

# Detection and Quantification of Oestrogenic Endocrine Disruptors in Water in Mwanza Gulf in the Lake Victoria Basin, Tanzania

Mdegela, R.H<sup>1\*</sup>, Mabiki, F<sup>2</sup>, Msigala, S<sup>2</sup>, Mwesongo, J<sup>2</sup>, Mhina, M.P<sup>3</sup>,  
Waweru, K<sup>4</sup>, Mbuthia, P<sup>4</sup>, and Byarugaba, D.K<sup>5</sup>

<sup>1</sup>Sokoine University of Agriculture  
Faculty of Veterinary Medicine  
Morogoro, Tanzania

<sup>2</sup>Sokoine University of Agriculture  
Faculty of Science Morogoro, Tanzania

<sup>3</sup>National Fish Quality Control Laboratory  
, Nyegezi, Mwanza, Tanzania

<sup>4</sup>University of Nairobi  
Faculty of Veterinary Medicine  
Nairobi, Kenya

<sup>5</sup>Makerere University  
College of Veterinary Medicine, Animal Resources and Biosecurity  
, Kampala, Uganda

Correspondence E-mail: [rmdegela@yahoo.com](mailto:rmdegela@yahoo.com) / [mdegela@suanet.ac.tz](mailto:mdegela@suanet.ac.tz)

**Abstract:** *The aim of this study was to detect the presence and quantify the total oestrogens (estriol (E1), estradiol (E2), and estrone (E3)) in Lake Victoria water with a view of assessing their contribution to the health status of fish. A total of 27 water samples; three from each of the nine sampling sites were collected in Mwanza gulf in the city in May 2012. Solvent extraction procedures were used to obtain extracts of pollutants that were further analysed using the competitive Enzyme-Linked Immunosorbent Assay (ELISA) technique to detect and quantify the total oestrogens. Overall, the concentration of total oestrogens was low and ranged from 10 – 200 pg/L. Concentrations of these chemicals decreased along the gradient, being highest (107±81.4 pg/L) in rivers before entering into the lake and lowest (19±5.4 pg/L) in water samples collected in the lake at about 100 meters from inshore (intermediate sampling points). Levels of total oestrogens were significantly different between categories of water sources (P = 0.009). Two most polluted rivers were Butimba and Nyakurunduma with concentrations at 150 pg/L and 200 pg/L respectively. Dumping of wastes in rivers without treatment was the most likely source of the pollutants. Findings from this study have revealed the existence of oestrogens with endocrine disrupting properties at different concentrations, and that rivers are the main sources of oestrogenic endocrine disruptors in Lake Victoria water near Mwanza City.*

**Key words:** ELISA, endocrine disruptors, oestrogens, pollution

## INTRODUCTION

Lake Victoria is the second largest freshwater lake in the world and is central to economic development in the East African region. Together with River Nile, this lake provides opportunities for socio-economic activities that are vital to the life of over 160 million people living in various countries including Egypt, Sudan, Uganda, Ethiopia, Eritrea, Kenya, Tanzania, Rwanda, Burundi and the Democratic Republic of Congo (Odada *et al.*, 2004). The economic development of these countries is primarily based on agriculture and agriculture-based industries, fisheries, mining and tourism.

Despite its potential contribution to the economic development, the lake is under considerable pressure from a variety of interlinked human activities. Pollution resulting from these activities is among important stressors that cause serious negative consequences to the functions and services of the lake including loss of biodiversity (Odada *et al.*, 2004). Among the pollutants perceived to be of concern are endocrine disruptors (Colborn *et al.*, 1993). These are exogenous agents with potential to interfere with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones present in the body for homeostasis, reproduction and developmental processes both in humans and animals including fish (Campbell *et al.*, 2006). Oestrogens (estrone (E1), estradiol (E2) and estriol (E3)), the three major naturally occurring estrogens in women are among important endocrine disruptors responsible for the maintenance of reproductive tissues, organs, breasts, skin, and brain. These female hormones are excreted through urine or faeces (Hanselman *et al.*, 2003) and often end up in the environment through sewage discharge or animal waste disposal (Wenzel *et al.*, 1998; Lintelmann *et al.*, 2003). Estradiol is the predominant estrogen during reproductive years both in terms of absolute serum levels as well as in terms of estrogenic activity. During menopause, estrone is the predominant circulating estrogen and during pregnancy, estriol is the predominant circulating estrogen in terms of serum levels. Although estriol is the most plentiful of the three estrogens, it is also the weakest while estradiol is the strongest with a potency of approximately 80x that of estriol.

According to Liu *et al.* (2009), E1, E2 and E3 contribute to about 74% of the total excreted chemicals and the remaining 26% is derived from other sources. These hormones (E1, E2 and E3) also contribute about 90% to the total estrogenic activity in surface water and sediments compared to other oestrogenic anthropogenic chemicals (Furuichi *et al.*, 2004; Pojana *et al.*, 2007 and Duong *et al.*, 2010).

