An integrated use of histological and ultra-structural biomarkers in *Mugil cephalus* for assessing heavy metal pollution in east Berbice-Corentyne, Guyana

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**Abstract:** Bioaccumulation of heavy metals and its associated histopathological perturbations were studied in various tissues of *Mugil cephalus* collected from polluted and compared with the fish collected from less polluted of Corentyne coast. The concentration of copper, lead, zinc, cadmium, manganese and iron were quantified in gills, liver and muscle. The results showed marked differences between the two sites as well as significant variations within the tissues. The decreasing trend of metals in the tissues of fish sampled from both polluted and less polluted sites was in the order of Fe > Mn > Zn > Cu > Pb > Cd. Overall, the highest metal concentrations were found in the fish collected from polluted site. The accumulation in the gills and liver of *M. cephalus* was found to be quite high in comparison with the muscle. These tissues were further investigated by light and electron microscopy and the results were compared with the reference site (Less polluted). The presence of large lipid droplets in liver and increase of mucous cell in gill were some of the most noticeable alterations observed and were related to heavy metal contaminates. It is concluded that histopathological and ultrastructural biomarkers provide reliable and discriminatory data to augment heavy metal pollution in polluted site of Corentyne coast. Therefore, long-term monitoring is necessary to assess the untreated waste water discharged directly and indirectly into the water ways of Corentyne coast led to a reduction in waste assimilative capacity of the coastal marine waters, which provide accurate, reliable measurements of environmental quality.

**Key words:** Bioaccumulation; Heavy metals; *Mugil cephalus*; Histopathology; Ultrastructure.

**INTRODUCTION**

The wide diversity of human activities introduce pollutants into the environment, as well as their magnitudes make the assessment of environmental impact a subject of utmost interest (Marcovecchio, 2004). The presence of metals in aquatic ecosystems originates from the natural interactions between the water, sediments and atmosphere (Kalay and Canil, 2000; Sankar et al., 2006). Heavy metals may enter an aquatic ecosystem from different natural and anthropogenic sources, including industrial or domestic sewage, storm runoff, leaching from landfills, shipping and harbor activities and atmospheric deposits (Nair et al., 2006). The natural resources of water like rivers, ponds, lakes and seas are heavy metal polluted with a variety of solid and liquid water. Every waste is ultimately dumped or emptied in natural water bodies (Garg et al., 2008). The study of organisms as pollutant monitors has several advantages over the chemical analysis of abiotic compartments (Fernandes et al., 2007). Organisms can only accumulate the biologically available forms of the pollutants that are always present in the environment, thus enabling the continuous monitoring of pollutants. Organisms integrate fluctuations of pollutant concentration through time and the magnification afforded by bioaccumulation may be advantageous concerning the accuracy and expense of analysis of trace pollutants near the limits of analytical detection. Heavy metals are considered the hazardous inorganic and organic pollutants in the coastal environment (Akar and Tunali, 2005; Obasohan, 2007; Sivaperumal, 2007).

The concentrations of heavy metals in the various parts of organisms are determined primarily indicative of the level of the pollution in the environment (Canbek et al., 2007). Aquatic organisms are widely used to monitor environmental health due to anthropogenic impacts (Evans et al., 1993; Hellawell, 1986; Rashed, 2001). Urban streams are one of the ecosystems most hit by the contamination resulting from human activity (Paul and Meyer, 2001). Agricultural, industrial and domestic effluents generally contain a wide variety of organic and inorganic pollutants, such as solvents, oils, heavy metals, pesticides, fertilizers and suspended solids (Pandey et al., 2003) and are, invariably, discharged into small rivers and streams, without proper treatment. Such contaminants change water quality and may cause many problems to fish, such as diseases and structural alterations (Chang et al., 1998). A variety of effects, including disease, have been observed in marine fishes, crustaceans, and molluscs from contaminated environments (Sindermann, 1979; Longwell et al., 1992, 1996; Fournie et al., 1996). Neoplastic diseases of the integument (epidermal papilloma, squamous cell carcinoma) and liver (hepatocellular adenoma and carcinoma, cholangioma and cholangiocarcinoma, mixed hepatobiliary carcinoma), particularly in bottom-dwelling fishes, are more prevalent in coastal areas than in areas which are relatively pristine (Deys, 1969; Peters, 1975; McCain et al., 1977; Smith et al., 1979; Malins et al., 1984; Vogelbein et al., 1990; Johnson et al., 1993; Myers et al., 1994; Moore et al., 1996; Vethaak and Wester, 1996). Liver neoplasia, sometimes in epizootic proportions, has been reported in at least 15 species of feral and hatchery-reared fish in North America, Japan, and Europe. Reviews by Couch and Harshbarger (1985), Mix (1986) detail numerous examples of the effects of environmental and experimental carcinogenic agents.
In the marine environment, toxic metals are accumulated in sediments, marine organisms, and subsequently transferred to man through the food web. Thus, it has become increasingly important to determine and assess levels of heavy metals in marine organisms because of nutritional and safety conditions. This is true especially for edible marine organisms as they are a potential dietary source of protein (Blasco et al., 1999). According to Zya'adah and Chouikh (1999), knowledge of the distribution of metals in isolated tissues of marine organisms is useful in order to identify specific organs that may be particularly selective and sensitive to the accumulation of heavy metals. Metals do not degrade in general; therefore, they accumulate throughout the trophic chain. Metals are introduced into the aquatic ecosystems such as lakes, river and sea in a number of ways. The essential metals can also produce toxic effects when the metal intake is excessively elevated (Wagner and Boman, 2004; Turkmen and Turkmen, 2005). Accumulation in living organisms leads to concentrations to several orders of magnitude higher than those of the surrounding water (Casas et al., 2008).

