

Original Research Article

Use of fish somatic indices to assess pollutant exposure and effects: A case of two urban rivers and a wastewater sedimentation ponds in Lake Victoria Basin, Kenya

ABSTRACT

Aquatic ecosystem health assessment is critical for early detection of disturbances and water habitat degradation. This study assessed the ecological status of two urban rivers as well wastewater sedimentation ponds in Kisumu City, Kenya using physico-chemical water quality parameters and African catfish, *Clarias gariepinus* somatic indices. Site association of somatic indices was derived from principal component analysis (PCA) whereas the relationship between water quality parameters was examined by redundancy ordination analysis (RDA). PCA results revealed an increase in gill somatic index (GSI) in the wastewater sedimentation ponds while Fulton's condition factor (CF) increased in the midstream of Auji river and Kisat river mouth. Similarly, RDA showed that dissolved oxygen, temperature, total phosphorus, alkalinity and total nitrogen influenced the condition indices at upstream of Kisat river, up-stream and midstream of Auji river. However more impact on somatic indices were recorded in the wastewater sedimentation ponds. Although specific pollutants other than the physico-chemical parameters were not identified, application of somatic indices in *C. gariepinus* demonstrated that the two rivers and wastewater sedimentation ponds were contaminated by pollutants that can compromise the ecological health of the aquatic systems. The results of this study emphasized the negative impacts of anthropogenic activities on the environment.

Keywords: Ecological status, Clarias gariepinus, somatic indices, anthropogenic activities, multivariate analysis

1. INTRODUCTION.

Freshwater systems are major receivers of pollutants which, over time, can have severe effects on the biota that might not become clear until changes occur either at the population or ecosystem levels [1]. In Kenya, the problems of rivers draining urban areas have extremely increased in the past years [2] where discharge of partially treated or untreated domestic, municipal, agricultural and industrial wastewaters deteriorate their water quality [3].

A great deal of effort has gone towards selecting the best biomonitor and/or indicator organisms [4]. It is well recognized that organisms differ greatly in their sensitivity to diverse pollutants, and that no single species or monitoring procedure is subtlest or best appropriate to detect all possible toxic pollutant [5]. At organismal level, fish are largely used as sentinel species for biological assessment to quantify ecological alterations caused by the

amalgamation of physical, chemical and biological stressors [6] because they have some exclusive features and advantages as indicators of freshwater ecosystem health [7].

Despite some restraints associated with mobility, fish are considered to be the most useful organisms for biomonitoring of environmental pollution [8] because they are located at the top of the food chain, are highly visible and are known to accumulate toxicants [7]. In addition, they are in direct contact with pollutants in the water via their gills and their body surface. Fish are excellent indicators of aquatic health because they live in water all their life, differ in their tolerance to amount and type of pollution, are easy to collect with the right equipment, live for several years, are easy to identify in the field, represent a broad spectrum of community tolerance from very sensitive to highly tolerant and respond to chemical, physical and biological degradation in characteristic response pattern [9]. Thus, fish can be used as a “warning system” to signal the occurrence of pollutants in natural waters [1a, 10]. Prior to mortality or clear sickness symptoms, fish may react to stress by changing physiological, behavioral and molecular responses.

Many studies have shown that fish condition factor and somatic indices can be employed in fish health and population assessment as first level screen to identify and provide satisfactory information on the physiological response of fish to environmental stressors and presence of contaminants [11]. Somatic indices typically express organ weight as a percentage of total body weight. These indices show the condition of organ systems, which may alter in size because of environmental factors and stressors.

Somatic indices are helpful markers of overall organ and fish health; however, their interpretation requires utmost care because they are not sensitive or specific and may be impacted by non-pollutant factors [8]. Somatic indices are used as initial screening biomarker to indicate exposure and effects [12].

For better management of freshwater systems, comprehensive assessment of water quality as well as determining the biological end points in aquatic biota is crucial for well-informed strategies aimed at maintaining ecological well-being and safeguarding both aquatic and human health. The aim of this study was therefore to examine the condition factor and somatic indices of *C. gariepinus* as aquatic pollution biomarkers in assessing fish health and environmental quality of rivers Kisat and Auji in Kisumu County, Kenya.

2. MATERIAL AND METHODS

2.1 Study area

This study focused on water quality and somatic indices of African catfish in two river systems, Kisat and Auji as well as a wastewater sedimentation ponds (figure 1). As these two rivers flow into Lake Victoria, they pass through agricultural farms, heavily polluted low dwelling centers (Obunga, carwash, Nyalenda and Nanga) which lack proper sanitation facilities thus streams of domestic wastewater and sewage is introduced into the rivers at various points. Immediately after the informal settlement areas, Kisat river flows all the way through the Kisumu industrial area where various industries such as motor vehicle garages, fish, soap processing factories, salt works and stores for an array of items, including xenobiotics among other are located [13,14]. There is no existence of effluents treatment facilities for the aforementioned industries thus leaving them to discharge their effluents either through the sewerage systems or directly into the river. The main conventional sewerage treatment plant (CSTP) in Kisumu City is located at the tail end of River Kisat, while another wastewater treatment facility (wastewater sedimentation ponds (WWSP) is located at the eastern part of the city adjacent to Nyalenda slums and discharges partially treated effluents into River Auji. At the river mouth of both the rivers are recreational centers, golf club and Hippo point for River Kisat and Auji respectively that discharges their wastes

directly into the rivers. Therefore, biomonitoring of the health of these riverine ecosystems is one of the most important ways to ensure their sustainability and proper management.

GPS Location of Sampled Points

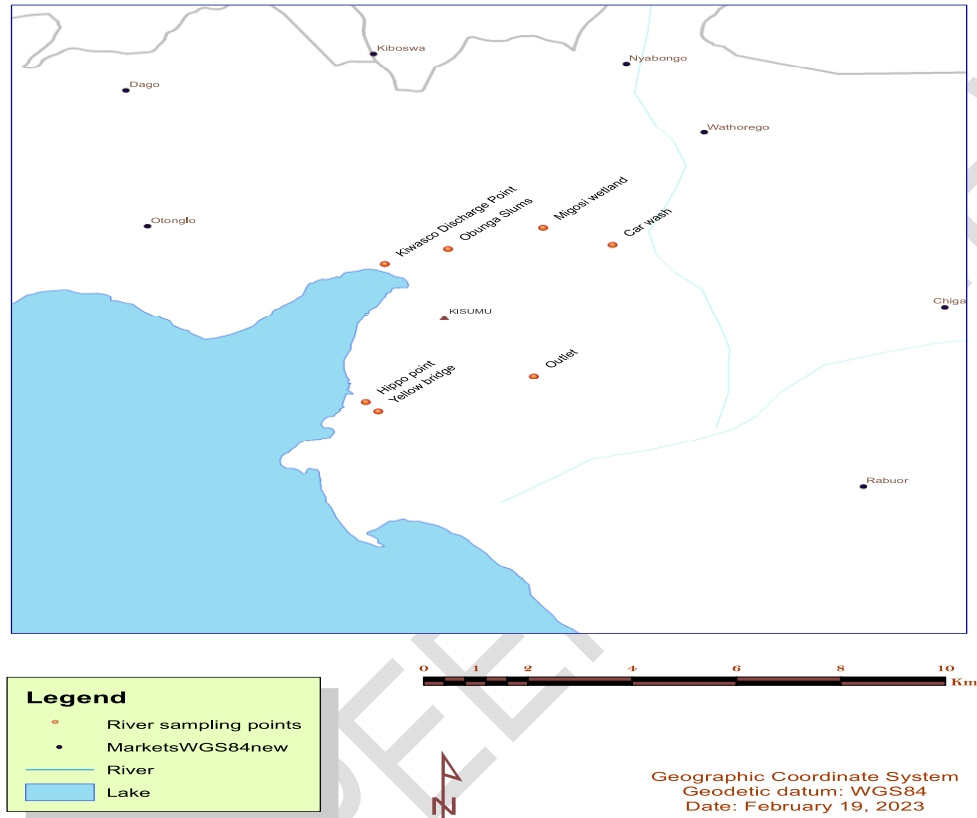


Figure 1. Location of sampling points in river Kisat, Auji and Nyalenda wastewater sedimentation ponds, Lake Victoria Basin, Kenya

