Levels and distribution of progesterone in receiving waters and wastewaters of a growing urban area

Hildegard R. Kasambala, Mwemezi J. Rwiza and Robinson H. Mdegela

ABSTRACT

This study aimed at investigating the levels and distribution of progesterone in receiving waters and wastewaters in Arusha, a fast-growing urban area and the third largest city in Tanzania. Specifically, the study was conducted along the Themi River and the adjacent waste stabilization ponds (WSPs). Progesterone was detected and quantified by using an enzyme-linked immunosorbent assay (ELISA) kit. For Themi River samples, the level of progesterone obtained ranged from ‘no detection’ to 439 ng/L with a mean value of 120.3 ng/L. The levels detected were significantly higher in the midstream than the upstream and downstream sections of the river (P<0.05). The higher values at the midstream were attributable to livestock, WSPs and household effluents; agricultural activities; and sewage infiltration. Progesterone levels in the present study, although not extremely high, correspond to those associated with harmful effects in other studies. Results for samples collected from the WSPs indicated a release of 215 ng progesterone per L of the receiving waters. Although progesterone removal efficiency by the WSPs was 75%, the amount released was still high enough to cause harm to aquatic organisms. Thus, more efficient techniques are required to adequately remove progesterone to the recommended levels.

Key words | endocrine disrupting compounds, progesterone, receiving waters, Tanzania, Themi River, waste stabilization ponds

INTRODUCTION

Numerous environmental chemicals are categorized as endocrine-disrupting compounds (EDCs) both globally and across Africa (Bergman et al. 2015). EDCs refer to any exogenous compound capable of interfering with the normal functioning of natural hormones (Zoeller et al. 2012). These compounds are so-named due to their ability to interrupt with natural hormonal activities in the body resulting in increased disease vulnerability in organisms. EDCs can directly interact with endogenous hormonal receptors and mimic their normal functioning either synergistically or antagonistically (Tyler et al. 1998). Exposure to an aquatic environment contaminated with EDCs can have negative effects on the nervous, immune, reproductive, and urogenital systems of both human and aquatic organisms. Moreover, exposure to EDCs can lead to infertility, abnormal prenatal development as well as abnormalities in child development (Diamanti-Kandarakis et al. 2009). Some studies have been conducted showing the quantification, effects, and distribution of other EDCs, e.g. estrogen, in the receiving rivers (Msigala et al. 2017b). However, in much of the environments in developing countries, there is limited information on the levels and distribution of EDCs. Even globally, research coverage on progesterone levels in aquatic systems is limited.

Progesterone is one of the EDCs which are sometimes categorized as progestogens or gestagens (King & Brucker 2010). Progesterone is among the steroid EDCs produced highly by both the female and male human body to help maintain pregnancy, regulate gamete maturation, organize reproductive behaviour, sperm capacitation and influence spermatogenesis (Bergman et al. 2013). Progestonic entities are commonly found in large quantities in aquatic environments because they are excreted through urine in great amounts by humans, administered to animals as growth...
promoters and excreted by animals as endogenous hormones. Moreover, progesterone is contributed by natural degradation of plant leaves present in the aquatic environment, which is the reason why sometimes progesterone may be found even in places with no anthropogenic activities.

Other studies on progesterone have covered sources and effects of progesterone, especially with regard to surface water, with little or no focus on the levels and distribution of progesterone in receiving rivers (DeQuattro et al. 2012). This study focuses on the distribution and levels of progesterone in its potential source, the WSPs, as well as the levels and distribution of progesterone in a corresponding receiving river, Themi River. Flowing across the eastern part of Arusha city, Themi River receives municipal wastewater effluents, untreated sewage effluents from industry and residences, and livestock effluents as well as agricultural effluents. Anthropogenic pollution in Themi River is known to cause harm to downstream aquatic environments (Lyimo 2015). Other sources of progesterone in Themi River may include municipal wastewater, paper mill effluents, and agricultural runoff and livestock effluents. It is known that most African WSPs are less efficient in removing EDCs (Madikizela et al. 2017), thus making the receiving rivers significantly polluted. Therefore, this study intended to assess the levels and distribution of progesterone in Themi River and how the WSPs might be contributing to progesterone pollution in the river.