Fishes have been proposed as sentinel species for the biomonitoring of land-based pollution because they may accumulate hydrophobic organic compounds in their tissues, directly from water, sediments and/or through their diets. Heavy metals accumulate in tissues and may pose a health risk to those who frequently consume fish. In the organism, xenobiotic compounds undergo a series of biotransformation reactions catalysed by different enzymatic systems, their activation may provide additional evidence for pollution exposure (Mormede and Davies, 2001). Intensive industrial and agricultural activities have inevitably increased the levels of heavy metals in natural waters (Jordao et al., 2002). For these reasons, it is important to determine the concentrations of heavy metals in commercial fish in order to evaluate the possible risk of fish consumption for human health (Cid et al., 2001).

Fish form an important part of human food and it is therefore not surprising that numerous studies have been carried out on metal pollution in different species of edible fish (Lakshman and Nambisan, 1983; Senthilnathan and Balasubramanian, 1998; Sultana and Rao, 1998). Fishes are often at the top of the aquatic food chain and may concentrate large amounts of some metals from the water (Mansour and Sidky, 2002). Heavy metals are taken up through different organs of fish and many are concentrated at different levels in different organs of the body (Scharenberg et al., 1994; Bervoets et al., 2001; Rao and Padmaja, 2000). Previous studies have employed the golden grey mullet (Liza aurata) as a model species for field studies (Oliveria et al., 2009) and under laboratory conditions (Oliveria et al., 2007). In fact, L. aurata has favourable features as a sentinel of contaminated particulate matter (SPM) in the water column. Moreover, mullets play an important role in the estuarine trophic web (Almeida, 2003).

An increasing amount of research is now incorporating histopathological biomarkers in practical ecological risk assessment methodologies (Wester et al., 2002). Histopathology has received increasing interest as an endpoint because histopathological changes are often the result of the integration of a large number of interactive physiological processes (Van der Oost et al., 2003). Histopathological analysis has already been tested and proposed as an efficient and sensitive tool in the monitoring of fish health and environmental pollution in natural water bodies (Teh et al., 1997). The studies on histopathological biomarkers are linked to the notion that they reflect fish health more realistically than biochemical biomarkers and can thus be better extrapolated to community and ecosystem-level effects of toxicity (Au et al., 1999). Cells have evolved different network of cellular stress responses to adapt during environmental changes and survive combating a wide variety of stress (Padmini and Usha Rani, 2010). Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory (Wester and Cantor, 1991; Thophon et al., 2003) and field studies (Hinton et al., 1992; Schwager et al., 1997; Teh et al., 1997). One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs, including gills, kidney and liver, that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish (Gernhofer et al., 2001). Furthermore, the alterations found in these organs are normally easier to identify than functional ones (Fanta et al., 2003), and serve as warning signs of damage to animal health (Hinton and Laurèn, 1990).

Electron microscopic studies (Carmona et al., 2004) have revealed marked variability between different teleost species in the morphology of the apical surface membrane of chloride cells, which all share a distinctive appearance that distinguishes them from adjacent pavement cells. Many studies have analyzed the effects of environmental salinity on the morphology and ultrastructure of cells that constitute the gill epithelium in teleosts (Carmona et al., 2004). Ultrastructural responses in different tissues of vertebrates and invertebrates are useful tools to characterize the health of organisms (Triebeskorn et al., 1997) and also to assess the impact of environmental contaminants on organisms exposed in the laboratory (Alazemi et al., 1996; Braunbeck and Appelbaum, 1999). Biomarkers have proven to be sensitive, short-term indicators of environmental pollution which display little temporal variation and integrate effects of a variety of different stressors including environmental contaminants (Triebeskorn et al., 1997). Previous studies reported that the exposure of fish to pollutants (agricultural, industrial and sewage) resulted in several pathological alterations in different tissues of fish (Abbas and Ali, 2007). The liver, as the major organ of metabolism, comes into close contact with xenobiotics absorbed from the environment and liver lesions are often associated with aquatic pollution. Histopathological changes were observed in the gills of many fish as a result of exposure to different toxicants (Camargo and Martinez, 2006 and Abbas and Ali, 2007).
In recent years, the discharge of effluents from major industries including fertilizers, motor vehicles, oil refineries and operations of the second major harbour for coal import, which includes a Guyuco thermal power plant and situated nearby, have been growing rapidly and industries like rum, beer, foundries and general engineering have converted Guyana into a major industrial hub severe stress on has imparted severe stress on marine ecosystem. Several countries, regions and cities have enacted legislation to ban or severely reduce the use of disposable plastic bags. Outright bans have been introduced in only a few countries, notably China, which banned very thin plastic bags in 2008. Data from City Council in 2009 shows that approximately 83,000 tons of waste is generated annually in Georgetown; of this, about 50 per cent is organic food and garden waste; 21 per cent comprises plastic bags and 10 per cent other plastic containers; three per cent plastic bottles; six per cent disposable diapers and two per cent Styrofoam. Waste is collected from approximately 42,000 households and about 2000 institutions and retail business (Guyana Times Feb 22, 2013). Due to an ever increasing population and the development of major industries during the past three decades, the ecosystem surrounding Corentyne coast has been distorted severely (Rajeshkumar et al., 2015). The present work is the first attempt to explore the influence of heavy metal mediated stress via histology and ultrastructural alterations in the vital tissues of grey mullets inhabiting a highly contaminated estuary which is challenged by several industries surrounding this site. Hence, the objective of the present study is, therefore, aimed at comparing heavy metal pollution Corentyne coast, east Berbice of Guyana.

