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Synergistic Effect of Nickel and Mercury on Fatty Acid Composition in the Muscle of Fish *Lates calcarifer*

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ABSTRACT

In this study, the effect of nickel (Ni), mercury (Hg) and nickel plus mercury (Ni+ Hg) on fatty acid composition in the muscle tissues of fish *Lates calcarifer* has been investigated. It was determined from the results of 96 h metal exposure period of fish, depending on Ni, Hg and Ni plus Hg concentration, the amount of saturated fatty, mono unsaturated fatty acid and poly unsaturated fatty acids in fish were decreased when compared with control group ($p < 0.05$). It was also found that Ni, Hg and Ni plus Hg had reduced effects on palmitoleic, linoleic, arachidonic acid, eicosapentaenoic, decosapentaenoic and docosahexaenoic belong to unsaturated fatty acid in muscle tissue. The data demonstrated the necessity for regular monitoring of the hazard quotient for food fish in wild conditions.

Key words: Heavy metals, mercury, nickel, fatty acid, *Lates calcarifer*

INTRODUCTION

Heavy metals are considered as the most important form of pollution in the aquatic environment because of their toxicity and accumulation by marine organisms. Heavy metal contents in aquatic environment have been increased by increased activities in industrial, domestic and agricultural sections (Khoshnoud *et al.*, 2011). The heavy metals are defined as metallic chemical elements that have a relatively high density and are toxic or poisonous at low contents. The contamination of heavy metals can enter from the water into fish body by different routes and accumulate in organisms (Olaifa *et al.*, 2004; Dobaradaran *et al.*, 2010). Some of the metals found in fish might be essential as they play important roles in biological systems of the fish as well as in the human being. Metals like iron and manganese are required for metabolic activities in organisms, but some other elements like mercury and nickel exhibit toxicity effects on aquatic organisms (Mason, 1991; Bhupander *et al.*, 2011). Fishes are major part of the human diet due to high protein content, low saturated fat and sufficient omega fatty acids which are known to support good health. Therefore, various studies have been taken worldwide on the contamination of different fish species by heavy metals (Mokhtar *et al.*, 2009; Bhattacharyya *et al.*, 2010). Fishes have been widely used as bio-indicators of pollution by metals. Muscle tissue of fish is the most frequently used for analysis because it is a major target tissue for metal storage and is the main edible part.

Fish is known to be a rich source of the unique polyunsaturated fatty acids of the omega-3 family, including both eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Fish provides ω-3 (n-3) fatty acids that reduce cholesterol levels and the incidence of heart

disease, stroke and preterm delivery (Daviglus *et al.*, 2002). Although ω-3 fatty acids in fish have been implicated for healthful benefits, there is increasing evidence that the heavy metal contents in some fishes, if consumed too frequently, can lead to adverse health effects (Rissanen *et al.*, 2000). During the last two decades, Polyunsaturated Fatty Acids (PUFA) have attracted a great interest among scientists for their medicinal and nutritional properties (Razak *et al.*, 2001). The abundance of the unsaturated fatty acids in fat is the most valuable characteristics of the fish (Hedayatifard and Moeini, 2007).

Polyunsaturated fatty acids of the ω-6 and especially of ω-3 family are recognized as the essential biochemical components of the human diet (Tawfik, 2009). Fish tissue is the main source of long chain polyunsaturated fatty acids especially the ω-3 and ω-6. These fatty acids have particular importance in human diet since their consumption contributes to the reduction of the appearance of the cardiovascular diseases (Nordoy *et al.*, 2001) as well as the improvement of learning ability (Suzuki *et al.*, 1998). Previous studies reported that the exposure of fish to heavy metals resulted in biochemical alterations in muscles of fish. Hence the present study is to investigate the effects of nickel, mercury and nickel plus mercury exposure on the fatty acid composition of the muscle tissues of fish *L. calcarifer*. This study also focused on the proximate composition and toxic metals assessment.

MATERIALS AND METHODS

In this study, clinically healthy *L. calcarifer* were procured from Rajiv Gandhi Center for Aquaculture (RGCA), Thirumullaivasal, Sirkali, Tamil Nadu, India and were acclimatized under laboratory conditions for 20 days prior to start of experiments. The fish were kept in tanks with daily partial water exchanges and fed rice bran and groundnut oil cake twice a day *ad libitum*.

