

Bioaccumulation of Heavy Metals in the Selected Commercially Important Edible Fish Species Gray Mullet (*Mugil cephalus*) from Negombo Estuary

**B. R. C. Mendis¹, M. M. M. Najim^{2, #},
H. M. P. Kithsiri¹ and S. A. M. Azmy¹**

¹National Aquatic Resources Research and Development Agency,
Colombo 15, Sri Lanka.

²Department of Zoology and Environmental Management,
Faculty of Science, University of Kelaniya, Kelaniya, Sri Lanka.

#Corresponding Author:
E-mail: mnajim@kln.ac.lk

ABSTRACT

Consumption of fish contaminated with non essential heavy metals such as Pb, Hg and Cd metals which exceed the required limits pose a risk to human health. Bioaccumulations of heavy metals in fish depend on several factors such as increased levels of industrial pollution and domestic solid waste dumping in the estuary. Based on the pollution inputs into the estuary, it is considered that different localities within the estuary are polluted at different rates. The objectives of this study were to determine the non essential heavy metals concentration in fish tissues and assess the seasonal variations in the ecosystem. For this study, fish tissue samples were collected from nine sampling locations along the North (n =3), South (n=2), West (n=2), and East (n=2) parts of the estuary for a period of one year from January to December 2014. Fish tissue heavy metals levels were analyzed employing standard methods. Results revealed that the average concentrations (mg/kg) varied from Pb 0 to 0.15 ± 0.06 , Cd 0.35 ± 0.16 to 1.04 ± 0.28 and Hg 0.0005 ± 0.0004 to 0.05 ± 0.046 mg/kg respectively. The detected levels were below the maximum permissible limits for hazardous substances in fish and fishery products for recommendation of FAO. The detected lead, mercury and cadmium contents in fish tissue were below the standard limits defined for each element < 0.5 mg/kg < 0.05 mg/kg and < 0.05 mg/kg respectively. Significant differences ($P < 0.05$) were observed for Pb and Hg depending on fish tissues. The levels of Pb and Hg metals in muscle tissue were lower than the standard limit for FAO. The value obtained on Cd 1.04 ± 0.28 mg/kg concentration was much higher than the permissible threshold limit observed in the north, south, east and west regions. The most prominent increases in Pb and Cd metal concentrations in fish tissue were found during the October, November and December study of 2014, which corresponded to peak periods from October, November and December which apparently coincided with the second inter monsoon of the island respectively.

KEYWORDS: *Bioaccumulation, Fish tissues, Heavy metals, Seasonal variation*

Introduction

Fish accumulate toxic chemicals such as heavy metals directly from water and diet, and contaminant residues may ultimately reach concentrations hundreds or thousands of times above those measured in the water, sediment and food (Goodwin *et al.*, 2003). Heavy metals are normal constituents of aquatic environment that occur as a result of pollution, principally due to the discharge of untreated wastes into rivers by many industries. Bioaccumulation of heavy metals in tissues of aquatic organisms has been identified as an indirect measure of the abundance and availability of metals in the aquatic environment (Kucuksezgin *et al.*, 2006). Fish have been the most popular choice as test organisms because they are presumably the best understood organisms in the aquatic environment and also due to their importance to man as a protein source (Buikema *et al.*, 1982).

Multiple factors including season, physical and chemical properties of water can play a significant role in metal accumulation in different fish tissues (Hayat *et al.*, 2007). With increased urbanization and industrialization, there has been a rapid increase in the discharge of municipal wastewater (sewage and industrial effluents), which in turn has intensified the environmental pollution. The disposal of industrial effluents and municipal wastes is therefore a major problem in urban cities. The major sources of contamination in surface water can be traced to industrial discharges, domestic waste disposal and application of agrochemicals on farm lands (Vutukuru, 2005).

