Environmental pollution of heavy metals as a cause of oxidative stress in fish: a review

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Abstract: This review summarizes the current information on the contribution of heavy metals as a result of pollution to the development of oxidative stress in fish. Metals are considered as important toxic pollutants and there is an extensive literature about their accumulation in aquatic ecosystems. Globally, there is now abundant evidence that anthropogenic activities have contaminated the environment with heavy metals from multiple sources. Heavy metals are important inducers of oxidative stress in aquatic animals, promoting formation of reactive oxygen species.Reactive oxygen species (ROS) are an unenviable part of aerobic life. The potential of oxygen free radicals and other reactive oxygen species (ROS) to damage tissues and cellular components, called oxidative stress, in biological systems has become a topic of concern for environmental pollution studies. Their steady-state concentration is a balance between production and elimination providing certain steady-state ROS level. The balance between prooxidant endogenous and exogenous factors (i.e., environmental pollutants) and antioxidant defenses (enzymatic and nonenzymatic) in biological systems can be used to assess the toxic effects under stressful environmental conditions, especially oxidative damage caused by various groups of chemical pollutants. Increased levels of reactive oxygen species lead to oxidative damage including lipid peroxidation, protein and DNA oxidation, and enzyme inactivation. The components of the antioxidant defence are used as a biochemical markers of oxidative stress. This knowledge extends to specific applications in fish because of their sensitivity to oxidative pollutants, their filtration capacity, and their potential for environmental toxicology studies.
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1.Introduction

Metals are considered very important and highly toxic pollutants in the various environmental departments. Inputs of metals to the environment as a result of anthropogenic activities is difficult to measure due to the huge inputs from the erosion or rocks, wind-blowing dusts, volcanic activity and forest fires. The world-wide emissions of metals to the atmosphere (thousands of tons per year) by natural sources is estimated as: Ni: 26, Pb: 19, Cu: 19, As: 7.8, Zn: 4, Cd: 1.0, Se: 0.4 , (tx103.yr-1). Whereas, from anthropogenic sources: Pb: 450, Zn: 320, Ni: 47, Cu: 56, As: 24, Cd: 7.5, Se: 1.1 (thousand t yr-1). It is obvious from these numbers that Pb, Zn, Ni and Cu are the most important metal pollutants from human activities (Clark et al., 1997).
Metals are classified into the essentials and non-essentials in classes A and B, and in a borderline class.2

A: Calcium (Ca), Magnesium (Mg), Manganese (Mn), Potassium (K), Sodium (Na), Strontium (Sr)
B: Cadmium (Cd), Copper (Cu), Mercury (Hg), Silver (Ag)
Borderline: Zinc (Zn), Lead (Pb), Iron (Fe), Chromium (Cr), Cobalt (Co) Nickel (Ni), Arsenic (As), Vanadium (V), Tin (Sn).

Ecotoxicologists and environmental scientists use the term “heavy metals” to refer to metals that have caused environmental pollution. The metals which have been studied extensively the last decades are: Cd, Hg, Zn, Cu, Ni, Cr, Pb, Co, V, Ti, Fe, Mn, Ag and Sn (Tin). Some metals that have received more attention are Hg, Cd, and Pb, because of their highly toxic properties and their effects on the environment and the living organisms.

The inputs of metals to the environment from anthropogenic activities is complicated to distinguish as there are very large natural inputs from the erosion, wind-blown dust, volcanic activity and forest fires. Atmospheric and river inputs, dredging spoil, direct discharges, industrial dumping and sewage sludge are some of the important contributors to metal pollution, which lead to the release of metals in the marine environment.

Some metals enter the sea from the atmosphere, e.g. natural inputs of metals, such as Aluminium (Al) in wind-blowing dust of rocks and shales, and mercury (Hg) from volcanic activity. Lead (Pb) inputs in the atmosphere from industrial and vehicular emissions are much greater than natural inputs. Few metals are deposited by gas exchange at the sea surface, by the fallout of particles (dry deposition) or are scavenged from the air column by

precipitation (rain) which is called wet deposition. Rivers are the main contributor of metals in the marine environment. The nature of metals depends on ore-bearing deposits in the catchment area and the discharge of human waste and discharges when the river passes through urban areas. Dredging of shipping channels produces large quantities of metal pollution. Much smaller quantities of metals are added to the sea by direct discharges of industrial and other waste and the dumping of sewage sludge Clark et al., 1997; Nieboer and Richardson, 1987; Depledge et al., 1998).

