

*Full Length Research Paper*

# Assessment of pollution impacts on the ecological integrity of the Kisian and Kisat rivers in Lake Victoria drainage basin, Kenya

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Macro-invertebrate assemblages were used as bioindicators to assess the ecological integrity of Rivers Kisat (influenced by urban development) and Kisian (influenced by agriculture) using community attributes and the Index of Biotic Integrity. Six stations, three per river, were selected to correspond to different impact types and intensities along the rivers. Physico-chemical parameters and nutrients were determined for each station on a monthly basis from November 2007 to April 2008. Two-way analysis of variance was used to compare water quality and nutrient parameters, and macro invertebrate community attributes between the two rivers, with the river and station as the main factors. Significant differences were accepted at 95% confidence level. There were inconsistencies in the variation of physico-chemical parameters along the two rivers. However, River Kisat recorded higher values for all physico-chemical parameters considered, except pH and DO. Different indices and metrics representing the structural and functional organization of macro invertebrates were computed and evaluated for responsiveness to physico-chemical parameters and nutrient levels. Macro invertebrate diversity, richness and evenness values failed to delineate stations according to the different levels of degradation they were experiencing. However, the differences were captured by the index of biotic integrity, which separated stations into different classes of quality. River Kisat stations in urban areas scored lowest index values, less than 15 out of 25, while two river Kisian stations scored the highest value, more than 19. The index provided evidence of response to changes in ecosystem integrity exhibited by resident macro invertebrate assemblages to pollution arising from both point and non-point sources.

**Key words:** Urban rivers, water quality, physico-chemical parameters, macro invertebrates.

## INTRODUCTION

In most developing countries, point and non-point source pollution are major environmental problems affecting water quality. The situation is exacerbated by lack of or scarcity of treatment for domestic wastes (Dudgeon, 1992) and poor agricultural practices (Iwata et al., 2003). In East Africa, land use changes caused by rapid urbanization and clearance of forests to create room for agriculture have emerged as major stressors of streams and rivers (Kibichii et al., 2007; Kasangaki et al., 2008). In Kenya, degraded water quality, losses of biodiversity and altered hydrography have been recorded among streams and rivers draining urban areas (Ndaruga et al.,

2004). On the other hand, deforestation and cultivation have been found to cause an increase in water temperature, conductivity, total suspended and dissolved solids and turbidity (Kibichii et al., 2007). Animal overuse on the riparian areas has been found to increase ammonia and nitrite as a consequence of increased run-off of animal wastes into streams (Kibichii et al., 2007). Near-stream human activities like sand mining, bathing, laundry and row crop agriculture have been reported to cause the greatest influence on stream habitat and biotic characteristics (Mathooko, 2001; Raburu et al., 2009).

In lower catchments of Lake Victoria Basin- Kenya, streams, rivers and the lake itself serve as the major source of freshwater to the riparian communities and their livestock. For town and city residents, portable water sup-

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ply by relevant authorities is less than 60% and most people use the water directly without prior treatment. Against this backdrop, increased intensity of agriculture and deforestation coupled with the rapid growth of urban centers and industrial activities pose a potential threat in degrading small streams and rivers that drain directly into the lake. Because of their urban set up these small ecosystems are often not protected by buffer zones that allow for the absorption of immense run-off from Jua Kali sheds and settlements on the riparian areas. The problem is worsened by the fact that most industries and malfunctional sewerage facilities discharge directly into the small streams and rivers which act as conduits for delivery of the wastes into the lake (Lung'aya, 2002). This has consequently led to sedimentation and eutrophication that have affected domestic and industrial water supply (Ntiba, et al., 2001).

Macro invertebrate assemblages have been used as bioindicators of stream biological integrity (Collins et al., 2008; Miltner et al., 2004; Stepenuck et al., 2002). Within this framework, the use of a multimetric approach that utilizes the index of biotic integrity (IBI) (Karr, 1981) has gained interest in biological assessment of rivers and streams in urban and suburban catchments (e.g. Collins et al., 2008; Miltner et al., 2004). In the upper reaches of Lake Victoria basin results have indicated the usefulness of the index in assessing the biological integrity of studied rivers and streams (Masese et al., 2009a, b; Raburu et al., 2009). In the lower catchments of Lake Victoria basin, urban and suburban developments are replacing agricultural land use in most of the catchments. Studies that have compared the two land use types indicate that a low level of watershed urbanization triggers a biological response of greater strength and magnitude than watershed agriculture (Wang and Lyons, 2003). However, in the tropics inferences about the relative importance of urban and agricultural land use are limited, especially where the independent influences of the two land use types on water and overall ecosystem integrity are to be distinguished.

Although a number of rivers highly influenced by anthropogenic activities drain their water directly into the lake, information on urbanization and agriculture, which are the prominent land use types, on lowland streams within the lower catchments of Lake Victoria Basin is minimal. This study, therefore, focused on two streams within the same ecoregion, one in an urbanizing catchment and the other in an agricultural catchment, with the aim of utilizing macro invertebrate community attributes and a Macro invertebrate Index of Biotic Integrity (M-IBI) in relation to water quality and nutrient parameters to assess the effects of the two land-use practices on water quality and overall ecosystem integrity.

## MATERIALS AND METHODS

### Study area

The study was conducted on Rivers Kisian and Kisat in the lower

catchments of Lake Victoria Basin, Kenya (Figure 1). The two rivers occur within the same ecoregion in which they share common relief features, altitude and longitude range and climate. The area occupied by Winam Gulf of the lake, into which the two rivers drain, has tertiary and alkali volcanic and sedimentary rocks (Johnson et al., 2000).

The Kisian River originates from Maragoli Forest and is surrounded by the catchments of Riat and Kodiaga hills. These are areas that have rocks that are continuously weathering and releasing ions ( $Mg^{2+}$  and  $Ca^{2+}$ ) in the river. There is sand harvesting in the river near the bridge (Otonglo-Kiboswa road). Activities on the river are relatively less intense, although farming activities along the river are evident (maize, tomatoes, millet, and kales). The communities living along this river extract water for domestic use. Before it drains its waters into the lake at Usoma Bay it passes through a swamp.

The source of River Kisat is a small swamp on the eastern suburbs of Kisumu city. Drainage and subsequent cultivation of the land has accelerated the drying up of the once vast swamp, leaving the river to trickle. Grazing fields for livestock mainly cattle, small farms of maize and vegetables and a few of millet and sorghum are in the vicinity. All these activities have modified most of the area that is also being converted for residential use. The middle reach of Kisat passes through an area with small farms of maize, sorghum, vegetables where cultivation is done up to the riverbanks. As it flows into the lake it passes through the densely populated Obunga slums, which lack sanitation facilities and streams of sewage and residues from makeshift distilleries of local brew (chang'aa), an illicit whisky, enters the river at various points. After the slums the river flows through the Kisumu industrial area and the main industries include textile mill, soap and fish processing factories, salt works, motor garages and stores for various items, including toxic chemicals. The factories have no facilities for treating effluents and are connected to the sewage drainage system or discharge their waste effluents directly into the river. The Kisumu city municipal sewage treatment plant is located at the lower part of the river. However, the sewage plant has not worked for several years, and untreated sewage was being discharged into the river. A golf course is located just before the mouth of the river at Kisumu Bay. The Kisat River is greatly overwhelmed by multiple wastewater discharges and chemical effluents right from the source to the mouth.

