Cadmium: Bioaccumulation, Histopathology and Detoxifying Mechanisms in Fish

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ABSTRACT

Metals are being utilized of ways in industries and agriculture; particularly heavy metals such as Mercury, Lead, Arsenic and Cadmium constitute a significant potential threat to human health because they are associated to many adverse effects on health. Due to the increasing pollution, concentrations of various chemical compounds have increased in aquatic environments. Water contaminants have a high potential risk for the health of populations and protection from toxic effects of environmental aquatic pollutants primarily involves considering the mechanism of low level toxicity and likely biological effects in organisms who live in these polluted waters. Fish have the ability to accumulate heavy metal in their tissues. Therefore the intake of fish should be regulated; information regarding the species of fish consumed and its possible levels of content of heavy metals can be of benefit to diminish the hazard to public health. In this mini-review we aimed to bring the main work on the harmful effects of cadmium on fish through its accumulation in the tissues, the main histological changes encountered in fish as well as the detoxifying systems against the toxicity of this metal.

Key words: Bioaccumulation, Cadmium, Histopathology, Metallothioneins

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1. Introduction

Aquatic pollution by organic and metal chemicals (inorganic) has been identified as one of the most important factors in the poisoning of marine organisms, such as fish

(Al-Ghais, 1995). Non-essential metals, such as mercury, lead and cadmium, are not known to play any metabolic function and can be toxic for humans, even at very low concentrations (Belitz and Grosch, 1999). Pollutants originating from the discharge of waste, specialized industries or mining activities are thought to be responsible for the reduction of fish resources in estuaries and coastal waters. In addition, coastal areas constitute nurseries, essential habitats for juvenile fish.

Heavy metals (essentially mercury, cadmium and lead) are toxic elements that can be assimilated, stored and accumulated by organisms, through the food chain, resulting in physiological damage (Pigott and Tucker, 1990; Ruiter, 1995). Cadmium as a toxic element might be a stressor agent for fish. Cadmium exposure may lead to the results of some pathophysiological damages including growth rate reduction in fish (Kaviraj and Ghosal, 1997; Hansen et al., 2002) and also in other aquatic organisms (Das and Khangarot, 2010). In order to protect aquatic wildlife, it is therefore necessary to determine contamination levels and water quality criteria. Chemical biomonitoring is often combined with evaluation of biomarkers that represent early indications of biological effects. During the past two decades, increasing emphasis has been placed on the need to develop physiological, biochemical, and molecular biomarkers that provide indicators of stress on organisms exposed to toxicants in the environment. However, in order to be ecologically relevant, these biomarkers must be linked to higher-level processes that reflect effects at the population and community levels of organization. The measurement of metabolic rate represents a useful indicator of environmental stress because it reflects both the energetic demands of lower-level processes and the availability of ingested energy that can be partitioned for growth and reproduction (Wu et al., 2012).

For this purpose, one of the aims of aquatic toxicology is to elucidate the subtler and most pronounced alterations induced by pollutants on aquatic organisms and their environment. In this way, we tend in this mini-review to resume the major toxic effects of cadmium following the bioaccumulation in tissues, histolopatholigical studies and detoxification mechanisms in fish.

2. Bioaccumulation of Cd in fish Tissues

Toxic elements such as heavy metal are known to bioaccumulate in the tissues of fish, marine mammals and seafood. In particular, the bioaccumulation of Cd, Arsenic,

Manganese, Mercury, and Lead in the trophic food chain is cause of concern since they can have deleterious effects on human health (Ersoy and Celik, 2009; Jarup and Akesson, 2009). Furthermore, fish and seafood are one of the main links between the heavy metal present in the environment and the human exposure (Verbeke et al., 2005; Burger and Gochfeld, 2009; Fraser et al., 2012). Under natural exposure conditions, prediction of toxic effects based on environmental or tissues concentrations remains difficult while many studies have examined the relationship between metal exposure, accumulation and toxicity under laboratory conditions (Peakall and Burger, 2003; Vijver et al., 2004). A growing number of evidence has shown that several factors influence Cd accumulation in fish tissues. These factors include the environmental metal concentration and time of exposure. It should be noted that several authors showed that animals tissues, contaminated in the laboratory, accumulate heavy metals in a concentration and contamination period dependent manner (Roméo et al., 1999; McGeer et al., 2000; Francis et al., 2004). Fish have the ability to accumulate heavy metal in their tissues by the absorption along the gill surface and gut tract wall to higher levels than the toxic concentration in their environment (Chevreuil et al., 1995).

