HEPATIC PATHOLOGIES IN THE BRACKISH WATER CATFISH (*CHRYSICHTHYS NIGRODIGITATUS*) FROM CONTAMINATED LOCATIONS OF THE LAGOS LAGOON COMPLEX

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Abstract. Several toxicological studies into the effects of aquatic pollutants on the liver of teleost fish exist in literature. The focus on the liver in these studies is predicated on its central nature in the scheme of biotransformation and excretion of xenobiotics following exposure in polluted water bodies. As a consequence of the latter primary role of the liver in these processes it is regarded as a predilective site for the sub lethal effects of xenobiotics on the organism usually detectable at histological level. Hepatic histopathology recorded in livers from feral populations of the brackish water catfish *Chrysichthys nigrodigitatus* from locations on the Lagos lagoon complex with significant anthropogenic inputs from denizen populations and industries are presented. Liver sections from sixty specimens from two locations on the Lagos lagoon complex (Badagry lagoon: $6^0$24'N, $2^0$56'E; and Lagos lagoon: $6^0$29'N, $3^0$22'E) were analysed. Observed pathologies included hydropic degeneration (58%), portal / sinusoidal congestion (33%), hepatic necrosis (26%), hemosiderosis (12%) and foci of cellular alterations (FCA's). No obvious oncologic features were observed; the presence of the hydropic Vacuolation lesion was taken as prelude to the development of neoplasms and discussed as such.
Keywords: *Liver, Pathology, Fish, Toxicology, Water quality*

Introduction

Histopathology provides a sensitive indicator of sublethal stress induced by xenobiotics. Due to the central role of the liver in the biotransformation of several chemical active compounds into the aquatic environment, the teleost liver has been the focus of toxicological studies and has indeed been shown to be very sensitive to pollutant exposure [7, 18, 26, 27]. Hepatic changes resulting as consequences due to exposure to certain chemicals, especially Poly Aromatic Hydrocarbons (PAH's), regarded as characteristic, have being included in the definitions of beneficial use impairment criteria [7]. Relevant aquatic xenobiotic sources in Nigeria in particular, Lagos, the commercial capital with its great (and ever growing) population and industries, include pesticides, plastic wastes, myriad industrial effluents, sawmill and pulp industry runoffs and shipping ballast. The importance of the Lagos lagoon complex to its satellite populations has been described by Olarinmoye [22]. Due to a dearth of

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temporal relevant pathological data relating anthropogenic inputs to fish health, an attempt was initiated in 2005 by the authors to document, and determine the significance of the severity of observed lesions to the different levels of xenobiotic inputs into the Lagos lagoon complex and also to define the baseline health status of the test species which is suggested for use as a biomarker species [21, 22]. The test species, *C. nigrodigitatus,* was selected for use as a local sentinel species for the investigation of the impacts of marine pollutants on fish on the basis of it's ubiquitousness in Nigerian inland waters, and, it's situation in benthic habitat on muddy substrate of river bottoms and channels. Its natural proclivity for the bottom of water bodies brings the fish into intimate contact with the sediments, in which substantial proportions of aquatic pollutants are bound. As part of our efforts to ascertain the deleterious effects of contaminated aquatic habitats on fish health, and to establish beyond conjecture, the latter fact, this study into the hepatic pathology of C. *nigrodigitatus* collected from polluted reaches of the Lagos lagoon complex was carried out.

Materials and methods

Specimen collection

One hundred adult *Chrysichthys nigrodigitatus* were collected, live, from early morning catches at Badagry lagoon ($6^0$24'N, 2^0 56'E), and Lagos lagoon ($6^0$29'N, 3^0 22'E), two locations in the Lagos Lagoon complex, between May and August 2006. Fish were selected using the criteria of size. The external appearance of the fish indicated few abnormalities. No sex selection was made. The fish were sacrificed by a pre-occipital severance of the spinal cord. Each specimen was weighed and the standard length was recorded. The fish were then dissected, necropsies done, and the livers excised. Livers were then examined using hand lens, to detect the presence of gross lesions.

