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Metal Induced Oxidative Stress in Fishes: A Review

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Abstract

Fisheswhen exposed to various contaminants, particularly metals in their habitat can induce oxidative stress. In fishes, metals contamination results in oxidative stress by promoting reactive oxygen species (ROS) formation through redox cycling and impairing antioxidant defenses. ROS are harmful to all forms of aquatic life and can damage tissues and cellular components.Higher amount ofROS lead to varying degree of oxidative damage to the fish tissues including lipid peroxidation, protein and DNA oxidation as well as enzyme inactivation through different mechanisms as observed in different fish species. The present comprehension of how metals contribute to the onset of oxidative stress in fish is summed up in this review.

Keywords: Fishes, metals, oxidative stress, Reactive Oxygen Species, oxidation

1. INTRODUCTION:

Like all other aerobic animals and their ancestors, fishes also obtain their energy from the reduction of molecular oxygen, which is then stored in ATP molecules. The great majority of cellular functions including those of motility, cell division and biosynthetic reactions are powered by these ATP molecules. However, similar to other animals, fishes while exposed to the presence of different pollutants or contaminants in their natural habitat, also experience oxidative stress as a result of contaminant-stimulated Reactive Oxygen Species (ROS) generation and subsequent initiation of toxicity pathway in their body [1].

As metals are well-known oxidative stressors, fish oxidative damage and antioxidant defence assessments could be a major way for evaluation of metal contamination of the aquatic environment. Besides,metals are also found to be accumulated in aquatic food chain involving the fishes and other aquatic organisms. As such, consumption of fishes collected from metal-contaminated water bodies poses a serious risk to human health. Being at the top of the different aquatic food chains, different species of fishes are considered to be good bio-indicators of metal contamination in aquatic habitats [2].

Light metals like Calcium (Ca), Sodium (Na), and Potassium (K) are crucial components of different biological systems. The transition metals such as Iron (Fe), Copper (Cu), Cobalt (Co) and Manganese (Mn) are necessary for living organisms but can be hazardous in large amounts. However, the heavy metals

constitute one of the major groups of environmental contaminant including aquatic ecosystems. Metals including mercury, lead, zinc, nickel, selenium, chromium and arsenic are hazardous to living organisms even in very small amounts and are typically not needed for metabolic processes [3] [4]. Presence of xenobiotics including various heavy metals in the aquatic environment put fishes and other aquatic life under oxidative stress through their capacity to perform redox cycles[5].This review focuses on the important metals and different aspects of their role in inducing oxidative stress in different species of fishes.

2. OXIDATIVE STRESS IN FISHES:

2.1. CAUSES OF OXIDATIVE STRESS:

Oxidative stress is an inevitable component of an aerobic life. 'Oxidative stress'has been defined as an imbalance between oxidants and antioxidants in favour of the oxidants, potentially leading to damage [6].Oxidative stress is a component of many extensive stresses. Oxidative stress occurs when the steady-state concentration of reactive oxygen species (ROS) is temporarily or persistently elevated. This disruption of cellular metabolism and its regulation can lead to damage of the cellular constituents [7]. It has been reported that a variety of environmental physical, chemical and biological variables can cause oxidative stress [1].

The primary endogenous source of ROS is mitochondrial respiration. In the mitochondrial electron transport chain, there are four steps involved in this reduction of molecular oxygen, and at the end of each step, a free radical is formed as an intermediate molecule [8]. Furthermore, ozonization used in aquaculture and environmental hypoxia are two important factors that might cause stress [2]. Likewise, under both natural and artificial conditions, variations in temperature, oxygen and salinity can induce an imbalance between the formation and removal of reactive oxygen species (ROS), leading to stress [1]. In eukaryotes, cytochrome oxidase in the mitochondrial electron-transport chain (ETC) uses four-electron processes to convert more than 90% of oxygen consumed directly to water under aerobic circumstances without releasing reactive oxygen species (ROS) [9] [10].

Despite the fact that over 90% of the oxygen consumed by organisms is used to produce energy in the form of ATP (via coupling of the ETC with oxidative phosphorylation), less than 10% of the oxygen consumed by organisms is reduced via one-electron transport pathways, which start with the conversion of molecular oxygen to the superoxide anion radical (O_2•–). Through a one-electron pathway, the latter can be further reduced to create water, hydroxyl radical (HO•) and hydrogen peroxide (H_2O_2). O_2•– and HO• are considered to be free radicals since they have unpaired electrons in their exterior electron orbital whereas $H_2O_2$is not a free radical as it does not have any electrons in its electron orbitals. ROS reactivity is known to decrease in the following order: HO• > O_2•-, > H_2O_2 [11].

ROS are also produced by various oxidases, such as those that can react with specific amino acids, carbohydrates, heterocyclic compounds and other substances [12] [13]. If the organisms have little control over the production of ROS through the aforementioned mechanisms, NADPH oxidase regulates it precisely [5]. Certain endogenous and exogenous small compounds, especially xenobiotics, may occasionally produce significant levels of ROS through autoxidation [14][15][16].