Skin and, especially, gills are the likely primary entry point of waterborne Cd, which is in accordance with the absence of lesions in the digestive tract, well known to be impacted in fish exposed to the metal via food items (Berntssen et al., 2001), even though changes to intestine epithelia have also been found in fish exposed to high concentrations of waterborne Cd (Giari et al., 2007; Isani et al., 2009). Iger et al. (1994) described a series of both dermic and epidermal alterations (from necrotic pavement epithelium to fibrous tissue alterations) in *Cyprinus carpio* L., without an obvious dose-effect relationship at earlier stages of exposure. Handy (1992), described reduced Cd bioaccumulation in skin compared to liver, kidney and especially gills, where highest Cd concentrations were observed, in trouts, but the gills were found to be the most efficient organ detoxifying the metal (Costa et al., 2012). Moreover, it was reported that Cd is rarely distributed uniformly within the fish body tissues, and it is nevertheless accumulated by particular target organs (Surech et al., 1993).

Several past studies investigated Cd accumulation and its distribution in organs. However, accumulated levels among organs differed following different treatment doses of Cd^{2+} and exposure times (Wu et al., 1999). The metal ion usually accumulated less in gills since they are a temporary target organ of Cd accumulation, and then Cd is

transferred to digestive and reproductive organs (Wu et al., 2007). Thus, the accumulation of Cd in ovaries was similar to those in kidneys, intestines, and liver after Cd exposure (Cattani et al., 1996; Wu et al., 2012). Cd accumulate in the gonadal tissues of fish, reaching a concentration of up to 1000-fold higher than in the surrounding water environment and becoming extremely harmful for reproduction (Kime et al., 1996; Dietrich et al., 2011). In addition, fish sperm may be exposed to Cd through bioaccumulation in the testis (Kime, 1995).

Fish exposed to the highest dose of Cd experienced a significant accumulation of the metal in liver and brain tissue. Vetillard and Bailhache (2005), showed that Cd levels found in liver follow the predictive linear pattern of metal accumulation in this tissue (Lange et al., 2002) and agree with observations on trout coming from contaminated rivers (Olsvik et al., 2000). Cd not only accumulated in the liver but was also increased in the brain, even if levels were far lower than those found in the liver. Accumulation in the brain seems to be dependent on the administration route. Oral treatments failed to induce any significant increase in trout (Melgar et al., 1997), but Cd has been shown to be taken up by olfactory epithelium and transported to the brain in pikes (Tallkvist et al., 2002), a route that could fit with the waterborne exposition. After waterborne exposure, Cd accumulated in the olfactory rosettes, nerves, and bulbs in rainbow trout but not in the brain (Scott et al., 2003; Vetillard and Bailhache, 2005). De Conto Cinier et al. (1997) stated on Cd uptake in fish liver and kidney can be divided into two groups according to the presence or absence of a plateau in the Cd accumulation kinetic curves. Entry of heavy metals into the organs of a fish mainly takes place by the adsorption and absorption and the rate of accumulation is a function of uptake and depuration rates (Sreedevi et al., 1992). McDonald and Wood (1993), suggest that, after the initial shock phase of metal exposure, fish physiologically adapts to compensate for ion losses by secreting mucus and altering gill structure at the cellular and subcellular level.

Reynders et al. (2008) report that on the tissue level, highest Cd concentrations were observed in kidneys of carp (*Cyprinus carpio*) and roach (*Rutilus Rutilus*), followed by gills, intestine and liver, while low concentrations were observed in carcass and muscle. Accumulation and toxicity under laboratory conditions, prediction of toxic effects based on environmental or tissues concentrations remains difficult under natural exposure conditions (Peakall and Burger, 2003; Vijver et al., 2004). Increased Cd concentrations in gills and intestine probably reflected the source of metals uptake from

water and food, since gills generally accumulate much higher metal concentrations during waterborne exposure, while intestine accumulate metal following dietary exposure (Chowdhury et al., 2004).