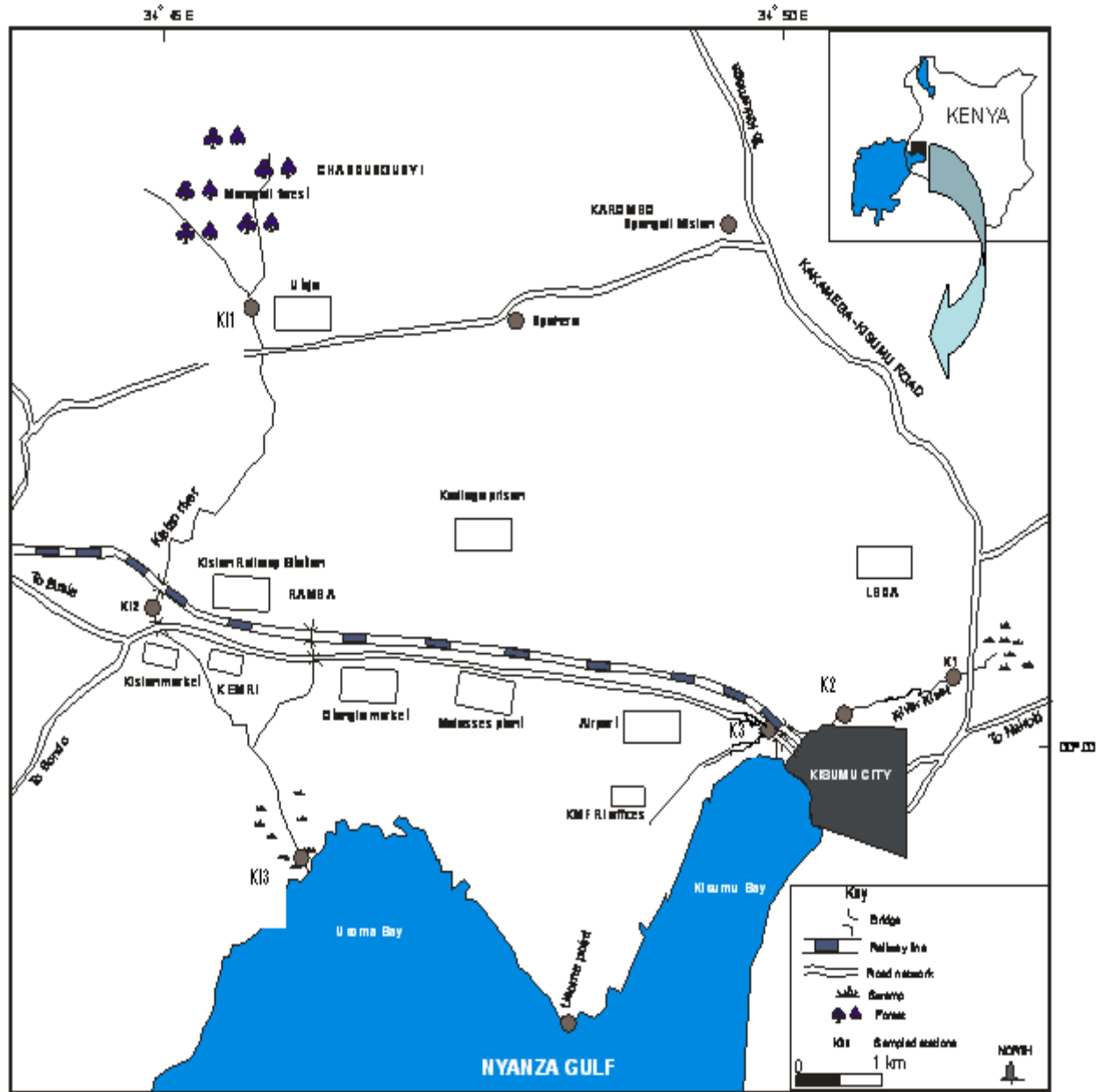
### Sampling design

#### Selection of sampling stations

The two rivers were selected on the basis of differing hydrology, habitat condition and human activity. Sampling stations were selected to represent different ecological and environmental variations within each river, in order to understand the influence of natural and human induced stress on physical, chemical and biological attributes of the water quality. Three sampling stations were selected along each river. In the Kisian River station K11 was located in a forested section at the source. Station K12 was located in an agricultural area where vegetable farming was the main activity while Station K13 was located after the river passes through a swamp before it enters into the lake. In Kisat River sampling station K1 was located near in area with minimal human activity, 0.5 km from the source. Station K2, 9 km from the source, was located at Obunga slums where domestic effluents and other wastes were being deposited into the river, and finally K3 was located after the industrial and municipal waste discharges.

### Data collection

Data on physico-chemical parameters and macro invertebrates were collected monthly for a period of six months from November



**Figure 1.** A map representing the study area showing the location of sampling points. Stations K1 - K3 are influenced by suburban and urban development while stations K11-K13 are influenced by agricultural development.

2007 to April 2008. The following physical-chemical parameters were measured *in situ*; pH and temperature were by microprocessor pH meter, conductivity by a WTW microprocessor conductivity meter LF6 and turbidity by Hach 2100P Turbidimeter. Triplicate samples for dissolved oxygen were fixed *in situ* before they were determined by the Winkler method in the laboratory (APHA, 1998). Triplicate samples for nutrients (nitrogen, silicates and phosphorous) were collected and analyzed according to Wetzel and Likens (2000). Samples for water quality were collected before sampling for macroinvertebrates to prevent contamination.

Sampling for macro invertebrates was done where water samples had been collected using a scoop net (0.5 mm mesh size). Quantitative triplicate samples were collected from runs, riffles and pools from each station. Sampling was done for a standard three minutes by disturbing a 1 m<sup>2</sup> area for each microhabitat. Samples

were sorted live in a white plastic tray and then poured into vials and preserved with 70% ethanol. The samples were then pooled to make one composite sample per station. In the laboratory samples were processed and identified to genus level according to Macan (1977), Merritt and Cummins (1996), Nilson, (1996, 1997), Quigley (1977) and Scholtz and Holm (1985). Taxonomic lists of species known to be present in Kenya were also useful (Johanson, 1992; Mathooko, 1998). Functional feeding groups were assigned according to (Merritt and Cummins, 1996) and assignments that have been used on Kenyan fauna (Dobson et al., 2002).