Histology

Liver samples of each specimen were fixed by placing in 10% formalin in phosphate buffer (Electron Microscopy Sciences, Hatfield, PA, USA) for 36 hours. Care was taken to ensure that onset of fixation was immediately post excision. Fixed specimens were dehydrated in graded ethanol (Sigma-Aldrich, St. Louis, MO, USA) and then transferred into xylene (Alfa Aesar GmbH & Co. KG, Karlsruhe Germany) for five minutes preparatory to embedding in paraffin (Sigma-Aldrich, St. Louis, MO, USA). Livers were then embedded in paraffin and histological sectioning subsequently done at 5 µm using a TBS® CUT™ (Cole-Palmer, UK) rotary microtome. Sections were randomly done but care was taken to ensure that a large as possible area of the livers were sectioned. Resulting sections were mounted on glass microscope slides and air dried prior to staining using Hematoxylin and Eosin stain, and cover slipped (Luna 1992). Stained sections were then analysed using light microscopy. Obtained sections were carefully observed under high magnification light microscopy at x350 and x450 magnification, for the presence and quantification of detectable stromal and parenchymal derangements including, but not limited to: 1. Vacuolation, 2. Macrophage aggregation, 3. Biliary duct proliferation, and, 4. Neoplasia. Various regions of each liver sample were sectioned to keep the investigative process as accurate as possible.

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Water analysis

(I) Sampling stations

Sites were selected for inclusion based on the presence of bottom feeding/dwelling, demersal fish, and, sampling locations were established approximately equidistant covering areas deemed representative of the study locations. They were, for Badagry lagoon ($6^0$24'N, 2^0 56'E): Akarakumo, Ajido, and the Marina, and for Lagos lagoon ($6^0$29N, $3^0$22'E): Ikorodu/Ibeshe, Iddo, and the Marina *(Fig. 1).* Each location was sampled every two weeks between May 2 and August 1, 2006.

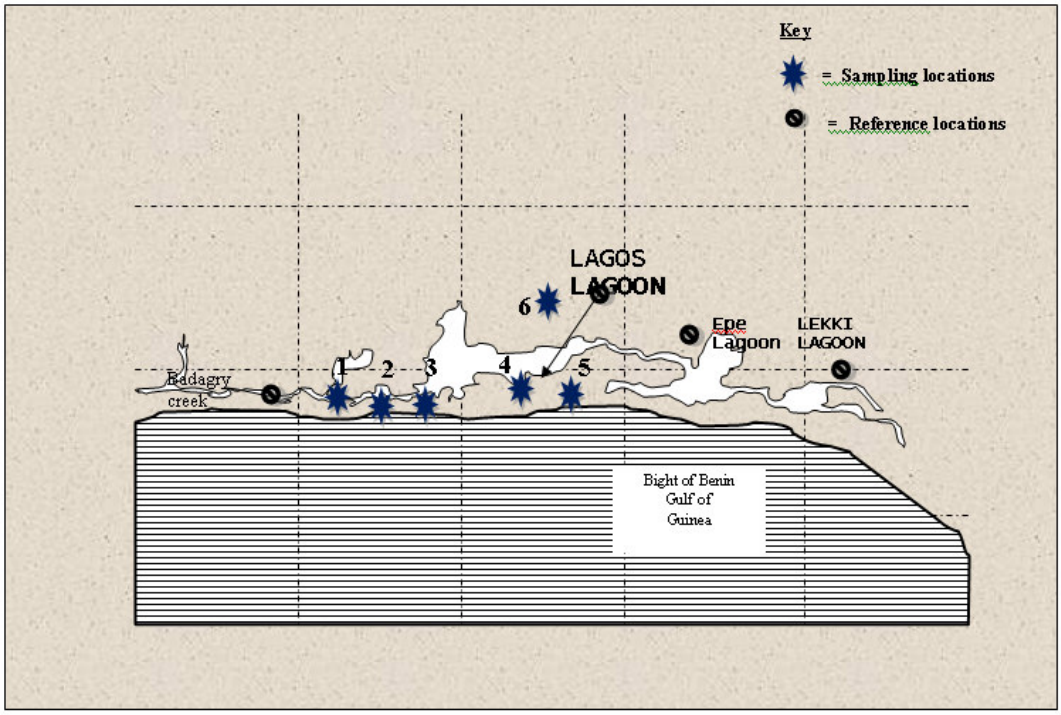

Figure 1. *Map of Lagos lagoon complex showing water sampling locations: 1-Marina, 2-Akarakumo, 3- Ajido, 4-Iddo, 5- Marina, 6-Ikorodu.*

(II) Water sample analysis

Temperature (equilibrated, Mercury in glass thermometer), turbidity, pH, salinity and conductivity measurements were conducted on site using the Horiba U-10 water quality checker. The determination of other parameters commenced in the laboratory within a few hours of collection [2].