Several studies in different parts of the world have shown that wild fish exposed to untreated sewage water exhibit reproductive abnormalities consistent with exposure to estrogenic pollutants (Purdom *et al.*, 1994; Harries *et al.*, 1996; Jobling *et al.*, 1998; 2003). Natural and synthetic hormones excreted by humans, as well as some alkylphenolic industrial chemicals in sewage effluents are the ones responsible for the majority of estrogenic activity (Desbrow *et al.*, 1998; Routledge *et al.*, 1998). High prevalence of feminised male roach (*Rutilus rutilus*), a cyprinid (carp) fish with abnormally high plasma vitellogenin concentrations (a female plasma protein), and intersex (development of eggs within their testes) in rivers throughout the

Detection and Qualification of Oestrogenic Endocrine Disruptors in Water  
*Mdegela, R. H<sup>1\*</sup>, Mabiki, F<sup>2</sup>, Msigala, S<sup>2</sup>, Mwesongo, J<sup>2</sup>, Mhina, M.P<sup>3</sup>,  
Waweru, K<sup>4</sup>, Mbuthia, P<sup>4</sup>, and Byarugaba, D.K<sup>5</sup>*

United Kingdom is one of the examples demonstrating the effect of exposure to effluents with oestrogenic endocrine disruptors (Jobling *et al.*, 1998; 2006). Other health effects in male fish include hermaphroditism and impaired testicular developments.

With the exception of South Africa (Swart and Pool, 2007; Olujimi *et al.*, 2010), the oestrogenic endocrine disruptors, an emerging group of pollutants have not been studied in most of the African countries including Tanzania. Mwanza City, located within the Lake Victoria basin is the second largest urban centre in Tanzania with a fast human population growth rate of 4.5%. With a human population of over 600,000, only 90% of the area is served with clean and safe water and 8% (about 35,000 people) are connected and use formal sewerage services. Thus, over 90% of the human population in the city has poor sewage disposal systems and as a result much of it ends up in rivers and in the Lake Victoria. Rapid urbanisation and industrialisation processes taking place in Mwanza, while improving the socio-economic conditions, they also lead to increased environmental pollution from untreated domestic and industrial waste discharges, pesticides and other toxic chemicals.

Currently, there is lack of information on oestrogenic endocrine disruptors released in the environment in Mwanza City. Due to increased global attention on the impact of endocrine disruptors in humans and wildlife, and in particular in fish, assessment of oestrogenic pollutants in rivers and in the lake within the Lake Victoria basin is important.

The aims of this study, therefore, were to detect the presence of total oestrogenic endocrine disruptors in the lake, quantify their levels and assess the contribution of different activities to the hormones that are released in the lake from different rivers.

## **MATERIAL AND METHODS**

### **Materials**

HPLC grade water, Ethanol, Methanol, Hexane and 2-propanol and ELISA KIT (E1/E2/E3) (Ecologena®) Japan Enviro Chemicals, Ltd.

### **Description of Study Area**

This study was conducted in Mwanza City, which has a total area of 1,324 km<sup>2</sup> out of which, 900 km<sup>2</sup> (68%) is covered by water and the remaining 424 km<sup>2</sup> (32%) is dry land. It lies along the southern shores of Lake Victoria, North-West of the United Republic of Tanzania. It has a human population of over 600,000 and is fast developing as a centre of regional economic development for the East African region. About 70% of the population live in unplanned settlements, mostly in hilly areas, without access to a central sewerage system services and generates pollutants that enter into the lake through rivers and rain wash outs.

This study was conducted in May 2012 both in the Lake Victoria in Mwanza City where the field based studies were carried out and at Sokoine University of Agriculture, in Morogoro, Tanzania where the method for detection and

quantification of total oestrogens was developed and validated. Validation of methods for water extraction and for the ELISA technique were carried out using a pristine water sample collected from the origin of Morogoro River and a polluted water sample from a sewage pond at Mzumbe University, in Mvomero district.

### **Collection of Water Samples**

In Mwanza City, water samples were collected in May 2012 from nine sampling points in Lake Victoria in Mwanza gulf (Table 1) during the end of rainy season. Three samples were collected from each of the nine sampling points. The sampling points in beaches included inshore (0 meter), intermediate (about 100 meters from the inshore) and offshore (about 500 meters from the inshore). Sampling in rivers was made at about 100 meters before entry into the lake; at entry point into the lake (0 meter) and at about 500 meters from the river entry point.

The water samples were collected in clean 2.5 litre glass containers and immediately transported to the laboratory at the National Fisheries Quality Control Laboratory (NFQCL) at Nyegezi, Mwanza for solvent extraction (liquid-liquid extraction) of organic pollutants including the endocrine disruptors that was done within 12 hours after sample collection. On arrival into the laboratory, water samples were stored at 4°C until extraction was done.

