

Metals as a cause of oxidative stress in fish: a review

M. SEVCIKOVA, H. MODRA, A. SLANINOVA, Z. SVOBODOVA

Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

ABSTRACT: This review summarizes the current knowledge on the contribution of metals to the development of oxidative stress in fish. Metals are important inducers of oxidative stress in aquatic organisms, promoting formation of reactive oxygen species through two mechanisms. Redox active metals generate reactive oxygen species through redox cycling, while metals without redox potential impair antioxidant defences, especially that of thiol-containing antioxidants and enzymes. Elevated levels of reactive oxygen species lead to oxidative damage including lipid peroxidation, protein and DNA oxidation, and enzyme inactivation. Antioxidant defences include the enzyme system and low molecular weight antioxidants. Metal-binding proteins, such as ferritin, ceruloplasmin and metallothioneins, have special functions in the detoxification of toxic metals and also play a role in the metabolism and homeostasis of essential metals. Recent studies of metallothioneins as biomarkers indicate that quantitative analysis of mRNA expression of metallothionein genes can be appropriate in cases with elevated levels of metals and no evidence of oxidative damage in fish tissue. Components of the antioxidant defence are used as biochemical markers of oxidative stress. These markers may be manifested differently in the field than in results found in laboratory studies. A complex approach should be taken in field studies of metal contamination of the aquatic environment.

Keywords: ROS; metallothioneins; glutathion; superoxide dismutase; antioxidant defence

List of abbreviations

ALA-D = aminolevulinic acid dehydratase; **BNF** = β -naphthoflavone; **CAT** = catalase; **GPx** = glutathione peroxidase; **GR** = glutathione reductase; **GSH** = glutathione; **GSSG** = glutathione disulphide; **GST** = glutathione S-transferase; **LPO** = lipid peroxidation; **MDA** = malondialdehyde; **MTs** = metallothioneins; **NADPH** = nicotinamidadeninedinucleotide phosphate (reduced); **ROS** = reactive oxygen species; **SOD** = superoxide dismutase

Contents

1. Introduction	4.2. Redox – inactive metals and metalloids
2. Oxidative stress and antioxidant defences	4.2.1. Cadmium
3. Mechanisms of metal-induced oxidative damage	4.2.2. Mercury
4. Metals involved in oxidative stress in fish	4.2.3. Lead
4.1. Redox – active metals	4.2.4. Arsenic
4.1.1. Iron	4.2.5. Selenium
4.1.2. Copper	4.2.6. Other metals
4.1.3. Chromium	5. Conclusions
	6. References

1. Introduction

Metals, especially heavy metals, are important contaminants of aquatic environments worldwide. Metal pollution has increased with the technologi-

cal 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,

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Grant No. MSM 6215712402).

and persist in water and sediments (Luoma and Rainbow, 2008a). Fish are an important component of human nutrition, and those from contaminated sites present a potential risk to human health. Since 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).

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 strongly depends on water pH. The interaction of metals can alter their toxic effects on aquatic organisms both positively and negatively (Jeziarska and Witeska, 2001). Different modes 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).

Various 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. Oxidative stress and antioxidant defences

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).

Mechanisms of antioxidant defences 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). Zinc 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 produce 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 to 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- κ B 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).

4. Metals involved in oxidative stress in fish

4.1. Redox – active metals

4.1.1. Iron

Iron is an essential element required for many physiological functions, and its homeostasis is strictly regulated by various mechanisms. In biological systems iron exists in three oxidation states (II, III, and IV). The majority of iron 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). Various substances capable of producing superoxide radicals can induce the oxidative potential of iron, since the metabolism of iron and the superoxide are interconnected. Elevated 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).

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

Copper plays an essential function in a variety of metabolic processes. It is a component of many enzymatic and structural proteins, including Cu-Zn SOD, cytochrome oxidase, and ceruloplasmin. Copper occurs naturally in soil and water. Mining, industrial discharges, and copper-based pesticides, especially algacides, are sources of water contamination (WHO, 1998). Copper 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 reduce the harmful effects of copper on the growth of Nile tilapia (Abdel-Tawwab et al., 2007).

