological effect of lead chloride on the brain of Gambusia Affinis. In: Journal of Physics: Conference Series. IOP Publishing 1818(1):012024
132. Shah N, Khan A, Ali R, Marimuthu K, Uddin MN, Rizwan M, Rahman KU, Alam M, Adnan M, Jawad SM, Hussain S (2020) Monitoring bioaccumulation (in gills and muscle tissues), hematology, and genotoxic alteration in Ctenopharyngodon idella exposed to selected heavy metals. BioMed Res Int 2020
133. Ando H, Ogawa S, Shahjahan M, Ikegami T, Doi H, Hattori A, Parhar I (2014) Diurnal and Circadian oscillations in expression of kisspeptin, kisspeptin receptor and gonadotrophin-releasing hormone 2 genes in the grass puffer, a semilunar-synchronised spawner. J Neuroendocrinol 26(7):459–467
134. Ando H, Shahjahan M, Kitahashi T (2018) Periodic regulation of expression of genes for kisspeptin, gonadotropin-inhibitory hormone and their receptors in the grass puffer: implications in seasonal, daily and lunar rhythms of reproduction. Gen Comp Endocrinol 265:149–153
135. Jaensson A, Scott AP, Moore A, Kylin H, Olsén KH (2007) Effects of a pyrethroid pesticide on endocrine responses to female odours and reproductive behaviour in male parr of brown trout (Salmo trutta L.). Aquat Toxicol 81(1):1–9
136. Jezierska B, Lugowska K, Witeska M, Sarnowski P (2000) Malformations of newly hatched common carp larvae. Electronic Journal of Polish Agricultural Universities. Ser Fish 3(2)
137. Celino FT, Yamaguchi S, Miura C, Miura T (2009) Arsenic inhibits in vitro spermatogenesis and induces germ cell apoptosis in Japanese eel (Anguilla japonica). Reproduction 138(2):279–287
138. Santos GS, Neumann G, de Nascimento CZ, Domingues CE, Campos SX, Bombardelli RA, Cestari MM (2018) Exposure of male tilapia (Oreochromis niloticus) to copper by intraperitoneal injection: DNA damage and larval impairment. Aquat Toxicol 205:123–129
139. Azab AM, Aly-Eldeen MA, Khalaf-Allah HMM, El-Battal MMA (2019) Effect of heavy metals on the ovary of Tilapia zillii in some canals of Nile Delta area, Egypt. Egypt J Aquat Biol Fish 23(3):329–345
140. Molina AM, Lora AJ, Blanco A, Monterde JG, Ayala N, Moyano R (2013) Endocrine-active compound evaluation: qualitative and quantitative histomorphological assessment of zebrafish gonads after bisphenol-A exposure. Ecotoxicol Environ Saf 88:155–162
141. Senarat S, Jiraungkoorskul W, Kettratad J (2017) Ovarian histology and reproductive health of short mackerel, Rastrelliger brachysoma (Bleeker, 1851), as threatened marine fish in Thailand. Songklanakarin J Sci Technol 39(2). https://doi.org/10.14456/sjst-psu.2017.26
142. Jangu S, Kumar D, Brraich OS, Baek KH, Mishra AK (2021) Alterations in ultra-structures and elemental composition of ovaries of Labeo rohita (Hamilton-Buchanan) as a pollution indicator in Harike Wetland (Ramsar Site), India. J Histotechnol 44(2):62–69
143. Du J, Zhou J, Li S, Shao J, Jiang P, Lei C, Song H, Bai J, Han L (2022) Timing of early gonadal differentiation and effects of estradiol-17β treatments on the sex differentiation in Largemouth bass (Micropterus salmoides). Fish Physiol Biochem 48(3):805–815
144. Hayati A, Pramudya M, Soepriandono H, Astri AR, Kusuma MR, Maulidah S, Adriansyah W, Dewi FRP (2022) Assessing the recovery of steroid levels and gonadal histopathology of tilapia exposed to polystyrene particle pollution by supplementary feed. Vet World 15(2):517
145. Luo Y, Shan D, Zhong H, Zhou Y, Chen W, Cao J, Guo Z, Xiao J, He F, Huang Y, Li J (2015) Subchronic effects of cadmium on the gonads, expressions of steroid hormones and sex-related genes in tilapia Oreochromis niloticus. Ecotoxicology 24:2213–2223
146. Hayati A, Giarti K, Winarsih Y, Amin MHF (2017) The effect of cadmium on sperm quality and fertilization of Cyprinus carpio L. J Trop Biodivers Biotechnol 2(2):45–50