Fish with a size range of 7-8 cm length and weighing 8-10 g were selected for use in the experiments. Water quality measurements taken throughout the experiments according to APHA methods (APHA, AWWA and WEF., 1998) were recorded as follows: dissolved oxygen (6.2±0.15 mg L^{-1}), pH (7.20±0.17), water temperature (25.2±0.6°C), salinity (28±0.00 ppt), total hardness (13.1±0.3 mg L^{-1}), calcium (5.0±0.1 mg L^{-1}), magnesium (8.0±0.1 mg L^{-1}) and total alkalinity (20.1±0.6 mg L^{-1}). Preliminary studies were carried out to determine the median lethal concentration (LC_{50}) of nickel, mercury and nickel plus mercury by 96 h bath exposure, according to the probit analysis method by Finney (1978).

The LC_{50} for 96 h nickel, mercury and nickel plus mercury bath exposure was determined to be 4.0, 0.8 and 2.0 mg L^{-1}. Four replicate test tanks with nickel at 4.0 mg L^{-1}, mercury at 0.8 mg L^{-1}, nickel plus mercury at 2.0 mg L^{-1} and a control tank with the same water quality and devoid of metals were used. All experimental tanks were filled with 20 L water and metals nickel, mercury and nickel plus mercury were added to each tank at a concentration of 4.0, 0.8 and 2.0 mg L^{-1} except control tank. Twenty fish were introduced into each tank. The toxicant was renewed daily in the experimental tanks. No mortality was observed throughout the experimental period. A total of 10 fish were collected from each experimental tank on a 24 h basis for biochemical studies.

Measurement of fatty acid composition: Extraction of the fish fillet lipids was done according to the method of Bligh and Dyer (1959). Each 100 g muscle sample was homogenized in a mixer blender for 2 min with a mixture of 100 mL chloroform and 200 mL methanol. To the mixture, 100 mL chloroform was added and subsequently blending for 30, 100 mL distilled water was added and continued blending for another 30. The homogenate was filtered and was transferred to a 500 mL graduated cylinder. After allowing a few min for complete separation and clarification, the

alcoholic layer was removed by aspiration while lower layer (chloroform layer) contains purified lipid. The extraction of lipids was done separately for each fish sample in each experiment. Subsequently, the oil was sponified to isolate the Free Fatty Acids (FFA). The oil was analyzed for fatty acid profile by using gas chromatograph (Shimadzu-A17, Japan) with a Flame Ionization Detector (FID) and attached to an integrator. The injected sample was 1 mL with carrier gas He (flow: 1.2 mL min^{-1}), column temperature 170°C (50 m×0.32 mm (ID-BPX 70×0.25 mm) cyanopropyle siloxan column), injection port temperature 210°C and detection port temperature 230°C.

Statistical analysis: Statistical analysis was carried out by using the SPSS for Windows, version. 10 (SPSS Inc. Chicago, IL, USA). Students t-test was used to determine whether differences between means were significant with $p < 0.05$ taken as the significant level.