2.2. EFECTS OF OXDATIVE STRESS IN FISHES:

It is commonly known that ROS can interact with almost every component of a cell including proteins, lipids, carbohydrates and nucleic acids. When fishes are exposed to waters contaminated with effluents fromthermal power plants, they develop hypoglycemia, hyperlipidemia and hypercholesterolemia. Besides, the fish's immune systems are weakened, leaving them more susceptible to illness. Other significant factors that could have an impact on the health of fish include how the fish are handled during transit and how densely they are stocked during caging. Lipids and carbohydrates that have been altered by ROS may break down or change into hazardous by-products. With a few exceptions, a similar scenario occurs with proteins when reduced oxidized proteins are handled by particular systems [17]. Although ROS-modified DNA is sometimes irreversibly repaired, ROS-modified RNA is likewise destroyed [18] [19] [20].

Fish oil has been a standard material for studying lipid oxidation or peroxidation processes [18] [19]. The stability of fish oil's polyunsaturated fatty acids and food deterioration were the initial two areas of considerable research on lipid peroxidation (LPO) [21] [22] [23]. Different studiesalso shows the possibility of occurrence of LPO products [24] [25] [26] [27][28] [29] [30] [31], [32] [33] [34][35]. Oxidatively changed proteins are often broken down, but occasionally they can build up and some of them can even start to form ROS.

One of the consequences of ROS in the cell is DNA oxidation. This kind of damage is extremely crucial for the functions of cells as it can lead to mutations. ROS-induced DNA alteration occurs more frequently in

mitochondria than in the nucleus and is typically repaired by certain systems. The most widely used indicator of DNA damage is 8-oxoguanine, which is produced when ROS assault DNA[36] [37] [38] [39] [40] [41] [42].
However, findings of different studies have shown that the degree of stress varies from species to species implying that different fish taxa have varying levels of stress tolerances[43]. A degraded aquatic environment thus, has a significant impact on over-all growth and development of fishes inhabiting therein [44].

2.3. ANTIOXIDANT DEFENSE SYSTEM:

Elimination of ROS provides control over their steady-state levels in addition to production. Different living organisms have different multilayered and intricate antioxidant systems which work to either minimize the harmful effects of ROS or prevent the creation of ROS and ROS-modified molecules. As such, antioxidants are typically categorized into two groups: high molecular mass antioxidants (which have molecular masses >10kd) and low molecular mass antioxidants (which typically have molecular masses less <1kd) [36].
The first line of defence or primary defencemechanism against ROS is made up of the enzymes SOD(Superoxide dismutase), catalase, and GPx(glutathione-dependent peroxidase)whereas the second line or secondary defence is formed by GR and G6PDH. The first line of defence involves the antioxidant enzymes that interact directly with ROS as substrates, while the second line of defence involves linked antioxidant enzymes, support the first line. The reducing equivalents (GSH, NADPH) required for the primary antioxidant enzymes' function are supplied by the second line antioxidant enzymes. Intermediate metabolic pathways provide the resources needed for the activity of secondary antioxidant enzymes [36].
Low molecular mass antioxidants encompass a wide range of chemical substances, such as carotenoids, anthocyanins, polyphenols, gluathione, Vitamin C (ascorbic acid) and Vitamin E (tocopherol). The majority of these are obtained by fish through their diet, but most aerobic organisms synthesize the tripeptide glutathione (γ-glutamyl-cysteinyl-glycine, or GSH), which is used to regulate ROS levels either directly through interactions with them or by acting as a cofactor for enzymes that detoxify ROS [45] [46]. Through a number of regulatory pathways, organisms including fishes can precisely modify the GSH level to suit particular physiological or environmental situations. Nevertheless, this regulation primarily occurs at the level of biosynthesis. The functioning of high molecular mass antioxidants, such as primary antioxidant enzymes that directly detoxify ROS and related enzymes that promote their action, is the subject of the majority of research. However, Superoxide dismutase (SOD, EC 1.11.1.11) which breaks down O_2•– into H_2O_2 and molecular oxygen, catalase (EC 1.11.1.6) which eliminates H_2O_2 and peroxidases such as glutathione-dependent peroxidase (GPx, EC 1.11.1.9) are examples of primary antioxidant enzymes [36].

