# Oxidative Stress in Fish induced by Environmental Pollutants

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**Abstract**
Environmental pollutants represent a risk factor for human and animals in all areas of occurrence. Environmental pollution caused by anthropogenic activities is a major problem in many countries. Numbers of studies deals with cumulation of xenobiotics in tissues but not all respond to the real impact on living organisms. Freshwater fishes are exposed to several anthropogenic contaminants. The most commonly studied are three metals: mercury (Hg), lead (Pb), cadmium (Cd). These contaminants could have several impacts to oxidative stress. In the normal healthy cell, ROS and pro-oxidant products are detoxified by antioxidant defences. Redox-active or Redox-inactive metals may cause an increase in production of reactive oxygen species (ROS). Mercury has a high affinity for thiol groups, and can non-specifically affect several enzymes, e. g. GSH (glutathione), which can induce GSH depletion and oxidative stress in tissue, also can induce lipid peroxidation, and mitochondrial dysfunction. The toxicity of Cd to aquatic species depends on speciation, with the free ion, $Cd^{2+}$ concentration being proportional to bioavailability. Cadmium toxicity worsened of Ca, Na, and Mg ions homeostasis. Lead can be toxic to nervous and skeletal systems; at cellular level can cause apoptosis, also can affect mitochondria, neurotransmitters, and can substitute for Ca.

**Keywords**: heavy metals, oxidative stress, pollutants

## 1. Introduction

Environmental pollutants represent a risk factor for human and animals in all areas of occurrence. Nowadays the most important and the most studied pollutants are heavy metals [1, 2], pharmaceuticals [3-5] and endocrine disruptors [6, 7]. Environmental pollution caused by anthropogenic activities is a major problem in many countries [8, 9]. The main concern of recent studies is essentially a consumer protection [10]. Numbers of studies deals with cumulation of xenobiotics in tissues [11] but not all respond to the real impact on living organisms (health status, oxidative stress). Probably the best models for xenobiotic research are aquatic animals (fishes), due to their continuous contact with contaminated environment.

There are several chemical, physical, and biological factors that influence the toxicity of chemicals to fish, including the properties of the chemical in water, the water quality conditions, the route of exposure, and the species and life stage of the fish being tested [12]. Freshwater fishes are exposed to several anthropogenic contaminants. The most commonly studied and tested are mainly three metals: mercury (Hg), lead (Pb), cadmium (Cd). These contaminants could have several impacts to oxidative stress. In the last years, there has been meaningful interest in the pollutants effect to “reactive oxygen species” (ROS).

I review the literature on oxidative stress effects of certain heavy metals (Hg, Pb, Cd). The review focuses on freshwater fishes.

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## 2. Oxidative stress

In the normal healthy cell, ROS and pro-oxidant products are detoxified by antioxidant defenses [13]. Redox-active or Redox-inactive metals may cause an increase in production of reactive oxygen species (ROS) such as hydroxyl radical, superoxide radical or hydrogen peroxide. Increased generation of ROS can exceed cells antioxidant defenses, result is known as "oxidative stress". Cells under oxidative stress showed various dysfunctions due to damage caused by ROS to lipids, proteins, and DNA [14].

Metals play importance role in the biochemistry of reactive oxygen species (influence enzyme systems). Superoxide dismutase, catalase, and glutathione peroxidase are the main enzymes that have role in ROS biochemistry (indicators of oxidative stress) [15]. Higher production of ROS can affect oxidation of proteins and lipids, alterations in gene expression, and changes in cell redox status [13]. Reduced glutathione (GSH) and oxidized glutathione disulphide (GSSG) play a key role in non-enzymatic antioxidant defense [16].

Metallothionein (MT) is a family of cysteine-rich, low molecular weight proteins. MTs have the capacity to bind both physiological (such as zinc, copper, selenium) and xenobiotic (such as cadmium, mercury, silver, arsenic) heavy metals through the thiol group of its cysteine residues, which represent nearly 30% of its constituent amino acid residues [17].

## 3. Metal-induced Oxidative Damage

Redox active metals (Fe, Cu, Cr, V) produced ROS through redox cycling. Metals without redox potential (Hg, Pb, Cd, Ni) disrupt antioxidant defenses, concretely thiol-containing antioxidants and enzymes [18].

Free radicals are molecules or molecular fragments containing one or more unpaired electrons. This unpaired electron(s) usually gives a considerable degree of reactivity to the free radical [19]. The most interesting radicals in living systems derived from oxygen [20]. Mechanism of free radical production explains the Fenton reaction [21]:

$$H_2O_2 + Fe^{2+} + H^+ \rightarrow HO^- + Fe^{3+} + H_2O$$

Hydroxyl radicals are cytotoxic and can be involved in the production of further active oxygen species such as singlet oxygen [21].

## 4. Heavy metals

### 4.1. Mercury (Hg)

Mercury exists naturally in the environment and exists in several forms. Mercury is a constituent element of the earth. In pure form, it is known alternatively as elemental (liquid form at room temperature) or metallic mercury. Mercury is rarely found in nature as the pure, liquid metal, but rather within compounds and inorganic salts. Mercury can exist as a cation in two oxidation states, monovalent (mercurous) or divalent (mercuric) mercury, and can also covalently bind to carbon (organomercuric compounds) [15].