RESULTS

In the present study, fatty acid levels in the muscle of fish *L. calcarifer* was statistically found significant when it exposed to nickel, mercury and nickel plus mercury compared with control fish. The SFA content of the muscles of fishes exposed to nickel, mercury was found to be decreased (44.45, 41.75%), which was comparatively less than the control fishes (47.69%) (Table 1 and Fig. 1).
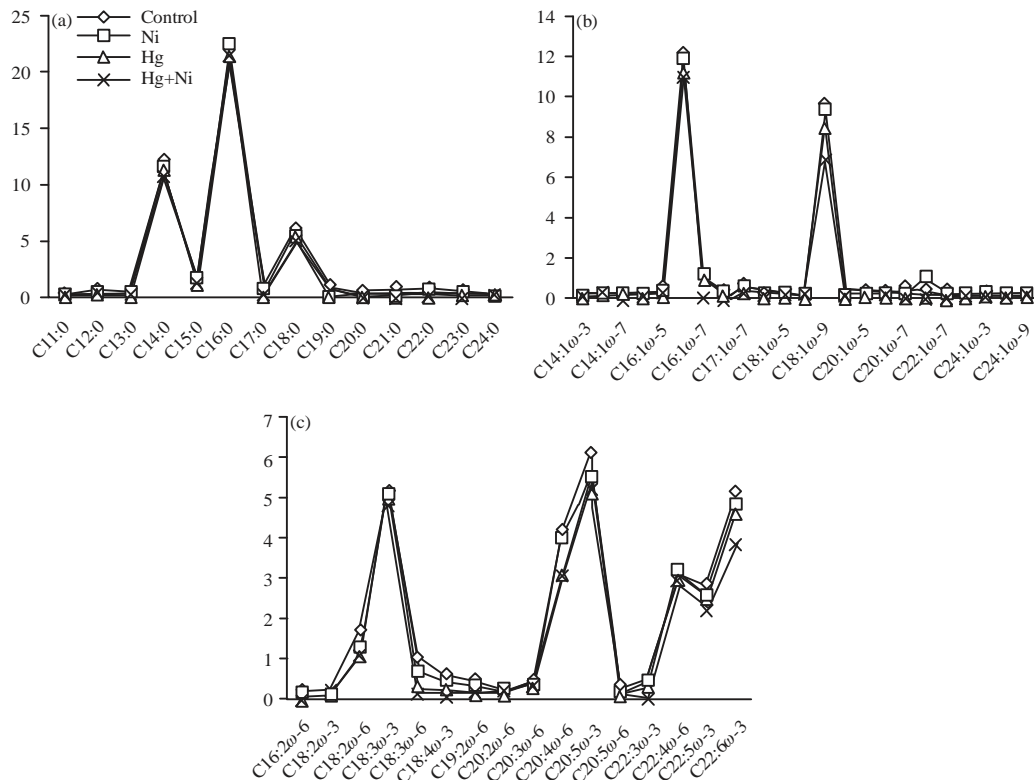

Fig. 1(a-c): Saturated, monounsaturated and polyunsaturated fatty acid in the muscles of *Lates calcarifer* during acute nickel, mercury and nickel plus mercury exposed to varying treatments, (a) Saturated fatty acids, (b) Mono unsaturated fatty acid and (c) Poly unsaturated fatty acids

Table 1: Saturated, monounsaturated and polyunsaturated fatty acid in the muscles of *Lates calcarifer* during acute nickel, mercury and nickel plus mercury exposed to varying treatments

