2015-09

Development of a suitable pragmatic and cost effective biomonitoring method for assessing water pollution in tropical African rivers

Elias, Julius Daud

NM-AIST

https://doi.org/10.58694/20.500.12479/224

DEVELOPMENT OF A SUITABLE PRAGMATIC AND COST EFFECTIVE BIOMONITORING METHOD FOR ASSESSING WATER POLLUTION IN TROPICAL AFRICAN RIVERS

Julius Daud Elias

A Dissertation Submitted in Partial Fulfilment of the Requirements for the Doctoral Degree in Environmental Science and Engineering of the Nelson Mandela African Institution of Science and Technology.

Arusha, Tanzania.

September, 2015

ABSTRACT

The needs of tropical African countries to develop their own biomonitoring indices using local macroinvertebrates as opposed to adoption or relying on non-tropical indices in biomonitoring programmes for assessing freshwater pollution were discussed. The discussion build-up involved: (i) reviewing of existing indices from non-tropical regions and their adoption setbacks in assessing pollution in tropical African rivers; (ii) testing of key review findings in the field at a small scale and then at a wide scope by characterizing macroinvertebrates while examining environmental variables; (iii) assigning pollution sensitivity ratings of macroinvertebrates based on related scoring systems and the help of PRIMER7 software; (iv) determination of macroinvertebrates response towards nutrients loading, and; (v) developing suitable and cost-effective biomonitoring method. The review paper, pilot and detailed studies and toxicity test study were aimed at laying the groundwork needed to develop a simple, quick and advanced tropical biomonitoring tool for initial application on Tanzanian rivers.

Collectively, ninety seven macroinvertebrate families belonging to seventeen orders were observed at 85 sampling stations, representing 46 reference and 39 monitoring sites along Tanzanian rivers. All observed orders were subjected to six stepwise criteria to identify the one with a potential of discriminating reference from monitoring sites. The criteria include: numerical truncate test, Mann-Whitney test, inter-quartile overlap levels in Box-and-Whisker plots, Spearman rank correlation (r_s) analysis, more diverse order (with > 10 taxa) and validation test. Ephemeroptera (E), Diptera (D), Odonata (O) and Trichoptera (T) orders that comprised the 55% (N = 97) of all Tanzanian families met all test criteria after being found with abundances > 2% upon truncate test, a p-value < 0.05 in a Mann-Whitney U test, a sensitivity score of 3, r_s < 75% (with $p < 0.005$), higher number of taxa (n > 10) and separating reference from monitoring sites and thus, chosen to develop EDOT index. Being developed using only four and most diverse local orders, minimizes data variability, needs for great expertise and time in the field, the credibility that is not hitherto possessed by existing indices.

DECLARATION

I, **Julius Daud Elias** do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Name and signature of candidate	**Date**

The above declaration is confirmed

Name and signature of supervisor 1	**Date**
Name and signature of supervisor 2	**Date**

COPYRIGHT

CERTIFICATION

The undersigned certifies that he has read and hereby recommend for acceptance by the Nelson Mandela Institution of Science and Technology a dissertation titled, ***Development of a Suitable Pragmatic and Cost Effective Biomonitoring Method for Assessing Water Pollution in Tropical African Rivers***, in fulfilment of the requirements for the Doctoral Degree in Environmental Science and Engineering (EnSE) of the Nelson Mandela Africa Institution of Science and Technology.

Name and signature of supervisor 1	**Date**
Name and signature of supervisor 2	**Date**

ACKNOWLEDGEMENTS

Writing a scientific report such as this is an incredible journey from start to finish, and along the path there individuals who have played a vital role. I am therefore wish to express my sincere gratitude to my God and all people who have contributed to the accomplishment of this research work in different ways.

First and foremost, I am thankful to my Almighty God for all his provision and grace.

To my wife (Felister) and our son (Bryan) and daughter (Karen) for love, patience, and encouragement during busy and frustrating time. Felister…..darling, I do appreciate for everything.

Heartfelt thanks are directed to my parents (Mr. and Mrs. Elias) and guardians (Mr. and Mrs. Mselle) for being part of my life and for their greatest support in my long education journey.

I would like to express my sincere gratitude to my supervisors Prof. Y. D. Mgaya and Dr. J. N. Ijumba, for accepting and taking the responsibility of supervising my research work. They even managed to turn some of my immature thoughts into scientific work.

Much appreciation should also go to Dr. Kithongo King'ondu, Dr. F. A. Mamboya, Dr. L. T. Kaaya and Dr. D. S. Kacholi for being considerate and friendly mentors.

I am grateful to the Commission for Science and Technology (COSTECH), Tanzania, through the Nelson Mandela scholarship, for funding my Ph.D. studies. Without this financial support, this work would have remained a dream to me. I also express my gratitude to the Department of Aquatic Sciences and Fisheries, University of Dar es Salaam for providing laboratory space and facilities to analyze water and macro-benthos samples.

Lastly, to Mr. Stephen Mselle, Mr. Amos Lugata, Eng. Martin G. Mkenda, Mzee Peter, Ms. Bertha Mrisha, Mr. Patrick and the people near the sampling sites, for their greatest support.

DEDICATION

With love and respect, I would like to dedicate this work to my family and my late grandfather Costantine Rikanga Mchau for endless love, encouragement, support and prayers.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS

B-IBI	Benthic Index of Biological Integrity
BMWP	Biological Monitoring Working Party
COSTECH	Tanzanian Commission for Science and Technology
EDOT	Ephemeroptera, Diptera, Odonata and Trichoptera
EPT	Ephemeroptera, Ptecoptera and Trichoptera
NM-AIST	Nelson Mandela African Institution of Science and Technology
SASS	South African Scoring System

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background Information

Tropical African rivers are subject to the most pressing requirement for improved attention to sustainable use due to the rapidly increasing anthropogenic pressure that threatens their ecological and socio-economic values (Karr and Chu, 1999; Revenga and Kura, 2003; Ellison, 2004). Deterioration of freshwater quality in tropical countries has reached alarming proportions (Hall *et al*., 2000; Jamil, 2001; Revenga and Kura, 2003) which might affect the capacity of riverine systems to support its biodiversity (Rosenberg and Resh, 1993; Malmqvist and Rundle, 2002; Boyle and Fraleigh, 2003) due to improper land use practices near river channels (Karr and Chu, 2000; Böhmer *et al*., 2004). Slopes of river banks are often perceived by man as fertile and productive lands suitable for agriculture (Elias, 2009). Similarly, man has considered freshwater bodies as a place to dump industrial, agricultural and domestic wastes because it is cheap and convenient (Hall *et al.,* 2000; Karr and Chu, 2000; Jamil, 2001; Böhmer *et al.,* 2004).

Overexploitation, improper land uses and regulation of freshwater resources have caused significant changes in the flow regimes of rivers while altering negative impacts on the environment and loss of ecosystem functioning (Hart, 1994). The discharge of pollutants with excessive nutrients and toxic substances, as well as destructive land use practices near and/or into freshwater ecosystems can impair water quality (Elias, 2009). In Tanzania, for example, Themi (near Arusha town), Karanga, Njoro and Rau (near Moshi town in Kilimanjaro) and Mzinga, Msimbazi, Yombo, and Kizinga (in Dar es Salaam) rivers were all found polluted by urban-based industrial and domestic wastes (Ak'habuhaya and Lodenius, 1988; Mkuula, 1993; Ngana, 2003; Laveque and Balian, 2005). Presence of human induced stressors (such as pollution, habitat destruction and hydrological alterations) can directly impact freshwater habitat by significantly changing the biotic integrity and functional ability of a vast number of riverine ecosystems (Zedler and Kercher, 2005; Verhoeven *et al.,* 2006).

Owing to these concerns, the *Dublin Principles and Rio Declaration on Environment and Development,* was enacted in 1992 as the first conservation turning point. The declaration agenda emphasized the need for all nations to conserve ecosystems and protect water

resources against pollution. Similarly, conservation efforts at national level focus on developing and implementing national laws and policies relating to conservation, protection, and sustainable use of fresh water resources while discouraging any sort of aquatic pollution (McCaffrey and Weber, 2005). Several African countries, for example, are actively addressing water conservation obligations through their respective water jurisdictions (i.e., South African Water Act, 1998; Zimbabwe Water Act, 1998; Kenya Water Act, 2002; Namibia Water Resources Management Act, 2004; Tanzania Water Resources Management Act, 2009; Uganda Water Resource Management Act, 2009 etc.) with emphasis on aquatic ecosystems. However, most of these legislations have set ecological quality objectives and demand long term status of water quality based on monitoring of the structure and functioning of aquatic ecosystems (Barbour *et al.,* 2000; Wente, 2000).

The second turning point was the introduction of the concepts and principles of local macroinvertebrates based biomonitoring indices in 1970s to complement traditional method of water quality assessment, which mainly was relying on physico-chemical aspects. Such new concepts were introduced after traditional methods have been hampered by lack of clear standards against which to judge the degree of environmental degradation and their inability to detect biological stressors that occurred over time and at multiple scales (Elias, 2009). Moreover, the biomonitoring application has ignored tropical African regions in favour of modifying and adopting the existing non-tropical indices (Elias *et al*., 2014a). Nonetheless, the indices are normally developed for specific regions in order to account for regional variation using local macroinvertebrates that exhibit regional variation based on organism's sensitivity or tolerance. Such variations might affect the capability, functioning, and reliability of the biomonitoring indices developed for non-tropical regions when applied in tropical rivers. Likewise, modification of non-tropical biomonitoring indices for use in tropical regions is usually hindered by incomplete taxonomical resolution and the barely known sensitivity levels of many tropical taxa (Jacobsen *et al*., 2008; Elias *et al*., 2014a). As a means of bridging this gap in knowledge, the present research has investigated macroinvertebrate assemblages in Pangani and Wami-Ruvu basins in Tanzania and developed suitable pragmatic and cost-effective biomonitoring index for assessing water pollution in Tropical African rivers. Preference was made in which only orders with frequently occuring macroinvertebrate species that offer a wide range of tolerance or sensitivity to pollution were used. In that regard, only four orders (Ephemeroptera (E),

Diptera (D), Odonata (O) and Trichoptera (T)) out of 17 recordings were used to develop species level EDOT biomonitoring index.

1.2 Statement of the Problem

In tropical African regions, research on the taxonomical status and trends of freshwater macroinvertebrates in rivers has not been given much attention. This has hindered the potential use of local benthic macroinvertebrates in developing advanced and less sophisticated biomonitoring indices that can accurately evaluate the integrity of tropical aquatic ecosystems. As a result, complex indices that have been developed inconsistently in different ecoregions of non-tropical region using their local macroinvertebrates are adopted and used for assessing pollution in tropical rivers. Presence of geographical differences between tropical and non-tropical countries may however contribute to differences in the physical and chemical characteristics of rivers between the regions. Consequently, these differences can as well lead to variation in macroinvertebrate taxa composition and their sensitivity levels to disturbance and general ecosystem impairment from one geographical region to another. Ecological and taxonomical variations between the regions might also affect the capability, functioning, compatibility and reliability of the existing non-tropical biomonitoring indices when opted and applied in tropical rivers. In that regard, there is a risk of having unreliable findings when non-tropical biomonitoring indices are applied or adopted to assess water pollution in tropical rivers. Apart from failing to minimize data variability, the existing indices also require users with great expertise and much time in the field as they had been developed by including all families of the identified orders. Such reasons call for user friendly and cost-effective tropical indices to be developed using local families of only most diverse orders to minimize data variability, needs for great expertise and time in the field, the credibility that is not hitherto possessed by existing indices.

1.3 Objectives

1.3.1 General Objective

The main objective of this study was to develop a suitable pragmatic and cost-effective biomonitoring tool for assessing water pollution in tropical African rivers.

1.3.2 Specific Objectives

The general objective was broken into four specific objectives as follows:-

(a) To characterize macroinvertebrate communities and environmental variables along Pangani and Wami-Ruvu river basins;

(b) To assess the comparability of the two river basins using characterized macroinvertebrate communities and environmental variables;

(c) To examine the response of some key macroinvertebrate organisms towards different concentration levels of selected nutrients (NO_3-N and PO_4-P), and;

(d) To develop a biomonitoring tool using key tropical macroinvertebrate species for water pollution assessment in tropical river basins.

1.4 Hypotheses

The following hypotheses were formulated to address the above specific objectives:

(a) Environmental variables determine macroinvertebrate community structure within and between the two basins;

(b) Mortality tallies increase with increased toxicant concentrations, and;

(c) Orders with more diverse families are key indicators for a suitable and cost-effective biomonitoring index.

1.5 Significance of the Research

The present study has provided the first user friendly and cost-effective biomonitoring index that will minize sampling time and need for specilized expertise as opposed to existing indices. Similarly, the importance of the local macroinvertebrates communities in determining the water quality of freshwaters has therefore been recognized by having cost-effective biomonitoring indices that cover tropical regions. Moreover, the developed EDOT index will provide guidelines and directions to meet current and anticipated future status of water quality along the basins towards the achievement of at least good ecological status for all surface waters.

1.6 Dissertation Organization

This dissertation comprises seven chapters. Chapter one describes the general introduction of the study and focuses on the research problem and justification, the general and specific objectives, research hypotheses and significance of the study. The effectiveness and compatibility of non-tropical biomonitoring indices for assessing pollution in tropical rivers are elucidated in Chapter two. Study on freshwater macroinvertebrates of Tanzania as a basis for developing biomonitoring index for assessing pollution in tropical African rivers is dealt with in Chapter three. A comparative study of macroinvertebrate communities and physico-chemical properties of water along Pangani and Wami-Ruvu basins in Tanzania is described in Chapter four. Chapter five reveals the response of three tropical macroinvertebrates toward nutrients. Advanced biomonitoring tool for assessing pollution in tropical African rivers is developed in Chapter six. Finally, Chapter seven integrates all chapters in a general discussion, conclusion and recommendations of the study.

CHAPTER TWO

2.0 EFFECTIVENESS AND BIOMONITORING INDICES FOR ASSESSING POLLUTION IN TROPICAL RIVERS – A REVIEW[1]

Abstract

In Tropical regions, biomonitoring indices for assessing pollution in streams and rivers are not yet in place. As a result, indices that have been developed inconsistently in different non-tropical regions using their local macroinvertebrate species are adopted and used for assessing pollution in tropical rivers. In Africa, only one review on existing non-tropical biomonitoring indices to assess river quality in southern Africa was previously reported in conjunction with comparisons with those developed in United States of America, Asia, Australia, Canada, and European countries. However, a comprehensive overview of the complete body of biomonitoring applications of these indices to streams and rivers in tropical African countries, particularly East and Central Africa was not addressed. Similarly, comparisons of the different sampling techniques, taxonomic resolutions and sensitivity of bio-indicators' species that were used in different studies to develop the existing indices were not covered in that review. In that regard, this review work has highlighted the geographical compatibility, effectiveness, and capability of existing non-tropical biomonitoring indices to assess pollution in tropical African rivers, in a view of improving biomonitoring programmes. The need for tropical African regions to have or develop their own biomonitoring index that will be more reliable than adopting indices from other geographical areas (non-tropical regions) cannot be overemphasized.

[1]International Journal of Ecosystem 2014, 4(3): 128-134 DOI: 10.5923/j.ije.20140403.05.

2.1 Introduction

Conventional methods which rely on chemical aspects to assess river pollution are becoming less suitable in monitoring programmes as they cannot detect physical and biological stressors that occurred over time and at multiple scales (Barbour *et al*., 2000; Bohmer *et al*., 2004; Chaves, 2008; Jacobsen *et al*., 2008; Elias, 2009). As such, the concepts and principles of biomonitoring indices, which are more efficient, effective, and of lower cost than the traditional methods have been developed and applied broadly around the world to assess river pollution. Nonetheless, this new initiative has exempted tropical regions in favour of modifying and adopting the existing indices that have been developed for non-tropical regions using their local freshwater organisms. These biomonitoring indices are normally developed for specific regions in order to account for regional variation using local biotic assemblages that exhibit regional variation based on organism's sensitivity or tolerance. Such variations might affect the capability, functioning, and reliability of the biomonitoring indices developed for non-tropical regions when applied in tropical rivers. Likewise, modification of non-tropical biomonitoring indices for use in tropical regions is usually hindered by incomplete taxonomical resolution and the barely known sensitivity levels of many tropical taxa (Jacobsen *et al*., 2008).

This paper presents a review on concepts, current use and anticipated future research directions of existing non-tropical biomonitoring indices with a view of improving biomonitoring programmes in Africa. The review focused on reliability and geographical compatibility of adopting non-tropical indices to assess water pollution in tropical African rivers. It also discussed the bottlenecks of adopting such indices while setting some basis for developing tropical biomonitoring index in the near future.

2.2 Historical Background and Overview of Biomonitoring Indices

United Kingdom was the first nation to officiate the use of Rapid Biomonitoring Methods (RBMs) for assessing river pollution in 1970. In response to criticisms about the inadequacy of the method, the Biological Monitoring Working Party (BMWP) index was developed in 1976 and recommended for use in river pollution surveys (Hawks, 1997). Later, a number of RBMs based on macroinvertebrates were developed and used worldwide as a response to the need in water management for quick and cost-effective methods for assessing water quality (Dallas, 1997). For instance in Germany, Kolkwitz and Marsson (1908) developed Saprobien or Saprobic System based on the Saprobien System, followed by other developments in the

United Kingdom (Wright *et al*., 1984; Wright, 1994), North America (Hilsenhoff, 1988), United States of America (Rosenberg and Resh, 1993; Barbour *et al*., 1999), Canada (Rosenberg *et al*., 1999), Australia (Simpson and Norris, 2000), Mexico (Henne *et al*., 2002), Thailand (Mustow, 2002), Brazil (Baptista *et al*., 2007), and Bolivia (Jacobsen and Marin, 2007).

In southern region of Africa, the South African Scoring System (SASS) in South Africa (Chutter, 1998; Day, 2000; Dickens and Grahm, 2002; Dallas *et al*., 2010), the Namibia Scoring System (NASS) in Namibia (Palmer and Taylar, 2004), the Okavango Assessment System (OKAS) in Okavango delta (Dallas, 2009), and the Zambia Invertebrate Scoring System (ZISS) in Zambia (Lowe *et al*., 2013) have been developed based on local freshwater macroinvertebrate families. NASS, OKAS, and ZISS have been modified from SASS5 index, which has been extensively tested in South Africa, and its capability and reliability proven for assessment of water quality and general river condition (Dallas, 1997; Dallas, 2004a, 2004b; Dallas *et al*., 2010).

On the contrary, on-going efforts in southern tropical African countries regarding biomonitoring indices development, testing, refinement, and validation are yet to be employed in rivers in tropical countries such as Kenya, Uganda, and Tanzania (Jacobsen *et al*., 2008). Apart from few ecological and taxonomical studies conducted in patches on the north-east of Africa (Tesfaye Berhe, 1988; Worku Legesse *et al*., 2000), in Kenya (Barnard and Biggs, 1988; Kinyau and Pancini, 1991; Mathooko, 2002; Ndaruga *et al*., 2004), Uganda (Tumiwesigye *et al*., 2000; Kasangaki *et al*., 2006) and Tanzania (Swarthout, 2003; Elias, 2009; IUCN/PBWB, 2010; Lyimo, 2012), there is no any effort that has led to the development of biomonitoring indices in tropical African countries. The presence of technical, financial and logistical constraints have hindered the potential use of macroinvertebrate communities as indicators of water quality and thus, making biomonitoring programs a remote possibility in tropical African region.

2.3 Review on Non-Tropical Biomonitoring Indices Applications in the Tropics

2.3.1 Sampling Techniques

A number of ecological and taxonomical studies together with those used to develop biomonitoring indices have been conducted within the same or different climatic-regions using different sampling protocols and sampling tools. These include the frequently used

sampling protocols (quantitative, semi-quantitative, and non-quantitative) and tools (substrates Hess samplers, surber sampler, substrate corer, grab sampler, and kick-net samplers) in developing Rapid Biomonitoring Methods (RBMs). The use of these sampling techniques interchangeably in the same or different studies may either produce data of unknown quality or yield conflicting data interpretations, even at the same ecoregions or biotopes. As a result, element of doubt may arise regarding the precision of developed biomonitoring tool, if the produced data were used to develop that particular index.

(a) Sampling Protocols

Different studies have been done using different types of sampling protocols to collect macro-benthic assemblage samples from different biotopes (Barbour *et al*., 1999; Dickens and Grahm, 2002; Baptista *et al*., 2007). These include quantitative method that used to collect benthic assemblage samples for developing BalkaN Biotic Index, Chutter's Biotic Index, Family-level Biotic Index, Quantitative Macroinvertebrate Community Index, and Hilsenhoff's Biotic Index. Both semi-quantitative and non-quantitative (qualitative) protocols were jointly employed to collected specimens during the development of Biological Monitoring Working Party Score System, Indice Biologique Global Normalisé, South African Scoring System, Version 4 (SASS4), South African Scoring System, Version 5 (SASS5), Namibian Scoring System, Version 1 (NASS), and Okavango Scoring System, Version 1 (OKASS) of Botswana (Ollis *et al*., 2006; Dallas *et al*., 2010). Likewise, non-quantitative (qualitative) method was preferred in Iberian BMWP, Florida Index, Beck's Biotic Index, Macroinvertebrate Community Index, Stream Invertebrate Grade Number - Average Level Biotic Index, and Trent Biotic Index development whereas, semi-quantitative protocol opted to develop Stream Invertebrate Grade Number – Average Level Weighted Biotic Index, Semi-Quantitative MCI, Rivers of Vaud Index, Rivers of Vaud Index, 1995 Version, Indice Biotique, Average Chandler Biotic Score, and Chandler's Biotic Score (Ollis *et al*., 2006; Dallas *et al*., 2010).