In Cd accumulation, plateaus have been reported in liver and kidney of zebrafish (*Danio rerio*) after 2 or 3 months (Rehwoldt and Karimian-Teherani, 1976) and in one-summer-old carp (*Cyprinus carpio*) exposed to Cd (De Conto Cinier et al., 1997). Continuing accumulation of Cd has been observed in rainbow trout (Oncorhyn*chus mykiss*) exposed for 178 days (Giles, 1988). However, a reduction of accumulation has been reported in many other taxonomic groups as a physiological mechanism for metal resistance and adaptation (Hall et al., 1979; Bariaud et al., 1985; Tsuchiya and Ochi, 1994; Yanagiya et al., 1999).

3. Histopathological Studies

Teleosts have long been targeted in toxicological studies involving aquatic pollutants due to their ecological relevance, availability and ability to act as surrogates for higher vertebrates. These studies involved either collecting feral animals or bioassays with locally exposed (caged) animals or even laboratory bioassays to test the toxicity of single or combined substances. In either case, histopathological assessment in fish as long been recognized as a highly valuable tool to identify the toxicopathic effects of substances since it may better reflect the true health condition of the animal than other biomarker/diagnosis methods (Au, 2004).

Still, fish histopathology is far to be as standardized, with respect to lesion detailing, identification and nomenclature, as in higher vertebrates (i.e. mammals, including humans), to which are added difficulties in establishing cause-effect relationships and the lack of specificity of most biomarker candidates. Furthermore, there are yet few studies with fish exposed to environmentally realistic concentrations of waterborne Cd and even fewer concerning histopathology. Giari et al. (2007) reported conclusive histopathological alterations in multiple organs of *Dicentrarchus labrax* exposed to waterborne Cd. In the few bioassay-based studies performed with fish within this range of contamination, Lizardo-Daudt and Kennedy (2008) found Cd-induced hatching and developmental abnormities in rainbow trouts (*Oncorhynchus Mykiss*) exposed for 28 days. In another study, Faucher et al. (2008) found that exposure to Cd

could alter the escape behaviour of *Dicentrarchus labrax*, presumably by affecting the lateral line system.

Cd is long known to disrupt hepatic carbohydrate metabolism, leading to a decrease in glycogen storage and increased plasma glucose, as observed by Soengas et al. (1996) in Atlantic salmon (*Salmo salar* L.). Gills were more prone to acquire histopathological lesions than skin as a result of exposure to low Cd concentrations, which should indicate differential response and defense mechanisms between the two organs. Regarding these, changes to skin goblet cell (size, distribution and chemical composition) as a consequence of external insult have already been reported. A reduction in skin goblet cell size in fish, including flatfish, has already been found to occur as a result of different factors, from bacterial infections (Yamamoto et al., 2011) to exposure to sediment bound contaminants (Mézin and Hale, 2000). The gill epithelium is the main apical entry surface for waterborne contaminants in fish. Although variable, Costa et al. (2012) found a trend to increase changes to gill epithelia with increasing concentrations of Cd, with especially regard to chloride cell hypertrophy by intraplasmatic fluid retention, an alteration that is likely to compromise ion excretion, a crucial physiological process in marine fish to maintain internal osmotic balance.

