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Vitellogenin induction and reduced fecundity in zebrafish exposed to effluents from the City of Bulawayo, Zimbabwe

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HIGHLIGHTS

- We tested effects of effluents from Bulawayo Zimbabwe on zebrafish reproduction.
- Sewage treatment plant effluent caused vitellogenin induction in male zebrafish.
- Textile effluent reduced fertilization success and gonad maturity of fish.
- Little data is available on EDCs in Developing Countries in Sub-Saharan Region.

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ABSTRACT

Industrial and municipal effluents regularly pollute water bodies and cause various toxic effects to aquatic life. Because of the diverse nature of industrial processes and domestic products, urban effluents are often tainted with various anthropogenic endocrine disrupting chemicals that may interfere with the reproductive physiology of aquatic fauna. In this study, we tested effluents from the City of Bulawayo for the presence of estrogenic endocrine disrupting chemicals and their effects on fish gonads and fecundity. Effluents were collected from two sewage treatment plants (STPs), which receive largest volume of industrial effluents from the City, and from a textile factory. Male and female zebrafish (*Danio rerio*) were exposed to effluents and analyzed for vitellogenin induction, gonad alterations, and fertility. Male zebrafish exposed to effluent from Thorngrove STP had significantly higher ($p \leq 0.05$) vitellogenin compared to control. Textile effluent caused adverse gonad alterations such as oocyte atresia (females) and increased proportion of spermatogonia (males) which could lead to reduced fertility. Textile effluent (5% v/v) and Thorngrove effluent also caused a decline in fertilization success of breeding groups of zebrafish. The results of this study show the potential effects of effluent pollution and the occurrence of EDCs in developing countries. This underscores the need to effectively prevent pollution of environmental water bodies from industrial and municipal sewage treatment plant effluents. We recommend a follow-up study to monitor the effects of the effluents on feral fish in effluent polluted downstream dams of Bulawayo.

1. Introduction

Some anthropogenic chemicals that may contaminate water bodies have received considerable attention from environmentalists and environmental protection agencies because they have been shown to interfere with reproduction of wild organisms. Some of their known effects have been linked to a decline of fecundity (e.g. Ankley et al., 2002; Zha et al., 2006) and gamete quantity (Haldén et al., 2010; Saillenfait et al., 2009), therefore their presence in the environment can affect processes of reproduction leading to a decline of populations of organisms. Urban effluents are well known as significant sources of anthropogenic chemicals such as endocrine disrupting chemicals (EDCs) to the environment (Gomes et al., 2003; Körner et al., 1999; Kusk et al., 2011; Purdom et al., 1994). This is because a variety of industrial processes in urban areas release chemicals which are known to interfere with reproductive physiology of organisms such as alkyl phenols (Bicchi et al., 2009; Céspedes et al., 2008), bisphenol-A (Spengler et al., 2001), nonyl-phenol (Soares et al., 2008), pharmaceuticals (Spengler et al., 2001) and flame retardants (Haldén et al., 2010).

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Many of the known EDCs are estrogenic, (Metcalfe et al., 2001; Routledge and Sumpter, 1996), acting as estrogen mimics or increasing blood estrogen levels, sometimes resulting in feminization of male organisms (Harris et al., 2011). Globally, reports have established the occurrence of EDCs in urban effluents (Fang et al., 2012; Kirk et al., 2002; Metcalfe et al., 2001; Nakada et al., 2004; Pawlowski et al., 2004; Purdom et al., 1994) and effluent polluted water bodies (Buchinger et al., 2013; Song et al., 2006; Wang et al., 2011). So far, the predominant sources of these chemicals have been municipal sewage treatment plants (Kusk et al., 2011; Nakada et al., 2004; Nakari, 2004).

Due to their known and perceived impacts, a number of highly sensitive early detection biomarkers have been developed to detect EDCs and are useful in understanding the mechanisms in which diverse chemicals exert their toxicities. These biomarkers include vitellogenin (Vtg) induction in male or juvenile fish (Folmar et al., 1996; Navas and Segner, 2006), the recombinant yeast estrogen screen (YES) tests (Beck et al., 2006; Routledge and Sumpter, 1996; Schultis and Metzger, 2004), estrogen responsive MCF-7 cells (Matsumura et al., 2005; Okubo et al., 2001), and histopathological assessments (Johnson et al., 2009).

The induction of Vtg in live fish has many advantages as a biomarker. Most importantly, unlike *in-vitro* tests such as MCF-7 and YES assays, live fish present realistic exposure routes and the entirety of metabolic pathways before any effects are detected. Therefore, Vtg induction in immature or male fish is a recommended biomarker of exposure to exogenous estrogens or estrogen mimics in the aquatic environment (Herbst et al., 2003; Okoumassoun et al., 2002; Örn et al., 2003). The zebrafish Vtg ELISA has been developed and recommended as a sensitive assay (Holbech et al., 2001) and commercial kits are now available for routine screening.

