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Consequences of oxidative damage and mitochondrial dysfunction on the fatty acid profile of muscle of Indian Major Carps considering metal toxicity

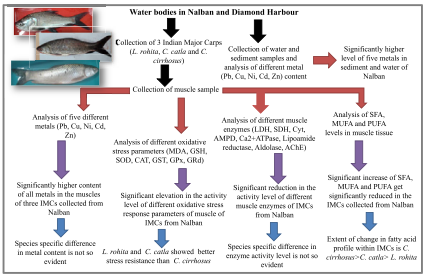
Debjit Das [a], Payel Das [b], Mahammed Moniruzzaman [a], Mousumi Poddar Sarkar [b], Joyita Mukherjee [c], Suman Bhusan Chakraborty [a, *]

[a] Department of Zoology, University of Calcutta, Kolkata, 700019, India
[b] Department of Botany, University of Calcutta, Kolkata, 700019, India
[c] Department of Zoology, Krishna Chandra College, University of Burdwan, Hetampur, Birbhum 731124, West Bengal, India

HIGHLIGHTS

- Metal pollution alters oxidative balance and affects mitochondrial enzymes activity.
- Metal exposure is likely to change the fatty acid composition of the carp muscle.
- Muscle mitochondrial enzymes regulate fish flesh quality against metal stress.
- Metal toxicity causes lipid peroxidation and subsequently alters carp muscle quality.
- Metal exposure alters PUFA, MUFA, omega fatty acid contents in carp muscle.

GRAPHICAL ABSTRACT



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ABSTRACT

Current study aims to find interrelation between mitochondrial enzyme function and fatty acid profile in fish muscle and role of antioxidant agents to maintain their balance in response to metal accumulation. Fishes (*Labeo rohita*, *Catla catla*, *Cirrhinus cirrhosus*) were collected from two sites (Nalban Bheri and Diamond Harbour, India). Concentrations of metals (lead, cadmium, copper, nickel, zinc), enzymatic and non-enzymatic antioxidant activity (malondialdehyde, superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, glutathione S-transferase), muscle enzyme activity (acetylcholinesterase, succinate dehydrogenase, lactate dehydrogenase, Ca^{2+}ATPase, AMP-deaminase, lipoamide reductase, cytochrome C oxidase, aldolase) and fatty acid composition in muscle tissues were analyzed. Metal concentrations were significantly higher ($P < 0.05$) in fish muscles from Nalban compared to those in Diamond Harbour. Increased activity of antioxidant enzymes was noted with diminished mitochondrial enzymes activity and altered fatty acid composition in response to higher metal accumulation. Higher metal concentration in fish muscle of Nalban seems to significantly ($P < 0.05$) affect poly and monounsaturated fatty acid content, possibly due to oxidative damage and accumulation of hazardous reactive oxygen species (ROS) molecules. Changes in fatty acid contents following metal accumulation

* Corresponding author. Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road Kolkata, 700019 West Bengal, India. Tel.: +91 9831189211.
E-mail addresses: sumanbc76@gmail.com, sbczoo@caluniv.ac.in (S.B. Chakraborty).

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were observed to be species specific. Current study is the first correlative study to illuminate the level of oxidative damage and possible consequences on muscle cellular integrity, mitochondrial functionality and flesh quality against bioaccumulation of different metals in carps. Future studies are needed to quantify the relative contributions of enzymatic and low-molecular-mass antioxidants in protecting mitochondrial function and maintenance of proper fatty acid oxidation during acclimation to long term metal exposure.

1. Introduction

Freshwater ecosystems are frequently exposed to a large extent of xenobiotic materials including different trace and heavy metals from diverse sources like agricultural run-offs, industrial and domestic sewages (Lo'pez-Galindo et al., 2010). Metals have a broad range of biological properties, from being elementary for several organismic functions to being extremely toxic for different metabolic pathways at the same time (Das et al., 2017). Thus, there is an absolute necessity to assess the toxic effects of such metals on aquatic ecosystems and organisms inhabiting there.

Though there is a difference in the bioavailability and distribution pattern of different trace and heavy metals in aquatic systems and aquatic organisms, fish is thought to be the most important bio-indicator of aquatic habitat regarding such pollution (Mukherjee et al., 2017). Fish accumulate heavy metals and all other xenobiotic pollutants in higher concentration and is consumed directly by human to a very large extent. There are few reports regarding toxic effects in fish due to metal contamination that includes neurotoxicity, genotoxicity, oxidative damage, depleted flesh quality, carcinogenicity and metabolic enzyme inhibition (Das et al., 2017). Metal deposition at fish muscle might have undesirable effects on quality and freshness of fish flesh, and be responsible for metal toxicity related health risks in humans during consumption of fish (Storelli, 2008).

