Association between Heavy Metals and Selected Reproductive Parameters in the Nile Tilapia, *Oreochromis niloticus*, along River Ruiru, Kiambu County, Kenya

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Authors’ contributions

This work was carried out in collaboration among all authors. Author OMK designed the study, performed the practical part including sample collection and supervising laboratory analysis and wrote the first draft of the manuscript. Authors SAO and JAS did over all supervision of the study and writing of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The Nile tilapia, *Oreochromis niloticus*, is a tropical fish species of commercial importance in both aquaculture and in the wild. It plays a great role in human nutrition and food security. River Ruiru is one of the rivers faced with pollution from nearby industries. It is inhabited by various species of fish such as tilapia and common carp, which, may be harvested by surrounding communities for food. High levels of heavy metals disrupt normal reproductive process in fish. Besides, it has been reported that edible fish contaminated with heavy metals has deleterious effects on the health of humans and other animals that consume them. There is no documented information on the association between heavy metals and the reproductive biology of *Oreochromis niloticus* in River Ruiru. This study was aimed at assessing the level of heavy metals in fish ovaries and their association with selected reproductive parameters in the reproductive cycle of *O. niloticus*. Fish
samples were collected monthly, for 8 months, from the downstream and upstream sections. Gonadosomatic index (GSI), serum 17β-estradiol (E₂) levels and levels of heavy metals lead, cadmium, copper, iron and zinc in ovaries were determined. The levels of the heavy metals were measured using Atomic Absorption Spectrophotometer. The level of E₂ was analyzed using Enzyme-Linked Immunosorbent Assay. There was no significant difference in the GSI between the upstream and the downstream sites (t=0.82, p=0.416). Similarly, there was no significant difference in the levels of E₂ between the downstream and the upstream sampling sections. In the downstream, the levels of lead and iron in fish ovaries were significantly higher compared to the upstream (lead: t = 3.36, p = 0.002; iron: t = 4.920, p=0.001). The results showed that levels of heavy metals did not associate with the selected reproductive parameters in the Nile tilapia, along River Ruiru. Levels of lead and cadmium were above allowable concentrations for fish consumption when compared to WHO levels. The study recommends that the Ministry of Environment and Natural Resources should put measures in place to stop discharging raw effluents into River Ruiru.

Keywords: Oreochromis niloticus; heavy metals; 17β-estradiol; gonadosomatic index; River Ruiru.

1. INTRODUCTION

Fish is of great importance for nutrition worldwide, employment and trade in developing countries. According to Food and Agricultural Organization (FAO), fish accounts for more than 40 percent of protein in the diet of two thirds of global population [1,2].

River Ruiru harbors various species of fish, which are potential sources of proteins for the surrounding communities. River Ruiru is affected by rural and urban effluents, agricultural and industrial effluents [3], which make the river polluted with heavy metals. Exposure of fish to various environmental toxicants cause gonadal changes such as decreased gonadosomatic index (GSI) due to stressed liver tissues resulting in reduced production of phosphoglycoproteins that form part of the egg yolk [4]. Other workers reported that exposure of O. niloticus to cadmium decreases GSI at the beginning probably due to inhibition of enzymes functioning in synthesis and release of reproductive hormones whereas prolonged exposure to this metal causes increase in GSI due to activation of synthesis of metal binding proteins in gonads [5].

Heavy metals are reported to stimulate or inhibit the endocrine system and cause overproduction or underproduction of hormones such as 17β-estradiol in fish [6]. Accumulation of heavy metals in the ovaries and other environmental pollutants is reported to disrupt the production of reproductive hormones such as 17β-estradiol, luteinizing hormone and follicle stimulating hormone, through changes in the physiological processes of the hypothalamic – pituitary – ovarian axis [7]. However, other researchers reported that estradiol levels significantly increased in dissolved cadmium exposed female O. niloticus [8].