**Materials and Methods**

**Study area**

The East Berbice-Corentyne (5º57’ to 10º N and 57º08’ 01 to 32º W) is one of six regions in Guyana covering the whole of the east coast of the Berbice. It borders the Atlantic Ocean to the north, Suriname to the east, Brazil to the south and the regions of Mahaica-Berbice, Towns in the region include New Amsterdam, Corriverton, Mara and Rose Hall. The Corentyne River forms the whole of the eastern border with Suriname, though the southern-most section is disputed territory known as the New River Triangle. It is a shallow area with an average depth of 5 to 7 m. The temperature and salinity if this Corentyne coast under study ranged between 25-30°C and 25-29 ppt respectively. The rapid development of Corentyne coast in the last two decades has put additional stress on the local aquatic environment. The main source of metal input to Corentyne coast is via the discharge of waste water effluents, chemicals, fertilizers and Guyusco thermal power plant situated very close to the creek which drains the effluent directly into it (Rajeshkumar and Munuswamy, 2013). Based on the criteria and strategic location of the stations, they are categorized as polluted (Station 1 & 2) and less polluted (Stations 3 & 4). Hence, throughout the study it will be detailed that stations 1 and 2 are polluted and station 3 and 4 less polluted of Corentyne coast (Fig.1).

![Location map of the study area - East Berbice - Corentyne Coast, Guyana.](image)

**Sampling**

*M. cephalus* (grey mullet), a natural inhabitant of the estuaries, was chosen as the experimental animal for the study according to the Food and Agriculture Organization (FAO) species identification sheets (Fischer and Bianchi, 1984), Grey mullet (n=12) were collected with an average length of 30-32 cm were collected from less polluted and polluted east Berbice-Corentyne Coast using baited minnow traps and brought to the laboratory on the same day. Samples of gills, liver and muscle from each specimen were dissected, washed with distilled water, weighed, packed in polyethylene bags and stored at -20 °C for 24h. After complete dryness the tissues were homogenized with mortar and pestle separately. The dried powder tissue samples were then weighed accurately to approximately 2 g. The samples were transferred to a 25 mL conical flask, to which 10 mL of 4:1 (v/v) nitric acid and perchloric acid mixture were added. Each comical flask was then covered with a watch glass and allowed to react overnight at room temperature. Then the simples were digested to near dryness by evaporating liquid at 90 °C on a hot plate and cooled to room temperature. The digested samples were then filtered through Whatman No. 1 filter paper and collected in 50 mL beakers. The filters were rinsed thoroughly with deionized water. Content of the beakers were determined by Atomic Absorption Spectrometer (Perkin-Elmer, AA 800) and are expressed as µg g⁻¹ dry weight of tissue (Kingston and Jassie, 1988 and Rajeshkumar et al., 2013). The accuracy of the analytical procedures was verified by analysis of appropriate CRMs using the same digestion and analytical methods. Quantitative results were obtained for each metal in each CRM (Table.1).
Table 1: Measured and certified values of heavy metal concentration, as µg g⁻¹ dry weight, in standard reference material BCSS-1 and DORM-2 (dogfish muscle).

<table>
<thead>
<tr>
<th>Reference material</th>
<th>Certified values</th>
<th>Measured value</th>
<th>Percentage of recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCSS-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>19</td>
<td>18.1</td>
<td>95.4</td>
</tr>
<tr>
<td>Lead</td>
<td>22.7</td>
<td>21.6</td>
<td>96.2</td>
</tr>
<tr>
<td>Zinc</td>
<td>119</td>
<td>115.6</td>
<td>96.2</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.25</td>
<td>0.24</td>
<td>96.0</td>
</tr>
<tr>
<td>Manganese</td>
<td>10.5</td>
<td>10.3</td>
<td>98.10</td>
</tr>
<tr>
<td>Iron</td>
<td>184</td>
<td>191</td>
<td>103.80</td>
</tr>
<tr>
<td>DORM-2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>2.34 ± 0.16</td>
<td>2.32</td>
<td>99.1</td>
</tr>
<tr>
<td>Lead</td>
<td>0.065 ± 0.007</td>
<td>0.0665</td>
<td>100</td>
</tr>
<tr>
<td>Zinc</td>
<td>25.6 ± 2.3</td>
<td>25.2</td>
<td>98.4</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.043 ± 0.008</td>
<td>0.042</td>
<td>99.5</td>
</tr>
<tr>
<td>Manganese</td>
<td>10.0 ± 0.005</td>
<td>10.3</td>
<td>98.10</td>
</tr>
<tr>
<td>Iron</td>
<td>184.0 ± 0.004</td>
<td>191.0</td>
<td>103.80</td>
</tr>
</tbody>
</table>