(III) Statistics

The water quality results were analysed using the Microsoft Excel 2007 software. Means, standard deviations of water parameter measurements, and heavy metal concentrations were determined and tabulated *(Tables 1 and 2).*

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Table 1. *Water quality parameters for Lagos lagoon during period of study*

Water quality of Lagos lagoon									
	Ibeshe/Ikorodu		Marina		Iddo		Total		FEPA Std
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Temperature (c)	30.27	0.64	30.17	0.06	39.8	1.76	33.42	0.82	15
Turbidity	3.63	1.31	8.27	0.46	65	3	25.63	1.59	10
conductivity	160.3	4.51	42.83	0.23	163.3	2.89	122.17	2.54	1500
Salinity	12.23	0.306	2.78	0.015	1.603	0.358	5.54	0.23	0
pH	8.07	0.067	8.19	0.047	8.53	0.1308	8.26	0.08	6.9
Fe(Mg/L)	0.407	0.067	2.01	1.296	0.407	0.067	0.94	0.48	0.001
Cu(Mg/L)	0.11	0.01	2.76	0.452	0.11	0.01	0.99	0.16	0.5
Pb(Mg/L)	≤ 0.1	0	1.26	0.08	≤ 0.1	0	0.42	0.03	0.5
Cd(Mg/L)	≤ 0.1	0	≤ 0.1	0	≤ 0.1	0	0.00	0.00	0.005
Zn(Mg/L)	0.22	0.035	1.75	0.454	0.217	0.035	0.73	0.17	5
Cr(Mg/L)	0.133	0.032	0.51	0.409	0.133	0.032	0.26	0.16	0.001
Ar(Mg/L)	0.1	0	0.14	0.0608	≤ 0.1	0	0.08	0.02	0.1
Mn(Mg/L)	≤ 0.1	0	0.26	0.056	≤ 0.1	0	0.09	0.02	5
Ni(Mg/L)	≤ 0.1	0	0.477	0.232	≤ 0.1	0	0.16	0.08	0.5

Table 2. *Water quality parameters for Badagry lagoon during period of study*

Water quality of Badagry lagoon									
	Akarakumo		Ajido		Marina		Total		FEPA Std
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Temperature (c)	27.28	0.95	24.58	6.92	28.04	1.12	26.63	2.996667	15
Turbidity	13.58	3.49	12	3.26	13.46	3.22	13.01	3.323333	10
conductivity	2366.33	3449.59	42.83	1964.73	3109.9	2260.04	4274.55	2197	3611.347
Salinity	0.94	1.66	0.91	1.67	0.74	1.32	0.86	1.55	0
pH	7.44	0.73	7.45	0.63	7.59	1.09	0.453333	0.816667	6.9
Fe(Mg/L)	0.42	0.2212	0.36	0.1593	0.24	0.9441	0.126833	0.441533	0.001
Cu(Mg/L)	0.25	0.1562	0.25	0.13	0.14	0.737	0.0954	0.341067	0.5
Pb(Mg/L)	≤ 0.1	0	≤ 0.1	0	≤ 0.1	0	0	0	0.5
Cd(Mg/L)	0.13	0.1033	0.15	0.137	0.15	0.137	0.0801	0.125767	0.005
Zn(Mg/L)	1.09	2.042	0.23	0.156	0.31	0.191	0.732667	0.796333	5
Cr(Mg/L)	≤ 0.1	0	≤ 0.1	0	≤ 0.1	0	0	0	0.001
Ar(Mg/L)	0.7	0.044	0.72	0.043	0.078	0.04	0.029	0.042333	0.1
Mn(Mg/L)	0.28	0.222	0.26	0.23	0.15	0.22	0.9	0.124	0.424
Ni(Mg/L)	0.1	0	0.1	0	0.1	0	0	0	0.5

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Results

Microscopic examination showed normal liver appearance in forty percent of the test population (n = 40). In the latter group, normal hepatocyte morphology including minimal vacuolation, lipid and glycogen storage, sparse biliary duct numbers, and normal arrangement of hepatic cords were a consistent finding *(Fig. 2).*