3. METAL INDUCED OXIDATIVE STRESS IN FISHES:

Speciation of metals, their solubility and complication are the most important factors that influence the toxicity of metals in the aquatic environment. The amount of dissolved metal strongly depends on p^H of water. The interaction of metals can alter their toxic effects on aquatic organisms both positively and negatively [47]. Likewise, modes of exposure to metals also play a role in metal toxicity. Fishesusually take up metals through the gills, digestive tract and body surface [48] [49]. Various metal ions are known to induceoxidative stress in fishes [50]. Metals generally produce free radicals in the following ways:

a) Redox cycling: Redox active metals generate ROS through redox cycling (iron, copper, chromium and vanadium).
b) Antioxidant defences: Redox inactive metals or metals without redox potential impair antioxidant defences, especially those involving thiol-containing antioxidants and enzymes (mercury, nickel, lead, and cadmium) [51].
c) Fenton reaction: A third important mechanism of free radical production is the Fenton reaction, by which ferrous iron (II) is oxidized by hydrogen peroxide to ferric iron (III), a hydroxyl radical, and a hydroxyl anion [52].Copper, chromium, vanadium, titanium, cobalt and their complexes can all be reduced by the superoxide radical and contribute to the Fenton reaction [53].

Different strategies can be used by metals to cause oxidative stress depending on their chemical characteristics. Metal ions can be categorised into two types based on their ability to generate ROS: ions with a variable valence state and ions with a constant valence state. Ions with constant valence states have the potential to disrupt metabolic processes in general. Bivalent magnesium, strontium and barium ions, for instance, may have an impact on zinc and calcium-related activities, jeopardizing the latter ones, such the creation of energy or the control of gene expression. They have the ability to either replace Zn^{2+} or Ca^{2+} ions

in enzymes or regulatory proteins, or they can interfere with processes that depend on these ions. But in some proteins and enzymes, the substitution of ions with variable valence, such as iron and copper, is linked to more serious occurrences. Due to their significant biological significance and ability to promote the generation of ROS through their participation in the Haber-Weiss reaction (1), the ions of iron, copper, manganese and chromium are the most studied of this group. The following is often the mechanism that describes hydrogen peroxide cleavage caused by the acceptance of one electron from metal ions with variable valence:

$H_2O_2 + Men+ \rightarrow HO\bullet + OH^- + Me(n+1)+$……..(1) ,where Me is any metal ion with a variable valence.

Oxidized metal ions may be further reduced in a reaction with $O_2\bullet^-$ (or other electron donors) to form molecular oxygen according with equation:

$O_2\bullet^- + Me(n+1) + \rightarrow O_2 + Men+$ …….(2)

A combination of these reactions (1) and (2) gives the net Fenton reaction:

$H_2O_2 + O_2\bullet^- \rightarrow HO\bullet + OH^- + O_2$ ……... (3) [2] [18] [36] [50]

3.1. REDOX- ACTIVE METALS:

3.1.1. Iron (Fe):

Many physiological processes depend on iron, a metal whose homeostasis is tightly controlled by a number of systems. Because of its electronic structure, iron (ferrum) can drive one-electron reactions. This, along with the element's relatively high content in cells, makes iron one of the main components in biological systems. Fish absorb iron from water through the gill epithelium and through the intestinal tract from food. Thus, there is only a trace quantity of free iron available [52] [53]. Fe exists in three different oxidation states in living systems (II, III, and IV) [5]. Fe^{3+} precipitates as oxyhydroxide polymers under physiological conditions, notably at neutral p^H, while Fe^{2+} is still soluble. But in physiological solutions, Fe^{2+} is prone to oxidation, especially by molecular oxygen, which produces the superoxide anion [2] [18]. Xenobiotics liberate bound iron and allow it to create free radicals. Since the metabolism of superoxide and iron are linked, several chemicals that can produce superoxide radicals can also induce the oxidative potential of Fe [51]. Free iron is released more readily when superoxide anions are produced in greater quantities [5].

It is thought that teleost fish species' iron metabolism and function are comparable to those of other vertebrates. Numerous studies have examined the metabolism of iron in rainbow trout fed both a normal diet and one lacking in iron [55] [56]. African catfish (*Clarias gariepinus)*, reduced their growth when given a greater dietary iron ration over a period of five weeks, suggesting that the metal was present at hazardous but sub-lethal levels [57]. The diet had no effect on tissue iron concentrations, but the amount of malonic dialdehyde in the liver and heart increases in direct proportion to the amount of dietary iron. α-tocopherol (vitamin E) is a fat-soluble antioxidant that was markedly reduced in the liver of fish fed a high-iron diet at the same period. The dynamics of the latter two parameters show that the high iron content of meals is causing oxidative stress to develop [57] [58].In gold fish, lipid peroxide concentration decreases while the levels of protein carbonyl group increaseswhen the fishes are treated with 500mM ferrous sulphate in water for 7 days. A rise in TBARS concentrations in their liver and kidney is also observed at the same time. Variations in these oxidative damage indicators all point to the emergence of mild oxidative stress in goldfish tissues [59] [60].