Generally, sampling variability among replicate samples collected from one site, at one time, and of similar biotope may lead to incorrect interpretation of data and conclusions (Hawkins *et al*., 2010). Environment Canada's Reference Condition Approach (Rosenberg *et al*., 1999), the US Environment Protection Agency (Barbour *et al*., 1999) and the South African Scoring System in South Africa (Dickens and Grahm, 2002), for example, have used a single-composite-sample method as their sampling protocol. This sampling protocol involves

sampling of dominant habitats or biotopes at a site and combining them into an overall site composite sample. As a result, only one-composite-sample per site is obtained and there is no replication (Clarke and Hering, 2006). Moreover, sampling variability becomes more critical in this protocol as single sample is expected to provide comparative information of reference conditions. This is due to the fact that, a sample is continuously collected from a particular habitat or biotope over either a fixed period of time (e.g. 2-5 minutes in stones) or fixed distance (2 meters for marginal vegetation) or fixed area (1 m^2 for aquatic vegetation) in such a way that the sample is a composite as it is being collected rather than collecting discrete replicate samples, which are then used to give one biotope sample (Clarke and Hering, 2006; Hawkins *et al*., 2010).

Another major concern in the use of single-sample per biotope is the possibility of not collecting all macroinvertebrates taxa occurring at a site hence affecting the biotic index metrics. With single composite sample protocol, under expected number of taxa from study sites are likely due to the small sample size collected from few sample areas (habitats). In order to compile relatively complete taxa lists at a given area, taxonomic studies should sample macroinvertebrate assemblages from more areas of different habitat types. Similarly, the use of standardized protocol together with available taxonomic knowledge is more recommended as they can easily govern the differentiation of site conditions or macroinvertebrate composition in the same biotope.

(b) Sampling Tools

Existing Rapid Biomonitoring Methods (RBMs) developed in/for the same climatic regions (temperate or Mediterranean) have been found to use different field-based sampling tools of different diameters and retention sizes (often varies from 250 µm to 1000 µm). The most commonly used sampling tools for collecting macrobenthic assemblages include kick (hand) net, substrate Hess sampler, surber sampler, grab net, and stovepipe coring device. In New Zealand, surber sampler was used in collecting macro-benthic assemblage for developing Macroinvertebrate Community Index (MCI) and Quantitative MCI (QMCI) while both surber sampler and hand-net of 500 µm were used in France for developing Indice Biologique Global Normalise (IBGN). In southern part of Africa, the macro-benthic assemblages that used to develop South African Scoring System (SASS) in South Africa (Chutter, 1998; Dickens and Grahm, 2002; Day, 2000; Dallas *et al*., 2010), the Namibia Scoring System (NASS) in Namibia (Palmer and Taylar, 2004), the Okavango Assessment System (OKAS)

in Okavango delta (Dallas, 2009) and the Zambia Invertebrate Scoring System (ZISS) in Zambia (Lowe *et al*., 2013) were collected using hand-net of 1000 µm mesh size. In Brazil, Ferreira *et al.* (2011) used surber sampler of 0.09 m^2 and 250 µm mesh size while Stone *et al*., (1998) used stovepipe coring device in Southern Illinois (USA) before Elias (2009) opted for substrates Hess sampler in Tanzanian rivers to collect macro-benthic assemblages. Hand net of different mesh sizes and diameters were also used in North America (Hilsenhoff, 1988) and United Kingdom (Wright *et al*., 1984; Wright, 1994) while both hand-net of 250 µm mesh size and grab net were used in Australia (Simpson and Norris, 2000).

However, application of these different sampling tools and protocols might produce errors and uncertainty to data collected in the same climatic ecoregions or biotopes (Clarke and Hering, 2006), underscoring the question of accuracy in biomonitoring programs. In Mediterranean climatic regions, for example, sampling tools used to collect macroinvertebrate assemblages to develop both South Africa Scoring System (SASS) and Iberian Peninsula indices were different in respect to their mesh sizes (Bonada *et al*., 2006). The influence of sampling tools of different mesh sizes on collected taxa may vary seasonally in response to size, temperament of habitat and development stage of a particular taxon. Therefore, organisms in stressed habitats (or temporary steams) and at earliest development stages, they cannot be retained by samplers of larger mesh sizes as opposed to others due to their small body sizes.

On the other hand, Daniel and Erika (2008) recommended kick (hand) net over surber sampler on account of its ability of collecting macroinvertebrate assemblages. Similarly, macro-benthic samples collected with the kick method had significantly higher richness and BMWP scores in relation to surber sampler (Daniel and Erika, 2008). The surber technique often underestimates macroinvertebrate richness (5 - 25 % less families than in kick net) and also is not an adequate device for streams with more than 30 cm depth because of difficulties in handling it in rocky and pebble-bottomed streams, commonly found in tropical and mountainous areas (Daniel and Erika, 2008). Additionally, inability to apply some of sampling devices (Surber and Hess sampler) to deep rivers meant that certain river stretches would not be sampled for any environmental impact using these devices (Mtetwa *et al*., 2002). Against that backdrop, hand-net, surber sampler, grab net, substrate Hess sampler, and sediment corer should be re-assessed for their effectiveness before any of them is recommended as a suitable sampling device.

2.3.2 Conflicting Features of Bio-Indicators' Species and Metrics

Generally, the basic principle of existing biomonitoring indices is that healthy rivers contain large numbers of different species at all levels of pollution tolerance while no single species dominates. Biomonitoring indices rank macroinvertebrate species relative to their levels of pollution sensitivity. Macroinvertebrate families that are very tolerant to pollution score a lower rank and vice versa for more sensitive organisms. However, low EPT (Ephemeroptera, Plecoptera and Trichoptera) percentages may prove good indicator of increased anthropogenic waste run-off as families of Ephemeroptera (Heptageniidae), and Plecoptera (Perlidae), are known to be sensitive to low dissolved oxygen concentrations (Thorn and Covich, 1991). On the other hand, it may not be an effective indicator of sediment pollution because some Trichopterans (Hydropsychidae), and Ephemeropterans (Caenidae), can thrive in heavily sedimented streams (Thorn and Covich, 1991). In that case, EPT percentage may not be the best measure of water quality if the two stressors occurred simultaneously and thus, there is a need of identifying macroinvertebrate families to the lowest taxonomic unit (genus or species level).

Another case is regarding the families that are considered to be sensitive to disturbance, while some taxa are considered somewhat facultative to disturbance. For example, the Midges, Baetidae, Simuliidae, Ceratopogonidae and Orthocladiinae can frequently be collected at highly impacted sites as well as in reference sites (Elias, 2009). Similarly, *Potomanthidae* families that are usually expected to be found in impacted sites due to their known low sensitive score, they have also been found in reference sites (Elias, 2009). Such absence of precise clear-cut distinction between these organisms and disturbance (pollution gradients) requires taxonomic studies to identify specimens further to the resolution of species rather than family level.

2.3.3 Indices' Taxonomic Resolution

Although species level biomonitoring indices produce great results in comparison with family level biomonitoring indices, yet many taxonomic studies identify specimen to the resolution of family rather than species. These include the South African Scoring System, Version 5 (SASS5); Namibian Scoring System (NASS); Okavango Scoring System (OKASS) of Botswana; and Biological Monitoring Working Party Score System (BMWP) mostly used in UK, Finland, and Sweden and Belgian Biotic Index (BBI) mostly used in Belgium. Others include Hilsenhoff's Biotic Index (HBI), Florida Index (FI), Chundler's Biotic Score (CBS),

and Average Chundler's Biotic Score (Avg. CBS) all used in USA; Family Level Biotic Index (FBI) in USA and Chile; Danish Stream Fauna Index (DSFI) used in Denmark and Sweden; Macroinvertebrate Community Index (MCI), Quantitative MCI (QMCI), and Semi-Quantitative MCI (SQMCI) all used in New Zealand; Indece Biotio Esteso (IBE) for Italy; Indice Biologique Global Normalise (IBGN) for France; Iberian BMWP (IBMWP) mostly used in Italy and Spain; BalkaN Biotic Index (BNBI) of Serbia and Stream Invertebrate Grade Number-Average Level Biotic Index (SIGNAL) and Stream Invertebrate Grade Number-Average Level Weighted Biotic Index (SIGNAL-W) mostly used in Australia (Dallas *et al*., 2010). Only Indice Biotique (IB) that was developed in France and Trent Biotic Index (TBI) in Australia were developed using macroinvertebrate data that classified up to species level (Dallas *et al*., 2010).

The choice of suitable taxonomic resolution is a compromise between the cost of obtaining data at high taxonomic resolutions and the loss of data at lower resolutions (Marshall *et al*., 2006). About 6% of data can be lost by identifying taxa to family as opposed to species (Marshall *et al*., 2006). The cost saved in identifying macroinvertebrates to develop family level indices may not be justified if precision cannot be met by such indices at family level. Likewise, the cost expended to obtain species level indices may also not be warranted if cheaper family level indices can evaluate accurately the status of aquatic ecosystems. Moreover, the distribution patterns of all analysed macroinvertebrate samples collected from reference and degraded sites showed that the use of species level indices (or indices developed by best available taxonomic level) perform better at a practical (fine) scale in comparison with family level indices (Verdonschot, 2006). For the sake of data accuracy and precision, any index development for tropical climatic regions should be downscaled to the lowest taxonomical unit.

2.3.4 Geographical Compatibility of Adopted Non-Tropical Indices to Tropical Regions

Macro-invertebrate assemblages exhibit regional variation because bio-monitoring indices are developed based on organism's sensitivity or tolerance for specific regions in order to account for regional variation. The indices have been developed as a response to the need in water management for quick and cost-effective methods for assessing water quality (Dallas, 1997). The wide spread use of biomonitoring indices have also been facilitated by regulatory authorities who appreciate the value of biomonitoring data and information on water resource management. To date, a number of biomonitoring indices have been developed (though in

patchy form) by different countries around the world to assess the rivers' health status in aquatic ecosystems (Chutter, 1998; Barbour *et al*., 1999; Tiller and Metzeling, 2002). However, the absence of such indices in tropical and mountainous climatic regions in fulfilment of effectiveness and efficiency regarding their application to wider area is still a problem. As a result, tropical regions are depending on existing non-tropical developed biomonitoring indices, which are geographically incompatible in assessing river pollution. These indices have used indicator species metrics (individual taxa) that are only relevant to the geographical range related to the distribution of the indicator species. For example, a Stonefly family (Perlidae) is absent in mountainous and tropical regions but abundantly found in temperate and Mediterranean biogeographical zones. Moreover, *Osmerus epertanus* is profusely used as a qualified indicator in North Sea estuaries but not qualified to be used in Mediterranean estuaries. Since these indices are area specific, existing non-tropical indices require substantial refinement and calibration on a variety of scales before being applied for use in tropical climatic-regions. This is due to the fact that some macroinvertebrate species might occur abundantly in temperate but not in tropical or Mediterranean climatic regions and vice versa. However, when they occur in all climatic regions, they tend to differ in diversity (for example, Plecoptera is a low diversity family in the tropics but of high diversity in temperate and Mediterranean regions) and thus, nullify them as key metric during biomonitoring index development. In that regard, locally available freshwater organisms of high diversity should be considered in developing biomonitoring index to represent that particular region after being downscaled to the lowest taxonomic unit. This suggests that current prevalence of developing indices within a single or the same biogeographical zone indicates the spatially restricted relevance of most of existing biomonitoring indices. In Tanzanian rivers for example, high diversified families of common Orders: Odonata, Diptera, Coleoptera, Ephemeroptera and Trichoptera should only be used as key metrics in developing a new biomonitoring index of that particular eco-region (Elias, 2009). For attainment of fully developed tropical biomonitoring indices that adhere to ISO standards, it requires committed and qualified experts, capacity building, government will and support to cater for field sampling and identification work.

2.4 Conclusion

Differences in climate, geology, longitude, and latitude between tropical and non-tropical countries may contribute to differences in the physical and chemical characteristics of rivers between the regions. Consequently, these differences can as well lead to variation in macroinvertebrate taxa composition and their sensitivity levels to disturbance and general ecosystem impairment from one geographical region to another. Such variation might affect the capability, functioning, and reliability of the existing non-tropical biomonitoring indices when opted and applied in tropical rivers. In that regard, there is a risk of having unreliable findings when non-tropical biomonitoring indices are applied or adopted to assess pollution in tropical rivers. Therefore, there is a need for tropical region to have or develop and validate their own indices that will be more reliable than adopting indices from other geographical areas which are inconsistence with regard to tools, research methods, taxonomic resolution and organisms involved in such studies. Additionally, field standard operating procedures and reference conditions for tropical rivers should also be developed and accredited by a recognized governing body or certified by ISO standard covering both surface and underneath benthic organisms.

CHAPTER THREE

3.0 STUDY ON FRESHWATER MACROINVERTEBRATES OF TANZANIA AS A BASIS FOR DEVELOPING BIOMONITORING INDEX FOR ASSESSING POLLUTION IN TROPICAL AFRICAN RIVERS[2]

Abstract

Macroinvertebrates and physicochemical parameters were assessed at 15 sites along five rivers in Kilimanjaro region, Tanzania, with the aim of understanding their ecological status and setting a base to the development of a biological index for tropical regions. Investigated rivers that occur within Pangani basin include Karanga, Rau, Lumbanga, Sere, and Umbwe. Sampling sites were categorized according to the level of water and habitat quality as follows: reference or least impacted (4 sites), moderately impacted (5 sites), and highly impacted (6 sites) sites. A total of 12 527 macroinvertebrates belonging to 13 orders and 48 families were recorded. The highest total abundance of 4110 individuals per m^2 was found in Karanga river, while Umbwe river had the lowest with 1203 individuals per m^2. Chironomidae was the most abundant family (2588 individuals per m^2) and the least were Hydridae and Thiaridae, each having 5 individuals per m^2. High numbers of taxa were noted among the orders: Ephemeroptera (8), Odonata (8), Diptera (7), and Trichoptera (6). In conclusion, orders with greater diversity of macroinvertebrate families offer a wide range of tolerance to pollution and, thus can potentially be used to develop a biomonitoring index for evaluating pollution in tropical African rivers.

[2]Journal of Ecosystems Volume 2014, Article ID 985389, 8 pp. http://dx.doi.org/10.1155/2014/985389.

3.1 Introduction

Freshwater macroinvertebrate species are at higher risk of extinction due to habitat degradation following overwhelming human activities (i.e., invasive industrialization, agriculture, and urban development) near rivers (Jamil, 2001; Leveque and Balian, 2005; Likens, 2010). It is unlikely that, there is a substantial number of freshwater bodies remaining that have not been irreversibly altered from their original state as a result of anthropogenic activities (Sala et al., 2000). In Tanzania, for example, most of the industries are located in Dar es Salaam city and mostly discharge their waste waters into Mzinga, Msimbazi, Yombo, and Kizinga rivers, which eventually discharge into the Indian Ocean (Ak'habuhaya and Lodenius, 1989; Leveque and Balian, 2005). This, in turn, affects the occurrence, composition as well as the distribution of freshwater macroinvertebrate species, depending on their levels of tolerance and adaptability (Hall *et al*., 2000; Karr and Chu, 2000; Bohmer *et al*., 2004; Suleiman and Abdullahi, 2011).

In Tropical African regions, researches on the status and trends of freshwater macroinvertebrates in rivers have not been given much attention compared to non-Tropical regions (Elias *et al*., 2014a; Umar *et al*., 2013). As a result, some species may already have become extinct even before they were taxonomically classified leading to lack of taxonomical information. This situation has hindered the potential use of benthic macroinvertebrates as indicators for water quality assessment and thus making biomonitoring programmes a remote possibility to these regions (Elias *et al*., 2014b). Alternatively, tropical biomonitoring studies are relying on indices that were developed for other regions (Elias *et al*., 2014b). Such adoption signalling the growing interest and recent need for the use of macroinvertebrates based indices in the tropics to assess streams and river health status. Unfortunately, recorded macroinvertebrates in temperate, Mediterranean, arid and semi-arid regions did not sufficiently match with those in the tropics to confirm the existence of general adopting rules among macroinvertebrates based indices from other regions (Masese *et al*., 2009; Blakely *et al*., 2010; Ngupula and Kayanda, 2010; Umar *et al*., 2013). Besides, differences in climate, altitude, combined with the longitudinal position of sites, appears to be important factors governing diversity and structure of macroinvertebrate communities among regions (Boulton *et al*., 2008; Pearson and Boyero, 2009; Umar *et al*., 2013). Given what has been described above, taxonomical and ecological information regarding tropical African macroinvertebrates remain of major importance. This study therefore seeks to characterize macroinvertebrate communities in some Tanzanian rivers, with the aim of understanding their taxonomical and

ecological status and set a base to the development of a biomonitoring index for tropical African regions.

3.1 Materials and Methods

3.1.1 Description of the Study Area

Macroinvertebrate community structures and physico-chemical parameters were assessed in five Tanzanian rivers located in Kilimanjaro region which flow into the Pangani basin. Investigated rivers include Karanga, Rau, Lumbanga, Sere, and Umbwe. Kilimanjaro region is located in the northern-eastern part of Tanzania mainland between 037^{O} 30' 0" E and 03^{O} 4' 59" S (Figure 3.1).

Karanga River flows from the foot of Mount Kilimanjaro southwards and empties into Nyumba ya Mungu dam. Three sites were identified along this river with the site near Kibo Match Industries being categorised as moderately impacted while the other two (Shirimatunda and Bonite Bottlers factory sites) being highly impacted. Major threats on this river are industrial and household wastes, agricultural activities, and habitat degradation by human activities.

Rau River flows southwards through Njoro and Kahe forests before discharging into Lake Jipe. Along the channel, one least impacted site (Mawela) and two highly impacted sites namely Majengo and Msaranga were used as sampling stations. Intense land use involving cultivation of cash and food crops cultivation (with application of fertilizers and pesticides), animal grazing and construction work have caused almost complete depletion of riparian vegetation.

Lumbanga River consists of two reference (least impacted) sites (Mweka and Singachini) and one moderately impacted site (Kirima). It drains extensively cultivated highlands, coffee estates and food croplands, settled areas and cultivated plains before emptying into Nyumba ya Mungu dam. Along its course there are alterations of areas with no or with minor degraded reaches whereas, in some areas intact riparian vegetation are still retained.

Sere River is found on the western part of Kilimanjaro National Park. In this river, Kombo site was regarded as being least impacted, whereas Narumu and Weruweru sites were considered to be moderately impacted. The presence of coffee plantations and human

settlements in the vicinity of the selected sites are the possible source of pollution that could affect the river water quality and biota.

In Umbwe River, one moderately impacted site (Umbwe upstream) and two highly impacted sites (Kwa-Rafael and Kindi) were identified and used for macrobenthic sample collection. Presence of extensive agricultural activities and human settlements in the area are the causes of watershed pollution and riparian zone degradation along the river.

3.1.2 Sampling Design

The five rivers were classed into three site categories namely reference (least impacted), moderately impacted, and highly impacted sites within which 15 sampling sites were established. The sampling sites were selected based on: i) ease of accessibility, ii) presence of high, low or absence of sustained anthropogenic land uses, iii) exhibition of high micro-scale heterogeneity, and iv) level of water and habitat quality.

(a) Physico-Chemical Data Collection

The physical parameters (pH, dissolved oxygen, temperature, turbidity, and conductivity) as well as the four major nutrients (soluble reactive phosphorus (SRP), nitrates, nitrites, and ammonia) were measured. Measurements of stream water temperature, conductivity, dissolved oxygen (DO), and pH were recorded *in situ* at each established site using a multi-sensor probe YSI Professional Plus Water Quality Instrument (Model 6 050 000). Turbidity was measured using turbidity meter by Hatch Instrument Limited. Determination of nutrients involved collection of water samples from running water at each site, filtering it using 0.45 µm glass fiber filters before being placed in hydrochloric acid washed polythene bottles. The samples were also preserved in a cool box at $\leq 10\ ^{O}C$ before transported to the Department of Aquatic Sciences and Fisheries Laboratory at the University of Dar es Salaam for analysis.

In the laboratory, nitrate (NO_3^--N), nitrite (NO_2^--N), ammonia (NH_4^+-N) and SRP (PO_4^{3-}-P) were analyzed using standard spectrophotometric methods described in APHA (1998). Nitrate and nitrite were determined using the cadmium reduction method followed by diazotization with sulphanilamide and coupling with N-(1 naphthl)-ethylenediamine to form a highly coloured azo dye that is measured spectrophotometrically at 545 nm wavelength. Ammonia was determined using a phenate method which forms a blue indophenol colour measured at wavelength of 640 nm whereby SRP was analyzed using the molybdate ascorbic

acid method which results in a formation of intense blue colour measured at wavelength of 880 nm.

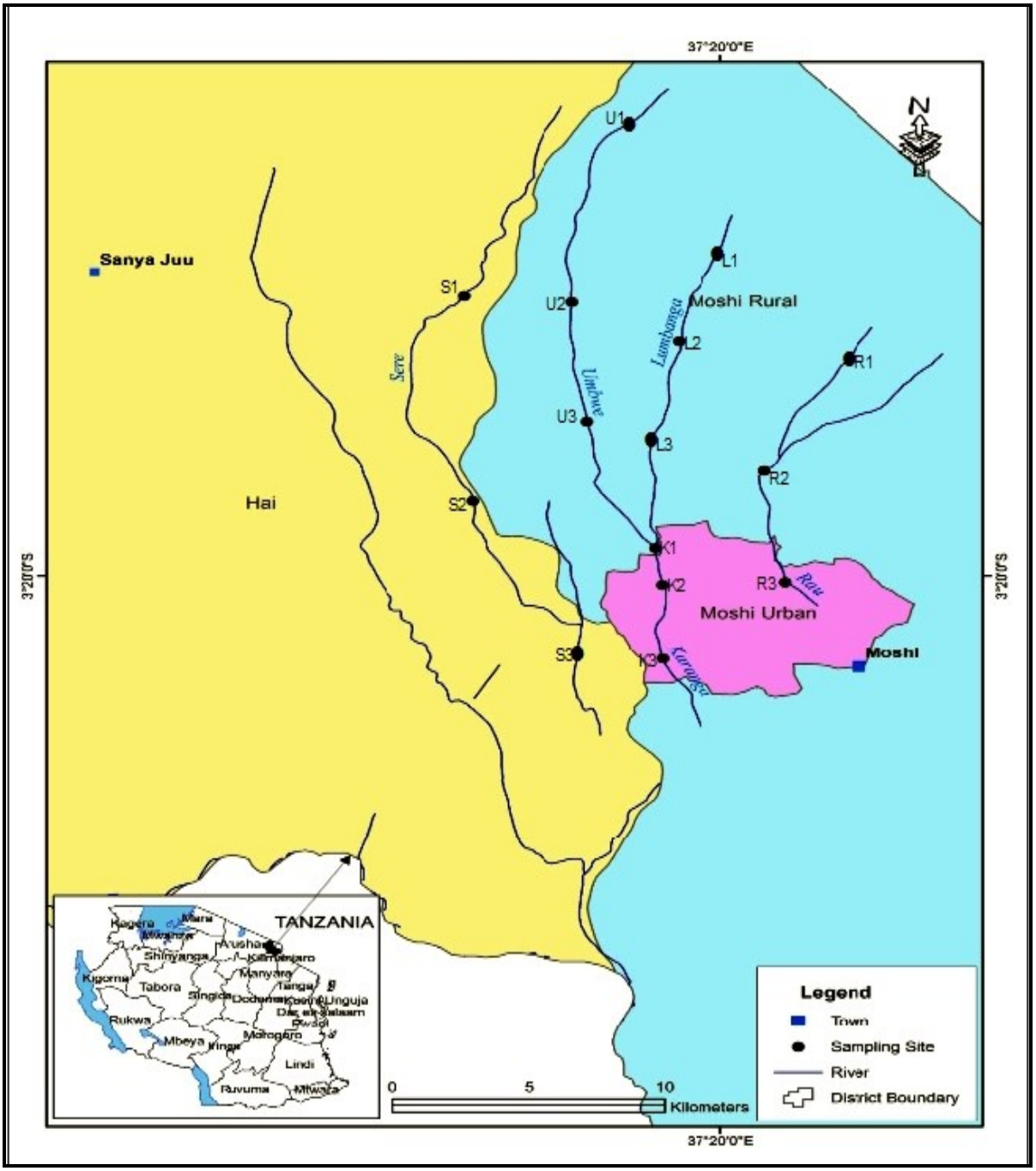


Figure 3.1: Map showing sampling sites along Karanga, Rau, Lumbanga, Sere, and Umbwe rivers.

