ORIGINAL RESEARCH ARTICLE

INTERNATIONAL JOURNAL OF BIOASSAYS
ISSN: 2278-778X
CODEN: IJBNHY
OPEN ACCESS

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

Sivakumar Rajeshkumar*[1], Dhivya Karunamurthy[2], Grayson Halley[1], and Natesan Munuswamy[3]

[1]University of Guyana, Faculty of Agriculture and Forestry, Berbice Campus, Johns/Tain, Guyana, South America.
[2]Dpartment of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agriculture University, Coimbatore, India.
[3]Unit of Aquaculture and Cryobiology, Department of Zoology, University of Madras, Guindy Campus, Chennai, India.

Received for publication: September 26, 2015; **Accepted**: October 26, 2015

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 Coretyne 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.

***Corresponding Author:**
Dr. S. Rajeshkumar,
Faculty of Agriculture and Forestry,
University of Guyana
Berbice Campus, Johns Guyana,
South America.

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 Zyadah and Chouikhi (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 activates 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 on field studies (Oliveria *et al.,* 2009) and under laboratory conditions (Oliveira *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 Canton, 1991; Thophon *et al.,* 2003) and field studies (Hinton *et al.,* 1992; Schwaiger *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 Laurén, 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 (Triebskorn *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 (Triebskorn *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 fertilizes, motor vehicles, oil refineries and operations of the second major harbour for coal import, which includes a Guysuco 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 (Rajeshkumat *et al.,* 2015). The present work is the first attempt to explore the influence of heavy metal mediated stress via histology and ultrastructure 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 effects on the bioaccumulation and histoarchitectural changes in *Mugil cephalus* collected from polluted and less polluted site. Based on the above mentioned information, it is obvious that not much research has been carried out on heavy metal pollution Corentyne Coast, east Berbice of Guyana.

Materials and Methods

Study area

The East Berbice-Corentyne (5^{o}.57. 44” to 10” N and 57^{o}.08’ 01 to 32^{o} 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^{o}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 Guysuco 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).

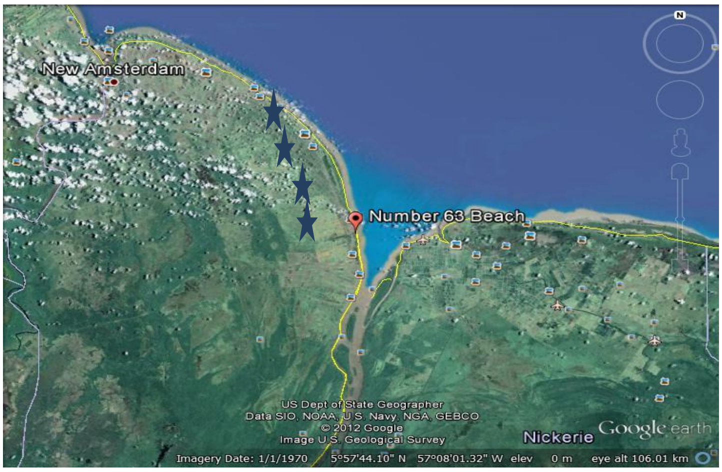

Fig. 1: Location map of the study area - East Berbice - Corentyne Coast, Guyana.

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-Corantyne 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 ^{o}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 ^{o}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 quantitatively transferred to the 10 mL volumetric flasks, and brought to volume with ultrapure water. Element contents in the samples were determined by Atomic Absorption Spectrometer (Perkin-Elmer, AA 800) and are expressed as $\mu g\ g^{-1}$ 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).

Reference material	Certified values	Measured value	Percentage of recovery
BCSS-1			
Copper	19	18.1	95.4
Lead	22.7	21.6	96.2
Zinc	119	115.6	96.2
Cadmium	0.25	0.24	96.0
Manganese	10.5	10.3	98.10
Iron	184	191	103.80
DORM-2			
Copper	2.34 ± 0.16	2.32	99.1
Lead	0.065 ± 0.007	0.0065	100
Zinc	25.6 ± 2.3	25.2	98.4
Cadmium	0.043 ± 0.008	0.042	99.5
Manganese	10.0 ± 0.005	10.3	98.10
Iron	184.0 ± 0.004	191.0	103.80

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 eosin. 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 CO_2, 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 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 Cu (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 ($p < 0.05$).

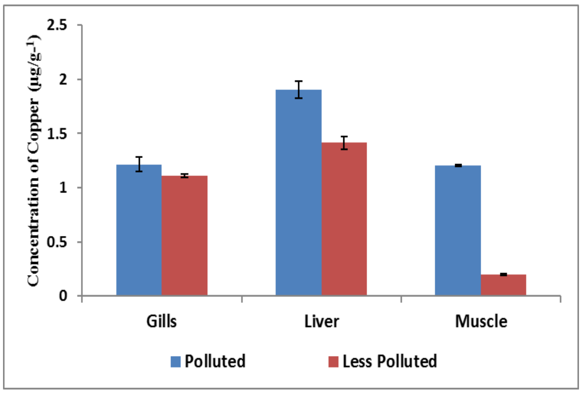

Fig 2a. Concentration (μg g^{-1}) of Copper in gills, liver and muscle of *M. cephalus* collected from Polluted and Less Polluted sites. The value in a bar with different letters denoted significant difference ($p<0.05$).

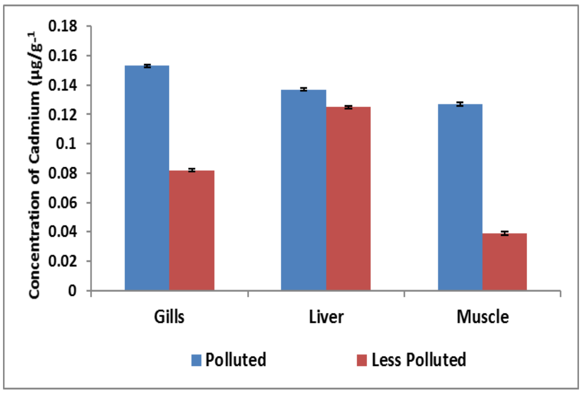

Fig 2b. Concentration (μg g^{-1}) of Cadmium in gills, liver and muscle of *M. cephalus* collected from Polluted and Less

Polluted sites. The value in a bar with different letters denoted significant difference ($p<0.05$).