In gold fishes, the activities of glutathione reductase in the kidney and catalase in the liver showed a high positive association with the levels of lipid peroxidation products, suggesting that these products may be up-regulating these enzymes. It was suggested that antioxidant mechanisms would respond cooperatively to mild oxidative stress brought on by elevated concentrations of ambient iron ion in goldfish tissues [58]. Lipid peroxide concentrations dropped while protein carbonyl groups, a sign of oxidative protein modification, increased in response to the treatment. Study on adult and embryonic medaka *Oryzias latipes* subjected to nano-iron also showed lipid peroxidation and changes in antioxidant enzyme activity in them [61]. Medaka embryos showed increased generation of malondialdehyde (MDA) and dose-dependent inhibition of SOD function. However, after being exposed to nano-iron, adult medaka's hepatic and cerebral SOD activity decreased at first but then increased with time. Thus, it is evident that medaka embryos are more vulnerable to nano-iron exposure than the adults as there has no evidence of iron-induced oxidative damage in adult fish [18] [50].

Despite the abundance of iron in the environment, it can be concluded that three mechanisms limit its toxicity to fish (mediated at least partially via Fenton chemistry):

i)Therapid auto-oxidation of iron (from Fe^{2+} to Fe^{3+}) and subsequent precipitation, which limits iron bioavailability;
ii)The presence of effective antioxidant mechanisms that prevent the development of negative effects; and
iii)The presence of iron sequestration mechanisms in the tissues that prevent iron from participating in ROS-mediated processes [36].

3.1.2. Copper (Cu):

Copper (cuprum) is an essential element for most living organisms. The possibility to exist in two valence states in Cu creates a $Cu^{2+/}Cu^{+}$ redox pair. This provides for the transfer of electrons in biological systems because of the high redox potential of this pair. Cupric ions ($Cu^{2+)}$ in the presence of biological reductants such as GSH or ascorbic acid can be reduced to cuprous ion (Cu^{+}), which is able to catalyze the formation of HO• during the decomposition of H_2O_2 via the Fenton reaction. Several studies have examined the effects of fish exposure to copper ions administered in waterborne, injected or dietary formats [36]. The physicochemical characteristics of water, such as p^H, alkalinity, suspended particles, organic component concentration and hardness affect copper's toxicity to fish and its bioavailability in the water [62]. Water acidity raises the concentration of free copper, or cupric ion (II). A number of investigations also indicate a high copper diet, waterborne exposure or even administration of copper could cause oxidative stress [36].
In the European eel, [63] found an increase in copper-associated LPO and DNA damage. Molecules containing thiols like glutathione are bound by copper. When $CuSO_4$ was administered to three-spined sticklebacks (*Gasterosteus aculeatus*), the liver showed an inhibition of total GSH. Enzymatic indicators like CAT, SOD and GPxfound to increase during the first week of exposure and then recovered, contemporaneous with Cu bioaccumulation in the liver, while GSH is being depleted. Metallothioneins may be involved in detoxification, as evidenced by the restoration of GSH and the return of antioxidant enzymes to baseline levels [64].
Similarly, the estuarine guppy (*Poecilia vivipara*) exhibited concentration-dependent development of oxidative stress in response to exposure to copper at ecologically relevant values of 5, 9 and 20 μg L^{-1}. There are conflicting findings about how salinity affects the way copper ions induce oxidative stress, and these findings may vary depending on the species under investigation [65]. However, the overall conclusions that can be made from their works are as follows:
i) Fish exposed to metal ions (especially Cu) exhibit increased activities of both primary (SOD, catalase, glutathione peroxidase) and secondary (glutathione reductase, metallothionein) enzymes/proteins;
ii) mRNA levels do not always match up with corresponding protein levels; and
iii) Metallothioneins are not always upregulated by the addition of metal ions like copper.
Treating rainbow trout gill cells with copper found to increased ROS production and cytotoxicity in a dose-dependent manner. It had no effect on the quantity of lipid peroxide but it dramatically enhanced DNA strand breakage [66]. On the other hand, zebrafishwhile exposed to copper found to increased protein carbonyl concentrations and superoxide dismutase activity whereas it suppressed the catalase activity and enhance the cytochrome c oxidase subunit 17 (COX-17) gene expressions [67].
Copper functions as a shield against oxidative damage brought on by various xenobiotics. Ceruloplasmin contributes to iron homeostasis and functions as an antioxidant in plasma via ferroxidase activity [68] [69] [70]. [68] also reported that fish serum ceruloplasmin activity is increased by Cu pre-exposure. Moreover, Cu has the ability to stimulate metallothionein production [71]. The liver of the freshwater snakehead*Channapunctatus* is protected against chronic copper exposure by oxidative defence mechanisms that include metallothionein production [72]. $CuCl_2$administration in gilthead sea-bream (*Sparusaurata*) found to increase TBARS concentrations [73]. Elevated waterborne Ca^{2+} and Na^{+} had protective effects on copper poisoning in zebra-fish (*Danio rerio*).Experiment hasalso showed that a higher salinity could lessen the severity of Cu-induced oxidative stress in adult killifish *(Fundulus heteroclitus)* [74]. In order to pinpoint molecular targets that may be altered to lessen the harmful consequences of exposure to this ion by the fishes, it is critical to investigate possible regulatory pathways that may mitigate Cu-induced oxidative damage [75].