Key: K1 = Karanga site at Kibo Match Industry Ltd; K2 = Karanga site at Shirimatunda; K3 = Karanga site at Bonite Bottlers Factory Ltd; R1=Rau site at Mawela; R2= Rau site at Majengo; R3 = Rau site at Msaranga; L1 = Lumbanga site at Mweka; L2 = Lumbanga site at Singachini; L3 = Lumbanga site at Kirima; S1 = Sere site at Kombo; S2 = Sere site at Narumu; S3 = Sere site at Weruweru; U1 = Umbwe upstream site; U2 = Umbwe site at Kwa-Rafael; U3 = Umbwe site at Kindi.

(b) Macroinvertebrates Sampling

Macroinvertebrates sampling was conducted in accordance with methods for assessing biological integrity of surface waters (Barbour *et al*., 1999). Three benthic samples were obtained from each site using Hess sampler. The Hess sampler was placed into the water while positioned against water flow direction. Stream substrate was disturbed ten times for 30 seconds in order to collect macroinvertebrate samples. Macroinvertebrate samples representative of the range of water flow conditions collected from all possible microhabitats were pooled into single sample for each site. To eliminate effects of substrate diversity biasing the semi-quantitative sampling, an effort was made to sample riffle habitats that afforded macroinvertebrates with the best arrangement or layering of cobble, gravel, and small boulders. Non-riffle habitats were sampled qualitatively to try to collect as many specimens as possible within the stream reach.

The collected benthic samples were stored in well labelled bottles and preserved in 10% formaldehyde (formalin) before being transported to the Department of Aquatic Sciences and Fisheries Laboratory at the University of Dar es Salaam. Prior to identification, each sample was rinsed thoroughly to remove all traces of formalin. In the laboratory, 500 and 100 μm sieves were used for sample fractioning and removal of excess sediment. For sites with high abundance of individuals like Kibo Match and Shirimatunda in Karanga river and Majengo in Rau river, the sub-sampling technique was used to isolate at least 200 individuals from the original composite sample. Fauna remaining in the composite samples were assessed and single individuals representing rare ones not already included in the 200+ individual-sub sample were added to original composite sample. All specimens collected were sorted, enumerated, and identified to family level with the help of available keys i.e., Aquatic Invertebrates of South African Rivers (Gerber and Gabriel, 2002) under a stereomicroscope at 10 x 45 magnification followed by listing and counting of individuals.

(c) B-IBI Scores

B-IBI (Benthic Index of Biological Integrity) was calculated at each site according to Barbour *et al.* (1999) using percentage composition of 14 metrics (including H-FBI), excluding abundance. These include: %Baetidae, %Dominant taxa, %Taxa richness, %Ephemeroptera, %Plecoptera, %Trichoptera, %Odonata, %EPT, %H-FBI, %Non-insect taxa, %Diptera, %Chironomidae and %Oligochaeta and Shannon Diversity Index. The range of numbers observed for each of these characteristics was divided into 3 categories

representing values expected from least stressed (reference sites), intermediate (moderately impacted sites), and most stressed communities (highly impacted sites). Depending on the range into which a specific characteristic at a particular site falls, a score of 5, 3, or 1 (that referred as "standardized scores") was assigned. The score of 5 stands for reference sites, 3 for moderately impacted sites and 1 representing highly impacted sites. Since B-IBI value is the sum of these character scores, it generates a maximum (least stressed) score of 70 (14 characters each with a maximal score of 5) and a minimum value (most stressed) of 14x 1 = 14. B-IBI values were calculated in this way for each site. The B-IBI values were then standardized to 100 point scale giving 100 (least stressed), 60 (moderate) and 20 (most stressed) B-IBI values. To categorize the sites into various impairment levels, the range of B-IBI numbers was divided into 3 sub-ranges, and then impairment levels were given as shown in Table 3.1. Therefore, the 100 point scale B-IBI values calculated at the family level may correspond to the following water quality assessments (Table 3.1).

Table 3.1: Methods of classification of water quality status based on impairment level from B-IBI data

B-IBI Value	**Water Quality Characterization**	**Impairment**
20-46	Very poor to Poor	Severe
> 46-72	Fair to Good	Moderate
> 72-100	Very good to Excellent	Very little to None

3.1.3 Statistical Analysis

Statistical software InStat version 3 (GraphPad), PASW Statistics 18 and Excel spreadsheet were used for analysis. Physico-chemical parameters were expressed as means ± standard error (M ± SE), macroinvertebrate count data were $\log_{10}$ (x+1) transformed to meet the statistical criteria for normality. One-way analysis of variance (ANOVA, $\alpha = 0.05$) and Pearson rank correlation were used to test whether the physico-chemical parameters and benthic macroinvertebrates differed among the rivers and site categories (Hayford and Ferrington, 2005; Lyimo, 2012). The macroinvetebrates data were subjected to Bray-Curtis similarity analysis to reveal resemblance among sites and rivers.

3.2 Results

3.2.1 Environmental Variables

The environmental variables that have been used in this study to understand the criteria defining the 3 site categories (classes) are physical and chemical (nutrients) parameters. They are the baseline against which the effectiveness of benthic macroinvertebrates to reflect the water quality is measured (Merritt *et al*., 2008; Likens, 2010; Bird, 2012). With exception of DO, there was a distinct trend for environmental variables at the highly impacted sites being higher than at other site categories (Figures 3.2 and 3.3). One-way ANOVA revealed higher significant differences in turbidity ($F_{(2,12)} = 25.962$; $p < 0.0001$), DO ($F_{(2,12)} = 14.022$; $p = 0.0007$), and nitrate ($F_{(2,12)} = 7.255$; $p = 0.0086$) between reference sites and highly impacted sites as well as at reference versus moderately impaired sites. However, pH ($F_{(2,12)} = 2.336$; $p = 0.1391$), temperature ($F_{(2, 12)} = 1.207$; $p = 0.3329$), nitrite ($F_{(2,12)} = 0.683\ 9$; $p = 0.5233$), SRP ($F_{(2,12)} = 5.373$; $p = 0.0216$), conductivity ($F_{(2,12)} = 5.781$; $p= 0.0175$) and ammonia ($F_{(2,12)} = 5.372$; $p = 0.0216$) values showed no or very slight variations among the three site categories with pH values being mostly close to neutral.

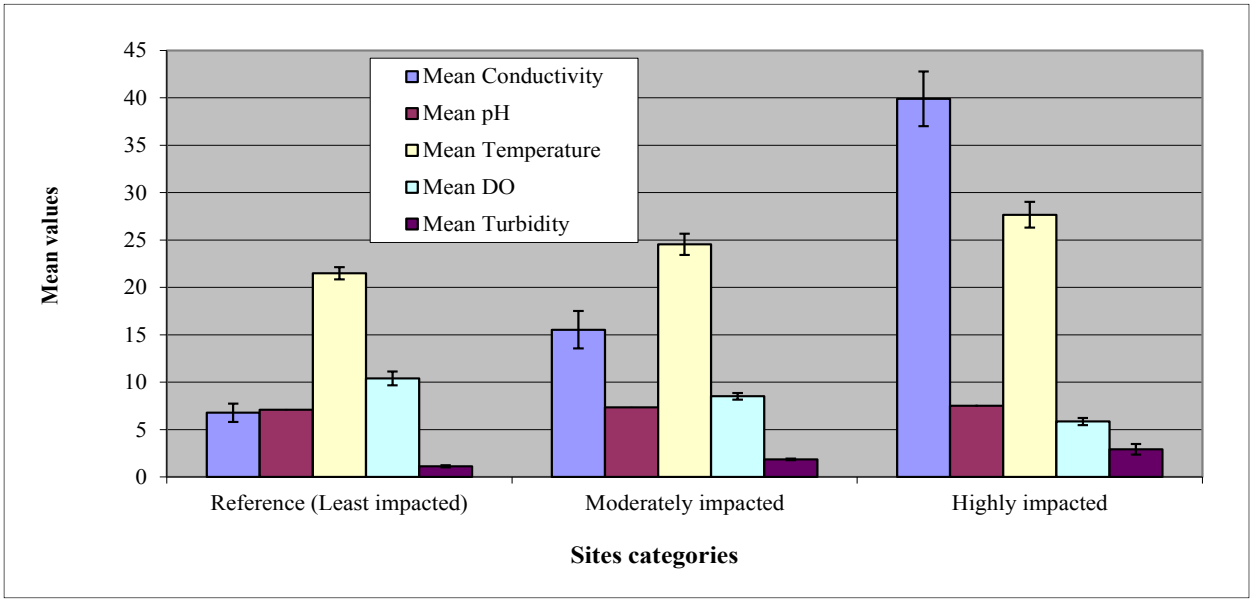


Figure 3.2: Mean physical parameter values recorded at three site categories

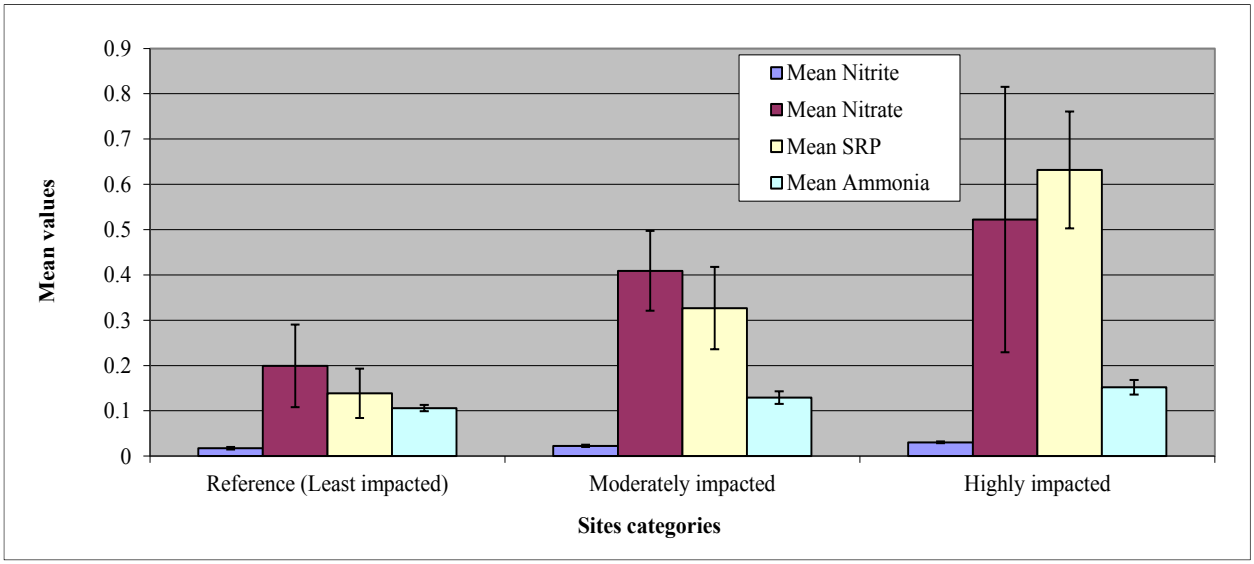


Figure 3.3: Mean chemical parameter values recorded at three site categories

3.2.2 Macroinvertebrates

A total of 12 527 macroinvertebrates belonging to 13 orders and 48 families from 15 sites of the 5 sampled rivers were collected, sorted and counted (Table 3.2). Among all identified families, 33 were common and 15 were rare. Most of the rare families were identified from reference sites. Ephemeroptera and Odonata were the most diverse taxa, consisting of 8 families each, followed by Diptera and Trichoptera with 7 and 6 families respectively. Arhynchobdellida, Decapoda, Hydroida, Plecoptera, Tubificida and Tricladida were orders found with the least diverse taxa consisting of one family each.

The highest total abundance of 4110 individuals per m^2 was counted at Karanga River while the lowest (1203 individuals per m^2) recorded within Umbwe River. Again the highest total abundances of 2057 and 608 individuals per m^2 were recorded at downstream sites of Karanga and Umbwe rivers, respectively. Chironomidae was the most abundant family collected with 2588 individuals, followed by Simuliidae (1955 individuals) and Baetidae (1898 individuals) families. Hydridae and Thiaridae were the least abundant families, found with only 5 individuals each, and followed by Planorbinae with 8 individuals.

Table 3.2: Macroinvertebrate families recorded at each site of the five studied rivers

ORDER	FAMILY	K1	K2	K3	R1	R2	R3	L1	L2	L3	S1	S2	S3	U1	U2	U3	Total
Arhynchobdellida	Hirudinidae	1	0	0	4	0	0	1	1	8	3	2	0	0	0	0	**20**
Coleoptera	Dytiscidae	0	15	7	3	17	13	14	0	21	7	5	12	6	1	3	**124**
	Dryopidae	0	0	2	19	7	11	38	29	17	3	10	7	16	17	87	**263**
	Gyrinidae	0	16	4	16	18	3	10	0	32	0	3	2	13	8	13	**138**
	Haliplidae	3	0	0	9	0	0	4	4	5	10	3	0	0	0	0	**38**
	Hydrophilidae	2	2	3	2	11	1	2	2	14	0	2	3	9	2	9	**64**
Decapoda	Potamonautidae	18	27	41	8	2	20	1	6	3	2	2	11	4	17	6	**168**
Diptera	Athericidae	34	73	123	21	64	81	3	4	11	11	34	26	1	43	19	**548**
	Ceratopogonidae	58	22	56	5	25	14	4	5	14	4	10	27	11	17	10	**282**
	Chironomidae	294	456	607	63	368	369	1	6	45	29	56	49	13	37	195	**2588**
	Muscidae	21	47	56	15	28	32	2	27	14	16	18	34	24	12	6	**352**
	Simuliidae	54	275	647	67	261	296	10	68	17	35	31	29	14	28	123	**1955**
	Tabanidae	42	53	106	20	37	64	3	15	6	16	32	38	20	16	11	**479**
	Tipulidae	45	30	85	4	18	24	16	15	13	29	27	66	27	34	16	**449**
Ephemeroptera	Baetidae	31	16	9	284	7	18	480	337	31	353	279	37	13	1	2	**1898**
	Caenidae	7	4	1	94	9	7	104	94	11	42	27	16	11	1	0	**428**
	Dicercormyzidae	14	0	0	9	0	0	54	28	11	9	16	0	0	0	0	**141**
	Ephemerythidae	2	0	0	16	0	0	2	4	7	4	2	0	0	0	0	**37**
	Heptageniidae	6	0	0	3	0	0	5	2	5	6	2	0	0	0	0	**29**
	Leptophlebiidae	8	0	0	2	0	0	11	6	2	3	7	0	0	0	0	**39**
	Oligoneuridae	7	0	0	10	0	0	16	8	2	5	1	0	0	0	0	**49**
	Polymitarcyidae	2	0	0	4	0	0	12	8	2	1	3	1	3	0	0	**36**
Gastropoda	Lymnaeidae	0	3	0	1	0	1	3	0	1	0	0	0	1	0	0	**10**
	Planorbidae	1	0	2	0	0	0	1	1	0	1	0	0	2	0	0	**8**
	Thiaridae	1	0	0	1	0	0	1	1	0	0	0	0	1	0	0	**5**
Hemiptera	Corixidae	5	18	11	7	23	22	17	7	45	6	54	31	23	17	9	**295**
	Gerridae	1	0	3	0	4	12	2	6	18	2	13	7	10	3	7	**88**
	Naucoridae	1	6	0	23	0	0	28	0	37	2	0	0	35	1	10	**143**
	Notonectidae	3	21	3	0	14	8	13	8	51	23	41	20	10	6	4	**225**
	Veliidae	1	3	10	4	1	4	7	2	14	5	2	9	5	2	0	**69**
Hydroida	Hydridae	0	0	0	1	0	0	1	1	1	1	0	0	0	0	0	**5**
Odonata	Aeshnidae	0	6	1	6	2	4	1	6	4	1	4	1	0	1	4	**41**
	Calapterygidae	1	1	1	1	3	0	0	0	2	1	1	1	1	1	0	**14**
	Chlorocyphidae	1	0	0	3	1	1	0	0	1	1	0	0	0	1	1	**10**
	Coenagrionidae	2	24	3	4	1	3	0	8	3	6	4	1	2	1	3	**65**
	Corduliidae	2	1	0	1	3	4	1	1	6	2	2	2	5	9	13	**52**
	Gomphidae	0	4	2	2	3	1	0	6	5	0	5	3	0	1	1	**33**
	Libellulidae	0	2	17	5	5	7	0	5	8	4	7	1	1	1	0	**63**
	Macroiidae	0	15	14	2	0	2	0	9	15	0	3	3	0	6	8	**77**
Plecoptera	Perlidae	8	0	0	11	0	0	4	6	15	0	12	21	1	0	0	**78**
Tubificida	Naididae	67	117	211	68	92	129	0	1	2	0	15	19	3	18	46	**788**
Tricladida (Turbellaria)	Planariidae	1	0	0	0	0	0	19	2	1	1	2	0	1	0	0	**27**
Trichoptera	Ecnomidae	0	1	0	5	1	0	14	13	1	19	12	1	1	1	0	**69**
	Hydropsychidae	0	0	1	11	1	0	7	7	1	13	4	0	3	0	0	**48**
	Lepidostomatidae	1	0	0	9	0	1	5	4	0	4	5	0	1	2	1	**33**
	Leptoceridae	2	0	0	1	0	0	13	6	1	14	9	1	0	0	0	**47**
	Philopotamidae	1	0	0	3	0	0	23	16	0	10	21	0	0	0	0	**74**
	Phryganeidae	3	0	0	8	0	0	7	4	2	5	2	3	1	0	0	**35**
TOTAL		**751**	**1258**	**2026**	**855**	**1026**	**1152**	**960**	**789**	**525**	**709**	**790**	**482**	**292**	**305**	**607**	**12527**

Key: K1 = Karanga site at Kibo Match Industry Ltd; K2 = Karanga site at Shirimatunda; K3 = Karanga site at Bonite Bottlers Factory Ltd; R1=Rau site at Mawela; R2= Rau site at Majengo; R3 = Rau site at Msaranga; L1 = Lumbanga site at Mweka; L2 = Lumbanga site at Singachini; L3 = Lumbanga site at Kirima; S1 = Sere site at Kombo; S2 = Sere site at Narumu; S3 = Sere site at Weruweru; U1 = Umbwe upstream site; U2 = Umbwe site at Kwa-Rafael; U3 = Umbwe site at Kindi.

B-IBI scores calculated from 14 biometric data have demonstrated a decreasing pattern from least (reference) to highly impacted sites (Figure 4). Lumbanga river had good water quality with significantly higher B-IBI value of 57% compared to Karanga that falls within a very poor water quality with B-IBI score of 21% (Figure 3.5). Moreover, statistical findings in Table 3.3 suggest that some reference sites were similar to moderately impacted sites and vice versa. A similar overlapping character was also found between moderately and highly impacted sites. Table 3 has also categorized the sites with less and/or very little impairment (L1, L2, L3, R1, S1 and S2) as reference (least impacted) sites; sites with moderate impairment (U1, U2 and S3) as moderately impacted sites and those with major disturbance (K1, K2, K3, R2, R3 and U3) as highly impacted sites.