**Histology**

Samples of muscle, gills and liver were quickly removed from the fish and fixed in 5% neutral buffered formaldehyde solution (pH 7.0). After fixation, the tissues were dehydrated through a graded alcohol series and embedded in paraffin wax. Tissues sections 6-8 µm thickness were taken and stained with haematoxylin and cosin. The stained sections were mounted using DPX and photomicrographs were taken Leica 2500 microscope (Germany) (Rajeshkumar and Munuswamy, 2011).

**Scanning electron microscopy**

Gill samples from the *M. cephalus* were removed and sectioned transversely into two halves and transferred to fixative (gluteraldehyde 4% in buffered phosphate, pH 7.5) for 1 h. Subsequently, the gills were quickly rinsed with distilled water and submitted to sequential ethanol/acetone dehydration. Afterwards, they were dried repeatedly in a critical point drying apparatus (Balzers) with liquid CO₂, then coated with gold, and finally examined with a Cambridge Stereoscan S100 scanning electron microscope.

**Statistical analysis**

Two- way analysis of variance was performed using SPSS 7.2 version statistical package to determine significant of heavy metal concentrations, among the tissues and between sites. A probability level below *p* < 0.05 was considered as statistically significant.

**RESULTS**

**Bio accumulation**

Levels of copper, lead, zinc, cadmium, manganese, and iron in gills, liver and muscle of *M. cephalus* caught in the polluted and less polluted sites from Corentyne coast waters are presented in Figs 2a-2f. The mean metal concentrations in gills of *M. cephalus* collected from polluted sites were recorded as 0.124 ± 0.007 µg Cu g⁻¹, 0.139 ± 0.007 µg Pb g⁻¹, 0.490 ± 0.099 µg Zn g⁻¹, 0.127 ± 0.014 µg Cd g⁻¹, 0.145 ± 0.013 µg Mn g⁻¹, 0.177 ± 0.040 µg Fe g⁻¹. The levels of Cu (1.906 ± 0.177 µg g⁻¹), Pb (0.122 ± 0.002 µg g⁻¹), Zn (3.880 ± 0.813 µg g⁻¹), Cd (0.137 ± 0.010 µg g⁻¹) Mn (0.364 ± 0.050 µg g⁻¹), Fe (4.498 ± 0.284 µg g⁻¹), appeared higher in liver, whereas the metal levels in muscle in muscle recorded as 1.206 ± 0.168 µg Cu g⁻¹, 0.058 ± 0.016 µg Pb g⁻¹, 0.324 ± 0.043 µg Zn g⁻¹, 0.153 ± 0.011 µg Cd g⁻¹, 0.278 ± 0.136 µg Mn g⁻¹, 0.152 ± 0.014 µg Fe g⁻¹.

However, the concentration of metals in gills of *M. cephalus* collected from less polluted sites were reported to be 0.099 ± 0.011 µg Cu g⁻¹, 0.127 ± 0.118 µg Pb g⁻¹, 0.306 ± 0.034 µg Zn g⁻¹, 0.082 ± 0.010 µg Cd g⁻¹, 0.121 ± 0.005 µg Mn g⁻¹, 0.142 ± 0.005 µg Fe g⁻¹. The accumulation of Ca (1.413 ± 0.232 µg g⁻¹), Pb (0.127 ± 0.010 µg g⁻¹), Zn (1.605 ± 0.458 µg g⁻¹), Cd (0.125 ± 0.007 µg g⁻¹), Mn (0.229 ± 0.025 µg g⁻¹), and Fe (3.775 ± 0.138 µg g⁻¹), were higher in liver than on other organs. In muscle the metal levels were comparatively low; Cu (0.157 ± 0.006 µg g⁻¹), Pb (0.035 ± 0.007 µg g⁻¹), Zn (0.223 ± 0.052 µg g⁻¹), Cd (0.039 ± 0.003 µg g⁻¹), Mn (0.194 ± 0.034 µg g⁻¹), Fe (0.142 ± 0.047 µg g⁻¹).

Different tissues showed different capacities for accumulating heavy metals. Overall, the fish collected from polluted sites showed higher levels for metals compared to samples from less polluted sites. According to organs, the highest metal concentration was found in the liver and gills of fishes from both sites followed by muscle. The mean concentration of trace metals analyzed in various organs of *M. cephalus* from polluted and less polluted sites were seen in the order of Fe > Zn > Mn > Cu > Pb > Cd. Analysis of variance showed that the mean concentrations of heavy metals between the sites and tissues were significantly different (*p* < 0.05).

**Fig 2a.** Concentration (µg g⁻¹) of Copper in gills, liver and muscle of *M. cephalus* collected from Polluted and Less Polluted sites. The value in a bar with different letters denoted significant difference (*p*<0.05).