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 copper and BNF. β -naphthoflavone was shown to increase the activity of ethoxyresorufin-O-deethylase in liver, causing a reduction in copper. This mechanism facilitates copper redox cycling, leading to enhanced levels of ROS.

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 copper sulphate. 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 copper accumulation 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

GSH synthesis, inhibition of glutathione reductase, and the participation of GSH as a substrate in detoxification reactions. The depletion of GSH in fish muscle after copper sulphate exposure has also been reported (Jena et al., 2009).

Copper plays a protective role against oxidative damage caused by variety of xenobiotics. The antioxidant effects of ceruloplasmin and metallothioneins seems to be the mechanism by which copper protects under these conditions (Pandey et al., 2001). Ceruloplasmin serves as a transport protein of copper in plasma. Parvez et al. (2003) reported that copper 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). Copper 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*. This study described induction of metallothionein biosynthesis after a 60-day exposure to paper mill effluent containing copper. Concurrent with the induction of MTs, the accumulation of copper was observed in the liver. Also, subchronic copper 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 livers of fish exposed to deltamethrin.

4.1.3. Chromium

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).

The most biologically important oxidative states of chromium are trivalent (Cr III) and hexavalent (Cr VI). The trivalent and hexavalent forms of chromium are involved in redox cycling (Stohs and Bagchi, 1995). Cell reducing agents such as GSH and nicotinamideadenine dinucleotidephosphate (NADPH) reduce Cr (VI) to the pentavalent state (V), which can participate in the Fenton reaction to produce hydroxyl radicals. The hexavalent form can

be reduced to the trivalent form. This transformation is considered to be a major means of detoxification of Cr (VI) in biological systems. Chromium (III) plays a stimulatory role in physiological glucose metabolism. Chromium (VI) actively enters cells through an anion (phosphate) transport mechanism. Chromium (III), meanwhile, is not able to use this mechanism (Valko et al., 2005). Fish mucus can reduce the oxidative state of Cr (VI) and decrease its penetration, providing fish protection against chromium pollution (Ariello and Melodia, 1990). Kubrak et al. (2010) compared the effects of hexavalent and trivalent ions in the goldfish; both ions were found to induce oxidative stress.

Chromium (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).

A study conducted by Kuykendall et al. (2006) also reported DNA damage following chromium 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 chromium in water and in the diet.

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

Cadmium is a non-essential metal with no known biological function. The source of cadmium in the aquatic environment is industrial activity (Stohs and Bagchi, 1995). Cadmium does not generate ROS directly, but can alter GSH levels and influence cell thiol status, inducing the expression of metallothioneins in the liver. Changes in GSH and MTs can lead to LPO of the cell membrane. Cadmium enters the electron transport chain in

mitochondria, leading to accumulation of unstable semiquinones which donate electrons and create superoxide radicals. Cadmium also affects antioxidant enzymes, especially SOD and CAT, and is able to displace copper and iron 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 cadmium, and this process is clearly organ-specific (De Smet et al., 2001). The induction of *de novo* synthesis of MTs following cadmium 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 cadmium, as described by Ghedira et al. (2010), is evidence of a genetic ability to synthesise MTs. Contradictory results 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 cadmium exposure on GSH levels vary with fish species, duration of exposure, and the chemical involved. Both increases and decreases in GSH have been observed, depending on field and experimental conditions (Kovarova et al., 2009; Cao et al., 2010; Jia et al., 2011).

4.2.2. Mercury

Mercury 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, 2008b). 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. Methylmercury is generally more toxic to fish than the inorganic forms (Houserova et al., 2006). Mercury reacts with the thiol groups of GSH, which can induce GSH depletion and oxidative stress in tissue (Stohs and Bagchi, 1995).

Monteiro et al. (2010) described changes in biomarkers of oxidative stress following exposure to inorganic mercury. Methylmercury was shown to induce oxidative stress in several field studies

(Larose et al., 2008; Mieirol 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 total mercury content and MT levels were described by Mieirol 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 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 Mieirol 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).