The macro invertebrate diversity, richness and abundance were determined for each sampling station and sampling occasion using number of taxa, total number of individuals and relative abundance of each taxon. Relative abundance (R.A) was calculated as the proportion (percentage by numbers) of each taxon in a station. The

relative abundance was calculated as:

$$R. A = \frac{\text{No. of individuals of one taxon} \times 100}{\text{Total no. of individuals in a station}}$$

The Shannon-Weaver diversity index (Shannon and Weaver, 1949) was used to assess diversity as follows:  $H' = -\sum ((n/N) * \ln (n/N))$ , where  $n$  = number of individuals of a taxon;  $N$  = total number of individuals in the station. An associated evenness  $H'/H'_{\max}$  (Pielou, 1975) was also calculated. The Simpson Index ( $D_s$ ) (Simpson, 1949) was used as a measure of taxon richness. The index is given by:

$$D_s = \sum_{i=1}^{i=n} \frac{\{n_i (n_i - 1)\}}{\{N (N - 1)\}}$$

Where;

$n_i$  is the number of species in the sample

$N$  is the total number of individuals in the station.

#### Macro invertebrate community attributes for index development

In order to develop a macro invertebrate based index of biotic integrity (M-IBI) for the two streams, 10 macro invertebrate assemblage attributes, termed metrics, were selected a priori and tested to determine their response to the different human impacts types. These attributes included number of taxa, EPT and intolerant taxa, percent Ephemeroptera + Plecoptera + Trichoptera (EPT), intolerant, tolerant, no-insects, and predator individuals and percent gatherer genera. These macro invertebrate metrics have been used widely in developing M-IBIs where they have proved their utility as discriminators of pollution gradients (Fore and Karr, 1996; Kerans and Karr, 1994; Thorne and Williams, 1997; Weigel et al., 2003; Masese et al., 2009a; Raburu et al., 2009). To assess point-source pollution from industries, domestic wastes and municipal sewage effluents as well as non-point agricultural inputs, the Biological Monitoring and Working Party's Average Score Per Taxon (BMWP-ASPT) biotic index, which was developed in Britain (ISO-BMWP, 1979), was used. The index has been used in several countries, including India (De Zwart and Trivedi, 1994), Australia (Chessman, 1995) and in Ghana (Thorne and Williams, 1997).

#### Index development

Metrics were screened for range, responsiveness to disturbance and redundancy with other metrics. On the basis of these results, we selected a set of non-redundant metrics that responded to a variety of disturbance types and included different metric classes. In order to combine the selected metrics into an index, each metric was transformed into a dimensionless number by scoring. The scored metrics were then summed to obtain the final index score. For the range test, all richness metrics with a range of 5 or less were eliminated while percentage metrics with a range of less than 10% were also eliminated (Klemm et al., 2003). Responsiveness of metrics to disturbance was evaluated using Pearson's correlation analysis with physico-chemical and nutrient parameters. Metrics not correlated with any of the parameters was eliminated. Redundancy in the remaining metrics was evaluated by Pearson correlation coefficients and visual inspection of scatter plots. Metrics with a correlation coefficient ( $r$ )  $\geq 0.85$  were considered redundant. Only one metric from a group of redundant metrics was included in the final index. Metrics that passed the screening process were included in the final index.

#### Scaling and scoring criteria

We used a 1, 3, 5 scoring system, which has been commonly used in developing fish and macro invertebrate IBIs (Karr, 1981; Kerans and Karr, 1994; Barbour et al., 1999; Raburu et al., 2009) (Table 2). Because all streams in the region are considered degraded in one way or another, reference sites were not used to establish the scoring criteria. Instead, the highest value for each metric across all sites was used as a reference (Karr and Chu, 1999). For positive metrics (that is those that increased with improving conditions), the upper expectation was the 95<sup>th</sup> percentile of the highest value of a metric across all sites. The ranges of values from 0 to the 95<sup>th</sup> percentile were then trisected. Values above the upper one-third received a score of 5; those in the middle received a score of 3 while those in the lower one-third received a score of 1, corresponding to unimpaired, intermediate and impaired biota respectively (Barbour et al., 1999; Raburu et al., 2009). For negative metrics, those that decreased with improving condition, the lower expectation was the 5<sup>th</sup> percentile. The range from the 5<sup>th</sup> percentile was trisected but scoring done in reverse, i.e. values above the upper one third received a score of 1, those in the middle a score of 3 while those in the lower one-third a score of 5. To calculate the M-IBI value for each station all the metric scores were added.

#### Condition categories

Criteria used for contrasting biological conditions at sites (e.g., good, fair, poor) using IBIs or single metrics may be established with many methods (Stevenson et al., 2004). In this study integrity classes for condition categories were defined using highest M-IBI value for all stations, which was considered to represent reference conditions at the study area. In this case the reference site was arrived at posterior based on the highest M-IBI value across all sites.

#### Data analysis

Data on physical-chemical parameters and nutrients were expressed as (means  $\pm$  SE) for each station. Macro invertebrate count data was log transformed ( $\log_{10} x+1$ ) while percentage data was Arcsine transformed before analysis. Two-way ANOVA was used to compare physico-chemical parameters and macro invertebrate community attributes between the two rivers with river and station as the main factors (Zar, 2001). Where there were no interactions, one-way ANOVA was re-run with stations as the only main factor and post hoc Duncan's Multiple Range Test (DMRT) performed to identify the stations that differed from one another. Pearson's correlation coefficients were used to determine the inter-relationships between physico-chemical and nutrient parameters and macro-invertebrate community attributes. Analysis was in Minitab for Windows (Version 13) and significant differences for all inference tests were accepted at  $p < 0.05$ .

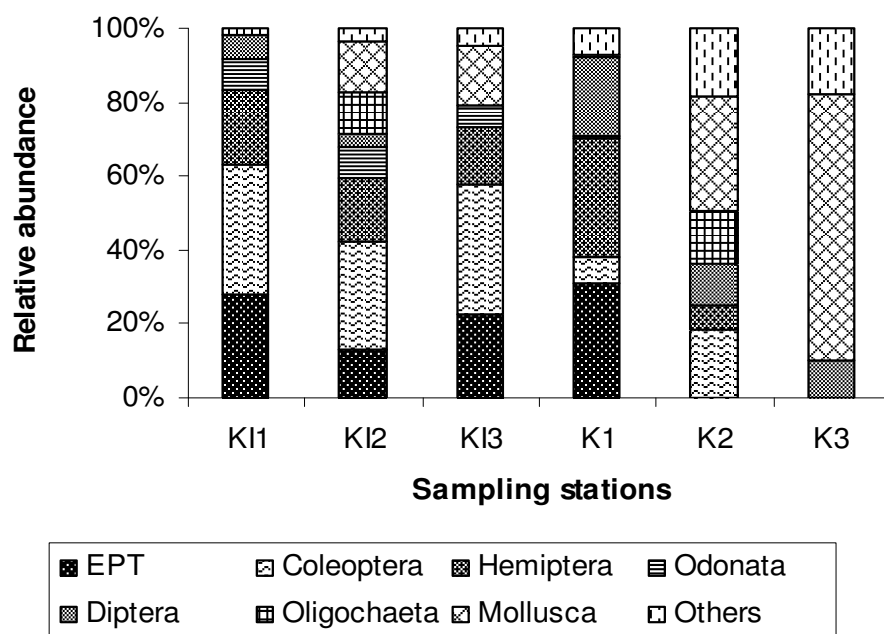
## RESULTS

### Physico-chemical parameters

Results for physico-chemical parameters and nutrients for the six stations in the two rivers are shown in Table 1. There were significant differences in all physico-chemical and nutrient parameters between the two rivers ( $p < 0.05$ ), except pH and silicates. While comparing the temperature range among the sampling stations in the two rivers, there was inconsistency in the mean values in

**Table 1.** Mean ( $\pm$  SE) value for physicochemical parameters and nutrients for the Kisian and Kasat Rivers during the study period, November 2007 to April 2008.