Metals, especially heavy metals, are important pollutants of aquatic environments worldwide. Metal pollution has increased with the technological progress of human society. Industry, mining, advanced agriculture, household waste, and motor traffic are all among the activities considered to be major sources of metal pollution. Metals can accumulate in aquatic organisms, including fish, and persist in water and sediments (Luoma and Rainbow, 2008). Fish are an important component of human nutrition, and those from polluted areas present a potential risk to human health. As fish occupy the top of the aquatic food chain, they are suitable bioindicators of metal contamination. Metals are well-known inducers of oxidative stress, and assessment of oxidative damage and antioxidant defences in fish can reflect metal contamination of the aquatic environment (Livingstone, 2003). Many metals are essential to living organisms but some of them are highly toxic or become toxic at high concentrations. Fe (haemoglobin), Cu (respiratory pigments), Zn enzymes), Co (Vitamin B_{12}), Mo and Mn (enzyme). Light metals such as Sodium (Na), Potassium (K) and Calcium (Ca) can play an important role in biological system. The transition metals like Fe, Cu, Co and Mn which are essential but may be toxic at high concentrations. Metals such as Hg, Pb, Sn, Ni, Se, Cr and As which are generally not required for metabolic activity and are toxic to living organisms at quite low concentrations (Forster and Whittmann, 1983; Meria. 1983).

The speciation of metals, their solubility and complexation, are important factors that influence the toxicity of metals in the aquatic environment. The amount of dissolved metal mainly depends on water pH. The interaction of metals can change their toxic effects on aquatic animals both positively and negatively (Jezierska and Witeska, 2001). Different ways of exposure to metals also play a role in metal toxicity. Fish take up metals through the gills, digestive tract and body surface (Tao et al., 2001; Kamunde et al., 2002).

Mostly, xenobiotics entering into the environment exert their effects through their ability to redox cycle (one electron reduction and oxidation reactions). This may be intended in order to gain maximum effect for the xenobiotics' purposes or may just be a by-product of their chemical structure. Negative side effects include the formation of reactive oxygen species with the ability to damage cellular molecules. These polluting compounds mostly retain these qualities in the aquatic environment as well, with the ability to cause oxidative stress in aquatic organisms. The physiological systems of many aquatic organisms, which are in place to metabolize these compounds and resulting byproducts, are evolutionarily similar to those in humans. Different metal ions are involved in oxidative stress in fish. This review concentrates on the most important and most studied metals (Fe, Cu, Cr, Hg and Pb) and metalloids (As, Se).

2. Reactive oxygen species, Oxidative stress and antioxidant defences

Oxidative stress is defined as a situation when steady-state ROS concentration is transiently or chronically enhanced, disturbing cellular metabolism and its regulation and damaging cellular constituents (Lushchak, 2011). The activation of oxidative manifestations leads to the response of antioxidants, activation of expression of genes encoding antioxidant enzymes, elevation of the concentration of ROS scavengers. Nevertheless, there are considerable gaps in our knowledge in response to oxidative stress, particularly in the feral animals. Indeed, in field studies, wide spectrum of inter-site differences (higher, equal or lower activities of various antioxidant enzymes with tissue peculiarities and disbalance) have been observed in polluted compared to clean areas reflecting both mild stress conditions of the location or strong oxidative damage. Different models of the aquatic animal response, therefore, need to be analyzed before conclusions can be drawn. In any case, the integrated approach with the appreciation of the balance between president manifestations and antioxidant defense (enzymatic and nonenzymatic) in biological systems needs to be a central point to assess the toxic effects under stressful environmental conditions. Oxidative stress is an unavoidable aspect of aerobic life. It is the result of an imbalance between the production of reactive oxygen species (ROS) and antioxidant defences in living organisms (Nishida, 2011). Reactive oxygen species are induced by substances such as transitional metal ions, pesticides, and petroleum pollutants (Slaninova et al., 2009; Lushchak, 2011). Free radicals are also produced by endogenous cellular sources during normal cell metabolism. Mitochondrial respiration is the main endogenous source of ROS. Elevated production of ROS can

cause oxidation of proteins and lipids, alterations in gene expression, and changes in cell redox status (Livingstone, 2003).

Fish, as all other aerobic organisms and their ancestors, use the reduction of molecular oxygen as the source of energy that will be stored in adenosine triphosphate (ATP) molecules. These will provide energy for the vast majority of cellular processes including biosynthetic reactions, motility and cell division. This reduction of molecular oxygen occurs in four steps in the mitochondrial electron transport chain, each of which results in the formation of an intermediate molecule, a free radical.

O_2 +e- → **O2•-** (superoxide radical)

O_2•- + e- + 2H+ + H_2O → **H_2O_2** (hydrogen peroxide)

H_2O_2 + e- + H+ → **OH•** (hydroxyl radical)

OH• + e- + H+ → H_2O

Other sources of ROS include irradiation, UV light, production of H_2O_2, NO and O_2•- by activated macrophages and phagocytes, metal catalyzed oxidation systems, air pollutants, and autooxidation of electron transport carriers (Stadtman and Levine, 2000). ROS have historically been thought only to cause tissue damage and disease, but researchers are now beginning to recognize the importance of ROS in maintaining normal cellular activity including inter- and intracellular signaling (Halliwell and Gutteridge 1999, Dröge 2002, Hensley and Floyd 2002).