It is reported that exposure of fish to high levels of the heavy metals zinc and copper (0.5 mg/l respectively, causes atrophy and cytoplasmic leakage in the ova leading to severe degeneration [9]. Other researchers observed that heavy metals cause atresia and necrosis in oocytes leading to a decrease in egg production [10]. [11], reported that exposure of an Indian teleost to copper, zinc, or lead, caused disappearance of oocytes in the ovaries Also, post hatch larvae of O. niloticus subjected to 2 and 5 ppm sub lethal levels of zinc for 30 days retained undifferentiated gonads with oogonial proliferation and ovaries of mature tilapia exhibited hyperemia and reduced oocyte number [12]. Cadmium prevents egg maturation and hence lowers the number of spawned ova [13].

Fish contaminated with heavy metals is unsafe for human consumption as this is associated with potential health disorders such as immunodeficiency, osteoporosis, neurodegeneration and organ failures [14]. There is no information on the association between heavy metals and reproductive biology of fish inhabiting river Ruiru. The current study was therefore undertaken to study the relationship between heavy metals and selected reproductive parameters in the reproductive cycle of O. niloticus to thereby establish correlations between metals in the ovaries and reproductive status of fish in river Ruiru.
2. MATERIALS AND METHODS

2.1 Study Location

The study was conducted in River Ruiru, Kiambu County, Kenya. The river passes through Ruiru Town in Ruiru Sub County, which is 3 kilometers away from Nairobi City County border as it joins Athi River. Two sampling sites along the river were considered during the study period. They are within longitudes 36° 54'E and 37° 3'E and latitudes 1° 12'S and 1° 8'S within Ruiru Sub County (Appendix 1, Fig. 1). There are several settlements along the river without proper sewage disposal system, despite the large population. The river also passes through areas where some industries discharge their wastes into it. Human activities along River Ruiru therefore, affect aquatic animals living along the River [15].

2.2 Sampling Sites and Collection of Fish Samples

Sampling sites were chosen based on the surrounding economic activities, proximity of effluent discharge into the river and habitat characteristics such as physical appearance of the river water, type of vegetation and substrate. The course of River Ruiru was divided into downstream and upstream sections with respect to Ruiru Town and one sampling site was chosen on each section. The upstream site was identified as 'A', located upstream along the course of River Ruiru, 3 kilometers past Ruiru Town while the downstream site, 'B', was at the downstream section of the river, 750 meters away from Ruiru Town (Appendix I, Fig. 1). Samples of *O. niloticus* were collected using a cast net. All fish samples were macroscopically examined and the sex of each sample was established based on the external morphologies of each sample using a magnifying lens [16]. All the males were returned to the sampling sites and the females were retained for subsequent studies. Same procedure was repeated for eight consecutive months (November 2014 to June 2015). It was not possible to collect six samples of female Nile tilapia from each sampling site once a month as was planned earlier because they were scarce in both sampling sites.

2.3 Blood Samples from the Females

Five milliliters of blood samples were withdrawn from the fish samples of stages III, IV, V and VI separately from both sampling sections, via cardiac puncture using medium sized heparinized needle and 5 milliliter Hindustan syringes. The blood was then transferred to micro-centrifuge tubes separately and centrifuged in revolutions per minute (rpm) to separate the blood serum from plasma. The serum was pipetted into Eppendorf tubes and then stored in a deep freezer at -20°C until analysis for the level of 17β-estradiol.

2.4 Determination of Sexual Maturity Stages

The fish samples collected from both sampling sites were dissected. Ovary samples were carefully excised and trimmed to remove connective tissues. The maturity stages of the fish gonads were determined separately through visual inspection of the appearance, size and texture, following the procedures by [17] and [18].

2.5 Gonadosomatic Index (GSI)

Gonadosomatic index is the calculation of gonad weight as a percentage of total body weight [19]. Weight, in grams, of the ovary samples of the fish samples obtained from both sampling sites were taken separately using an electric weighing balance (Model AAA Adam Co Limited). The GSI for the fish samples were calculated separately using the formula by [20]:

\[
GSI = \frac{\text{weight of gonad}}{\text{Weight of fish}} \times 100
\]

2.6 Fecundity (F)

Fecundity is the number of eggs ripened by a female during spawning season [21]. Ovary samples in stages IV and V were carefully dissected separately using a scalpel. The eggs were spread on the dissecting tray and the egg samples physically counted and recorded [22].