The histopathological changes observed occur in a progressive time- and concentration-related series: organs that form the organism-water boundary (gills and skin) were the least affected, followed by the spleen, trunk kidney and finally the liver as the most affected. The differences in the severity and the dissemination degree of histopathological lesions and alterations are a probable function of tissue-species defenses and sensitivity. Interestingly, the mechanisms of Cd translocation within the organism, from water to gills, then to blood which conveys the metal to other organs (especially the liver followed by the kidney and the spleen) is likely impacted through the recycling of Cd-affected blood cells. By its turn, the kidney is impacted especially by the impairment of tubular active transport, as in gill chloride cells (van Kerkhove et al., 2010). Additionally, these data indicate that, although no specific biomarkers of exposure to Cd could be distinctively pin-pointed, the analyses of multiple organs may reveal an histopathological pattern. This pattern could be an indicative of exposure to low concentrations of a toxic metal that include identifiable traits (such as focal cell death, inflammation), changes that reveal alterations to carbohydrate metabolism (as glycogen depletion and lipidosis), osmotic balance impairment (inferred from gill

chloride cell hypertrophy and kidney tubule lesions) and skin goblet cell engorgement revealing increased mucous production as a response/defense mechanism. Overall, the histopathological lesions and alterations observed are consistent with chronic disease and may be surveyed in future research for qualitative or semi quantitative assessment (as histopathological indices) of metal-induced injury either in the laboratory or, most importantly, in field studies, where low background levels of Cd contamination apply (Costa et al., 2012).

Although it is generally considered that Cd is primarily nephrotoxic and then hepatotoxic as a consequence of chronic exposure (Nordberg, 2009; Nawrot et al., 2010), Costa et al. (2012) reported that the liver sustained histological lesions and alterations more clearly relatable to Cd concentration and time of exposure. The alterations correspond especially to inflammation-related responses (as hyperaemia and macrophage intrusion). Direct lesions such as necrosis had a low prevalence, although a trend to increase with time and concentration of exposure was identified, showing that exposure to low concentrations of this toxic metal may in fact elicit chronic hepatic damage to juvenile *Solea senegalensis*.

According to our laboratory exposure of *Gambusia affinis* to Cd, histopathological investigations revealed greater changes in gills, kidney and liver tissues after chronic exposure than those recorded during acute Cd exposure (Annabi et al., 2011). The changes in gills were characterized by epithelial lifting, total and partial lamellar fusion, epithelial necrosis as well as telangiectasis. In kidney tissue, necrosis of epithelial cells of renal tubules, glomerular contraction and reduction of Bowman's space were observed in exposed fish. In addition, the liver hepatocytes showed cytoplasmic vacuolization with lipid droplets and glycogen accumulation. Congestion of the hepatic central vein, desquamation of hepatic tissue and an increase in sinusoidal space were also noted in the liver tissue.

4. Metallothioneins and detoxification mechanism

Metal homeostasis has to be carefully regulated by the cell to prevent production of toxic free radicals (Radisky and Kaplan, 1999), thus triggering oxidative stress. Metallothioneins (MTs) is a low-molecular-weight metal binding protein and is known to play an important role in protection against heavy metal toxicity. MTs is known to be a protein involved in protection against oxidative stress (Andrews, 2000; Gourgou et al.,

2010), including heat-induced stress (Ivanina et al., 2009; Guinot et al., 2012). A possible role for MT in the oxidative stress reaction has also been documented (Manuel et al., 1992). It has been shown to be an effective free radical scavenger, important because the release of various species of oxygen metabolites is thought to be indirectly responsible for the initiation of apoptosis. However, MTs has itself been implicated as a causal factor in apoptosis (King et al., 1997), and has intrinsically toxic effects in itself when bound to Cd (Sabolic et al., 2002; Thompson and Bannigan, 2008). In addition to the detoxification of toxic metals such as Cd, MTs is involved in the maintenance of homeostasis of essential trace elements such as Zinc and Copper (Coyle et al., 2002). Its role in the protection against xenobiotics or in the cellular protection against oxidative stress should be underlined (Coyle et al., 2002). Although its synthesis is related to the metal exposure, its levels can be affected by endogenous and exogenous factors such as the reproductive cycle or the temperature (Van Cleef-Toedt et al., 2001). Furthermore, variations in the water temperature could directly or indirectly modify the behavior of this protein as regards the bioaccumulation of metals, as well as its participation in toxicokinetic processes (Gorbi et al., 2005; Baykan et al., 2007; Guinot et al., 2012). The effect of temperature on MTs levels in liver could be a direct thermal response, or may be related to the increase in metal content. MTs synthesis is considered one of the best-known biochemical detoxification mechanisms for metal and it is widely demonstrated that its induction may be influenced by metal contamination. There are only a few studies especially designed for elucidating the effect of temperature on MTs synthesis in fish and it is very common to try to relate seasonal variability to temperature, but in this case the reproductive state may also be involved (Gorbi et al., 2005). Van Cleef-Toedt et al. (2001) demonstrated that non-spawning *Fundulus heteroclitus* exposed to thermal stress exhibited significantly elevated liver, gill, and intestine MT- mRNA expression compared with controls. Riggio et al. (2003) reported that after exposure of Zebrafish (*Danio rerio*) with Cd, the MTs content increased around 30-fold, and MTs synthesis was induced in oocytes during vitellogenesis and at the blastula stage of embryos. Previous reported data that the rate of Mt-mRNA in the liver of different species are sensitive to acute contamination, but not to chronic exposure (Quirós et al., 2007; Navarro et al., 2009). Wangsongsak et al. (2007) showed that the hepatic expression of Mts-mRNA increases significantly after the exposure of *Puntius gonionotus* to three increasing amounts of Cd (0.012, 0.06 and 0.12 mg/L). This increase