Carbon chain	Fatty acid	Control	Ni	Hg	Hg+Ni
C10:0	Capric acid	0.12	0.06	0.16	-
C11:0	Undecyclic acid	0.16	0.14	0.12	0.11
C12:0	Lauric acid	0.58	0.48	0.39	0.31
C13:0	Tri decyclic acid	0.51	0.42	0.21	-
C14:0	Myristic acid	12.11	11.61	11.51	10.72
C15:0	Penta decyclic acid	1.73	1.61	1.22	1.11
C16:0	Palmitic acid	22.11	22.41	21.59	21.40
C17:0	Margaric acid	0.87	0.77	0.16	0.14
C18:0	Stearic acid	6.05	5.41	5.11	4.91
C19:0	Nonadecyclic acid	1.01	0.19	0.18	0.71
C20:0	Arachidic acid	0.44	0.16	0.31	-
C21:0	Heneicosanoic acid	0.72	0.18	0.18	-
C22:0	Pehinic acid	0.66	0.51	0.19	0.16
C23:0	Tricosanoic acid	0.44	0.33	0.31	-
C24:0	Lignoceric acid	0.18	0.17	0.11	0.10
Σ of SFA		47.69	44.45	41.75	39.67
C14:1ω-3	Cis-3 myristoleic acid	0.16	0.11	0.11	0.04
C14:1ω-5	Trans-5 myristoleic acid	0.34	0.31	0.29	0.22
C14:1ω-7	Cis-7 myristoleic acid	0.22	0.19	0.17	-
C15:1ω-6	Cis-6-pentadecenoic	0.17	0.11	0.12	0.10
C16:1ω-5	Cis-5-palmitoleic acid	0.44	0.24	0.19	0.18
C16:1ω-6	Cis-6-palmitoleic acid	12.11	11.89	11.11	10.84
C16:1ω-7	Trans-7-palmitoleic acid	1.10	1.06	1.00	-
C16:1ω-9	Trans-9-palmitoleic acid	0.35	0.29	0.27	-
C17:1ω-7	Cis-7-heptadecenoic acid	0.60	0.51	0.48	0.31
C17:1ω-8	Trans-8-heptadecenoic acid	0.22	0.14	0.13	0.13
C18:1ω-5	Cis-5-octadecenoic acid	0.21	0.18	0.15	0.11
C18:1ω-7	Cis-7-octadecenoic acid	0.16	0.16	0.14	0.11
C18:1ω-9	Oleic acid	9.54	9.41	8.52	6.82
C19:1ω-8	Nonadecenoic acid	0.15	0.14	0.11	0.06
C20:1ω-5	Cis-5-eicosenoic acid	0.33	0.19	0.16	0.20
C20:1ω-6	Cis-6-eicosenoic acid	0.28	0.21	0.21	0.20
C20:1ω-7	Cis-7-eicosenoic acid	0.51	0.18	0.17	-
C20:1ω-9	Trans-9-eicosenoic acid	0.33	1.02	0.11	-
C22:1ω-7	Trans-7-docosenoic acid	0.21	0.11	-	-
C22:1ω-9	Cis-9-docosenoic acid	0.18	0.18	0.11	0.14
C24:1ω-3	Cis-3-tetracosenoic acid	0.33	0.21	0.21	0.16
C24:1ω-6	Cis-6-tetracosenoic acid	0.21	0.18	0.11	0.12
C24:1ω-9	Trans-9-tetracosenoic acid	0.18	0.16	0.14	0.20
Σ of MUFA s		28.33	27.18	24.01	19.94
C16:2ω-6	Hexadecenoic	0.16	0.11	-	-
C18:2ω-3	Trans-3-linoleic	0.16	0.11	0.14	0.15
C18:2ω-6	Linoleic	1.71	1.21	1.12	1.11
C18:3ω-3	Alfalinolenic	5.12	5.01	5.03	4.91
C18:3ω-6	Gammalinoleinic	1.00	0.72	0.31	0.15
C18:4ω-3	Stearidonic	0.56	0.44	0.22	0.11
C19:2ω-6	Octadecenoic	0.44	0.31	0.14	0.13
C20:2ω-6	Eicosadienoic	0.22	0.21	0.14	0.19
C20:3ω-6	Dihomogammalinoleic	0.41	0.34	0.33	0.31
C20:4ω-6	Arachidonic acid	4.16	4.00	3.11	3.01
C20:5ω-3	Eicosapentaenoic	6.12	5.51	5.11	5.12
C20:5ω-6	Cis-6 eicosapentaenoic	0.31	0.14	0.13	0.12
C22:3ω-3	Docosatrienoic	0.51	0.44	0.31	-
C22:4ω-6	Docosatetraenoic	3.16	3.16	3.01	2.84
C22:5ω-3	Decosapentaenoic	2.81	2.53	2.51	2.21
C22:6ω-3	Docosahexaenoic	5.12	4.81	4.64	3.84
Σ of PUFAs	31.97	29.05	26.25	24.20	

However, the SFA in nickel plus mercury (39.67%) was comparatively lower than nickel and mercury treated tanks and control. Palmitic acid (C16:0%) was the primary saturated fatty acid followed by myristic acid (C14:0%).

Total MUFA content of lipids of *L. calcarifer* in control (28.33%) was higher than the treatment tanks such as nickel (27.18%), mercury (24.01%) and mercury plus nickel (19.94%). Palmitoleic acid (C16:1w-6) and oleic acid (C18:1w9) were identified. Total PUFA content of *L. calcarifer* in control (31.97%) was higher than the treatment tanks of nickel (29.05%), mercury (26.25%) and mercury plus nickel (24.2%).

DISCUSSION

Fatty acid composition and lipid metabolism depend on peroxidation in animals (Ramirez and Gimenez, 2002). In the present study, it was showed that lipid levels decreased in muscle of fish exposed to nickel, mercury and nickel plus mercury. Lipid peroxidation is the reaction of oxidative deterioration of membrane polyunsaturated fatty acids. The reduction of unsaturated fatty acid in muscle of fish in this study showed similarities to the findings of Kawamoto *et al.* (2007) and Konar *et al.* (2010).

