

Diatoms as Bioindicators in Monitoring Water Quality, River Sosiani, Kenya

Namwaya, David W.
University of Eldoret, Department of
Fisheries & Aquatic Science
namwayadavid@yahoo.com

Raburu, O. Philip
University of Eldoret,
Department of Fisheries &
Aquatic Science

Lubanga Lunaligo
University of Eldoret,
Department of Fisheries
& Aquatic Science

Abstract

The purpose of the study was to investigate the potential use of diatoms as biological monitors of environmental quality in Urban R. Sosiani in view of diatom distribution being affected by chemical, biological and physical characteristics of an aquatic system. In determination of Abundance, distribution and diversity of diatoms in relation to changing water quality variables, diatoms from benthic substrate in the riffle, littoral and the run with physical and chemical data were collected for a duration of six months from four strategically located sites covering a variety of anthropogenic activities along the river. Water quality parameters for instance total nitrogen whose changes are accounted for by the changing human activities but affecting diatom population were studied. During the study, a total of 9 diatoms genera unevenly distributed along the river were identified. In the upstream, the highest species diversity and relative abundance at 36.19% were recorded reducing to a relative abundance of 11.135% downstream. Pollution sensitive species; *Gomphenema*, *Navicula* and *Tabellaria* sp were identified in the upstream while pollution tolerant species among them *Melosira* and *Nitzschia* dominating downstream that was characterized with high nutrient levels. It is reputable that human activities influenced water quality along the river continuum accredited to the changing human activities and water quality parameters resulting to less species diversity downstream necessitating pollution management measures unavoidable.

Keywords: Diatoms, Diversity, Bio indicators

Introduction

Water from various aquatic systems worldwide is used for Agricultural, domestic and industrial purposes. The various uses water is subjected to results to changing its biological, chemical and physical characteristics. Despite the disturbances, there is a great need for good quality water throughout the world (Guy *et al.*, 2004) not only by man but other organisms either terrestrial or aquatic. The interference with the ecology of rivers has been exacerbated by increase in human population, industrialization and its associated pressure on aquatic resource (Ntiba *et al.*, 2001; Raburu, 2003; Njiru *et al.*, 2008). Anthropogenic activities identified to degrade water quality include; deforestation, agricultural activities and discharge of untreated waters from storm sewers (Njiru *et al.*, 2008) that alters the chemical, biological and physical characteristics of aquatic systems so influencing water utilization. Living organisms utilize water to a given set of limits beyond which they tolerate, migrate or die in case of aquatic organisms.

Monitoring water pollution in rivers has been approached by different methods (Lee *et al.*, 1967) with Chemical tests and other water chemistry data tests determining the quality of surface water (Raburu *et al.*, 2009) being used. Bio-monitoring using phytoplankton (Wu, 1985), fish (Karr, 1981) and macro invertebrates (Aura *et al.*, 2010a) has been used. Blue green algae dominates as a bio monitor in the phytoplankton but diatoms having a well-known ecological tolerance (Roger, 2005) ability to reflect the overall health of the ecosystem they inhabit, short generation time, sensitivity to changes in nutrient levels, diverse assemblages (de la Rey, 2004), limited ability of movement between habitats, ubiquitous nature, relative ease of identification and generally well-known aetiological relationships with driving forces such as pH (Whitmore, 1989), specific conductance (Pan & Stevenson, 1996) and nutrients (Winter & Duthie, 2000) make diatoms an important tool in monitoring and assessing aquatic resource condition.

Benthic algae distribution in a river is as a result of a complex series of interactions between hydrological, water quality and biotic factors (Rey *et al.*, 2004). Like other living organisms Diatom distribution can be affected by chemical, biological and physical characteristics of the environment they occupy. The goal of the study was to determine the diatom abundance, distribution and diversity along the river in relation to changing water qualities. The research objectives included: (i) Determining the abundance and distribution of diatoms along river Sosiani, (ii) Determining the diversity of diatoms, (iii) Relating changes in abundance and diversity of diatoms to changes in Physico-chemical water quality parameters. Such information is necessary for managing the urban river Sosiani and other similar river elsewhere in the World.