remains significantly important in the liver during the first 14 days of exposure. However, in the renal tissue, the most important expression levels of mRNA-Mt was noticed after 28 days. In the same context, Hollis et al. (2001) demonstrated that Cd induced, at low concentrations (0.012 and 0.06 mg/L), an important expression level of MTs in the liver tissue compared to the kidney. Nevertheless, Wangsongsak et al. (2007) reported that, at the highest concentration of Cd (0.12 mg/L), the expression of MTs was higher in the kidney than the liver. Taking together, these data suggest that the expression of mRNA-Mts in the liver tissue would be dependent on the time of exposure, while in the renal tissue, it seems to be dose-dependent.

Induction of MTs (Piersma et al., 1993) is an alternative mechanism by which protection against Cd toxicity could be conferred. This low-molecular weight protein binds to Cd, limits its availability to cells and tissues (King et al., 1997), and plays a role in transport, detoxification, and storage (Kang, 2006; Thompson and Bannigan, 2008). After absorption, Cd is transported to the liver, bound to albumin (Nordberg et al., 1992), where it induces the synthesis of MTs, a class of small cysteine-rich heavy metal binding proteins. Changes within the liver itself following parenteral administration of $CdCl_2$ are dose- and time-dependent, ranging from moderate diffuse hepatocellular degeneration through to multifocal necrosis (Sauer et al., 1997). Following release from the liver, MT-bound Cd enters the plasma. MT-bound Cd appears in the glomerular filtrate, from where it is re-absorbed intracellularly by renal tubule cells. In the latter, the Cd is cleaved from the MT by lysosomal action, and Cd^{2+} ions are re-excreted into the tubular fluid and then the Cd is eliminated in the urine. The ability of Cd to induce hepatic and renal lesions exacerbates its toxic effects, and compounds its propensity to accumulate over years (Thompson and Bannigan, 2008).

Several studies on fishes have shown 2 metallothionein isoforms in salmonids, carp, gudgeon (*Gobio gobio*), and other species, whereas other fishes possess a single metallothionein isoform (Bonham et al., 1987; Bargelloni et al., 1999; Knapen et al., 2005). Where present, the 2 metallothionein isoforms may show different sensibility to exogenous inputs, such as metals and oxidative stress (Zafarullah et al., 1990; Scudiero et al., 2001). In addition, cold stress resulted in a significantly higher induction of Mt-1 than of Mt-2 (Hermesz et al., 2001). Sato and Kondoh (2002) reveal that both Mt-1 and Mt-2 are expressed in nearly all organs of the body and are related to metal regulation and detoxification in mammals. Mt-3 is localized in the brain, while Mt-4 is specifically

expressed in epithelial cells of the skin (Quaife et al., 1994). For example, in avian species, Mt-1 had no correlation with any metal concentrations, and Mt-2 expression was positively correlated with only Cu (Nam et al., 2007). All of these MT isoforms showed different functions and roles in their subsequent organs.