Collectively, these data suggest that GSH has a key role in oxidative-induced toxicity caused by mercury.

4.2.3. Lead

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).

Lead accumulation in sediment is of significance for aquatic organisms. Lead is not a transition metal and cannot readily undergo valence changes. Lead 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 aminolevulinic 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

Arsenic is a known carcinogen in human. Arsenic forms inorganic and organic complexes in the environment. Arsenite (III) and arsenate (V) are inorganic forms that can be methylated. The trivalent arsenite is biologically more active than pentavalent arsenate. Glutathione plays a key role in the cell redox status induced by arsenic. Glutathione is an electron donor in the reduction of arsenate to arsenite. Arsenic cell metabolism generates ROS, although the mechanisms are not clear. Reactive nitrogen species are also involved in oxidative damage associated with arsenic (Bhattacharya and Bhattacharya, 2007).

The central role of GSH in arsenic toxicity was described in several studies. Allen et al. (2004) described 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.

Oxidative stress-induced apoptosis was suggested by Seok et al. (2007) as a possible mechanism of arsenic toxicity in a zebrafish *Danio rerio* liver cell line.

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

Selenium 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 organoselenide. 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. CONCLUSIONS

The above mentioned studies document 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 components of antioxidant defences are diversely influenced by metals. Both increases and decreases in enzyme activities and also enhanced and reduced levels of non-enzymatic components have been described after metal exposure. A specific biomarker of oxidative stress caused by metals does not exist, and for that reason a complex approach should be taken. Metallothioneins seem to be a suitable biomarker of metal exposure, especially under laboratory conditions. In field studies the applicability of MT content in fish tissues as a biomarker is questionable following chronic metal exposure. In several field studies there were no significant correlations found between MT content and cadmium as well as between MT content and mercury in fish tissue.

Frequently, aquatic contamination involves various chemicals that interact with one another. For that reason studies on 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 deltamethrin 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.

The experimental conditions in field studies vary with a season and weather, and the physicochemical

properties of water play an important role in metal solubility. Organic substances in 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.

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.

Acknowledgement

The authors would like to thank Dr. Alan Pike (Lucidus Consultancy) for manuscript improvement and English correction.

6. REFERENCES

- Abdel-Tawwab M, Mousa MAA, Ahmad MH, Sakr SFM (2007): The use of calcium pre-exposure as a protective agent against environmental copper toxicity for juvenile Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture* 264, 236–246.
- Ahmad I, Hamid T, Fatima M, Chand HS, Jain SK, Athar M, Raisuddin S (2000): Induction of hepatic antioxidants in freshwater catfish (*Channa punctatus* Bloch) is a biomarker of papermill effluent exposure. *Biochimica et Biophysica Acta* 1523, 37–48.
- Ahmad I, Maria VL, Oliveira M, Pacheco M, Santos MA (2006): Oxidative stress and genotoxic effects in gill and kidney of *Anguilla anguilla* L. exposed to chromium with or without pre-exposure to beta-naphthoflavone. *Mutation Research* 608, 16–28.
- Allen T, Singhal R, Rana SV (2004): Resistance to oxidative stress in a freshwater fish *Channa punctatus* after exposure to inorganic arsenic. *Biological Trace Element Research* 98, 63–72.
- Amiard JC, Amiard-Triquet C, Barka S, Pellerin J, Rainbow PS (2006): Metallothioneins in aquatic invertebrates: Their role in metal detoxification and their use as biomarkers. *Aquatic Toxicology* 76, 160–202.