### **Extraction of Water Samples**

Extraction of water samples was carried out according to the protocol described by Swart and Pool (2007) with minor modifications customized to our laboratory settings.

In brief, before extraction using C18 SPE columns (Abraxis, LLC, 54 Steamwhistle Drive, Warminster, PA 18974, Pennsylvania, USA), each water sample (2 litres) was first filtered through a cotton wool and thereafter through a filter paper facilitated by a vacuum pump. The filtered water samples were then extracted on C18 SPE columns following the extraction procedure described in previous similar studies (Swart and Pool, 2007). Initially, the C18 SPE columns were pre-washed with 4 ml of solvent mixture (40% hexane, 45% methanol and 15 %, 2-propanol), followed by another wash with 4 ml of ethanol. The columns were then washed with one column volume of HPLC grade water after which the water samples were applied onto the column, then air-dried with a vacuum pump. The bound hydrophobic substances were eluted with solvent mixture. The elutes were air-dried and then reconstituted in 1/1000<sup>th</sup> of the original sample volume with ethanol. The samples were stored at -20°C until analysis. To evaluate efficiency of the extraction procedure, samples were spiked with known amounts of 17 $\beta$ -estradiol (E2) and 17 $\alpha$ -ethynylestradiol (EE2).

### **Detection and quantification of total oestrogens using competitive ELISA method**

The detection and quantification of total oestrogens (E1, E2 and E3) was carried out using Oestrogen (E1/E2/E3) ELISA kit (Ecolqiena®) from Japan Enviro Chemicals, Ltd.

### **Detection of Total Oestrogens**

Before carrying out the analysis, the antigen-enzyme conjugate solution was reconstituted following manufacturer's recommendation. Reconstituted conjugate solution was stored at 4°C and was used within 5 days after reconstitution. A total of 100 µl of E2 standard (or sample) prepared in 10% (v/v) methanol solution and 100 µl of conjugate solution were mixed in each well of uncoated microplate using microtitre pipette. One hundred µl aliquots of the above mixture was dispensed into each of the coated well of the microplate. The plate was lightly tapped to make the liquid level horizontal. The microplate was incubated for 60 minutes at controlled room temperature (25°C). After incubation, the wells were washed three times using a 6-fold concentrated solution (about 1.2 ml solution per well). To remove the solution from the wells, the microplates were firmly tapped out. This was further assisted by blotting the plates against a clean paper towel.

After proper washing, 100 µl of colour solution was dispensed into each of the microplate well. This was followed by incubation of the microtitre plate at room temperature (25°C) for 30 minutes. After 30 minutes of incubation, the reaction was terminated by adding 100 µl of stop solution into each of the wells. The reaction was measured immediately using a microtitre plate reader.

### **Quantitative Analysis**

The standard curve, from a dose-response curve obtained from known concentrations of E2 standards (0, 0.05, 0.15, 0.5 and 3.0 µg/L in 10% methanol) determined from the absorbance at 450 nm was used for quantification of unknown samples. The concentration of total oestrogenic endocrine disruptors in each sample was calculated by interpolation using the absorbance intensity obtained from the standard curve. Following the manufacturer's recommendations, the assays were performed within concentrations that did not exceed 3 µg/l. Samples with concentration beyond 3µg/l were diluted further with 10% methanol and re-tested.

### **Survey to establish sources of oestrogenic endocrine disruptors**

While collecting water samples, direct observations and descriptive of the sampling sites were made in addition to collection of qualitative information using formal and informal interviews with key informants regarding major economic activities, possible sources of pollutants in rivers and in the lake, major environmental challenges and control measures currently being taken. Within the City Council, key representatives working in the Agriculture, Fisheries, Urban Planning, Environment and Tourism, Health and Water and Sewerage Authority departments were interviewed. Further interviews were made to the officials of the Lake Victoria Environmental Management Project (LVEMP), Nile Perch Industry, pharmacy and veterinary drug stores, Zonal Veterinary Investigation Centre in Mwanza, National Fish Quality Control Laboratory as well as the main industrial wastewater disposal facility in the city.

### **Data analysis**

Data were analyzed using the SPSS; PASW Statistics 18 (2009) statistics software (IBM SPSS). Differences in the mean of total oestrogens were analyzed by one-way

analysis of variance (ANOVA). Graphical presentation of the mean was made using Microsoft Excel for Windows 2010. A significance level of  $\alpha = 0.05$  was chosen and Tukey HSD was used for multiple comparison of means.

## RESULTS

### Validation of the assay

The absorbance against concentrations of total oestrogens is presented in Figure 1(a) with correlation coefficient of 0.986. A similar finding presenting the absorbance against different concentrations of total oestrogens from an extract of oestrogens obtained in water from a sewage pond in Morogoro, Tanzania is presented in Figure 1(b) with a correlation coefficient of 0.931. Comparisons of the two correlation coefficients resulted into goodness of fit of 94.5%. Based on these agreement results, the method was selected for further use.