3.1.3. Chromium (Cr):

The two most common valence states of chromium (Cr) in the environment are trivalent (Cr^{3+}) and hexavalent (Cr^{6+}). Trivalent chromium salts (such as chromium picolynate, chromium chloride, and niacin-bound chromium) are utilized as micronutrients and dietary supplements while hexavalent chromium compounds are also widely used in different fields. Like other metal ions, chromium simply changes forms in ecosystems and can be hazardous. Due to its variable valence (II, III, IV, V, and VI), this element can undergo a Haber-Weiss-type reaction that produces the •OH radical [76] [77] [52] [78].

Chromium may bioaccumulate either directly or indirectly in exposed organisms, which can have an impact on individual animals as well as the ecosystems as a whole. It is worth mentioning here that-

i) Chromium reduction in cells is required for the production of HO•, and
ii) Chromium's ability to undergo reversible oxidation may act as a catalyst.

Consequently, it is well acknowledged that the production of ROS is somewhat related to the biological effects of chromium [36].

Fish health is considerably harmed by high chromium concentrations. Observation on Cr^{6+} conversion in the liver tissue of the European eel (*Anguilla anguilla*) using electron paramagnetic resonance (EPR) [79] showed the presence of a significant amount of Cr^{5+}while $Cr^{6+}$is converted to Cr^{3+}. It is also noted thatdespite the development of antioxidant defences, lipid oxidative degradation persisted after exposure to Cr^{6+}. However, the observed cellular damage is not directly or predominantly caused by Cr^{5+}, a species with a limited life span. Moreover, studies conducted on rainbow trout (*O. mykiss*) exposed to Cr^{6+}revealed that *in vitro* testing using tissue homogenates are more sensitive than *in vivo* tests for identifying and assessing harmful effect of Cr^{6+} [80]. Hexavalent and trivalent Cr ions arealso found to induce oxidative stress in goldfish.

Detrimental effects of chromium on DNA in different fish specieshave also been observed by various workers. [82] reported on the chromium genotoxicity in the kidney and gills of the European eel *Anguilla anguilla*. Fathead minnows produced substantial amounts of DNA-protein crosslink (DPX) in their erythrocytes when exposed to 2 mg/L Cr^{6+}.Likewise, largemouth bass exposed to similar dose of chromium showed a 62% increase in DPX levels after four days of exposure. Moreover, DPX levels in largemouth bass erythrocytes significantly increased when the fish were fed with a diet consisting of minnows administered with 20 mM Cr^{6+} for five days. Thus, it is evident that erythrocytes of predatory fish species including bass may develop DPX as a result of exposure to Cr^{6+}. Similarly, Chinook salmon (*Oncorhynchus tshawytscha*) tissues showed evidence of DNA damage and an increase in LPO after long-term exposure to hexavalent chromium in water. DNA damage after exposure to Cr is alsonoted[83] [84]. Thus, chromium exposure triggered lipid peroxidation in the tissues of Chinook salmon (*O. tshawytscha*) [87].

Hexavalent chromium's genotoxicity and development of oxidative stress have been recently demonstrated in common carp (*Cyprinuscarpio*) [85]. Chromium toxicity in goldfish (*Carassius auratus*) hasalso reportedby [86] where the kidney appearing to be more susceptible to Cr-induced toxicity than the liver. The kidney showed increased lipid peroxidation as measured by thiobarbituric acid reactive substances (TBARS).

Cr^{6+} induced oxidative stress in goldfish tissues, including statistically significant increases in SOD and GPx activity along with metallothionein (MT) expressionhas also beenobserved by [86]. Their findings showed that 1 mM dichromate enhanced glutathione peroxidase activity and lowered glutathione (GSH) contents in gills of gold fish but had no effect on catalase or glutathione-transferase activities. One possible mechanism for increasing antioxidant capability in response to Cr^{6+} exposure in juvenile of gold fish was demonstrated to be up-regulation of genes encoding antioxidant enzymes [88]. Applying certain chemicals or combinations of them can sometimes provide insight into the processes underlying tolerance-boosting. For instance, [89] investigated whether antioxidants like taurine (TAU), N-acetylcysteine (NAC), curcumin (CUR), or alpha-lipoic acid (LA) could shield the liver and kidney tissues of common carp (*Cyprinus carpio carpio* L.) against chromium-induced toxicity in vivo. The creation of modest oxidative stress, which by definition is linked to an increase in the activity of first-line antioxidant enzymes, can explain these observations [90] [91]. N-acetylcysteine was an extremely powerful antioxidant, most likely because of its effects on GSH redox state and metal-reducing activity [89]. [92] found that administering propolis and Cr^{6+} concurrently improved the functional state of *C. carpio* parameters, indicating that propolis may be able to lessen oxidative damage caused by chromium. Propolis contains certain polyphenols that may function similarly to curcumin in stimulating the Nrf2/Keap1 signalling pathway, which in turn up-regulates the expression of antioxidant enzymes and counteracts the harmful effects of oxidative stress caused by chromium [36].