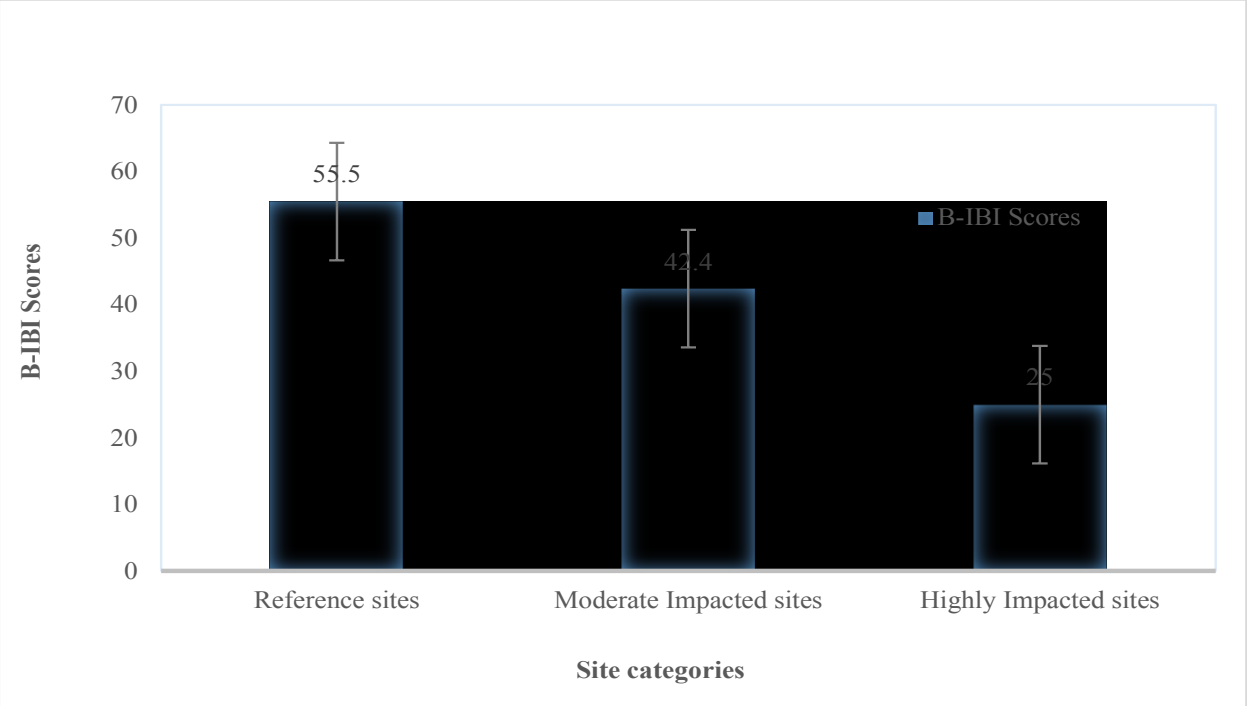


Figure 3.4: B-IBI scores of the site categories showing a decreasing pattern from reference to highly impacted site

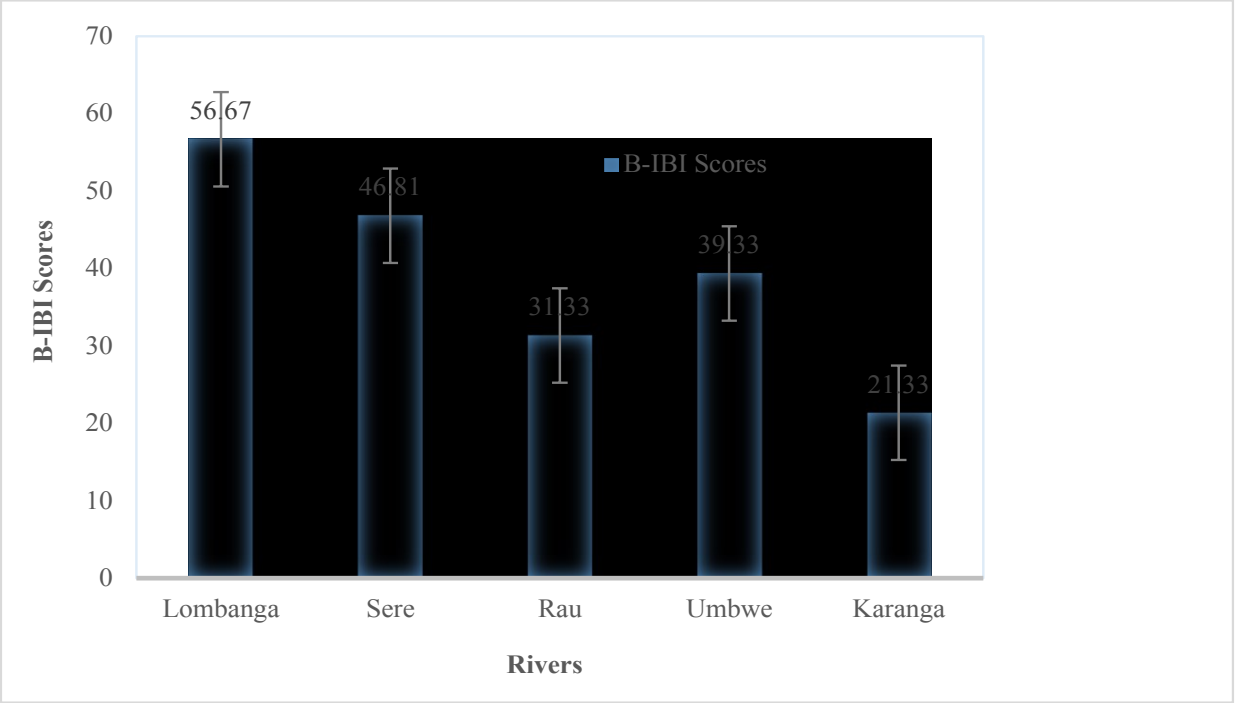


Figure 3.5: B-IBI scores of studied rivers showing their impairment levels

Table 3.3: Categorization of sites into different impairment levels based on B-IBI results

B-IBI Value	Water Quality Characterization	Impairment	Sites fall at each impairment level
20-46	Very poor to Poor	Severe to Slight	U3, K1, R2, K2, R3 and K3
46-72	Fair to Good	Moderate to Less	U1, U2 and S3
72-100	Very good to Excellent	Very little to None	L1, L2, L3, R1, S1 and S2

Cluster analysis done using Bray-Curtis similarity dendrogram revealed some similarities between macroinvertebrates among sites and among rivers (Figures 3.6 and 3.7). R2 and R3 showed a very close similarity of macroinvertebrate abundance as compared to other sites of about 80%. S1 and S2 showed a close similarity in terms of abundance of macroinvertebrates of about 75%. Generally, all sites showed a close similarity with regard to abundance of macroinvertebrates of more than 55% excluding sampling site U3 (Figure 3.6).

River Sere and Lumbanga showed a close similarity of number of macroinvertebrates of about 65%. River Rau and Karanga showed a similarity of abundance of macroinvertebrates of about 55%. River Umbwe had a similarity of abundance of macroinvertebrates with the rest of other rivers at less than 55% (Figure 3.7).

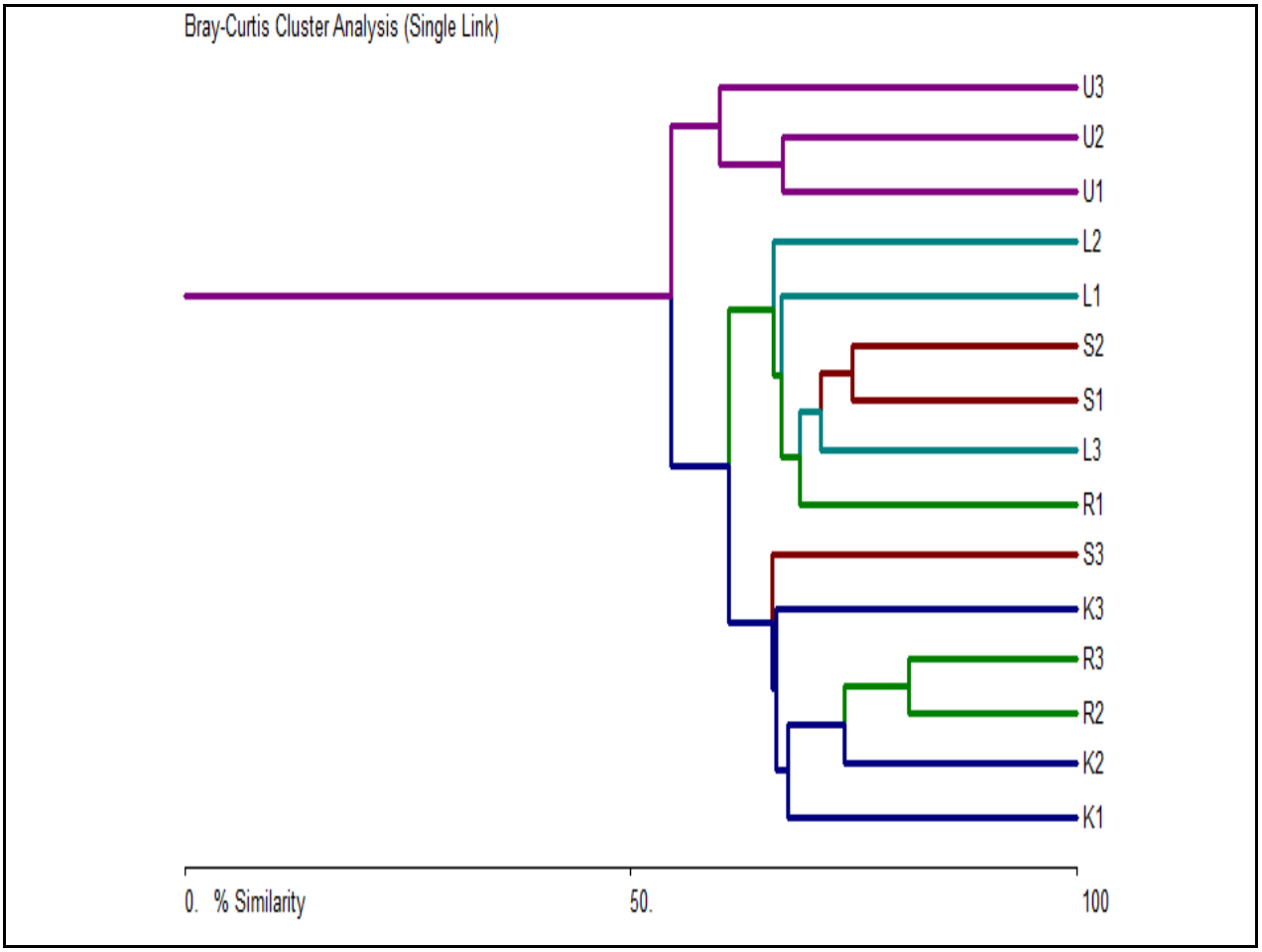


Figure 3.6: Bray-Curtis similarity dendrogram showing similarities of sampling sites in abundance of macroinvertebrates.

Key: K1 = Karanga site at Kibo Match Industry Ltd; K2 = Karanga site at Shirimatunda; K3 = Karanga site at Bonite Bottlers Factory Ltd; R1=Rau site at Mawela; R2= Rau site at Majengo; R3 = Rau site at Msaranga; L1 = Lumbanga site at Mweka; L2 = Lumbanga site at Singachini; L3 = Lumbanga site at Kirima; S1 = Sere site at Kombo; S2 = Sere site at Narumu; S3 = Sere site at Weruweru; U1 = Umbwe upstream site; U2 = Umbwe site at Kwa-Rafael; U3 = Umbwe site at Kindi.

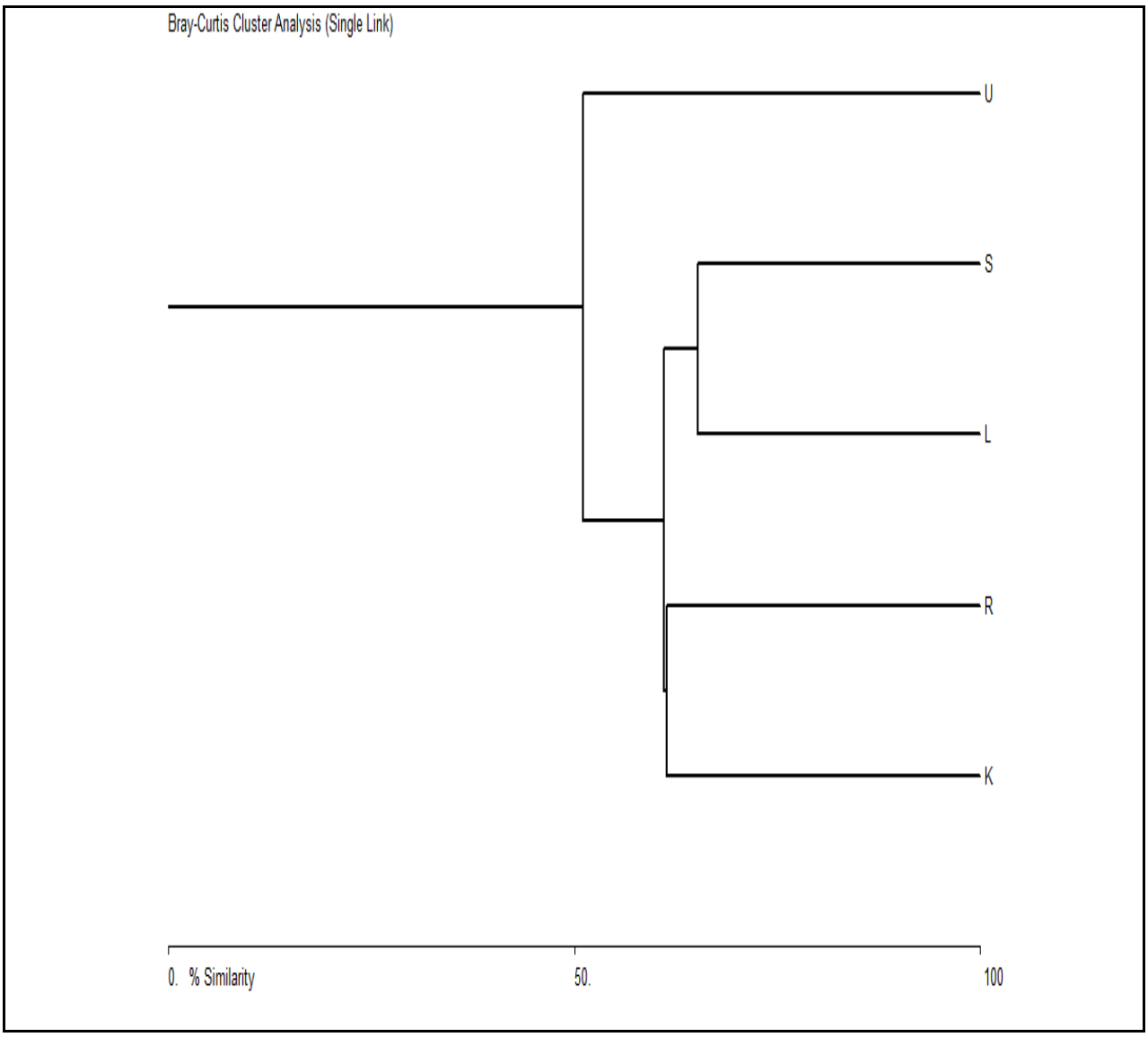


Figure 3.7: Bray-Curtis similarity dendrogram showing similarities of sampled rivers in abundance of macroinvertebrates.

Key: K = Karanga river; R =Rau river; L = Lumbanga river; S = Sere river; U = Umbwe river.

3.3 Discussion

Presence or absence of macroinvertebrates in any given freshwater ecosystem is a function of habitat quality, physico-chemical parameters, and the regional taxonomic pool (Merritt *et al*., 2008; Akasaka *et al*., 2010; Likens, 2010; Suleiman and Adbullahi, 2011). Consequently, a wide variety of freshwater habitat and water chemistry offers the potential for a high diversity of freshwater macroinvertebrates (Cushing and Allan, 2001; Merritt *et al*., 2008; Akasaka *et al*., 2010; Likens, 2010; Sharma and Chowdhury, 2011). Macroinvertebrate communities at degraded sites are characterized by either absence of any sensitive taxa or presence of few if

any; greater dominance of only few taxa; and larger numbers of macroinvertebrates that are tolerant to pollution (Lyimo, 2012). Indeed, a strong relationship observed in this study between the families found and the degradation of the watercourses, appears to support such contention. Families of Orders Ephemeroptera (Dicercormyzidae, Ephemerythidae, Heptageniidae, Oligoneuridae, and Polymitarcyidae) and Trichoptera (Lepidostomatidae, Leptoceridae, Philopotamidae and Phryganeidae) for example, disappeared or their numbers reduced drastically in impacted sites as opposed to some Diptera and Odonata taxa which were observed in all sites. The complete absence of these taxa from impacted sites is probably related to the differences of in-stream environmental degradation along rivers as a result of human activities i.e., agriculture, urbanization, and industrialization. However, total disappearances of these taxa from all disturbed sites and the continuous presence of the Ephemeroptera (8 taxa), Odonata (8 taxa), Diptera (7 taxa), and Trichoptera (6 taxa) in all sampled sites, suggest their potential use as key indicators of water quality assessment for biomonitoring programmes.

The study also showed that, increased total abundance does not necessarily depict better environment but rather might be due to mild disturbance that favors some tolerant taxa with subsequent reduction of sensitive taxa. Presence of least sensitive taxa to pollution (i.e., Chironomids) in all site categories, further suggesting the notion that they are good colonizers and appear under a range of conditions (Likens, 2010) Since highly impacted sites cannot provide habitat suitable for very sensitive macroinvertebrates, the Chironomids which are able to withstand high levels of organic pollution due to their high haemoglobin affinity (Thorn and Covich, 1991; Likens, 2010; Ngupula and Kayanda, 2010) can thrive there. Moreover, the abundance of Chironomids also correlates with the amount of detritus or fine particulate organic matter in the sediment as they are considered tolerable (Boyle and Fraleigh, 2003). As reported by Eggermont and Vershuren (2003), the Dipteran family Chironomidae and midge larvae may also be more effective indicators of increased stress, due to their abundance domination in impacted sites compared to other families. However, general response among macroinvertebrate families toward pollution levels necessitate identification to be done to a possible lower level for more precision before being used to develop an index for Tropical African regions.

B-IBI scores have shown the wide range in water quality along the rivers, with upstream sites having good water quality compared to middle and downstream sites. The decreased trend of

DO and elevated trend of other recorded physico-chemical parameters towards river mouth might be associated with watershed disturbances as well as rural and urban organic loading (Akasaka *et al*., 2010). Streams in which highly impacted sites were found to have the worst metric scores, had lost much of their capacity to support diversity of pollution sensitive taxa. This however has led to adverse change in faunal structure within the rivers and thus calls for biomonitoring programmes that aim at ecosystem restoration.

3.5 Conclusion

Generally, this study has provided the first comprehensive set of published ecological and taxonomical data describing macroinvertebrate communities at reference, moderately, and highly impacted sites in Tanzania. Macroinvertebrate organisms were shown to be potentially good quality indicators in Tropical African regions and the remarkably high number of taxa collected could be an interesting source of information. However, there is a need for more intensive study on the entire length of other Tanzanian river basins to fully comprehend the general freshwater organisms of the rivers involved. As macroinvertebrates remained a key indicator of pollution in aquatic ecosystems, orders with more diverse taxa (i.e., Ephemeroptera, Odonata, Diptera, and Trichoptera) that offer a wide range of pollution tolerance or sensitivity have the potential to be part of Tropical African biomonitoring programmes.

CHAPTER FOUR

4.0 COMPARATIVE STUDY OF CHARACTERIZED MACROINVERTEBRATE COMMUNITIES AND PHYSICO-CHEMICAL PROPERTIES OF WATER ALONG PANGANI AND WAMI-RUVU BASINS IN TANZANIA[3]

Abstract

In this inventory, macroinvertebrates and physico-chemical variables were used as surrogates to determine levels of impairment among site categories and differences between Pangani and Wami-Ruvu basins. It was hypothesized that, there would be differences in macroinvertebrate assemblages and human-induced environmental variables within and between the basins. Taxonomically, Pangani was observed with more diverse and abundant taxa compared to Wami-Ruvu basin. Similarly, the absence of Nepidae, Notonectidae and Lumnichidae families at Wami-Ruvu might be associated with differences in geo-hydrological patterns and levels of impairment between the basins. It was also recognized that, the spatial distribution of macroinvertebrate communities in the basins were significantly influenced by varied levels of environmental variables as a result of geomorphology and improper land uses. More diverse orders with a wider range of occurrences and tolerance to pollution (Ephemeroptera (E), Diptera (D), Odonata (O) and Trichoptera (T)) can be considered as potential bio-indicators in developing biomonitoring index for Tropical African Rivers as they showed a significant discriminating power that separated reference from monitoring sites.

[3]Manuscript submitted to Elsevier (International Journal of Aquatic Sciences)

4.1 Introduction

Over the past decades, various types and diverse macroinvertebrates have been reported in tropical (Kibichii *et al*., 2007; Elias, 2009; Kidan, 2010), temperate (Dalas, 2004; Bonada *et al*., 2007), and Mediterranean (Thorn and Covick, 1991; Bonada *et al*., 2006; Bonada and Resh, 2013) regions. This is due to the fact that macroinvertebrate communities differ greatly taxonomically from one region to another and within a region due to differences in ecological degradation, biogeographical and climatical conditions (Elias *et al*., 2014a, 2014b). Tropical African rivers, for example, are known to be more specious compared to other regions but their taxonomical and ecological knowledge is still incomplete. Besides, habitat modification and pollutants discharged into tropical freshwater systems have led to habitat degradation and thus threatening the existence of some species. Macroinvertebrate species along Pangani and Wami-Ruvu basins, for instance, are facing more threats of extinction due to increased human activities and habitat modification (Elias *et al*., 2014b).

Increased human settlement and associated anthropogenic activities near river banks is exerting more pressure on aquatic ecosystems as basins are considered by people to be convenient and cheap means of disposing agricultural, domestic and industrial waste (Mkuula, 1993; Ngana, 2001). River banks, for example, are often perceived by man as fertile and productive lands suitable for agriculture. Similarly, human induced activities (such as pollution, habitat transformation of landscape and hydrological alterations) have direct impacts on freshwater habitat as they significantly change biotic integrity and functional ability of many river ecosystems worldwide, particularly in urban and agricultural areas (Zedler and Kercher, 2005; Verhoeven *et al*., 2006).

Owing to these concerns in most African countries, conservation efforts should start at national levels and focus on developing and implementing national environmental laws and policies relating to conservation, protection, and sustainable use of fresh water resources while discouraging pollution of water bodies (McCaffrey and Weber, 2005). Legislations should set ecological standards and quality objectives and make biomonitoring programmes of aquatic ecosystems mandatory. This study has therefore, been designed to characterize macroinvertebrates along Pangani and Wami-Ruvu basins in order to increase biomonitoring programme base in the tropical African region.

4.2 Materials and Methods

4.2.1 Description of Study Areas

Eighty five (85) sampling sites of varying degradation levels along Pangani and Wami-Ruvu river basins were selected for sampling to ensure the characterization of macroinvertebrates and determination of physico-chemical parameters (Figure 4.1). The basins provide a wide range of riverine systems, climate, geology, topography and human disturbance within different hydrological patterns. Pangani river basin is located in the north-east of Tanzania mainland, 36°23′ - 39°13′ E and 03°03′ - 05°59′ S with an altitude ranging from 0 - 4500 m. The basin has an estimated area of about 43 650 km^2 that covers Arusha (2369.76 km^2), Manyara (17 911.35 km^2), Kilimanjaro (10 346.76 km^2), and Tanga (10 223.17 km^2) regions. Land use practices along Pangani basin range from small-scale farming to large-scale mechanized agriculture, overexploitation of riparian vegetation, construction of dam and hydro power projects, grazing, bathing and washing, dumping of industrial and domestic wastes and human settlement.