- Arillo A, Melodia F (1990): Protective effect of fish mucus against Cr (VI) pollution. *Chemosphere* 20, 397–402.
- Ates B, Orun I, Talas ZS, Durmaz G, Yilmaz I (2008): Effects of sodium selenite on some biochemical and hematological parameters of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) exposed to Pb²⁺ and Cu²⁺. *Fish Physiology and Biochemistry* 34, 53–59.
- Bagnyukova TV, Chahrak OI, Lushchak VI (2006): Coordinated response of gold fish antioxidant defenses to environmental stress. *Aquatic Toxicology* 78, 325–331.
- Baker RTM, Martin P, Davies SJ (1997): Ingestion of sublethal levels of iron sulphate by African catfish affects growth and tissue lipid peroxidation. *Aquatic Toxicology* 40, 51–61.
- Bhattacharya A, Bhattacharya S (2007): Induction of oxidative stress by arsenic in *Clarias batrachus*: Involvement of peroxisomes. *Ecotoxicology and Environmental Safety* 66, 178–187.
- Campana O, Sarasquete C, Blasco J (2003): Effect of lead on ALA-D activity, metallothionein levels, and lipid peroxidation in blood, kidney, and liver of the toadfish *Halobatrachus didactylus*. *Ecotoxicology and Environmental Safety* 55, 116–125.
- Cao L, Huang W, Liu J, Yin X, Dou S (2010): Accumulation and oxidative stress biomarkers in Japanese flounder larvae and juveniles under chronic cadmium exposure. *Comparative Biochemistry and Physiology C* 151, 386–392.
- Ciardullo S, Aureli F, Raggi A, Cubadda F (2010): Arsenic speciation in freshwater fish: Focus on extraction and mass balance. *Talanta* 81, 213–221.
- De Smet H, De Wachter B, Lobinski R, Blust B (2001): Dynamics of (Cd,Zn)-metallothioneins in gills, liver and kidney of common carp *Cyprinus carpio* during cadmium exposure. *Aquatic Toxicology* 52, 269–281.
- Di Giulio RT, Meyer JN (2008): Reactive oxygen species and oxidative stress. In: Di Giulio RT, Hinton DE (eds.): *The Toxicology of Fishes*. CRC Press, Taylor and Francis Group. 273–324.
- Elia AC, Dorr AJM, Mantilacci L, Taticchi MI, Galarini R (2000): Effects of mercury on glutathione and glutathione-dependent enzymes in catfish (*Ictalurus melas* R.). In: Markert B, Friese K (eds.): *Trace Elements-Their Distribution and Effects in the Environment: Trace Metals in the Environment*. Elsevier Science, Amsterdam. 411–421.
- Elia AC, Galarini R, Taticchi MI, Dorr AJM, Mantilacci L (2003): Antioxidant responses and bioaccumulation in *Ictalurus melas* under mercury exposure. *Ecotoxicology and Environmental Safety* 55, 162–167.
