

Full Length Research Paper

# Performance of wastewater stabilization ponds in treatment of endocrine disrupting estrogens in Morogoro urban and peri-urban, Tanzania

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The aim of the study was to assess the performance of wastewater stabilization ponds (WSPs) in treatment of endocrine disrupting estrogens particularly estradiol (E2), estrone (E1) and ethinylestradiol (EE2). The study was conducted at Mafisa and Mzumbe wastewater stabilization ponds located in Morogoro Urban and Peri-urban areas respectively. The endocrine disrupting estrogens in low quality water and sludge were detected and quantified using competitive Enzyme Linked Immunosorbent Assay. The recovery of estrogens in this study ranged from 65% to 90%. The EE2, E1 and E2 were detected in all samples of low quality water and sludge from anaerobic to maturation ponds in both study sites. The overall estrogen reduction at Mafisa was 95.8%, 95.3%, 94.9% for EE2, E1 and E2 respectively. At Mzumbe the overall estrogen reduction was 81.6%, 89.3% and 82.5% for EE2, E1 and E2 respectively. The mean concentrations of estrogens in influent to effluent at Mafisa ranged from 35.6 to 1.5, 60.7 to 2.9 and 85.6 to 4.4 ng/L for EE2, E1 and E2 respectively. The corresponding mean concentration at Mzumbe WSPs ranged from 25.5 to 2.7, 23.8 to 4.4 and 39.4 to 6.9 ng/L for EE2, E1 and E2 respectively. Sludge from anaerobic pond contained significant high amount of estrogens, whereas in other ponds median concentrations were significant low. The endocrine disrupting estrogens were significantly reduced in the wastewater stabilization ponds.

**Keywords:** Ethinylestradiol, estradiol, estrone, mafisa, mzumbe, micropollutants reduction.

## INTRODUCTION

Endocrine disrupting estrogens are among the emerging pollutant in water. The occurrence of these compounds in the environment is of major concern because they exert endocrine disruption at low concentrations usually at ng/L range (Gross-Sorokin et al., 2004; Hoffman et al., 2003; Christiansen et al., 2002; Panter et al., 1998; Ingram et

al., 2011). The compounds disrupt the action of endogenous hormone, hence may induce abnormal reproduction, stimulate cancer growth and cause dysfunction of neuronal and immune system (Lee et al., 2013). Detection and quantification of natural and synthetic estrogens in wastewater treatment systems is fundamental for risk assessment in aquatic environment (Singhal et al., 2009). Ethinylestradiol is even more potent than the natural estrogens in inducing reproduction abnormalities. It can induce vitellogenin

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formation in some male fish species at 1 ng/L and induce intersex of fish at 4 ng/L. On the other hand 17 $\beta$ -estradiol can induce vitellogenin formation at 5 ng/L and induce intersex at 10 ng/L (Metcalf et al., 2001; Thorpe et al., 2001). Scientific investigations in the UK, Europe, USA and Japan have demonstrated the occurrence of intersex and elevated concentrations of plasma vitellogenin in freshwater fish and in estuarine environments for instance zebrafish, fathead minnow and medaka (Gross-Sorokin et al., 2004; Länge et al., 2012).

This high potency at low concentrations necessitates understanding of the fate and effects of these compounds in the aquatic environments.

The influent entering wastewater treatment system contains several chemicals and biological contaminants. In the course of the treatment, chemicals may be reduced in amount or converted to less harmful form(s). There are several wastewater treatment systems for instance activated sludge, wastewater treatment plants, and wastewater stabilization ponds which can be used. Several published research works verified that effluent from domestic wastewater treatment systems contain natural and synthetic estrogens (Limpiyakom *et al.*, 2011; Pessoa et al., 2014; Liu et al., 2015; Cook et al., 2016). The levels of estrogens in the effluent depend on the efficiency of the treatment system. Therefore, the performance of wastewater treatment system in reduction of endocrine disrupting estrogens determine water quality of effluent receiving water bodies such as rivers.

Wastewater stabilization ponds (WSPs) have been reported to be slightly less effective in reducing estrogens compared to other wastewater treatment systems (Coleman et al., 2010; Pessoa et al., 2014). Treatment of wastewater with wastewater stabilization ponds is the method of first choice in developing nations such as Tanzania. The method has its merits such as simplicity in design and construction, low capital and operating cost, are very reliable and sustainable technology (Phuntsho et al., 2007; Rozkošný et al., 2014). Other treatment processes such as activated sludge and biofilms are used seldom in Africa including Tanzania due to lack of reliable energy and financial resources (Wang et al., 2014).

Wastewater stabilization ponds consist of a series of three or more ponds with influent being transferred from the anaerobic pond to facultative pond and maturation pond. Anaerobic and facultative ponds are mainly designed for reduction of organic matter measured as Biological Oxygen Demand (BOD) and maturation ponds mainly for pathogen reduction (Mara and Pearson, 1998). Anaerobic ponds essentially serve to settle undigested material and non-degradable solids as bottom sludge, dissolve organic material and break down biodegradable organic material. Whereas the facultative pond serves for

further treatment of wastewater through sedimentation and oxidation of organic material, reduce odour, reduce some disease-causing microorganism and store residues as bottom sludge (Tilley et al., 2014).

The performances of wastewater stabilization ponds are generally measured by comparing the concentrations of the target substance between treated and raw sewage. The performance of WSPs is affected by natural factors such as temperature, wind velocity, sunlight and rainfall. In addition, the performance is also affected by pond design, maintenance and physical chemical parameters such as pond surface area and water depth, pH and dissolved oxygen. The activity in the WSP is a complex symbiosis of bacteria and algae, which stabilizes the waste and reduces pathogens. The result of this biological process is to convert the organic content of the influent to more stable and less offensive forms (Phuntsho et al., 2007). Some pollutants are mainly reduced through sedimentation.