Aquatic ecosystems are very much vulnerable to water pollution. Notably aquatic ecosystems are often polluted with anomalously high levels of toxicants (organic and inorganic substances), which find their way into the aquatic systems with wastewater and effluents generated from industrial enterprises. Heavy metal accumulations in aquatic ecosystems show that they are accumulated either in aquatic organisms or in the sediments. In estuarine ecosystem, sediments are not only functioning as heavy metal scavenger, but also as one of potential sources for heavy metals to the ecosystem. Due to the capability of aquatic organisms to accumulate heavy metals, there is a possibility of heavy metals to exert their toxic effect towards the organisms. In recent years, there has been an increasing interest in the utilization of fishes as bioindicators of the integrity of aquatic environmental system. Several studies have indicated enhanced levels of both non-essential and essential heavy metal load in muscle tissues of fishes (Nayar, 2006). Thus, the contamination of fish and the aquatic environment by heavy metals is viewed with serious concern (Malik *et al.*, 2010).

These metals, after accumulation by the body of aquatic organisms, enter into food chain and extremely consumed by human. Reactions of these elements depend on the concentration, physico-chemical properties, chemical bonds and their solution on the absorption, accumulation, distribution in body and physiological effects on metals. Human mediation activities have locally and episodically introduced numerous potentially hazardous metals to the environment since the onset of

industrial revolution. These chemicals accumulate in the tissues of aquatic organisms at concentrations many times higher than concentrations in water and may be biomagnified in the food chain to levels that cause physiological impairments at higher trophic levels and in human consumers (Raposo *et al.*, 2009). Urban and industrial activities in estuary areas introduce significant amounts of heavy metals into the environment, causing permanent disturbances in estuarine ecosystems, leading to environmental and ecological degradation and constitute a potential risk to a number of flora and fauna species, including humans, through food chains (Boran and Altinok, 2010).

Water is polluted by many sources, such as accidental spillage of chemical wastes, discharge of industrial or sewerage effluents, agricultural drainage, domestic wastewater and gasoline from fishery boats (Handy, 1994). Water pollution is one of the principal environmental and public health problems in Negombo estuary in Sri Lanka (Silva, 1996). Based on the levels of Hg, Pb and Cd in the fish, edible muscle of *Etroplus suratensis* and *Ambassis commersoni* fish species collected from the estuary was found to be safe for human consumptions (Indrajith *et al.*, 2008). However, Negombo estuary is becoming polluted due to rapid industrialization and urbanization in the area. Industries, houses, boat repair stations, fuel supply stations and fish market are located along the estuary. With changing environmental conditions under increasing anthropogenic influences, especially from municipal and industrial sources pollutants including heavy metals may enter the food chain, accumulate in organisms and affect their survival. High concentration of some essential trace metals may become toxic at concentrations which exceeds the required limits (Wright and Welbourn, 2002). The metal levels in many aquatic ecosystems have been increased due to anthropogenic activities in recent years, which raise the concerns of metal bioaccumulation and related human health hazards.

Objectives

The objectives of this study were to determine concentration of non essential heavy metals in fish tissues and assess the seasonal variations of these heavy metals (rainy and non rainy periods) in the ecosystem.

Methodology

Study Site

Negombo estuary (Figure 1) is a shallow basin estuary of approximately 3,164 ha in extent, located between latitude 7° - 7°12' N and longitude 79°-79°53' E in the West coast of Sri Lanka. The exchange of water in the estuary is influenced by the tides from the ocean and freshwater supply from the island. The estuary has been indiscriminately exploited for commercial, residential and industrial development and as a sink for industrial and domestic waste. Sampling sites were selected covering the entire estuary based on the channels and pollutant inputs from

industrial, (Ja-ela and Katunayake free trade zones) tourism, domestic and municipal sources. Nine sampling locations were selected based on wastewater inputs (Figure 1).



Figure 1: Sampling sites in Negombo estuary

Three sampling locations were selected in the North region (site 1) (Figure 1). This region is being polluted due to various anthropogenic activities such as solid waste dumping, waste from industries, slaughter houses, domestic sewage outlets, shrimp farms, hatcheries and boat yards (Table 1). A South region (site 2) receives water from Hamilton canal and Dundugam Oya and carries various effluents from Ekala industrial zone (CEA, 1994). West region (site 3) receives waste from hotels, shrimp farms and fish processing industries. Eastern region (site 4) receives effluent from mainly Katunayake industrial zone, hotels and a housing scheme (Table 1).

