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Gross pathology, physiological and toxicological responses in relation to metals and persistent organic pollutants (POPs) burden in tilapia species from Ogun River, Nigeria

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ABSTRACT

We have investigated gross pathology, physiological (steroid precursors) and toxicological responses (oxidative stress and phase II biotransformation) in relation to tissue contaminant burden in Tilapia species along the entire length (320 km) of Ogun River, Nigeria. The Ogun River is the longest and largest river in Southwestern Nigeria located along heavily industrialized cities and receives complex mixtures of effluents. A total of 1074 tilapias were collected from three sampling points (Abeokuta, Isheri and Ikorodu) and from an upstream control point (Igboho) and evaluated for gross pathological changes, hepatic transcript levels for oxidative stress and phase II biotransformation responses. Trace metal concentrations and POPs in muscle samples were analyzed using ICP-MS and GC-MS respectively. Evaluation of gross pathological changes showed a 50-, 33-, 17 and 0% prevalence of hepatic tumors at the Ikorodu, Abeokuta, Isheri and Igboho sites, respectively. Plasma concentrations of cholesterol and pregnenolone showed apparent significant decreases at downstream sites of the control point in both male and female fish (except for pregnenolone levels of male fish at Ikorodu). Inversely, *gst, ugt*-1, *ZuCu-sod* and *sod* significantly increased in fish collected from downstream sites, compared with the control site and these increases paralleled the significant increase in trace metal and POPs concentrations at these sites. PCA revealed a site related association between measured toxicological responses and contaminant burden, indicating a potential cause-and-effect relationship. Thus, the possible adaptation of Ogun River Tilapia species to contaminants may have significant consequences on cellular, physiological and biochemical processes regulating metabolism, growth, development and reproduction, and also have serious human health consequences, since the Ogun River is used for fisheries and domestic water supply for surrounding neighborhoods.

1. Introduction

The increasing release of environmental contaminants including heavy metals from industrial, agricultural and domestic sources into the aquatic environment possess a serious threat to aquatic biota (Wang, 2002; Dautremepuits et al., 2004; Arukwe et al., 2015). These potential threats have attracted serious scientific and societal attention, both in developed and developing countries because heavy metals are not biological degradable, and as such have the ability to accumulate in the environment, biota and humans who depends on aquatic organisms including fish as sources of food (Farombi et al., 2007; Croom, 2012). In Nigeria and many other developing countries, the contamination of aquatic ecosystems, mainly due to improper waste disposal such as the discharge of largely untreated effluent from anthropogenic sources, has resulted in the widespread pollution of most rivers, lakes and streams (Adeogun et al., 2016a,b,c). Despite the alarming potential environmental, human health and wildlife consequences of contaminant exposure, information on the biological effects is limited in Nigeria and many other developing countries. For example, the Ogun River is the longest and largest river in

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Southwestern Nigeria; located along heavily industrialized cities and receives industrial, agricultural and domestic effluents. Previous scientific reports from the Ogun River have mainly focused on measuring biota heavy metal concentrations in relation to hydrological conditions (Jaji et al., 2007; Adeniyi et al., 2008), with limited data on the biological effects of contaminant exposure along this large river (Ibor et al., 2016).

Exposure of organisms to environmental contaminants may modulate the activities and expression of enzymes that activate biological processes in order to manage or eliminate these foreign chemicals through a process generally referred to as oxidative stress and biotransformation responses (Galloway et al., 2004; Arukwe et al., 2015). Phase II biotransformation usually involves the introduction of a covalent linkage between the functional group of a xenobiotic and an endogenous water-soluble conjugate such as glucuronic acid to facilitate excretion (Lech and Vodicnik, 1984). Once conjugated, the xenobiotic compound becomes more water-soluble and is easily excreted. Several classes of environmental contaminants such as heavy metals, polyaromatic hydrocarbons (PAHs) polychlorinated biphenyls (PCBs), organochlorinated pesticides (OCPs) act as prooxidant stressors increasing the intracellular generation of reactive oxygen species (ROS) causing oxidative stress conditions via the mitochondrial electron transport chain, which in turn affect the levels and functions of redox-sensitive signaling proteins and antioxidants (Osburn and Kensler, 2008). A group of cytoprotective enzymes such as the glutathione transferase (GST), uridine diphospho-glucuronosyltransferase (UDPGT), glutathione reductase, catalase, Cu/Zn-superoxide dismutase (SOD) and glutathione peroxidases (GPx) that are coded through the antioxidant responsive element (ARE) form a network of protective machinery against oxidative stress (Osburn and Kensler, 2008; Giuliani et al., 2013; Regoli and Giuliani, 2014).