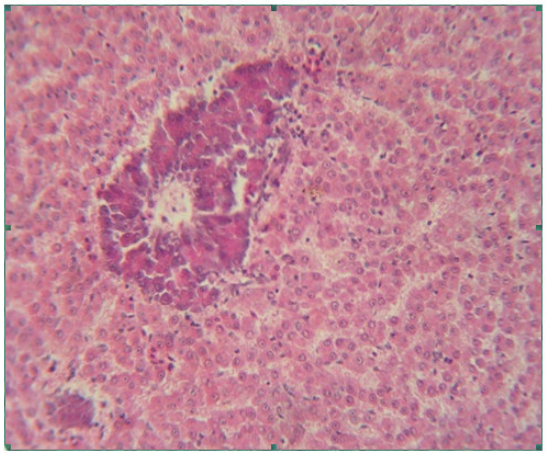

Figure 2. *Normal liver histology of Chrysichthys nigrodigitatus (H & E; x350)*

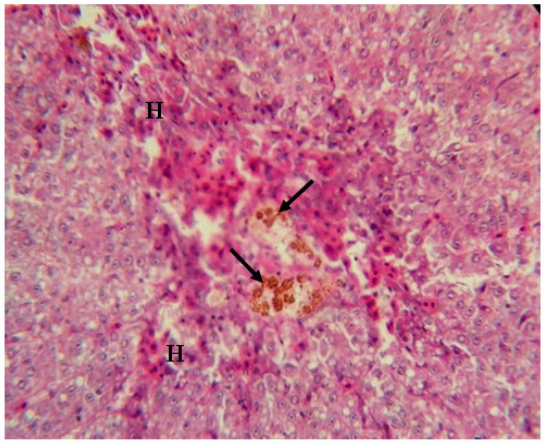

Figure 3. *Portal congestion, vacuolar degeneration of hepatocytes, pancreatic necrosis, heterophilic (H) infiltration and haemosiderin-laden melano-macrophages (arrows) (H & E; x350)*

Mild portal congestion and sinusoidal congestion (33%) ranging from mild to severe were a relatively consistent finding in all pathologic specimens. Vacuolar hepatocellular degeneration and necrosis and pancreatic necrosis (58%) and architectural disruption and dissociation of the Bilroth cords (26%) were a regular finding with the majority of the observed lesions occurring in livers from Lagos lagoon specimens (Hydropic Vacuolation: 31%; Bilroth cord thinning:14%) *(Fig. 3, 4 & 5).* In most of the specimens from the latter location, the observed degeneration was severe and widespread *(Fig. 4)*, in contrast to the mild to moderate vacuolation seen in the Badagry lagoon specimens.

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Affected hepatocytes were enlarged with clear staining vacuoles which compressed the cytoplasm and nuclei to the cell margins *(Fig. 3).* In some specimens, the cordlike layout of the hepatocytes was maintained. However, in severe cases, there were severe disruptions in the normal Bilroth cord layout *(Fig. 5).* Coagulative hepatic necrosis, hemosiderosis (12%), and Kupffer cell hyperplasia (11%) as a group of lesions and, individually as unit observations was also observed. An abnormal proliferation of megalocytes *(Fig. 5)*, probably pre-neoplastic or an indication hepatic regeneration, was seen. No neoplastic features were however observed. Tables 1 and 2 set out the water quality determination results.

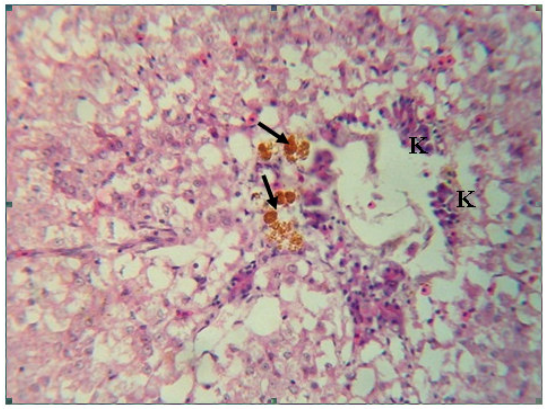

Figure 4. *Severe widespread vacuolar degeneration and necrosis of hepatocytes, pancreatic necrosis and presence of melano-macrophages (arrows) and Kupffer cells (**K**) (H & E; x450)*

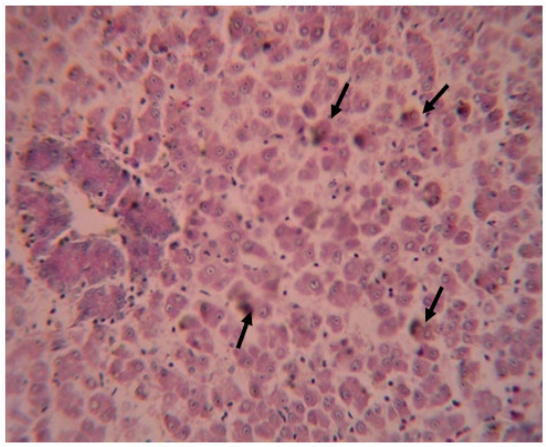

Figure 5. *Hepatocellular and pancreatic necrosis, severe disruption of hepatic cord architecture and presence of megalocytes (arrows) (H & E; x350).*

Discussion

Histopathology is widely accepted as a useful method for the assessment of injury in fish due to the adverse short term and chronic effects of xenobiotic exposure. Several liver lesions have been established as putative tissue biomarkers consistent with the exposure of fish to xenobiotics. These include pigmented macrophage aggregations [24,