Mechanisms of antioxidant defenses in fish include the enzyme system and low molecular weight antioxidants, similar to those in mammals, although the specific isoforms of enzymes in various fish species have not been well identified (Di Giulio and Meyer, 2008). Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-s-transferase (GST) are the main antioxidant enzymes and important indicators of oxidative stress. Reduced glutathione (GSH) and oxidized glutathione disulphide (GSSG) play a key role in non-enzymatic antioxidant defence. Metal-binding proteins such as ferritin, ceruloplasmin, and metallothioneins (MTs) have special functions in the detoxification of toxic metals, and also play a role in the metabolism and homeostasis of essential metals (Kelly et al., 1998).

Metallothioneins are low molecular weight proteins rich in cysteine residues that can bind various metals, including mercury, silver, copper, cadmium, lead, zinc, and cobalt, with varying affinities (Hamer, 1986). It has been reported that different fish species possess different isoforms of MTs (Smirnov et al., 2005). Metallothioneins are involved in the regulation of the essential metals copper and zinc and in the detoxification of non-essential metals (Amiard et al., 2006). Zn has an essential function in the activation of metal-regulated transcription factors which initiate expression of the MT genes (Roesijadi, 1996).

3. Mechanisms of metal-induced oxidative damage

The involvement of metals in oxidative damage is multi-faceted. In general, metals produces free radicals in two ways. Redox active metals such as iron, copper, chromium, and vanadium generate ROS through redox cycling. Metals without redox potential, such as mercury, nickel, lead, and cadmium, impair antioxidant defences, especially those involving thiol-containing antioxidants and enzymes (Stohs and Bagchi, 1995). 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 (Valko et al., 2005). The superoxide radical can reduce iron in its ferrous form. Copper, chromium, vanadium, titanium, cobalt, and their complexes can also be involved in the Fenton reaction (Lushchak, 2011).

Activation of redox-sensitive transcription factors such as AP-1, p53, and NF-κB is another mechanism by which metals can participate in producing oxidative stress. These transcription factors control the expression of protective genes which repair DNA and influence apoptosis, cell differentiation, and cell growth (Valko et al., 2005).

The depletion of antioxidant defenses and the changes in the activities of various antioxidant enzymes indicative of lipid and protein oxidation have been implicated in oxidative tissue damage. ROS are known to convert amino groups of proteins and thereby, change protein structure and function. An increase in the number of modified carbonyl groups correlates with protein damage caused by oxidative stress (Halliwell and Gutteridge, 1999; Hermes-Lima2004; Lushchak and Bagnyukova, 2006). Ulcerative dermal necrosis (UDN) seem to be quite capable of causing oxidative stress in the livers, muscle tissues, hearts, and spawn of brown trout (Kurhalyuk et al. 2009, 2010).

4. Metals

4.1. Redox – active metals

4.1.1. Iron (Fe):

Up to date, the metabolism of iron in eukaryotes is rather well studied (Aisen et al., 2001; Ponka, 1999). In fact, all studied organisms depend on iron for survival. The paradox of iron-related life is connected with the hazards of both, iron deficiency and iron overload, each with serious or even fatal consequences. It is considered that Fe metabolism and

its function in aquatic animals is similar to other ones. For example, fish obtain iron from water by uptake across the gill epithelium and by intestinal uptake from food (Bury et al., 2003). Fe metabolism in rainbow trout in normal and iron deficient diets has been studied in details (Walker and Fromm, 1976; Carriquiriborde et al., 2004). It is concluded now that Fe is essential element involved in many living processes. However, because it undergoes a redox cycle it is involved also in the initiation and propagation of free radical processes, but the information on free radical processes induced by this element in hydrobionts is limited.

Iron is an essential element required for many physiological functions, and its homeostasis are strictly regulated by various mechanisms. In living systems Fe present in three oxidation states (II, III, and IV). The majority of Fe in the organism is bound to haemoglobin, transferrin, ferritin, and iron-containing enzymes. Therefore, only a trace amount of free iron is present (Valko et al., 2005). Excessive uptake of iron or disturbances in its regulation can be toxic which is related to its ability to catalyze ROS formation via the Fenton reaction. Iron may also potentiate the toxicity of chemicals such as paraquat or 2,3,7,8-tetrachlorodibenzo-*p*-dioxine. Xenobiotics release bound iron and enable it to produce free radicals (Stohs and Bagchi, 1995). Different substances capable of producing superoxide radicals can induce the oxidative potential of Fe, since the metabolism of iron and the superoxide are interconnected. Increased production of superoxide anions increases the release of free iron (Emerit et al., 2001). The deleterious effects of iron include DNA damage, lipid peroxidation (LPO), and oxidation of proteins (Valko et al., 2005). The effects of waterborne Fe on free radical processes in goldfish *C. auratus* liver and kidney were studied in our laboratory (Bagnyukova et al., 2006). The treatment increased the levels of protein carbonyl groups, a marker of oxidative modification of proteins, but decreased the concentrations of lipid peroxides.