Materials and Methods

Study Area

River Sosiani ($0^{\circ}29'54''N$ $35^{\circ}17'58''E$), a tributary to R. Nzoia, is a medium-sized bed Rock river system discharging in Lake Victoria with a catchment in the Rift Valley. The area is characterized by several developments and other human-generated disturbance along the river, contributing a great deal of nutrients and organic pollution. The study having interest in nutrients and diatoms, four sites were strategically located along the river with the first station in Nyaru catchment area, the second within the agricultural zone before Two Rivers Dam with the third station in the heart of Eldoret town. The last station; Hurumais located at a point where Sewage wastewater treatment facility deposits its effluents to the river contributing a large amount of treated sewage to an already nutrient-rich river ecosystem.

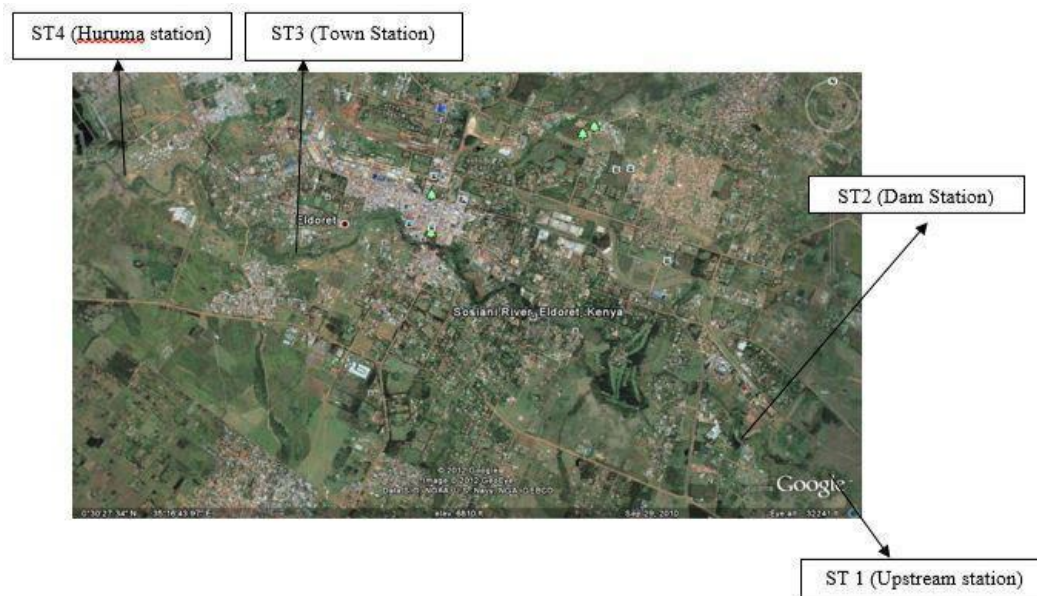


Plate 1. Study area (Source: Google map)

Methods

Between, October 2011 and April 2012 Diatoms were collected monthly using the 'First Approach' method according to Round, 1991 involving direct sampling of natural substrate in the stream then preserved for laboratory identification. Five rocks or substrata (surface area $\leq 15 \text{ cm}^2$) were collected per station with a least five substrate from the riffles, the run and the littoral. Using a tooth brush as described by Kelly *et al.* (1998), the upper surface was scrubbed for diatoms then washed into 100ml plastic bottles using river water flowed by fixation in 0.1 ml Lugol's solution. After sedimentation for 24 hours and concentration to 50 ml, numerical identification was carried out using a compound microscope (Olympus-BHS, Japan; 400X magnification) from a Sedwick-Rafter cell. Diatoms identification was done using Sanet *et al.* (2006) keys.

Dissolved oxygen was determined and analyzed using the Wrinkler's method (Bryan *et al.*, 1976), soluble reactive phosphorus (SRP) using ascorbic acid method while levels of total nitrogen determined using Kjeldahl digestion method as described in APHA 1998. TN and SRP were considered in the study as they are indicators of human-generated organic pollution. Other water quality parameters were measured *in-situ*

Statistical Analysis

With a goal of examining samples for statistical significance, raw data was entered into Microsoft excel spread sheet from which Diatom abundance was obtained using the formula:

$$RA = \frac{n}{N} \times 100$$

Where: n= individual species

N= Total number of diatoms observed

Species diversity was worked out using Shannon and Weaver (1983) index H' calculated using

$$H' = -\sum P_i \ln P_i \text{ Where}$$

P_i = Proportion abundance of i^{th} species in a population

P= Total number of individual in a population

Significance difference between and within the stations was calculated through Minitab 14 program.