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10], hepatocyte hydropic vacuolation (*HydVac)* [29], pre-neoplastic foci of cellular alteration [3], and liver neoplasms [6, 26]. These biomarkers have also been conclusively linked with certain factors e.g. macrophage aggregations have been shown to increase with age [7], and stress [10], and, hydropic degeneration with level of exposure to PCB's [29] etc. HydVac was a consistent finding in this investigation and was the most frequently observed and widespread lesion. The preponderance of this lesion in fish from contaminated waters bordering urban locations similar to our test locations has been firmly established and described in detail for, Winter flounder *Pleuronectes americanus* [4, 17, 19], and, white perch *Morone americana* [8], among others. Augspurger *et al*. [4] presented a compilation from various sources documenting the prevalence of HydVac in fish resident in the waters of the northeast United States Atlantic coast, and conclusively established a direct relationship between the pervasiveness and severity of this lesion, hepatic neoplasms and levels of site contamination, noting the absence or low prevalence of HydVac in relatively uncontaminated areas, and the positive relations between the lesion and hepatic neoplasia in highly contaminated areas. Also corroborating this locational correlation, [23] reported that the risk of liver lesion occurrence in Sole *Pleuronectes vetulus*, from non urban, relatively unpolluted locations was lower than for urban and near urban sites on along Puget sound. The latter observations and deductions were consistent with our findings, and conclusively indicative of the relationship between locational pollution indices and lesion prevalence. The prevalence of cases occurring in specimens from the Lagos lagoon could be attributable to the very large influx of all manner of untreated sewage, industrial effluent and other point source pollutants into this water body, the tidal flushing effect of the Atlantic notwithstanding. The converse of the latter situation applies for Badagry, where the satellite population, industrial activity, and, concomitantly, effluent and waste generation and dumping is significantly lower than in Lagos. The water quality information in tables 1 and 2, set out clearly the differences in habitat quality between Lagos lagoon and Badagry. The readings for Lagos consistently exceed, those for Badagry, and, Federal Environmental Protection Agency of Nigeria (FEPA) standards. The stresses of exposure of fish resident in this location seems to adequately explain the preponderance of observed lesions in this location. Certain uncertainties as to whether HydVac is part of a proliferative process or apoptopic in nature have emerged [15, 19]. There is a consensus, however, that vacuolated hepatocytes are frequently found proximal to neoplasms and that tumor prevalence is associated with increasing numbers of vacuolated liver cells and that, the extent of deformity and cell injury is also consistent with hepatotoxicant action [4, 11]. PAH's have been implicated in the development of liver carcinogenesis [5, 14, 30] and a cause and effect relationship between PAH's and liver tumors or preneoplastic lesions in fish has been established by in-vitro studies [16, 28]. Our observations in the present study could be attributable in part to this chemical group, as measurable levels of heavy metals, hydrocarbons, organo-chlorines, PAH's and PCB's have been reported in the Lagos lagoon complex by Ajao *et al.* [1]. In spite of the latter, analytic studies on the lagoon complex ascertaining the true composition and relative concentrations of individual chemicals and chemical groups, in the xenobiotic cocktails present are lacking and have been initiated by this research team as of the present. The absence of frank tumors in the test population should not be taken as given that no such end state lesions of oncogenesis exist in these waters. More realistically, it could be presumed that the levels of xenobiotic contamination in the test waters are significant, and that the

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exposure of resident feral fish populations could lead to the development of tumors. Such absences could also be a result of limited test specimen numbers, few test locations and migrant proclivities of feral fish populations away from heavily polluted locales. These are some of the experimental limitations to be considered in later screening exercises. Vascular aberrations exhibiting as congestion of sinusoidal vessels was the second most common lesion. Sinusoidal congestion has been reported as pathognomonic of exposure to some toxicants, including insecticides [9]. However the presence of focal and in some cases, widespread areas of hepatic necrosis, as reported in our results would be regarded alongside HydVac, as a more demonstrative indication of contaminant induced hepatoxicity [27]. This study, the third in a series, establishes the significant pollution index of the Lagos lagoon complex and the inducibility of oncogenesis and frank neoplasms in resident finfish, especially benthic species, as a result of exposure to sediment bound xenobiotics. It is planned for the future that analytic studies are carried out on these waters to establish the identities of present pollutants, the relative preponderance of these pollutants, and to establish a definite cause and effect relationship between observed lesions and identified pollutants.

This study has established to some degree the disruptive and pathological potential of polluted Nigerian estuaries on resident aquatic fauna, and corroborates earlier work described by the authors.

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