147. Kime DE (1995) The effects of pollution on reproduction in fish. Rev Fish Biol Fish 5:52–95
148. El-Hak HNG, Ghobashy MA, Mansour FA, El-Shenawy NS, El-Din MIS (2022) Heavy metals and parasitological infection associated with oxidative stress and histopathological alteration in the Clarias gariepinus. Ecotoxicology 31(7):1096–1110
149. Ni X, Shen Y (2021) Transgenerational effects of hexavalent chromium on marine medaka (Oryzias melastigma) reveal complex transgenerational adaptation in offspring. Biomolecules 11(2):138
150. Bhat RA, Saoca C, Cravana C, Fazio F, Guerrera MC, Labh SN, Kesbiç OS (2023) Effects of heavy pollution in different water bodies on male rainbow trout (Oncorhynchus mykiss) reproductive health. Environ Sci Pollut Res 30(9):23467–23479
151. Gárriz Á, Miranda LA (2020) Effects of metals on sperm quality, fertilization and hatching rates, and embryo and larval survival of pejerrey fish (Odontesthes bonariensis). Ecotoxicology 29(7):1072–1082
152. Cousins RJ, Liuzzi JP, Lichten LA (2006) Mammalian zinc transport, trafficking, and signals. J Biol Chem 281(34):24085–24089
153. Santos DCMD, Cupertino MDC, Matta SLPD, Oliveira JAD, Santos JADD (2015) Histological alterations in liver and testis of Astyanax aff. bimaculatus caused by acute exposition to zinc. Rev Ceres 62(2):133–141
154. Cao J, Wang G, Wang T, Chen J, Wenjing G, Wu P, He X, Xie L (2019) Copper caused reproductive endocrine disruption in zebrafish (Danio rerio). Aquat Toxicol 211:124–136
155. Liao CY, Fu JJ, Shi JB, Zhou QF, Yuan CG, Jiang GB (2006) Methylmercury accumulation, histopathology effects, and cholinesterase activity alterations in medaka (Oryzias latipes)

following sublethal exposure to methylmercury chloride. Environ Toxicol Pharmacol 22(2):225–233
156. Alkahemal-Balawi HF, Ahmad Z, Al-Akel AS, Al-Misned F, Suliman EAM, Al-Ghanim KA (2011) Toxicity bioassay of lead acetate and effects of its sub-lethal exposure on growth, haematological parameters and reproduction in Clarias gariepinus. Afr J Biotech 10(53):11039
157. Pinto GL, da Silva Castro J, Val AL (2021) Copper and cadmium impair sperm performance, fertilization and hatching of oocytes from Amazonian fish Colossoma macropomum. Chemosphere 266:128957
158. Authman MM, Zaki MS, Khallaf EA, Abbas HH (2015) Use of fish as bio-indicator of the effects of heavy metals pollution. J Aquac Res Dev 6(4):1–13
159. Rai S, Yadav KK, Trivedi SP (2019) Ovarian impairment in fish, Channa punctata (Bloch) on exposure to mercuric chloride, Caldan 50 SP and their mixture. J Ecophysiol Occup Health 19:65–70
160. Shukla JP, Pandy K (1984) Impaired ovarian functions in arsenic treated fresh water fish, Colisa fasciatus (bl and sch.). Toxicol Left 20:1–3
161. Elgaml SA, Saad TT, Hamed M, Zaki VH (2019) Effects of heavy metal pollutants on the reproduction of Nile tilapia. Int J Fish Aquat 7(5):542–547
162. Chen H, Cao J, Li L, Wu X, Bi R, Klerks PL, Xie L (2016) Maternal transfer and reproductive effects of Cr (VI) in Japanese medaka (Oryzias latipes) under acute and chronic exposures. Aquat Toxicol 171:59–68
163. Mishra AK, Mohanty B (2014) Acute spill-mimicking exposure effect of hexavalent chromium on the pituitary-ovarian axis of a teleost, Channa punctatus (Bloch). Environ Toxicol 29(7):733–739. https://doi.org/10.1002/tox.21799
164. Hani YMI, Turies C, Palluel O, Delahaut L, Bado-Nilles A, Geffard A, Dedourge-Geffard O, Porcher JM (2019) Effects of a chronic exposure to different water temperatures and/or to an environmental cadmium concentration on the reproduction of the threespine stickleback (Gasterosteus aculeatus). Ecotoxicol Environ Saf 174:48–57