Lipid peroxidation and alterations in antioxidant enzyme activity in embryonic and adult medaka *Oryzias latipes* exposed to nano-iron was reported by Li et al. (2009). Dose-dependent inhibition of SOD activity and increased production of malondialdehyde (MDA) was observed in medaka embryos. Activity of hepatic and cerebral SOD in adult medaka was initially reduced following nano-iron exposure but subsequently increased with exposure time. There was no evidence of oxidative damage in adult fish; therefore, this study suggested that medaka embryos are more sensitive to nano-iron exposure than are adults. Also, according to Baker et al. (1997), an iron-enriched diet in the African catfish *Clarias gariepinus* induced LPO in the liver and heart.

Significant increases in SOD activity and higher levels of LPO were observed in erythrocytes of cichlid fish from a metal-contaminated river, with the highest levels in spring, when the concentration of iron in water was elevated (Ruas et al., 2008). Bagnyukova et al. (2006) observed increases in protein carbonyls, a marker of protein oxidation, in the goldfish *Carassius auratus* liver and kidney after waterborne ferrous sulphate exposure.

4. 1.2. Copper (Cu):

Copper homeostasis in aquatic animals entails on regulated uptake, transport and excretion similarly to mammals. However, there is some specificity of these processes in hydrobionts related with the possibility of branchial uptake along with intestinal. Both ways are efficient, but depend on many factors and are highly regulated processes (Kamunde et al., 2002). Cu toxicity to fish and its bioavailability in water vary with physicochemical properties of water, i.e., pH, alkalinity, suspended solids, organic compound content, and hardness (Di Giulio and Meyer, 2008). The concentration of free copper, cupric ion (II), increases with water acidity. Copper hydroxide predominates in water of pH 8.0 and higher (Tao et al., 2001). Calcium, as a contributor of water hardness, was shown to decrease the hazardous effects of Cu on the growth of Nile tilapia (Abdel-Tawwab et al., 2007).

The oxidative stress was demonstrated to be induced by both diet and waterborne exposure to high Cu, or even by copper injection. The oral Cu exposure of grey mullet *Chelon labrosus*, hepatic-tocopherol concentrations were 63% lower, while malondialdehyde increased by 304% when fish were fed a high Cu diet for 67 days, which along with other parameters led to conclude that the diet high in Cu could cause oxidative stress in this fish (Baker et al., 1998). In gilthead seabrams *Sparus aurata* injection with $CuCl_2$ increased the concentrations of thiobarbituric reactive substances and oxidized proteins (Pedrajas et al., 1995). The treatment also increased the specific activity of superoxide dismutase a The cellular toxicity of copper can be explained through its participation in the Fenton reaction. Cuprous (I) ion can catalyse the formation of hydroxyl radicals. Copper-induced oxidative damage can be augmented by various substances. Gravato et al. (2006) observed an increase in copper-associated LPO and DNA damage in the European eel *Anguilla anguilla* pre-exposed to β-naphthoflavone (BNF), a polynuclear aromatic hydrocarbon-like compound. This study suggests a synergistic relationship between Cu and BNF. β-naphthoflavone was shown to increase the activity of ethoxyresorufin-*O*-deethylase

in the liver, causing a reduction in Cu. This mechanism facilitates copper redox cycling, leading to enhanced levels of ROS. Hansen et al. (2006a,b) investigated the effects of water contamination on the activities and steady-state concentrations of mRNA of certain antioxidant enzymes in brown trout tissues.

Copper binds thiol-containing molecules such as glutathione. The inhibition of total GSH was observed in the livers of three-spined sticklebacks *Gasterosteus aculeatus* exposed to $CuSo_4$. Concurrent with the depletion of GSH, enzymatic biomarkers such as CAT, SOD, and GPx increased within the first week of exposure and then recovered, concomitant with Cu bioaccumulation in the liver. The recovery of GSH and a return of antioxidant enzymes to basal levels suggest that metallothioneins play a role in detoxification (Sanchez et al., 2005). Gravato et al. (2006) attribute the depletion of GSH to direct copper interference with and resulted in the appearance of two new Cu, Zn-SOD isoforms. The general conclusions, which may be drawn from their studies are: (i) fish exposure to metal ions, particularly to Cu, increase the activities of primary (SOD, catalase, glutathione peroxidase) and secondary (glutathione reductase, metallothionein) enzymes/proteins; (ii) the level of mRNA do not always correspond to the respective protein level and (iii) metallothioneins does not necessarily are up-regulated by the addition of metal ions like copper.