Results

Diatom Distribution and Abundance

Of the nine diatom taxa identified, *Melosira spp.*, *Nitzschia spp.* and *Eunotia spp.* were observed from all the four stations and within all the microhabitats. *Gomphenema spp.*, *Synadara spp.*, *Navicula spp.*, *Cycotella spp.*, *Cymbella spp.*, and *Tabellaria spp.* were identified in small numbers in the upper stations only.

Table 1. List of diatoms identified from Sosiani River within the various stations

Taxa name	ST1			ST2			ST3			ST4		
	Z1	Z2	Z3	Z1	Z2	Z3	Z1	Z2	Z3	Z1	Z2	Z3
<i>Alacosira.</i>	*	*	*	*	*	*	*	*	*	*	*	*
<i>Nitzschia.</i>	*	*	*	*	*	*	*	*	*	*	*	*
<i>Eunotia.</i>	*	*	*	*	*	*	*	*	*	*	*	*
<i>TabellariaSpp.</i>	*	—	—	—	*	—	—	—	—	—	—	—
<i>Cymbella.</i>	*	—	*	*	—	*	—	—	—	—	—	—
<i>Cycotella.</i>	*	*	*	—	—	—	*	—	*	—	—	—
<i>Navicula.</i>	*	*	—	*	*	—	—	—	*	—	—	—
<i>Synadara.</i>	*	*	—	—	—	—	*	—	—	—	—	—
<i>Gomphenema.</i>	—	—	—	—	—	—	*	—	—	—	—	—

(* Indicates diatom presence, ST1=Upstream station, ST2= Two rivers Dam station, ST3= Town station, ST4=Huruma station, Z1=Littorall, Z2=Run, Z3=Riffle)

The upper station (ST1) registered the highest mean number of diatoms per volume at 22 ± 1.9 diatoms per ml while the last station (ST4) recording the lowest mean number of diatoms at 7 ± 1.2 diatoms per ml of water (Table 2).

Table 2. The mean $\pm$ SE number of diatoms per ml of sampled water from the sampling stations during the study period

Diatom	ST1		ST2		ST3	ST4	
<i>Melosira</i>	7.17	± 1.34	9.73	± 0.32	3.83 ± 0.07	2.10	± 0.10
<i>Nitzschia</i>	8.20	± 0.98	4.10	± 0.56	4.90 ± 0.10	2.50	± 0.23
<i>Eunotia</i>	3.37	± 0.09	4.20	± 1.23	3.80 ± 0.30	1.93	± 0.15
<i>Tabellaria</i>	0.03	± 0.03	0.00	± 0.00	0.00 ± 0.00	0.00	± 0.00
<i>Cymbella</i>	0.07	± 0.03	0.07	± 0.03	0.00 ± 0.00	0.00	± 0.00
<i>Cycotella</i>	0.07	± 0.03	0.00	± 0.00	0.07 ± 0.03	0.00	± 0.00
<i>Navicula</i>	0.03	± 0.03	0.07	± 0.03	0.00 ± 0.00	0.00	± 0.00
<i>Synadara</i>	0.07	± 0.03	0.00	± 0.00	0.07 ± 0.03	0.00	± 0.00
<i>Gomphenema</i>	0.00	± 0.00	0.00	± 0.00	0.00 ± 0.00	0.00	± 0.00

Along the river, the littoral zones recorded the highest diatom numbers while the riffle registering the lowest total numbers (Fig. 1). Diatom abundance of 14.87 diatoms per ml in the littoral was the highest followed by the run at 13.69 diatoms per ml while the riffle having the lowest abundance at 12.63 diatoms per cm^2 as shown below

Along the river, the littoral zones recorded the highest diatom numbers while the riffle registering the lowest total numbers (Fig.1). Diatom abundance of 14.87 diatoms per ml in the littoral was the highest followed by the run at 13.69 diatoms per ml while the riffle having the lowest abundance at 12.63 diatoms per cm^2 as shown below.