Ahmed and Abdallah, (2016) reported that the emerging pollutants in most developing nations are poorly characterized compared to developed nations. In addition, Miraji et al., (2016) reported that research coverage on emerging pollutants such as endocrine disrupting estrogens are very limited in Tanzania. The wastewater treatment systems are the potential sources of emerging pollutants to the aquatic ecosystems.

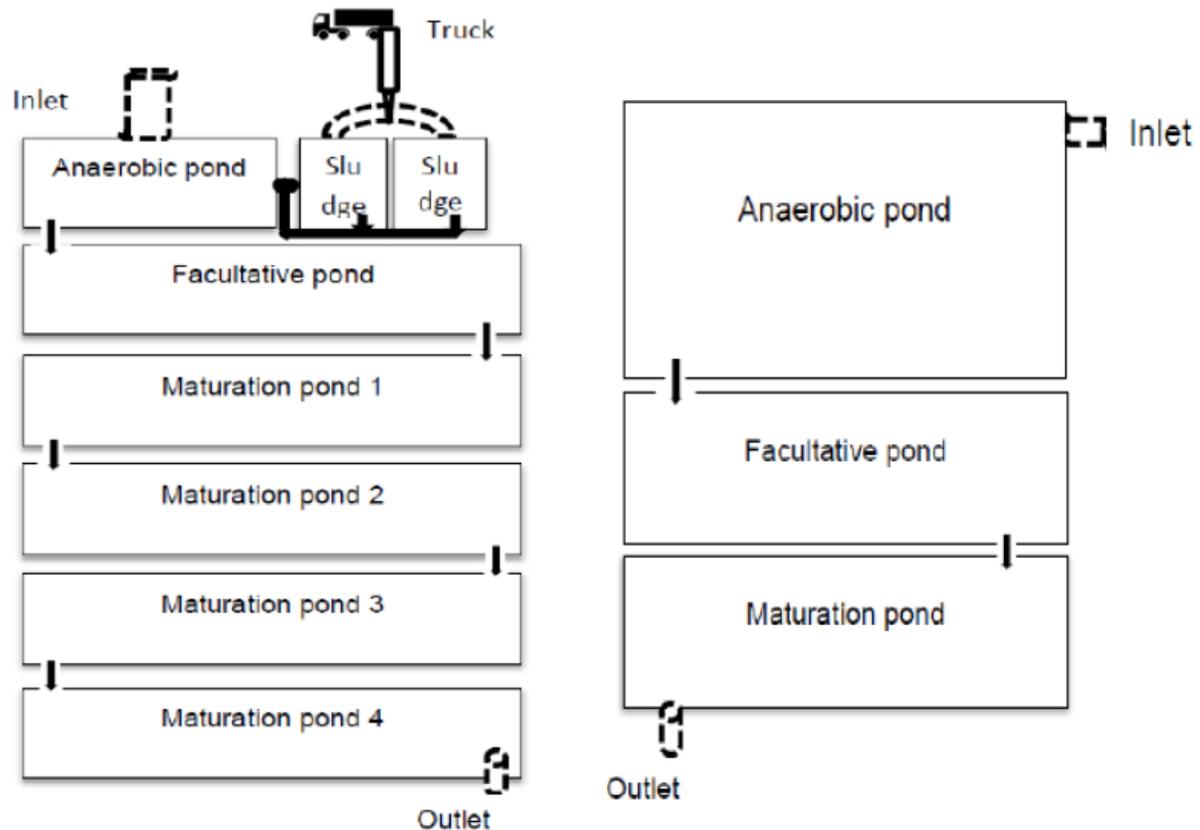
This study aimed at assessing the performance of WSPs in the reduction of endocrine disrupting estrogens in Morogoro urban and peri-urban areas in Tanzania.

## MATERIALS AND METHODS

### Description of the Study Area

This study was carried out in Morogoro in Tanzania, particularly at Mafisa Wastewater Stabilization Ponds in Morogoro Municipal and Mzumbe Wastewater Stabilization Ponds. Mzumbe is situated 25 kilometers from Morogoro town and Mafisa is within Morogoro Municipality.

Mafisa WSPs treat domestic wastewater for Morogoro municipality with the combined ponds volume of 58,000m<sup>3</sup>. There are eight ponds, one anaerobic, one facultative, four maturation ponds and two of them are sludge ponds receive sewage by trucks ( Figure 1A). The ponds are connected to sewer trunk of 30.02 km, a total of 1,120 customers are directly connected with the sewerage. The customers connected to the sewer trunk are families, institutions and commercial places (MORUWASA, 2013). Majority of customers are not directly connected to the sewerage, they use sewage trucks to carry sewage to the ponds. Eventually the



A. Mafisa WSPs Sketch Map

B. Mzumbe WSPs Sketch Map

Figure 1. Mafisa and Mzumbe Wastewater Stabilization Ponds.

effluent from Mafisa ponds flows into Morogoro river as the natural receiving water body.

Mzumbe WSPs consist of three ponds, one anaerobic, one facultative and one maturation pond (Figure 1B). The WSPs treat the wastewater from the Mzumbe University main campus students' hostels and staff resident houses. The campus accommodates a total of 4700 students and 268 staff families. Staff families make a population of approximately 1,206 making an estimate total of 6000 people served by the Mzumbe WSPs. The effluent from the ponds drained into Mlali river as natural receiving body but vegetable farmers use the effluent for irrigation agriculture especially during dry season.