The synthesis of steroid hormones and their primary precursor (cholesterol) are important and critical factors in the physiological development of organisms including growth, development and reproduction (Sayed et al., 2012). Moreover, it has been established that the generation of ROS and their derived free radicals, due to contaminant exposure may perturb their regulatory roles in many different process, such as hormonal responses in animals, alter membrane lipid profile, cellular damage, development of tissue/organ injury and physiological consequences such as disease and aging (Murphy et al., 2005; Arukwe et al., 2008; Winterbourn and Hampton, 2008; Lushchak, 2011; Olufsen et al., 2014). An alteration in steroid hormone synthesis, gross pathology of tissue, organ damage that is coupled with transcriptional changes in antioxidant defense system are important biological responses, from a toxicological standpoint. The reason for this is because, the biological effects of pollutant exposure in organisms can be investigated through several molecular and biochemical biomarkers reflecting the onset of various cellular alterations (Valavanidis et al., 2006; Osman et al., 2010; Pereira et al., 2013). Further, in biomonitoring studies, changes in antioxidant enzyme system, both at transcriptional (mRNA) and functional (enzyme activity) levels may indicate exposure to group of environmental contaminants, including heavy metals (Valavanidis et al., 2006). These responses have been widely adopted as sensitive and reliable early warning signals of biological exposure to pollutants (Regoli et al., 2011; Regoli and Giuliani, 2014). Therefore, the aims of this study were to investigate the changes in general health condition (organ pathology and biometrics), enzyme protective systems (oxidative stress and biotransformation responses) and alterations in precursor molecules (cholesterol and pregnenolone) for steroid hormone synthesis, in relation to trace metal burden in the muscle of three Tilapia species from Ogun River, Nigeria.

2. Materials and methods

2.1. Chemicals and reagents

Trizol reagent was purchased from Gibco-Invitrogen Life Technologies (Carlsbad, CA, USA). iScript™ cDNA synthesis kit, iTaq DNA polymerase, dNTPmix, iTaq™ Sybr® Green supermix with ROX and EZ Load 100 bp Molecular Ruler were purchased from Bio-Rad Laboratories (Hercules, CA, USA). GelRed™ Nucleic Acid Gel Stain was purchased from Biotium (Hayward, CA, USA). All other chemicals were of the highest commercially available grade.

2.2. Study area

Ogun River is the largest and longest River in southwest Nigeria with the characteristics of a tropical climate (Fig. 1). It flows from Igboho in Oyo state through Abeokuta and Isheri in Ogun state and empties into the Lagos lagoon at Ikorodu. The River serves as a source of drinking water, supports major artisanal fishing activities, especially in Oyo, Ogun and Lagos States of Nigeria and is used for other domestic purposes by local communities. The Ogun River receives untreated industrial effluents, domestic sewage and waste disposal from several point sources. The entire length of the river studied span about 320 km from its source in Igboho to Ikorodu where it empties into the Lagos Lagoon. Four sampling points were selected (i.e Abeokuta, Isheri and Ikorodu) including a putative control point at Igboho. Brief descriptions of the major activities at all the sampling points are presented below.

Igboho: Chosen as a putative control point, because there is no evidence of industrial or domestic waste discharge at this point. Fishing and agricultural activities were the only activities observed. It is about 237.9 km away from the second sampling point, namely Abeokuta.

Abeokuta: Domestic effluents from residential areas and Lafenwa market are discharged at this point and industries located at this portion of the river include – those that produce paints, bottle water, products for human and veterinary pharmaceutical and consumer health, personal care products, beverages, sawmill, car wash and abattoir. Activities such as dumping of wood shavings from the sawmill industry and the discharge of warm effluent (consisting of kerosene and detergent for burning and washing burnt cattle) employed by the abattoir industry in washing roasted cattle's are discharged into the river at this point. Other effluents that are characterized by pungent smell and appear very viscose and turbid are also discharged at this point. The site is about 46.7 km away from the next sampling point, Isheri.

Isheri: Domestic effluents from residential areas and the popular Kara (Isheri) cow market are discharged here and industries located along this point include; (a plastic industry involved in the production of plastics, nylon bags, paper, security doors and plastic shoes), pulp mill effluent, abattoir and a mechanic workshop. This point is about 25.5 km away from the last sampling point Ikorodu.

Ikorodu: Domestic and agricultural effluents are discharged here and major construction and sand mining activities were observed at this point.

2.3. Sample collection

A total of 1074 live Tilapias [*T. guineensis* (n = 578, mean weight: 148.7 ± 20.1 g), *S. galileaus* (n = 278, mean weight: 139.5 ± 39.2 g) *and O. niloticus* (n = 218, mean weight: 99.5 ± 23 g)] were collected across the entire length of Ogun River. Fish samples were collected with a cast net of 50–55 mm mesh size with the aid of local fishermen between January 2013 and April 2014. Fish were anaesthetized on ice and liver was harvested immediately and preserved