2.7 Quantitative Determination of 17β-estradiol (E₂) Concentrations in Serum

The sex steroid 17β-estradiol in the blood serum was analyzed using Enzyme-Linked Immunosorbent Assay (ELISA) following the assay kit procedures and methods by [23] and [24]. The concentration of 17β-estradiol in serum
samples were interpolated from the standard curve (Appendix II, Fig. 2).

### 2.8 Digestion of Ovary Samples

Ovary samples were wet digested separately where 1 g of the wet digest was accurately weighed using an electronic balance (Model AAA Adam Co limited). Ten milliliters (10 ml) of concentrated nitric acid was first added into the ovaries in separate glass beakers and allowed to stand overnight. They were then gently heated on hot plates until dense brown fumes began to appear. Hydrogen peroxide was added drop wise to clear the brown fumes and improve the dissolving power of nitric acid. Digested fish ovaries were allowed to evaporate to about 5 ml to get rid of excess water from the mixture. This was cooled and filtered (using Whatman number 42 filter paper into 100 ml different clean and dry volumetric flasks and then diluted to the mark with distilled water [25]. The ovary samples from upstream and downstream were separately digested in triplicates then transferred into separate plastic bottles, labeled and stored awaiting analysis of the heavy metals lead, cadmium, copper, zinc and iron. For background correction, six blanks were digested as pre-test samples and each analyzed for lead, cadmium, copper, zinc and iron by atomic absorption spectrophotometer [26]. Blank solutions were free of the heavy metals lead, cadmium, copper, zinc and iron. Hence they were used to test if the AAS was free from contamination of lead, cadmium, copper, zinc and iron before analysis of the digested water and ovary samples.

### 2.9 Analysis of Heavy Metals

Analysis of the heavy metals lead, cadmium, copper, zinc and iron, in the digested ovary samples were determined separately using Atomic Absorption Spectrophotometer, at wavelengths 283.3 nanometers (nm), 228.8 nm, 324.8 nm, 213.9 nm and 248.3 nm respectively. The concentrations were read from the standard curves generated, using the standards prepared based on atomic absorption standards made. Each sample was assayed in triplicate (the average values calculated from triplicates were used in statistical analysis). The minimum level of detection for each of the metals (lead, cadmium, copper, zinc, and iron) was 0.001 mgkg⁻¹.

### 2.10 Statistical Analysis

Statistical analysis of data was carried out using Minitab software version 13. A two sample t-test was used to compare the difference in mean levels of the heavy metals in the ovaries, means of gonadosomatic index, serum level 17β-estradiol, standard length and fecundity of the fish from the downstream and upstream sections of the river. To establish the relationship between the level of heavy metals in the ovaries and gonadosomatic index (GSI), 17β-estradiol and fecundity, a Pearson moment correlation was conducted. One way analysis of variance (ANOVA) was used to test if there were significant differences in GSI and levels of 17β-estradiol between different months. The results were expressed as mean ± S.E. Difference in mean values were accepted as being statistically significant at p<0.05.

### 3. RESULTS

#### 3.1 Maturity Stages

A total of thirty O. niloticus samples were collected from the upstream and seventeen from the downstream sampling sites. Maturity status of each fish in each month was determined and assigned as stages I, II, III, IV, V and VI (Table 1).

| Maturity stages | Appearance of ovaries                                      |
|-----------------|----------------------------------------------------------|
| I.              | Tiny and transparent                                     |
| II.             | White                                                    |
| III.            | Slightly yellow                                          |
| IV.             | Highly vascularized, dark yellow ovaries                 |
| V.              | Ripe and loosened eggs from ovary walls                  |
| VI.             | Shrunkened, flaccid and sac-like ovaries                  |


3.2 Gonadosomatic Index (GSI) for Sexually Mature Samples

Twenty one ovary samples were mature (stages III, IV, V and VI) from fish sampled from the upstream sampling section while seventeen ovary samples were from fish from the downstream sampling site (Appendix III, Fig. 3). The mean monthly overall GSI of ovary samples in stages III, IV, V and VI from fish sampled from the upstream sampling section (2.39 ± 0.064) was slightly lower than the mean monthly GSI of the ovary samples in stages III, IV, V and VI of fish sampled from the downstream sampling section (2.88 ± 0.051). However, there was no statistically significant difference (t=0.82; p >0.05) in these means.