- Emerit J, Beaumont C, Trivin F (2001): Iron metabolism, free radicals, and oxidative injury. *Biomedicine and Pharmacotherapy* 55, 333–339.
- Ercal N, Gurer-Orhan H, Aykin-Burns N (2001): Toxic metals and oxidative stress part I: Mechanisms involved in metal induced oxidative damage. *Current Topics in Medicinal Chemistry* 1, 529–539.
- Farag AM, May T, Marty GD, Easton M, Harper DD, Little EE, Cleveland L (2006): The effect of chronic chromium exposure on the health of Chinook salmon (*Oncorhynchus tshawytscha*). *Aquatic Toxicology* 76, 246–257.
- Garcia-Medina S, Razo-Estrada AC, Gomez-Olivan LM, Amaya-Chavez A, Madrigal-Bujaidar E, Galar-Martinez M (2010): Aluminium-induced oxidative stress in lymphocytes of common carp (*Cyprinus carpio*). *Fish Physiology and Biochemistry* 36, 875–882.
- Ghedira J, Jebali J, Bouraoui Z, Banni M, Guerbej H, Boussetta H (2010): Metallothionein and metal levels in liver, gills and kidney of *Sparus aurata* exposed to sublethal doses of cadmium and copper. *Fish Physiology and Biochemistry* 36, 101–107.
- Gravato C, Teles M, Oliveira M, Santos MA (2006): Oxidative stress, liver biotransformation and genotoxic effects induced by copper in *Anguilla anguilla* L. – the influence of pre-exposure to β -naphthoflavone. *Chemosphere* 65, 1821–1830.
- Gutteridge JM (1985): Inhibition of the Fenton reaction by the protein ceruloplasmin and other copper complexes. Assessment of ferrioxidase and radical scavenging activities. *Chemico-Biological Interactions* 56, 113–120.
- Hamer DH (1986): Metallothionein. *Annual Review of Biochemistry* 55, 913–951.
- Harkabusova V, Macharackova B, Celechovska O, Vitoulova E (2009): Determination of arsenic in the rainbow trout muscle and rice samples. *Czech Journal of Food Sciences* 27, S404–S406.
- Houserova P, Kuban V, Spurny P, Habarta P (2006): Determination of total mercury and mercury species in fish and aquatic ecosystems of Moravian rivers. *Veterinarni Medicina* 51, 101–110.
- Jebali J, Banni M, Guerbej H, Almeida EA, Bannaoui A, Boussetta H (2006): Effects of malathion and cadmium on acetylcholine esterase activity and metallothionein levels in the fish *Seriola dumerilli*. *Fish Physiology and Biochemistry* 32, 93–98.
- Jena SD, Behera M, Dandapat J, Mohanty N (2009): Non-enzymatic antioxidant status and modulation of lipid peroxidation in the muscles of *Labeo rohita* by sublethal exposure of CuSO₄. *Veterinary Research Communications* 33, 421–429.