The Wami-Ruvu river basin is elongated and extends from the central part of Tanzania towards the eastern part between 36°00′ - 39°00′ E and 05°00′ - 07°00′ S with an altitude of 0 - 2500 m before draining into the Indian Ocean at Saadani village. It extends through Dodoma, Morogoro, Coast and Dar es Salaam regions covering a total area of 72 930 km^2 of wide plains and mountain ranges.

The two basins experience equatorial type of climate with mean annual rainfall between 1100 and 3000 mm per annum, with a maximum mean temperature ranging from 32-35°C in the dry season and lowest of 14-18 °C during the wet season. Human activities that are impacting the Wami-Ruvu River basins include mining activities, brick making, poor agricultural practices involving application of agrochemicals, saline water intrusion, uncontrolled and illegal water abstraction for irrigation, bathing and washing along river basins, fauna droppings and disposal of untreated industrial and domestic wastes into the two rivers.

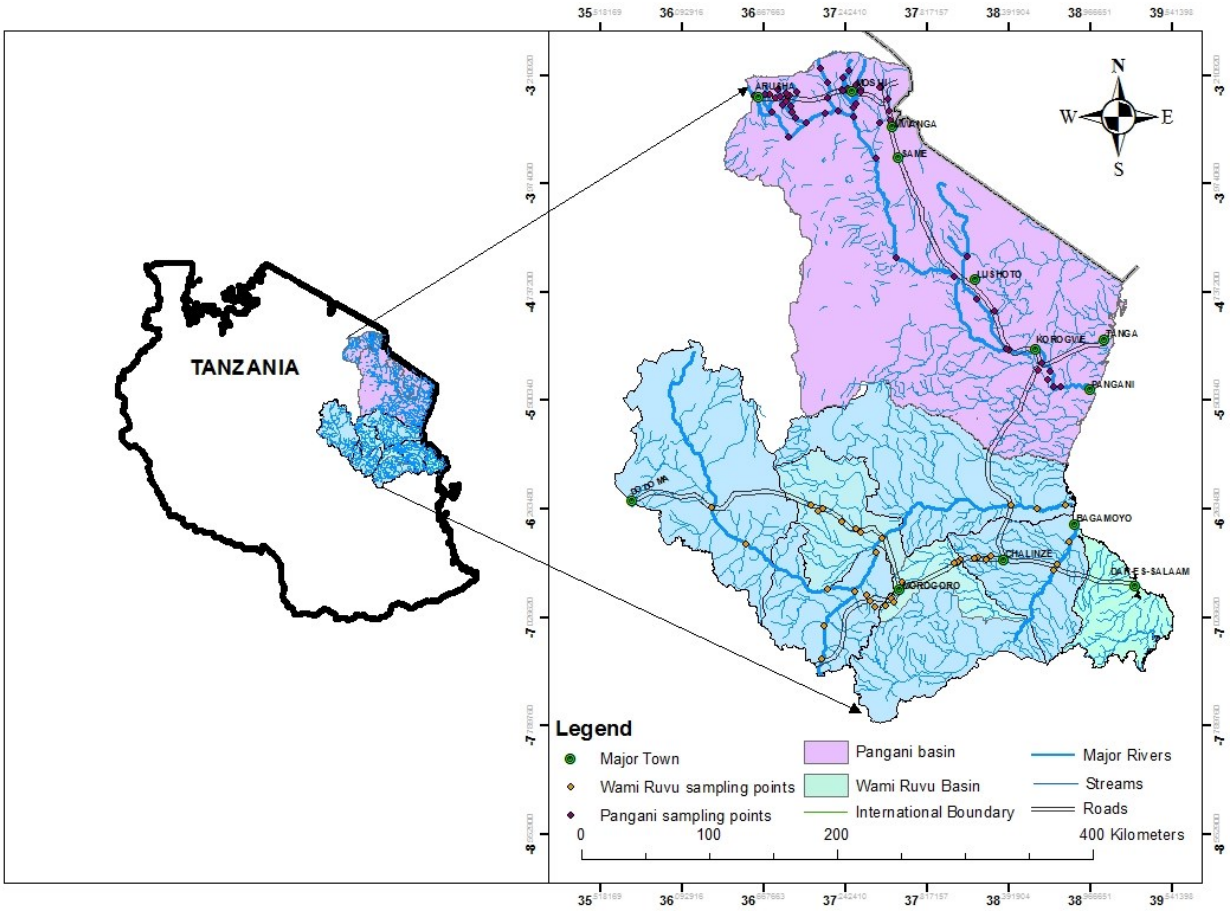


Figure 4.1: Tanzanian map showing sampling sites along Pangani and Wami-Ruvu river basins

4.2.2 Sampling Design

The two river basins were divided into two site categories in which 39 reference (least impacted) and 46 monitoring (highly impacted) sites were established. Triplicate water and macroinvertebrate assemblage samples were collected at each site near the end of dry and wet seasons to capture the effect of respective seasons. The sampling sites were selected based on their ease of accessibility, presence and/or absence of sustained anthropogenic disturbances, pools, riffles and runs, and degree of water physico-chemical and habitat degradation.

(a) Physico-chemical Data Collection

Water physico-chemical parameters i.e., pH, dissolved oxygen (DO), temperature, turbidity, conductivity, total dissolved solids (TDS), ammonia (NH_4^+-N), potassium (K^+), sulphate (SO_4^{2-}), soluble reactive phosphorus (SRP (PO_4^{3-}-P), nitrate (NO_3^--N) and nitrite (NO_2^--N) plus Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) were measured. Water temperature, conductivity, DO, TDS, and pH were measured and recorded

in situ at each site using a multi-sensor probe YSI Professional Plus Water Quality Instrument (Model 6 050 000) while turbidity was measured using a turbidity meter. Laboratory analysis for water chemistry variables involved filtering of collected water samples using 0.45 μm glass fiber filters and placed in hydrochloric acid washed polythene bottles before being preserved in a cool box at $\leq 10\ ^{O}C$. The samples were then taken to the Department of Aquatic Sciences and Fisheries Laboratory of the University of Dar es Salaam for analysis.

Nitrate (NO_3^--N), nitrite (NO_2^--N), ammonia (NH_4^+-N) and SRP (PO_4^{3-}-P) were analyzed using standard spectrophotometric methods described in APHA (1998, 2000). Ammonia was determined using a Phenate method, nitrate and nitrite concentrations by Cadmium reduction method, SRP analyzed using molybdate ascorbic acid method, SO_4^{2-} by turbid-metric method, BOD by instrumental (BOD track) method and COD using Instrumental (semi-automated calorimetry) method (ALPHA, 2000; Wetzel and Likens, 2000).

(b) Macroinvertebrate Samples

Macroinvertebrate samples were collected at the end of wet (May and June, 2014) and dry (September and October, 2014) seasons to capture the effect of respective seasons. Samples were collected throughout each sampling reach of 100 m by the same operator using a 30 x 30 cm kick-net with a 250 μm mesh size according to Barbour *et al*. (1999), Dickens and Graham (2002) and Lowe *et al*. (2013) methods. To avoid bias due to spatial variations or patchiness, samples were collected in triplicates and at random locations within 200 m reach, making nine samples per reach or sampling site. The nine individual samples were pooled as one composite sample and sorted grossly in the field at order level before preservation in 10% formaldehyde solution prior to transportation to the laboratory for identification. In the laboratory, macroinvertebrate specimens were identified to the lowest possible taxonomic level) under the help of dissecting microscope (100 x magnification) according to Day *et al*. (2001a, 2001b), Thorp and Covich (2001), Day and De Moor (2002a, 2002b), Day *et al*. (2003), De Moor *et al*. (2003a, 2003b) and Stals and De Moor (2007) identification methods and/or keys in relation to the local conditions, followed by listing and counting of individuals.

(c) B-IBI Scores

Percentages of 14 metrics including H-FBI (Hilsenhoff, 1988) were used to calculate Benthic Index of Biological Integrity (B-IBI) at each site according to Barbour *et al*. (1999), with their abundances being excluded. These include: % Ephemeroptera, % Plecoptera,

%Trichoptera, %Baetidae, % Odonata, % Dominant taxa, % Taxa richness, % EPT, % H-FBI, % Diptera, % Chironomidae and % Oligochaeta, % Non-insect taxa, and Shannon Diversity Index.

Metrics were standardized into three score ranges based on the degree of impairment. Maximum score of 5 was assigned to slightly impaired sites, 3 for moderately impaired sites, and 1 for severely impaired sites. These scores are simply arbitrary standards (Karr and Chu, 1999). Standardized metric scores were then added to produce the B-IBI score on a 70-point scale (involving 14 characters each with a maximum score of 5) and 14-point score (involving 14 characters with minimum value of 1). The B-IBI values were then standardized to 100-point scale: giving 100 (slightly impaired), 60 (moderately impaired), and 20 (severely impaired) as shown in Table 4.1 based on actual rating criteria prescribed by Pond *et al*. (2003)**.** For purposes of this study, streams/rivers B-IBI values below a score of 68 would be impaired (i.e., fair, poor and very poor).

Table 4.1: Classification of water quality status based on impairment levels from B-IBI score data

B-IBI score	Water quality characterization	Impairment level
20 to 46	Poor water quality	Severely impaired
>46 to 68	Fair water quality	Moderately impaired
>68 to 100	Good water quality	Slightly impaired

4.2.3 Statistical Analysis

MS Excel, Community Analysis Package version 4 (CAP IV), Species Richness and Diversity IV (SDR IV), and Instat® version 3 (GraphPad®) softwares were used to analyze the data. All data were organized using MS Excel spreadsheet and saved in appropriate format acceptable by particular software. Any variable that failed the normality test was transformed (to either log (x+1), square root, or arcsine), where appropriate. Species diversity and species accumulative curves were performed by Species Richness and Diversity IV (SDR IV) software (Seaby and Henderson, 2006a). Significance tests were performed by one-way analysis of variance (ANOVA) followed by the post hoc Tukey's multiple comparisons test, with p set at 0.05 to determine the differences between and within the site categories based on their mean biotic and abiotic data. Relationships between and within site categories of the

two basins were calculated using Instat version 3 (GraphPad®) and Community Analysis Package version 4 (CAP IV) software (Seaby and Henderson, 2006b).

4.4 Results

4.4.1 Macroinvertebrate Assemblages

A combined list of 12 629 macroinvertebrate communities representing 79 families of 17 orders collected from Pangani and Wami-Ruvu basins during the rainy and dry seasons is abridged in Appendix 1. Of that total, 60.95% (N = 12 629) was observed at Pangani with a preponderance of dipterans and 39.05% for Wami-Ruvu basin dominance is attributed to ephemeropterans. Collectively, dipterans and ephemeropterans represented 97.39% and 48.82% of the observed organisms along Pangani and Wami-Ruvu basins, respectively. Trichoptera was the most diverse order, found with 11 families, followed by Ephemeroptera, Diptera, and Odonata, with 10 families each. Hydroida, Lepidoptera, Plecoptera, Tubicifida and Turbellaria were the least diverse orders, each being represented by one family. The remaining orders had intermediate numbers of families that were rather uniform among sites, ranging from 2 to 9. Besides, 19 rare families (with abundances ≤ 0.3% in all site categories) were registered at Pangani compared to 15 at Wami-Ruvu basin and the absence of Nepidae, Notonectidae and Lumnichidae families. Ephemeropterans and trichopterans were observed at all reference sites (regardless of the seasonality) and in monitoring sites during the wet season as opposed to dipterans and Odonata.

Approximately 15 metrics were calculated separately to characterize macroinvertebrate assemblages for Pangani and Wami-Ruvu data. These metrics were categorized according to their taxonomical and ecological characteristics. These include: B-IBI, %EPT, H-FBI, percentages of Ephemeroptera, Trichoptera, Baetidae, Plecoptera, Oligochaeta, Chironomidae, Odonata, Diptera, Dominant Taxa, SDI, Non-Insecta and Relative Taxa Richness. Reference sites were dominated by Baetidae, Ephemeroptera, Trichoptera, Plecoptera, and B-IBI scores while Odonata, Diptera, Oligochaeta, and Chironomidae dominated the monitoring sites (Table 4.2). However, the low value of B-IBI scores and % EPT and higher H-FBI could be taken as an indicator of degraded water quality within a basin.

Table 4.2: Mean ± standard error of mean (SEM) of biometric data in Pangani and Wami-Ruvu sites

PANGANI REFERENCE	Relat TR	% Baet	% Ephem	% Trico	% Pleco	% Odon	% EPT	SDI	%Dom. Taxa	% Diptera	H-FBI	% Oligoch	% Chiron	%Non Ins	B-IBI
Mean	27.22	28.89	45.23	20.77	1.42	7.44	66.68	2.64	29.15	11.75	4.37	0.00	2.78	2.35	89.33
SEM	0.88	1.85	1.86	2.68	0.77	0.64	1.94	0.05	1.70	1.42	0.09	0.00	0.85	0.61	0.80
PANGANI REFERENCE	**Relat TR**	**% Baet**	**% Ephem**	**% Trico**	**% Pleco**	**% Odon**	**% EPT**	**SDI**	**%Dom. Taxa**	**% Diptera**	**H-FBI**	**% Oligoch**	**% Chiron**	**%Non Ins**	**B-IBI**
Mean	22.43	11.55	20.84	3.08	0.00	8.63	24.45	2.26	35.05	40.58	5.31	0.88	24.86	4.27	57.83
SEM	0.58	1.83	1.89	0.57	0.00	0.78	2.07	0.03	1.16	1.80	0.04	0.20	0.76	0.96	0.93
WAMI-RUVU REFERENCE	**Relat TR**	**% Baet**	**% Ephem**	**% Trico**	**% Pleco**	**% Odon**	**% EPT**	**SDI**	**%Dom. Taxa**	**% Diptera**	**H-FBI**	**% Oligoch**	**% Chiron**	**%Non Ins**	**B-IBI**
Mean	19.36	17.75	41.62	24.16	2.14	12.59	67.93	2.44	19.57	8.60	4.09	0.00	3.10	1.66	82.95
SEM	0.59	1.68	2.16	1.40	0.63	0.73	2.22	0.03	1.40	1.22	0.08	0.00	0.50	0.62	1.15
WAMI-RUVU MONITORING	**Relat TR**	**% Baet**	**% Ephem**	**% Trico**	**% Pleco**	**% Odon**	**% EPT**	**SDI**	**%Dom. Taxa**	**% Diptera**	**H-FBI**	**% Oligoch**	**% Chiron**	**%Non Ins**	**B-IBI**
Mean	25.5	6.6	19.2	10.6	0.4	14.9	30.3	2.7	19.9	29.9	5.0	0.2	18.4	3.1	68.57
SEM	0.4	1.1	1.1	0.6	0.1	1.0	1.1	0.0	0.9	1.5	0.0	0.1	1.1	0.3	0.97

4.4.2 Physico-chemical parameters

Generally, higher mean values of recorded physico-chemical variables followed the order: TDS > COD > BOD > Temperature > Conductivity and TDS > COD > BOD > SO_4^{2-} > Turbidity at Pangani and Wami-Ruvu, respectively. However, there was also a trend for most variables being higher at monitoring sites relative to reference sites. For instance SO_4^{2-}, K^+, NH_4^+-N, Conductivity, and BOD were 45, 40, 15, 8 and 6 times higher, respectively at Wami-Ruvu monitoring sites while, TDS and COD were 38 and 5 times higher at Pangani monitoring sites compared to reference sites. Moreover, mean DO was higher at all reference sites and there was no NO_2^--N or PO_4^{3-}-P detected at Wami-Ruvu reference sites (Table 4.3).

Table 4.3: Mean ± standard error of mean (SEM) of environmental variables in the site categories of Pangani and Wami-Ruvu river basins

PANGANI REFERENCE	Temp ^{O}C	pH -	DO mg/l	Turb NTU	TDS mg/l	Cond µS/cm	NO_3 mg/l	NO_2 mg/l	NH_4-N mg/l	SRP mg/l	K mg/l	$SO4^{2-}$ mg/l	BOD mg/l	COD mg/l
Mean	20.38	7.61	7.82	0.87	26.92	14.62	0.25	0.66	0.10	0.51	0.33	0.27	9.52	22.47
SEM	0.18	0.12	0.08	0.15	2.51	2.18	0.05	0.11	0.03	0.06	0.05	0.05	1.08	2.53
PANGANI MONITORING	**Temp ^{O}C**	**pH -**	**DO mg/l**	**Turb NTU**	**TDS mg/l**	**Cond µS/cm**	**NO_3 mg/l**	**NO_2 mg/l**	**NH_4-N mg/l**	**SRP mg/l**	**K mg/l**	**$SO4^{2-}$ mg/l**	**BOD mg/l**	**COD mg/l**
Mean	23.64	7.80	6.97	7.37	1031.42	37.10	0.37	4.39	0.43	0.39	1.13	0.94	40.75	110.84
SEM	0.47	0.10	0.20	1.51	239.95	2.83	0.04	0.91	0.05	0.04	0.55	0.19	5.79	18.44
WAMI-RUVU REFERENCE	**Temp ^{O}C**	**pH -**	**DO mg/l**	**Turb NTU**	**TDS mg/l**	**Cond µS/cm**	**NO_3 mg/l**	**NO_2 mg/l**	**NH_4-N mg/l**	**SRP mg/l**	**K mg/l**	**$SO4^{2-}$ mg/l**	**BOD mg/l**	**COD mg/l**
Mean	22.51	7.88	8.89	26.33	72.33	103.35	0.00	0.14	0.08	0.00	0.02	0.60	6.76	15.08
SEM	0.60	0.09	0.79	11.29	35.53	58.09	0.00	0.05	0.04	0.00	0.00	0.40	2.16	3.89
WAMI-RUVU MONITORING	**Temp ^{O}C**	**pH -**	**DO mg/l**	**Turb NTU**	**TDS mg/l**	**Cond µS/cm**	**NO_3 mg/l**	**NO_2 mg/l**	**NH_4-N mg/l**	**SRP mg/l**	**K mg/l**	**$SO4^{2-}$ mg/l**	**BOD mg/l**	**COD mg/l**
Mean	24.39	8.14	5.97	35.23	651.19	808.33	0.38	2.67	1.23	0.38	0.79	22.29	38.55	82.80
SEM	0.48	0.14	0.61	6.56	293.52	384.42	0.20	1.35	0.76	0.10	0.25	18.39	20.56	37.63

4.4.3 Differences between the basins and site categories

(a) Sites versus biometric data

Percentages of B-IBI score, EPT, Ephemeroptera, Trichoptera, Baetidae, and Plecoptera were weighted toward isolated reference sites on the right site of PCA ordination (Figures 4.2 and 4.3). It is not surprising that B-IBI score was also weighted toward reference sites (on right side) in ordination since by definition all reference sites have good water and/or habitat quality. On the contrary, percentages of Oligochaeta, Chironomidae, Odonata, Diptera, Dominant Taxa, Non-Insecta and Relative Taxa Richness vectors pointed toward monitoring sites (on left side) in ordination for each basin. Graphically, the biometric data had clearly discriminate reference sites from monitoring sites at each basin and consequently demonstrate the differences in water quality and physical habitat between the site categories.

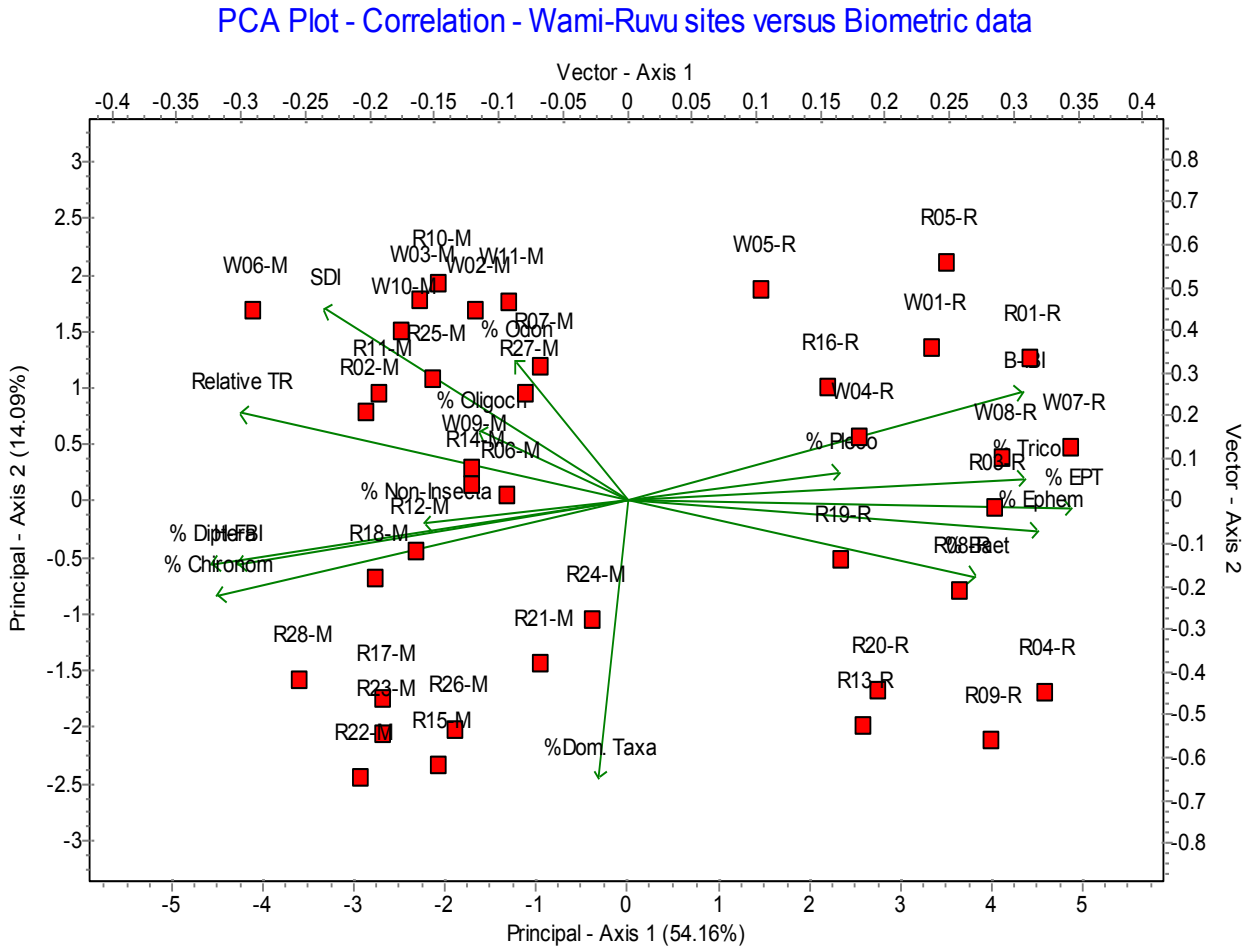


Figure 4.2: Principal Components Analysis (PCA) ordination based on biometric data (vectors) among Wami-Ruvu site categories. The codes with the symbol % stand for biometric data and any site codes with R (i.e., W01-R) and M (i.e., W03-M) refer to Reference and Monitoring sites, respectively.