2.2 Choice of fish species for studies

Clarias gariepinus (hereafter the African catfish), was chosen as the ideal species for this study because it exhibits hardiness and can therefore be found even in the most polluted waters. Due to their tolerance to pollutants and their tendency to develop toxicopathic gill, kidney and liver lesions, catfish have been broadly used for ecotoxicological studies [15]. Consequently, this makes it a good candidate for studies on never-ending environmental pressure, since it can reflect both past and recent cumulative effect of environmental quality changes. Catfish is probably the most widely distributed fish species in Kenya, inhabiting swamps, streams and rivers. It is tolerant to low oxygen concentrations and can withstand desiccation due to the possession of accessory air breathing organ (pseudo branch). It is omnivorous, utilizing various kind of food resources available in their habitat. However, the application of its somatic indices as an indicator for ecological and fish health in Kenyan aquatic system are rare (most of the studies on aquatic monitoring have focused majorly on micro- and macro- invertebrates), it makes for an ideal test organism with which to conduct pollutant exposure and effects response investigations for studies described herein because of its availability and apex predator status.

2.3 Measurements of physico-chemical parameters

Water samples were collected and analyzed monthly during February 2021 to October 2021 from eight sampling sites (Figure 1). Ten physico-chemical parameters were determined. These included: temperature, dissolved oxygen, electrical conductivity, total dissolved solids, pH, turbidity, alkalinity, hardness, total nitrogen and total phosphorus. In situ measurements of water temperature, dissolved oxygen (DO), conductivity, total dissolved solids (TDS), salinity, pH and turbidity were performed using portable YSI Professional Plus multi – parameter instrument (YSI, 35C). Water samples for nutrient analysis were collected in 1 litre polyethylene sample bottles pre-cleaned with (double distilled water). The water was directly drawn from the two rivers and the wastewater sedimentation pond (WWSP) and put in bottles that were then labeled and later stored in cooler boxes at temperature of 4°C to await further laboratory analysis. The total nitrates (TN) and total phosphorus (TP) concentration, total alkalinity and total hardness were determined using standard methods according to [16].

2.4 Fish sampling and organ collection for somatic index determination

Sampling was done between 07.00 am to 11.00 am to minimize the effect of abrasion and all landed fish were directly transferred to oxygenated water tanks. However, for the determination of somatic indices, only live fish with usual opercula tendencies such as intact central cartilaginous core were used. The fish were physically restrained, total length (cm) and whole-body wet weight (g) measured after which each was killed by cervical dislocation and pithing, and organs (gills, kidney and liver) removed and blotted dry with a tissue paper. The weight of each organ was taken using an electric weighing balance (Scanvaegt salter, model 323; A very Weigh – Tronk Ltd, West Midland, UK).

2.5 Determination of Condition Factor and Somatic Indices

2.5.1 Determination of Condition Factor

Fulton's condition factor (CF) that relate weight and length to an indicator of the "condition" [17], "well-being" or "fitness" of fish [18] for each fish was calculated using the formula:

$$CF = 100 \times \frac{W}{L^3} \dots \dots \dots \text{Equation 1}$$

Where W and L = body weight (g) and total length in cm, respectively [19].

2.5.2 Determination of somatic indices

The somatic index of each organ was calculated according to [20] using the formula.

$$\text{Somatic index \%} = \frac{\text{Weight of organ (g)}}{\text{Weight of fish (g)}} \times 100 \dots \dots \dots \text{Equation 2}$$

Data were tested for normality and homogeneity of variance using Levene's tests. The variations in physico-chemical parameters and somatic indices were tested by one way analysis of variance (ANOVA), considering sites as variable. Whenever the ANOVA revealed significance differences Tukey's post hoc multiple comparison between sites was done to determine which site differed significantly and where the statistical demand of normality was not met, non-parametric test (Kruskal- Wallis) was used to test for the significance. The significance of the results was ascertained at $p < 0.05$. To determine site association of somatic indices, Principal component analysis (PCA) was computed. Redundancy ordination analysis (RDA) [21] was used to assess how much response variable (somatic indices) were

explained by the variation in environmental variables (physico-chemical parameters). All statistical analyses were done using statistical software PRIMER 6.

3. RESULTS

3.1 Physico-chemical parameters

The water quality parameters measured at each sampling site on River Kisat and Auji as well as wastewater sedimentation ponds are summarized in Table 1. The study demonstrated substantial changes in the parameters as the rivers flowed to Lake Victoria. During the sampling period, temperature varied accordingly with highest temperature recorded in river Auji and the lowest in River Kisat. The lowest value 22.20 ± 0.01 °C was obtained upstream of river Kisat while the highest 27.69 ± 2.76 °C was recorded at the midstream of river Auji. The levels of temperature between sites exhibited no significance differences. The lowest dissolved oxygen (DO) level of 1.63 ± 0.70 mg/l was recorded at pre-treatment pond while the highest value of 8.07 ± 0.24 mg/l was recorded upstream of river Kisat. The mean levels of DO between sites showed significance differences ($P = 0.05$). The electrical conductivity was lowest 329.51 ± 29.94 μ S/cm upstream of River Kisat. The highest electrical conductivity of 837.07 ± 39.59 μ S/cm was observed at pre-treatment pond of the WWSP. Significance differences in electrical conductivity was observed between the sites ($P = 0.05$). The lowest recorded value of TDS 245.55 ± 76.76 mg/l was recorded at Auji river mouth while the highest value of 526.01 ± 36.51 mg/L was observed at pre-treatment pond of the WWSP. TDS displayed significance difference between sites ($P = 0.05$). The levels of salinity were significantly higher ($P = 0.05$) at the pre-treatment sampling site than any sampling site during the study period. The lowest pH levels 3.88 ± 1.46 during the sampling period were recorded at Auji post-treatment pond, while the highest level of 7.85 ± 0.48 was recorded upstream of river Kisat with most sampling stations having a near neutral pH, which falls within the ranges of 6.5 to 8.5 levels for natural water bodies recommended by the European Union. However, pH levels between the sites showed no significance differences. The highest value of turbidity 222.17 ± 310.77 NTU was recorded at Kisat mid-stream and lowest value 43.2 ± 45.16 NTU was recorded at upstream of River Kisat. The turbidity levels displayed significance difference between the sites ($P = 0.05$). Total nitrogen (TN) and total phosphorus (TP) increased downstream of river Kisat. TN ranged from 218.12 μ g/L to 1622.97 μ g/L, whereas the TP ranged from 89.98 μ g/L to 1088.98 μ g/L. On the other hand, TN and TP decreased downstream of river Auji. TN value of 1614.10 μ g/L was obtained upstream of river Auji while 767.66 μ g/L was recorded at the river mouth. The highest level of TP of 966.94 μ g/L was obtained upstream of river Auji and the lowest value (542.29 μ g/L) was recorded downstream. TN value of 3451.41 μ g/L was obtained at the pre-treatment of the wastewater sedimentation pond while a reduction in TN value, 1445.55 ± 365.71 was obtained at the post-treatment pond. The TP values increased from 1876.64 ± 434.09 μ g/L at the pre-treatment pond to 2201.80 ± 748.46 μ g/L at the post-treatment pond respectively. While TN levels showed significance between the sampling sites ($P = 0.05$) there was no significance difference in the mean values of TP.