- Jeziarska B, Witeska M (2001): Summary of metal-induced disturbances in fish organism. In: Metal Toxicity to Fish. Wydawnictwo Akademii Podlaskej, Siedlce. 214–243.
- Jia X, Zhang H, Liu X (2011): Low levels of cadmium exposure induce DNA damage and oxidative stress in the liver of Oujiang colored common carp *Cyprinus carpio* var. *color*. *Fish Physiology and Biochemistry* 37, 97–103.
- Kamunde C, Clayton C, Wood CM (2002): Waterborne vs. dietary copper uptake in rainbow trout and the effects of previous waterborne copper exposure. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 283, R69–R78.
- Kandemir S, Dogru MI, Orun I, Dogru A, Altas L, Erdogan K, Orun G, Polat N (2010): Determination of heavy metal levels, oxidative status, biochemical and hematological parameters in *Cyprinus carpio* L., 1785 from Bafra (Samsun) fish lakes. *Journal of Animal and Veterinary Advances* 9, 617–622.
- Kelly SA, Havrilla CHM, Brady TC, Abramo KH, Levin ED (1998): Oxidative stress in toxicology: Established mammalian and emerging piscine model systems. *Environmental Health Perspectives* 106, 375–384.
- Kovarova J, Celechovska O, Kizek R, Adam V, Harustiakova D, Svobodova Z (2009): Effect of metals, with special attention of Cd, content of the Svitava and Svratka rivers on levels of thiol compounds in fish liver and their use as biochemical markers. *Neuroendocrinology Letters* 30, 167–169.
- Kubrak OI, Lushchak OV, Lushchak JV, Torous IM, Storey JM, Storey KB, Lushchak VI (2010): Chromium effects on free radical processes in gold fish tissues: Comparison of Cr(III) and Cr(VI) exposures on oxidative stress markers, glutathione status and antioxidant enzymes. *Comparative Biochemistry and Physiology C* 152, 360–370.
- Kuykendall JR, Miller KL, Mellinger KN, Cain AV (2006): Waterborne and dietary hexavalent chromium exposure causes DNA-protein crosslink (DPX) formation in erythrocytes of largemouth bass (*Micropterus salmoides*). *Aquatic Toxicology* 78, 27–31.
- Larose C, Canuel R, Luccote M, Di Giulio R (2008): Toxicological effects of methylmercury on walleye (*Sander vitreus*) and perch (*Perca flavescens*) from lakes of the boreal forest. *Comparative Biochemistry and Physiology C* 147, 139–149.
- Li HC, Zhou Q, Wu Y, Fu J, Wang T, Jiang G (2009): Effects of waterborne nano-iron on medaka (*Oryzias latipes*): Antioxidant enzymatic activity, lipid peroxidation and histopathology. *Ecotoxicology and Environmental Safety* 72, 3684–3692.
- Livingstone DR (2003): Oxidative stress in aquatic organism in relation to pollution and agriculture. *Revue de Medecine Veterinaire* 154, 427–430.
- Luoma SN, Rainbow PS (2008a): Sources and cycles of trace metals. In: *Metal Contamination in Aquatic Environments: Science and Lateral Management*. Cambridge University Press, Cambridge. 47–66.
- Luoma SN, Rainbow PS (2008b): Methyl mercury. In: *Metal Contamination in Aquatic Environments: Science and Lateral Management*. Cambridge University Press, Cambridge. 368–392.
- Lushchak VI (2011): Environmentally induced oxidative stress in aquatic animals. *Aquatic Toxicology* 1, 13–30.
- Luza SC, Speisky HC (1996): Liver copper storage and transport during development: Implications for cytotoxicity. *American Journal of Clinical Nutrition* 63, 812–820.
- Maiti AK, Saha NK, Paul G (2010): Effect of lead on oxidative stress, Na⁺K⁺ATPase activity and mitochondrial electron transport chain activity of the brain of *Clarias batrachus* L. *Bulletin of Environmental Contamination and Toxicology* 84, 672–676.
- Mieiro CL, Ahmad I, Pereira ME, Duarte AC, Pacheco M (2010): Antioxidant system breakdown in brain of feral gulden grey mullet (*Liza aurata*) as an effect of mercury exposure. *Ecotoxicology* 19, 1034–1045.
- Mieiro CL, Bervoets L, Joosen R, Blust R, Duarte AC, Pereira ME, Pacheco M (2011): Metallothioneins failed to reflect mercury external levels of exposure and bioaccumulation in marine fish – Considerations on tissue and species specific responses. *Chemosphere* 85, 114–121.
- Miller LL, Wang F, Palace WP, Hontela A (2007): Effects of acute and subchronic exposures to waterborne selenite on the physiological stress response and oxidative stress indicators in juvenile rainbow trout. *Aquatic Toxicology* 83, 263–271.
- Monteiro DA, Rantin FT, Kalinin AL (2010): Inorganic mercury exposure: toxicological effects, oxidative stress biomarkers and bioaccumulation in the tropical freshwater fish matrinxa, *Brycon amazonicus* (Spix and Agassiz, 1829). *Ecotoxicology* 19, 105–123.
- Navarro A, Quiros L, Casado M, Faria M, Carrasco L, Benejam L, Benito J, Diez S, Raldua D, Barata C, Bayona JM, Pina B (2009): Physiological responses to mercury in feral carp populations inhabiting the low Ebro River (NE Spain), a historically contaminated site. *Aquatic Toxicology* 93, 150–157.
- Nishida Y (2011): The chemical process of oxidative stress by copper (II) and iron (III) ions in several neurodegenerative disorders. *Monatshefte fur Chemie* 142, 375–384.