3.3 Levels of Heavy Metals (mg/kg) in the Ovaries of Mature Tilapia from the Upstream and Downstream Sites (Appendix V, Tables 2 and 3 respectively)

The mean level of lead (0.707±0.05) was significantly higher in the fish ovary samples found downstream than those found upstream (t = 3.36; P < 0.05). The mean level of iron (3.87±0.03) was highly significant in the downstream (t=4.92; p<0.01) than in the upstream (Appendix VI, Table 4). There was no significant difference in the mean levels of cadmium, copper and zinc between the upstream and downstream ovary samples (p>0.05).

3.4 Relationship between the Level of Heavy Metals in the Ovaries and the Gonadosomatic Index (GSI) of Mature Tilapia Upstream and Downstream

There was no significant correlation (P > 0.05) between the levels of heavy metals (lead, Cadmium, Copper zinc, iron) and the GSI of the Nile tilapia in samples from both the upstream (Appendix VI, Table 5) and downstream sites (Appendix VI, Table 6).

3.5 Serum Level of 17β-estradiol (E2) (pg/ml) in Mature Tilapia Upstream and Downstream

Twenty one serum samples were taken from mature fish sampled from the upstream sampling section, while seventeen were from the fish sampled from the downstream section. During the month of November 2014, the level of E2 in serum recorded was 127.08 ± 0 pg/ml upstream while in the downstream; the level was 498 ± 155 pg/ml (Appendix III, Fig. 4). There was a drop in the preceding month (December) which recorded 106.35 ± 0 pg/ml in the upstream and 80.36 pg/ml ± 0 pg/ml in the downstream site. This was followed by a drastic rise in serum level E2 (1143 ± 0 pg/ml) in the upstream whereas there was a drop in the downstream section (72.52 ± 0 pg/ml) in January 2015. The months of February and March recorded a sharp drop of the hormone in the upstream section. However, low serum level E2 was recorded from fish sampled in the downstream section during the month of February 2015 (105.494 pg/100 ml) and March (66 ±7.79 pg/ml). The months of April, May and June 2015 recorded a rise in the level of serum E2 in both sampling sites (Appendix III, Fig. 4). The mean level of serum 17β-estradiol (E2) in the upstream was 504.90 ± 187.74 pg/ml while, in the downstream it was 304.08±188.88 pg/ml. However, the two means were not statistically different (t = 1.14; P >0.05).

3.6 Relationship between Levels of Heavy Metals in Ovary Samples and the Level of 17β-estradiol in Mature Tilapia in the Upstream and Downstream

Pearson moment correlation analysis showed that there was no significant correlation between levels of heavy metals (lead, cadmium, copper, zinc and iron) and the levels of E2 of the mature O. niloticus from both the upstream and downstream sampling sites (P > 0.05) (Appendix VII, Table 7) and (Appendix VII, Table 8).

3.7 Fecundity of Oreochromis niloticus

Ripe ova were sampled from eleven ovary samples of fish in stages IV and V fish samples from the upstream sampling section and eight ovary samples in stages IV and V fish samples from the downstream sampling sections. The sampled number per month is represented in the bar graph in Appendix IV, Fig. 5. Fecundity of O. niloticus in the downstream section was 921 eggs per female. Fish from the upstream showed lower fecundity at 603 eggs per female. However, a two sample t-test showed that there was no significant difference in fecundity...
between the downstream and upstream (t= -0.19, p >0.05).