Table 1: Mean values +SD for physico-chemical parameters for rivers Kisat and Auji as well as Nyalenda wastewater sedimentation ponds

Physico-chemical Parameter	River Kisat			River Auji			Nyalenda WWSPs	
	Upstream	Midstream	River mouth	Upstream	Midstream	River mouth	Pre-treatment	Post-treatment
Temp (°C)	22.20± 0.01	26.65± 3.10	26.85± 2.48	25.90± 1.40	27.69± 2.76	25.90± 1.24	26.15± 0.43	26.35± 2.11
DO (mg/L)	8.07± 0.24	3.95± 1.21	3.08± 1.13	3.78± 1.05	4.13± 0.59	5.33± 0.47	1.63± 0.70	4.87± 0.94
EC (µS/cm)	329.51± 29.94	421.73±157.9	497.83± 209.19	422.67± 110.67	476.01± 102.42	364.07± 127.17	837.07± 39.59	614.61± 77.13
TDS (mg/L)	272.04±94.4	283.01± 15.56	345.97± 110.90	291.26± 59.73	307.62± 38.72	245.55± 76.76	526.01± 36.51	388.14± 78.49
Sal (PSU)	0.10± 0.03	0.18± 0.08	0.20± 0.05	0.20± 0.05	0.21± 0.04	0.16± 0.06	0.40± 0.02	0.30± 0.04

pH	7.85± 0.48	7.04± 0.79	6.50± 0.39	7.19± 0.60	7.03± 0.71	7.73± 0.34	3.88± 1.46	6.65± 0.42
Turb (NTU)	43.2± 5.16	222.17± 10.77	116.47± 4.38	190.16± 7.03	89.62± 5.74	55.60± 1.79	194.87± 7.89	183.73± 4.90
Alk (mg/L)	95.0± 17.40	113.14±24.19	100.0±(47.93)	114.86±31.2 6	103.14±26.3 3	110.29±41.1 4	103.50±14.6 4	108.0± 42.24
Hard (mg/L)	94.0± 5.29	93.43± 19.62	76.29± (30.03)	83.14± 17.16	79.71± 17.26	86.85± 19.32	92.50± 36.64	80.57± 28.09
TN (µg/L)	218.12± 80.54	1003.07±302. 6	1622.97± 464.6	1614.10± 673.5	1348.26± 558.60	767.66± 211.40	3451.41± 585.80	1445.55± 365.71
TP ((µg/L)	89.98± 54.35	492.37±234.5 1	1088.98± 438.4	966.94± 358.97	756.94± 345.41	542.29± 225.02	1876.64± 434.09	2201.80± 748.46

Temp, DO, EC, TDS, Sal, Turb, Alk, Hard, TN and TP refer to Temperature (⁰C), Dissolved oxygen (mg/L), Electrical conductivity (µS/cm), Total dissolved solids (mg/L), Salinity (PSU), Turbidity (NTU), Alkalinity (mg/L), Hardness (mg/L) Total nitrates (µg/L) and Total Phosphorus (µg/L)

UNDER PEER REVIEW

3.2 Condition Factor and somatic indices

The Fulton's condition factor of *Clarias gariepinus* from the respective sampling sites is shown in Table 2. Mean CF values were highest for fish samples collected midstream of River Auji and lowest at pre-treatment pond. Significance differences between the sites were observed for Fulton's condition factor ($P= 0.05$)

3.2.1 Gill somatic Index

The gill somatic index (GSI) showed ranges from 0.69 to 6.63 during the sampling period. The highest mean value was obtained from fish sample at post-treatment pond while the lowest mean value was recorded for fish samples collected from Kisat river mouth (Table 2). The percentage GSI showed significance differences between the sites ($P = 0.05$)

3.2.2 Kidney somatic index

The mean values of kidney somatic index (KSI) are presented in Table 2. In the fish samples, the lowest mean KSI value 0.53 (0.48) was recorded in fish sampled midstream of Auji River. The highest mean value of 1.81 was recorded at post-treatment pond. There was no significance difference observed in KSI between the sites.

3.2.3 Liver somatic index

The mean values of liver somatic index (LSI) calculated at each sampling site are summarized in Table 2. During the sampling period, post-treatment and pre-treatment sedimentation ponds recorded the highest mean value LSI 3.64 (0.70) and 3.43(0.42) respectively. The lowest mean LSI value 0.79 (0.38) was observed in fish sampled at station Hippo point (Auji river mouth). There were no significance differences observed in LSI for fish samples between the sites.

Table 2: Mean percentage value + SD for somatic indices of *C. gariepinus* from rivers Kisat and Auji as well as Nyalenda WWSPs

Rivers	Sampling Sites	Somatic Indices			
		CF	GSI	KSI	LSI
Kisat	Upstream	0.69± 0.17	1.51± 1.12	0.93± 0.38	1.39± 0.33
	Midstream	0.65± 0.17	1.60± 1.70	1.05± 0.98	1.86± 0.78
	River Mouth	0.96 ±0.38	0.69± 0.52	0.72± 0.54	1.63± 1.40
Auji	Upstream	0.67± 0.15	2.24± 1.86	1.02± 0.28	1.75± 0.76
	Midstream	1.31± 0.44	1.13± 0.59	0.53± 0.48	0.93 ±0.66
	River Mouth	0.71± 0.12	1.34± 1.81	0.58± 0.20	0.79± 0.38
Nyalenda WWSP	Pre-treatment	0.23± 0.05	3.05± 1.13	1.39± 0.36	3.43± 0.42
	Post-treatment	0.24± 0.03	6.63± 2.48	1.81± 0.57	3.64± 0.70

KF, GSI, KSI and LSI refer to Fulton's condition factor, gill somatic index, kidney somatic index and liver somatic index

3.3 Principal Component Analysis

Principal component analysis performed on the somatic indices accounted for 89.4% of the variability Figure 2. The analysis showed GSI increasing towards pre-treatment and post-treatment sedimentation ponds. On the other hand, condition factor increasing towards River Auji midstream and Kিসাত river mouth.