Copper treatment of rainbow trout gill cells resulted in a dose dependent elevation in cytotoxicity and enhanced ROS formation (Bopp et al., 2008). It significantly increased DNA strand breaks, but did not affect lipid peroxide level. The exposure of zebrafish to copper increased protein carbonyl concentrations, the activity of superoxide dismutase, while suppressing catalase activity and enhanced gene expression of cytochrome c oxidase subunit 17 (COX-17) (Craig et al., 2007)

Copper plays a protective role against oxidative damage caused by different xenobiotics. The antioxidant effects of ceruloplasmin and metallothioneins seems to be the mechanism by which Cu protects under these conditions (Pandey et al., 2001). Ceruloplasmin serves as a transport protein of copper in plasma. Parvez et al. (2003) reported that Cu pre-exposure increases the activity of ceruloplasmin in fish serum. Ceruloplasmin, through ferroxidase activity, is involved in iron homeostasis and acts as an antioxidant in plasma (Gutteridge, 1985; Luza and Speisky, 1996). Cu is able to induce the biosynthesis of metallothioneins (Roesijadi, 1996). Ahmad et al. (2000) reported that metallothionein induction plays a role in the oxidative defence against chronic copper exposure in the liver of a freshwater catfish *Channa punctatus*. Concurrent with the induction of MTs, the accumulation of Cu was observed in the liver. Also, subchronic Cu pre-exposure reduced LPO in the liver of endosulfan-exposed fish (Pandey et al., 2001). Parvez and Raisuddin (2006), meanwhile, observed that sublethal copper pre-exposure had effects on non-enzymatic antioxidants in the liver of fish exposed to deltamethrin.

4.1.3. Chromium (Cr):

Chromium compounds are used in ferrochrome production, electroplating, pigment production, and tanning. These industries, together with the burning of fossil fuels, and waste incineration are sources of chromium in air and water and chromium is ubiquitous in nature (WHO, 1988). Cr present in various forms of oxidation, ranging from +2 to +6, and its forms +3 and +6 are the most stable in the environment and biologically important. Being an element with changeable valence (II, III, IV, V and VI) it can enter Haber–Weiss-type reaction resulting in generation of •OH radical and at least several types of this reaction may be found in the literature (Halliwell and Gutteridge, 1989; Shi and Dalal, 1990; Valko et al., 2005; Lushchak 2008). Kubrak et al. (2010) compared the effects of hexavalent and trivalent ions in the goldfish; both ions were found to induce oxidative stress. Cr (IV) is known to be carcinogenic in humans (WHO, 1988), and harmful effects of chromium on DNA have been described in fish. Ahmad et al. (2006) described genotoxicity of chromium in the gill and kidney of the European eel *Anguilla anguilla*. DNA damage and an elevation of LPO were observed in the tissues of Chinook salmon *Oncorhynchus tshawytscha* during chronic exposure to hexavalent chromium in water. According to the authors, the accumulation of Cr (VI) in the kidney led to macroscopic and microscopic abnormalities and negatively affected fish growth and survival (Farag et al., 2006; Sevikova et al., 2011).

Kuykendall et al. (2006) also reported DNA damage following Cr exposure. The formation of DNA-protein crosslinks was observed in erythrocytes in the fathead minnow *Pimephales promelas* and largemouth bass *Micropterus salmoides* exposed to hexavalent Cr in water and in the diet (Sevikova et al., 2011).

Most results on Cr effects on living organisms have been reported in mammals and their effects on hydrobionts are very limited. However, the question cannot be ignored with aquatic animals. Cr has both beneficial and deleterious effects on organisms being essential trace element involved in the regulation of a broad array of biological processes, particularly in glucose metabolism. In experiments with guppies *Poecilia reticulata* Perez-Benito (2006) found that low concentrations (<10−4

M) of Cr^{6+} increased the maximum lifespan in both mails and females. The toxicity of chromate was substantially decreased by antioxidant d-mannitol. The latter might indicate the ROS involvement in describing the effects of chromium (Perez-Benito, 2006). The exposure to potassium dichromate clearly induced oxidative stress in gills and kidney of European eel *Anguilla anguilla* L. (Ahmad et al., 2006). In gills, 1m Mdichromate did not affect catalase and glutathione-Stransferase activities, but increased glutathione peroxidase activity and decreased glutathione (GSH) concentrations. Lipid peroxidation, assessed as thiobarbituric acid reactive substances (TBARS), was intensified in the kidney, but no other changes were found in this tissue. DNA integrity, evaluated as DNA strand breaks, was lower in both tissues of dichromate-treated animals (Ahmad et al., 2006).