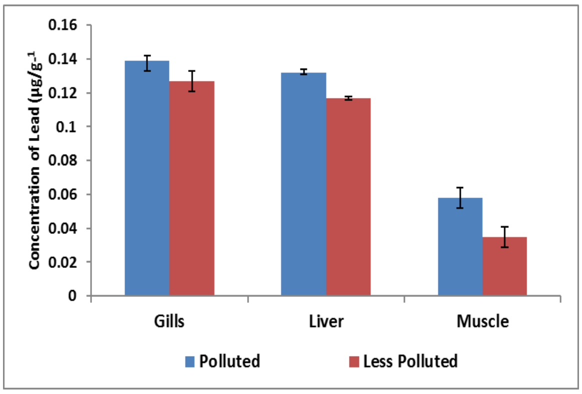

Fig 2c. Concentration (µg g^{-1}) of Lead in gills, liver and muscle of *M. cephalus* collected from Polluted and Less Polluted sites. The value in a bar with different letters denoted significant difference ($p<0.05$).

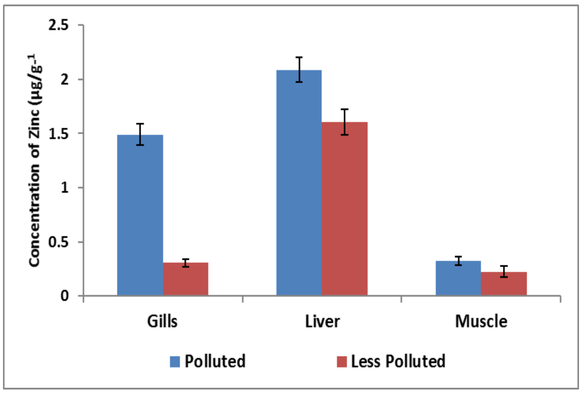

Fig 2d. Concentration (µg g^{-1}) of Zinc in gills, liver and muscle of *M. cephalus* collected from Polluted and Less Polluted sites. The value in a bar with different letters denoted significant difference ($p<0.05$).

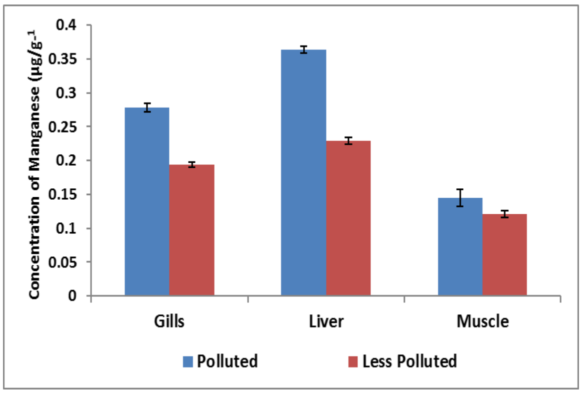

Fig 2e. Concentration (µg g^{-1}) of Manganese in gills, liver and muscle of *M. cephalus* collected from Polluted and Less Polluted sites. The value in a bar with different letters denoted significant difference ($p<0.05$).

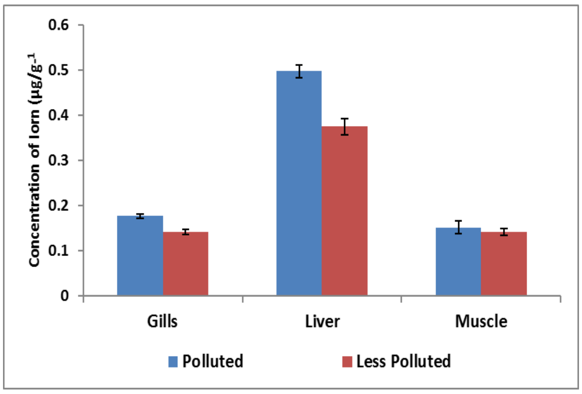

Fig 2f. Concentration (µg g^{-1}) of Iron in gills, liver and muscle of *M. cephalus* collected from Polluted and Less Polluted sites. The value in a bar with different letters denoted significant difference ($p<0.05$).

Histological and ultrastructural observations

Gills: The histoarchitecture 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).

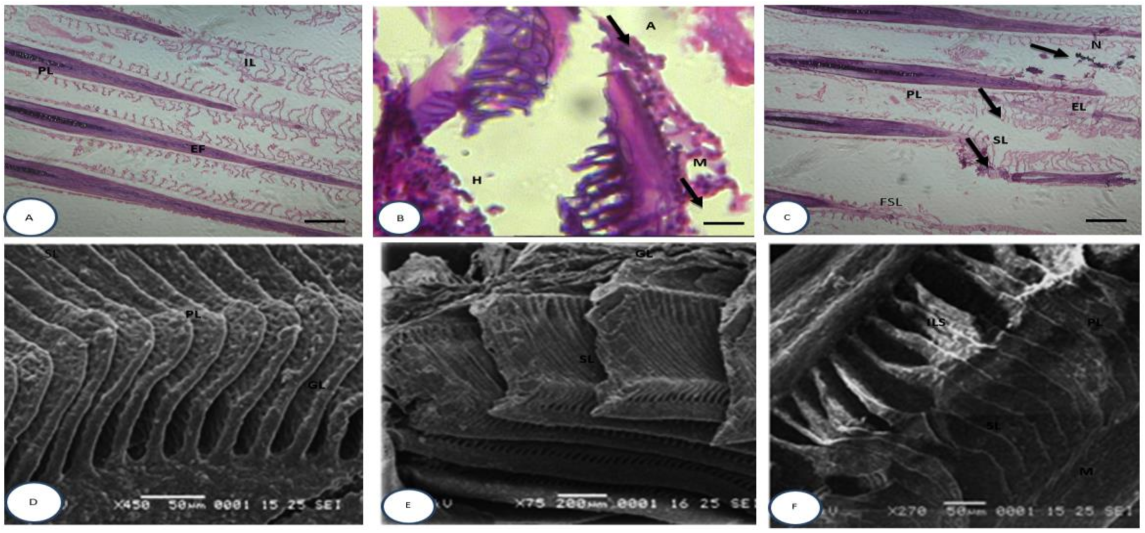

Fig. 3. (A-C) Photomicrographs of gills of M. cephalus stained with hematoxylin and eosin. Control (Gills of fish) showing the normal architecture of gill filaments such as Primary lamellae (PL) and secondary lamellae (SL) covered by filamentary epithelium (EF) which is perpendicularly intersected by 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).