### Chemicals and Materials

Two standards ethinylestradiol (EE2) and  $\beta$ -estradiol (E2) hormones were supplied by Santa Cruz Biotechnology, Texas, USA. Other chemicals used were n-heptane(99%) , methanol (99%) ,acetone(99.8%) and hydrochloric acid

(37%, 1.18M) supplied by Carlo Erba Reagenti and Sigma Aldrich, Germany. Glass fiber filter papers of MN 615, size  $\varnothing$  150mm and 2576 size ;  $\varnothing$  240 mm from Macherey-Nagel GmbH & Co.KG ,Duren-Germany and Munktell & Filtrak GmbH, Barenstein Germany respectively. Solid phase extraction C-18 cartridges (130mg, 3mL) by Varian and NH<sub>2</sub>-cartridges (500mg, 3ml) by Chromabond, Germany and Multi-parameter water quality meter with probe for pH, conductivity, dissolved oxygen (DO), temperature, and total dissolved solids(TDS). ELISA kits for EE2, E1 and E2 were supplied by Cloud-Clone Corp. 1304 Langham Creek Dr. Suite 226, Houston, TX 77084, USA.

### Measurement of Pond Dimensions and Physical-Chemical Parameters

Since the performance of the WSPs is influenced by dimensions and physical-chemical parameters, it was necessary to measure them. The width and length of the ponds were measured using tape measure, whereas

graduated pole was used to measure pond depth, local boat was used to cross the ponds for measuring depth at different positions. Hence, the pond area and volume could be calculated. Flow rate meter was used to measure flow rate of the wastewater; hence retention time of wastewater could be calculated. Multi parameter water quality meter was used to measure pH, dissolved oxygen, total dissolved solids, electric conductivity and temperature.

### Collection of Water Samples from WSPs

Low quality water (LQW) samples were collected twice in each sampling point in an interval of one month. Samples were drawn from inlet and outlet of each pond. At each sampling point three water samples (each 500 ml) were thoroughly mixed in a clean 2.5 liters glass bottle to form 1.5 litres of composite sample. The pH of water samples adjusted to about 3 by adding concentrated hydrochloric acid so as to fix the estrogens (Hansen et al., 2011). Then, the samples were carried in cool box packed with ice packs to the Ecotoxicology and Natural Products research Laboratory in the faculty of Veterinary Medicine at Sokoine University of Agriculture. In the laboratory pretreatment and solid phase extraction of estrogens was done within 12 hours after sample collection.

### Extraction of Estrogens from Water Samples

Extraction of estrogens from water samples was carried out according to the protocol described by (Hansen et al., 2011) with some modifications customized to our laboratory settings. Each water sample (1.5 litres) was first filtered twice using GFC filters papers to ensure removal of debris. The C-18 cartridges were conditioned with 2x3 mL heptane, 3 mL acetone, and lastly with 3ml of distilled water. Thereafter, solid phase extraction (SPE) was performed with C-18 cartridges (Bond Elut 500 mg, 3cc reservoir, Varian Agilent Technologies, USA) facilitated by vacuum manifold. After extraction the cartridges were dried in air using vacuum manifold for about half an hour. Then, elution of the analyte was achieved by using 10 ml of heptanes and acetone (65:35). Thereafter, the elute was air dried at 30°C, followed by reconstitution using 5 ml methanol. The samples were stored at -20°C, until analysis by ELISA competitive technique was done

### Collection of Sludge Samples

Systematic random sampling of the sludge samples was adopted; A pond with at least eighty metres long was divided into two portions, thereafter each portion was divided into four subsections and a sample was collected

after every ten meter of a pond, followed by thorough mixing to form a composite sludge sample of about 400g. A total of twelve composite samples were collected from seven ponds at Mafisa WSPs and four composite samples from Mzumbe WSPs.

### Pretreatment of Sludge Samples and Extraction of Endocrine Disrupting Estrogens

The sludge samples were air dried for four days in shade area. The samples were homogenized by grinding using motor and pestle. This study adopted a technique used by Kawakami and Montone, (2002) to extract steroids from marine sediments using soxhlet extraction technique. Some modifications were made for the technique to suit our laboratory setting. Estrogens from sludge were extracted as shown in (Figure 2). For each sludge sample a duplicate of soxhlet extraction was set.

### Detection and Quantification of Endocrine Disrupting Estrogens by ELISA Competitive Technique

The detection and quantification of ethinylestradiol, estradiol and estrone was carried out using ELISA kit from Cloud-Clone Corp. 1304 Langham Creek Dr. Suite 226, Houston, TX 77084, USA. Manufacturer instructions were followed; Thereafter, immediately measurement on ELISA reader was conducted at 450 nm.