	KI1	R. Kisian	Stations	K1	R. Kasat	K3
Physico-chemical parameters		KI2	KI3		K2	
Temperature ( $^{\circ}$ C)	23.5 $\pm$ 0.9	23.4 $\pm$ 0.6	23.2 $\pm$ 0.5	24.2 $\pm$ 0.9	25.2 $\pm$ 9.3	24.6 $\pm$ 0.9
DO (mg/l)	7 $\pm$ 0.2	6.7 $\pm$ 0.4	5.0 $\pm$ 0.6	5.8 $\pm$ 0.3	0.1 $\pm$ 0.1	2.4 $\pm$ 0.1
pH	7.8 $\pm$ 0.2	7.8 $\pm$ 0.3	7.7 $\pm$ 0.2	7.7 $\pm$ 0.2	7.3 $\pm$ 0.1	7.2 $\pm$ 0.2
Conductivity ( $\mu$ S/cm)	114.9 $\pm$ 3.3	105 $\pm$ 2.3	146.6 $\pm$ 10.8	435.6 $\pm$ 48	795.2 $\pm$ 31.8	612.9 $\pm$ 3.8
Turbidity (NTUs)	80.0 $\pm$ 5.2	70.5 $\pm$ 1.4	63.1 $\pm$ 2.5	98.9 $\pm$ 17.4	325.4 $\pm$ 40.3	348.1 $\pm$ 37.2
Nutrients						
TP (mg/l)	0.9271 $\pm$ 36.3	0.8264 $\pm$ 41.9	0.9348 $\pm$ 63.2	0.8512 $\pm$ 60.7	1.0906 $\pm$ 41.5	1.0069 $\pm$ 42.5
TN (mg/l)	4690 $\pm$ 38.1	5856 $\pm$ 131	5730 $\pm$ 438	4948 $\pm$ 208	7116.7 $\pm$ 79.7	6068.4 $\pm$ 91.7
Silicates (mg/l)	13.6 $\pm$ 0.6	12.8 $\pm$ 0.4	13.0 $\pm$ 0.2	14.0 $\pm$ 0.4	11.6 $\pm$ 0.3	12.3 $\pm$ 0.6

**Figure 2.** Relative abundance of macro invertebrate groups in Rivers Kisian and Kasat during the study period.

both rivers with K2 in river Kasat recording the highest ( $25.2 \pm 9.3^{\circ}$ C) whereas KI3 in river Kisian recorded the lowest ( $23.2 \pm 0.5^{\circ}$ C). Comparing the DO levels in the two rivers, stations K2 had the lowest than any of the stations in both rivers, with a mean value of  $0.1 \pm 0.1$ mg/l. DO in river Kisian decreased downstream while in River Kasat there was a sharp decrease at station K2. There were significant differences in both conductivity and turbidity among all the stations sampled ( $p < 0.001$ ). Sampling station K2 had the highest mean value of conductivity ( $795 \pm 31.8 \mu$ S/cm) while station KI2 had the least ( $105 \pm 2.3 \mu$ S/cm). The lowest mean value for turbidity ( $63.1 \pm 2.5$  NTUs) was recorded at station KI3 while the highest was recorded at station K3 ( $348.1 \pm 37.2$  NTUs).

### Nutrients

River Kasat recorded the higher TP and TN concentrations as compared to River Kisian. Station K2 recording the highest mean value for TP ( $1.0906 \pm 41.5$  mg/l) while K2 recorded the least ( $0.8264 \pm 41.9$  mg/l). Station K1 recorded the lowest TN mean value ( $4.690 \pm 38.1$  mg/l) while KI2 recorded the highest ( $7.1167 \pm 79.7$  mg/l). Both TN and TP showed a significant difference in the sampling dates ( $p < 0.001$ ). The month of March recorded the highest concentration of TN whereas the higher concentration of TP was recorded during the month of April. Silicates for the two rivers showed no significant difference. However, there were significant differences among the

**Table 2.** List of macro invertebrates sampled and their relative abundances in Kisian and Kijat Rivers during the study period

Order	Family	Genus	R. Kisian	R. Kijat
Coleoptera	Elmidae	<i>Elmis</i> sp.	30.80	7.12
	Hydraenidae	<i>Hydraena</i> sp.	2.81	
Pulmonata	Lymnaeidae	<i>Lymnae</i> sp.		9.99
Hemiptera	Corixidae	<i>Corixina</i> sp.	6.78	2.97
	Leuctridae	<i>Leuctra</i> sp.	10.13	0.20
	Velidae	<i>Velia</i> sp.	0.68	3.07
	Nepidae	<i>Nepus</i> sp.		1.08
	Notonectidae	<i>Notonecta</i> sp.		0.56
Bivalvia	Sphaeriidae	<i>Sphaerium</i> sp.		10.30
	Sphaeriidae	<i>Pisidium</i> sp.		27.46
Odonata	Aeshnidae	<i>Aeshna</i> sp.	4.59	0.10
	Gomphidae	<i>Gomphus</i> sp.	2.87	
Diptera	Limnionidae	<i>Dicronata</i> sp.		6.15
	Simuliidae	<i>Simulium</i> sp.	1.37	
	Chironomidae	<i>Chironomus</i> sp.	2.33	6.40
Plecoptera	Nemouridae	<i>Nemoura</i> sp.	6.43	
Oligochaeta	Lumbriculidae	Lumbriculidae sp.	4.59	5.48
	Tubificidae	<i>Tubificida</i> sp.		0.10
Arachnedeae	Aranida	<i>Acari</i> sp.	1.30	
Ephemeroptera	Baetidae	<i>Baetis</i> sp.	14.65	3.43
Platyhelminthes	Dendrocoelidae	<i>Dendrocoelum</i> sp.		0.31
Hirudinae	Erpobdellidae	<i>Erpobdella</i> sp.	0.89	10.96
	Glossiphonidae	<i>Glossiphonia</i> sp.		4.30

sampling stations ( $p < 0.05$ ) with the highest station, K1 recording  $14.05 \pm 0.4$  mg/l and the lowest, K2  $11.6 \pm 0.3$  mg/l. Also, there was a significant difference for the sampling dates ( $p < 0.05$ ) with the highest mean value being recorded during the month of April.