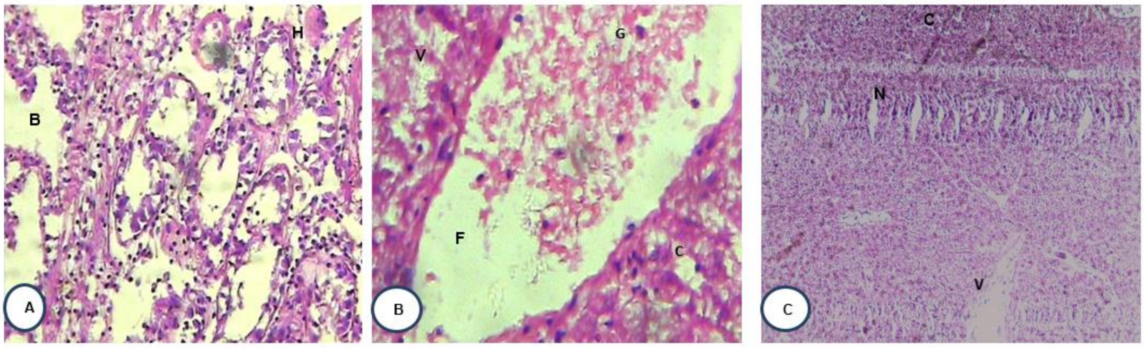

Fig. 4. (A-C) Photomicrographs of liver of *M. Cephalus* stained with haematoxylin and eosin. (A) Control fish liver showing normal parenchymal architecture of hepatocytes (HP) and blood sinusoids (BS. (B) Fish collected from polluted sites showing vacuolization in the hepatocytes (HP), fibroblast proliferation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

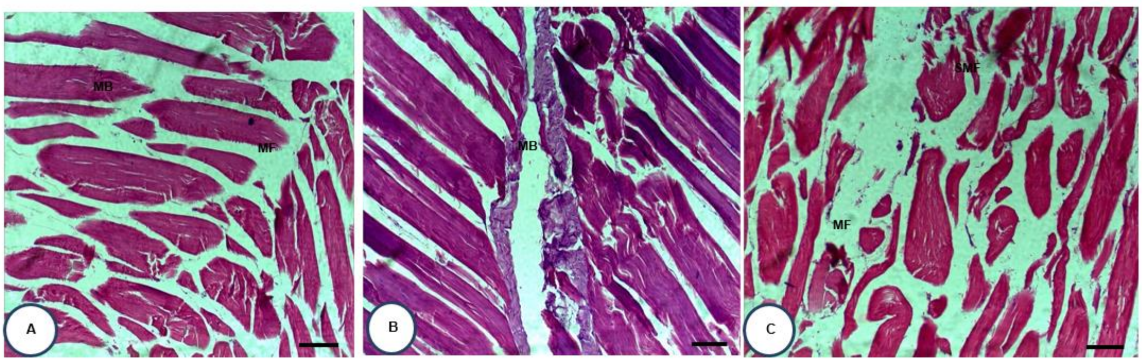

Fig. 5. Section through the muscle of *M. cephalus* stained with heamatoxylin and eosin. (A) Control muscle showing normal arrangement of muscle fiber (MF) and uniform muscle bundles (MB). (B) Muscles of fish collected from polluted sites showing loss and swelling of muscle fiber layer (MF), (C) Breakdown 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 nectosis 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 arrangements 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 (Arickia 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; Dyrynda *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 Protasowicki, 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 *Cathorops spixii* 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 metallothionine (Kargin and Erdem, 1991; Hogstrand and Haux, 1991). Thus, liver and gill are more often recommended as environmental indicator organs of water pollution then 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 Atli, 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 $\mu g\ g^{-1}$ 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 (Karadede 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. macrolepis* 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-Rodríguez *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; Rosety-Rodríguez *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 (Temmink, 1983). Winkaler 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 Hebibi, 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-Norrgren *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.

Acknowledgments

We would like to thank the director of the University of Guyana, Berbice Campus, Tain, for providing lab facilities for this study. We also thank the authority of Personnel Division, University of Guyana, Georgetown, Guyana for their cooperation.