MATERIALS AND METHODS

Site description

This study was carried out in the Themi River and the Arusha municipal WSPs in the northeastern part of Tanzania (see Figures 1 and 2). A zoomed-in layout of the Arusha municipal WSPs is indicated in Figure 1. Themi River has a length of 46.4 kilometres and serves a population of 416,442 (National Bureau of Statistics 2012). It lies between latitudes 2° and 6° south and 34.5° and 38° east of the Greenwich meridian. Themi River originates from the slopes of Mount Meru at Olgilai village about 1,700 metres above sea level and flows through Arusha city at an altitude of 1,254 m (Lyimo 2012). Human activities taking place along the river include agriculture, livestock keeping, industrial activities, car washing, bathing, washing of clothes, etc.

Themi River and Arusha city WSPs were selected as the sampling sites because of the anthropogenic activities taking
place within the area which have high chances of producing effluents with progesterone. The control samples were collected from the river source in the Machame sub-village of the Olgilai area at the foot of Mount Meru. The river source is free from anthropogenic activities. This area is used as a catchment point for the Arusha Urban Water and Sanitation Authority (AUWSA), from where water used for domestic and industrial purposes in the city is piped. Along the river, there are several densely populated unplanned settlements, e.g. Daraja Mbili and Lemara. Apart from human settlements, several industrial and agricultural activities take place along the river as Figure 2 indicates. The WSPs’ influents and the resulting effluents from anaerobic, facultative and maturation ponds were assessed for progesterone levels.

Materials used

The materials used in this study included: progesterone AccuBind® ELISA Test System Kit (Lake Forest, CA 92630, USA); and methanol (99%) of high-performance liquid chromatography (HPLC) grade from Sigma Aldrich (Germany) for progesterone extraction, hydrochloric acid (37%, 1.18 M) (Sigma Aldrich, Germany) for pH adjustment and minimization of microbial degradation of the sample, and distilled water for washing purposes. Solid phase extraction (SPE) C-18 cartridges (500 mg, 6 mL) supplied by J & K Scientific (Beijing, China) were used for hormone concentration and matrix removal but n-heptane (99%) and acetone (99.8%) used for elution and membrane filter DSO210-4045 used for debris and particle removal were all supplied by Fischer Scientific (Waltham, MA, USA). A multiparameter water quality meter with probe (HI 9829) and pH meter (GHM 3500 series), HANNA Instruments, Woonsocket, RI, USA were used to measure pH, conductivity, dissolved oxygen (DO), temperature, and total dissolved solids (TDS). An air vacuum pump Manford 2546C-02 B (Welch, Germany) was also used.

Sample collection and preparation

The water samples were collected for 5 days in the end of March 2019. The days were sunny with water temperature ranging between 25.8 and 26.8 °C. One-litre capacity bottles were used for the collection of samples which were taken at circa 20 cm below the water surface. For the WSPs’ effluent
and influent water, a water sampler was used. The collected samples were adjusted to pH 3 by adding hydrochloric acid. Then the samples were stored in a cool-box packed with ice packs to keep the samples below 4 °C and transported to the NM-AIST laboratory for further analysis. Samples were analyzed within 10 days after sampling while stored at −20 °C. Prior to extraction, wastewater samples were filtered twice by using GF/C and membrane filter of pore size 0.45 μm for the purpose of removing suspended solids and debris, while the Themi River samples were filtered once by using a membrane filter.

**Extraction of hormones**

After filtration, SPE was carried out according to the protocol of Hansen *et al.* (2011) with some modifications made in the laboratory as described in Figure 3. The water samples were thawed at room temperature before commencing the extraction process. The SPE was conducted using C-18 cartridges (500 mg, 6 mL) facilitated by a vacuum manifold pump. The C-18 cartridges were conditioned with 2 × 3 mL n-hexane, 3 mL acetone, and finally with 3 mL of distilled water adjusted to a pH of 3 to remove impurities. The samples were loaded into the extraction system supported by the Manford pump at the rate of 6 mL/min in which the particles were trapped by the glass fiber filter. The cartridges were washed with 1 mL methanol at the rate of 2 mL/min to remove hydrophobic substances. Subsequent to extraction the cartridges were dried out in air using a vacuum manifold for about 30 min and then eluted using a mixture of 10 mL of heptane and acetone (65:35). The elutes were dried in air at 30 °C, and then reconstituted in 5 mL methanol and subsequently stored at −20 °C for ELISA analysis.