**Fig 2b.** Concentration (µg g⁻¹) of Cadmium in gills, liver and muscle of *M. cephalus* collected from Polluted and Less
Polluted sites. The value in a bar with different letters denoted significant difference ($p<0.05$).

**Fig 2c.** Concentration ($\mu g \text{g}^{-1}$) of Lead in gills, liver and muscle of *M. cephalus* collected from Polluted and Less Polluted sites. The value in a bar with different letters denoted significant difference ($p<0.05$).

Histological and ultrastructural observations

Gills: The histarchitecture of the gills of fish collected from less polluted offshore showed the primary lamellae arranged in double rows, projecting towards the lateral side with a series of alternately arranged secondary lamellae (Fig. 3A). This is common for unaffected teleost gills. The gills of fish collected from polluted showed aneurism or nodule formation in the secondary lamellae and hypertrophy is observed with the enlargement of the tissues. The lamellae fused together and necrosed with mucoid depositions along the surface. Damage was more pronounced with swelling of lamellae and epithelial lifting in the interfilamentar regions. The cartilaginous rod at the core of primary lamellae was seen to be disrupted (Fig. 3B and C).

Scanning electron micrographs confirmed the results obtained by light microscopy. The ultra-thin section of control fish (collected off less polluted) gills showed a smooth surface topography and organized arrangement of primary secondary lamellae with uniform inter lamellar space. Each one supports many filaments (primary lamellae) in two rows called hemi-branches (Hughes, 1966). The gills arches, filaments and lamellae are seen without any marked surface damage (Fig. 3D). However, the fish collected from polluted showed distorted appearance of primary and secondary lamellae are broken. Overproduction of mucous resulting in the formation of a sheath over the lamellae. The inter lamellar space are shown to be filled with sponge-like tissue and also forming the sheath over the adjoining lamellae onsets at the distal part of the secondary lamellae, leading to local hyperplasia (Fig 3E and F).
Fig. 3. (A–C) Photomicrographs of gills of *M. cephalus* stained with hematoxylin and eosin. Control (Gills of fish) showing the normal architecture of gill filaments such as Primary lamellae (PL) and secondary lamellae (SL) covered by filamentary epithelium (EF) which is perpendicularly intersected by lamellae (IL). (B) Fish collected from polluted sites showing aneurism (A) mucous deposition and hypertrophy (H). (C) Fusion of secondary lamellae (FSL), ruptured epithelial layer (EL), Lifting of Primary lamellae (PL), lamellar swelling (L) and necrosis in the inter filamental region (N). Scale bar = 50μM. (D and F) Scanning electron micrographs of gills of *M. cephalus* collected from less polluted site showing normal architecture and surface topography of gills with sequential arrangement of primary lamellae (PL), secondary lamellae (SL), uniform inter lamellae space (ILS) and Gill lamellae (GL). (E). Fish from polluted site showing disrupted primary lamellae (PL) and broken secondary lamellae (SL). (F) Hyperplasia (H), swelling and enlargement of irregular inter lamellar space (ILS) and mucous deposition (M).

Fig. 4. (A–C) Photomicrographs of liver of *M. Cephalus* stained with haematoxylin and eosin. (A) Control fish liver showing normal parenchymal architecture of hepatocytes (HP) and blood sinusoids (BS. (B) Fish collected from polluted sites showing vacuolization in the hepatocytes (HP), fibroblast proliferation (F), vacuole formation (V) and granular degeneration (GD). (C) Necrosis (N) and vacuolization (V). Scale bar = 50μM.

Fig. 5. Section through the muscle of *M. cephalus* stained with hematoxylin and eosin. (A) Control muscle showing normal arrangement of muscle fiber (MF) and uniform muscle bundles (MB). (B) Muscles of fish collected from polluted sites showing loss and swelling of muscle fiber layer (MF), (C) Breakdown of muscle bundles (MB) and Splitting of muscle fibers (SMF). Scale bar = 50μM.
Liver

Section through the fish liver from the reference site exhibited normal parenchymal architecture of hepatocytes, which contained homogenous cytoplasm with a centrally placed nucleus. Liver composed of masses of hepatocytes organized in distinct lobules and were interrupted by sinusoids and endothelial cells lining the sinusoidal lumen (Fig. 4A). Fish liver collected from polluted showed vacuolization in the hepatocytes and proliferation of fibroblast. There was an increase in fat vacuolation and granular degeneration. Hepatocellular necrosis was obvious in the hepatocytes. The hepatocellular were shrunken with engorged sinusoidal blood spaces and granular degenerations became evident in most of the hepatocytes (Fig. 4B and C).

Muscle

The section of muscle of fish from reference site exhibited normal arrangement of muscle bundles and muscle fibres with well-organized connective tissues (Fig 5A). In contrast, the fish collected from polluted sites exhibited degenerative deformities and necrotic changes in the muscle tissue include connective tissue damage, splitting of muscle fibres and formation of edema between muscle bundles (Fig.5B and C).