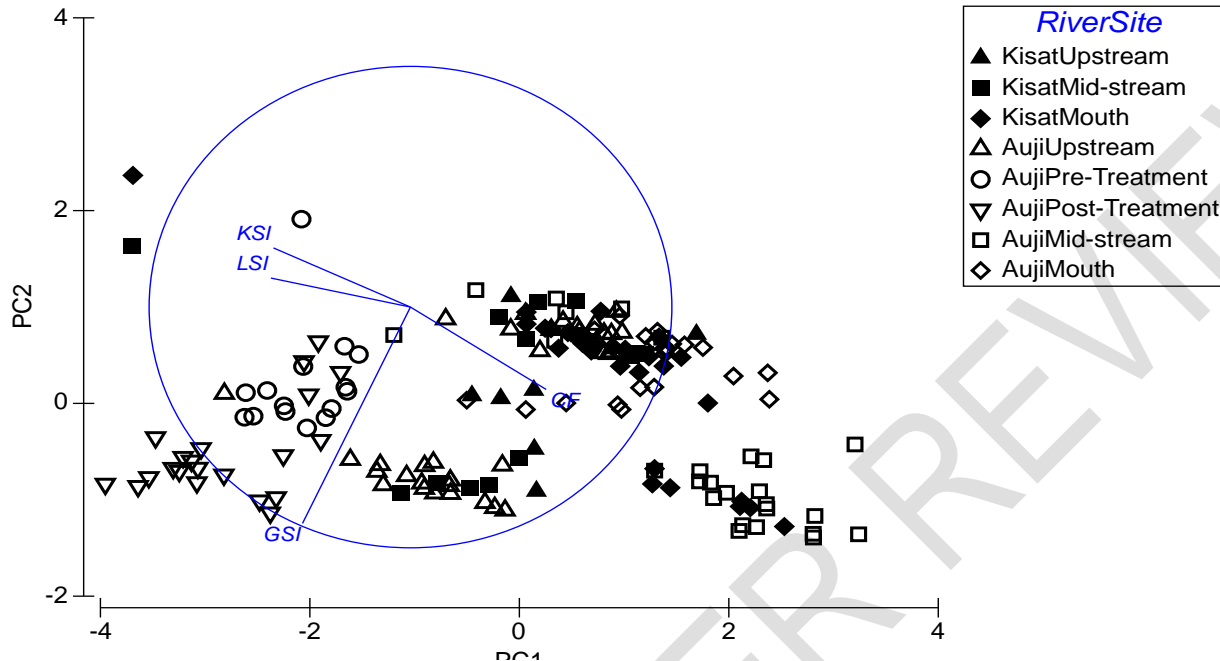


Figure 2. Principal component analysis showing the relationship between the somatic indices and the sampling sites.

3.4 Relationship between the condition factor, somatic indices and the water quality parameters

The results of the relationship between the somatic indices and the water quality parameters are shown in Figure 3. Dissolved oxygen, temperature, total phosphorus, alkalinity and total nitrates impacted the somatic indices at river Kিসাত upstream, Auji river upstream and midstream. However, more impact on the somatic indices was recorded in Nyalenda WWSP.

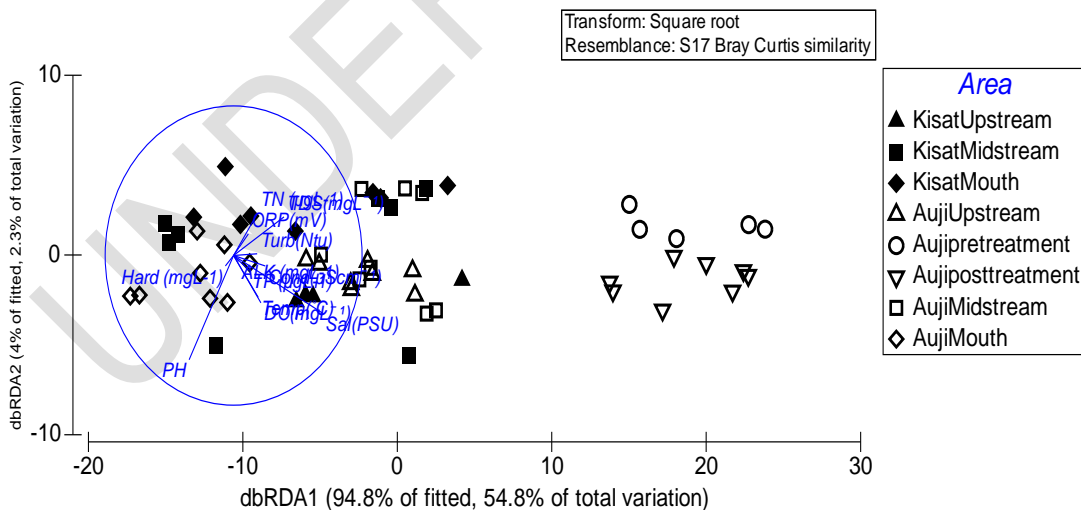


Figure 3. Redundancy ordination analysis showing the relationship between somatic indices and the water quality parameters.

4.0 Discussion

In the present study, we set out to apply Fulton's condition factor and fish somatic indices to assess the ecological health of urban rivers in the Lake Victoria Basin (LVB) of Kenya.

4.1 Variation of physico-chemical water characteristics in rivers and the wastewater sedimentation ponds.

Variation in physico-chemical water quality parameters in rivers Kisat and Auji as well as the wastewater sedimentation ponds strongly suggest that pollution is the primary factor contributing to the deterioration of water quality. The undesirable impact of diverse sources of pollutants discharged into these rivers was further confirmed by the highest values in all water quality parameters with an associated decrease in dissolved oxygen.

In an established system, water temperature controls the chemical and physical characteristics of water [22] as well as upsetting fish immunity, growth and reproduction. As expected, the highest levels of temperature were obtained midstream of rivers Kisat, Auji, Kisat river mouth as well as the wastewater treatment ponds attributable to direct heating of the open waters by solar radiation since the sampling sites had very little vegetation [14]. Furthermore, the higher temperature observed in the sampling sites along River Kisat i.e., midstream and river mouth could be due to high turbidity of the water which absorbs and retain solar energy and also from thermal heat from the factories just before the sampling sites. This is supported by the results of the present study where TDS and turbidity were relatively high in Kisat river mouth and midstream sampling sites.

Overall, low dissolved oxygen was recorded in all the sampling sites apart from upstream of river Kisat and this might have been due to the decomposition of suspended organic matter in these sampling sites. The low dissolved oxygen levels observed could also be attributable to the relative rise in temperature which might have affected the retention ability of the water [23]. A rise in temperature affects concentration of oxygen in water column [24] as evidenced in sampling sites where dissolved oxygen is inversely proportional to temperature levels. The discharge of excess organic matter particularly in sampling sites midstream and Kisat river mouth, upstream of River Auji and wastewater sedimentation ponds might have contributed to the depletion of oxygen in these sites [25]. Protracted exposure of fish to low dissolved oxygen levels ($< 5\text{-}6\text{ mg/L}$) has been documented to increase the vulnerability of fish to other stressors and has a calamitous consequence for the existence of fish and other aquatic animals [26]. A reduction in dissolved oxygen levels provokes physiological regulatory mechanisms involved in the maintenance of oxygen gradient from water to tissues which is vital to maintaining metabolic aerobic pathways [24].