**ELISA analysis**

Prior to analysis, samples and ELISA kits were thawed at room temperature (20–27 °C) for 30 minutes. This was in accordance with the manufacturer’s recommendations. For ELISA techniques a buffer solution was prepared by diluting the content of wash solution to 1,000 mL distilled water and stored at 2–8 °C. All microplates’ wells for each of the reference calibrators, samples, and control were assayed in duplicate. Seven reference standards (1–7) and 76 water samples (8–84) of 25 μL were pipetted into assigned wells. Progesterone enzyme reagent of 50 μL was added to all wells and swirled

![Figure 3](https://iwaponline.com/wst/article-pdf/80/6/1107/629267/wst080061107.pdf)
for 10–20 s, then covered and incubated for 60 minutes at room temperature. Then the microplates were decanted and dried by using a wash machine, then 350 μL of wash buffer was added three times. Then 100 μL of substrate solution was added to all wells at the same time to minimize time differences between wells and then incubated at room temperature for 20 min. Lastly, 50 μL of stop solution was added in each well at the same time and gently mixed for 15–20 s. The quantification of the sample was done within 15 min after the reaction stopped in which sample absorbance was measured with a microplate reader at 450 nm. The progesterone standard curve was drawn by taking common logarithms of standard concentrations against absorbance to obtain the linear equation which was used to calculate the concentrations of progesterone based on their corresponding absorbance.

For statistics, GenStat 15th edition (VSNi, Rothamsted, England) and OriginPro 9 (Originlab Corporation, Wellesley Hills, USA) were used for statistical analyses. Inferential statistics one-way analysis of variance (ANOVA) with post hoc Tukey’s was engaged for multiple comparisons of progesterone levels between sampling clusters with significance difference between groups reported as at $P<0.05$.

## RESULTS AND DISCUSSION

### Sampling area characteristics

Table 1 presents a summary of the important environmental, social and economic characteristics of the sampling area.

### Progesterone levels

#### Overall patterns

In Table 2 and Figure 4, the mean concentrations of progesterone in Themi River are presented. The results indicate that the midstream section progesterone levels were significantly higher ($P<0.05$) than those at the upstream and downstream sections. Comparison between the upstream and downstream samples shows that the downstream was more polluted by progesterone than the upstream ($P<0.05$). Overall, the levels of progesterone in the current study are relatively low compared with those reported in the literature (Jenkins et al. 2003; Payus et al. 2016).

#### Upstream progesterone levels

Overall, the lowest level of progesterone was found upstream in the samples collected close to the river source, with an average of 23.4 ng/L as indicated in Figure 5.
and Table 2. It would be expected that at the river source there would be very little to no progesterone levels but as the results from this study indicate, this was not the case. Even at the river source there were measurable amounts of progesterone. Progesterone at the river source was probably due to the degradation of natural products in leafy and other plant materials (Jenkins et al. 2003). Moreover, it was observed that there were small plots of celery farming in areas near the river source. This small-scale vegetable farming probably contributed to the levels of progesterone found in the water samples. Also, informal discussion with some farmers in the study area revealed that although the use of synthetic fertilizers in vegetable production in the area is prohibited, it is not uncommon for some farmers to smuggle fertilizers and other chemicals into their plots to boost smallholding horticultural production.

**Progesterone in waste stabilization ponds**

The WSPs' progesterone concentrations were also assessed and the highest concentrations were found in anaerobic pond influent whereas the lowest concentrations were found in the maturation pond effluent (Figure 6).

The Arusha WSPs receive a maximum of 2,488 ± 1.3 ng/L of progesterone at influent with a mean concentration of 862.9 ng/L. They release into the Themi River a minimum of 215 ± 1.4 ng/L progesterone in the effluent. Similar results have been reported by other authors who evaluated the levels of EDCs elsewhere as indicated in Table 3 (Manickum & John 2014; Madikizela et al. 2017; Zhang et al. 2017). Other researchers, e.g. Payus et al. (2016), however, reported higher levels of progesterone with a mean of 6,556 ng/L in waters associated with WSPs (Table 3). The relatively low levels of progesterone in the WSPs found in the present study may be due to natural attenuation of progesterone. Normally, progesterone undergoes biodegradation but can still be detected in the effluent of both industrial and municipal wastewater and even in the receiving rivers. Receiving rivers have been

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The image includes graphs and diagrams to illustrate the variations and concentrations of progesterone in different sections of the Themi River and at various sampling points. The key figure definitions are also provided for reference.
reported to have high levels of progesterone compared with other rivers which did not receive the wastewater effluent (Corrales et al. 2015). This may be attributed to the fact that WSPs and many other wastewater treatment systems cannot adequately remove progesterone.