### Macro invertebrate assemblages

In total 3185 macro invertebrate individuals belonging to 12 orders, 22 families and 23 genera were counted. The taxonomic composition and distribution of the groups in the two rivers is shown in Table 2. Orders Hemiptera and Diptera were the most diverse taxa, consisting of five and three families respectively. The order Hemiptera had 5 genera, hence the most diverse among the study stations. Orders Pulmonata, Plecoptera Arachnida, Ephemeroptera and Platyhelminthes were the least diverse tax consisting of one family each. The order Hemiptera were the most abundant macro invertebrates in River Kijat, station K1 (42.8%), whereas the most abundant macro invertebrates in River Kisian were recorded at station K13 (28.6%). Pollution tolerant group of the Bivalvia recorded total percentage of 40% in River Kijat at station K3 (Figure 2). Results of macro invertebrate composition measures for the six stations are shown in Table 3. The

attributes showed significant variation between the two rivers ( $p < 0.05$ ), except Shannon-Weaver diversity and percent individuals in the most dominant 1-3 taxon (taxa). Stations in Kisian River recorded lower mean abundances, Simpson richness and Shannon-Weaver diversity than stations in Kijat River.

### Metric selection

Numbers of EPT and intolerant genera were eliminated on the basis of range. The 8 metrics remaining showed significant correlation with the physico-chemical and nutrient parameters. However, percent predator individuals were eliminated because they did not show clear separation of stations scored similar values for all except one station. Redundancy tests showed that numbers of genera were redundant with percentage of predator individual, percent EPT individuals were redundant with percent intolerant individuals and percent tolerant individuals with percent non-insect individuals.

For metrics that were redundant with one another, consideration was given to ones with ecological significance, interpretability, wide response to a range of physico-chemical and nutrient parameters and clear separation of sites. We chose number of genera, percent EPT indivi-

**Table 3.** Macro invertebrate composition measures for the six study stations for the period November 2007 to April 2008.

Diversity measures	Stations					
	R. Kisian			R. Kisat		
	KI1	KI2	KI3	K1	K2	K3
No. of taxa	10	11	9	15	11	6
Mean abundance	80.8±9.3	85.8±9.3	76.8±16.9	54.5±10.7	127.0±17.2	103.8±18.4
Simpson richness (1/D)	3.3±0.1	4.3±0.3	3.3±0.4	4.7±0.2	5.5±0.6	4.4±0.4
Diversity index (H')	1.4±0.02	1.6±0.06	1.3±0.07	1.6±0.07	1.8±0.06	1.5±0.08
Evenness index (e)	0.32±0.01	0.36±0.01	0.32±0.02	0.43±0.02	0.38±0.02	0.33±0.01

**Table 4.** Five component metrics of the Macro invertebrate Index of Biotic Integrity (M-IBI) and metric values corresponding with scores based on the 1, 3, 5 scaling system.

Metrics	Scoring criteria		
	5	3	1
Number of taxa	>10	6-10	<6
Percent EPT individuals	>20.2	10.1-20.2	<10.1
Percent tolerant individuals	<21.2	21.2-42.2	>42.4
BMWP-ASPT	>3.2	1.6-3.2	<1.6
Percent gatherers genera	<22.2	22.2-44.4	>44.4

duals and percent tolerant individuals for inclusion in the final index. The metrics and the scoring criteria are shown in Table 4.

### Metric responses to degradation

The correlations between the final metrics and physico-chemical and nutrient parameters that depicted the degradation gradient in the study area are shown in Table 5. Number of taxa was negatively correlated with turbidity. Percent EPT individuals and percent tolerant individuals were negatively correlated and this reflected on their relationships with environmental parameters considered. While the EPT individuals were negatively correlated with conductivity, turbidity, TP and TN, the tolerant individuals were positively correlated with the same parameters. Percent EPT and BMWP-ASPT were sensitive to poor water quality where they displayed a negative correlation with conductivity, turbidity, TP and TN. Percent gatherer genera were positively correlated with temperature, conductivity, turbidity, TP and TN indicating that they were being favoured by poor water quality. The M-IBI index displayed a significant relationship with all parameters indicating that it was responding to the degradation gradient in the study area.

### Condition categories

All sites in this study were grouped into three condition categories, good, fair, and poor, using the highest M-IBI

score (25). This score was trisected such that the 75<sup>th</sup> percentile (19) was used to separate “good” from “fair” conditions while the 50<sup>th</sup> percentile (13) was used to separate “fair” from “poor” sites (Table 6). We interpreted biological responses to human influence, establishing narrative descriptions for multiple ranges of M-IBI values using physico-chemical and nutrients data, individual metrics, final M-IBI and human activity (Table 6). A notable response was the association of poor water quality caused by industrial domestic and municipal waste disposal with either low numbers of EPT individuals or high numbers and dominance of tolerant individuals. Chironomids, oligochaetes and tolerant molluscs were the dominant groups at stations K2 and K3, which were classified under “poor” sites.

On the other hand, “good” sites, Like KI1, KI3 and K1 were characterized by low temperature, conductivity, turbidity, TP and TN and high DO. This favoured higher numbers and dominance of EPT and intolerant taxa and this was registered by the high BMWP-ASPT values at the stations. Stations KI1 and K1, which were located in a forested and wetland area respectively, recorded the highest abundance of EPT. Station KI2 was under the fair category. The station was mainly affected by vegetable farming on the riparian zone. Slight to moderate levels of nonpoint source pollution appeared to influence sites categorized as “good”. Despite the high index values at station K1, farming activities on the riparian zone were expected to affect the index values. Percent EPT (minus baetidae), tolerant individuals, and BMWP-ASPT were strong indicators of environmental condition.

**Table 5.** Pearson's correlation coefficients between physico-chemical parameters and nutrients, and the component metrics and the final M-IBI for Rivers Kisian and Kisat in lower catchments of Lake Victoria Basin. Units for physico-chemical and nutrient parameters as in previous table.\* designate significant relationships at  $p < 0.05$ .