The high electrical conductivity (EC) observed in wastewater treatment plants, Kisat river mouth, midstream of Auji river as well as midstream of Kisat and Auji rivers may be attributed to the run-off from the catchment and strongly links industrial and sewage sources. This concurs with a report of conductivity being a direct indicator of anthropogenic impact [27].

The measurements of total dissolved solids (TDS) incorporate all anions and cations in the sample and some ions, or a combination of ions are considerably more poisonous than other ions or a mixture of ions. A species could be more sensitive to TDS toxicity at certain life stages, as many fish are during fertilization [28]. In the present study, relatively high TDS was reported at both pre-treatment and post-treatment of the WWSP as well as Kisat river mouth. This is attributable to municipal and industrial effluents discharges into these systems. TDS can cause toxicity in aquatic environment through variations in ionic composition of water, individual ion and surge in salinity.

The highest salinity levels were recorded at the pre-treatment sampling site, while the lowest was recorded upstream of River Kisat. Salts are highly used in the food and chemical industries; therefore, the discharge from chemical and food industries within Kisumu municipality into the wastewater sedimentations ponds could have been the reason for the high salinity wastewater recorded. and may result in high salinity as reported in wastewater. A high salt concentration could lead to exclusion of less – tolerant species, cause acute or chronic effects at specific life stages in an organism, cause a change in biotic communities, and limit biodiversity [29]. Moreover, high salinity may lead to higher osmotic pressure hence cause water loss from bacterial cells and lower enzymatic activities leading to reduction in breakdown of organic pollutants [30]. Salt stress can hinder many enzymes of aquatic organisms and influence cellular activities [31]. [29] noted that salinity and aquatic biodiversity are inversely correlated in freshwater ecosystem.

pH is undoubtedly by far the most critical parameter governing the behaviour of other water quality parameters as well as pollutant concentrations in the aquatic environment [32,33]. The relatively low pH observed in wastewater sedimentation ponds could be attributed to the large amounts of different pollutants discharged in it. pH has immense effect on water quality influencing the capability of bacteria which require slightly acidic pH to degrade toxic materials to less harmful forms [24]. Generally, very high or very low pH can make water unsuitable for certain uses. At very high pH, chemicals such as ammonia become poisonous to aquatic life and in alkaline condition, water tend to have unpleasant smell and taste [34]. At low pH, solubility of pollutants tends to be high, chemicals like sulphide and cyanide become more lethal [33].

Therefore, pollutants in water with low pH tend to be more toxic since they become more soluble and bioavailable [35]. Therefore, the determination of pH could serve as a sensitive indicator for water pollution. Turbidity is an extremely useful indicator that can yield valuable information quickly, relatively cheaply and on an ongoing basis. Measurement of turbidity is applicable in a variety of settings from low – resource small systems all the way to large and sophisticated water treatment plants. The lowest turbidity was recorded upstream of River Kisat while the highest turbidity was recorded midstream of the same river. The wetland vegetations near the upstream sampling site acted as a filter, removing most of the sediments before they could be deposited into the river, which is why the recorded turbidity was the lowest. The cumulative effects of re-suspension of sediments conveyed by the river flow from Obunga informal settlement and the associated activities, such as the deposition of domestic wastes and industrial effluents into the river, may have caused the highest turbidity measured during this study period. High turbidity in the water samples is an indication of pollution and usually due to direct discharge of wastewater into the river. The results of turbidity reported in this study were lower than [2] reported but somewhat comparable to what [14] reported in River Kisat. Furthermore, the levels reported exceeded WHO [36] permissible limit of 25 NTU for surface freshwater bodies. The levels were also higher than 5 NTU recommended by National Environment Management Authority, Kenya. The high turbidity observed indicates that the rivers and the WWSP contain some dissolved substances that can pose problems to aquatic biota [37].

Although nutrients such as nitrogen and phosphorus are essential for supporting river ecosystem, excessive nutrients can cause diverse deleterious effects. Variation in nutrient levels in surface water systems are attributed to domestic and industrial inputs, municipal and domestic sewage discharges, agricultural runoff, rainfall frequency, atmospheric sources, leachate from refuse dumps and vegetation types and size of the catchment [38]. In the present study, an increase in concentration of TN was obtained in sampling stations downstream River Kisat compared to the upstream sampling sites suggesting possible eutrophication of Lake Victoria. High levels of TN in midstream of river Kisat, Kisat river mouth, upstream and midstream of river Auji sampling sites may be attributed to agricultural runoff from agricultural farms in the catchment in which synthetic fertilizers are applied to improve crop productivity, domestic effluents which constitute biodegradable household wastes such as vegetable and animal matter dumped in the rivers and along the riverbanks which is later washed off into the river. The lowest mean values of TN recorded upstream of river Kisat and Auji river mouth sampling sites can be explained by minimal agricultural activities as opposed to domestic, municipal and industrial discharges that have greater loads of nutrients and sequestration by the wetland plants covering an expansive part of the sampling sites [2, 14]. Consequently, the high mean levels of TN obtained at pre-treatment sampling site was mainly due to the discharge of municipal and domestic wastewater rich in nutrients. The low levels of TN recorded at post-treatment sampling site were expected since WWSP are effective in removing organic pollutants.

Phosphorous that enter the aquatic environment through anthropogenic sources such as fertilizers run-off and domestic wastewater inflow could be incorporated into either organic or inorganic components. Once phosphorous accumulate within an aquatic system, it can cycle through its water column and promote algal bloom indefinitely [39]. In the current study, the levels of TP increased downstream river Kisat with the highest concentration recorded at Kisat river mouth. This is attributable to the industrial discharges especially from the nearby soap industries as well as cumulative effects of discharges from domestic wastes originating from Obunga informal settlements as well as fertilizers from the Kisumu golf club. The low levels of phosphorus upstream of river Kisat may be as a result of minimal human activities. On the other hand, the levels of TP decreased downstream river Auji with the highest level recorded upstream of the river. The high level of TP in this site can be attributed to discharge of grey water from residential areas high in detergents into the river. The use of detergents rich in phosphorus are the main sources of phosphorus in surface waters especially in urban areas [40]. The lowest levels of TP at sampling site Auji river mouth can be attributed to uptake of the nutrients by the high population of wetland plants as wetland vegetations are known to actively take up nutrients [41]. Additionally, it was noted that the concentration of TP increased rather than decreased in WWSP. This can be attributed to continuous addition and cumulative effects of partially treated effluents from the primary oxidation ponds.

While nutrients concentrations are hardly detected at levels poisonous to aquatic biota [42], low dissolved oxygen is sometimes linked with an upsurge in ammonia which can be noxious at certain concentrations and temperature. Excess nutrients are also associated with the occurrence of harmful algal blooms which can produce toxins that affect fish and humans [43].

4.2 Somatic indices as indicator of aquatic pollution

The somatic indices determined in the present study included CF, GSI, KSI and LSI which are useful in evaluating the health status of fish and lethal effects of pollutants. Although physiological activities such as reproduction, secretion and growth may influence the values of these indices under certain environmental conditions, they may act as initial screening indicators to detect exposure and effects of pollutants [8]. Moreover, these indices are simple, cost – effective and basic measures of status and condition of aquatic biota and as such may be applied to evaluate the capability of animals to withstand toxic challenges or other environmental stressors [44].