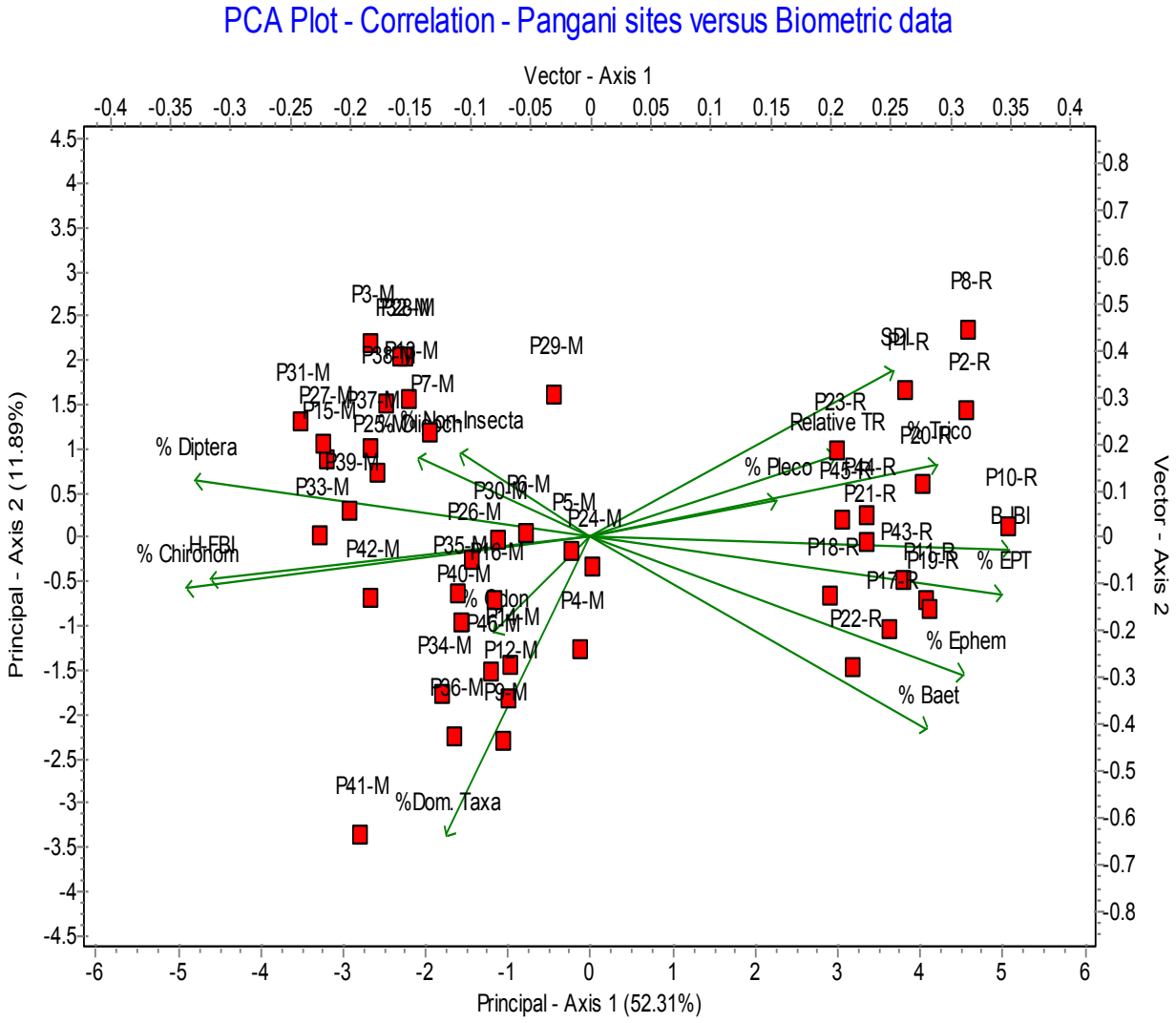


Figure 4.3: Principal Components Analysis (PCA) ordination based on biometric data (vectors) among Pangani site categories.

(b) Differences between the basins and site categories

Seven of the 30 biometric and environmental variables tested differentiated the basins and site categories significantly, with p values < 0.05 (Table 4.4).

Table 4.4: One way ANOVA results for biotic and abiotic variables that showed significant effect in all compared classes between the basins

S/N.	Variable	Compared Classes	Probability	Differences between the classes
1.	B-IBI scores	Pr versus WRr	P = 0.0017	Very significant
		Pm versus WRm	P < 0.0001	Extremely significant
		Pb versus WRb	$F_{(3,81)}$ = 197.55; P<0.05	Significant
2.	F-IBI scores	Pr versus WRr	P = 0.02	Significant
		Pm versus WRm	P = 0.0002	Extremely significant
		Pb versus WRb	$F_{(3,81)}$ = 86.29; P<0.05	Significant
3.	Turbidity	Pr versus WRr	P = 0.038	Significant
		Pm versus WRm	P = 0.0007	Extremely significant
		Pb versus WRb	$F_{(3,81)}$ = 8.22; P<0.05	Significant
4.	% SDI	Pr versus WRr	P = 0.013	Significantly different
		Pm versus WRm	P < 0.0001	Extremely significant
		Pb versus WRb	$F_{(3,81)}$ = 30.34; P = 0.05	Significant
5.	% Odonata	Pr versus WRr	P = 0.0036	Very significant
		Pm versus WRm	P < 0.0001	Extremely significant
		Pb versus WRb	$F_{(3,81)}$ = 15.92; P<0.05	Significant
6.	% Dominant taxa	Pr versus WRr	P < 0.0001	Extremely significant
		Pm versus WRm	P < 0.0001	Extremely significant
		Pb versus WRb	$F_{(3,81)}$ = 39.89; P<0.05	Significant
7.	% Taxa richness	Pr versus WRr	P < 0.0001	Extremely significant
		Pm versus WRm	P = 0.001	Very significant
		Pb versus WRb	$F_{(3,81)}$ =24.38; P<0.05	Significant

Key: Pb= Pangani basin; WRb = Wami-Ruvu basin; Pr = Reference sites at Pangani; WRr = Reference sites at Wami-Ruvu; Pm = Monitoring sites at Pangani and WRm = Monitoring sites at Wami-Ruvu.

(c) Water quality status of the basins

The B-IBI score was used for this study as it combines several distinctive, stress-influenced community characteristics into a single aggregate value that can be used to compare the level of stress evidenced by communities from different river localities. The B-IBI scores calculated from 14 biometric data resulted in categorization of sites based on their

impairment levels with reference sites out-scoring monitoring sites at each basin. The scores indicated that 43.53% of the sampling sites analyzed in the catchment basin presented very good water quality, 56.47% fair to good water quality (Table 4.5).

Table 4.5: Categorization of sites into different impairment levels based on B-IBI score results

B-IBI Scores	Water Quality	Impairment	Pangani Basin	Wami-Ruvu Basin
20-46	Very Poor to poor	Severe to Slight	-	-
>46-72	Fair to Good	Moderate to Less	All 31 monitoring sites	17 monitoring sites (W06, W09,W10, W11, R02, R11, R12, R14, R16, R17, R18, R21, R22, R23, R24, R26 and R28)
>72-100	Very Good to Excellent	Very Little to None	All 15 reference sites	All 15 reference sites and 7 monitoring sites (W02, W03, R06, R07, R10, R25 and R27)

4.5 Discussion

Macroinvertebrate communities have become somewhat out of balance among site categories and the basins, both taxonomically and ecologically. Composition of macroinvertebrates and environmental variables was different not only between site categories and the basins but also presented seasonal variation. When comparing the taxonomic list of these two basins, macroinvertebrate organisms in Pangani seem to be more diverse and abundant. It was also possible to observe a greater representativeness of some sensitive taxa like Coleoptera, Ephemeroptera, and Trichoptera in all site categories of Pangani and Wami-Ruvu, respectively, which were not exposed to high impacts. The results are in line with similar findings by Rosenberg and Resh (1993), Compin and Céréghino (2003), Morse *et al*. (2007), Song *et al*. (2009) and Foto Menbohan (2012) who associated presence of coleopterans, ephemeropterans and trichopterans with good water quality and habitat suitability. The occurrence of these sensitive taxa in all site categories further suggests a possible improvement in the environmental conditions related to the decrease in concentrations of nutrients and changes in some of the physico-chemical parameters (e.g., dissolved oxygen,

pH, temperature and electrical conductivity), and hence, reflecting greater richness of macroinvertebrate assemblages.

Maximum values of water conductivity (7854.20 µS/cm), TDS (5838.70 mg/l), COD (928.01 mg/l), BOD (500.80 mg/l), SO_4^{2-} (444.51 mg/l) and turbidity (104.44 NTU) and depletion of DO from 16.97 to 0.58 mg/l were registered at Wami-Ruvu monitoring sites, and could have been responsible for the absence of Nepidae, Notonectidae and Lumnichidae families. Indeed, absence of these families in Wami-Ruvu basin is undoubtedly due to differences in hydrological patterns between Pangani and Wami-Ruvu basins and levels of impairment caused by the uncontrolled discharge of domestic sewage, agrochemical inputs and industrial wastes in the rivers (Morris *et al*., 2003). These results are consistent with those of Compin and Céréghino (2003), Song *et al*. (2009) and Foto Menbohan (2012) who showed that a decrease in Coleoptera richness (i.e., Lumnichidae family) in human impacted rivers is clearly related to changes in water quality and habitat suitability. It can therefore be hypothesized that these taxa would have historically been present at Wami-Ruvu before human disturbances, as most of the environmental variables i.e., TDS and DO were found with values above recommended limits of 500 mg/l and 5 mg/l, respectively (Chapman *et al*., 1996; WHO, 2000). Moreover, the results also agree with studies of Sousa *et al*. (2006) and Fuji (2007) who reported the effect of environmental variables on the occurrence and distribution of macroinvertebrate organisms in freshwater ecosystem.

Statistical tests revealed correlation of the metrics related to impacts with TDS, turbidity, COD, BOD, DO, temperature, conductivity, potassium, sulphate, nitrogen, and phosphorus contents associated with improper land uses near basins (Kidan, 2010; Masese *et al*., 2013). The two site categories of Pangani and Wami-Ruvu basins were clearly separated on PCA plot ordination, with two distinct patterns of biometrics that represented least and more pollution tolerant macroinvertebrate communities (Figures 4.2 and 4.3). Although, sensitivities of macroinvertebrates to pollution do vary (Yuan, 2004), increase or decrease of physico-chemical variables beyond required limits is considered harmful to least tolerant living biota (Kimmel, 1983; Thorp and Covich, 1991; Angelier, 2003; Yuan, 2004). For instance, the increase in nutrients beyond required limits is likely to lead to reduced occurrences of intolerant taxa (trichopterans and plecopterans) and favour the tolerant taxa (dipterans i.e., Chironomidae), which can survive better in low oxygenated conditions (Thorp and Covich, 1991; Lake, 2003; Elias *et al*., 2014b). However, the dominance does not always

reflect better environment, as mild disturbance may favour some tolerant taxa with subsequent reduction in sensitive taxa.

Reference sites of the two basins were prominently located in riffles and undercut banks of stone substrates with subsequent waters of high DO and lower nutrient levels compared to fine substrate (gravel, sand and mud) of monitoring sites. These fine substrates contain loose sediments and decomposed organic matter of low DO, as a result, support only tolerant macroinvertebrate communities (Koel and Stevenson, 2002) compared to those accommodated by stone substrates. Heptageniidae and Baetidae (Ephemeroptera), Chironomidae (Diptera) and Hydropsychidae (Trichoptera) for example, can survive under serious environmental stresses with low DO waters because of their ability to oxidize mud on the river bottom and produce haemoglobin (Ikonomov, 1963; Simic, 1999; Paunovic *et al*., 2007). Their diverse nature and ability to tolerate a wider range of tolerance towards varied environmental conditions might have contributed to their distribution. However, the dominance of dipterans (Chironomidae) in fine bottom substrates and Trichoptera and Ephemeroptera on riffles and hard substrate sites, which was revealed in this study is also consistent with previous taxonomical and ecological studies conducted by Lyimo (2012), Elias *et al*. (2014b), and Kaaya (2014) in some Tanzanian rivers. Contrary to Heptageniidae and Hydropsychidae, Plecoptera and Trichoptera were only found at the reference sites and were totally absent at monitoring sites (especially in dry season) because they have predilection for habitats of good water quality (Richards *et al*., 1997). Moreover, Ephemeroptera, Plecoptera and Trichoptera were also reported by Morse *et al*. (2007) as taxa that are very sensitive to pollutants i.e., nutrients, sediments, heavy metals, chemicals and organic nutrients.

Monitoring sites were dominated by pollution tolerant biometrics while intolerant biometrics dominated the reference sites despite the basins being located in different geo-hydrological pattern. Hilsenhoff Family-level Biotic Index (H-FBI) findings have also indicated the slightly enriched type of water quality in reference sites with monitoring sites demonstrating a deterioration from slightly enriched to enriched water quality (Table 4.2). The H-FBI results concur with B-IBI score in which reference sites were segregated from monitoring sites (Table 4). H-FBI and B-IBI scores have suggested the slight deterioration of water quality in monitoring sites compared to reference sites as a consequence of improper land use and habitat degradation (Salomoni *et al*., 2007; Bahar *et al*., 2008; Masese *et al*., 2013;

Abrehat *et al*., 2014). Moreover, the dominance of intolerant taxa (Ephemeroptera and Trichoptera) in the reference sites as opposed to tolerant taxa (Diptera and Odonata) and absence or fewer Plecoptera in monitoring sites also corroborates findings from other studies e.g., Kasangaki *et al*. (2008), Masese *et al*. (2009), and Aura *et al*. (2010) in tropical African rivers. Similarly, the observed fewer numbers of Plecoptera at Pangani (0.25%, N = 19) and Wami-Ruvu (0.81%, N = 40) are also in line with most other studies conducted in tropical African rivers (Ndaruga *et al*., 2004; Sitotaw, 2006; Kibichii *et al*., 2007; Kasangaki *et al*., 2008; Aure *et al*., 2010; Elias *et al*., 2014b), as *Perlidae sp.* was rarely encountered and totally absent in severely degraded sites. Furthermore, the dominance of certain taxa (i.e., Chironomidae and Naididae), and absence of others (e.g., Plecoptera), at some sites can also be associated with habitat modification. In summary, this provides further evidence to support the notion that, presence of human induced activities discharging various forms of pollutants especially nutrients into watersheds, can predict macroinvertebrate structure and function.

4.6 Conclusion

With the aid of measured physico-chemical variables, all identified macroinvertebrate orders were useful in detecting disparities between site categories and basins at a family level. However, by increasing their taxonomic resolution to genus or species levels might improve or enhance the ability to detect differences among site categories and the two basins with respect to their macroinvertebrate assemblages and environmental variables. Updated ecological inventory and the taxonomic list (including distribution records and descriptions of new taxa) generated from this study will contribute to new effort of documenting existing macroinvertebrate species and development of regional identification guides and cost-effective biomonitoring index. It was recognized that, more diverse orders with wider range of occurrences and tolerance to pollution can be considered as bio-indicators in developing species level biomonitoring index for Tropical African Rivers as they have significant power of discriminating reference sites from impacted sites.

CHAPTER FIVE

5.0 RESPONSE OF THREE TROPICAL MACROINVERTEBRATES TOWARD NUTRIENTS[4]

Abstract

Although increased nitrate and phosphorus concentrations are known to be toxic to aquatic life as they stimulate productivity and increase eutrophication, there were no established thresholds of harm or standard limits specifically for these nutrients to safeguard riverine ecosystems. Toxicity effects of these nutrients were therefore experimented in the laboratory using static method as part of the process in developing threshold limits toward the mortality response of tested macroinvertebrates. Three native tropical African organisms of different sensitivity levels responded differently to treatments, with toxicants affecting least tolerant species more strongly than moderate and most tolerant ones. The observed lethal effects and/or mortality increased with concentration, and exposure time among tested species of different sensitivity. The results demonstrated that both nitrates and phosphorus are toxic to the three aquatic organisms studied, under the test conditions utilized with *Neorpela spio* displaying the highest acute effect in water with nitrate and phosphate than *Baetis harrisoni* and *Tubifex* spp. The 100% cumulative mortality were experienced at 3.2 mg NO_3-N/L and 2.4 mg PO_4-P/L for *Neorpela spio*, 5.6 mg NO_3-N/L and 4.8 mg PO_4-P/L for *Baetis harrisoni* and 128 mg NO_3-N/L and 24 mg PO_4-P/L for *Tubifex* spp. However, *Neorpela spio* and *Baetis harrisoni* showed a high mortality at the Tanzanian nitrate recommended lower and maximum limits of 10 and 75 mg NO_3-N/L respectively for drinking water and a significant mortality at the recommended limits of nitrite (20 mg NO_3-N/L) and phosphorus (6 mg PO_4-P/L) concentrations for municipal and industrial wastewaters. Therefore, there is a need for these Tanzanian recommended nitrate ranges for drinking water of 10 to 75 mg NO_3-N/L and 20 mg NO_3-N/L and 6 mg PO_4-P/L for municipal and industrial wastewaters to be refined for the betterment of protecting both human health and riverine organisms.

[4]Manuscript submitted to Ambio (International Journal of Ecotoxicology)

5.1 Introduction

In the developing world, nutrient loads are increasingly deteriorating riverine ecosystems as a consequence of rapidly growing human populations, land use changes, intensified agriculture, and increasing urbanization and industrialization (Gruber and Galloway, 2008). The elevation of nutrient concentrations into rivers is associated with overgrowth of algal blooms, a phenomenon known as "*eutrophication"* that deplete the oxygen levels needed by aquatic organisms for survival (Camargo and Alonso, 2006). Eutrophication induces a disruption of the normal functioning of the ecosystem by favouring simple algae and plankton which proliferate and prevent light from penetrating the water column, thus severely affecting water quality and aquatic life (Carpenter *et al*., 1998; Camargo and Alonso, 2006). In excess amounts, however, nutrients cause hyper-eutrophication resulting in an over-abundance of primary producers and decline of the biological community when present at levels above biologically-tolerable concentrations (Camargo and Alonso, 2006). Moreover, the dynamics of macroinvertebrate communities in freshwater systems are caused by the consequence of human induced pollution (particularly nutrients loading) of different magnitudes (Heiskary and Markus, 2003). Similarly, the protection of aquatic environment is often neglected when pursuing socio-economic development and food security objectives due to increased pressures from induced human activities. It will, therefore, be a great challenge for developing countries to be able to achieve these objectives without compromising the future health of aquatic ecosystems.

Evidence of increased algal abundance and decreased biological integrity can be associated with increased phosphorus and nitrogen concentrations in aquatic environment (Heiskary and Markus, 2003). Despite its association with pollution into freshwater ecosystems, toxicity test on the response of local macroinvertebrates toward nutrients loading from fertilizers application into agricultural farms have been rarely conducted in tropical African rivers. Where conducted, only the most pollution sensitive macroinvertebrates are used as test organisms with least and moderate sensitive organisms being exempted. These most sensitive species to pollution have been preferred in ecological protection on the grounds that their protection would automatically protect the entire freshwater ecosystem (ASTM, 1978; Kenega, 1978; Buikema and Benfield, 1980). Since macroinvertebrate species have different physical and chemical preferences in which they can survive, the conservation measures should also be rational. Irrational grounds of favouring only the most sensitive organisms i.e., *Dalphnia magma* would hurt exempted moderate and/or most tolerant organisms and

eventually led to their extinction. Moreover, the knowledge regarding sensitivity or response of different macroinvertebrate groups towards toxicants would only provide an informed choice of suitable species and help to bolster biomonitoring programmes if both least and most sensitive organisms are involved.

A number of developing African countries have identified the specific concentration levels at which nutrient over-enrichment occurs in their waters, but they have not adopted such nutrient criteria into their national water quality standards. In Tanzania for instance, the standard limits for surface water (rivers) that would help to protect local freshwater species are not prescribed by Tanzanian Bureau of Standards compendium. As a result, nutrient over-enrichment problems in rivers are underestimated and the responsible authorities dealing with freshwater quality are not fully engaged. Only nitrate standard limits for portable (drinking) water and nitrate and phosphorus limits for discharged wastes from point sources were included but only for the purpose of protecting people's health and monitoring municipal and industrial wastewaters. For such reasons, there is a need of having integrated approach that would incorporate both least, moderate and most pollution sensitive organisms to provide the toxicant threshold limit ranges from which protection and management efforts of riverine ecosystems would rely on. This study has therefore defined the numerical threshold limits and/or thresholds of harm as a basis in formulating Tanzanian nutrients guideline limits specifically for surface (river) water, protective for all freshwater macroinvertebrates. Findings of this study can be used as baseline information towards management of water quality in the studied area and may be replicated in other watersheds with similar agricultural activities and population scenarios.

5.2 Materials and Methods

5.2.1 Description of specimen sampling sites

Being located in the socio-economic strategic areas, Tanzanian rivers flow through farm and/or estate areas where nutrients loading as a result of fertilizers application are a possibility. Community diversity and land uses (particularly agriculture) along the basins can suggest the basins modification (Chapter four; Elias *et al*., 2014b), and for such a reason had prevented the sensitive species i.e., Ephemeropterans, Plecopterans and Trichopterans to thrive in some impaired sites before emptying into Indian Ocean. However, water quality of the downstream sites is more modified compared to upstream sites and considered poor, with

the main impact being caused by agricultural activities and hydro-power project and to a lesser extent industrial pollution.