165. Farag AM, Harper DD, Cleveland L, Brumbaugh WG, Little EE (2006) The potential for chromium to affect the fertilization process of Chinook salmon (Oncorhynchus tshawytscha) in the Hanford Reach of the Columbia River, Washington, USA. Arch Environ Contam Toxicol 50:575–579. https://doi.org/10.1007/s00244-005-0010-2
166. Yamaguchi S, Miura C, Ito A, Agusa T, Iwata H, Tanabe S, Tuyen BC, Miura T (2007) Effects of lead, molybdenum, rubidium, arsenic and organochlorines on spermatogenesis in fish: monitoring at Mekong Delta area and in vitro experiment. Aquat Toxicol 83(1):43–51
167. Lemly AD (2004) Aquatic selenium pollution is a global environmental safety issue. Ecotoxicol Environ Saf 59(1):44–56
168. Shelke AD (2014) A study on histopathological changes in the gonads of a freshwater teleost fish. *A. mola exposed to the heavy metals*. J Glob Biosci 3:763–771
169. Ruby SM, Jaroslawski P, Hull R (1993) Lead and cyanide toxicity in sexually maturing rainbow trout, Oncorhynchus mykiss during spermatogenesis. Aquat Toxicol 26(3–4):225–238
170. Łuszczek-Trojnar E, Drąg-Kozak E, Szczerbik P, Socha M, Popek W (2014) Effect of long-term dietary lead exposure on some maturation and reproductive parameters of a female Prussian carp (Carassius gibelio B.). Environ Sci Pollut Res 21:2465–2478
171. El-Agri AM, Emam MA, Gaber HS, Hassan EA, Hamdy SM (2022) Integrated use of biomarkers to assess the impact of heavy metal pollution on Solea aegyptiaca fish in Lake Qarun. Environ Sci Eur 34(1):74
172. Abdel-Warith AWA, Younis ESM, Al-Asgah NA, Rady AM, Allam HY (2020) Bioaccumulation of lead nitrate in tissues and its effects on hematological and biochemical parameters of Clarias gariepinus. Saudi J Biol Sci 27(3):840–845
173. Flores MI, Bravo-Thais SC, Romero MB, Guzman MG (2023) Evaluation of heavy metal removal using Phragmites australis (Cav.) and Schoenoplectus californicus (CA Mey.): a comparison of the dry ashing and wet digestion method. Anal Bioanal Chem Res 10(1):97–109
174. Naicker K, Mahlambi P, Mahlambi M (2023) Comparison of ultrasonic and microwave assisted digestion methods for the determination of heavy metals in soil and sediment: the effect of seasonal variations on metal concentrations and risk assessment. Soil Sediment Contam: An Int J 32(3):320–336
175. Sheehan A, Furey A (2024) Advanced review of the contributing factors for the microwave digestion of food matrices for trace elemental analysis. Talanta Open 9:100309
176. Adolfo FR, do Nascimento PC, Leal GC, Bohrer D, Viana C, de Carvalho LM, Colim AN (2019) Simultaneous determination of iron and nickel as contaminants in multimineral and multivitamin supplements by solid sampling HR-CS GF AAS. Talanta 195:745–751
177. Eskina VV, Dalnova OA, Filatova DG, Baranovskaya VB, Karpov YA (2020) Direct precise determination of Pd, Pt and Rh in spent automobile catalysts solution by high-resolution continuum source graphite furnace atomic absorption spectrometry. Spectrochim Acta, Part B: At Spectrosc 165:105784
178. Elik A, Demirbas A, Altunay N (2020) Analysis of zinc and chromium in grain samples using ionic liquid-based ultrasound-assisted microextraction followed by flame-AAS after microwave digestion. Biol Trace Elem Res 198:697–706
179. Vinodhini R, Narayanan M (2008) Bioaccumulation of heavy metals in organs of fresh water fish Cyprinus carpio (Common carp). Int J Environ Sci Technol 5:179–182
180. Ali Z, Yousafzai AM, Sher N, Muhammad I, Nayab GE, Aqeel SAM, Shah ST, Aschner M, Khan I, Khan H (2021) Toxicity and bioaccumulation of manganese and chromium in different organs of common carp (Cyprinus carpio) fish. Toxicol Rep 8:343–348
181. Szebedinszky C, McGeer JC, McDonald DG, Wood CM (2001) Effects of chronic Cd exposure via the diet or water on internal organ-specific distribution and subsequent gill Cd uptake kinetics in juvenile rainbow trout (Oncorhynchus mykiss). Environ Toxicol Chem: An Int J 20(3):597–607