Fulton's condition factor has been used extensively in fish health and population assessments as an indicative tool of the overall condition and nutritional status of an individual [45]. The significant high CF values reported in fish collected from

midstream of Auji river compared to the upstream site of the WWSP might have been caused by the high consumption of food to meet the enhanced demand to cope with stress imposed by both sewage effluents from the WWSP as well as direct discharges from the informal settlement area [46]. Moreover, [47] stated that the occurrence of few larger and healthier individuals can be a sign of abnormal conditions. Conversely, high CF in fish at disturbed sites has also been credited to chronic exposure [48]. The lowest CF values were obtained in fish collected from WWSP. This can be attributed to three factors; firstly, the presence of pollutants can affect food intake and absorption by fish such that even if food is available, a significant portion of nutrients from the catabolism of food could be converted to energy to help the fish cope with energy demand caused by the chemical stress induced by pollutants; secondly, low dissolved oxygen observed in the WWSP might have been a key factor affecting the growth and by extension survival of the fish. In hypoxic conditions such as those recorded in the WWSP, energy may be diverted away from expenditures of growth, resulting in a significant fitness cost [49], which could explain the low CF values recorded. Thirdly, the low CF might suggest that contaminants could have affected the health of fish through indirect effect on food chain. Studies on the macroinvertebrate communities in WWSPs indicate that the quantity and quality of food organisms available for consumption in wastewater sedimentation ponds may be impacted by the domestic, municipal and industrial discharges [50]. Thus, pollutants could indirectly affect the health status of fish in the WWSP by reducing the nutritional quality or quantity of food items. A decrease in the CF value of fish exposed to pollutants was also reported by [1b] in Lake Nasser and [51] in Lake Naivasha, Kenya who reported low CF values in lakes polluted by chemical pollutants. [52] while studying the health status of *Clarias gariepinus* in Lake Kallar Kahar receiving wastewater from a WWTP reported a CF value below 1 in stations heavily impacted by pollutants.

Somatic indices for fish gills were significantly higher in WWSP sampling station compared to the rivers. It could possibly be associated with the continuously low oxygen saturation of the WWSP. In addition, increase in the gill mass could be a consequence of other morphological anomalies in fish gills such as hyperplasia of the lamellar epithelium, which could be induced by a variety of factors such as environmental pollutants. According to [53], hyperplasia inhibits the respiratory gas exchange by increasing diffusion distance and decreasing interlamellar distance, and therefore could have negative effect on respiratory functions of fish. The low GSI value measured at sampling site Kisat river mouth could be attributed to increased gill area as a result of respiratory disturbances which increased metabolic cost such as energetic cost of ventilation, gill maintenance, and osmoregulation.

A measurement of liver weight in fish, in relation to the whole-body weight, has been documented in numerous studies as a valuable indicator of exposure to pollutants, as well as the well-being of fish [54]. Liver somatic index can be higher or lower depending on the toxicity of discharges. Exposure of fish to low concentration of pollutants normally leads to higher LSI levels [55, 56, 57], attributable to induction or activation of biotransformation oxidase enzymes which enables the fish to develop increased ability to metabolize pollutants. On the other hand, pollutants with high toxicity generally leads to lower LSI values due to hepatocellular injury related to cell necrosis [58, 59]. In the present study, LSI was significantly higher in fish from WWSP than fish sampled from two rivers. The elevated values of LSI for fish from these sites could have been due to hyperplasia and hypertrophy of the liver cells, which is commonly associated with exposure of fish to discharges with sublethal concentration of pollutants. It is unlikely that enlarged livers from the wastewater sedimentation ponds are the result of lipid and glycogen accumulation, because previous studies have shown that lipid metabolism is impaired in fish exposed to sublethal toxicants resulting in reduced body stores of lipids [60]. Another possible reason for the high levels of LSI is that high microcystin levels might have been present at the time of sampling as algal blooms were still visible. Microcystin are known to accumulate in the liver [61] and cause cellular changes. It is therefore likely that higher LSI of fish from NWWSP could be related to the microcystin toxins present as a result of eutrophication, which are in turn caused by high phosphorus levels reported in the same sampling sites during this study. [62] reported LSI values greater than three from African catfish obtained from Roodeplaat and Hartbeespoort dam impoundments which receive discharges from wastewater facilities. [63] reported an association between sewage effluents and an increase in liver size of fish. The noticed significance low values of LSI in fish sampled at Auji midstream and river mouth may be due to mobilization of stored energy reserves biomolecules towards maintaining the homeostasis instead of utilizing them for their somatic growth. Thus, the evident low value of LSI in the present study indicates that chronic exposure of fish to sublethal environmental pollutants could lead to a decline in weight of vital tissues in fish and this might hamper the overall fitness and growth. [64] stated that exposure of fish to multiple stressors affecting energy storage and limiting available energy can be demonstrated as reduced LSI. [65] noted a reduction in the LSI in the Guinean tilapia and African catfish inhabiting cotton basin heavily impacted by micropollutants in West Africa.

4.3 Relationship between the water quality parameters, concentrations of pharmaceuticals and fish somatic indices

Redundancy analysis (RDA) was chosen and executed to assess the association between the water quality parameters and the fish somatic indices. Knowledge of these relationships can help assess the condition of the ecosystem and identify human activities that significantly contribute to pollution as well as areas that are at risk and promote management practices to reduce non-point sources of pollution.

The result showed that dissolved oxygen, temperature, alkalinity, TN and TP impacted the somatic indices particularly upstream of river Kisat, up and midstream of river Auji with much impact felt at wastewater sedimentation ponds suggesting these parameters as well large quantities of domestic, municipal, agricultural runoff and industrial effluents without sufficient treatment are affecting the fish health in these rivers and by extension the environmental health of these aquatic systems.

5. CONCLUSION

The results of this study demonstrated that both rivers Kisat and Auji as well as the wastewater sedimentation ponds are polluted with various pollutants due to the continuous release of diverse pollutants. Similarly, the study revealed that these pollutants are inducing changes in various fish organs as evidenced by the somatic indices suggesting that fish somatic indices are sensitive markers despite these indices being at a higher level of organization. The integrated use of both chemical and biological environmental monitoring approach can provide easy, cost-effective and a quick method of assessing the ecological health of urban rivers receiving both point and non-point sources of pollution. The results also shows that wild *C. gariepinus* is a suitable species for aquatic pollution investigations in tropical region.