In the present study, the SFA decreased in the muscles tissues in fish exposed to nickel, mercury and nickel plus mercury compare with control. The above supported by Konar *et al.* (2010) reported that the amounts of palmitoleic acid, oleic acid, linoleic acid, arachidonic acid, eicosapentaenoic acid, docosapentaenoic acid and docosahekzaenoic acid decreased in the skin and muscle tissues in fish exposed to the cadmium compared with control groups. The fatty acid composition might be related to the changes in nutritional habits of the fish.

Kitts *et al.* (2004) reported that the fatty acid composition and lipid contents of fish were affected by the species, sex, age, water temperature, degree of pollution, nutritional condition, seasonal variation and fish origin. The nutritional benefits of fish are mainly due to the content of high quality protein and high content of two kinds of ω-3 polyunsaturated fatty acids. Toxic heavy metals in fish can damage the positive effects of the ω-3 fatty acids present in fish and may leads to heart disease (Chan and Egeland, 2004).

Rahayu *et al.* (2014) observed that highest content of SFA was found in muscle of *Leiognathus lineoatus*, while the lowest value can be found in muscle of *Selaroides leptolepis*. Dominant fatty acid in saturated fatty acids detected was almitic acid (C16:0), which the palmitic acid content in muscle of *Leiognathus lineoatus* was 24.19%. The cadmium had reduced effects on palmitoleic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid, eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid belong to unsaturated fatty acid in muscle tissues Konar *et al.* (2010).

The total mono and polyunsaturated fatty acids significantly decreased after nickel plus mercury treatment compared to control group. This decrease may be due to metals induction of prostaglandin biosynthesis pathway (Choi *et al.,* 2002). The metabolic pathways alteration could play a significant role in decreasing polyunsaturated fatty acids levels. Weinberg (2006) suggested that the triglycerides serve primarily a storage function with toxicity deriving mainly from long chain nonesterified fatty acids and their products such as ceramides and diacylglycerols.

Saturated fatty acid level was decreased in muscles of fish exposed to nickel, mercury and nickel plus mercury. This result showed similarity with the results of Newairy *et al.* (2007) and Larregle *et al.* (2008). The decrease in saturated fatty acids content may be due to inhibition of some desaturase enzymes. Supporting this finding cadmium treatment suppressed activity of hepatic steroyl-COA desaturase (Alvarez *et al.,* 2007) as well as the activity of microsomal Δ9 desaturase (Kudo and Wake, 1996). The changes in the distribution of lipids should be associated to a change in the turnover of lipids in a medium high of oxidative stress which is known to modify the properties of membranes (Nigam *et al.,* 1999).

The generalized the EPA and DHA values in the fish *Lates calcarifer* living in polluted water were found as 0.22 and 5.12% with similar observation have been reported by Bayır *et al.* (2006) EPA and DHA values in some marine fish species living in Turkish water were 6.18 and 12.15% for bluefish, 8.74 and 20.55% for gilthead sea bream, 11.68 and 28.85% for anchovy, 7.48 and 10.57% for horse mackerel, 8.7 and 22.71% for grey mullet, 8.21 and 19.61% for atlantic bonito, 10.22 and 12.7% for mackerel, 6.14 and 34.92% for garfish, respectively. Khoshnoud *et al.* (2011) observed the first time correlation of metals concentrations with fatty acids ratios in both species was statistically analyzed and showed no correlation except for Pb mean value which had negative correlation with PUFA percentage in tiger tooth croaker species. As fish is staple food for human, the accumulation of metals exceeding the permissible limits is a serious health concern. The present study thus highlighted the heavy metal concentrations of fish *L. calcarifer* to evaluate their nutritional value.

CONCLUSION

The heavy metals in muscles of fish samples could be attributed to the industrial activities and other anthropogenic metal sources affecting aquatic habitats. Although the results revealed that fish *L. calcarifer* species are safe from the human health point of view. It is suggested that monitoring studies be periodically performed to examine the metal concentrations especially in commercial fish. As a conclusion, it may be stated that nickel, mercury and nickel plus mercury application has effects on the fatty acid components in exposed tissues.

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