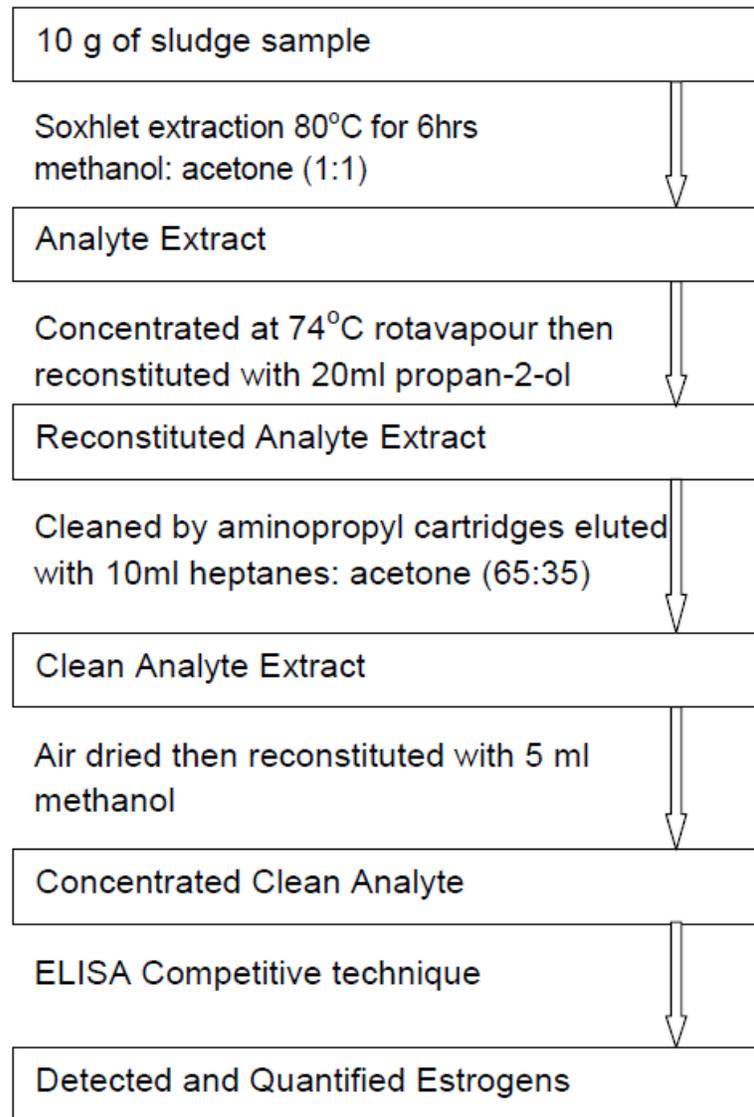
### Quantitative Analysis

The concentrations of the standards (2000 ng/L, 670 ng/L, 220 ng/L, 74 ng/L, and 25 ng/L) were transformed into natural logarithm to obtain linear calibration curve, and therefore natural logarithm of concentration for each hormone was drawn against the respective absorbance. The linear equation obtained in the curve was used to interpolate the concentration of estrogens in samples

### Recovery Studies

Four different concentrations (2,000 ng/L, 1,330 ng/L, 130 ng/L and 13 ng/L) of mixed standard ethinylestradiol and estradiol each was made by dissolving in 1500 ml distilled water. The same pretreatment and analysis steps were followed as were done for low quality water samples.

To establish recovery in sludge samples, about 100g of sludge sample was soaked in a mixture of 100 ml acetone and methanol overnight for removal of estrogens. After decantation of the soaked sludge sample was heated for four hours in furnace at 350°C to decompose the remaining estrogens. After cooling, the



**Figure 2.** Summary protocol for extracting and analysing estrogens from sludge.

sludge sample was divided into four portions each with 10 g of sludge, 2,000 ng/L of a mixture of ethinylestradiol and estradiol was spiked in three of the four samples, the other was treated as blank. Thereafter, soxhlet technique was used to extract the estrogens, the same steps as were done for sludge samples.

#### **The Extent of Endocrine Disrupting Estrogens Reduction in WSP**

The estrogens treatment efficiency of the WSP was computed by applying equation 1 with assumption that

estrogens derived from estrogen conjugates were negligible (Liu et al., 2015). The mean concentration of each estrogen in the inlet and outlet for each pond were used to calculate the treatment efficiency in the pond series

$$RE = \frac{E_{in} - E_{out}}{E_{in}} * 100 \quad \dots\dots\dots \text{equation 1}$$

where

RE stand for estrogens reduction efficiency in percentage  
 $E_{in}$  estrogens mean concentration in the pond's inlet in ng/L

**Table 1.** Recovery data for E2 and EE2.

Concentration of EE2 & E2 spiked in distilled water ng/ml	Recovered Concentration EE2 ng/ml	% Recovery EE2	Recovered Concentration E2 ng/ml	% Recovery E2
2	1.34	67	1.30	65
1.3	0.98	75.4	0.89	68.5
0.133	0.112	84.21	0.12	90.22
0.05	0.043	86	0.045	90

$E_{out}$  estrogens mean concentration in the pond’s outlet in ng/L

**Statistical Analysis**

IBM SPSS version 20 was used for statistical analysis of the results; both descriptive and inferential statistics were carried out. For descriptive statistics, means, standard deviation, median and range were calculated. Inferential statistics one way ANOVA with post hoc Tukey’s-b was employed for multiple comparisons of the mean concentrations of estrogens between sampling points in WSP. For sludge sample nonparametric test particularly Krustal- Wallis was employed for comparison of median concentrations of the estrogens in WSP. Level of significance between groups was reported at  $p < 0.05$ .

**RESULTS**

**Estrogens standard curves**

Natural logarithms of standard concentrations were plotted against absorbance to obtain linear curves (Figure 9). The  $R^2$  for EE2, E2 and E1 was 0.9707, 0.9851 and 0.982 respectively. Hence, the linear equations were used to quantify the estrogens based on their respective absorbance.

**Recovery data**

The recovery of EE2 and E2 were assessed for solid phase extraction and ELISA technique analysis. The results are shown in Table 1

**Detected and Quantified Endocrine Disrupting Estrogens in Low Quality Water**

Figure 3 shows the mean concentrations of the endocrine disrupting estrogens in LQW in Mafisa WSP. The influent had significant high amount of estrogens but in the course of treatment decreased significantly ( $p < 0.05$ ). Hence, the mean concentrations of the estrogens in the

effluent were significantly lower than those of influent at  $p < 0.05$ . The mean concentrations for EE2, E2 and E1 ranged from 35.6 to 1.5 ng/L, 85.6 to 4.4 ng/L and 60.7 to 2.9 ng/L respectively.