References

1. Alan G Heath. "Water Pollution and Fish Physiology". CRP press, Florida (1987): P. 245.
2. Almeida. "Feeding ecology of Liza ramada (Risso, 1810) (Pisces, Mugilidae) in a south-western estuary of Portugal". Estuarine Coastal and Shelf Science 57 (2003): 313-323.
3. Al-Yousuf, El-Shahawi, and Al-Ghais. "Trace metals in liver, skin and muscle of Lethrinus lentjan fish species in relation to body length and sex". Science of the Total Environment 256 (2000): 87-94.
4. Anderson, Law, Shariff, and Nash. "A parvo-like virus in the giant freshwater prawn, Macrobrachium rosenbergii". Journal of Invertebrrate Pathology 55 (1990): 447-449.
5. Bernet, Schmidt, Meier, Burkhardt-Holm, and Wahli. "Histopathology in fish: proposal for a protocol to assess aquatic pollution". Journal of Fish Diseases 22 (1999): 25-34.
6. Bernet, Schmidt-Posthaus, Wahli, and Burkhardt Holm. "Evaluation of two monitoring approaches to assess effects of waste disposal on histological alterations in fish". Hydrobiology 524 (2004): 53-66.
7. Bervoets, Blust, and Verheyen. "Accumulation of metals in the tissues of three spined stickelback (Gasterosteus aculeatus) from natural fresh waters". Ecotoxicology Environmental Safety 48(2) (2001): 117-127.
8. Blasco, Arias, and Saenz. "Heavy metals in organisms of the River Guadalquivir estuary: possible incidence of the Aznalcollar disaster". Science of the Total Environment 242 (1999): 249-259.
9. Braunbeck, and Appelbaum. "Ultrastructural alterations in the liver and intestine of carp Cyprinus carpio induced orally by ultra-low doses of endosulfan". Disease of Aquatic Organisms 36 (1999): 183-200.
10. Canbek Mediha, Temir Ali Demir, Mustafa Uyanoglu, Go¨khan Bayramoglu, O¨ zgu¨r Emiroglu, and Naime Arslan, et al. "Preliminary assessment of heavy metals in water and some cyprinidae species from the Porsuk river". Turkey Journal of Applied Biological Science 1(3) (2007): 91-95.
11. Carmona, Garcia-Gallego, Sanz, Domezain, and Ostos-Garredo. "Chloride cells and pavement cells in gill epithelia of Acipenser naccaril: ultrastuctural modifications in seawater acclimated specimens". Journal of Fish and Biology 64 (2004): 553-566.
12. Casas, Gonzalez, Andral, and Cossa. "Relation between metal concentration in water and metal content of marine mussels (Mutilus galloprovincialis): impact of physiology". Environmental Toxicology and Chemistry 27 (7) (2008): 1543-1552.
13. Cerqueira, and Fernandes. "Gill tissue recovery after cooper exposure and blood parameter responses in the tropical fish Prochilodus scrofa". Ecotoxicological and Environmental Safety 52 (2002): 83-91.
14. Chale. "Trace metal concentration in water, sediments and fish tissue from lake Tanganyika". Science of the Total Environment 299 (2002): 115-121.
15. Chang, Zdanowicz, and Murchelano. "Associations between liver lesions in winter flounder (Pleuronectes americanus) and sediment chemical contaminants from north-east United States estuaries". Journal of Marine Science 55 (1998): 954-969.
16. Chen YC, and Chen MH. "Heavy metal concentrations in nine species of fish caught in coastal waters off Ann-Ping, SW Taiwan". Journal of Food Drug Analysis 9 (2001): 107-114.
17. Cid, Bioa, Pombo, and Rebelo. "Determination of trace metals in fish species of the Ria de Aveiro (Portugal) by electro thermal atomic adsorption spectrometry". Food Chemistry 75 (1) (2001): 93-100.
18. Couch, and Harshbarger. "Effects of carcinogenic agents on aquatic animals: an environmental and experimental overview". Environmental Carcinogenesis Reviews 3 (1985): 63-105.
19. Coutinho, and Gokhale. "Selected oxidative enzymes and histopathological changes in the gills of Cyprinus carpio and Oreochromis mossambicus cultured in secondary sewage effluent". Water Research 34 (2000): 2997-3004.
20. David E Hinton, and Lauren OJ. "Liver structural alterations accompanying chronic toxicity in fishes": potential biomarkers of exposure. In: Biomarkers of environmental contamination. J. F. McCarthy and L. R. Shugart (Eds.,) MI Lewis Publishers 1990, p.12- 68.
21. David E Hinton, Baumann PC, Gardner GR, Hawkins WE, Hendricks JD, Murchelano RA, and Okihiro MS. "Histopathologic biomarkers". In: Hugget, R, Kimerle, R,

Mehrle P, H. Bergman (Eds.). Biomarkers - biochemical, physiological and histological markers of anthropogenic stress. Boca Raton, Lewis Publishers 1992, p.155-195.

22. David Evans, Dedoo, and Hanson. "Trace element concentrations in fish livers. Implications of variations with fish size in pollution monitoring". Marine Pollution Bulletin 26(6) (1993): 329-334.

23. David Evans. "The fish gill: site of action and model for toxic effects of environmental pollutants". Environmental Health Perspective 71 (1987): 47-58.

24. Deys. Papillomas in the Atlantic eel, Anguilla vulgaris. "In A Symposium on neoplasms and related disorders of invertebrate and lower vertebrate animals". Ed. by C. J. Dawe and J. C. Harshbarger. National Cancer Institute Monograph 31 (1969): 187-193.

25. Doris Au. "The application of histo-cytopathological biomarkers in marine pollution monitoring: a review". Marine Pollution Bulletin 48 (2004): 817-834.

26. Dyrynda, Pipe, and Ratclitte. "Sub populations of haemocytes in the adult and developing marine mussel, myticus edulis, identified by use of monoclonal antibodies". Cell Tissue Research 289 (3) (1997): 527 - 36.

27. Eateves. Fundanentod de limologia Rio de Janeiro, Interciencia. FINEP (1988).

28. Eckwert, and Kohler. "The induction of stress proteins (HSP) in Oniscus asellus (Isopoda) as a molecular marker of multiple heavy metal exposure". Toxicology to Assessment of Ecotoxicology 6 (1997): 249-262.

29. Ekamaram Padmini, and Usha Rani Munuswamy. "Thioredoxin and HSP 90α modulate ASK1-JNK1/2 signalling in stressed hepatocytes of Mugil cephalus". Comparative Biochemistry Physiology Part C. 151 (2010): 187- 193.

30. Ekamaram Padmini, Kavitha Murgan. "Contaminant induced stress impact on biochemical changes in brain of estuarine grey mullets". Pollution Research 24 (2005): 647-651.

31. Emilio Carpene, and Vasak Milan. "Hepatic Metallothionein from Goldfish (Carassius auratus)". Comparative. Biochemistry and Physiology 92B (1989): 463-468.

32. Engelhardt, Wong, and Duey. "Hydromineral balance and gill morphology in rainbow trout Salmo gairdneri, acclimated to fresh and sea water as affected by petroleum exposure". Aquatic Toxicology 1 (1981): 175-186.

33. Eu. Commission Regulation (Ed.), No 466/2001. Setting Maxiumum Levels for Certain Contaminants in Food Chemistry 75 (1) (2001): 93-100.