3.8 Relationship between Fecundity and the Level of Heavy Metals (mg/kg) in the Mature Ovary Samples of Nile tilapia from the Upstream and the Downstream

In establishing the relationship between fecundity with the levels of heavy metals in the ovaries of fish samples from the upstream, the result showed that there was no significant relationship between the fecundity with any of the heavy metals (P > 0.05) (Appendix VII, Table 9).

Similarly, in downstream sections, there was no significant relationship between the fecundity and any of the heavy metals in the ovary samples of O. niloticus sampled from the downstream section of the River (P > 0.05) (Appendix VII, Table 10).

4. DISCUSSION

Metals may enter the body of fish through three possible ways: the body surface, the gill, and the alimentary tract [27]. Ovaries are reported to have a tendency of accumulating heavy metals in them [28]. Mean higher levels of lead and iron in the fish ovaries sampled from the downstream site than from the upstream section can be associated with wastes from industrial and urban centers that are closer to the section. Runoff from carwash and petrol stations in Ruiru Town gain access into the river at the downstream section. The levels of lead and cadmium exceeded the recorded permissible level of 0.05 mg/kg for the fish and fish products [29-31] (Appendix VIII, Table 11). This means that consumption of fish from River Ruiru is dangerous to man considering their levels.

Slightly high gonadosomatic index (GSI) in June (upstream) and December (downstream) was due to presence of more eggs in the ovaries in stages III, IV and V. These ovary stages contained ova in stages III, IV and V indicating breeding season [32]. Low GSI recorded during the month of November 2014 in both sampling sites indicated immature (stage II) O. niloticus in terms of developing oocytes [33]. In this study, results show that there was no correlation between the mean GSI and the mean levels of heavy metals both in upstream and downstream of River Ruiru. This could be due to an adaptive response to drastic conditions concerning various heavy metal pollutants in the river [34]. The mean GSI in both sampling sites were similar to the one recoded by [35] but below the one obtained by [11].

There was no significant relationship between the levels of heavy metals and 17β-estradiol in mature Oreochromis niloticus from both sampling sites. This can be attributed to tolerance of fish to the heavy metals [36]. Mature fish tissues and body fluids are reported to contain certain proteins that react with harsh environmental antigens and provide natural immunity to fish [37]. In the upstream, serum levels of 17β-estradiol gradually increased from December to January (peak). Thereafter it dropped in February and March 2015, whereas in the downstream, it dropped in December and almost remained constant in the months of January and February 2015. This may be due to a decline in steroidogenic postovulatory follicles (stage VI). It also suggests that this period corresponds with the major mouth brooding phase of female Oreochromis niloticus [38], though fries were never found in the mouths of stages VI O. niloticus during sampling. Gonadal estradiol levels gradually increased from March to reach a peak in June 2015 in both sampling sites. This is related to a response of the developing ovaries to gonadotrophin hormones, produced during the prespawning (stage IV) and spawning (stage V) time, to secrete 17β-estradiol [39]. It is reported that E2 stimulates the synthesis of yolk lipid in the oocytes and its increase in levels confirms an increase in the immediate pre-spawning activity. It also reflects a continuous maturing (stages III and IV) of females to prepare for the following spawning cycle [40]. The initial estradiol peak observed in January 2015 in female O. niloticus in the upstream section may result in the oocytes being maintained through a protective effect. This protection prevents the oocytes from becoming atretic [37]. The second estradiol peak in June 2015 could be due to response to rapid vitellogenic growth phase in the stages IV oocytes [41].

Based to the results of this study, there was no correlation between mean levels of heavy metals and fecundity of fish from both sampling sites. This could be due to adaptability of tilapia to heavy metals. Fish is reported to have Kupffer cells, responsible for detoxification and elimination of toxic ions [42]. Fecundity recorded in this study was lower than in other similar water
systems, which could be due to other factors such as environmental factors and body size [43,44].