It is also important to note that usually WSPs receive influents that have a combination of multiple micropollutants. The micropollutants are usually converted into other forms. This transformation process may result in reduced levels of the original pollutants. Moreover, the level of progesterone at effluent mostly depends on the removal efficiency of the WSPs. High efficiency in removing progesterone lead to high quality of effluent received by surface waters and determine the quality of groundwater. In the present study, progesterone removal efficiency was measured by comparing the amount of progesterone at influent and effluent of each pond as shown in Equation (1):

\[
\text{Removal efficiency (\%)} = \left(\frac{[\text{Progesterone}]_i - [\text{Progesterone}]_e}{[\text{Progesterone}]_i}\right) \times 100
\]  

where \([\text{Progesterone}]_i\) stands for the influent progesterone concentration and \([\text{Progesterone}]_e\) stands for the effluent progesterone concentration.

Figure 6 shows the mean concentration of progesterone in the Arusha city WSPs. The amount of progesterone at influent is significantly high compared with the effluent \((P < 0.05)\). The highest removal efficiency was observed at the two maturation ponds, which are 70.8% and 75.7%, respectively, with the lowest progesterone removal found at the anaerobic pond with the efficiency of 22.3%. Probably the high removal efficiency of progesterone at maturation ponds was due to rapid biodegradation facilitated by high DO.
(see Figure 7). Also the shallowness of the maturation ponds compared with the anaerobic pond probably helped to increase oxygen transfer which in turn led to increased progesterone biotransformation and biodegradation (Koumaki et al. 2018). Moreover, low removal efficiency of progesterone in the anaerobic pond was probably due to shorter retention time and hence the low degree of biotransformation and biodegradation caused by excessive flowrates into the anaerobic pond. The pond was designed for an inflow rate of 86 m$^3$/day but it has been reported to receive inflow rates of up to 6,500 m$^3$/day (AUWSA 2015). This means that the WSPs are too overloaded to perform at their expected standard. The removal efficiency of WSPs may also be influenced by many other natural factors including temperature, wind speed, sunlight, and rainfall. Moreover, the performance of WSPs may be affected by pond design, poor maintenance and physical–chemical parameters such as pond surface area, water depth, pH and DO (Msigala et al. 2017a).

Midstream progesterone levels

General patterns in progesterone levels at midstream

The levels of progesterone in the midstream portion of the river were higher than those found in the upstream and downstream portions (Figure 5). The mean progesterone concentration obtained at midstream was $166 \pm 115$ ng/L. The high standard deviation value was probably due to the many varied sources of progesterone at the midstream section of the river. High levels of progesterone in the midsection of the river were possibly attributable to the various anthropogenic activities taking place in that section (Table 1) (Liu et al. 2012).

The influence of WSPs on progesterone levels

At midstream, the river also received effluent from WSPs, with a concentration of 177 ng/L at the point where WSP effluent entered the river (Table 2). This was almost four times lower than the progesterone concentration values obtained in the WSP influent. However, the amount of progesterone in the receiving waters was comparable to those found in previous studies (Kolpin et al. 2002; Chang et al. 2011).

Livestock effluents’ progesterone contribution

The results of this study indicate the highest progesterone concentration (439 ± 0.2 ng/L) at the point where effluents from livestock-keeping entered the river. Some studies conducted elsewhere have also found elevated amounts of hormonal pollutants at points where effluents from livestock enter the receiving waters (Liu et al. 2012). The mean progesterone concentration obtained from pure livestock effluent was 908 ng/L which is two times higher than the levels found at the entry point into the river. This decrease in hormonal concentrations may be attributable to hormonal degradation by sunlight, sedimentation or infiltration.

Figure 7 | Levels of dissolved oxygen (DO) at different zones in the Arusha municipal WSPs. The encircled portion marks the maturation with high DO levels.
into soil as water flows towards the river. The high levels of progesterone in livestock effluents may also be indicative of hormonal chemicals being used in livestock production in the area. Hormonal supplements are probably given to livestock as growth promoters, weight and yield improvers, and/or immunity enhancers. This poses a threat to animal, human and environmental health. If released in high doses, hormonal pollutants may easily find their way into air, soil, surface water and groundwater, causing contamination in the fate media (Kabir et al. 2015).