Metrics	Temperature (°C)	DO (mg/l)	pH	Conductivity (µS/cm)	Turbidity (NTUs)	TP (µg/l)	TN (µg/l)	Silicates (mg/l)
No. of genera	-0.03	-0.16	0.50*	-0.06	-0.45*	-0.46*	-0.30	0.50*
Percent EPT individuals	-0.66*	0.47*	0.84*	-0.68*	-0.86*	-0.69*	-0.88*	0.95*
Percent Tolerant individuals	0.88*	-0.72*	-0.98*	0.87*	0.97*	0.73*	0.73*	-0.77*
BMWP-ASPT	-0.83*	0.67*	0.98*	-0.85*	-0.98*	-0.79*	-0.76*	0.83*
Percent gatherer genera	0.63*	-0.49*	-0.93*	0.68*	0.90*	0.76*	0.74*	-0.84*
M-IBI	-0.85*	0.71*	0.96*	-0.98*	-0.87*	-0.79*	-0.83*	0.87*

**Table 6.** Total M-IBI scores, their integrity classes and narrative description based on the current study

Integrity classes, M-IBI score and stations in the category	Narrative description based on the current study
Good, >19 (K11, K13, K1)	Minimal human activity within 100 m of the riparian zone, natural vegetation intact along the river, macro invertebrates dominated by EPT, few non-insects, in-stream substrate dominated by stones and boulders. Turbidity less than 100 NTUs. BMWP-ASPT > 3.2.
Fair, 15-23 (K12)	Minimal human activity within 50m of the riparian zone, bottom substrate dominated by sand and stones, EPT if present dominated by Baetidae. Chironomids present but in low numbers. Buffer zone maintained along the streams. BMWP-ASPT 1.6-3.2.
Poor <13 (K2 and K3)	Human activity up to the edge of the stream, no buffer zone, collapsed and eroded river banks, human activity include dumping of domestic wastes, municipal and industrial discharges, EPT absent, % tolerant taxa >42 and dominated by chironomids, oligochaetes and molluscs. Bottom dominated by sand and organic materials. Turbidity > 100 NTUs. BMWP-ASPT < 1.6.

## DISCUSSION

### Physico-chemical and nutrient parameters

The difference in water quality status between the two rivers and among the stations can be attributed to land-use practices. Most of the parameters considered, for instance, were lower in River Kisian where land use was mostly under agriculture and sub-urban settlement as compared to River Kisat where most land was under urban settlement and industrial use. Low DO values and higher temperature at stations K2 and K3 were due to organic and industrial discharges into the river whose decomposition utilizes most oxygen in the water creating anoxic conditions. Higher temperatures might have been due to the open channel being heated and also thermal blooms from the industries.

The spatial differences in mean turbidity along the rivers reflected human activity in the vicinity of the sampling stations. The lowest turbidity at station K13 can be attributed to the filtering effect of the wetland before the station, which removed most of the sediments. High values at K2 and K3 can be attributed to sediments from

the Obunga slums and associated activities like brewing and deposition of wastes into the river.

Stations in Kisat River, except station K1, recorded higher total nitrogen values than stations in Kisian River. Domestic wastes, raw sewage effluent and industrial wastes deposited in the river before stations K2 and K3 can explain the high values for Kisat River. The least mean value for TN recorded for Station K1 can be explained by the nature of human activities. In this station, as in stations along Kisian River, agriculture was the major source of nutrients as opposed to domestic, municipal and industrial discharges that have higher loads of nutrients. A similar trend was observed for TP whereby station K1 and K2 in River Kisat recorded the lowest and highest mean values respectively. At station K12 in River Kisian near-stream human activities and the associated physical disturbance and chemical inputs impacted negatively on macroinvertebrate assemblages reducing the index values, a situation that has been observed in other studies in Kenya (Lung'ayia, 2002; Mathooko, 2001; Masese et al., 2009a, Raburu et al., 2009).

High concentrations of phosphates and low DO indicate organic pollution. This was the case in the study area, as



higher values for total phosphorus and low DO were recorded below points of industrial discharges and municipal effluents. On the other hand, high levels of TP in Kisian River might have originated from washing and leaching of phosphate fertilizers used on the riparian zone. Lack of significant differences in silicates between the two rivers can be explained by the similar geological conditions, considering that their major source into water bodies is through weathering processes (Lampert and Sommer, 1997).

### **Macro invertebrates assemblages and land-use activities**

Macro invertebrates are known to display structural and functional responses to environmental changes caused by pollution. In the current study, while evenness values remained low across all stations, the diversity and richness indices were higher in River Kisat stations, despite the higher levels of degradation. Thus, the indices failed to delineate the degradation gradient across the stations. Higher diversity and richness at station K1 can be attributed to the intermediate disturbance hypothesis (Ward and Stanford, 1984), which predicts that diversity and richness will be highest at stations experiencing moderate levels of pollution. Moderate pollution has also been found to cause an increase in abundance without excluding species, with the result that the index values actually go up (Cook, 1976). Because of these reasons, diversity indices are insensitive to and give poor site discrimination over the moderate range of pollution (Thorne and Williams, 1997).

There were marked shifts in relative abundance and composition of macro invertebrate assemblages among the stations. Though stations K12 and K2 recorded the same number of genera, the two stations differed in composition. At K12 Elmidae, Baetidae, Nematouridae, Hydracnidae, Gomphidae and Leuctridae dominated the samples, groups that did not occur at station K2. The rich organic wastes at station K2, from the slum dwellings on the riparian zone, acted as food for tolerant groups like chironomids, oligochaetes, platyhelminthes and gastropods and this increased the overall diversity. Considering that macroinvertebrate groups that dominated station K12 and their sensitivity to pollution range from moderate to high, their characteristic absence in stations K2 and K3 indicated heavy pollution. The lowest genera number at Station K3 is attributed to industrial and municipal wastes that reached their peak at the station. This caused a high abundance of few tolerant groups such as Bivalvia, Pulmonata, Oligochaeta, Hirudinae, and Platyhelminthes as compared to the orders Plecoptera, Hemiptera, Odonata, Coleoptera and Ephemeroptera, which dominated River Kisian stations.

In overall land use practices at the vicinity of sampling stations, like human settlement, industrial and municipal discharges, were the major factors that influenced water

quality and macroinvertebrate assemblage characteristics in the study area. Agriculture, direct watering of animals in the river and grazing also affected water quality and macro invertebrate assemblages but the response they elicited was less than that associated with urbanization. For instance, the relative abundance of the pollution sensitive EPT was high at stations K11 and K1, in an agricultural area, while the groups were replaced at stations K2 and K3, in an urban area, by tolerant groups like Mollusca and Oligochaeta. This response was a clear indication that macro invertebrate communities in the two rivers were good candidates for delineating effects caused by agriculture and urbanization on water quality and overall ecosystem integrity.

### **The M-IBI and metric performance**

In Kenya unpublished results have demonstrated the utility of macro invertebrate-based index of biotic integrity in assessing water quality and overall ecosystem integrity of rivers and streams in the upper reaches of Lake Victoria Basin. In order to develop a similar index for the lower catchments of the Lake that will capture the effects of land use practices, especially urbanization and its constituent impact types, on water quality and macro invertebrate assemblages, six of the ten metrics passed the selection criteria and were included in the final index.

The BMWP-ASPT biotic index is particularly important in assessing organic pollution (Armitage, 1983; Thorne and Williams, 1997). This metric was objectively selected for registering organic pollution in the streams and rivers which emanate from livestock grazing and watering, raw sewage disposal, industrial effluents and dumping of domestic wastes. In the two streams, the metric recorded low values below point sources of pollution and also registered non-point source pollutants from riparian agriculture. Considering these results, the biotic index shows promise as a tool for assessing and monitoring organic pollution, which is still a serious problem in Kenya. Most cities and upcoming towns lack sewage treatment facilities and the existing ones are either not functioning properly or are overwhelmed by a rapidly increasing human population. This is a case of Kisumu City where a sewerage facility initially constructed for a population of 50,000 people still serves the current population of more than 500,000 inhabitants.