Indeed, it is well known that teleost MTs also include some isoforms, for instance, Rainbow trout (*Oncorhynchus mykiss*), Zebrafish (*Danio rerio*), Carp (*Cyprinus carpio*), icefish (*Chionodraco hamatus*), tilapia (*Oreochromis mossambicus*) and mediterranean killifish (*Aphanius fasciatus*) (Annabi et al., 2012; Wu et al., 2012). Past studies found that MT isoforms of fish were like those of mammals and avian species in that they showed various functions and responses to different stresses. Generally regarded as unrelated to the reproductive system, MTs constitute a family of proteins whose expression has been linked to exposure to heavy metals and, secondarily, to other forms of stress in essentially all animal species (Cajaraville et al., 2000, Bourdineaud et al., 2006, Sarkar et al., 2006). For example, in common Carp, both Mt-1 and -2 levels increased with Cd concentrations in time- and dose-dependent manners, but the expression of Mt-2 was more responsive to a high dose of Cd. In Zebrafish, genes of *mt2* and *mt1* were related to heavy metal detoxification (Gonzalez et al., 2006) and regulated by essential metals such as zinc and copper during development (Chen et al., 2004). However, comparison of sequences between *mt1* and *mt2* revealed 96.7% identity after gene alignment (Wu et al., 2008b). Previous studies showed that *mt1* and *mt2* genes are up-regulated after Cd exposure in Zebrafish (Gonzalez et al., 2006). In addition, it was suggested that *mt1* has an important physiological role in gills in low-level contaminated water (Gonzalez et al., 2006). Wu et al. (2008b) found that Zebrafish *mt2* mRNA existed at the 6h post-fertilization (hpf) stage, and had rapidly increased at the 24 hpf stage. It also increased gradually with further larval growth. Both MT isoforms were measured in the early stages of Zebrafish embryos. Furthermore, at 72-hpf Zebrafish larvae, *mt2* signals were observed in mitochondrion-rich (MR) cells, olfactory pits, the pronephric duct, retinas, and the ventricular zone upon Cd exposure and cold shocks. Additionally, MT isoforms showed different tissue-specific expression in fish. Furthermore, our results provide the first evidence of gonadal stress of Mediterranean killifish (*Aphanius fasciatus*) on background of high increase of Mt-2 mRNA levels in testes (some 100-fold) in Cd polluted sites (Annabi et al., 2012). These data suggesting that this probe may be a good marker for pollution in gonads.

This variety of cytotoxic agents including Cd have been shown to up-regulate proteins especially Heat shock proteins (Hsps). HSPs are a family of highly conserved molecular chaperones that aid in the proper folding, transport, and degradation of cellular proteins. The 70 kDa Hsp subfamily, the best characterized of the Hsps, contains a highly stress-inducible isoform encoded by the *hsp70* gene. For this purpose, *hsp70* expression has been examined as a potential marker in toxicologic screening for a number of vertebrate species (Blechinger et al., 2002).

5. Conclusion and perspectives

The levels of contamination by Cd in fish are of considerable interest because fish consumption is an important source of intake Cd for the general population. Most of the Cd content in fish or other seafood is highly absorbable in $CdCl_2$ form; in humans, the efficiency of gastrointestinal absorption of Cd has been reported to be approximately 3–8% of the ingested load. In the perspective way, acute toxicological and physiological effects to aquatic organisms following waterborne Cd exposure can be altered by some others parameters: water hardness (Davies et al., 1993), water concentration of calcium (Meyers, 1999), Zinc (Brando-Netro et al., 1995) and Selenium (Lin and Shiau, 2007; Abdel-Tawwab et al., 2007; Messaoudi et al., 2010a, 2010b). While there have been reports on metal-resistance in fish and on the underlying changes in metal accumulation, mechanisms underlying metal-resistance have not been studied in fish populations for which there is unequivocal evidence that the resistance differences are genetically based. Future investigations should be designed to evaluate the association of metabolic rate with specific physiological, biochemical, and cellular responses as well as the protective effects of the supplements.

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