3.2. REDOX INACTIVE METALS:

3.2.1. Mercury (Hg):

Mercury is a cation and is found in two cationic states- Hg^{2+} and Hg^{1+}. Thus, there are two oxidation states for mercury: +2 (mercuric) and +1 (mercurons). Mercury may exist in the environment as methylmercury, which is mostly created when microorganisms in soil and water methylate inorganic (mercuric) forms of mercury [92]. Fishes, like other animals, may develop high tissue concentrations of mercury [93] [94]. High levels of mercury in freshwater fishes are linked to an elevated risk of acute myocardial infarction andcoronary heart disease in humans who consume such fishes and subsequently accumulate mercury in their bodies. Consequently, it need to be highlighted that even while there is logic to using fatty acids produced

from fish oil to lower the risk of acute coronary events, the protective impact of fish oil may be diminished by high mercury concentration [92].

Fishes are typically more sensitive to methyl mercury than they are to inorganic forms [95]. When mercury (Hg) combines with GSH's thiol groups, tissue may experience oxidative stress and GSH depletion [96]. At the location of the organic anion transporters, mercuric conjugates of glutathione and cysteine are transportable species. Due to the strong affinity of mercury for glutathione, the first can cause intracellular GSH pool depletion, which can lead to oxidative stress either directly or indirectly [97]. Studies shows mercury induces regional brain pathology and behaviourial alterations [98]. Methyl mercury greatly accumulated in the brains of Atlantic salmon (*Salmo salar* L. parr) while exposed to mercuric chloride for four months though neither death nor reduced growth was observed [99]. However, this led to a notable rise in lipid peroxidation products (measured as TBARS) and a fall in SOD and glutathione peroxidase activity. [100] have confirmed the neurotoxic effects of methyl mercury (MeHg) on zebrafish embryos. They opined that MeHg interfered with cellular homeostasis, which in turn affected CNS function. The disruptions impacted the expression of genes essential in normal neuronal activity, and they were at least partially linked to the generation of oxidative stress [2].

[102] examined the impact of the antioxidant β-carotene on the haematological parameters and antioxidant response of fish treated with mercury on Nile tilapia (*Oreochromis niloticus*). It's noteworthy to be mentioned here that β-carotene is effective as an immunostimulant, reducing the immune-depressing effects of mercury exposure in fish. Notably, a decrease in glutathione peroxidase and SOD activity while an increase in lipid peroxidation products (measured as TBARS) observed. It is important to note that the antioxidant enzyme SOD activated in the brain in response to modest dietary quantities of mercury, indicating the development of protective redox defenses [99].

The wolf fish's superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, and glutathione reductase activities are found to be increased by inorganic mercury obtained from the prey fish. In reaction to mercury exposure, metallothioneins (MT) also provide protection. The liver of common carp (*Cyprinus carpio*) from a mercury-contaminated river expressed two MT genes through messenger RNA. The tissue exhibited no biochemical indications of oxidative damage linked to these alterations. This implies that, in situations when there is elevated metal concentration and no indication of oxidative damage in fish tissue, quantitative examination of the MT genes' mRNA expression may be a useful biomarker of subtoxic metal exposure [103].

3.2.2. Cadmium (Cd):

Different industrial activities have been recognized as the major source of cadmium in the aquatic environment [104]. Cadmium is released into the atmosphere from the burning of domestic garbage, industrial emissions from mining, and the burning of coal to produce energy. Because cadmium particles can travel great distances in the air, even far from the source of the emissions, the soil and water may get poisoned. Water and soil contain cadmium, which is firmly bonded to other substances [105].

Although Cd does not directly cause ROS to be produced, it can change GSH levels and cell thiol status, which can cause the liver to express metallothioneins. The cell membrane's LPO may result from modifications in GSH and MTs. When cadmium enters the mitochondrial electron transport chain, unstable semi-ubiquinones build up, donate electrons and produce superoxide radicals.

Cadmium accumulation in aquatic animals has been documented in a number of studies [106] [107] particularly in the liver, spleen, gills and muscle tissue where they verified the cytotoxicity and oxidative stress caused by Cd in such animals. It has also observed that cadmium cause damage to the kidneys by way of releasing MT (metallothionein) into the cytoplasm of the proximal tubule cell where it causes oxidative stress, after being released from the cell by an endo-lysosome [108]. When cadmium enters the mitochondrial electron transport chain, unstable semi-ubiquinones accumulate, contribute electrons, and produce superoxide radicals. Additionally, cadmium has an impact on antioxidant enzymes, particularly SOD and CAT, and it can replace iron and copper in a variety of proteins, allowing those metals to take part in the Fenton reaction [109]. [110] showed decreased CAT activity in the kidney of the sea bass *Dicentrarchus labrax* after exposure to Cd. Such a decrease in CAT activity could be attributed to cadmium's direct binding to CAT [50]. Moreover, it is also evident that the detoxification of mercury is an organ-specific process, and metallothioneins play an important role in this process [111]. Numerous investigations have reported on the activation of de novo synthesis of MTs after exposure to Cd [112] [113]. The kind of fish exposed, the length of exposure and the chemical involved, all affect GSH levels differently when exposed to Cd. Depending on the field and experimental settings, GSH has been shown to rise and decrease [114] [115] [116].