34. Evans Fanta, Rios Fanta, Romão Vianna and Freiberger. "Histopathology of the fish Corydoras paleatus contaminated with sublethal levels of organophosphorus in water and food". Ecotoxicology and Environmental Safety 54 (2003): 119-130.

35. Fernandes, Fontainhas, Monteiroand, and Salgado Ali. "Changes in plasma electrolytes and gill histopathology in wild Liza saliens from the Esmoriz-Paramos coastal lagoon". Bulletin Environmental Contamination and Toxicology 79 (2007): 301-305.

36. Folmar Gardner, Schreibman Magluilo-Cepriano, Mills Zaroogian, Gutjahr-Gobell Haebler, and Horowitz Denslow. "Vitellogenin-induced pathology in male summer flounder (Paralichthys dentatus)". Aquatic Toxicology 51 (2001): 431-441.

37. Food and agricultural organization (FAO). Compliation of legal limits for hazardous substances in fish and fishery products. FAO Fish Crick 464. (1983): 5-100.

38. Fournie, Summers, and Weisberg. "Prevalence of gross pathological abnormalities in estuarine fishes". Transactions of the American Fisheries Society 125 (1996): 581-590.

39. Francis Henry, Amara Rama, Courcot, Lacouture Daniel, and Bertho. "Heavy metals in four fish species from the French coast of the eastern English Channel and southern bight of the North Sea". Environmental International 30 (2004): 675-683.

40. Gernhofer, Pawet, Schramm, Müller, and Triebskorn. "Ultrastructural biomarkers as tools to characterize the health status of fish in contaminated streams". Journal of Aquatic Ecosystems 8 (2001): 241-260.

41. Gil, Allen and Martynov. "Heavy metals burdens in nine species of freshwater and anadromous fish from the Pechora River, northern Russia". Science of the Total Environment 160-161 (1995): 653-659.

42. Guven K, Ozbay C, Unlu E, Star A. "Acute lethal toxicity and accumulation of copper in Gammarus pulex (L.) (Amphipoda)". Turkey Journal of Biology 23 (1999): 513-521.

43. Guyana Times. Kaieteur News, February 2013; 23: Guyana. P 5.

44. Haaparanta, Valtonen, and Hoffmann. "Gill anomalies of perch and roach from four lakes differing in water quality". Journal of Fish Biology 50 (1996): 575-591.

45. Halim, Abbas, and Ali Fagr. "Study the effect of hexavalent chromium on some biochemical, cytological and histolopathological aspects of Orechromis spp Fish". Pakistan Journal Biological Sciences 10 (2007): 3973-3982.

46. Hogstrand C, and Haux C. "Binding and detoxification of heavy metals in lower vetrebrats with reference to metallothionein". Comparative Biochemistry and Physiology 100 (1/2). (1991): 137-141.

47. Hughes DA. "The role of responses to light in the selection and maintenance of microhabitat by the nymphs of two species of mayfly". Anita Bahavan 14 (1966): 17-33.

48. Hulya Karadede, and Unlu Erhan. "Concentrations of some heavy metals in water, sediment and fish species from the Ataturk Dam Lake (Euphrates), Turkey", Chemosphere 41 (9) (2000): 1371-1376.

49. Hulya Karadede, Oymak Seyit Ahmet, Unlu Erthan. "Heavy metals in mullet, Liza abu and Cat fish, Silurus triostegus, from the Ataturk Dam Lake Euphrates, Turkey". Environmental International 30 (2004): 183-188.

50. Humphry Bowen. "Environmental chemistry of the elements". London: Academic Press 1979, pp. 269.

51. Jiraungkoorskul W, Sahaphong S, and Kangwanrangsan N. "Toxicity of copper in butterfish (Poronotus triacanthus): tissues accumulation and ultrastructural changes", Environmental Toxicology 22 (2007): 92-100.

52. JM Hellawell. "Biological Indicators of Freshwater Pollution and Environmental Management". Amsterdam: Elsevier Publications (1986): 546-554.

53. Jordao CP, Pereira MG, Bellato CR, Pereira JL, and Matos AT. "Assesssment of water systems for contaminants from domestic and industrial sewages". Environmental Monitoring Assessment 79 (1) (2002): 75-100.

54. Jordi Floch, Lees, Stanley Scoane. "A simple method for the isolation and purification of total lipids from animal tissues". Journal of Biological Chemistry 226 (1957): 497-508.

55. Kalay M, and Canil M. "Elimination of essential (Cu, Zn) and non-essential (Cd, Pb) metals from tissue of a freshwater fish Tilapia zilli". Turkish Journal of Zoology 24 (2000): 429-436.

56. Kargin, and Erdem Cahit. "Accumulation of copper in liver, spleen, stomach, intestine, gill and muscle of Cyprinus carpio. Doga. Turkey". Journal of Zoology 15 (1999): 306-14.

57. Karlsson-Norrgren L, Runn P, Haux C, and Forlin L. "Cadmium induced changes in gill morphology of zebrafish, Brachydynio rerio (Hamilton-Buchanan), and rainbow trout, Salmo gairdneri". Journal of Fish Biology 27 (1985): 81-95.

58. Kikuchi M, Wakabayashi M, Kojima H, and Yoshida T. "Uptake, distribution and elimination of sodium linear alkylbenzene sulfonate and sodium alkyl sulfate in carp". Ecotoxicology and Environmental Safety 2 (1978): 115-127.

59. Kumaraguru AK, Beamish FWH, and Ferguson HW. "Direct and circulatory paths of Permethrin (NRDC-143) causing histopathological changes in the gills of rainbow trout, Salmogairdneri Richardson". Journal of Fish Biology 20 (1982): 87-91.

60. Lakshmanan PT, and Nambisan RNK. "Seasonal variation in trace metal content in bivalve molluscs, Villorita cyprinoides var. cochinensis (Hanley), Meretrix casta (Chemnitz) and Perna viridis (Linnaeus)". Indian Journal of Marine Science 12 (1983): 100-103.

61. Laurent P, and Hebibi N. "Gill morphometry and fish osmoregulation". Canadian Journal of Zoology 67 (1988): 3055-3063.