### *Detection and quantification of total oestrogenic endocrine disruptors in water*

The total oestrogenic endocrine disruptors were detected in all of the water samples collected at different sampling sites at levels that ranged from 10 to 200 pg/L (Table 2). The difference of total oestrogenic endocrine disruptors concentrations was highly significant ( $P = 0.009$ ) between various categories of water sources (Figure 2). Concentrations of these chemicals decreased along the gradient, being highest ( $107 \pm 81.4$  pg/L) in water samples collected from rivers before entering into the lake and the lowest ( $19 \pm 5.4$  pg/L) in water samples collected at the intermediate sampling points (Figure 2).

Based on these findings (Table 2), Nyakurunduma and Butimba rivers were the most polluted ones with total oestrogens. Such high level of pollution was ascribed to the type of activities carried out in river catchments (Table 1), and the nature of pollutants released into the water bodies. In descending order, after Nyakurunduma and Butimba rivers the levels of total oestrogens were followed by Old Igombe landing site and Mirogo River. Sources with moderate contribution to the total oestrogens released into the lake were Nyashishi River, Shede beach, Maganga beach and Butuja waste disposal area. The lowest concentrations of total oestrogens were found in Kigongo ferry.

### *Sources of pollutants with potential to cause endocrine disruption*

Based on the results from direct observations, and formal and informal interviews with key informant's, direct discharges of untreated effluents from unplanned settlements with poor sanitation and sewage disposal systems were identified as the main source of pollutants in the Lake Victoria at the study sites. In addition, garages that do not have interceptors, poor disposal and discharges from hospital, health centres, persistent halogenated compounds from industries and agricultural activities, and other chemical pollutants such as mercury in soap and cosmetics were also identified as important sources of pollution in the area.

## DISCUSSION

Steroidal estrogens, originating principally from human excretion are believed to play a major role in causing widespread endocrine disruption in fish populations in

Lake Victoria. Given the extent of this problem, risk assessment models are needed to identify potential sources and magnitude in river catchments and to identify hotspot areas where interventions will be considered necessary. Oestrogens constitute a group of important emerging environmental pollutants with potential to induce estrogenic effects in aquatic organisms exposed to them. However, in developing countries like Tanzania; studies of oestrogenic endocrine disruptors in water bodies are limited. To the best of our knowledge, this is the first study conducted in Lake Victoria in the East African region to demonstrate the presence and quantify the total oestrogens (E1, E2 and E3) in water. All water samples collected and analysed were positive for total oestrogens (E1, E2 and E3) (Table 2). Overall, however, the levels detected were at low concentrations as reported by Campbell *et al.* (2006). Considering their weak potency as endocrine disruptors, these levels are considered rather quite low. Despite being detected at relatively low concentrations, (Campbell *et al.*, 2006), their potential to cause pathological changes in fish health must be considered (Jobling *et al.*, 2004).

Mwanza City located in the Lake Victoria basin has a rapidly growing economy largely due to developments in agriculture, chemical industries, mining and fisheries. The City, however, is largely faced with challenges of proper disposal of sewage and other wastes which are discharged into the lake while still raw. Wastes from fish and oil processing plants, textile plants, and tanneries are the ones of the main concern. The lack of a central sewage collection, management and disposal system in Mwanza means that domestic wastes are dumped directly into the lake. Thus, Lake Victoria receives significant pollutants from natural and anthropogenic point and non-point (diffuse) sources which often act as endocrine disruptors in particular those that are likely to be oestrogenic in nature. Unlike point source pollution, which enters the lake at a specific site through a defined point such as pipe discharges, diffuse pollution has no specific point. Leaching of potential pollutants into surface waters and groundwater as a result of rainfall, soil infiltration and surface runoff are examples of diffuse or non-point source of pollution.

Proper planning of measures to be instituted to reduce environmental deleterious effects of pollutants in the Lake Victoria requires an understanding of the nature of pollutants and the route through which such chemicals are released. In this study, presence of total oestrogens was confirmed and rivers contributed significantly to the total load (Table 2, Figure 2).

Since the differential concentration of total oestrogens observed corresponds to the type of activities carried out in the catchment area and along the rivers, it is likely that much of the pollutants are anthropogenic in nature. Though wastewater from households and industries are usually treated before they are released into the river systems, where studies have been conducted, they have shown that, despite the treatments done, they still contain oestrogenic endocrine disruptors with potential to cause contamination of water surfaces and water tables. Subsequently, they cause health effects in aquatic animals. Findings from this study, therefore, calls for intervention that will minimize the discharge of domestic wastewater into the rivers that eventually enters into the lake. Measures or wastewater treatment methods that are efficient in reducing the concentration of oestrogenic endocrine disruptors in

effluents or in rivers before entering into the lake in Mwanza gulf in the City should consider Butimba and Nyakurunduma rivers.