- Orun I, Talas ZS, Ozdemir I, Alkan A, Erdogan K (2008): Antioxidative role of selenium on some tissues of (Cd²⁺, Cr³⁺)-induced rainbow trout. *Ecotoxicology and Environmental Safety* 71, 71–75.
- Pandey S, Ahmad I, Parvez S, Bin-Hafeez B, Haque R, Raisuddin S (2001): Effect of endosulfan on antioxidants of freshwater fish *Channa punctatus* Bloch: 1. Protection against lipid peroxidation in liver by copper preexposure. *Archives of Environmental Contamination and Toxicology* 41, 345–352.
- Parvez S, Raisuddin S (2006): Copper modulates non-enzymatic antioxidants in the freshwater fish *Channa punctata* (Bloch) exposed to deltamethrin. *Chemosphere* 62, 1324–1332.
- Parvez S, Sayeed I, Pandey S, Ahmad A, Bin-Hafeez B, Haque R, Ahmad I, Raisuddin S (2003): Modulatory effect of copper on non-enzymatic antioxidants in freshwater fish *Channa punctatus* (Bloch.). *Biological Trace Element Research* 93, 237–248.
- Rana SVS, Singh R, Verma S (1995): Mercury-induced lipid peroxidation in the liver, kidney, brain and gills of a fresh water fish *Channa punctatus*. *Japanese Journal of Ichthyology* 42, 255–259.
- Roesijadi G (1996): Metallothionein and its role in toxic metal regulation. *Comparative Biochemistry and Physiology C* 113, 117–123.
- Romeo M, Bennani M, Gnassia-Barelli M, Lafaurie M, Girard JP (2000): Cadmium and copper display different response towards oxidative stress in the kidney of the sea bass *Dicentrarchus labrax*. *Aquatic Toxicology* 48, 185–194.
- Ruas CBG, Carvalho CD, Araujo HSS, Espindola ELG, Fernandes MN (2008): Oxidative stress biomarkers of exposure in the blood of cichlid species from a metal-contaminated river. *Ecotoxicology and Environmental Safety* 71, 86–93.
- Sanchez W, Palluel O, Meunier L, Coquery M, Porcher JM, Ait-Aïssa S (2005): Copper-induced oxidative stress in three-spined stickleback: relationship with hepatic metal levels. *Environmental Toxicology and Pharmacology* 19, 177–183.
- Seok SH, Baek MW, Lee HY, Kim DJ, Na YR, Noh KJ, Park SH, Lee HK, Lee BH, Ryu DY, Park JH (2007): Arsenite-induced apoptosis is prevented by antioxidants in zebrafish liver cell line. *Toxicology in Vitro* 21, 870–877.
- Slaninova A, Smutna M, Modra H, Svobodova Z (2009): A review: Oxidative stress in fish induced by pesticides. *Neuroendocrinology Letters* 30, 2–12.
- Smirnov LP, Sukhovskaya IV, Nemova NN (2005): Effects on environmental factors on low-molecular-weight peptides of fishes: A Review. *Russian Journal of Ecology* 36, 41–47.
- Stohs SJ, Bagchi D (1995): Oxidative mechanisms in the toxicity of metals ions. *Free Radical Biology and Medicine* 2, 321–336.
- Tao S, Wen Y, Long A, Dawson R, Cao J, Xu F (2001): Simulation of acid-base condition and copper speciation in fish gill microenvironment. *Computers and Chemistry* 25, 215–222.
- Ternjej I, Mihaljevic Z, Stankovic I, Kerovec M, Sipos L, Zeljezic D, Kopjar N (2010): Estimation of DNA integrity in blood cells of eastern mosquitofish (*Gambusia holbrooki*) inhabiting an aluminium-polluted water environment: an alkaline comet assay study. *Archives of Environmental Contamination and Toxicology* 59, 182–193.
- Valko M, Morris H, Cronin MTD (2005): Metals, toxicity and oxidative stress. *Current Medicinal Chemistry* 12, 1161–1208.
- Wang CH, Zhao Y, Zheng R, Ding X, Wei W, Zuo Z, Chen Y (2006): Effects of tributyltin, benzo[a]pyrene, and their mixture on antioxidant defense systems in *Sebastes marmoratus*. *Ecotoxicology and Environmental Safety* 65, 381–387.
- WHO (1988): Environmental health criteria, Chromium. WHO, Geneva.
- WHO (1998): Environmental health criteria, Copper. WHO, Geneva.

Received: 2011–11–14

Accepted after corrections: 2011–11–23

Corresponding Author:

Marie Sevcikova, University of Veterinary and Pharmaceutical Sciences, Faculty of Veterinary Hygiene and Ecology, Department of Veterinary Public Health and Toxicology, Palackeho 1–3, 612 42 Brno, Czech Republic
Tel. + 420 541 562 781, Fax + 420 541 562 790, E-mail: H10374@vfu.cz