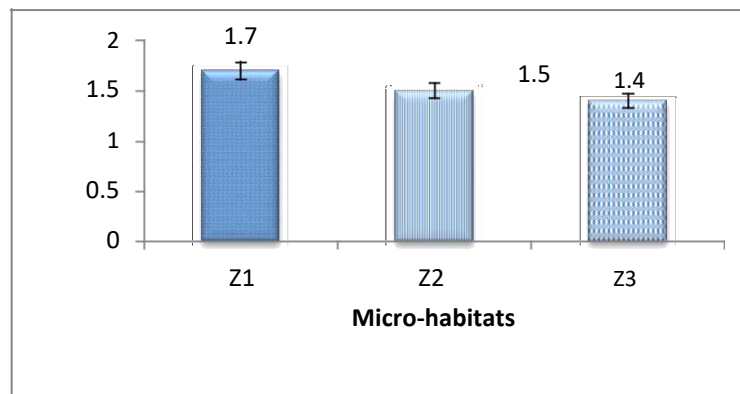


Figure 1. Abundance of diatoms in different macro-habitats in R. Sosiani (Z1 $\equiv$ Littoral, Z2 $\equiv$ Run and Z3 $\equiv$ Riffle)

Table 3. Average number of diatom as per the river zones

	<i>Littoral</i>	<i>Run</i>	<i>Riffle</i>
<i>Melosira</i>	6.17 $\pm$ 1.73	5.72 $\pm$ 1.71	4.82 $\pm$ 1.90
<i>Nitzschia</i>	4.82 $\pm$ 0.96	5.06 $\pm$ 1.19	4.55 $\pm$ 1.64
<i>Eunotia</i>	3.71 $\pm$ 0.83	2.78 $\pm$ 0.66	3.16 $\pm$ 0.61
<i>Tabellaria</i>	0.04 $\pm$ 0.03	0.00 $\pm$ 0.00	0.00 $\pm$ 0.61
<i>Cymbella</i>	0.04 $\pm$ 0.02	0.02 $\pm$ 0.01	0.05 $\pm$ 0.03
<i>Cycotella</i>	0.02 $\pm$ 0.01	0.04 $\pm$ 0.02	0.04 $\pm$ 0.02
<i>Navicula</i>	0.03 $\pm$ 0.01	0.04 $\pm$ 0.02	0.00 $\pm$ 0.00
<i>Synaderra</i>	0.04 $\pm$ 0.02	0.02 $\pm$ 0.01	0.01 $\pm$ 0.00
<i>Gomphenema</i>	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00

Melosira and *Nitzschia* accounted for 75.42% of the total abundance but *Melosira* dominating in all the stations at the rate of 23 organisms per ml of water then *Nitzschia* at the rate of 19 diatoms per ml.

A high relative mean abundance for *Melosira* (40.46%) was noted from all stations as compared to the rest with a high mean relative abundance in the run (Z2) at 41.90%. The highest abundance was observed in Two Rivers Dam station (ST2) with a mean relative abundance of 53.6 % which kept reducing along the river with the lowest mean relative abundance of 32.10% in Huruma.

Nitzschia, the second most abundant genera with a high mean relative abundance of 34.97% in general. The upstream (ST 1) recorded an abundance of 43.10% as compared to 22.70%, 38.70% and 28.03% in ST2, ST3 and ST4 respectively. Z2 recorded the highest abundance at 36.83 % in the microhabitats. Distribution of the major abundant genera in the river is as shown in below.

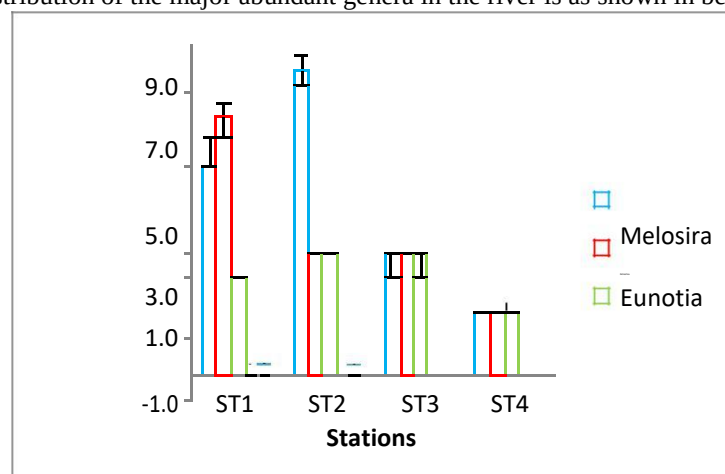


Figure 2. Diatom distribution and abundance of most abundant genera in R. Sosiani for six months
The other seven taxa had individual mean relative abundance of less than 25% with *Gomphenema* registering the lowest combined relative mean abundance from sampled stations at 0.02%.