Table 1. Description of study sites at Negombo estuary

Sampling Locations	Section of the Estuary	Input Sources (If Any)
Locations 1, 2 and 9	Northern Estuary Site 1	Municipal solid waste, industrial effluents, urban wastes, hotel outlets, fishing harbor and boat repair stations and domestic sewage outlets.
Locations 5 and 6	Southern Estuary Site 2	Two fresh water canals, Ekala industrial zone, seaplane landing site, various effluents in Hamilton canal outlet.
Locations 7 and 8	Western Estuary Site 3	Hotels, shrimp farm and fish processing industries.
Locations 3 and 4	Eastern Estuary Site 4	Katunayake industrial processing zone, hotels and housing schemes.

Sample Collection

Fresh fish samples (*Mugil cephalus*) were collected by using cast net from the sampling sites (Table 1) during the one year study period from January 2014 to December 2014. Fish samples were collected in monthly intervals during each sampling, 7 to 8 fish samples were collected from each sampling location.

Analysis of Fish Tissues

All fish samples were collected, labeled and placed in clean polyethylene bags with ice to maintain the freshness and immediately taken to laboratory. In the laboratory, the total length and weight of the fish were recorded. The fish samples were dissected to remove muscle and removed muscles were placed in glass petri dishes. The muscles were oven dried to constant weight at 105°C.

The dried fish tissues were crushed and powdered in an agate mortar and then the samples were kept in polyethylene bottles for analysis. One gram (01g) portions of fish tissues were digested by means of a microwave digestion after addition of nitric acid (65% v/v) and hydrogen peroxide (30% v/v). The digested samples were transferred into sterile sample bottles, labeled and kept for digestion and analysis of heavy metals. After complete digestion, samples were stored in pre-cleaned polyethylene bottles until analysis using atomic absorption spectrophotometer (AOAC, 2002).

Statistical Analysis

One-way ANOVA was done using Minitab version 14.0. Where differences were significant, mean values were compared by Turkey's test. The significance level was tested at the $p < 0.05$. Microsoft Excel was used to calculate mean value and standard errors.

Results

Heavy Metals in Fish

The mean concentrations and standard error of heavy metals in the muscles of *Mugil cephalus* are presented in Table 2.

In the present study, results of measured metals in the edible muscle tissues that were collected from Negombo estuary range from Pb 0 to 0.3, Cd 0.35 to 1.04, and Hg 0.0005 to 0.05 mg/kg, respectively. The highest level of Cd level in tissues were found at the sampling site 1, whereas levels of Hg was high in the site 4 located in the east region (Table 2).

Table 2. Mean concentrations \pm SE of heavy metal in muscle tissue (mg/kg) of fish species

Sites	Pb (mg/kg)	Cd (mg/kg)	Hg (mg/kg)
Site 1	0.15 \pm 0.06	1.04 \pm 0.28	0.03 \pm 0.018
Site 2	0.30 \pm 0.19	0.45 \pm 0.28	0.0005 \pm 0.0004
Site 3	0.25 \pm 0.04	0.55 \pm 0.48	0.001 \pm 0.0005
Site 4	0.0 \pm	0.35 \pm 0.16	0.05 \pm 0.046

The detected lead and mercury contents in fish tissues were below the standard limits defined by FAO for each element are < 0.5 mg/kg, and < 0.05 mg/kg, respectively (FAO, 1983). The mean concentration of Cd was above the standard limits for all the sites.