Mercury is an extended pollutant of freshwater worldwide. There are three main forms of Hg in the aquatic environment, inorganic $Hg^o$, $Hg^{2+}$ [Hg(II)], and organic $CH_3Hg^+$ [methylmercury or MeHg(I)], with MeHg(I) being of particular concern because of its potent neurotoxicity. Waterborne or dietborne exposures of fish to Hg(II) and MeHg(I) affect their growth, development, and reproduction, even in remote ecosystems [22].

Oxidative stress/damage following exposure to mercury was shown in several studies [23, 24]. Mercury has a high affinity for thiol groups, and can nonspecifically affect several enzymes [15], e. g. GSH (glutathione), which can induce GSH depletion and oxidative stress in tissue [25], also can induce lipid peroxidation, and mitochondrial dysfunction [15]. Organic and inorganic forms of mercury participate in the formation of ROS (reactive oxygen species) [16].

Acute aqueous exposures to Hg(II) can affect the expression of antioxidant enzymes or non-enzymatic scavenger molecules [22]. Monteiro et al. [26] exposed for 96 h at 150 mg/L of Hg(II) and showed significant increase superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione peroxidase (GSH-Px), glutathione reductase (GR), glutathione (GSH) and metallothionein (MT).

4.2. Lead (Pb)

Lead is toxic element found in food, air, water [27] and soil [28]. Lead can be toxic to nervous system; at cellular level can cause apoptosis and excitoxicity, affect mitochondria, alter neurotransmitter dynamics, and can also substitute for calcium [15].
Lead intoxication has multiple hematological effects, including lead-induced anemia where red blood cells are microcytic and hypochromic as seen in iron deficiency [29]. Numerous studies observed hematological parameters demonstrated anemia in a variety of fish following waterborne Pb exposure, including brown trout (*Salmo trutta*) [30], common carp (*Cyprinus carpio*) and European catfish *(Sylurus glanis*) [31], and tench (*Tinca tinca*) [32]. Lead can induce oxidative damage by direct effects on the cell membrane [16], interactions between lead and δ-aminolaevulinic acid, SOD (superoxide dismutase), and methemoglobin [33]. Ercal et al. [14, 34] described lead induced a decrease in the concentration of GSH (reduced glutathione) and an increase in the concentrations of GSSG (oxidized glutathione), malondialdehyde, and lead induced auto-oxidation of hemoglobin, interactions with GR (glutathione reductase), by complexation with selenium, which decrease GPx (glutathione peroxidase) activity.

4.3. Cadmium (Cd)

In the environment, cadmium is present in air due to incineration of household wastes, through emission from industry (mining), and from energy production based on coal combustion. Cadmium particles can be transported in air long distances and thus the ground and water could be contaminated far from the emission source. Cadmium remains in the soil and water strongly bound to other compounds [35].
The toxicity of Cd to aquatic species depends on speciation, with the free ion, $Cd^{2+}$ concentration being proportional to bioavailability. Toxicity is reduced by complexation of $Cd^{2+}$ by inorganic and organic anions and through competitive interactions between $Ca^{2+}$ and $Cd^{2+}$. Cadmium toxicity worsened of Ca, Na, and Mg ions homeostasis [36].
Numerous studies describe cumulation of cadmium in aquatic animals, especially in tissue liver, spleen, gills, and muscle [37, 38]. They confirmed Cd-induced oxidative stress and cytotoxicity. Cadmium also induced kidney damage; inside the proximal tubule cell is dissociated from MT (metallothionein) by endo-lysosome and is released into cytosol where it induces oxidative stress [15].
Verbost et al. [39] showed that $Cd^{2+}$ had a high affinity for $Ca^{2+}$ binding sites on the ATPase and that there was a direct competition between $Ca^{2+}$ and $Cd^{2+}$. Cd exposure can be also associated with disruption of Na ions (acute toxicity) [36], inhibited $Na^{+}/K^{+}$-ATPase activity and influenced liver catalase [40].

## 5. Conclusions

Oxidative stress is an imbalance between the production of oxidants and antioxidants. The increased concentration of oxidants, i. e. reactive oxygen species causing oxidative damage in cells and can lead to various diseases. Antioxidant enzymes protect the organism against the harmful effects of free radicals. Increased levels of the enzymes activity indicate the oxidative stress.
The involvement of heavy metals in oxidative stress was confirmed, especially in aquatic organisms. Published studies described effect of metals to increase superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione peroxidase (GSH-Px), glutathione reductase (GR), glutathione (GSH) and metallothionein (MT).
For this reason, fishes can be used as model animal and/or bioindicator of oxidative stress induced by environmental pollutants, especially heavy metals. The future research (*in vitro* and *in vivo*) about mechanisms and impact of environmental pollution to oxidative damage is still need to be investigated.

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