The relative abundance of *Synaderra* was the same in ST1 and ST2 but reduced drastically in ST3 & ST4. *Cymbella* and *Cycotellahada* relative abundance of 0.4% in ST1 reducing to 0.3% in ST2 then to 0 in the subsequent stations while *Navicula Sp.* maintained a low mean relative abundance with less variation from the stations.

Diversity

Species diversity varied downstream and within the zones (Figure 3).

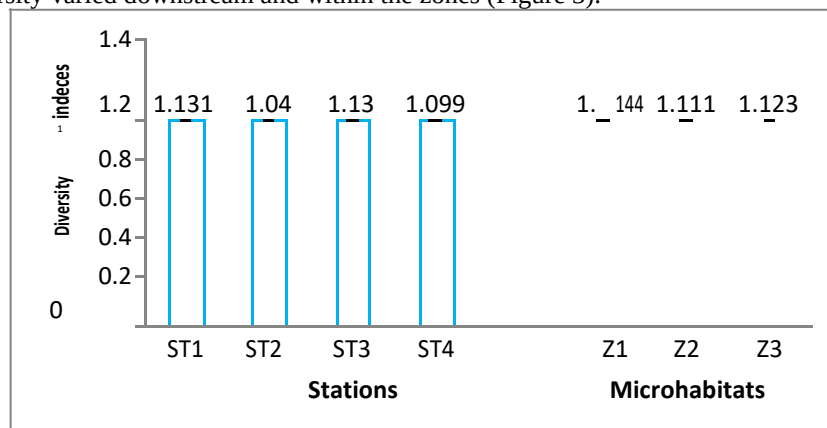


Figure 3. Shannon & Weiner diversity index for diatoms along R. Sosiani within the stations and micro-habitats

Relationship between Diatom Species Abundance and Water Quality

Major water quality variables varied as in table below.

Table 5. Water quality changes along the river

Site	TN	TP	DO	BOD
ST1	0.300	0.0198	4.107	2.744
ST2	0.367	0.0325	3.883	2.576
ST3	0.525	0.0395	3.304	2.277
ST4	0.625	0.0645	5.413	2.800

A positive correlation in water quality variables and sampled sites was observed with nutrients whose concentration directly affect growth and distribution of phytoplankton. TP concentration were statistically significant from within the study stations and showed a positive correlation ($R^2=0.938$) as shown in graph below.

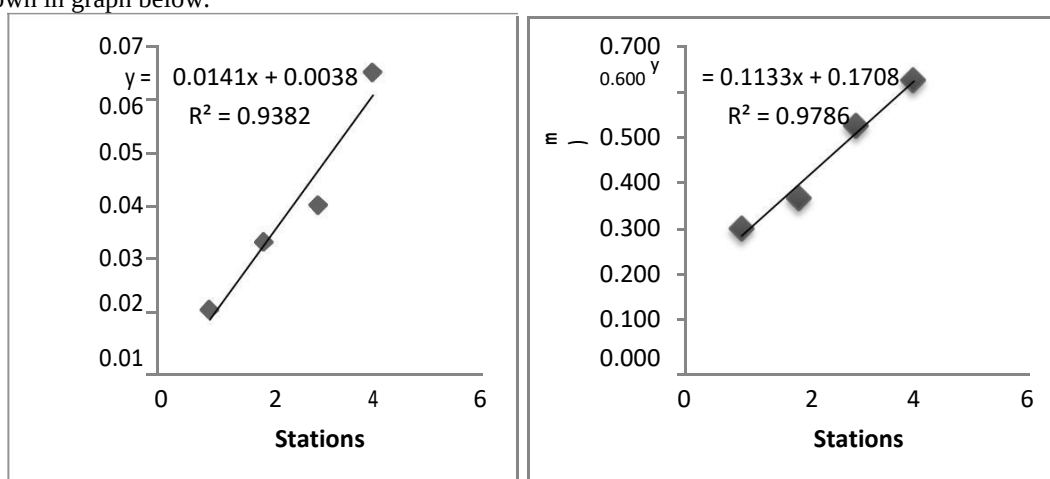


Figure 7. Total phosphate and total nitrogen concentration along R. Sosiani

Nitrates concentrations increased downstream with a positive correlation of $R^2=.98$ observed between stations (Fig. 7). The other water quality variables including Total dissolved solids, Dissolved Oxygen and Biological Oxygen Demand also showed a positive correlation an indication that the stations location directly affected the levels of water quality parameters.