Although Vtg is a reliable biomarker of exposure to (xeno) estrogens, it cannot be used to indicate reproductive dysfunction (Mills et al., 2003) and thus other assessments such as gonad histopathology are indispensable (Johnson et al., 2009) and valuable if assessed along molecular biomarkers.

Histological alterations in gonads subsequent to exposure to water pollutants have been noted. In particular, some gonad alterations have been demonstrated to be strongly correlated to (xeno) estrogen exposure (Jobling et al., 2006; Mlambo et al., 2009). Due to the importance of gonad histological data in assessing reproductive dysfunction, harmonized guidelines have been developed to assess these alterations in order to have consistency in data outputs and interpretation (Johnson et al., 2009).

Municipal sewage treatment plants have a fundamental role of safeguarding the environment from pollution by mixtures of diverse complex effluents. However, some of the pollutants are not completely removed during the effluent treatment process. Some scientific reports have demonstrated that some pollutants are released in treated effluents at concentrations that are potentially toxic to some organisms (Körner et al., 2001; Lee et al., 2008).

Developing Countries such as Zimbabwe face the challenges of dilapidated STPs; insufficiently treated effluents flow into peri-urban water bodies (BCC, 2014) which could have serious environmental consequences. Despite the worldwide attention and concern given to water pollution, Developing Countries have noticeably scant literature on water pollution and assessment of its ecological risk.

The City of Bulawayo is an industrial hub of the Republic of Zimbabwe. Textile factories are among the prevalent industry in Bulawayo. Due to an economic crash the country has experienced within the last decade, Bulawayo, like other cities, has been facing serious challenges in maintaining its aging sewage reticulation system, leading to excessive pollution of downstream water bodies from both raw and inadequately treated effluents. Most of the sewage treatment plants were constructed when the City had a human population of 350 000 (BCC, 2014), yet the current human population has reached approximately 700,000 (Zimstat, 2013). At the time of the study (2014), it was estimated that at least 70% of the effluents from the City (approximately 50 MLd^{-1}) were directly flowing into downstream peri-urban dams (BCC, 2014) raising concerns that this could lead to serious adverse ecological consequences downstream. To date, no studies have assessed the (potential) environmental effects of these effluents except for the occurrence of heavy metals in one of the downstream dams (Siwela et al., 2010, 2009).

In this study, we used zebrafish to assess textile and STP effluents from the City of Bulawayo, Zimbabwe for the presence of estrogenic EDCs (using Vtg as a biomarker) and their effects on fish fecundity.

2. Materials and methods

2.1. Materials

Tricaine methane sulfonate and 17α-ethinyl estradiol were sourced from Sigma Aldrich, Germany. Zebrafish Vtg ELISA kits (product number: V01008402) were sourced from Biosense, Denmark. Mayer's hematoxylin, Eosin-Y and histology mounting media (Bio Mount) were sourced from, Bio-Optica, Italy. Paraffin wax (56 °C-58 °C) was sourced from, MEDITE® GmbH, Germany. Methanol (99.9%) and xylene (99%) were sourced from Merck, South Africa; formaldehyde (37–40%) was sourced from Associated Chemical Enterprises, Johannesburg South Africa.

A microplate spectrophotometer (Spectramax® 340pc) was used for ELISA assays (Molecular Devices, USA). Histological sections (5 μm) were prepared using a rotary microtome (Jung Heidelberg, Germany), using disposable rotary microtome blades (Shandon, Thermo Electron Corporation, USA).

2.2. Test model fish

Adult healthy zebrafish were purchased from a local pet shop. The fish were adapted for three weeks to laboratory conditions before performing any studies. The laboratory conditions included an artificial 12/12 photoperiod regulated by an automated lights switch and regulated temperature of 27 ± 1 °C to mimic optimal tropical conditions for zebrafish. The fish were fed on frozen *Artemia nauplii* and freeze dried larvae three times a day in the morning, afternoon and evening. During feeding, the fish were also monitored for health status. The fish were kept in activated carbon filtered municipal potable tap water in 100 L glass aquaria. Carbon filtration was done to remove residual chlorine and to adsorb organic contaminants using granular activated carbon (1 kg) filled in-line in circulatory water pumps. The municipal potable tap water in Bulawayo was drawn from 'pristine' dams with no history of water contamination. Fish densities in aquaria were kept at no more than one fish per liter of water. Water quality was maintained by air pumps (to maintain high oxygen levels) and use of water pumps fitted with in-line physical filter (to remove physical wastes), biological filter (to remove ammonia) and activated carbon. After three weeks of acclimatization, fish were used for experiments. All experimental procedures were performed in compliance with relevant laws, institutional guidelines, and the Zimbabwe Parks and Wildlife Authority. Our methods also complied with the U.K. Animals (Scientific Procedures) Act, 1986 and the ARRIVE guidelines.