The use of taxon richness is common in biological assessments (Collins et al., 2008; Kerans and Karr, 1994; Roy et al., 2003). In the upper reaches of Lake Victoria Basin, the metric has been used to discriminate between impacted and relatively unimpacted sites (Masese et al., 2009a; Raburu et al., 2009). Urbanization has been found to reduce macro invertebrate taxa richness in rivers and streams (Collins et al., 2008; Roy et al., 2003). Because of the diverse impacts that accompany urbanization, like sedimentation, industrial and municipal wastes, decline in taxa richness caused by elimination of

sensitive species is among the major responses of the community. This was the case in the study area as sites below discharge points, like K3, had low numbers of genera and stations minimally impacted, like K11, had higher numbers.

The relative abundance of EPT and pollution tolerant individual metrics performed well and separated different stations according to the level of degradation. The two metrics were inversely correlated with physico-chemical and nutrient parameters whereby percent EPT individuals metric was sensitive to poor water quality whereas percent tolerant individuals were not. Stations heavily impacted by discharges, like station K2 and K3, recorded zero EPT while high values were recorded at stations K11 and K1 indicating that they were less impacted. Contrary to expectations, station K1 recorded the highest abundance for EPT despite the effects arising from human activities witnessed in the area. However, only family Baetidae, which is the most tolerant of the EPT (Thorne and Williams, 1997; Buss et al., 2002; Masese 2009a, b), was recorded indicating that the station was suffering from intermediate levels of organic pollution.

There was an increase in relative abundance of tolerant individuals at adversely degraded stations K2 and K3, with total exclusion of the EPT. Non-insect individuals in the study area, especially oligochaetes and mollusca, responded more strongly to organic pollution, as it has been observed in other studies (DeShon, 1995; Kerans and Karr, 1994). Their high abundance at the two stations, receiving industrial wastes, domestic effluent and raw sewage, indicate that the groups can be used as indicators of poor water quality.

Among the functional metrics tested only percent gatherer genera met the test criteria. The highest numbers of gatherer genera were recorded at stations K2 and K3. These stations were below point sources of pollution receiving sewage and domestic wastes. On the other hand Station K11 in a relatively pristine area recorded the lowest number of gatherer genera. According to the River Continuum Concept (Vannote et al., 1980), dominance of gatherers at low order streams are expected to be due to accumulation of fine particulate organic matter (Cummins, 1988; Rabeni et al., 1985). Changes in land use that cause an increase in sediments and suspended inorganic solids hamper the scrapers and shredders more than gatherers, in turn increasing the number of gatherer genera (Wallace and Webster, 1996). To be effective, a multimetric system must incorporate metrics which accurately reflect the environmental changes of interest, and to be efficient, these metrics must not supply duplicate information (Barbour et al., 1992). The five metrics can be used to develop a multimetric system for low order streams in the lower catchments of Lake Victoria basin. Overall, there was good correspondence between the results of the multimetric index and the degradation gradient, which is a key ingredient in bioassessment (Karr, 1999). However, because of the steep pollution

gradient and lack of purely pristine conditions against which individual metrics could be tested, sites were grouped into three categories of good, fair and poor. To improve the utility and performance of the index, region specific modifications are needed for the BMWP-ASPT. This can be done by qualitative tolerance determinations for local macroinvertebrate fauna to a wide range of impact types, as opposed to organic pollution tests on which the index was initially based. Addition of metrics, after demonstration of their utility, could further improve the performance of the multimetric index. However, the multimetric index developed in this study is adequate for monitoring low order streams in urban, sub-urban and agricultural areas in the lower catchments of Lake Victoria Basin, Kenya.

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## REFERENCES

- APHA (American Public Health Association) (1998). Standard methods for the examination of water and wastewater. American Public Health Association, American Water Works Association, and Water Pollution Control Federation. Washington, D.C.
- Armitage PD, Moss D, Wright JF, Furse MT (1983). The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-water sites. *Wat. Res.* 17 (3):333-347.
- Barbour MT, Gerritsen J, Snyder BD, Stribling JB (1999). Rapid Bioassessment Protocols For Use in Wadeable Streams and Rivers: Periphyton, Benthic Invertebrates, and Fish (2<sup>nd</sup> edn). Report EPA 841-B-99-002. US Environmental Protection Agency, Office of Water, Washington DC.
- Buss DF, Baptista DF, Silveira MP, Nessimian JL, Dorville LFM (2002). Influence of water chemistry and environmental degradation on macroinvertebrate assemblages in a river basin in south-east Brazil. *Hydrobiologia* 481: 125-136.
- Chessman BC (1995). Rapid assessment of rivers using macroinvertebrates: a procedure based on habitat-specific sampling, family level identification and a biotic index. *Australian J. Ecol.* 20: 122-129.
- Collins CL, Mullen MW, Stewart PM, Webber EC (2008). Validation of an invertebrate community index for urban streams for an Alabama coastal plains watershed. *J. Am. Wat. Resour. Assoc.* 44(3):663-669.
- Cook SEK (1976). Quest for an index of community structure sensitive to water pollution. *Environ. Poll.* 11: 269-288.
- Cummins KW (1988). The study of ecosystems: a functional view. In: Pomeroy LR, Alberts JJ (eds) *Concepts of Ecosystem Ecology*, SPRINGER-verlag, New York. pp. 247-462.
- De Zwart D, Trivedi RC (1994). Manual on Integrated Water Quality Evaluation. Report 802023003, National Institute of Public Health and Environmental Protection (RIVM), Bilthoven, The Netherlands.
- Dobson M, Mathooko JM, Magana A, Ndegwa FK, (2002). Detritivores in Kenyan highland streams: more evidence for the paucity of shredders in the tropics? *Freshwat. Biol.* 47:909-919.
- Fore LS, Karr JR (1996). Assessing invertebrate response to human activities: evaluating alternative approaches. *J. Am. Benthol. Soc.* 15(2): 212-231.
- Griffith MB, Hill B, McCormick H, Kauffman R, Herlily T, Selle AR (2005). Comparative application of indices of biotic integrity based on