Figure 4 shows the mean concentrations of the endocrine disrupting estrogens from LQW in Mzumbe WSP. The mean concentrations for EE2, E2 and E1 ranged from 23.6 to 4.4 ng/L, 39.4 to 6.9 ng/L and 25.5 to 2.7 ng/L respectively. The mean concentrations of EE2 and E2 decreased significantly from influent to effluent. But for E1 mean concentrations at anaerobic and facultative ponds were not significantly different.

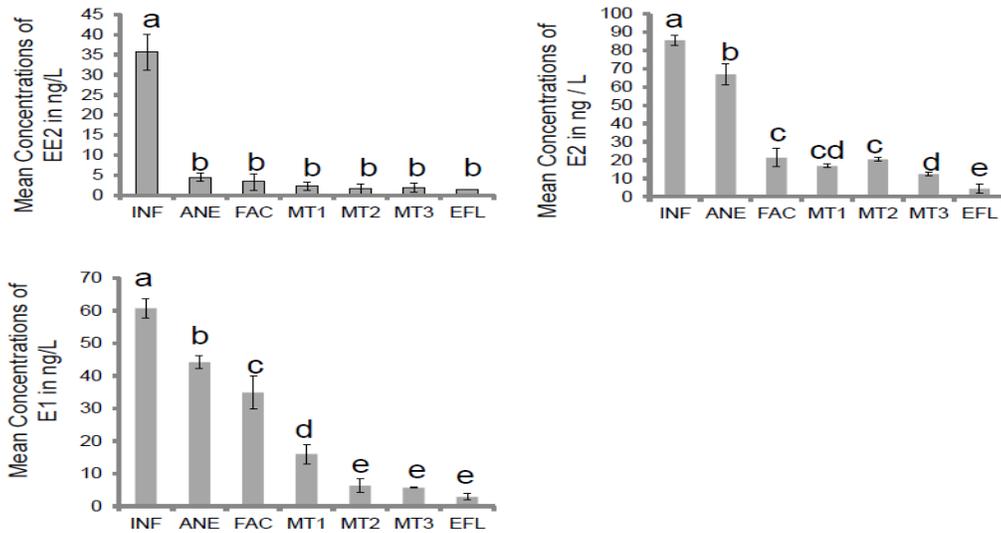
**Detection and Quantified Endocrine Disrupting Estrogens in Sludge**

Figure 5 and 6 display the median concentrations of the detected endocrine disrupting estrogens in sludge from Mafisa and Mzumbe WSPs respectively. The estrogens concentrations in the sludge from both study sites were not normally distributed.. The median concentrations of EE2 and E2 in anaerobic pond for both study sites were significantly different from other ponds ( $p < 0.05$ ). In both study sites the median concentrations of E1 in anaerobic and facultative ponds were comparable ( $p > 0.05$ ), but were significant different ( $p < 0.05$ ) to those in maturation ponds.

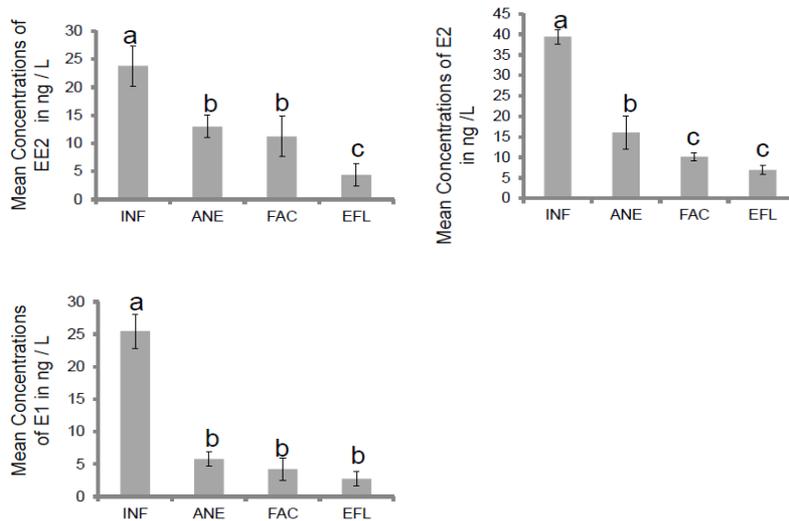
**The Extent of Endocrine Disrupting Estrogens Reduction in WSP**

Figure 7 and 8 display the percentage reduction of the endocrine disrupting estrogens in consecutive ponds at Mafisa and Mzumbe WSPs respectively. In each subsequent pond significant estrogen reduction occurred except in some ponds an increase in concentrations occurred. For instance at Mafisa WSPs percentage reduction of EE2 in the third maturation pond was negative. Likewise, percentage reduction of E2 in MT2 at Mafisa WSPs was negative due to increase in concentrations of E2.

Recovery data for estrogens in sludge sample was 63% and 68% for EE2 and E2 respectively.