Presence of different metals at high concentration might generate physiological abnormality in fish including temporary or permanent damage in oxidative homeostasis and metabolic imbalance (Moniruzzaman et al., 2017). Collective and harmonic activity of all the antioxidant and protective enzymes in different tissue provide the defensive shield against detrimental effects of superoxide ion accumulation, redox imbalance or metabolic disorder (Das et al., 2017). A stable equilibrium of enzymatic as well as non-enzymatic antioxidants in different tissues of an organism might alter following exposure to metals. Therefore, changes in the activity of enzymatic antioxidants [superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GRd)], non-enzymatic antioxidant reduced glutathione (GSH) and alterations of lipid peroxidation product malondialdehyde (MDA), may indirectly indicate the levels of toxic contaminants and be ideal markers of pollutant-mediated oxidative damage in fish (Das et al., 2017; Moniruzzaman et al., 2017). Inducible heat shock protein 70 (HSP70) can also be considered as biomarker of protein damage due to oxidative stress or any other metabolic dysfunction, especially after trace metal exposure in fish (Moniruzzaman et al., 2017). The enhanced levels of HSP70 in stressed cells shields the destruction of protein machinery, commence the progression of renaturation of denatured proteins, confirm protein aggregation, target the damaged proteins to breakdown and finally facilitate biosynthesis and translocation of new proteins (Moniruzzaman et al., 2017). Hence, change in HSP70 protein expression may also be regarded as an indicator of metal-induced stress in fish. But unfortunately, no study was yet undertaken to correlate the changes in fatty acid composition in fish muscle against changes in HSP.

Mitochondria are regarded as the main source of free radical formation (Cadenas and Davies, 2000) in the tissue systems of living animals. The mitochondrial electron transport chain and its reactions generate superoxide anion radicals that can be subsequently converted to hydrogen peroxide. This can lead to the formation of hydroxyl radicals that react with muscle and other tissue systems impacting on the well-being or the performance of animals. There are two possible outcomes from free radical reactions. The first is chemical modification of the surrounding compounds such as oxidation of amino acids, lipids, or vitamins. This can promote loss of physiological function in living animals or initiation of undesirable changes in the muscle tissue system leading to deterioration of quality attributes such as color stability, flavor, or nutritive characteristics after death. Second, the affected compound can become a radical causing a series of electron exchanges among several molecules leading to DNA damage or oxidation of lipids and proteins. Lipid composition of muscle is one of the determining factors to maintain muscle quality and its freshness. Fish muscle is reported to be enriched with eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and polyunsaturated fatty acid (PUFA). EPA and DHA are very much beneficial for functional improvement of cell membrane transport, enzyme activity and cell signaling processes (Calder and Yaqoob, 2009). Rare studies showed that bioaccumulation of different metals in muscle are reported to have negative consequences against fatty acid content of fish muscle (Senthamilselvan et al., 2016). This negative correlation can lead to depressed storage quality of fish fillet sourced from metal contaminated waterbodies.

Mitochondrial enzymes are the key factors to preserve the muscle structural and functional integrity and thereby regulate the quality of fish flesh. The enzymes like acetylcholinesterase, lactate dehydrogenase, succinate dehydrogenase and other enzymes of glycolytic pathways are found to regulate the functions like neurotransmission and bioenergetics in muscle (Belyaeva et al., 2012). Some other metabolic enzymes like lactate dehydrogenase, Ca^{2+} ATPase, Cytochrome C oxidase are also key factors to sustain the glycolytic pathways which in turn regulate fatty acid biosynthesis and quality indices of fish muscle (Kastaniotis et al., 2017). No noteworthy reports are available regarding the activity of metalloenzyme like lipoamide reductase in maintenance of fish muscle quality. However, very few studies have noted that enzymes like AMP deaminase is essential for fatty acid biosynthesis through generation of acetyl-CoA (Ratledge, 2004).

Indian Major Carps (IMCs) such as *Labeo rohita*, *Catla catla* and *Cirrhinus cirrhosus* have high consumer demand and are often cultured in waterbodies contaminated with different metals. These three carps may have differential metal accumulation pattern in muscle as they show differences in their habitat; *L. rohita* is a column feeder, *C. catla* is a surface planktnivore and *C. cirrhosus* is a benthic fish which largely depend on decomposed organic matter of the bottom (Jayaram, 1981). Different metal accumulation may affect differentially against the quality of fish flesh. The aim of the