In Tanzania, endocrine disruptors are among the group of pollutants of significant importance that have been neglected or not well understood and, consequently not included in the monitoring programmes. It is evident from various studies that the oestrogenic endocrine disruptors are capable of initiating health disorders in aquatic organisms including fish. For instance, the impact of 17- $\beta$  estradiol (E2) to stimulate vitellogenin (VTG) on *Xenopus laevis* has been reported (Barnhoorn *et al.*, 2004). Nonetheless, before thorough assessment of potential ecological and health impacts of total oestrogens are made, investigation of their levels and distributions in aquatic ecosystems is required.

The detection of estrogenic activity in surface waters in the rivers is related to poor disposal of untreated domestic and industrial wastes directly or indirectly in water bodies. Additionally, the differences in concentration of the chemicals in different rivers demonstrate the variations of activities carried out in the catchment area, the length of the river passing through and the type of chemicals deposited in the rivers. In view of the differences observed, intervention measures to minimize the challenge should take into account the variations observed. For the time being, focus should be on Butimba and Nyakurunduma rivers that contribute significantly to the total oestrogenic load. Based on these observations, the oestrogenicity of water in rivers in Mwanza gulf in the City deserve further investigations to elucidate potential sources and to assess estrogenic risk in detail. In any case, domestic discharges, especially from human and animal wastes need to be sufficiently treated in order to reduce estrogenic activity in water bodies.

All of the rivers in Mwanza City serve as a dumping site of raw sewage and other wastes (MCP, 2008). This is due to the fact that, only about 8% of the human population in Mwanza City receive clean water and is connected to the formal sewerage system served by trunk sewer, lateral and stabilization ponds (MCP, 2008). The low coverage of sewerage system and inability of the majority of the city residents to get connected to the sewerage network aggravate the challenge (Odada *et al.*, 2004).

While collecting water samples for this study, a large number of fish in particular the fry and fingerlings of different fish species were found in highly polluted rivers. This gives an indication that, high pollution of rivers and the presence of oestrogenic endocrine disruptors may interfere with the important breeding sites directly or through long term exposure of fish by these chemicals with potential to cause effect in different development stages of fish. The higher concentrations of these estrogenic compounds recorded in urban areas suggest that improperly treated discharges in these areas are the main source. In addition, the high concentrations of estrogens detected in surface water influenced by municipal discharges suggest that human and animal wastes are not sufficiently treated or are not treated at all before being discharged into the environment.



In studies conducted on fish in the Mekong Delta in Vietnam, (Yamaguchi *et al.* 2004) lower Gonadosomatic Index (GSI) in fish from urban area than those from rural water bodies were detected. Fish from the urban area had higher E1 levels than their rural counterparts. This gave an indication that occurrence of oestrogenic compounds in particular E1 led to disruption of reproductive performance in fish, and consequently reduced population and productivity. Probably the same conditions occur in the study area, a fact that needs further studies before evidence-based conclusions can be made.

The detection of total oestrogens in water though at relatively low concentrations, merits further studies towards characterization of different types of oestrogens and their attributable oestrogenic risks. It has been demonstrated, exposure of fish even to extreme low concentrations of estrogens such as 110 ng E2/L, or as low as 0.1 ng/L in the case of EE2 (17 $\alpha$ -Ethinylestradiol), may lead to reproductive abnormalities in fish (Purdom *et al.*, 1994; Young *et al.*, 2002; Johnson and Williams, 2004). Different estrogen guidelines have been proposed to protect aquatic animals (Purdom *et al.*, 1994; Kramer *et al.*, 1998; Metcalfe *et al.*, 2001; Seki *et al.*, 2005; Beck *et al.*, 2006; Caldwell *et al.*, 2008). A tentative long-term Predicted No Effect Concentration (PNEC) for freshwater life of 1 ng E2/L proposed by Young *et al.*, (2002) is regularly used to evaluate the estrogen risk in surface water. Thus, after detection of specific type of oestrogens present, appropriate interventions and remediation strategies will be proposed and agreed by relevant stakeholders before being implemented.

One of the possible remediation processes is oxidative treatments which are very efficient in eliminating estrogens present in water. However, while planning for intervention strategies using oxidative treatment procedures, precautions should consider risks from by-products with endocrine disrupting properties (Pereira *et al.*, 2011).

In conclusion, this study has reported the total oestrogens in rivers and in the Lake Victoria in Mwanza City for the first time. Although all water samples demonstrated the presence of total oestrogens, levels were relative low. Two rivers, Butimba and Nyakurunduma had the highest concentration of total oestrogens that warrant further studies on methods that can effectively remove the chemicals in water or prevent their pollution. Future studies should be directed towards establishment of steroid hormones profiles and other pollutants with oestrogenic properties; oestrogenicity of water through *in vivo* and *in vitro* studies and minimum concentration of oestrogenic endocrine disruptors that can cause changes in fish.