REFERENCES

- 1a. Gaber HS, El-Kasheif MA, Ibrahim SA, Authman MMN. Effects of water pollution in El-Rahawy drainage canal on hematology and organ of freshwater fish *Clarias gariepinus*. *World Applied Sciences Journal*. 2013a; 21(3): 329-341
- 1b. Gaber HS. Fish health as a biomarker of the condition of Lake Nasser. *Journal of applied sciences Research*. 2013b; 9(1): 5794-5810
2. Kobingi N, Raburu PO, Mases FO, Gichuki J. Assessment of pollution impact on the ecological integrity of the Kisian and Kisat rivers in Lake Victoria drainage basin, Kenya. *African Journal of Environmental Sciences and Technology*. 2009; 3: 97-107
3. Achieng AO, Mases FO, Coffey TJ, Raburu PO, Agembe SW, Febria CM et al. Assessment of the ecological health of Afrotropical rivers using fish assemblages: a case study of selected rivers in the Lake Victoria Basin, Kenya. *Frontiers in Water*. 2021; 2: 620704
4. Salanki J, Farkas A, Kamardrina T, Rozsa KS. Molluscs in biological monitoring of water quality. *Toxicology Letters*. 2003; 140-141(11): 403 – 410
5. Forbes VE and Forbes TL. *Ecotoxicology in theory and practice*. Chapman & Hall, London; 1994
6. Oberdoff T, Hughes RM. 1992. Modification of an index of biotic integrity based on fish assemblages to characterize rivers on the Seine Basin, France. *Hydrobiologia*. 1992; 228: 117-130
7. Streit B. Bioaccumulation of contaminants in fish. In: Braunbeck T, Hinton DE, Streit B, editors. *Fish ecotoxicology*. Basel: BirkhauserVerlag; 2008
8. Van der Oost R, Beyer J, Vermeulen NPE. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology* 2003; 13: 57- 149
9. United States Environmental Protection Agency (USEPA). Use of biological information to tier designated aquatic life uses in state tribal water quality standards. Office of Water, U.S. Environmental Protection Agency, Washington, D.C; 2005.
10. Nussey G, Van Vuren JHJ, du Preez HH. Effects of copper on the haematology and osmoregulation of the Mozambique tilapia, *Oreochromis mossambicus* (Cichlidae). *Comparative Biochemistry and Physiology C*. 1995; 111(3): 369-380
11. Teubner D, Paulus M, Veilh M, Klein R. Biometric parameters of the bream (*Abramis, brama*) as indicators for long-term changes in fish health and environmental quality data from German ESB. *Environmental Science Pollution Research*. 2014; 22 (3): 1620-1627
12. Mayer FL, Versteeg DJ, McKee MJ, Folmar LC, Graney RL, McCune DC and Rattner BA. 1992. Metabolic products as biomarkers. In: Hugget, R.J., Kimerly, R.A., Mehrle, P.M., Jr, Bergman, H.L. editors. *Biomarkers: Biochemical, physiological and histological markers of anthropogenic stress*. USA: Lewis Publishers; 1992.
13. K'oreje KO, Vergeynst L, Ombaka D, De Wispelaere P, Okoth M, Van Langenhove H et al. Occurrence patterns of pharmaceuticals residues in wastewater, surface water and ground water of Nairobi and Kisumu City, Kenya. *Chemosphere*. 2016; 149:238-244
14. Otieno AA, Kitur EL, Gathuru G. Physico-chemical properties of River Kisat, Lake Victoria Catchment, Kisumu County, Kenya. *Environmental Pollution & Climate Change*. 2017; 1: 137
15. Crafford D, Avenant-Oldewage A. Application of a fish health index and associated parasite index to *Clarias gariepinus* (Teleostei: Clariidae) in the Vaal River system. *South Africa Journal of Aquatic Sciences*. 2009; 34(3): 261-272
16. APHA. Standard methods for the examination of water and wastewater. 22nd edition, American Public Health Association, American Water Works Association, Water Environment Federation; 2012

17. Nash RDM, Valencia AH, Audrey JG. The origin of Fulton's condition factor – setting the record straight. *Fisheries*. 2006; 31(5): 236 – 238
18. Bolger T, Connolly PL. The selection of suitable indices for the measurement and analysis of fish condition. *Journal of Fish Biology*. 1989; 34: 171 – 182
19. Fulton TW. The rate of growth of fishes. In: Twenty-second annual report part 111. Fisheries Board of Scotland, Edinburgh; 1904
20. Dekic R, Savic N, Manojlovic M, Golub D, Pavlicevic J. Condition factor and organosomatic indices of rainbow trout (*Onchorhynchus mykiss*) from different brood stock. *Biotechnology in Animal Husbandry*. 2016; 32(2): 229-237
21. Paliy O, Shankar V. Application of multivariate statistical technique in microbial technology. *Molecular Ecology*: 2016; 25, 1032 – 1057
22. Beniwal VD, Kumari R, Jain S. Physico-chemical parameter: an indicator of water pollution. *Journal of Environmental Research*. 2021; 5(5): 7857
23. UNEP. A snapshot of the world's water quality: Towards a global assessment. United Nations Environmental Programme, Nairobi, Kenya. 2016
24. Adeogun, AO. Impact of industrial effluent on water quality and gill pathology of *Clarias gariepinus* from Alaro stream, Ibadan, Southwest Nigeria. *European Journal of Scientific Research*. 2012; 76 (1): 83-94
25. Veado MARV, de Oliveria AH, Revel G, Pinte G, Ayrault S, Teulhoat P. Study of water and sediment interactions in the Das Velhas River, Brazil-major and trace elements. *Water SA*. 2000; 26(2): 255-262
26. Osman AGM, Al-Awadhi RM, Harabawy ASA, Mahmoud UM. Evaluation of the use of protein electrophoresis of the African catfish (*Clarias gariepinus* Burchell, 1822) for biomonitoring aquatic pollution. *Environmental Research*. 2010; 4(3): 235-243
27. de Sousa DNR, Mozeto AA, Carneiro RL, Fadini PS. Electrical conductivity and emerging contaminants as markers of surface freshwater contamination by wastewater. *Science of the Total Environment*. 2014; 484: 19-26
28. Weber- Scannell PK, Duffy LK. Effects of total dissolved solids on aquatic organisms: a review of literature and recommendation for salmonid species. *American Journal of Environmental Sciences*. 2007; 3(1): 1-6
29. Derry AM, Prepas EE, Hebert PDN. A comparison of zooplankton communities in saline lake water with variable anions composition. *Hydrobiologia*. 2003; 505: 199-215
30. Liu M, Li Q, Sun H, Jia S, He X, Li M, et al. Impact of salinity on antibiotic resistance genes in wastewater treatment bioreactors. *Chemical Engineering Journal*. 2018; 338: 557 – 563
31. Song W, Li Z, Ding Y, Liu F, You H, Qi P, et al. Performance of a novel hybrid membrane bioreactor for treating saline wastewater from mariculture: Assessment of pollutants removal and membrane filtration performance. *Chemical Engineering Journal*. 2018; 331: 695-703
32. Weiner ER. Application of environmental aquatic chemistry: a practical guide. CRC Press, Boca Raton, Florida, United States. 2008.
33. Saalidong BM, Aram SA, Out S, Lartey PO. Examining the dynamics of the relationship between water pH and other water quality parameters in ground and surface water systems. *PLoS ONE*. 2022; 17 (1): e0262117
34. Pal P. Industrial water treatment process technology: Butterworth- Heinemann, Elsevier, Oxford, United Kingdom. 2017
35. Ohoro CR, Adeniji AO, Elsheikh EAE, Al-Marzouqi A, Otim M, Okoh OO, et al. Influence of physicochemical parameters on PPCP occurrence in the wetlands. *Environmental Monitoring and Assessment*. 2022; 194: 339
36. World Health Organization. Guidelines for drinking water quality, 4th edition. WHO press, Geneva, Switzerland. 2011
37. Agedah EC, Ineyougha ER, Izeh SC, Orutugu LA. Enumeration of total heterotrophic bacteria and some physico-chemical characteristics of surface water used for drinking sources in Wilberforce Island, Nigeria. *Journal of Environmental Treatment Techniques*. 2015; 3: 28-34
38. Brightbill RA, Munn MD. 2008. Environmental and biological data for the nutrients enrichment effects on stream ecosystem projects of the natural water quality assessment program, 2003 – 2004. US Geological Survey Data Series. 2008; 345: 13
39. Al-Afify ADG, Othman AA, Ramadan MF. Characterization of chemical microbial quality of Nile River surface waters at Cairo (Egypt). *Rendiconti Lincei Scienze Fisiche Naturali*. 2018; 29 (3): 725-736
40. Munn MD, Frey JW, Tesoriero AJ, Black RW, Duff JH, Lee K, et al. Understanding the influence of nutrients on stream ecosystems in agricultural landscape. *US Geological Circular*. 2018; 1437: 80
41. Musungu CP, Ogoche IJ, Lalah J, Onger D, Chepkui R, Kiema F. The extent of nutrients removal by wastewater treatment plants along Nyalenda Wigwa stream and the river Kisat (Kenya). *Ecology & Hydrobiology*. 2013; 13: 236-240
42. Rankin ET, Miltner RJ, Voder CO, Mishne D.A. Association between nutrients, habitat and the aquatic biota in Ohio rivers and streams: Columbus, Ohio EPA Technical Bulletin; 1999.
43. Graham JL, Loftin KA, Meyer MT, Ziegler AC. Cyanotoxin mixtures and taste-and-odor compounds in cyanobacterial blooms from the midwestern United States. *Environmental Science and Technology*. 2010; 44: 7361-7368
44. Mdegela RH, Marte B, Resto DM, Janneche US, Morten S.A. Assessment of pollution in sewage ponds using biomarkers response in wild African sharp tooth catfish, *Clarias gariepinus* in Tanzania. *Ecotoxicology*. 2010; 19: 722-734