62. LL Johnson, Stehr CM, Olson OP, Myers MS, Pierce SM, Wigren CA, McCain BB, and Varanasi U. "Chemical contaminants and hepatic lesions in winter flounder (Pleuronectes americanus) from the northeastern coast of the United States". Environmental Science and Technology 27 (1993): 2759-2771.

63. Longwell AC, Chang S, and Gadbois DF. "A complete analysis of winter flounder (Pleuronectes americanus) egg contaminants with respect to early reproduction with a review of their toxicity and other environmental concentrations". Reviews in Fisheries Science 4 (1996): 339-386.

64. Longwell AC, Chang S, Hebert A, Hughes JB, and Perry DM. "Pollution and developmental abnormalities of Atlantic fishes". Environmental Biology Fishery 35 (1992): 1-21.

65. Lourdraj Arockia Vasanthi, Revathi Peranandam, Mini Jayaprakash, Munuswamy Natesan. "Integrated use of histological and ultrastuctural biomarkers in Mugil cephalus for assessing heavy metal pollution in Ennore estuary, Chennai". Chemosphere 91(8) (2013): 1156-1164.

66. Malins D, McCain BB, Brown DW, Chan SL, Myers MM, Landahl J, Prohaska P, Friedman A S, Rhodes L, Burrow DG, Gronlund W, and Hodgins H. "Chemical pollutants in sediments and diseases of bottomdwelling fish in Puget Sound, Washington". Environmental Science and Technology 18 (1984): 705-713.

67. Mallatt J. "Fish gill structural changes induced by toxicants and other irritants: a statistical review". Canadian Journal of Fish Aquatic Science 42 (1985): 630-648.

68. Mansour SA, and Sidky MM. Ecotoxicological studies: "Heavy metals contaminating water and fish from Fayoum Governorate Egyptian". Food Chemistry 78 (2002): 15-22.

69. Marcovecchio JE. "The use of Micropogonias furnieri and Mugil liza as bioindicators of heavy metals pollution in La Plata river estuary, Argentina". Science of the Total Environment 323 (2004): 219-226.

70. Marina M P Camargo, and Claudia B R Martinez. "Biochemical and physiological biomarkers in Prochilodus lineatus submitted to in situ tests in an urban stream in southern Brazil". Environmental Toxicology and Pharmacology 21 (2006): 61-69.

71. Martinez CBR, Nagae MY, Zaia CTBV, and Zaia DAM. "Morphological and physiological acute effects of lead in the neotropical fish Prochilodus lineatus. Brazil". Journal of Biology 64 (4) (2004): 797-807.

72. Mazon AF, Pinheiro GHD, and Fernandes MN. "Hematological and physiological changes induced by short-term exposure to copper in the freshwater fish,

Prochilodus scrofa. Braz". Journal of Biology 62 (4A) (2002): 621-631.

73. Mc Donald DG, Freda J, Cavdek V, Gonzalez R, and Zia S. "Interspecific differences in gill morphology of freshwater fish in relation to tolerance of low-pH environments". Physiology of Zoology 64 (1) (1991): 124-144.

74. McCain BB, Pierce KV, Wellings SR, and Miller BS. "Hepatomas in marine fish from an urban estuary". Bulletin of Environ Contamination Toxicology 18 (1977): 1-12.

75. Mendil D, Uluozlu OD, Hasdemir E, Tuzen M, Sari H, and Suicmez M. "Determination of trace metal levels in seven fish species in lakes in Tokat, Turkey". Food Chemistry 90 (2005): 175-179.

76. Mix MC. "Cancerous diseases in aquatic animals and their association with environmental pollutants: a critical review". Marine Environmental Research 20 (1986): 1-141.

77. Mohammed A, May T, Echols K, Walther M, Manoo A, Maraj D, Agard J, and Orazio C. "Metals in sediments and fish from Sea Lots and Point Lisas Harbors, Trinidad and Tobago". Marine Pollution Bulletin 64 (2012): 169-173.

78. Monteiro Fernandes, and Mazon Fernandes. "Environmental pollution and fish gill morphology". In: Val, A. L. & B. G. Kapoor (Eds.). Fish adaptations. Enfield, Science Publishers (2003): 203-231.

79. Moore MJ, Shea D, Hillman R, and Stegeman JJ. "Trends in hepatic tumours and hydropic vacuolation, fin erosion, organic chemicals and stable isotope ratios in winter flounder from Massachusetts, USA". Marine Pollution Bulletin 32 (1996): 458-470.

80. Mormede S, and Davis IM. "Heavy metal concentrations in commercial deep sea fish from the Rockall Trough". Contamination of Shelf Research 21 (8-10) (2001): 899-916.

81. Morsey MG, and Protasowicki M. 1990. "Cadmium bioaccumulation and its effects on some hematological and histological aspects in carp, Cyprinus carpio (L.) at selected temperature". Acta Ichthyology of Piscat XX Fasc. 1.

82. Murat Karakoc. "Effects of salinity on the accumulation of copper in liver, gill and muscle tissues of Tilapia nilotica. Turkey". Journal of Zoology 23 (1999): 299-303.

83. Murthy RKV, and Rao BK, "Survey of meiofauna in the Gautami - Godavari estuary". Journal of Marine Biology and Association of India 29 (1987): 37-44.

84. Mustafa Canli, and Atli Guluzar. "The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and size of six Mediterranean fish species". Environmental Pollution 121 (1) (2003): 129-136.

85. Myers MS, Stehr CM, Olson OP, Johnson LL, McCain BB, Chan SL, and Varanasi U. "Relationships between toxicopathic hepatic lesions and exposure to chemical contaminants in English sole (Pleuronectes vetulus), starry flounder (Platichthys stellatus), and white croaker (Genyonemus lineatus) from selected marine sites on the Pacific coast, USA. Environmental Health Perspectives 102 (1994): 200-215.

86. Nair M, Jayalakshmy KV, Balachandran KK, and Joseph T. "Bioaccumulation of toxic metals by fish in a semi-enclosed tropical ecosystem". Environmental Forensics 7 (2006): 197-206.