Multivariate analysis for *Melosira* recorded a variance of 5.62 accounting for 62.5% of the total variance showing a negative correlation within all the stations for water quality parameters apart from

TDS. *Nitzschia* had a positive correlation with DO and BOD but negative with the other water quality parameters. This indicates that the different diatoms are affected by a specific water quality.

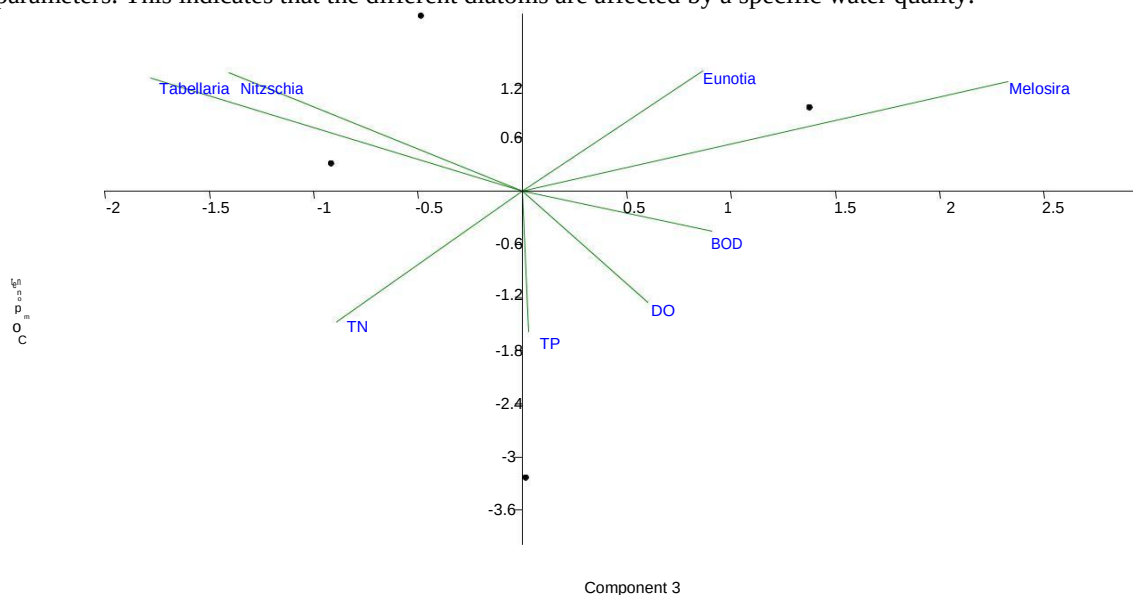


Figure 9. Principle component of the diatoms and water quality

Discussion

Diatom Distribution and Abundance

A variety of phytoplankton is found in most aquatic environment (Kelly *et al.*, 1998) or damp habitats. In Upstream station, diatom abundance was highest as compared to downstream stations a situation accounted for by low water quality parameters termed suitable for a wide variety of diatoms to survive. Water quality parameters were low in ST1 with SRP and TN reaching the highest levels in ST4 meanwhile Low diatom abundance was observed downstream with most pollution tolerant species for instance *Melosira* and *Nitzschia spp.* observed. With increasing water pollution, diatom abundance is altered in species structure composition (Wu, 1996) and reduces diatom abundance. It has been identified that nutrient concentration is one of factors shaping the abundance and distribution of diatoms (Bere *et al.*, 2009)

Melosira and *Nitzschia* species were observed from all stations and microhabitats. They are tolerant diatom species to water pollution (Wu, 1985) with their abundance only decreasing with increasing pollution. Their ability to withstand a polluted aquatic environment makes it possible to get them in these environments a reason why these species are identified although in small numbers in ST4. Huruma station is considered polluted compared to other stations after recording the highest nutrient levels enhanced by deposited effluents from the Sewage wastewater treatment facility contributing a large amount of treated sewage to an already nutrient-rich river ecosystem from agricultural activities along the river.

Gomphenema and *Tabellaria* species (less tolerant to adverse water quality parameters) were only identified in ST1; station with low nutrient levels. Due to their sensitivity, they are only found in small numbers as water quality parameters continues to increase downstream. The distribution pattern of diatoms in water habitually indicate the type of environment they inhibit (Wu, 1980).