Chromium exposure activated lipid peroxidation in tissues of Chinook salmon *O. tshawytscha* and high chromium concentrations significantly impaired fish health (Farag et al., 2006). The kidney was the target organ during chromium exposure—it had gross and microscopic lesions (e.g. necrosis of cells lining kidney tubules) and, levels of products of lipid peroxidation were elevated. These changes were associated with increased chromium concentrations in kidney, and reduced fish growth and survival. It has been proposed that accumulated chromium induced the lipid peroxidation pathway where fatty acid oxidation and DNA damage (expressed as chromosomal changes) occurred and caused cell death and tissue damage (Farag et al., 2006). These reports suggest that oxidative-induced alterations of DNA are the main effect of chromium in the studied fish species

4.2. Redox – inactive metals and metalloids

4.2.1. Cadmium (Cd):

Cadmium is a non-essential metal with no known biological function. The source of Cd in the aquatic environment is industrial activity (Stohs and Bagchi, 1995). Cd does not generate ROS directly, but can alter GSH levels and influence cell thiol status, inducing the expression of metallothioneins in the liver. The changes in GSH and MTs can lead to LPO of the cell membrane. Cd enters the electron transport chain in mitochondria, leading to accumulation of unstable semi ubiquinones which donate electrons and create superoxide radicals. Cd also affects antioxidant enzymes, especially SOD and CAT, and is able to displace Cu and Fe in various proteins, freeing these metals to then participate in the Fenton reaction (Ercal et al., 2001). Reduced CAT activity following Cd exposure has been reported by Romeo et al. (2000) in the kidney of the sea bass *Dicentrarchus labrax*. This decreased activity was explained by the authors as the direct binding of cadmium to CAT.

Metallothioneins play a major role in the detoxification of Cd, and this process is clearly organ-specific (De Smet et al., 2001). The induction of de novo synthesis of MTs following Cd exposure has been described in several studies (Jebali et al., 2006; Ghedira et al., 2010). According to De Smet et al. (2001), MT induction following intraperitoneal injection of Cd as described by Ghedira et al. (2010), is evidence of a genetic ability to synthesise MTs. Contradictory findings were reported from a field study conducted by Kovarova et al. (2009), where no significant correlation between cadmium liver content and MT concentration was observed.

The effects of Cd exposure on GSH levels vary with fish species, duration of exposure, and the chemical involved. The increase and decrease in GSH has been observed, depending on field and experimental conditions (Kovarova et al., 2009; Cao et al., 2010; Jia et al., 2011).

4.2.2. Mercury (Hg):

Mercury exists as a cation with an oxidation state +1 (mercurons) and +2 (mercuric). In the environment, Hg may be present in methylmercury form, produced mainly as the result of methylation of inorganic (mercuric) forms by microorganisms in soil and water (Valko et al., 2007). The biological effects of inorganic or organic Hg are related to their interaction with sulfhydryl-containing residues (Rooney, 2007). Mercuric conjugates of cysteine and glutathione are transportable species at the site of the organic anion transporters. Because of the high affinity of Hg to glutathione, the first can deplete intracellular GSH pool and directly or indirectly cause intracellular GSH pool and directly or indirectly cause oxidative stress (Sevikova et al., 2011).

Hg is an important pollutant of water worldwide. A variety of human activities are connected with mercury pollution (silver and gold mining, coal combustion, dental amalgams) (Luoma and Rainbow, 2008). Organic methylmercury and inorganic (mercurous, mercuric) forms exist in nature. Organic forms are the result of methylation of inorganic mercury by microorganisms in sediments and water. Methyl mercury is generally more toxic to fish than the inorganic forms (Houserova et al., 2006). Hg reacts with the thiol groups of GSH, which can induce GSH depletion and oxidative stress in tissue (Stohs and Bagchi, 1995).

Fish as other animals may accumulate higher concentration of Hg (Salonen et al., 1995; Guallar et al., 2002). In experiments with Atlantic salmon *Salmo salar* exposed for four months to mercuric chloride, methyl mercury was accumulated significantly in brain and did not cause mortality or

growth reduction (Berntssen et al., 2003). But it significantly increased levels of lipid peroxidation products (evaluated as TBARS) and decreased the activities of SOD and glutathione peroxidase. Comparing with other organs, the brain was particularly susceptible to dietary mercury, while kidney and liver were less sensitive. It should be also noted that low dietary concentrations of mercury induced protective redoxdefenses in the brain evidenced by the induction of antioxidant enzyme SOD (Berntssen et al., 2003). Monteiro et al. (2010) explained the changes in biomarkers of oxidative stress following exposure to inorganic mercury. Methylmercury was shown to induce oxidative stress in several fields (Larose et al., 2008; Mieiro et al., 2010). The data presented in these studies suggest that both organic and inorganic forms of mercury participate in the formation of ROS.