DISCUSSION

The present study documents heavy metals contamination in impact on histological and ultra-structural changes in M. cephalus inhabiting the polluted and less polluted sites of Corentyne Coast. However, the concentrations may be raised in coastal ecosystems due to the release of industrial waste, agricultural and mining activities. As a result, aquatic organisms were exposed to elevated levels of heavy metals (Kalay and Canil, 2000; Sankar et al., 2006). Knowledge of heavy metal kinetics in fish is important for natural resource management and the use of fish for human consumption (Karadede et al., 2004). The aquatic organisms exposed to heavy metals from the run-off water tend to accumulate it in their body but fishes are more commonly affected than other species (Guven et al., 1999; Henry et al., 2004).

The high metal concentration in the tissues of fish inhabiting Corentyne Coast is probably related to a high influx of metals as a result of pollution from the surrounding industries thereby increased bioavailability to the fish. Some authors have previously demonstrated the pollution stress status of Corentyne Coast and accumulation of heavy metals in fish inhabiting such coastal during different seasons (Rajeshkumar and Munuswamy, 2013). Consistent with these, in this study, we provide evidence that heavy metal contaminants differentially modulate the structure of vital organs of M. cephalus inhabiting in polluted sites of Corentyne Coast. During direct contact with contaminants, most of the chemicals were taken up into the organism by diffusion or actively through semi-permeable membranes of the gills and gut epithelia (Artickia Vasanthi et al., 2013; Fanta et al., 2003).

Histopathological damage and structural changes are evident in M. cephalus by especially with metals. In gills, fusion of primary, secondary lamellar epithelium and adjacent secondary lamellae are evident. Through the gills, as the main site of xenobiotic transfer, the toxins are distributed through their bodies accumulating in tissues and organs and may have deleterious effects (Arockia Vasanthi et al., 2013). Earlier reports explain varying degrees of histopathological changes in the gills filament (Jiraungkoorskul et al., 2002). Muscles of fish collected from the polluted sites showed loss of muscle fibre as well as swelling of muscle fibre layer. Normal architecture of muscle bundle seem to be distorted with breakdown of muscle bundles. Earlier studies have demonstrated that muscles are very sensitive to contaminants and their immune system can act as an early warning system of stress (Anderson, 1990; Dyrnyda et al., 1997; Sauves et al., 2002).

Similarly, the other vital organ ‘liver’ showed vacuolization, necrosis and nuclear condensation in hepatocytes of the fish collected from polluted sites. Similar observations on histological responses have been reported in the liver of various fish species (Cyprinus carpio) (Morsey and Protsawwicki, 1990). Hepatocytes showed increased vacuolation associated with lipid accumulation and congestion of blood vessels, in the fish liver, probably due to pollutants. However, these histological changes were associated with the response of hepatocytes to toxicants (Hinton and Lauren, 1990). The liver being the site for detoxification of pollutants, it is more susceptible for damage (Bernet et al., 1999). The observations recorded in the present study are similar to that reported on Liza Salien by Fernandes et al., (2007).

The concentration of metals was observed to be significantly higher during dry than during the rainy. These seasonal low values may be attributed to freshwater input following rain as well as due to the release of surplus water from the Surinam reservoir into the sea via Corentyne Coast, while the higher values in summer were due to evaporation raising the metal concentrations (Guyana Times Feb 22, 2013; Murthy and Rao, 1987). In an earlier study, lower metal concentrations were observed during rainy and higher concentrations during dry (Caccia et al., 2003). Once metals passed through the penetration barriers, they were transferred to the blood stream. From the results, it become obvious that the bioaccumulation was highly pronounced in gills and liver compared to muscle. This situation was also determined experimentally in Liza macrolepis (Chen and Chen, 1999). Relatively high concentrations of heavy metals in liver and gills were also found in various tissues of fish Cathangis spicai inhabiting the Point Lisas Harbors, Trinidad and Tobago (Mohammed et al., 2012).

The concentration of metals in the gill reflects the concentrations of metals in the waters where the fish species live, whereas the concentrations in liver represent storage of metals (Rao and Padmaja, 2000). The absorption of metals at the gill surface, as the first target for pollutant in water, could also have an important influence on total metal levels within gills (Heath, 1987). Studies carried out with different fish species have shown that heavy metals
accumulate mainly in metabolic organs such as liver that stores metals to detoxify by producing metallothioneine (Kargin and Erdem, 1991; Hogstrand and Haux, 1991). Thus, liver and gill are more often recommended as environmental indicator organs of water pollution than other organs. This is possibly attributed to the tendency of liver and also the gills to accumulate pollutants at different levels from their environment as previously reported (Al-Yousuf et al., 2000; Canli and Atlı, 2003). The accumulation of lead, zinc and iron are high in the gills due to body’s defense mechanism and this organ forms the principal route for entry of pollutants from water. The metal concentration in muscle tissue is important for the edible parts of the fish. The mean concentrations of heavy metals analysed in the fish collected from less polluted sites was lower than the maximum permissible limits proposed by FAO (1983).