Diatom Diversity

Suitable water quality conditions supporting a wide range of diatoms lead to high species diversity in ST1 with increasing nutrients levels and river load noted in the river continuum resulting to reduction of species diversity. It has been illustrated that a decrease in species diversity is accounted for by increasing water pollution (Rey *et al.*, 2004). The low Shannon Index of diversity (1.099) from ST4 is as a result of the high nutrient levels conquering with what Hillbrand and Sommer (2000) found out that a decrease in diversity are directly affected by increase in nitrate and phosphate levels. The levels reduce tolerant species with the sensitive species missing completely from nutrient elevated sites.

Water Quality

Water quality changed downstream with human activities ranging from agricultural activities to industrial. Among activities affecting water quality included direct animal watering with Cattle grazing

damaging river ecosystems through erosion, loss of riparian cover, nutrient enrichment and loss of stream habitat (Armour, Duff & Elmore, 1994; Fleischner, 1994), car washing, laundry and bathing contributing to nutrient increase due to detergents. These activities directly or indirectly affect the characteristics of the riparian ecosystem contributing to the high turbidity and solids in the river. Industrial discharge in the river also introduces a variety of pollutants.

Phosphate concentration increased downstream doubtless due to the use of inorganic fertilizers from the several farming activities along the river. Runoffs from agricultural land accompanied by erosion increases the potential for soil and nutrients including phosphates into rivers (Lung'ayia, 2002). Phosphate concentrations also increased in a river as a result of effluents from industrial discharges and sewage discharges as observed from the results obtained during the study.

Nitrates concentration increased downstream as a result of increased usage of inorganic fertilizers by farmers to increase yield. The sharp change in TN concentration noted from ST 2 and the subsequent stations is as a result of the change in human activities from farming to industrial activities that could realize in the river from its discharges. The upstream station with less human activities reducing load discharge to the river had the lowest total dissolved solids compared to other stations. The area is also covered by good riparian vegetation reducing or controlling the load flowing in to the river through surface runoff. ST2 located in an agricultural zone recorded the highest TDS levels as the stream load increased through erosion due to poor soil conservation activities seen around the area. Watering of the animals at this station also contributed to the increased load as a result of the animal activities. Decrease in TDS level in ST3 from ST2 is clarified by the change of anthropogenic activities from farming to industrial. River load reduction also reduced as it flows through the Two Rivers Dam where most of the load is deposited as it continued to flow on a rock bed in ST4.

High DO level in ST4 is accounted for by the increased velocity of the river as a result of elevated river terrain and the rocky bed rock resulting to the river water mixing so increasing dissolved oxygen levels; high flows and turbulence increases the potential of re-aeration and saturation of water with oxygen (Jeffries & Derek, 1990). The high BOD in ST4 is due to increased biological activities in the water enhanced by sewage plant located above the sampling station. Town station had the lowest DO levels as compared to the rest of the stations. The river at this station has less or rather little riverine cover and increased discharge from industrial activities exposing the river to high temperature which then reduces the amount of dissolved oxygen in the river.

Relationship between Diatom Species Abundance and Water Quality

A positive correlation ($r=0.938$) of diatoms and phosphates was obtained during the study. The levels of nutrients affect diatom distribution and availability with distribution determined by the specific tolerance to polluted water (Lavoie *et al.*, 2007). High nitrogen concentration has been associated with the changing abundance in pollution sensitive diatoms (Patrick & Reimer, 1996).

Conclusion and Recommendations

Abundance and distribution of diatoms is directly related to the changing water quality downstream qualifying the usage of diatoms as indicator of levels of pollution. The various human activities along the river should be done carefully considering the riparian vegetation to reduce nutrient enrichment to the ecosystem.