45. Schmitt CJ, Dethloff GM. Biomonitoring of environmental status and trends (BEST) program: selected methods for monitoring chemical contaminants and their effects in aquatic ecosystem. Information and Technology Report USGS/BRD-2000-005. Columbia (MO): US Geological Survey, Biological Resource Division; 2000
46. Lucky M. Determining the health condition of fishes. In: methods for diagnosis of fish diseases. Amerind Publishing Co. Ltd, New Delhi, Bombay; 1977
47. Morado CN, Araujo FG, Gomes ID. The use of biomarkers for assessing the effects of pollutants stress on fish species from a tropical river in Southern Brazil. *Acta Scientiarum Biological Sciences*. 2017; 39(4): 431-439
48. Hamid MA, Mansor M, Nor SAM. Length-weight relationship and condition factor of fish population in Temengor reservoir: indication of environmental health. *Sains Malaysiana*. 2015; 44 (1): 61 - 66
49. Samanta P, Im H, Na J, Jung J. Ecological risk assessment of contaminated stream using multi-level integrated biomarker response in *Carassius auratus*. *Environmental Pollution*. 2018; 233: 429-438
50. Adams SM, Ryon MG. A comparison of health assessment approaches for evaluating the effects of contaminants-related stress on fish population. *Journal of Aquatic Ecosystem Health*. 1994; 3: 12-25
51. Keyombe JL, Waithaka E, Obegi B. Length-weight relationship and condition factor of *Clarias gariepinus* in Lake Naivasha, Kenya. *International Journal of Fisheries and Aquatic Studies*. 2015; 2(6): 382-385
52. Furhan I, Raza N, Ali M, Athas M. Contamination of Kallar Kahar lake by inorganic elements and heavy metals and their temporal variations. *Journal of Applied Sciences & Environmental Management*. 2013; 10(2): 95-98
53. Santos DMS, Melo MRS, Mendes DCS, Roch BS, Silva JPL, Cantanhede SM et al. Histological changes in gills of two fish species as indicators of water quality in Jansen lagoon (Sao Luis, Maranhao State, Brazil). *International Journal of Environmental Research in Public Health*. 2014; 11: 12927 - 12937
54. Yang X, Baumann PC. Biliary PAH metabolites and the hepatosomatic index of brown bullhead from Lake Erie tributaries. *Indian Journal of Ecology*. 2006; 6: 567-574
55. Barse AV, Chakrabarti T, Ghosh TK, Pal AK, Jadhao SB. One-tenth dose of LC50 of 4-tert-butylphenol causes endocrine disruption and metabolic changes in *Cyprinus carpio*. *Pesticide Biochemistry and Physiology*. 2006; 86: 172-179
56. Abdel-Hamid NH. Physiological and histopathological alterations induced by phenol exposure in *Oreochromis aureus* juveniles. *Turkish Journal of Fisheries & Aquatic Sciences*. 2007; 7: 131 - 138
57. Carrola J, Fontainhas-Fernandes A, Mates P, Rocha E. Liver histopathology in brown trout (*Salmo trutta*) from the Tinhela River, subjects to mine drainage from abandoned Jales mine (Portugal). *Bulletin of Environmental Contamination and Toxicology*. 2009; 83: 35-41
58. Shailaja MS, D'silva C. Evaluation of impacts of PAH on tropical fish, *Oreochromis mossambicus* using multiple biomarkers. *Chemosphere*. 2003; 53: 835-841
59. Ma T, Won X, Huang Q, Wang Z, Liu J. Biomarker responses and reproductive toxicity of the effluent from Chinese large sewage treatment plant in Japanese medaka (*Oryzias latipes*). *Chemosphere*. 2005; 59: 281-288
60. Adams SM, Brown AM, Goede RW. 1992. A quantitative health assessment index and rapid evaluation of fish condition in the field. *Transaction of the American Fisheries Society*. 1992; 122: 63-73
61. Deblois CP, Giani A, Bird DF. Experimental model of microcystin accumulation in the liver of *Oreochromis niloticus* exposed sub chronically to a toxic bloom of microcystin sp. *Aquatic Toxicology*. 2011; 103: 67-70
62. Van Dyk JC, Cochrane MJ, Wagenaar GM. Liver histopathology of the sharp tooth *Clarias gariepinus* as a biomarker of aquatic pollution. *Chemosphere*. 2012; 87: 301-311
63. Galloway BJ, Munkittrick KR, Currie S, Grey MA, Curry RA, Wood CS. Examination of the response of slimy sculpin (*Cottus cognatus*) and white sucker (*Catostomus commersoni*) collected on the Saint John River (Canada) downstream of pulp mill, and sewage discharges. *Environmental Toxicology & Chemistry*. 2003; 22: 2898-2907
64. Gibbons WN, Munkittrick KR. A sentinel monitoring framework for identifying fish population responses to industrial discharges. *Journal of Aquatic Ecosystem Health*. 1994; 3(3): 227-237
65. Agbohesis PT, Toko II, Ouedraogo A, Jauniaux T, Mandiki SNM, Kestemont P. Assessment of the health status of wild fish inhabiting a cotton basin heavily impacted by pesticides in Benin (West Africa). *Science of the Total Environment*. 2015; 506 - 507: 567 - 584

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