However, the metal concentrations in the fish obtained from polluted sites showed more than the permissible limit. The concentration of cadmium obtained in the fish from pollutes sites crossed the upper limit of 1.0 µg g⁻¹ for fish used for human consumption set by EU (2001). Among the metals, Fe had the highest mean value in liver and Cd was lowest in muscle tissue. There are several possible reasons explaining lower accumulation of metals in muscle. Firstly, the muscle does not come into direct contact with the toxicant medium as it is totally covered by skin which helps the organism avoiding the penetration of the toxicant. Similar results have been reported from a number of fish species that the muscle in not active tissue in accumulating heavy metals (Karade and Unlu, 2000). The very high Fe level recorded in the present study could also be attributed to haemoglobin found in highly vascularised liver tissues of M. cephalus. Similarly, the maximum level of Fe and Cu were recorded in liver of L. macrocephis collected from the coastal waters off Ann-Ping (Chen and Chen, 2001). The results showed higher accumulation than those reported in the mullet, M. cephalus, in the Gulf of Antalya (Yazkan et al., 2002).

These results are similar to those reported earlier in fishes from lakes of Turkey (Mendil et al., 2005). However, Zn level in the present study was lower than those reported in the fishes from Ataturk Dam Lake and Lake Kasumigaura (Alam et al., 2002; Karadede and Unlu, 1998) and higher than those given in fish from Lake Tanganyika (Chale, 2002). The values of Cu in fish samples obtained in the present study were higher than those reported by Mendil et al. (2005). Copper and zinc are essential elements and are carefully regulated by physiological mechanisms in most organisms (Bowen, 1979). However, they are regarded as potential hazards that can endanger both animal and human health. The low concentrations of Cu and Zn in the muscle of the examined fish species may reflect the low levels of these binding proteins (metallothioneins) in the muscle (Allen-Gil and Martynov, 1995). In general, the aquatic environment near urban areas is exposed to a number of pollutants. Effluent from sewage treatment plants as well as drainage from urban and agricultural areas contain pollutants that may damage aquatic life (Wright, 2001). Fish species are most sensitive to aquatic pollutants during their early life stages (Folmar et al., 2001). This is true with the present observation on metal accumulation in various tissues of fish collected from the polluted sites of Corentyne Coast. Muscles of fish collected from the polluted sites showed loss of muscle fibre as well as swelling of muscle fibre layer. Normal architecture of muscle bundle seems to be distorted with breakdown of muscle bundles. Earlier studies have demonstrated that muscles are very sensitive to contaminants and their immune system can act as an early warning system to stress (Anderson, et al., 1990).

The gills, which participate in many important functions in fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment, and particularly sensitive to changes in the quality of the water, are considered the primary target of the contaminants (Poleksic and Mitrovic-Tutundzic, 1994; Mazon et al., 2002; Fernandes and Mazon, 2003). Alterations like epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae are examples of defense mechanisms, since, in general, these result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants (Mallatt, 1985; Hinton and Laurén, 1990; Poleksic and Mitrovic-Tutundzic, 1994; Fernandes and Mazon, 2003). These alterations were most common found in the gills of fish caged in the polluted site from Corentyne coast. Coutinho and Gokhale (2000) found epithelial lifting in the gills of carps (Cyprinus carpio) and tilapias (Oreochromis mossambicus) exposed to the effluents of a wastewater treatment plant. Engelhardt et al. (1981) observed epithelial lifting and lamellar fusion in rainbow trouts (Oncorhynchus mykiss) exposed to petroleum residues. Similar alterations in the gills have also been reported in the fishes exposed to metals (Oliveira Ribeiro et al., 2000; Cerqueira and Fernandes, 2002; Martinez et al, 2004) and organic contaminants (Rosety-Rodriguez et al., 2002; Fanta et al., 2003). According to Mallat (1985) such alterations are non-specific and may be induced by different types of contaminant (Mallatt, 1985). As a consequence of the increased distance between water and blood due to epithelial lifting, the oxygen uptake is impaired. However, fishes have the capacity to increase their ventilation rate, to compensate low oxygen uptake (Fernandes and Mazon, 2003).

Most part of the gill lesions caused by sublethal exposures affects lamellar epithelium (Hinton and Laurén, 1990); however, some alterations in blood vessels may also occur, when fishes suffer a more severe type of stress. In this case, damaged pillar cells can result in an increased blood flow inside the lamellae, causing dilation of the marginal channel, blood congestion or even an aneurysm (Takashima and Hibiya, 1995; Rosey-Rodriguez et al., 2002). The formation of an aneurysm is related to the rupture of the pillar cells (Heath, 1987; Martinez et al., 2004) due to a bigger flow of blood or even because of the direct effects of contaminants on these cells. This is a severe type of lesion, recovery from which is possible, but more difficult than the epithelial changes (Poleksic and Mitrovic-Tutundzic, 1994). Several animals caged in the polluted stream showed vascular alterations, the most frequent being...
dilation of the marginal channel and congestion. Some animals confined at station from polluted sites showed aneurysms which indicate the impaired condition of the water in these sites. In the present study, one fish confined polluted sites in the winter showed rupture of the gill epithelium, which caused hemorrhage. Like aneurysm, this lesion can be interpreted as a reflection of the direct action of toxic agents on the tissue (Temminck, 1983). Winkler et al. (2001) found anomalies such as hyperplasia, hypertrophy, dilation of the marginal channel and aneurysms in another Neotropical fish, Astyanax altiparanae, collected in Cambé stream, which corroborates the hypothesis that the water of this stream is really contaminated and that exposure to this water causes structural damage to the fish gill.