#### **ACKNOWLEDGEMENT**

We cordially acknowledge the financial and logistical support received from the Lake Victoria Research Initiative (VicRes), (VIC/P14/07), Sokoine University of Agriculture, University of Nairobi, Makerere University and National Fisheries Quality Control Laboratory (NFQC). Cooperation and support received from communities and fishermen in the sampling sites is also highly acknowledged. Permission to carry out this study in Mwanza was granted by the National Institute for Medical Research (NIMR) Ref. NIMR/HQ/R.8a/Vol.IX/713.

## References

- Barnhoorn, I.E.J., Borman, M.S., Pieterse, G.M. and van Vuren, J.H.J. (2004). Histological evidence of intersex in feral sharptooth catfish (*Clarias gariepinus*) from an estrogen polluted water source in Gauteng, South Africa. *Environmental Toxicology*, 19: 603-608.
- Beck, I.C., Bruhn, R., and Gandrass, J. (2006). Analysis of estrogenic activity in coastal surface waters of the Baltic Sea using the yeast estrogen screen. *Chemosphere*, 63: 1870-1878.
- Caldwell, D.J., Mastrocco, F., Hutchinson, T.H., Länge, R., Heijerick, D., Janssen, C., Anderson, P.D. and Sumpter, J.P. (2008). Derivation of an aquatic predicted no effect concentration for the synthetic hormone, 17 $\alpha$ -Ethinyl Estradiol. *Environmental Science and Technology*, 42: 7046-7054.
- Campbell, C.G., Borglin, S.E., Green, F.B., Grayso, A., Wozei, E. and Stringfellow, W.T. (2006). Biologically directed environmental monitoring, fate and transport of oestrogenic disrupting compounds in water: A Review. *Chemosphere*, 65:1265 – 1280.
- Colborn, T., Vom Saal, F.S. and Soto, A.M. (1993). Developmental effects of endocrine disrupting chemicals in wildlife and humans. *Environmental Health Perspective*, 101: 378-384.
- Desbrow, C., Routledge, E.J., Brighty, G.C., Sumpter, J.P. and Waldock, M. (1998). Identification of estrogenic chemicals in STW effluent: Chemical fractionation and *in vitro* biological screening. *Environmental Science and Technology*, 32: 1549–1558.
- Duong, C.N., Ra, J.S., Cho, J., Kim, S.D., Choi, H.K., Park, J., Kim, K.W., Inam, E. and Kim, S.D. (2010). Oestrogenic chemicals and oestrogenicity in river waters of South Korea and seven Asian countries. *Chemosphere*, 78: 286–293.
- Furuichi, T., Kannan, K., Giesy, J.P. and Masunaga, S. (2004). Contribution of known endocrine disrupting substances to the estrogenic activity in Tama River water samples from Japan using instrumental analysis and *in vitro* reporter gene assay. *Water Research*, 38: 4491-4501.
- Hanselman, T.A., Graetz, D.A. and Wilkie, A.C. (2003). Manure-borne estrogens as potential environmental contaminants: A review. *Environmental Science and Technology*, 37: 5471-5478.
- Harries, J., Sheahan, D., Jobling, S., Matthiessen, P., Neall, P., Routledge, R., Rycroft, R., Sumpter, J. and Tylor, T. (1996). A survey of estrogenic activity in United Kingdom inland waters. *Environmental Toxicology and Chemistry*, 15: 1993–2002.
- Jobling, S., Casey, D., Rodgers-Gray, T., Oehlmann, J., Schulte-Oehlmann, U., Pawlowski, S., Baunbecke, T., Turner, A.P. and Tyler, C.R. (2003). Comparative responses of molluscs and fish to environmental estrogens and an estrogenic effluent. *Aquatic Toxicology*, 65: 205- 220.
- Jobling, S., Casey, D., Rodgers-Gray, T., Oehlmann, J., Schulte-Oehlmann, U., Pawlowski, S., Baunbecke, T., Turner, A.P. and Tyler, C.R. (2004). Comparative responses of molluscs and fish to environmental estrogens and an estrogenic effluent. *Aquatic Toxicology*, 66: 207–222.

Detection and Qualification of Oestrogenic Endocrine Disruptors in Water  
Mdegela, R. H<sup>1\*</sup>, Mabiki, F<sup>2</sup>, Msigala, S<sup>2</sup>, Mwesongo, J<sup>2</sup>, Mhina, M.P<sup>3</sup>,  
Waweru, K<sup>4</sup>, Mbuthia, P<sup>4</sup>, and Byarugaba, D.K<sup>5</sup>