5. CONCLUSION

Based on the results obtained in this study, there was no significant correlation between the levels of heavy metals (lead, Cadmium, Copper, zinc, and iron) and both gonadosomatic index and levels of 17β-estradiol of the O. niloticus from both upstream and downstream sampling sites. There was no correlation between the levels of the heavy metals and fecundity in both sampling sites. This implies that there is no relationship between heavy metals and the selected reproductive parameters in Nile tilapia, along River Ruiru.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX I

Fig. 1. Map of Ruiru Sub-county showing the location of the sampling sites
(Source: Kiambu Topo Map and Kiambu County Government, 2010) [45]

APPENDIX II

Fig. 2. Standard curve for 17β-estradiol
APPENDIX III

Fig. 3. Monthly means of GSI for mature Oreochromis niloticus from the upstream and downstream sections of River Ruiru

Fig. 4. Mean monthly level of 17β-estradiol (pg/ml) in mature tilapia sampled from upstream and downstream sections of River Ruiru
APPENDIX IV

Fig. 5. Mean monthly ripe ova in the upstream and downstream sections

APPENDIX V

Table 2. Levels of heavy metals (mg/kg) in the ovaries in the mature tilapia sampled from the upstream section

| Month      | Lead  | Cadmium | Copper   | Zinc   | Iron |
|------------|-------|---------|----------|--------|------|
| November 2014 | 0.67  | 0.285   | 0.65     | 0.65   | 1.3  |
| December 2014 | 0.89± 0.06 | 0.25± 0.04 | 1.33 ±0.48 | 1.08± 0.21 | 2.35± 0.35 |
| January 2015  | 0.42 ±0.22 | 0.15±0.06 | 1.70±0.20 | 1.17± 0.45 | 3.35± 0.65 |
| February 15   | 0.37± 0.13 | 0.44±0.19 | 0.70±0.37 | 0.52± 0.26 | 1.52± 0.51 |
| March 2015    | 0.37± 0.16 | 0.19±0.13 | 1.71±0.68 | 0.70± 0.36 | 1.50± 0.52 |
| April 2015    | 0.49± 0.07 | 0.26±0.11 | 1.97±0.58 | 1.03± 0.21 | 1.53± 0.96 |
| May 2015      | 0.31± 0.17 | 0.20±0.07 | 2.15±0.12 | 0.83±0.19 | 1.93± 0.68 |
| June 2015     | 0.1    | 0.099   | 1.9      | 1.2    | 1.3  |
Table 3. Monthly mean levels of heavy metals (mg/kg) in the ovaries from the mature tilapia sampled from the downstream

| Month          | Lead   | Cadmium | Copper | Zinc     | Iron    |
|----------------|--------|---------|--------|----------|---------|
| November 2014  | 0.74±0.09 | 0.19±0.09 | 1.45±0.45 | 0.71±0.26 | 4.65±0.35 |
| December 2014  | 0.25   | 0.275   | 2      | 0.88     | 1.3     |
| January 2015   | 0.25   | 0.21    | 0.35   | 0.88     | 1       |
| February 2015  | 0.83   | 0.1     | 1.9    | 0.88     | 3       |
| March 2015     | 0.83±0.00 | 0.35±0.06 | 0.50±0.00 | 0.59±0.37 | 4.85±0.15 |
| April 2015     | 0.80 0.03 | 0.31±0.03 | 2.35±0.25 | 0.76±0.20 | 4.85±0.15 |
| May 2015       | 0.75±0.04 | 0.41±0.02 | 1.92±0.12 | 1.23±0.29 | 4.35±0.30 |
| June 2015      | 0.76±0.07 | 0.41±0.00 | 1.95±0.05 | 0.83±0.22 | 3.33±0.00 |

APPENDIX VI

Table 4. Comparison of the mean levels (mg/kg) of heavy metals in the ovaries of sexually mature tilapia from upstream and downstream

|          | Lead    | Cadmium | Copper | Zinc     | Iron    |
|----------|---------|---------|--------|----------|---------|
| Downstream | 0.707±0.051 | 0.318±0.029 | 1.627±0.18 | 0.887±0.11 | 3.87±0.34 |
| Upstream  | 0.433±0.064 | 0.252±0.049 | 1.560±0.19 | 0.839±0.10 | 1.83±0.25 |
| t-value   | 3.36    | 1.15    | 0.25   | 0.32     | 4.92    |
| P-value   | 0.002*  | 0.258   | 0.751  | 0.001*   |