Histological responses that have previously been reported in the liver of various fish species exposed to cadmium showed atrophy and necrosis of hepatic cells, decrease in the size of nuclei and nucleoli and indistinguishable cell membranes (Cyprinus carpio) (Morsey and Protasowicki, 1990). Gills and liver were chosen as target organs for assessing metal accumulation. The concentrations in liver represent storage of metals. Induction of metallothioneins in liver is the main form of storage and detoxication of metals in fish. Increased metal concentrations in liver may represent storage of sequestered products in this organs. This is also true of muscle with less metal content. It is well known that muscle is not an active tissue in accumulating heavy metals (Sunlu et al., 2001; Unlu et al., 1996). However, no research has been reported on the residue of metals in fish skin which is consumed by humans. The present study shows that the histopathological changes in the liver cause metabolic problems as well. Evidence for this is the bile stagnation in liver of most fish studied. This lesion, characterized by the remains of the bile in the form of brownish-yellow granules in the cytoplasm of the hepatocytes (Pacheco and Santos, 2002), indicates that the bile is not being released from the liver. This accumulation of bile indicates possible damage to the hepatic metabolism (Fanta et al., 2003).

Besides, the fusion of lamellae and the loss of microridges may decrease in the total respiratory area of the gills, resulting in decreased oxygen - uptake capacity of fish gills collected from polluted sites. The hepatocytes showed a number of lysosomes and electron dense bodies, dilation of the rough endoplasmic reticulum, cristae or outer or inner membranes with mitochondrial swelling. The changes consisted of extensive proliferation of the smooth endoplasmic reticulum and dilation of the rough endoplasmic reticulum, suggesting an active detoxification attempt by the liver of fish collected from polluted sites (Carpene and Vasak, 1989; Karadede et al., 2004). The histological changes observed in the gills, liver and muscle of the M. cephalus in the present study indicate that the fish were responding to the direct effects of the contaminants as much as to the secondary effects caused by stress. The analysis of the seasonal variation in the histological parameters leads to the conclusion that the changes observed in the three organs were not apparently related to the seasons, and neither were the distribution or the severity of the lesions. Such information confirms that histopathological alterations are good biomarkers for field assessment, in particular in tropical areas that are naturally subject to a multiplicity of environmental variations. It must be emphasized that histopathology is able to evaluate the early effects and the responses to acute exposure to chemical stressors.

The scanning electron micrographs document the surface topography of the gills of M. cephalus. In this context, fish gill arches, filaments and lamellae showed sufficient damage in the fish collected from polluted sites. This could be explained by increased excretion or adaptive processes to different ionic environments (Laurent and Hehibi, 1988; McDonald et al., 1991). Besides, there exists fusion of primary and secondary lamellae as well as complete loss of primary lamellae in the fish collected from polluted sites. A comparable phenomenon has also been described by others (Pratap and Wendelaar Bonga 1993; Haaparanta et al., 1996; Karlsson-Norr gren et al., 1985). Hyperplasia of secondary lamellae could often be observed in animals exposed to highly polluted sites (Karakoc, 1999). Fish gills are the main target of several aquatic pollutants (Kikuchi et al., 1978), an excellent model to examine the effects of dissolved substances in the tissues (Evans, 1987). The morphological perturbations of gill, liver and muscle are results of defensive mechanism or adaptive changes to heavy metal contamination in the study area (Au, 2004). Our findings leave us to suppose that, the structural modifications in the tissues at the contaminated sites might be associated to change at the membrane level that implied in tissue perturbations. Relatively high concentrations of heavy metals were found in liver and gill of the examined species caught from the polluted sites of Corentyne Coast, which suggests the possibility of using these two organs, as bio-indicators of metals present in the surrounding environment. However, it is believed that monitoring of these species should be repeated on similar-sized populations on more occasions and over a longer period to test whether the results and associated correlations were sufficiently consistent and robust for monitoring purposes.

**CONCLUSIONS**

In conclusion, there was a clear difference between the concentrations of heavy metals within tissues and between sites. The fish M. cephalus caught from polluted sites of Corentyne Coast were heavily burdened with metals especially in gills and liver. Furthermore, fish of polluted sites, chronically exposed to toxic metals, responded with various signs of lesions and the secretion of mucus of increased rigidity. Hence the integrated use of histological and ultrastructural changes in the fish tissues can be taken as efficient biomarkers for the assessment of metal contamination in the ecosystem and precautions need to be taken in order to present the future heavy metal pollution. Therefore, East Berbice-Corentyne coast is one of the most important pristine wetland ecosystems of Guyana. Efforts should be needed to protect Corentyne Coast from pollution and also to reduce environmental risk. This study the valuable data will pave the way for future research on Corentyne Coast.
Recommendation

1. I recommended that the information provided in this study be used when formulating heavy metal control protocols for Guyana’s coastal plane.
2. I recommended that the effluents from the industries should be checked and treated before discharging in to the coastal water.
3. I recommended the implementation and use of Bioremedial measures to protect the coastal ecosystem from further degradation.
4. I recommended that further studies be carried out by the Ministry of Public health to ascertain the impact of these pollutants on the health and wellbeing of the citizens.

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