- Jobling, S., Nolan, M., Tyler, C.R., Brighty, G.C. and Sumpter, J.P. (1998). Widespread sexual disruption in wild fish. *Environmental Science and Technology*, 32: 2498–2506.
- Jobling, S., Williams, R., Johnson, A., Taylor, A., Gross-Sorokin, M., Nolan, M., Tyler, C.R., van Aerle, R., Santos, E. and Brighty, G. (2006). Predicted exposures to steroid estrogens in U.K. rivers correlate with widespread sexual disruption in wild fish populations. *Environmental Health Perspective*, 114: 32- 39.
- Johnson, A.C. and Williams, R.J. (2004). A model to estimate influent and effluent concentrations of estradiol, estrone, and ethinylestradiol at sewage treatment works. *Environmental Science and Technology*, 38: 3649-3658.
- Kramer, V.J., Miles-Richardson, S., Pierens, S.L. and Giesy, J.P. (1998). Reproductive impairment and induction of alkaline-labile phosphate, a biomarker of oestrogen exposure, in fathead minnows (*Pimephales promelas*) exposed to waterborne 17 $\beta$ -estradiol. *Aquatic Toxicology*, 40: 335–360.
- Liu, Z.H., Kanjo, Y. and Mizutani, S. (2009). Removal mechanisms for endocrine disrupting compounds (EDCs) in wastewater treatment-physical means, biodegradation, and chemical advanced oxidation: A review. *Science of the Total Environment*, 407: 731-748.
- MCP (2008). Mwanza City Profile. [www.lakezonedesire.com](http://www.lakezonedesire.com). Accessed on 20<sup>th</sup> February 2013.
- Metcalf, C.D., Metcalf, T.L., Kiparissis, Y., Koenig, B.G., Khan, C. and Hughes, R.J. (2001). Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by *in vivo* assays with Japanese medaka (*Oryzias latipes*). *Environmental Toxicology and Chemistry*, 20: 297-308.
- Odada, E.O., Olago, D.O., Kulindwa, K., Ntiba, M. and Wandiga, S. (2004). Mitigation of environmental problems in Lake Victoria, East Africa: causal chain and policy option analyses. *Ambio*, 33: 13 - 23.
- Olujimi, O.O., Fatoki, O.S., Odendaa, J.P. and Okonkwo, J.O. (2010). Endocrine disrupting chemicals (phenol and phthalates) in the South African environment: a need for more monitoring. *Water SA*, 36: 245-252.
- Pereira, R.O., Postigo, C., de Alda, M.L., Daniel, L.A. and Barceló, D. (2011). Removal of estrogens through water disinfection processes and formation of by-products. *Chemosphere*, 82:789-799.
- Pojana, G., Gomiero, A., Jonkers, N. and Marcomini, A (2007). Natural and synthetic endocrine disrupting compounds (EDCs) in water, sediment and biota of a coastal lagoon. *Environment International*, 33: 929-936.
- Purdum, C.E., Hardiman, P.A., Bye, V.J., Eno, N.C., Tyler, C.R. and Sumpter, J.P. (1994). Estrogenic effects of effluents from sewage treatment works. *Chemistry and Ecology*, 8: 275-285.
- Routledge, E., Sheahan, D., Desbrow, C., Brighty, G., Waldock, M. and Sumpter, J. (1998). Identification of estrogenic chemicals in STW effluent. *In vivo* responses in trout and roach. *Environmental Science and Technology*, 32: 1559–1565.
- Seki, M., Yokota, H., Maeda, M. and Kobayashi, K. (2005). Fish full life-cycle testing for 17 $\beta$ -estradiol on medaka (*Oryzias latipes*). *Environmental Toxicology and Chemistry*, 24: 1259-1266.

Swart, N. and Pool. E. (2007). Rapid detection of selected steroid hormones from sewage effluents using an ELISA in the Kuils River water catchment area, South Africa. *Journal of Immunoassay and Immunohistochemistry*, 28:395-408.

Wenzel, A., Küchler, T. and Müller, J. (1998), Fraunhofer-Institut für Umweltchemie und Ökotoxikologie (IUCT): Konzentrationen estrogen wirksamer Substanzen in Umweltmedien. (Concentrations of estrogenic substances in the environment). Forschungsbericht 21602011/11, im Auftrag des Umweltbundesamtes

Young, W.F., Whitehouse, P., Johnson, I. and Sorokin, N. (2002). Proposed predicted no effect concentration (PNECs) for natural and synthetic steroid oestrogens in surface waters. Environment Agency Research and Development Technical Report P2-T04/1. England and Wales Environment Agency, Bristol; 172 pp.

**Legends for figures**

**Figure 1:** The absorbance against concentrations of total oestrogens (figure 1a) with correlation coefficient of 0.986. The absorbance against different concentrations of total oestrogens from an extract of oestrogens obtained in water from a sewage pond in Morogoro, Tanzania (figure 1b) with a correlation coefficient of 0.931.

**Figure 2:** Concentration (pg/L) of total oestrogens (mean ± SD) in water samples collected in rivers and in the Lake Victoria in Mwanza City.