**Downstream progesterone levels**

The level of progesterone hormone at downstream was lower than midstream as indicated in Figures 4 and 5. This would not usually be the case as higher contaminant levels are usually expected in downstream samples. Low levels of progesterone downstream were probably due to a dilution effect because there were springs in the downstream area. Spring water entered and mixed with Themi River at various points in the downstream section of the river. Furthermore, low levels of progesterone in the downstream section might have been due to the metabolic activities of some bacteria which transform progesterone into other androgens such as 17α-hydroxyprogesterone (17α-OHP), androstenedione, testosterone, estrone, and estradiol (Jenkins et al. 2004). Generally, EDCs are reduced due to different natural processes taking place in the water, including biodegradation, sorption and photolysis. Moreover, low concentrations of progesterone may be attributed to various microbial activities which lead into pollutant biotransformation (Lu et al. 2009).

**Socio-economic and environmental factors vs EDC levels**

**Poor sanitation facilities**

The levels of EDCs are likely to vary from place to place due to variation in the types of sources and in production rates of EDCs. According to this study, high progesterone pollution at the midstream section of the river may be attributed to the following sources: wastewater effluent, livestock effluent, industrial effluent, agricultural runoff and infiltration of untreated sewage from residential pit latrines. High rates of human settlement along the river play an important role in the release of progesterone into the environment. Most of the households in Arusha city and other growing African urban areas are not connected to the central sewage and sewerage system. According to the AUWSA, the authority responsible for water supply and wastewater management in Arusha, only 7% of households are connected to the municipal wastewater infrastructure (Thomas et al. 2015). It would therefore follow that wastewater from residential areas is commonly released untreated into the nearby streams and rivers.

**Poor solid, liquid and chemical waste management**

Moreover, it was observed that along the river various waste materials, e.g. plastics, hospital wastes, and solid domestic wastes, were deposited in the river and along the river. Although not measured, the wastes deposited in and along the river may have contributed to some extent to progesterone loading in Themi River. Another important factor to consider is lax control in medicine, medication and drug use. Unlike in the developed countries where hospital prescription is important before administration of drugs, in most developing countries delivery, administration and use of drugs is not strictly controlled. In many developing countries, e.g. Tanzania, the general public has easy access to contraceptives, antibiotics, veterinary drugs and agrochemicals. These medicines or their byproducts may enter the environment and lead to hormonal and other kinds of environmental pollution (Boxall 2004; Madikizela et al. 2017).

This study also revealed high levels of progesterone at a section of the river in vicinity to the industrial area. High progesterone levels in this section may be due to untreated or partially treated industrial discharges that are released into the receiving waters. Many industries in Tanzania do not have wastewater treatment facilities and they are also not connected to the central wastewater infrastructure. Probably some industries avoid conventional wastewater treatment practices as these practices usually come at a cost. It also known that most conventional means of wastewater treatment do not adequately remove EDCs from wastewater.

**CONCLUSIONS**

The results obtained from this study indicate that Themi River and the associated waste stabilization ponds have significant levels of progesterone. These appreciable levels of progesterone in the water and wastewaters of Arusha may be attributed to anthropogenic activities, e.g. livestockkeeping, municipal wastewater effluents, farming and
infiltration from untreated sewage. The levels of progesterone that we have reported in the current study may have effects on aquatic organisms' sperm motility, gene expression, fecundity, fecundity rate as well as hermaphroditic behaviour (DeQuattro et al. 2012). There was no progesterone detected in tap water. To date, there are no internationally accepted guidelines for progesterone in water. Therefore, the results of this study may help to contribute baseline information for the establishment of such guidelines in receiving waters and wastewater effluents. Further studies are needed on cost-effective techniques that can be used to sufficiently remove progesterone from water and wastewater.

ACKNOWLEDGEMENTS

This work has been supported by the African Development Bank (AfDB) project ID No. Z1–IAO–06 under Grant No. 2100155032816. We thank the Nelson Mandela African Institution of Science and Technology (NM-AIST) for providing further support related to laboratory work, sampling and analysis.

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First received 24 July 2019; accepted in revised form 13 October 2019. Available online 23 October 2019.