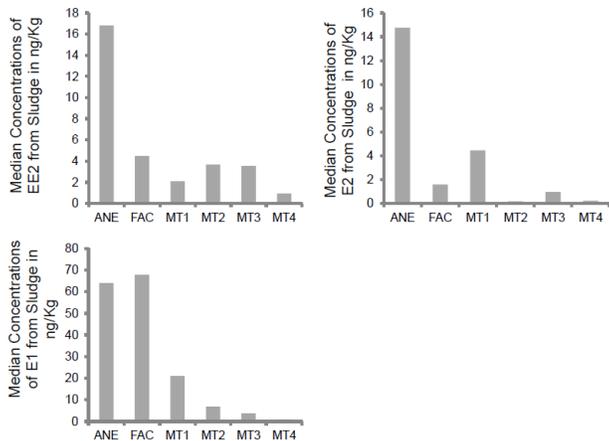
**Figure 3.** Mean Concentrations of endocrine disrupting estrogens (EE2, E2 & E1) in LQW from Mafisa WSP (n= 2 each being composite sample of three samples)  
 Legend; INF = influent ANE = anerobic pond outlet FAC = facultative pond outlet  
 MT1 = first maturation pond MT2 = second maturation pond MT3 = third maturation pond outlet  
 EFL = Effluent LQW = Low quality water.



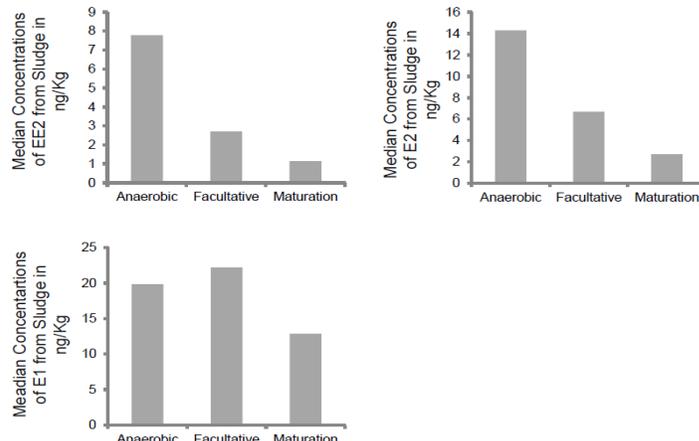
**Figure 4.** Mean Concentrations of endocrine disrupting estrogens (EE2, E2 & E1) in LQW from Mzumbo WSP. (n= 2 each being composite sample of three samples).  
 Legend; INF = Influent ANE = anaerobic pond outlet FAC = facultative pond outlet EFL = effluent LQW = Low quality water.

At Mafisa WSPs with six pond system percentage overall reduction of EE2, E1 and E2 was 95.8 %, 95.3% and 94.9% respectively. The corresponding overall estrogens

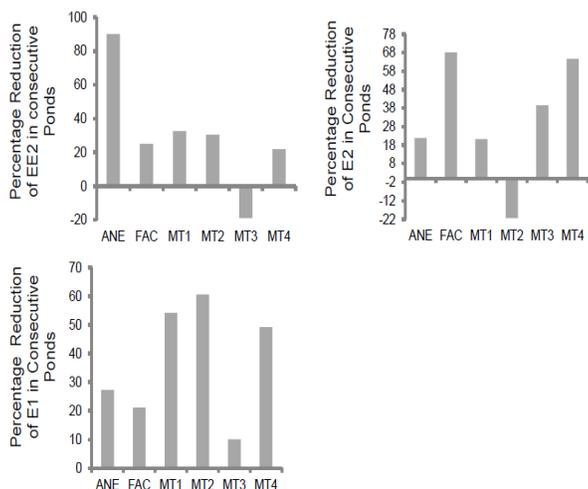
reduction at Mzumbo WSPs with three pond system was 81.56 %, 89.3% and 82.5 % for EE2, E1 and E2 respectively.



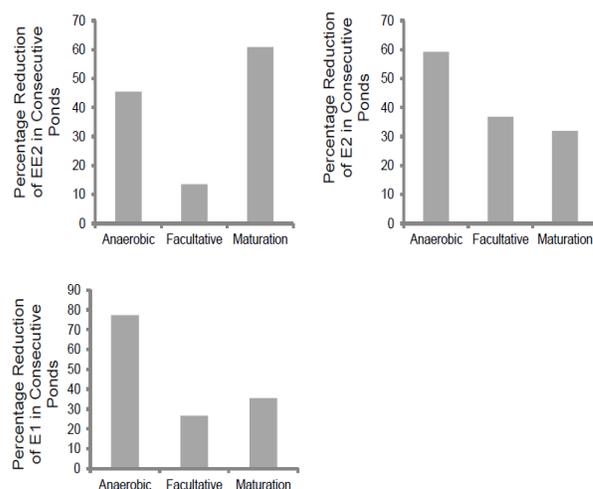
**Figure 5.** Median Concentrations of endocrine disrupting estrogens (EE2, E2 & E1) in sludge samples from Mafisa WSP (n = 4 for each pond)  
 Legend; ANE; Anaerobic pond FAC = facultative pond MT1 = first maturation pond MT2 = second maturation pond MT3 = third maturation pond MT 4 = fourth maturation pond



**Figure 6.** Median Concentrations of endocrine disrupting estrogens (EE2, E2 & E1) in sludge samples from Mzumba WSP (n= 4 for each pond).



**Figure 7.** Percentage Estrogens (EE2, E2 & E1) reduction in consecutive pond at Mafisa WSPs.  
 Legend: ANE = anaerobic pond FAC = facultative pond MT1 = the first maturation pond MT 2 = the second maturation pond MT3 = the third maturation pond MT 4 = the fourth maturation pond.



**Figure 8.** Percentage Estrogens (EE2, E2 & E1) reduction in consecutive pond at Mzumba WSPs.

**DISCUSSION**

In both study sites significant concentrations of natural and synthetic estrogens were obtained. The observation concur with several studies in Europe, Asia and USA which clearly identified natural (estrone and 17β-estradiol) and synthetic estrogen (17α-ethinylestradiol) as the main estrogenically active substances present in domestic effluents (Gross-

Sorokin et al., 2004; Coleman et al., 2010). In addition, presence of endocrine disrupting estrogens in sludge indicated that sedimentation was among the mechanisms of estrogens reduction. The mean concentrations of endocrine disrupting estrogens decreased from anaerobic pond to maturation pond except in some cases where the increase in concentration occurred. For instance E2 at Mafisa its concentration increased in second maturation and EE2

increased in third maturation pond. Estrogens conjugates can be transformed into free estrogens during sewer transit (Kumar et al., 2012). Therefore, the increase in concentration could be due to deconjugation of estrogen conjugate into free estrogens.