- periphyton, macroinvertebrates and fish to Southern Rocky Mountain streams. *Ecol. Indicators* 5: 117-136.
- ISO-BMWP (1979). Assessment of the biological quality of rivers by a macroinvertebrate score. ISO/TC147/SC5/WG6/N5, International Standards Organization.
- Iwata T, Nakano S, Inoue E, (2003). Impacts of past riparian deforestation on stream communities in a tropical rain forest in Borneo. *Ecol. Appl.* 13: 461-473.
- Johanson KA (1992). A catalogue of the caddisflies of East Africa (Insecta, Trichoptera). *Steenstrupia* 18:113-141.
- Johnson TC, Kelts K, Odada E (2000). The Holocene History of Lake Victoria. *Ambio* 29(1): 2-11.
- Karr JR (1981). Assessment of Biotic Integrity Using Fish Communities. *Fisheries* 6: 21-27.
- Karr, JR (1999). Defining and measuring river health. *Freshwat. Biol.* 41:221-234.
- Karr JR, Chu EW (1999). Restoring Life in Running Waters. Better Biological Monitoring. Island Press, Washington, D.C.
- Kasangaki A, Chapman LJ, Balirwa J (2008). Land use and the ecology of benthic macroinvertebrate assemblages of high-altitude rainforest streams in Uganda. *Freshwatat. Ecol.* 53: 681-697.
- Kerans BL, Karr JR (1994). A benthic index of biotic integrity (B-IBI) for rivers of the Tennessee Valley. *Ecol. Appl.* 4 (4): 768-785.
- Klemm DJ, Blocksom KA, Fulk FA, Herlihy AT, Hughes RM, Kaufmann PR, Peck DV, Stoddard JL, Thoeny WT, Griffith MB, Davis WS (2003). Development and Evaluation of a Macroinvertebrate Biotic Integrity Index (MBII) for Regionally Assessing Mid-Atlantic Highlands Streams. *Environ. Manage.* 31(5):656-669.
- Lung'ayia HBO (2002). Assessment of water quality using diatoms as bio-indicators in catchments of lake Victoria, Kenya. PhD thesis, Vrije universiteit Brussels. Faculty of science laboratory of general botany and nature management, Belgium.
- Macan TT (1977). A guide to freshwater invertebrate animals. Longman publishers, London.
- Masese FO, Raburu PO, Muchiri M (2009a). A preliminary benthic macroinvertebrate index of biotic integrity (B-IBI) for monitoring the Moiben River, Lake Victoria Basin, Kenya. *Afr. J. Aquat. Sc.* In press.
- Masese FO, Muchiri M, Raburu PO (2009b). Macroinvertebrate assemblages as biological indicators of water quality in the Moiben River, Kenya. *Afr. Journ. Aquat. Sc.* In press.
- Mathooko JM (2001). Disturbance of a Kenyan Rift Valley stream by daily activities of local people and their livestock. *Hydrobiology* 458: 131-139.
- Mathooko JM (1998). Mayfly diversity in East Africa. *Afr. J. Ecol.* 36:368-370.
- Merritt RW, Cummins KW (1996). An introduction to the Aquatic Insects of North America. Kendall/Hunt Publishing Company, Dubuque, IA.
- Miltner RJ, White D, Yoder C (2004). The biotic integrity of streams in urban and suburbanizing landscapes. *Landscape and Urban Planning* 69:87-100.
- Morgan RP, Cushman SF (2005). Urbanization effects on stream fish assemblages in Maryland, USA. *J. Am. Benthol. Soc.* 24:643-655.
- Ndaruga AM, George GN, Nathan NG, Wamicha WN, (2004). Impact of water quality on macroinvertebrate assemblages along a tropical stream in Kenya. *Afr. J. of Ecol.* 42: 208-216.
- Nilson A (ed) (1996). Aquatic Insects of North Europe. A taxonomic Handbook Vol 1. Ephemeroptera-Plecoptera-Heteroptera-Neuroptera-Megaloptera-Coleoptera-Trichoptera-Lepidoptera. Apollo Books Aps.
- Nilson A (ed) (1997) Aquatic Insects of North Europe. A taxonomic Handbook Vol 2. Odonata-Diptera. Apollo Books Aps.
- Ntiba MJ, Kudoja WM, Mukasa CT (2001). Management issues in the Lake Victoria watershed. *Lakes Reserv. Res. Manage.* 6:211-216.
- Pielou EC (1975). Ecological diversity. Wiley and Sons, New York.
- Quigley M (1977). Invertebrates of streams and Rivers: a key to identification. Edward Arnold Ltd. London.
- Rabeni CF, Davis SP, Gibbs KE (1985). Benthic invertebrate response to pollution abatement: structural changes and functional implications. *Wat. Resour. Bull.* 21: 489-497.
- Raburu PO, Masese FO, Mulanda CA (2009). Macroinvertebrate Index of Biotic Integrity (M-IBI) for monitoring rivers in the upper catchment of Lake Victoria Basin, Kenya. *Aquat Ecos. Health Manage.* 12 (2): 1-9.
- Roy AH, Rosemond AD, Leich DS, Paul MJ, Wallace JB (2003). Stream macroinvertebrate response to catchment urbanisation (Georgia, U.S.A.). *Freshwat. Biol.* 48:329-346.
- Scholtz CH, Holm E (1985). Insects of Southern Africa. Butterworths, Durban.
- Shannon CE, Weaver W (1949). The mathematical theory of communication. University of Illinois Press, Urbana, IL.
- Simpson EH (1949). Measurement of diversity. *Nature* 163: 688.
- Stepenuck KF, Crunkilton RL, Wang L (2002). Impacts of urban landuse on Macroinvertebrate communities in southeastern Wisconsin streams. *J. Am. Wat. Resour. Assoc.* 38:1041-1051.
- Stevenson RJ, Bailey BC, Harass MC, Hawkins CP, Alba-Tercedor J, Couch C, Dyer S., Fulk FA, Harrington JM, Hunsaker CT, Johnson RK (2004). Interpreting results of ecological assessments. Pages 85-111 in M. T. Barbour, S. B. Norton, H. R. Preston, and K. W. Thornton (editors). Ecological assessment of aquatic resources: linking science to decisionmaking. Society of Environmental Toxicology and Chemistry, Pensacola, Florida.
- Thorne RSJ, Williams WP (1997). The response of benthic macroinvertebrates to pollution in developing countries: a multimetric system of bioassessment. *Freshwat. Biol.* 37:671-686.
- Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE (1980). The River Continuum Concept. *Can. J. Fish. Aquat. Sci.* 37:130-137.
- Wallace JB, Webster JR (1996). The role of macroinvertebrates in stream ecosystem function. *Ann. Rev. Entomol.* 41: 115-139.
- Wang L, Lyons J (2003). Fish and Benthic Macroinvertebrate Assemblage as Indicators of Stream Degradation in Urbanizing Watersheds. In: Simon TP (ed) Biological Response Signatures: Indicator Patterns Using Aquatic Communities. CRC Press, Boca Raton, FL. pp. 113-120
- Ward JV, Stanford JA (1983). The Serial Discontinuity Concept of lotic ecosystems. Dynamics of Lotic Ecosystems. Ann Arbor Science, Collingwood, MI.
- Weigel BM, Henne LJ, Martinez-Rivera LM (2002). Macroinvertebrate-based index of biotic integrity for protection of streams in west-central Mexico. *J. Am. Benthol. Soc.* 21(4), 686-700.
- Wetzel RG, Likens GE (2000). Limnological analyses. Spinger-Verlag Inc. New York.
- Zar JH (2001). Biostatistical Analysis. Prentice Hall, Englewood Cliff, New Jersey