3.2.3. Lead:

Lead (Pb) has been considered as a significant environmental contaminant. Food, air, water, and soil all contain the hazardous element lead [117] [118]. The accumulation of lead in sediment has implications for aquatic life as lead is not a transition metal and it cannot easily change valence. Lead has the potential to be hazardous to the nervous system; it can replace calcium in cells, impact mitochondria, induce apoptosis and excitoxicity, and change the dynamics of neurotransmitters [119].

Lead can cause oxidative damage in several ways, including direct effects on the cell membrane, interactions with GR, auto-oxidized δ-aminolevulinic acid, haemoglobin auto-oxidation, and complex formation with selenium that lowers GPx activity. Because of the unclear LPO induction shown in the kidney and the observed drop in MDA levels in the liver, it is suggested that lead is not a good inducer of LPO [109].A number of studies have showedthe occurrence of anaemia in a variety of species including brown trout (*Salmotrutta*) [120] common carp (*Cyprinus carpio*), European catfish (*Sylurusglanis*) [121] and tench (*Tincatinca*) after being exposed to Pb in water [122]. Studies on toadfish *Halobatrachus didactylus* for the effects of intraperitoneal administration of lead on the activity of aminolevulenic acid dehydratase (ALA-D), MT levels, and LPO in the liver, kidney, and blood over a course of seven days, reveals no significant changes in ALA-D due to exposure to lead [123]. However, [124] reported higher MDA levels in the brains of a catfish, *Clariasbatrachus* when exposed to waterborne lead for a period of 60 days. These findings imply that lead-induced oxidative stress is influenced by the mode and length of exposure to the metal [125].

Lead can cause oxidative damage through interactions with SOD (superoxide dismutase), methemoglobin, δ-aminolaevulinic acid, and direct effects on the cell membrane [97] [98]. [99]reported that lead induced auto-oxidation of haemoglobin, interactions with GR (glutathione reductase), by complexation with selenium, which lower GPx (glutathione peroxidase) activity, and lead induced a decrease in the concentration of GSH (reduced glutathione) and an increase in the concentrations of GSSG (oxidised glutathione), malondialdehyde, and lead induced auto-oxidation of haemoglobin [126].

3.2.4. Arsenic:

The most prevalent arsenic oxidation values are +5, +3, and −3. It has the ability to produce both organic and inorganic molecules in cells and the environment. Arsenite (As^{3+}) and arsenate (As^{5+}) are examples of inorganic arsenic. The inorganic arsenics can be either methylated in vivo (monomethylarsonic acid, MMA) or dimethylated (dimethylarsinic acid, DMA) [52]. Oxidation of biological components, including lipids, DNA, and proteins, may be caused by arsenic compounds that produce both ROSs and RNSs. In terms of biological activity, pentavalent arsenate is less active than trivalent arsenite. The redox state of cells that arsenic induces is significantly influenced by glutathione. When arsenate is reduced to arsenite, glutathione acts as an electron donor. Although the exact mechanisms are unknown, ROS are produced during the metabolism of arsenic cells. Arsenic-related oxidative damage also involves reactive nitrogen species [127]. Interestingly, under physiological conditions, the oxidation of As^{3+} to As^{5+} produces H_2O_2 [52].

$H_3AsO_3 + H_2O + O_2 \rightarrow H_3AsO_4 + H_2O_2$

It has been reported that ROS are involved in arsenite-induced apoptotic cell death since antioxidants, N-acetylcysteine and dithiothreitol, greatly reduced apoptosis in TF cells [128]. Fish contain safe organic forms of arsenic called arsenobetaine and arsenocholine. Arsenobetaine contains most of the total arsenic in fish tissue [129]. [130] reported on the metabolic toxicity of arsenite in *Channapunctatus.*Based on the findings of changes in levels of GSH, GSSG and LPO in the liver and kidneys of fishes exposed to arsenic for a period of 90 days suggests adaptive response of the fish species to arsenic exposure is found to be duration-dependent changes in GSH levels, with positive peaks at seven, thirty, and ninety days of exposure [130]. The course of LPO also revealed a similar trend. The catfish *Clariasbatrachus* subjected to nonlethal dosages of arsenic for 10 days showed the induction of LPO, an elevated GSSG/GSH ratio, and excess generation of hydrogen peroxide. The increased hydrogen peroxide content is mainly attributed to changes in peroxisomes brought on by arsenic [127]. [131] investigated the effects of lipid peroxidation and hepatic metalloprotein expression in channel catfish exposed to arsenite, arsenate and the herbicide monosodium methyl arsenate.Their findings shows hepatic lipid peroxidation and glutathione concentrations remained unchanged by all of the arsenical treatments while metallothionein levels increased in a dose-dependent manner. This indicates that oxidative stress and metallothionein expression are uncorrelated [36].