The results indicate that the main mechanisms for EE2 reduction was through sorption in sludge. At Mafisa WSPs ninety percent (90%) of the EE2 was reduced through sedimentation in anaerobic pond, likewise at Mzumbe WSPs seventy seven percent (77%) of EE2 was reduced at anaerobic pond. Small proportion of EE2 may be biodegraded, this may be due to its resistance to biodegradation (Ivanov et al., 2010). For E1 and E2 in both study sites were reduced significantly through biodegradation. However, sorption in the sludge also took place.

The estrogens mean concentration in the influent from Mzumbe WSP was lower than those obtained from Mafisa WSP influent. But, at Mzumbe WSPs estrogens treatment efficiency was also lower than that of Mafisa WSPs. The results indicate that six pond systems may be more effective than three pond system, since provided larger surface area for further treatment of the sewage. On the other hand, at Mafisa WSPs the four maturation ponds apart from sedimentation mechanism of the estrogens, ensure significant reduction of estrogens through biodegradation, each contained enough dissolved oxygen to support the process (Table 4). In addition, estrogens which could be formed in the course of deconjugation of metabolites were biodegraded in subsequent ponds, as result enhanced the estrogen reduction at Mafisa WSPs (Andersen et al., 2004).

In the course of sewage treatment significant reduction of the endocrine disrupting estrogens occurred. The extent of estrogens reduction in these study sites were similar to those reported by Belhaj et al., (2014) in Tunisia WSP could reduce estrogens E1, E2 and EE2 at 80%, 92% and 84% respectively. In addition, Zhou et al., (2012) also reported estrogens reduction efficiencies at Gaobeidian WWTP in Beijing, China to be 83%, 96%, and 93% for E1, E2 and EE2, respectively. Furthermore, Cicek et al., (2007) reported that E1 & E2 reduction efficiency was 91% whereas EE2 was 75%. Moreover, Andrew et al., (2007) reported that Japanese wastewater treatment system could reduce estrogens in the range of 74% and more than 90%. On the other hand, the estrogens reduction efficiencies in this study were higher than those reported by Pessoa et al., (2014), in which estrogens reduction efficiencies of Brazilian WSP ranged from 54 to 80%. The differences in the estrogens reduction could be attributed to the differences in the concentrations of estrogens in the influents.

Moreover, the mean concentrations of EE2 and E1 in the effluent were slightly higher to those reported in other studies that could significantly induce vitellogenin

formation in male fish (Fenske et al., 2001; Thorpe et al., 2001). Therefore, proper routine maintenances of the ponds are necessary to improve their estrogen treatment efficiency.

### Health Implication

Tanzania National Bureau of Standards (TBS) has not yet set a tolerance limit of endocrine disrupting estrogens from WSPs effluents (TBS, 2005). Even WHO, EPA of the United Nations have not yet established guidelines for safe levels of estrogens to be discharged into surface waters from WSPs effluents (WHO and UNEP, 2012; Marti and Batista, 2014). The endocrine systems are very similar across vertebrate species and the effects of endocrine disrupting chemicals including estrogens manifest themselves independently of species. The effects are endocrine system related and not necessarily species dependent (WHO and UNEP, 2012).

In this study the effluent contained trace levels of estrogens yet these concentrations may be biologically active in the ecosystem and contribute to estrogenicity of surface water (Clouzot et al., 2008). If the receiving rivers are not polluted with estrogens, dilution effect could lower the concentrations consequently reduce health risks to aquatic organisms and human as well. Maintaining the WSPs properly, for instance removal of sludge to ensure the designed pond depth is maintained certainly will enhance the performance of the pond in treatment of endocrine disrupting estrogens.

### CONCLUSION

Both natural and synthetic endocrine disrupting estrogens particularly estradiol, estrone and ethinylestradiol have been detected and quantified in sludge, influent, anaerobic pond, facultative pond, maturation ponds as well as in effluent. Fortunately, the treatment in wastewater stabilization ponds reduced them significantly; hence the effluent had concentrations of those estrogens with low health risks to aquatic organisms and human. Comparing with the previously studies, the levels of estrogens in this study particularly in effluent from both study sites could induce feminization of male fish without causing intersex in the exposed fish. However, long term exposure to such low concentrations could lead to adverse effects. Routine maintenance of WSPs is necessary for improving their treatment performance so that estrogens concentrations in effluent become the lowest possible.

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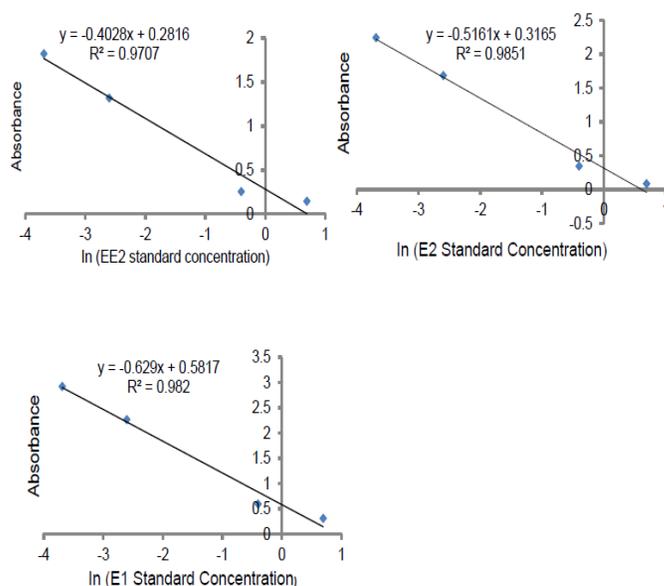
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## SUPPLEMENTARY MATERIALS

### Calibration Curves for EE2, E2 and E1

Mean absorbances for each standard estrogens were drawn against the natural logarithms of the standard concentrations (Figure 9).