Table 3. Mean concentrations \pm SE of monthly variation of heavy metal concentration in muscles (mg/kg) of fish species

Months	Pb(mg/kg)	Cd(mg/kg)	Hg(mg/kg)
January	0.157 \pm 0.631	0.06 \pm 0.006	0.03 \pm 0.002
February	0.3 \pm 1.94	0.45 \pm 0.04	0.0005 \pm 0
March	0.25 \pm 0.044	0.55 \pm 0.05	0.001 \pm 0.0005
April	0.1 \pm 0.01	0.35 \pm 0.01	0.05 \pm 0.004
May	0.5 \pm 0.05	1.1 \pm 0.11	0.01 \pm 0.005
June	0.5 \pm 0.05	0.43 \pm 0.04	0.09 \pm 0.008
July	0.1 \pm 0.01	1 \pm 0.19	0.04 \pm 0.003
August	0.003 \pm 0.002	0.008 \pm 0.065	0.01 \pm 0.002
September	0.03 \pm 0.002	0.09 \pm 0.008	0.04 \pm 0.002
October	0.4 \pm 0.03	1.6 \pm 0.19	0.08 \pm 0.007
November	0.6 \pm 0.06	1.4 \pm 0.15	0.09 \pm 0.01
December	0.03 \pm 0.003	1.2 \pm 0.35	0.05 \pm 0.045

Most prominent increments in Pb and Cd metal concentrations in fish tissues were found during October, November and December, which corresponds to second inter monsoon.

Discussion

Fish is an important food resource for human consumption and a major component of the brackish ecosystem, thus assessment of the heavy metal content in fish species is particularly important (WHO/FAO, 2011). The estuary is susceptible to chemical pollution due to ongoing development activities of Negombo and Katunayake areas (CEA, 1994). Monitoring of heavy metal pollution in Negombo estuary would be important to human health because of the fishery of the estuary (Indrajith *et al.*, 2008). Heavy metals may pollute fish in different ways and absorbed metals could accumulate in various organs of the fish body. The increase in mean concentration of heavy metals in the samples could be attributed to more bioaccumulation due to metal concentration arising from reduced water volume during the dry season (Goodwin *et al.*, 2003). This high level of Pb and Cd accumulated by the fish species may be attributed to the fact that Pb and Cd occur at high levels in organic matter at the bottom of the estuary (Yilmaz, 2009).

The present study revealed that levels of heavy metal variations exist in different regions of the estuary, with respect to Pb, Hg and Cd contents in fish tissue. The levels of Pb, Cd and Hg in fish tissues collected from some sampling sites located in the North region were higher compared to the other regions. Northern region of the estuary is being polluted due to various anthropogenic activities such as effluents and solid waste dumping from industries and households. The status of sediments in the Northern region is affected by tidal waves, sedimentation and human activities. The Southern region receives water mainly from the Jaela canal and Dandugam oya and they carry various effluents from Ekala industrial zone (CEA, 1994). Eastern region receives effluents from Katunayake industrial processing zone, hotels and housing schemes. Hence the levels of metals in Pb and Cd were high in this region. Lead, Cd and Hg are taken up passively from the water and deposited in the organisms and hence the organisms contain more of these metals than the concentrations in water. Seasonal variation could influence the accumulation of metals by the fish species and could increase bioaccumulation of these heavy metals in fish tissues increasing the actual dose of metal to which the local population is exposed (Chakraborty *et al.*, 2003).

Conclusion

In conclusion, the present study found that the fish surviving at highly polluted areas accumulate higher levels of toxic heavy metals than those living at less polluted areas. Finding of this study is revealed that heavy metal levels in the selected fish species from Negombo estuary followed an increasing order of accumulation as $Cd > Pb > Hg$. Based on the levels of toxic non essential heavy metals viz. Pb, Cd and Hg are biologically non essential metals which are accumulated in human tissue and harmful to human health. Hence, there is a necessity to regularly monitor heavy metal pollution in Negombo estuary as there are multiple sources of heavy metal contamination in the vicinity of the estuary. Concentration of heavy metals in the Negombo estuary are of concern in the inter-

monsoonal period. The high concentrations of heavy metals are mainly due to the discharge of untreated effluents from different industries. Based on the levels of heavy metals, Hg and Pb in the edible muscle of fish collected from the estuary were found to be safe for human consumption.

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