87. Obasohan EE. "Heavy metals concentrations in the offal, gill, muscle and liver of a freshwater mudfish (Parachanna obscura) from Ogba River, Benin city, Nigeria". African Journal of Biotechnology 6(22) (2007): 2620-2627.

88. Oliveira Ribeiro CA, Pelletier E, Pfeiffer WC, and Rouleau C. "Comparative uptake, bioaccumulation, and gill damages of inorganic mercury in tropical and nordic freshwater fish". Environmental Research 83 (2000): 286-292.

89. Oliveria M, Maria VL, Ahmad L, Serafim A, Bebianno MJ, Pacheco M, and Santos MA. "Contamination assessment of a coastal lagoon (Ria de Aveiro, Portugal) using defence and damage biochemical indicators in gill of Liza aurata an integrated biomarkers approach". Environmental Pollution 157 (3) (2009): 59-967.

90. Oliveria M, Pacheco M, and Santos MA. "Cytochrome P4501A, genotoxic and stress responses in golden grey mullet (Liza aurata) following short-term exposure to phenanthrene". Chemosphere 66 (2007): 1284-1291.

91. Pacheco M, and Santos MA. "Biotransformation, genotoxic and histopathological effects of environmental contaminants in European eel (Anguilla anguilla L.)". Ecotoxicology and Environmental Safety 53 (2002): 331-347.

92. Pandey S, Parvez S, Sayeed I, Haque R, Bin-Hafeez B, and Raisuddin S. "Biomarkers of oxidative stress: a comparative study of river Yamuna fish Wallago att (Bl. & Schn.)". Science of the Total environment 309 (2003): 105-115.

93. Paul MJ, and Meyer JL. "Streams in the urban landscape". Annual Review of Ecology and Systematic 32 (2001): 333-65.

94. Peters G. "Seasonal fluctuations in the incidence of epidermal papillomas of the European eel (Anguilla anguilla)". Journal of Fish Biology 7 (1975): 415-422.

95. Poleksic V, and Mitrovic-Tutundzic V. 1994. "Fish gills as a monitor of sublethal and chronic effects of pollution". Pp. 339-352. In: Müller, R. & R. Lloyd (Eds.). Sublethal and Chronic effects of pollutants on freshwater fish. Oxford, Fishing News Books.

96. Pratap HB, and Wendelaar Bonga SE. "Effect of ambient and dietary cadmium on pavement cells, chloride cells and Na+ K+ ATPase activity in the gills of the freshwater

Teleost Oreochromis mossambicus at normal and high calcium levels in the ambient water". Aquatic Toxicology 26 (1993): 133-150.

97. Rao LM, and Padmaja G. "Bioaccumulation of heavy metals in M. cyprinoids from the harbour waters of Visakhapatnam". Bulletin of Pure and Applied Science 19A (2) (2000): 77-85.

98. Rashed MN. "Monitoring of environmental heavy metals in fish from Nasser Lake". Environ. Internation 27 (2001): 27-33.

99. Roe JH. "The determination of sugar in blood and spinal fluid with anthrone reagent". Journal of Biological Chemistry 212 (1955): 335-343.

100. Rosety-Rodríguez M, Ordoñez FJ, Rosety M, Rosety J M, Ribelles A, and Carrasco C. "Morpho-histochemical changes in the gills of turbot, Scophthalmus maximus L., induced by sodium dodecyl sulphate". Ecoloxicology and Environnemental Safety 51 (2002): 223-228.

101. Sankar TV, Zynudheen AA, Anandan R, and Viswanathan Nair PG. "Distribution of organochlorine pesticides and heavy metal residues in fish and shellfish from Calicut region, Kerala, India". Chemosphere 65 (2006): 583-590.

102. Sauves S, Handaui M, Brousseau P, and Fournier M. "Phagocytic response of terrestrial and aquatic invertebrates following in vitro exposure to trace elements". Ecoloxicology and Environnemental Safety 52 (1) (2002): 21-9

103. Scharenberg W P, and Gramann WH. "Bioaccumulation of heavy metals and organochlorines in a lake ecosystems with special reference to bream (Abramis brama. L)". Science of the Total. Environment 155(2) (1994): 187-97.

104. Schwaiger J, Wanke R, Adam S, Pawert M, Honnen W, and Triebskorn R. "The use of histopatological indicators to evaluate contaminant-related stress in fish". Journal of Aquatic Ecosystem, Stress and Recovery 6 (1997): 75-86

105. Senthilnathan S, and Balasubramanian T. "Heavy metal concentration in oyster Crassostrea madrasensis (Bivalvia anisomyaria) from the Uppanar, velar and Kaduviar estuaries of Southeast Coast of India". Journal of Marine Science 27 (1998): 211-216.

106. Sindermann CJ. "Pollution-associated diseases and abnormalities of fish and shellfish: a review". Fishery Bulletin, U.S 76. (1979): 717-748.

107. Singh V, and Chandel CPS. "Analytical study of heavy metals of industrial effluents at Jaipur, Rajasthan, India". Journal of Environmental Science and Engineering 48 (2006): 103-108.

108. Sivakumar Rajeshkumar, and Munuswamy Natesan. "Impact of metals on histopathology and expression of HSP70 in different tissues of Milk fish (Chanos chanos) of Kaattuppalli Island, South East Coast, India". Chemosphere 83 (2011): 415-421.

109. Sivakumar Rajeshkumar, and Munuswamy Natesan. "Infection of acanthocephalan parasite Neoechinorhynchus agilis sp in the grey mullet, (Mugil cephalus) a candidate species from - Corentyne coast, Berbice, Guyana". World Journal of Pharmaceutical Research 2 (2013): Issue 6.

110. Sivakumar Rajeshkumar, Dhivya Karunamurthy and Munuswamy Natesan. "Heavy metal pollution in East Berbice-Corentyne Coast, Guyana". ID #120590, ISBN 978-3-659-69368-7). In: Lambert Academic Publishing, Germany. (2015) P. 1-72

111. Sivakumar Rajeshkumar, Mini Jayaprakash, and Munuswamy Natesan. "Effects of heavy metals on antioxidents and expression of HSP70 in different tissues of Milk fish (Chanos chanos) of Kaattuppalli Island, Chennai, India". Ecotoxicology and Environmental Safety 98 (2013): 8-18.