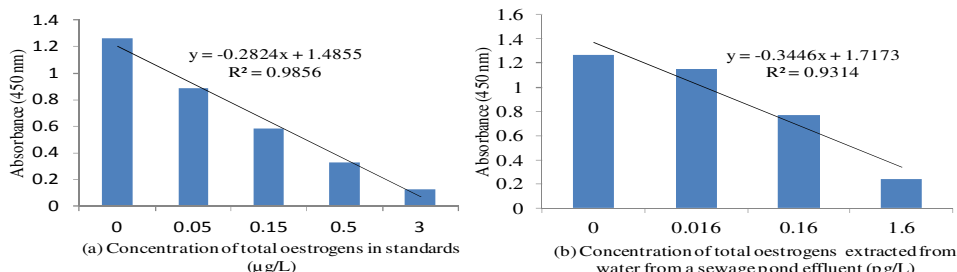


Figure 1

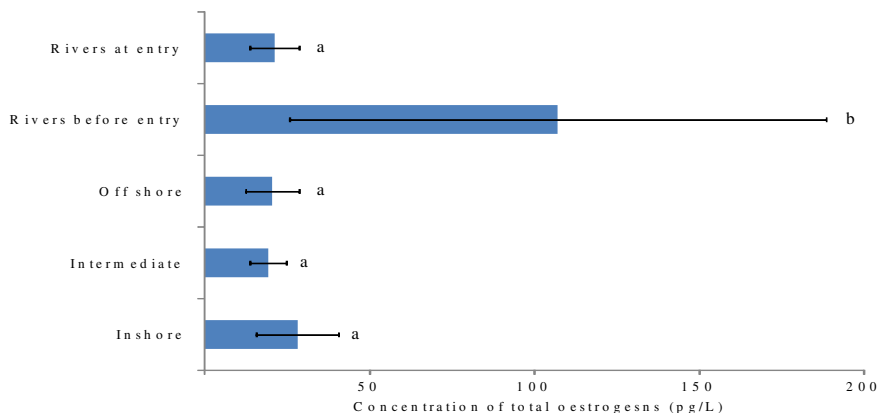


Figure 2

**Tables**

**Table 1:Water sampling sites for determination of oestrogenic endocrine disruptors in Mwanza Gulf, in the City**

<b>ID</b>	<b>Name of the site</b>	<b>GPS position</b>	<b>Description of the site</b>
M	Maganga beach	Elevation: 3706 feet South: 02°35.391' East: 032°52.819'	A beach under development with minimal recreational activities. It serves as a small landing site for artisanal fishers
N	Nyashishi River	Elevation: 3712 feet South: 02°39.159' East: 032°52.909'	This river pours its water at Shadi area in the Lake Victoria, a place with minimal domestic activities and limited number of small scale fishers.
B	Butimba River	Elevation: 3708 feet South: 02°35.061' East: 032° 53.938'	This river pours its water at Nyegezi Fisheries area, there is no landing site nearby and fishing activities are not common in the area. The origin of this river is far away from its pouring point hence it carries a lot of pollutants in particular wastes from houses, industries and agricultural activities.
NYK	Nyakurunduma River	Elevation: 3755 feet South: 02°33.647' East: 032°54.808'	This river pours its water at Mkuyuni area, originating far away from its pouring point hence carrying a lot of pollutants in particular wastes from houses, industries, agricultural activities and car washing among others.
ML	Mirongo River	Elevation: 3727 feet South: 02°30.451' East: 032°53.666'	This river pours its water off-shore of Kirumba Fish Market and is considered among the most polluted rivers from domestic and industrial wastes.
OIG	Old Igombe Landing site	Elevation: 3731feet South: 02°23.686' East: 032°57.809'	This is the site where most of the lake dwellers are fishers and fish dealers. The place is also famous as the local fish market.
BT	Butuja	Elevation: 3670 feet South: 02°27.670' East: 032°54.529'	This site has a mixture of wastewater , some from the Butuja water treatment plant and some from fish processing factories
SH	Shede	Elevation: 3724 feet South: 02°32.868' East: 032°54.314'	This is a very small beach with a capacity to hold 5-8 canoes. The beach is mostly used for repairing fishing gears and canoes
KF	Kigongo Ferry	Elevation: 3735 feet South: 02°42.783' East: 032° 53.613'	This is the crossing point from Mwanza to Sengerema and fishing activities are prohibited in the area. There is a small landing site for small canoes owned by people who live near to the ferry.

**Table 2: Concentration (pg/L) of total oestrogenic endocrine disruptors in extracts from water collected in Mwanza Gulf, in the City at different water sampling points**

Location	Type of water surface	Before entry	Entry point	After entry
Nyashishi	River	31	15	NA
Nyakurunduma	River	200	31	30
Mirongo	River	47	23	NA
Butimba	River	150	16	16
		Inshore	Intermediate	Offshore
Shede	Beach	28	20	24
Butuja	Waste disposal	23	22	NA
old Igombe	Landing site	48	24	NA
Kigongo	Ferry	14	20	NA
Maganga	Beach	28	10.1	12.1

NA = Not analysed