Table 5. Relationship between the level of heavy metals in the ovaries and the gonadosomatic index (GSI) of mature tilapia upstream

| Heavy metals | r-values | p-values |
|--------------|----------|----------|
| Lead         | 0.317    | 0.445    |
| Cadmium      | 0.113    | 0.789    |
| Copper       | -0.243   | 0.562    |
| Zinc         | 0.229    | 0.585    |
| Iron         | 0.270    | 0.518    |

Table 6. The relationship between the level of heavy metals in the ovaries and the gonadosomatic index (GSI) of mature tilapia downstream

| Heavy metals | p-values | r-values |
|--------------|----------|----------|
| Cadmium      | -0.248   | 0.145    |
| Lead         | 0.282    | 0.096    |
| Copper       | 0.111    | 0.519    |
| Zinc         | 0.178    | 0.298    |
| Iron         | 0.222    | 0.192    |

APPENDIX VII

Table 7. Relationship between the level of heavy metals in ovary samples and the level of 17β-estradiol in mature tilapia in the upstream site

| Metals      | r-value | p-value |
|--------------|---------|---------|
| Lead        | -0.577  | 0.175   |
| Cadmium     | 0.415   | 0.355   |
| Copper      | 0.302   | 0.510   |
| Zinc        | 0.421   | 0.347   |
| Iron        | 0.441   | 0.322   |
Table 8. Relationship between level of heavy metals in ovary samples and the level of 17β-estradiol in mature tilapia in the downstream

| Metals  | r-value | p-value |
|---------|---------|---------|
| Lead    | 0.412   | 0.359   |
| Cadmium | 0.420   | 0.348   |
| Copper  | 0.437   | 0.446   |
| Zinc    | 0.232   | 0.617   |
| Iron    | 0.222   | 0.632   |

Table 9. Relationship between fecundity and the level of heavy metals (mg/kg) in the ovaries of mature tilapia from the upstream River Ruiru

| Heavy metals | r-values | p-values |
|--------------|----------|----------|
| Lead         | -0.306   | 0.556    |
| Cadmium      | 0.329    | 0.525    |
| Copper       | 0.654    | 0.159    |
| Zinc         | 0.052    | 0.922    |
| Iron         | -0.198   | 0.707    |

Table 10. Relationship between fecundity and level of heavy metals (mg/kg) in the ovaries of mature tilapia from the downstream River Ruiru

| Heavy metals | r-values | p-values |
|--------------|----------|----------|
| Lead         | -0.542   | 0.345    |
| Cadmium      | -0.059   | 0.925    |
| Copper       | 0.763    | 0.133    |
| Zinc         | -0.173   | 0.780    |
| Iron         | -0.419   | 0.483    |

APPENDIX VIII

Table 11. Maximum allowable concentration of selected water quality variables in drinking water and fish, by various organizations

| Variable | In Drinking Water (mg/l) | In Fish (mg/kg) |
|----------|--------------------------|-----------------|
|          | EU (1998) | USEPA (2006) | WHO (2008) | FAO/WHO (1989) | FAO 2008 |
| Pb       | 0.01 mg/l | 0.015      | 0.01      | 0.0005       | -        |
| Cd       | 0.005     | 0.005      | 0.003     | 0.0005       | -        |
| Cu       | 2.0       | 0.03       | 2.0       | 0.03         | 0.03     |
| Zn       | NG        | 5          | NM        | 0.04         | 0.04     |
| Fe       | 0.2       | 0.3        | 0.3       | -            | 0.1      |
| EC (µS/cm)| 2500µS/cm | NM         | 250µS/cm  | -            | -        |
| PH       | 6.5 – 9.5 | 6.5 – 8.5  | 6.5 – 8.5 | -            | -        |

NM: Not mentioned; NG: No guidelines

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