5.2.2 Selection of the test organism

Since there is probably no universal and/or standard test organism(s) specifically known for toxicity testing (Buikema and Benfield, 1980; Buikema *et al*., 1982; Rand and Petrocelli, 1985; Standard Methods, 1992; Primbas, 2005), the selection of test organisms for this study was therefore based on: (i) pollution tolerance criteria, (ii) habitat preferences and feeding habits, (iii) compatibility to and ease for use in toxicity testing, (iv) availability (diverse and/or rarity) and distribution, and (v) ecological and taxonomical relevance. The selected test organisms involved: least pollution tolerant organisms (stonefly, Perlidae family, *Neorpela spio*), moderately tolerant organisms (mayfly, *Baetis harrisoni*) and most tolerant organisms (*Tubifex* worms).

Among the aquatic macroinvertebrates, stonefly and mayfly were chosen for this study because they are known to be relatively sensitive to changes in water quality and play an important role in the commonly used EPT Index (Lenat and Penrose, 1996). The absence of *Neorpela spio* and *Baetis harrisoni* at some sites found with poor water quality does imply presence of poor water quality. *Neorpela spio* was chosen as a test organism because only few of them were counted along Tanzanian river basins and thus affirming their rarity and/or extinction due to presence of fewer reference sites (Chapter four). Likewise, the presence of *B. harrisoni* in both least and moderately impacted sites and their absence in polluted downstream sites of Pangani river basin (Elias *et al*., 2014b), point to the moderate response of the species towards nutrients, or the physical parameters, or both. Moreover, *Baetis harrisoni* for example, has been mostly preferred as a test organism by the United States Environmental Protection Agency (US EPA), the American Society for Testing and Materials (ASTM) (Persoone and Janssen, 1993) and the Institute for Water Research, Grahamstown (Palmer *et al*., 1996) in different toxicity testing. Based on results in Chapter four, *Tubifex* worms and Chironomidae had exhibited their tolerance character as they are found dominating impaired sites of Tanzanian river basins. Ability of these organisms to inhibit and survive better at sites with higher levels of human induced pollution (extremely polluted waters of very low DO and highly turbid habitats) has therefore qualified them as test organisms representing most pollution tolerant organisms (Peckarsky *et al*., 1990). *Tubifex* worms and Chironomidae have been mostly preferred as test organisms by the APHA, ASTM,

FAO and the US EPA in different toxicity testing protocols. Therefore information of their (*Neorpela spio*, *B. harrisoni* and *Tubifex* spp.) response against nutrients would improve knowledge base and be useful for future comparative studies between regions.

5.2.3 Collection, transfer and quarantine of test organisms

All test organisms were collected using kick net from the same source to avoid differences in acute sensitivities as a result of using organisms of the same species from different sources. Organisms were collected in the field and handled properly during transportation to the laboratory for quarantine and acclimation according to ASTM E-729 (2002; 2007) to avoid unnecessary stress and/or injuries. During quarantine and acclimation, organisms were carefully observed daily for signs of stress, physical damage, mortality, disease, and external parasites (ASTM E-729, 2002). Abnormal, dead, and injured individuals were disqualified for the test (ASTM E-729, 2002; 2007). Since it was not easy to determine the exact age of test organisms, immature organisms of the same weight and length were chosen. For instance, selected instars of mayflies and stoneflies were in their early stages and *Tubifex* worms at their second or third instar. Immature organisms were more preferred because they are more sensitive to toxicants than older individuals of the same species (ASTM E-729, 2002; 2007).

5.2.4 Experimental design

Pilot toxicity test was conducted to set ranges for each toxicant nutrient at three test concentrations according to ASTM E-720 (2007) and previously reported toxicity tests. Preliminary NO_3-N and PO_4-P concentration ranges of 0.4 - 3.2 mg/l for *Neorpela spio*, 0.8 - 6.2 mg/l for *Baetis harrisoni* and 2 - 128 mg/l for *Tubifex* spp. were projected prior to testing. Static toxicity test began when test organisms were placed in treatment chambers within 30 min after the test material had been added into dilution water. The test organisms were exposed to two toxicants (Nitrates (NO_3-N) and Phosphates (PO_4-P)), of different concentrations and a control, using static and renewal procedures prescribed by ASTM E-729 (2002; 2007). The selected test organisms representing least, moderate and most pollution tolerant organisms were separately exposed to their respective nutrients ranges resulting from preliminary test for 96 hours. 96-hr static-removal nutrient toxicity test was preferred because it assumed that macroinvertebrates can survive in such duration without food. Both control and toxicant for each test were duplicated, resulting in 18 treatments or test chambers. Therefore, a total of 54 treatments were conducted covering the three toxicants tested for both least and most sensitive organisms. Only ten organisms were subjected to each treatment to

maintain test organisms in good condition and avoid unnecessary stress, be crowded or subjected to rapid changes in temperature or water quality. Water temperature change of not more than 3°C and dissolved oxygen concentration between 40 and 100 % were maintained throughout the test. Because death of some invertebrates is not easily distinguished from immobilization, an EC_{50} was usually determined rather than an LC_{50}. Dead and/or affected organisms in each test chamber were counted and recorded after every 12 hours from the start of each test. Delayed effects test were also determined after experiment by placing the live test organisms in water containing no toxicant for 2 days.

Lastly, a benchmark approach similar to Cormier *et al*. (2008) was used to identify the thresholds of harm while defining a concentration where a small change produces a large mortality response (Dodds *et al*., 2010). For purposes of this study, a concentration at which mortality has just exceeded 10% was considered as minimum limit and that exceeding 50% as maximum limit. The average of recorded concentrations causing 10% mortality for the three organism groups of different sensitivity levels and that caused just above 50% mortality for the groups represented the ranges of threshold limits. Although this concept is still in its infancy, threshold limits were translated into standards (such as numerical limits of quantifiable stressor variables) and thus, be relevant for policy-making (Andersen *et al.*, 2009).

5.3 Results

The cumulative mortalities observed from the control (blank) treatments were below 10% for all tested macroinvertebrate groups of different sensitivity levels. Results of acute (96 h) toxicity responses for riverine *Neorpela spio*, *Baetis harrisoni* and *Tubifex* spp. showed transient effects with 10% cumulative mortality at 0.8, 1.6 and 6.0 mg NO_3-N/L and 0.4, 0.8 and 2.0 mg PO_4-P/L respectively, as lowest thresholds of harm (Figures 5.1, 5.2 and 5.3). The 100% cumulative mortality tallies were experienced at 3.2 mg NO_3-N/L and 2.4 mg PO4-P/L for *Neorpela spio*, 5.6 mg NO_3-N/L and 4.8 mg PO_4-P/L for *Baetis harrisoni* and 128 mg NO_3-N/L and 24 mg PO_4-P/L for *Tubifex* spp. However, 100% cumulative mortalities that were attained by *Neorpela spio* after being subjected to LC_{50} of 3.2 mg NO_3-N/L and 2.4 mg PO_4-P/L for 96 hours are considerably higher than 65 and 68% for *B. harrisoni* and <10% for *Tubifex* spp.

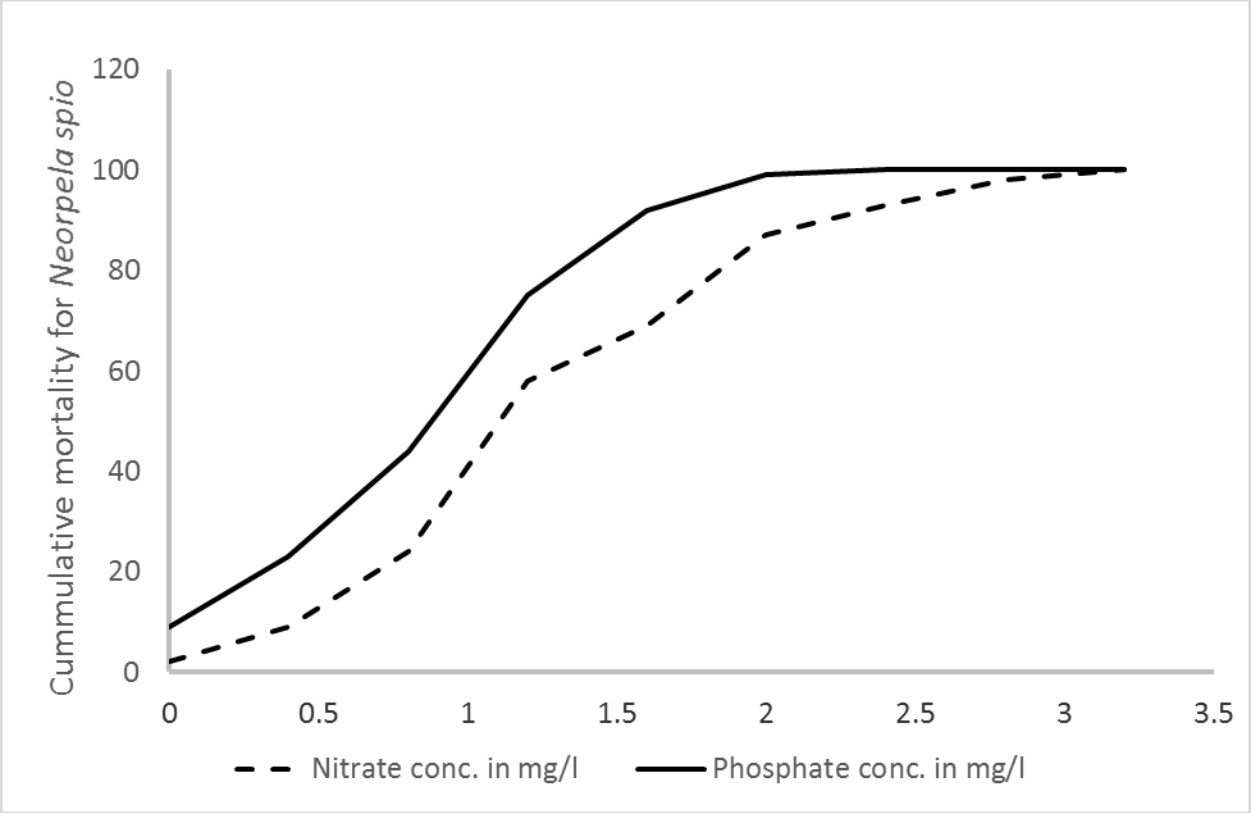


Figure 5.1: 96 hours concentration response curves showing cumulative mortality for *Neorpela spio*

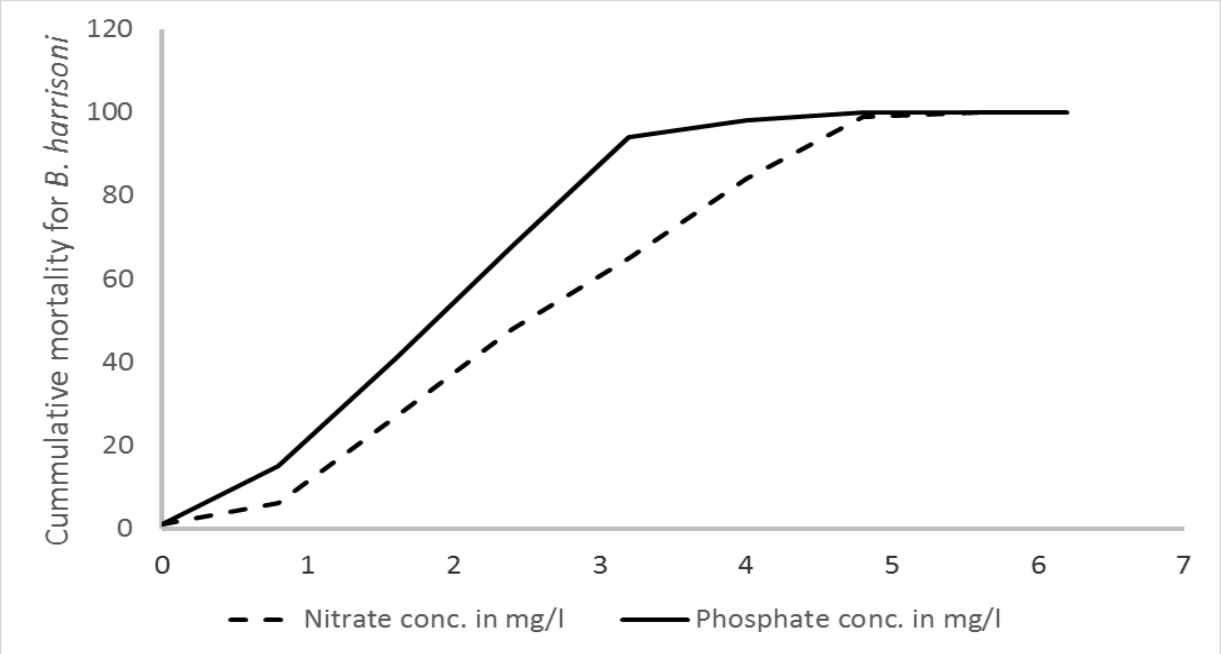


Figure 5.2: 96 hours concentration response curves showing cumulative mortality for *Baetis harrisoni*

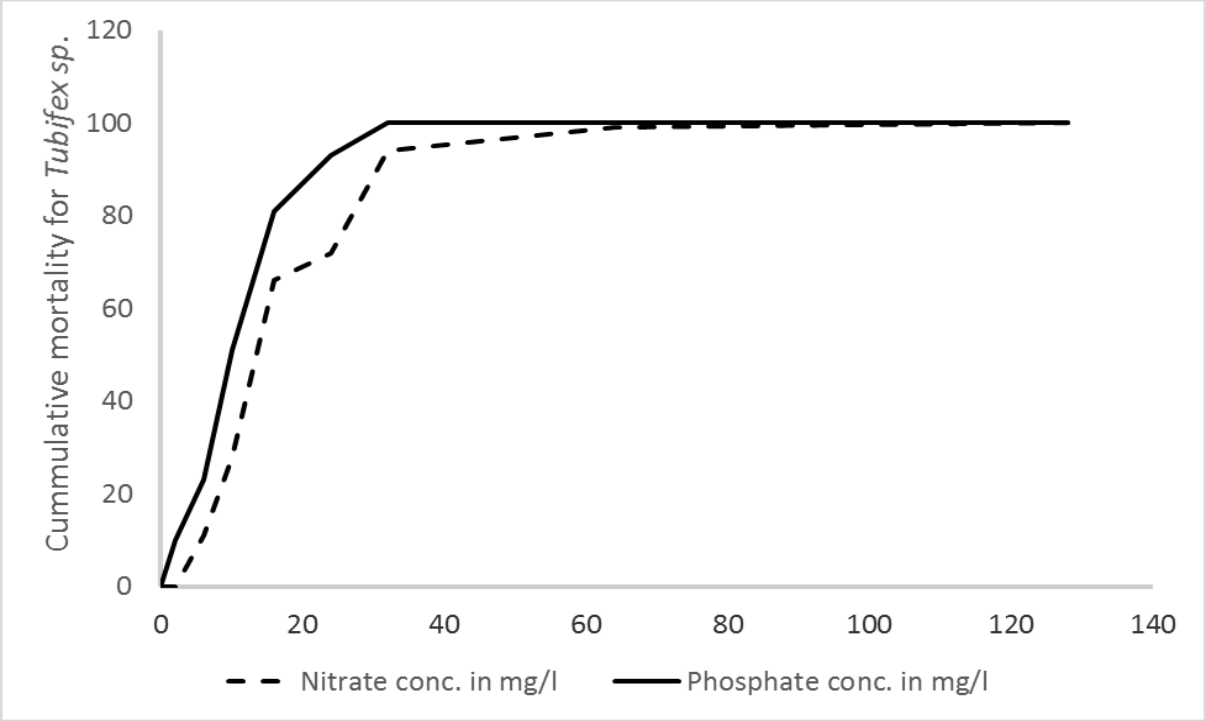


Figure 5.3: 96 hrs concentration response curves showing cumulative mortality for *Tubifex* spp.

5.4 Discussion

Elevated nutrient loads into riverine ecosystems as a result of agricultural activities may result into varied toxicological consequences. Macroinvertebrate organisms, for example, are well adapted to habitats with relatively low levels of nutrients compared to unpolluted or severely polluted habitats (Wetzel, 2001; Constable *et al*., 2003; Jensen, 2003; Camargo *et al*., 2005). Resulting acute or chronic toxicity from elevated levels of phosphorus and nitrogenous compounds can impair the ability of aquatic organisms to reproduce, grow and survive (Philips *et al*., 2002; Constable *et al*., 2003; Jensen, 2003; Camargo *et al*., 2005). However, the effects of these nutrients might vary widely between the tested species with respect to their differences in nutrients uptake, body size, developmental stage, and stress adaptation (Rouse *et al*., 1999; Camargo *et al*., 2004). Likewise, the complex relationship between a multitude of abiotic variables and community structure might obscure the effects of the toxicants, with some confounding variables tending to mask the effects of toxicants (Liess *et al*., 1999; 2008; 2009). For instance, calcium carbonate and chloride concentrations in rivers act as binders and thus decrease the effects of nitrate and phosphorus respectively to exposed organisms.

Increased mortality tally and discrepancies observed among tested macroinvertebrate groups are the consequences of differences in experimental-toxicant concentrations, exposure time and sensitivity of a group to toxicant. The 96 hours response curves for phosphorus fall to the right side of nitrate (Figures 5.1, 5.2 and 5.3), suggesting that the former is more harmful to tested specimens than nitrate. This indicates that NO_3-N is less toxic than PO_4-P to all test organisms, with *Tubifex* spp. being more tolerant than *Baetis harrisoni* whereas *Neorpela spio* is the least tolerant compared to *B. harrisoni*. These toxicity results are in line with Galdean *et al*. (2000) who reported Plecopterans as the most vulnerable group to pollution than other insects. Likewise, *Baetis harrisoni* which has been associated with agricultural inputs (Rae, 1989), is thought to be in a group with intermediate pollution tolerant taxa in rivers impacted by agricultural activities (Stone *et al*., 2005) more than that of Plecoptera, *Neorpela spio* but lower than *Tubifex* spp. However, stressed conditions are likely to make tested organisms more susceptible to toxicants while the previously exposed organisms tend to build up tolerance and become less susceptible to toxicants (Rand and Petrocelli, 1985). Moreover, the combined effects of predation, intimidation by predators and competition for food can exert distinct stress by weakening the fitness of organisms (Bolnick and Preisser, 2005) and significantly increasing their vulnerability to toxicants.

According to Figures 5.1, 5.2 and 5.3, the threshold limits found ranged from 0.8 to 1.2 mg NO_3-N/L (for Plecoptera, *Neorpela spio*), 1.6 to 3.2 mg NO_3-N/L for *Baetis harrisoni* and 6 to 16 mg NO_3-N/L for *Tubifex* worms, whereas 0.4 to 0.8 mg PO_4-P/L, 0.8 to 2.4 mg PO_4-P/L and 2 to 10 mg PO_4-P/L were the limits for *Neorpela spio*, *Baetis harrisoni* and *Tubifex* spp. respectively, with differences of standard deviations from these experiments being significant. These study results are strongly supported by the thresholds of harm ranging from 2.9 to 3.6 mg NO_3-N/L (to protect freshwater and marine life (Canadian Council of Ministers of the Environment, 2003), and Camargo *et al*. (2005) who proposed a maximum level of 2 mg NO_3-N/L for the protection of sensitive aquatic animals. Although the concept of threshold limits is still in its infancy, it can be translated into standards (such as numerical limits of quantifiable stressor variables) and thus, be relevant for policy-making (Andersen *et al.*, 2009). Therefore, the concentration ranging from 2.8 to 6.8 mg NO_3-N/L and 1.1 to 4.6 mg PO_4-P can be suggested as the thresholds of harm to protect stream condition. Moreover, it is necessary to set threshold limits as an effort towards resource management, protection and sustainability because it may be difficult to reverse degradation and extinction of rare species (Scheffer and Carpenter, 2003). However, it was much more difficult to detect the

immediate result in acute cumulative mortality at low concentrations compared to high concentrations, where the induced high rate of acute mortality was observed easily.

5.5 Conclusion

Introduction of nutrient loads in lakes and rivers, even at considerable dilution might cause the varied toxicological consequences which eventually affect the distribution of macroinvertebrate communities in riverine ecosystems. Both nitrates and phosphorus have shown toxic effects on the experimented organisms, with observed mortality increasing with increased concentration and exposure time among tested species. However, *Neorpela spio* displayed the highest acute effect in solution with nitrate and phosphate than *Baetis harrisoni* and *Tubifex* spp. under the test conditions. The marked increase of cumulative mortality of least pollution tolerant organisms with increasing toxicants concentrations compared to moderate and tolerant species suggests the need for practising safe and proper disposal of agrochemical inputs as a way to control nutrient loads on the stream bed.

CHAPTER SIX

ADVANCED BIOMONITORING TOOL FOR ASSESSING POLLUTION IN TROPICAL AFRICAN RIVERS[5]

Abstract

In Tanzania, non-sophisticated, cost-effective and locally based macroinvertebrates index for evaluating river health that can minimize data variability, needs for great expertise and time in the field is not yet in place. As such, this study was designed with a view to redressing the problem. Resident macroinvertebrates assemblages were collected from 85 sampling sites representing reference (39) and monitoring (46) sites along Tanzanian river basins. Validation on existing bioassessment protocols was done to fit study objective before being used to collect and process macroinvertebrate samples and key associated environmental variables during the end of long rain, short rain and dry seasons of 2014 and 2015. Seventeen identified orders were subjected to six stepwise criteria to evaluate their discriminatory power in separating reference sites from monitoring sites. The criteria included: numerical truncate test, Mann-Whitney U test ($p < 0.05$), the degree of inter-quartile (IQ) overlap in Box-and-Whisker plots, Spearman's rank correlation (r_s) analysis, more diverse order (with > 10 taxa) and validation test. Ephemeroptera, Diptera, Odonata and Trichoptera (EDOT) orders (that comprised the 55% (N=97) of all Tanzanian families) met all test criteria after exhibiting abundances >2% upon truncate test, a p-value < 0.05 in a Mann-Whitney U test, a sensitivity scores of 3, r_s <75% (with $p<0.005$) and higher number of taxa ($n>10$). EDOT index can be considered to be simple, robust, quick and advanced macroinvertebrate scoring tool developed for evaluating the ecological condition of Tanzanian rivers and other related tropical African riverine environment, where aquatic resources are under high pressure as a result of human activities particularly improper land uses.