References

- Armour C.L., Duff D.A. & Elmore W. (1994). The effects of livestock grazing on riparian and stream ecosystems. *Fisheries*, 19, 9–12.
- Aura, C. M., Raburu, P. O., & Hermann, J. (2010). A Preliminary Macroinvertebrate IBI for bioassessment of the Kipkaren and Sosiani Rivers, Nzoia River Basin, Kenya. *Lakes and Reservoirs*, 15(2), 119-128.
- Bere, T., & Tundisi, J. G. (2009). The role of diatoms change. *Environmental Science and Technology*, 26, 23-33.
- Bryan, J. R., Riley, J. P., Williams, (1976). A Winkler procedure for making precise measurements of oxygen concentration for productivity related studies. *Exp Mar. Biol. Ecol*, 21, 191-197.
- De La-Rey, P. A., Taylor, J. C., Laas, A., Rensburg, L., & Van, V. A. (2004). Determining the possible application value of diatoms as indicators of general water quality: a comparison with SASS 5. *Water SA*, 30(3), 325-332.
- Fleischner T.L. (1994) Ecological costs of livestock grazing in western North America. *Conservation Biology*, 8, 629–644.
- Guy, B., Pat, S., & Janine A. (2004). A water quality index for use with diatoms in the assessment of rivers. University of Port Elizabeth, Port
- Hillebrand, H., & Sommer, U. 2000b. Diversity of benthic microalgae in response to colonization time and eutrophication. *Aquat. Bot.* 67: 221-236.
- Jeffries, & Derek, (1990).
- <https://www.google.co.ke/maps/@0.514827,35.2707074,15z>

- Karr, J. R. (1981). Assessment of biotic integration using fish communities. *Fisheries*, 6(6), 21–27.
- Kelly, M. G. (1998). Use of community-based indices to monitor eutrophication in European rivers. *Environ. Conserv.*, 25(1), 22–29.
- Lavoie, I., Campeau, S., Grenier, M., & Dillon, P. (2006). A diatom-based index for water quality assessment in eastern Canada: an application of Canonical Analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, 63, 1793–1811.
- Lee, C. D., Kuo, C. L., & Wang, S. P. (1967). Benthic macroinvertebrates and fish as biological indicator, with reference to the community diversity index. *Sci. Technol. Rep Taiwan Poll. Contr. Agency*, 1, 1-44.
- Lung'aya, H.B. (2002). Assessment of water quality using Diatoms as bio-indicators in catchment of Lake Victoria, Kenya. PhD thesis, Virje Universitert Brussels. Faculty of Science laboratory of general botany and nature management, Belgium.
- Njiru, M., Kazungu, J., Ngugi, C. C., Gichuki, J., & Muhoozi, L. (2008). An overview of the current status of Lake Victoria fishery: Opportunities, challenges and management strategies. *Lakes Reserv. Res. Manage.*, 13, 1-12.
- Ntiba, M. J., Kudoja, W. M., & Mukasa, C. T. (2001). Management issues in the Lake Victoria watershed. *Lakes Reserv. Res. Manage.*, 6, 211-216.
- Pan, Y., & Stevenson, R. J. (1996). Gradient analysis of diatom assemblages in Western Kentucky Wetlands. *Journal of Phycology*, 32, 222-232.
- Patrick, R., & Reimer, C. W. (1966). *The Diatoms of the United States*, vol. 1. Monographs of the Academy of Natural Sciences of Philadelphia.
- Raburu, P. O., Masese, F. O., & Christopher, A. M. (2009). *Macroinvertebrate Index of Biotic Integrity (M-IBI) for monitoring rivers in the upper catchment of Lake Victoria Basin, Kenya*. Taylor & Francis London. pp 197-206.
- Roger, J. F. (2005). *A review of diversification trends in diatom research with special reference to taxonomy and environmental applications using examples from Lake Baikal and elsewhere*. University College.
- Sanet, J. V., Jonathan, T., Carin, V. G., & Annelise, G. (2006). *Easy identification of most common freshwater algae. A guide for the identification of microscopic algae in South Africa fresh waters*. ISBN 0-621-35471-6.
- Shannon, C. E., & Weaver, W. (1948). *The mathematical theory of communication*. Univ. Illinois Press Urbana III.
- Whitmore, T. J. (1989). Florida diatom assemblages as indicators of trophic state and pH. *Limnol. Oceanogr.*, 34(5), 882-895.
- Winter, J. G., & Duthie, H. C. (2000). Epilithic diatoms as indicators of stream total N and total P concentration. *Journal of North American Benthological Society*, 19(1), 32-49.
- Wu J.T.(1996). Relation of change in River diatom assemblages to water pollution: Institute of Botany, Academia Sinica Nankang, Taipei, Taiwan 11529, Republic of China.
- Wu, J.T. & Suen, W.C (1985). Change of algal associations in relation to water pollution. *Bol. Bull. Academia Sinina* 26:203-212.