182. Filipoiu DC, Bungau SG, Endres L, Negru PA, Bungau AF, Pasca B, Radu AF, Tarce AG, Bogdan MA, Behl T, Nechifor AC (2022) Characterization of the toxicological impact of heavy metals on human health in conjunction with modern analytical methods. Toxics 10(12):716
183. Balcaen L, Bolea-Fernandez E, Resano M, Vanhaecke F (2015) Inductively coupled plasma–Tandem mass spectrometry (ICP-MS/MS): a powerful and universal tool for the interference-free determination of (ultra) trace elements–a tutorial review. Anal Chim Acta 894:7–19
184. Penanes PA, Galán AR, Huelga-Suarez G, Rodríguez-Castrillón JÁ, Moldovan M, Alonso JIG (2022) Isotopic measurements using ICP-MS: a tutorial review. J Anal At Spectrom 37(4):701–726
185. Kagaya S, Maeba E, Inoue Y, Kamichatani W, Kajiwara T, Yanai H, Saito M, Tohda K (2009) A solid phase extraction using a chelate resin immobilizing carboxymethylated pentaethylenehexamine for separation and preconcentration of trace elements in water samples. Talanta 79(2):146–152
186. Pei J, Zuo J, Wang X, Yin J, Liu L, Fan W (2019) The bioaccumulation and tissue distribution of arsenic species in Tilapia. Int J Environ Res Public Health 16(5):757
187. Douvris C, Vaughan T, Bussan D, Bartzas G, Thomas R (2023) How ICP-OES changed the face of trace element analysis: review of the global application landscape. Sci Total Environ 905:167242

188. Petrean IA, Micle V, Sur IM, Șenilă M (2023) Characterization of sterile mining dumps by the ICP-OES analytical method: a case study from Baia Mare mining area (Maramures, Romania). Sustainability 15(2):1158
189. Aldoghachi MA, Rahman MM, Yusoff I, Sofian-Azirun M (2016) Acute toxicity and bioaccumulation of heavy metals in red tilapia fish. JAPS: J Anim Plant Sci 26(2)
190. Gupta R, Srivastava D, Sahu M, Tiwari S, Ambasta RK, Kumar P (2021) Artificial intelligence to deep learning: machine intelligence approach for drug discovery. Mol Diversity 25:1315–1360
191. Venkatesan D, Elangovan A, Winster H, Pasha MY, Abraham KS, Satheeshkumar J, Sivaprakash P, Niraikulam A, Gopalakrishnan AV, Narayanasamy A, Vellingiri B (2022) Diagnostic and therapeutic approach of artificial intelligence in neuro-oncological diseases. Biosens Bioelectron: X 11:100188
192. Ucun Ozel H, Gemici BT, Gemici E, Ozel HB, Cetin M, Sevik H (2020) Application of artificial neural networks to predict the heavy metal contamination in the Bartin River. Environ Sci Pollut Res 27:42495–42512
193. Venkatramanan S, Chung SY, Selvam S, Son JH, Kim YJ (2017) Interrelationship between geochemical elements of sediment and groundwater at Samrak Park Delta of Nakdong River Basin in Korea: multivariate statistical analyses and artificial neural network approaches. Environ Earth Sci 76:1–12
194. Altunkaynak A, Basakin E (2018) River flow estimation using time series and comparison with different methods. J Erzincan Univ Inst Sci Technol 11:92–101
195. Sarkar A, Pandey P (2015) River water quality modelling using artificial neural network technique. Aquat Procedia 4:1070–1077
196. Zhou X, Sun J, Tian Y, Yao K, Xu M (2022) Detection of heavy metal lead in lettuce leaves based on fluorescence hyperspectral technology combined with deep learning algorithm. Spectrochim Acta Part A Mol Biomol Spectrosc 266:120460
197. Zhao L, Hu YM, Zhou W, Liu ZH, Pan YC, Shi Z, Wang L, Wang GX (2018) Estimation methods for soil mercury content using hyperspectral remote sensing. Sustainability 10(7):2474
198. Liu M, Liu X, Li M, Fang M, Chi W (2010) Neural-network model for estimating leaf chlorophyll concentration in rice under stress from heavy metals using four spectral indices. Biosys Eng 106(3):223–233

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.