In addition, heat shock protein 70 (HSP70)- a chaperone protein, found to be activated by arsenite in a zebrafish liver cell line. However, cells are efficiently protected against arsenite insult by pretreatment with NAC or DTT [132]. The up-regulation of antioxidant enzymes and elevated levels of oxidative stress indicators were closely associated with neurological abnormalities. Furthermore, As_2O_3-exposed zebrafish

showed increased transcription factor Nrf2 mRNA levels, suggesting that Nrf2 may be involved in the protective response in these circumstances [133].

3.2.5. Nickel:

Nickel is considered to be an ultra-trace metal. Sometimes nickel (niccolum) pollutes the environment being widely used for different industrial purposes.Although compounds of Ni^{+} and Ni^{3+} are also well-known, the most prevalent oxidation state of nickel is Ni^{2+}. Nickel may participate in Fenton chemistry because of its ability to engage redox reactions. While single-strand break formation is thought to be caused by the production of hydroxyl radicals in solution, hydrogen peroxide and Ni^{2+} incubation of DNA is found to cause site-specific formation of double-strand breaks and, to a lesser extent, 8-OHdG and putative intra-strand cross-links. White muscle and liver tissues experienced very little oxidative stress from Ni^{2+} exposure, while the heart, brain, and gills are effectively shielded against the development of Ni^{2+} induced oxidative stress [134] [135]. When goldfish are exposed to waterborne Ni_2O, only the kidney and spleen showed signs of oxidative stress induction [136]. Exposure of goldfish to waterborne nickel caused apoptosis in the liver through the JNK pathway whereas similar exposures to rainbow trout resulted in both oxidative stress and neurotoxic effects with obvious histopathological abnormalities [137]. Studies on the neotropical fish *Prochiloduslineatus* also showed nickel ion-induced oxidative stress is found to be associated with genotoxic effects [138] [139].

3.2.6. Selenium (Se):

Selenium can build up to hazardous levels in aquatic environments. Mining coal from rocks rich in selenium and industrial discharges are the important sources of selenium and selenium derived form. Selenate is converted by plants into selenite and organo-selenide. As a necessary component, selenium contributes to antioxidant defences and functions as a cofactor for GPx. Selenite's protective properties against stress caused by heavy metals in rainbow trout was also reported [140] [141]. When selenium levels are high, fish may become poisonous. Se toxicity has been linked to a number of processes, one of which is ROS production [142].

3.2.7. Other metals:

Tributyltin and aluminium are two common pollutants. The induction of oxidative stress may also play some role in the mechanisms of their toxicity in fish, but more research is needed to explore this possibility. Other metals, besides the metals and metalloids mentioned above, are also connected with oxidative stress (vanadium and cobalt) [104] and can be detected in aquatic environments [143].

4. CONCLUSION

Metal-induced oxidative stress is a significant problem for aquatic life including fishes.As mentioned above, findings of the studies carried out on different fish taxa by various workers suggestmetal-induced oxidative stress in them. Different strategies can be used by metals to cause oxidative stress depending mainly on their chemical characteristics and mode of exposure.There are varying degrees ofoxidative damageto their tissuesthrough the generation of ROS (which caninteract with almost every component of a cell) when the fishes have been exposed to different contaminants in water including metals like chromium (Cr), lead (Pb), arsenic (Ar), mercury (Hg), cadmium (Cd), etc. However, it is also quite interesting to note that fishes and other aquatic life forms have been shown to have different mechanisms of adaptive response to oxidative stress.Defence mechanism against ROS is mainly attributed to the enzymes likeSOD, catalase and GPx. Different living organisms have different multi-layered and intricate antioxidant systems which work to either minimize the harmful effects of ROS or prevent the creation of ROS and ROS-modified molecules.By virtue of such mechanisms, fishes can limit the toxicity resulting from metal-induced oxidative stress. It is important to carry out studies on different aspects of operation of ROS metabolism systems and other associated mechanisms metal-induced oxidative stress in order to have a deeper comprehension of the mechanisms underlying metal toxicity in aquatic environments and their impact on different aquatic life forms including fishes.Certain metals can't be found in water because they are quickly bonded to biological materials but they can eventually turn up in fish meal. Thus, different fish species can be utilized as model organisms for various scientific studies for metal pollution in water bodies based on metal-induced oxidative stress,. Moreover, fishes may serve as potential bioindicators forpresence of hazardous metals in aquatic environment though further research is required in this aspect.

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