Metallothioneins also play a protective role in response to mercury exposure. The mRNA expression of two MT genes was noted by Navarro et al. (2009) in the liver of feral carp Cyprinus carpio from a mercury-contaminated river. No biochemical evidence of oxidative damage associated with these changes was found in the tissue. This suggests that quantitative analysis of the mRNA expression of MT genes can be a suitable biomarker of subtoxic metal exposure in cases of elevated levels of metals and no evidence of oxidative damage in fish tissue. No significant correlations between the total mercury content and MT levels were described by Mieiro et al. (2011) in different fish tissues from a mercury-contaminated area.

The induction of MTs in the liver, gill, and heart of the tropical freshwater fish Brycon amazonicus was measured by Monteiro et al. (2010) following a 96 h exposure to inorganic mercury. Significant alterations in the expression of the antioxidant enzymes SOD, CAT, GST, GPx, and GR were observed, leading to oxidation of lipids and proteins. Induction of the SOD-CAT systems represents a rapid adaptive response to mercury exposure. As mentioned, mercury influences the GSH concentration. In this study, an increase in GSH content was observed without changes in GSSG levels in the liver and gill. The authors explained this as enhanced hepatic uptake of amino acid substrates and activity of biosynthetic enzymes leading to the protection of the fish from oxidative damage. Other authors have also observed increases in GSH levels following mercury exposure (Rana et al.,1995; Elia et al., 2000). Depletion of GSH was reported by Elia et al. (2003) and Mieiro et al. (2010). Metal-induced decreases in GSH levels could be the result of direct binding of the metal to GSH through its SH group (formation of metal-SG complexes) or of enhanced oxidation of this thiol (Elia et al., 2003).

4.2.3. Lead (Pb):

Lead is a major environmental pollutant. Paint, cosmetics, human medicines, food supplements, and petroleum-based fuels are sources of lead pollution (Stohs and Bagchi, 1995). Pb accumulation in sediment is of significance for aquatic organisms. Pb is not a transition metal and cannot readily undergo valence changes. Pb can induce oxidative damage through direct effects on the cell membrane, interactions between lead and haemoglobin, which increase the auto-oxidation of haemoglobin, auto-oxidized δ-aminolevulinic acid, interactions with GR, or through the formation of complexes with selenium, which decrease GPx activity (Ercal et al., 2001).

An intraperitoneal injection of lead was administered to the toadfish Halobatrachus didactylus and its effects on aminolevulenic acid dehydratase (ALA-D) activity, MT levels, and LPO in the liver, kidney, and blood were investigated over seven days (Campana et al., 2003). The results showed an increase in MT levels, suggesting that lead can induce the synthesis of MTs, although to a lesser degree than some other metals. The authors proposed that lead is not a good inducer of LPO, because a decrease in MDA levels was measured in the liver, and the induction of LPO observed in the kidney was ambiguous. No significant variations in ALA-D as a result of lead exposure were reported. Maiti et al. (2010) described elevated MDA levels in the brains of walking catfish Clarias batrachus following a 60-day exposure to waterborne lead. These results suggest that the manner and duration of exposure are important factors in lead-induced oxidative stress.

4.2.4. Arsenic (As):

The most common oxidation numbers of arsenic are +5, +3 and −3. It can form both inorganic and organic compounds in the environment and cells. Inorganic arsenic includes arsenite (As^{3+}) and arsenate (As^{5+}). The inorganic arsenics can be either methylated (monomethylarsonic acid, MMA), or dimethylated (dimethylarsinic acid—DMA) in vivo (Valko et al., 2005).

Several ROS and RNS are knownto be involved into generation of dimethylarsinic peroxyl ($(CH_3)_2AsOO$) and dimethylarsinic ($(CH_3)_2As$) radicals as well as some intermediary arsine reactive species (Rin et al., 1995). It is interesting to note that the oxidation of As^{3+} to As^{5+} under physiological condition results in H_2O_2 formation (Valko et al., 2005):

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

Therefore, arsenic compounds generating both ROSs and RNSs may be involved in oxidation of

cellular components particularly lipids, DNA and proteins. In two cell lines, TF (fin cells of *Therapon jarbua*) and TO^{-2} cells (ovary cells of tilapia) treated with sodium arsenite time- and concentration-dependent line-specific cell death was found (Wang et al., 2004). The DNA-fragmentation analysis and the flow cytometric analysis of cell cycle progression demonstrated clearly that most cells were killed via apoptosis. Since antioxidants, N-acetylcysteine and dithiothreitol, significantly prevented apoptosis in TF cells, it was concluded that ROS were involved in arsenite-induced apoptotic cell death (Wang et al., 2004).