**Figure 9.** Calibration Curves for EE2, E2 and E1.

### Mafisa and Mzumbe WSPs Ponds Dimensions and Retention Time of Wastewater

Table 2 and 3 present wastewater stabilization ponds dimensions and retention time. The designed wastewater retention time at Mafisa WSPs was 25 days, but the measured retention time was 18.9 days which was lower than the expected. High flow rate and amount of sludge could have affected the retention time to some extent.

**Table 2.** Retention time of wastewater and dimensions of Mzumbe WSPs.

Type of Pond	Pond Width Meters (m)	Pond Length Meters (m)	Measured Average Pond Depth, meters (m)	Designed pond depth Meters (m)	Flow Rate m <sup>3</sup> /sec	Retention time days
Anaerobic	49	81.5	1.73	2.5	0.008	10
Facultative	49	37.9	1.157	1.5	0.004	6.2
Maturation	49	37.9	0.773	1	0.003	5.5
<b>Total Retention time</b>						22

**Table 3.** Retention time of wastewater and dimensions of Mafisa WSPs.

Type of Pond	Pond Width Meters (m)	Pond Length Meters (m)	Measured Average Pond Depth, meters (m)	Designed pond depth Meters (m)	Flow Rate m <sup>3</sup> /sec	Retention time days
Anaerobic	48	72.5	1.6	2.5	0.034	1.9
Facultative	59	133	1.5	2	0.031	4.5
1 <sup>st</sup> Maturation	59	133	1.1	1.2	0.031	3.1
2 <sup>nd</sup> Maturation	59	133	1.1	1.2	0.038	2.7
3 <sup>rd</sup> Maturation	59	133	1.2	1.2	0.039	2.7
4 <sup>th</sup> Maturation	59	133	1.2	1.2	0.027	4
<b>Total Retention Time</b>						18.9

**Table 4.** Physical-chemical parameters of wastewater at Mafisa WSPs.

Pond Type	Mean pH (n = 4)	Mean Electrical Conductivity (EC) Scm <sup>-1</sup> (n = 4)	Mean Total Dissolved Oxygen (mg/L) (n = 4)	Mean Temperature (TDS) °C (n = 4)	Mean Dissolved Oxygen DO mg/L (n = 4)
Anaerobic	5.25±0.07	1250 ± 5	623.3±15.8	25.13±0.84	0.4±0.1
Facultative	6.4±0.14	1296.7±23.68	608.3±13.16	24.8±1.1	0.5±0.2
1 <sup>st</sup> Maturation	6.55±0.07	1279.7±5.8	598.3±8.42	25.1±1.16	6.95±0.21
2 <sup>nd</sup> Maturation	7.5±0.14	1271.7±10.53	588.3±5.68	25.23±1.16	11.45±0.93
3 <sup>rd</sup> Maturation	7.65±0.07	1219.7±5.79	575±6.42	25.47±0.79	16±0.71
4 <sup>th</sup> Maturation	7.60±0.36	1196.7±15.79	570±10.58	25.57±1.06	16.3±2.6

For instance the depth of anaerobic pond was designed at 2.5 meters but the average measured depth was 1.6 meters, indicating that the pond contained sludge of about 0.88meters deep. In facultative pond the designed pond depth was 2 meters but the average depth was 1.5 meters, implied that 0.5 meters was the level of sludge. However, other ponds depth differed slightly from what was designed thus contained little sludge. The wastewater retention time at Mzumbe WSPs was a bit higher than that of Mafisa (Table 2 & 3). This was attributed by lower flow rate at Mzumbe. The flow rate at Mafisa was higher than that of Mzumbe by a factor that ranged between 4.3 to 9.

**Mafisa and Mzumbe WSPs- Physical-Chemical Parameters**

Table 4 and 5 depict the physical-chemical parameters at Mafisa and Mzumbe WSPS respectively. The physical chemical parameters (dissolved oxygen, temperature, TDS, ECs and pH) at both Mafisa and Mzumbe WSPs were within the allowable limits (TBS, 2005). Except some few cases for example dissolved oxygen in the anaerobic pond in Mzumbe WSPs which was higher than the allowable limit consequently worked as aerobic pond. This deviation could be due to water weeds in ponds as well as high amount of sludge.

**Table 5.** Physical-chemical parameters of wastewater at Mzumbe WSPs.

Pond Type	Mean pH  (n = 4)	Mean Electrical Conductivity (EC) Scm <sup>-1</sup> (n = 4)	Mean Total Dissolved Oxygen (TDS) mg/L (n = 4)	Mean Temperature °C  (n = 4)	Mean Dissolved Oxygen DO mg/L  (n = 4)
Anaerobic	5.8±0.14	390±10.2	210±14.4	23.1±0.8	7.45±1.26
Facultative	6.5±0.07	386±7.8	175± 5.79	22.7±1.1	10.3±3.32
Maturation	7.2±0.24	320±5.3	180± 2.92	23.2± 2.0	14.6±1.12