112. Sivaperumal P, Sankar TV, and Viswanathan Nair PG. "Heavy metal concentrations in fish, shellfish and fish products from internal markets of India vis-à-vis international standards". Food Chemistry 102 (2007): 612-620.

113. Smith C, Peck T, Klauda R, and McLaren J. "Hepatomas in Atlantic tomcod, Microgadus tomcod (Walbaum), collected in the Hudson River estuary in New York". Journal of Fish Diseases 2 (1979): 313-319.

114. Sultana R, and Rao DP, "Bioaccumulation patterns of zinc, copper, lead and cadmium in grey mullet Mugil Cephalus from harbour waters of Visakhapatnam India". Bulletin Environmental Contamination and Toxicology 60 (1998): 949-955.

115. Sunlu U, Ozdemir E, and Basaran A. "The red mullet Mullus barbatus (Linnaeus 758) as an indicator for heavy metal pollution in Izmir Bay (Turkey)". 36th Ciesm Congress Proceedings, Monaco: Monte Carlo (2001).

116. Takashima F, and Hibya T. 1995. "An atlas of fish histology: normal and pathological features", 2nd ed. Tokyo, Kodansha.

117. Teh SJ, Adams SM, and Hinton DE. "Histopathological biomarkers in feral freshwater fish populations exposed to different types of contaminant stress". Aquatic Toxicology 37 (1997): 57-70.

118. Temmink J, Bowmieister P, Jong P, and Van der Berg J. "An ultra-structural study of chromate-induced hyperplasia in the gill of rainbow trout, Salmo gairdneri". Aquatic Toxicology 4 (1983): 165-179.

119. Thophon S, Kruatrachue M, Upathan ES, Pokethitiyook P, Sahaphong S, and Jarikhuan S. "Histopathological alterations of white seabass, Lates calcarifer in acute and subchronic cadmium exposure". Environmental Pollution 121 (2003): 307-320.

120. Triebskorn R, Kohler, HR, Honnen W, Schramm M, Adams SM, and Muller EF. "Induction of heat shock proteins, changes in liver ultrastructure, and alterations of

fish behaviour: Are these biomarkers related and are they useful to reflect the state of pollution in the field". Journal of Aquatic Ecosystems Stress Recovery 6 (1997): 57-73.

121. Turkmen M, Turkmen A, Akyurt I, Tepe Y. Limpet. "Patella caerulea Linnaeus, 1758 and Barnacle, Balanus sp., as biomonitors of trace metals availabilities in Iskenderun Bay, Northern East Mediterranean Sea". Bulletin Environmental Contamination and Toxicology 74 (2005): 301-307.

122. Valentina G. Caccia, and Frank J. Millero. "The distribution of trace metals in Florida Bay sediments". Marine Pollution Bulletin 46 (2003): 1420-1433.

123. Vander Oost R, Beyer J, and Vermeulen NPE. "Fish bioaccumulation and biomarkers in environmental risk assessment: A review". Environmental Toxicology and Pharmacology 13 (2003): 57-149.

124. Vethaak AD, and Wester PW. "Diseases of flounder (Platychthys flesus) in Dutch coastal and estuarine waters, with particular reference to environmental stress factors 2 Liver histopathology". Diseases of Aquatic Organisms 26 (1996): 99-116.

125. Vogelbein WK, Fournie JW, van Veld PA, and Hugett RJ. "Hepatic neoplasms in the mummichug (Fundulus heteroclitus) from a creosote-contaminated site". Cancer Research 50. (1990): 5978-5986.

126. Wagner A, and Boman,J. "Biomonitoring of trace elements in Vietnamese freshwater mussels". Spectrochimica Acta 59 (2004): 1125-1132.

127. Wester PW, and Canton JH. "The usefulness of histopathology in aquatic toxicity studies". Comparative Biochemistry and Physiology 100 (1991): 115-117.

128. Wester PW, Van der Ven LTM, Vethaak AD, Grinwis GCM, and Vos JG. "Aquatic toxicology: opportunities for enhancement through histopathology". Environmental Toxicology and Pharmacology 11 (2002): 289-295.

129. William Fischer, Bianchi George. "FAO Identification Sheets for Fishery Purposes". Western Indian Ocean, FAO, Rome (1984).

130. William W Au, Carlos H Sierra Torres, Salazar Nohelia Cojas, and Salama A Salama. "Inheritance of polymorphic metabolizing genes and environmental disease and quality of life". Mutation Research 428 (1999): 131-140.

131. Winkaler EU, Silva AG, Galindo HC, and Martinez CBR. "Biomarcadores histológicos e fisiológicos para o monitoramento da saúde de peixes de ribeirões de Londrina, Estado do Paraná". Acta Scientiarum 23 (2001): 507-514.

132. Wright RT. 2001. Environmental Science toward Sustainable Future. Ninth Edition: Prentice - Hall of India Private Limited, New Delhi. 11p.

133. Yazkan M, Ozdemir F, and Golukcu M. "Cu, Zn, Pb and Cd content in some fish species caught in the Gulf of Antalya". Turkey Journal of Veterinary and Animal Science 26 (6) (2002): 1309-1315.

134. Zyadah M, and Chouikhi A. "Heavy metal accumulation in Mullus barbatus, Merluccius merluccius and Boops boops fish from the Aegean Sea, Turkey". International journal of food sciences and nutrition 50 (6) (1999): 429-434.

Cite this article as:
Sivakumar Rajeshkumar, Dhivya Karunamurthy, Grayson Halley *et al.*, An Integrated Use of Histological and Ultra-structural Biomarkers in *Mugil cephalus* for Assessing Heavy Metal Pollution in East Berbice-Corentyne, Guyana. *International Journal of Bioassays* 4.11 (2015): 4541-4554.

Source of support: Nil
Conflict of interest: None Declared