The central role of GSH in arsenic toxicity was described in several studies. Allen et al. (2004) describing the biochemical toxicity of arsenite in *Channa punctatus*. Levels of GSH, GSSG, and LPO in the liver and kidney were measured during 90 days of exposure. The authors reported duration-dependent changes in GSH levels, with positive peaks at seven, 30, and 90 days of exposure, as an adaptive response of fish to arsenic. The progression of LPO showed a similar pattern.

The induction of LPO, an increased GSSG/GSH ratio, and excess production of hydrogen peroxide were observed in the Indian catfish *Clarias batrachus* exposed to nonlethal doses of arsenic for 10 days (Bhattacharya and Bhattacharya, 2007). The authors explained the elevated concentration of hydrogen peroxide as arsenic-induced alterations of peroxisome.

Arsenobetaine and arsenocholine are non-toxic organic forms of arsenic present in fish. According to Ciardullo et al. (2010), the majority of total arsenic in fish tissue is present as arsenobetaine. This is similar to the conclusions of Harkabusova et al. (2009). The data presented suggest that an assessment of arsenic speciation needs to be included in studies dealing with arsenic pollution, especially in field conditions, taking into consideration the risk to humans of fish consumption.

4.2.5. Selenium (Se):

elenium as an essential element plays a role in antioxidant defences and is a cofactor for GPx. Preventive effects of selenite on heavy metal-induced stress in rainbow trout Oncorhynchus mykiss were described by Ates et al. (2008) and Orun et al. (2008). Selenium can be toxic to fish at high doses. A source of selenium is coal mining from Se-rich rocks; selenate is the main form of Se originated from industrial discharges and selenium accumulates to toxic levels in the aquatic environment. Plants transform selenate to selenite and organo-selenide. Various mechanisms have been suggested for Se toxicity, one of which is the generation of ROS (Miller et al., 2007).

4.2.6. Other metals

Apart from the above mentioned metals and metalloids, other metals are also connected with oxidative stress (nickel, vanadium, and cobalt) (Stohs and Bagchi, 1995) and can be detected in aquatic environments (Kandemir et al., 2010). Tributyltin and aluminium are widespread pollutants. The induction of oxidative stress may also play some role in the mechanisms of their toxicity in fish, but more studies need to be performed to explore this possibility (Wang et al., 2006; Garcia-Medina et al., 2010; Ternjej et al., 2010).

5. Conclusion

The above mentioned research findings suggests that oxidative stress induced by metals is an important issue in aquatic ecosystems. The response of fish to oxidative damage after acute and also chronic metal exposure is evident under laboratory conditions as well as in field studies.

The metabolism of ROS in aquatic animals possesses, in fact, all characteristics found in other groups, because in this review I focused on ectotherms, the generation of ROS. The increase in environmental oxygen level causes oxidative stress in this group, and although unexpectedly, hypoxia also was among inducers of this state. Many xenobiotics including metal ions also were found to be inducers of oxidative stress. Therefore, it can be concluded that virtually all strong enough stresses are accompanied by oxidative stress. Interestingly, even principal mechanisms of adaptive response to oxidative stress were found in aquatic animals which makes them not only important group to study, but may help to clarify some general principles of operation of systems of ROS metabolism.

Frequently, aquatic contamination involves various chemicals that interact with one another. For that reason studies of metal-metal interactions are required. According to the above mentioned studies selenium pre-exposure reduces oxidative damage caused by lead, copper, cadmium and chromium. On the other hand, metal-induced oxidative damage can be augmented by various substances. A synergistic relationship has been described between β-naphthoflavone and copper and also BNF and chromium. Copper reduces the toxicity of delthametrin and endosulfan and calcium has a protective role against copper toxicity. Laboratory studies dealing with multiple metal interactions should be performed to enable a better understanding of mechanisms of metal toxicity in the aquatic environment. Properties of water play an important role in metal solubility. Organic substances in the water influence the availability of metal to fish and reduce metal toxicity. Some metals are rapidly bound

to organic substances and thus cannot be detected in water; however, they can later become accessible in fish food. It should be borne in mind that fish of different species, sex, size, and age are involved in field studies. Another factor that can influence a representative sampling is fish migration. Therefore, field studies should be designed in such a way as to take into account the complexities of the aquatic environment.

The quality of water may be a key determinant for future development of hydrobiology. Biological diversity and the physiologicalstate are direct indices of water quality. The free radical approach is one of the most commonly used to develop markers of environmental pollution and in addition, it can be used in model experiments to evaluate effects of natural and polluted waters, as well as potential and virtual pollutants.

Fish can be used as bioindicators of metals in the environment by studying the induction of oxidative stress; however, the specific forms of biomarkers and mechanisms of their action still need to be investigated.

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