[5]Manuscript submitted to Hydrobiologia (International Journal of Aquatic Sciences)

6.1 Introduction

Over exploitation and pollutants discharge on aquatic resources as a consequence of anthropogenic activities have impacted the health status of freshwater ecosystems (Dudgeon, 1992; Ramírez *et al*., 2008). The decrease in water quantity and quality due to escalating population growth and improper land uses, pose a serious threat to sustainability of aquatic resources and conservation efforts all over the world (Ashton, 2002). Likewise, physico-chemical monitoring programmes which were previously initiated mostly in African countries to monitor riverine health have failed to deliver a systematic and sufficient data for interpreting water quality status and trends due to financial constraints and insufficient technical capacity (Kaaya, 2014). Such a setback has highlighted the need for using bioassessment approaches as an alternative way in the protection and management of freshwater ecosystems for short and long-term sustainability and utilization of water resources.

Studies on the use of macroinvertebrates in bioassessment and for developing biomonitoring indices to assess pollution in rivers have been widely reported in the literature (Rosenberg and Resh, 1993; Mason and Parr, 2003; Jacobsen *et. al*., 2008) for the past three decades. Despite the worldwide popularity and longevity of the concept, the regional share and application of the indices between tropical and non-tropical regions have become somewhat out of balance, taxonomically and ecologically. Taxonomically, tropical region is known to be more diverse than other regions but yet, the recognition and use of their local macroinvertebrates in biomonitoring programmes is still debatable. To keep in line with biomonitoring programmes, tropical African region is relying on indices that were developed specifically for other regions to assess aquatic pollution in their localities (Elias *et al*., 2014a). These adoption efforts met with varied success due to differences in ecological degradation, biogeographical and climatical conditions between the regions. For instance, one ephemeropteran family (Teloganodidae), and five trichopteran families (Barbarochthonidae, Glossosomatidae, Hydrosalpingidae, Petrothrincidae and Sericosostomatidae) are prevalent in the southwest cape of South Africa representing temperate regions, as opposed to ephemeropterans (Ephemerythidea and Dicercomyzidae) which are endemically widespread in both afro-tropical and tropical regions (Dickens and Graham, 2002; Palmer and Taylor, 2004). Presence of these regional differences have affected the capability, reliability, and functioning of the adopted indices because they are regional or area specific tools reflecting local species diversity and environmental conditions (Elias *et al*., 2014b).

Varying regional complexities have increased the recognition among ecologists on the new demands of having regional specific indices to render data accuracy in biomonitoring programmes (Elias *et al*., 2014a). Owing to such concerns, many countries i.e., United Kingdom (Wright, 1994), North America (Hilsenhoff, 1988), United States of America (Barbour *et al*., 1999), Canada (Rosenberg *et al*., 1999), Australia (Simpson and Norris., 2000), Mexico (Henne *et al*., 2002), Thailand (Mustow, 2002), Brazil (Baptista *et al*., 2007; Ferreira *et al*., 2011), Bolivia (Jacobsen and Marın., 2007), Netherlands (Tolkamp and Gardiener, 1977), Belgium (De Pauw and Vanhooren, 1983), Denmark (Andersen, 1984), Switzerland (Lang and Reymond, 1995) and South Africa (Dickens and Grahm, 2002) are now using their own family level biomonitoring indices developed by using their local macroinvertebrates to assess river pollution. Moreover, other scoring systems have come into existence, including the Namibian Scoring System (NASS) the Okavango Assessment System (OKAS) in the Okavango Delta (Dallas 2009) and the Zambia Invertebrate Scoring System (ZISS) in Zambia (Lowe et al. 2013) and of recently the TARISS (Kaaya, 2015) and ETHbios (Aschalew and Moog, 2015) which were developed in Africa based on the modification of the South African Scoring System version 5 (SASS5). Conversely, these indices had included all identified orders and/or families and thus, make them more complicated, resulting in a need for greater expertise, much more time in the field (Dickens and Grahm, 2002) and possible errors due to fatigue.

As a means towards bridging all gaps in practice, the present study was designed to develop a simple, robust and cost-effective biomonitoring index for assessing water pollution in Tropical African rivers. Compared to the existing indices, only few orders with more diverse families having a significant discriminating power of separating reference sites from monitoring sites were used to enhance the simplicity, robustness and cost-effectiveness. The novelty of the approach lies in the number of validation and calibration processes done during sampling, selection criteria for potential bio-indictors, and the use of a model to simulate sensitivity weightings for taxa with unknown sensitivity score ratings.

6.2 Materials and Methods

6.2.1 Study Areas

Pangani and Wami-Ruvu basins provide a wide range of riverine systems, climate, geology, topography and human disturbance within different hydrological patterns (Figure 6.1). Pangani river basin is located in the north-east of Tanzania mainland, 36°23′ - 39°13′ E and 03°03′ - 05°59′ S with an altitude ranging from 0 - 4,500 m. The basin drains an area of 43,650 km^2 (Elias *et al*., 2014b). Wami-Ruvu river basin is extended from central to eastern part of Tanzania between 36°00′ - 39°00′ E and 05°00′ - 07°00′ S with an elevation of 0 - 2,500 m. It flows through Dodoma, Morogoro, Coast and Dar es Salaam regions covering an area of 72,930 km^2 and drains into the Indian Ocean at Saadani village.

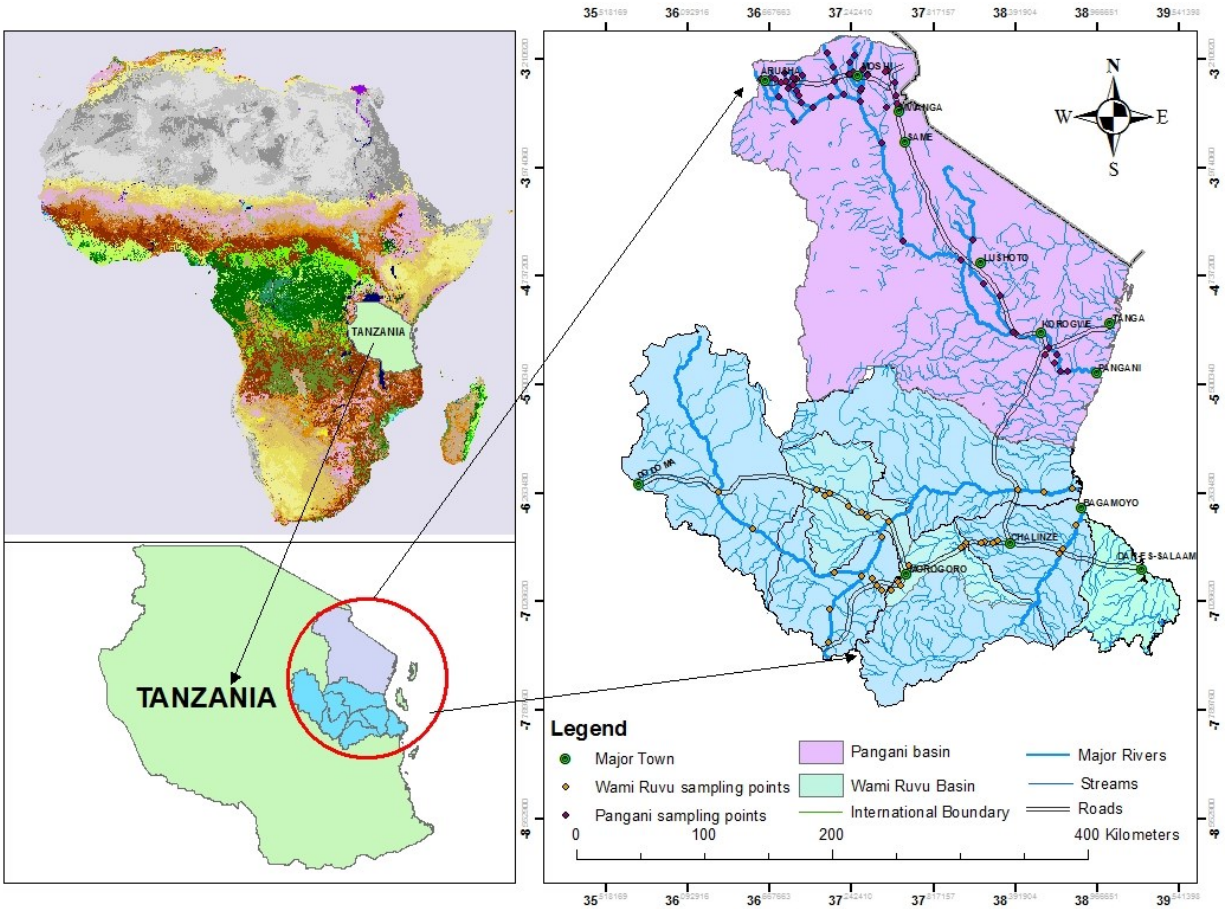


Figure 6.1: A map showing sampling stations along Tanzanian river basins

The two basins are experiencing equatorial type of climate with mean annual rainfall between 1 100 and 3 000 mm per annum, with a maximum mean temperature ranging from 32-35°C in the dry season and lowest of 14-18 °C during the wet season. The main impacts along these two basins are caused by land-use systems and practices ranging from small-scale

farming to large-scale mechanized agriculture, removal of riparian vegetation, construction of dams for hydro-electrical power generation and supply, animal grazing, disposal of raw domestic, mining and industrial wastes, brick making, saline water intrusion, uncontrolled and illegal water abstractions for irrigation, poor agriculture practices i.e., unsafe use and disposal of pesticides. As a consequence of these impacts, there is a considerable loss of quality of the water and freshwater biodiversity in several stretches of the basin (Elias *et al*., 2014b).

6.2.2 Sampling design and criteria for selecting potential bio-indicators

(a) Classification and validation of sampling site categories

The studied sites along Pangani and Wami-Ruvu basins were divided into two site categories based on their ability to segregate macroinvertebrate assemblages (i.e., those preferring least disturbed sites (as reference sites) and those which can survive in a wide range of varied environmental conditions (as monitoring sites)), as quantified by the USEPA protocol (USEPA, 2002). Of the 85 sampling stations used to collect macroinvertebrates dataset, 39 were designated as reference and 46 as monitoring sites. The samples were collected near the end of dry season as well as long and short rains to capture the effects of each season. Basins size (Spatial, temporal and longitudinal heterogeneity), morphological types (riffle/run or glide/pool), biotopes (fine, stony and vegetation), ecoregions (ecoregions-geomorphologic slopes, ecoregions-geomorphologic landforms), and habitat and water quality were considered as possible rivers classification variables (Thieme *et al*., 2005).

(b) Macroinvertebrates sampling protocol

Macroinvertebrates were sampled according to sampling methods developed by Dickens and Graham (2002) and Lowe *et al*. (2013), which were refined prior to use in order to match the study objective and reflect tropical aquatic environment. The modification include the tighter description of the technique, sampling and analytical methods and procedures. The protocol ensured macroinvertebrate assemblages are sampled at the end of each season (April and May for long rainy season, November and December for short rainy season and September and October for dry season) using kick-net (30 m x 30 m) to capture the effect of the respective seasons on macroinvertebrates and the recovery of the riverine ecosystems. *In situ*, all the collected macroinvertebrate communities were preserved in 10% alcohol for subsequent laboratory processing and identification.

(c) Macroinvertebrates identification and data refinement protocol

In the laboratory, macroinvertebrates identification guides, photographic and keys (Day *et al*., 1999; Day *et al*., 2001a, 2001b; Day and De Moor, 2002a, 2002b; Day *et al*., 2003; De Moor *et al*., 2003a, 2003b; Stals and De Moor, 2007) for freshwater macroinvertebrates were used to identify all organisms to family level.

(d) Selection criteria for potential orders (families) for use in BMI

Numerical and statistical redundant criteria were employed to select key bio-indicator families with a potential of separating reference sites from monitoring sites for use in developing a quick and accurate index (Figure 6.2). The selection was done by performing numerical truncate test, a non-parametric Mann-Whitney U test ($p < 0.05$), the degree of inter-quartile (IQ) overlap in Box-and-Whisker plots, Spearman's rank (r_s) correlation analysis and more diverse orders (with > 10 taxa) criterion. Orders with abundances >5%, a p-value < 0.05 in a Mann-Whitney U test, a sensitivity score of 3, r_s <75% and higher number of taxa were considered to be a good discriminator of separating reference sites from monitoring sites and thus, considered as potential orders for inclusion in developing BMI (Barbour *et al*., 1996; Baptista *et al*., 2007).

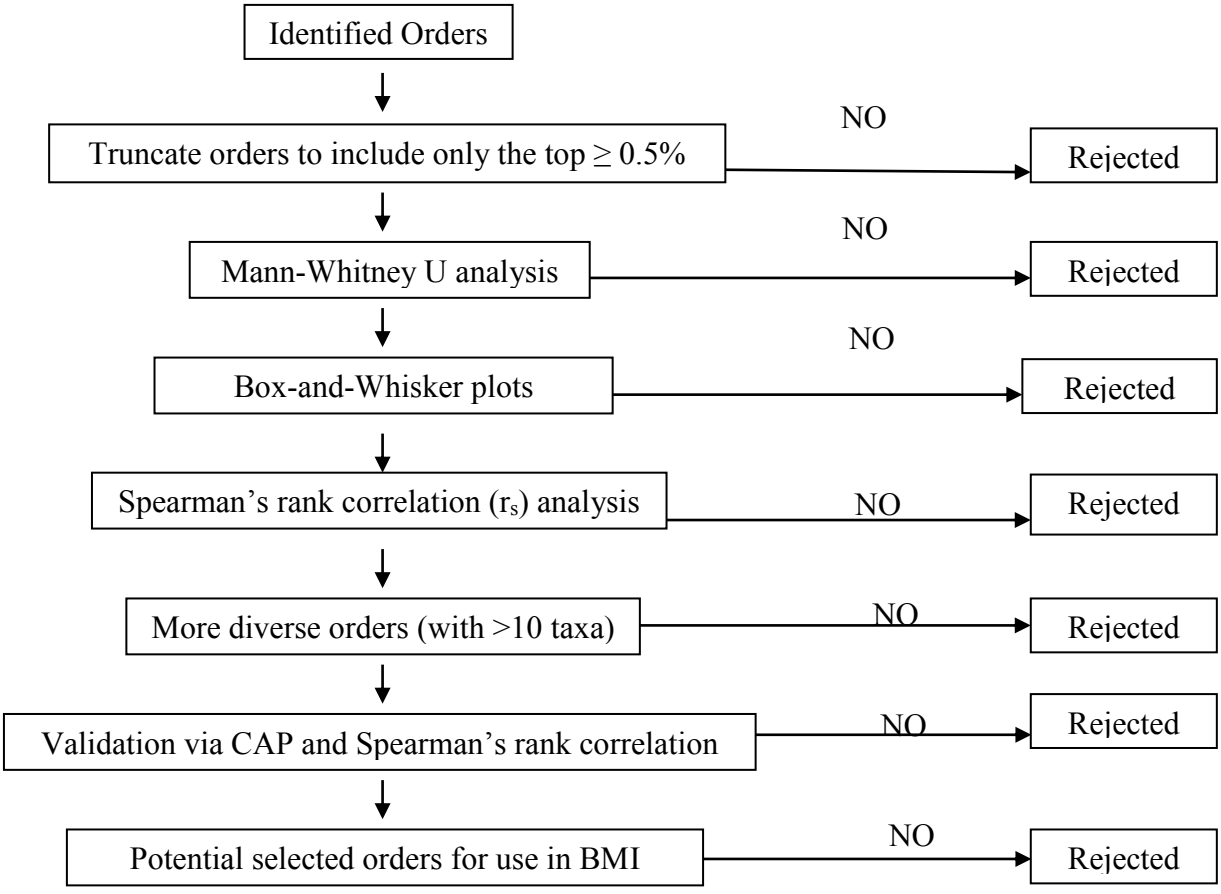


Figure 6.2: Selection criteria for potential BMI's orders

(i) Truncate test

Numerically, orders were truncated in order to eliminate the rare taxa (with < 0.5% of total macroinvertebrate abundances) that would only contribute noise to other statistical analyses. To reduce variability in the dataset, only dominant taxa (with total macroinvertebrate abundance of > 0.2%) were retained for further statistical analysis (Gauch, 1982).

(ii) Mann-Whitney U test with p value <0.05

Non-parametric Mann-Whitney U test was used as statistical testing criteria to eliminate the resulting orders that exhibited no significant differences ($p > 0.05$) after pairwise comparison of abundances for orders observed in reference sites with those in monitoring sites. In that regard, orders found to have a p-value < 0.05 in the test were considered to be strong discriminators of reference and monitoring conditions (Barbour *et al*., 1996; Baptista *et al*., 2007; Ferreira *et al*., 2011).

(iii) Box-and-Whisker test

Sensitivity scores of the orders were based on the levels of overlapping of the interquartile ranges of Box-and-Whisker plots (Figure 6.3) according to the modified procedures prescribed by Barbour *et al*. (1999), Baptista *et al*. (2007), Ferreira *et al*. (2011). Box-and-Whisker plots of reference and monitoring sites were examined to determine if there was significant vertical separation between their interquartile ranges of the corresponding conditions. For each order, sensitivity scores of 3, 2 and < 2, with the thresholds of median ranges between 25^{th} and 75^{th} percentiles of the reference site was used as selection criterion for potential orders representing a pivotal assessment tool. A sensitivity score of 3 (which meets the reference condition) was given if there was no overlap in the interquartile range (IQ) of Box-and-Whisker plots (Barbour *et al*., 1996; Baptista *et al*., 2007; Ferreira *et al*., 2011). A score of 2 (that represents an intermediate condition) was scored if there was a partial overlap of the IQ range with both medians being outside of the overlap (Barbour *et al*., 1996; Baptista *et al*., 2007; Ferreira *et al*., 2011). Likewise, sensitivity scores < 2 were given if the orders' abundances were below the 25th percentile. These scores were attained if: (i) there is moderate overlap of IQ range but one median appeared outside the IQ range overlap; (ii) one range is completely overlapping the other IQ range but one median is outside the IQ range overlap; and (iii) both medians were inside IQ range overlap.

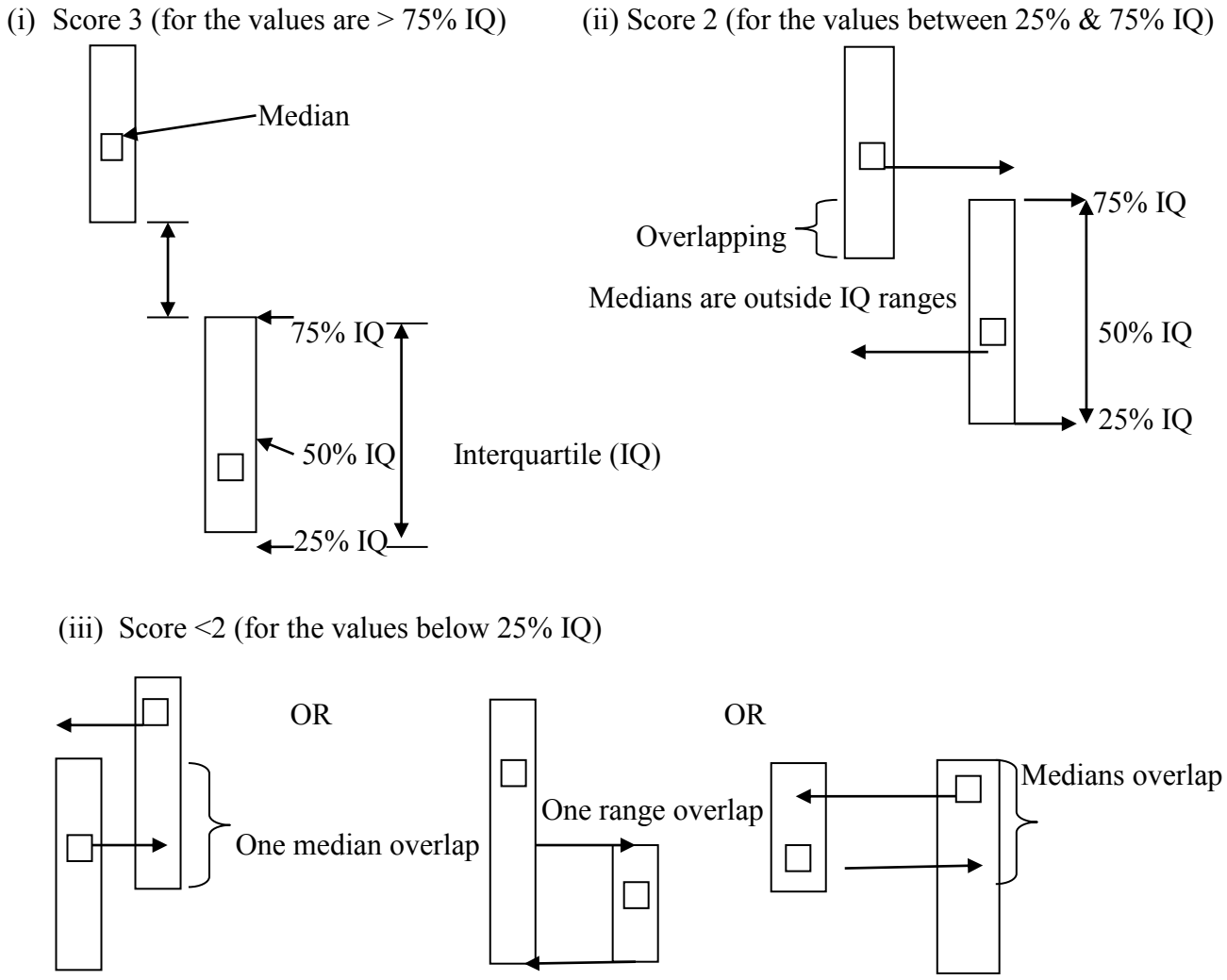


Figure 6.3: Sensitivity scores of Box-and-Whisker plots according to modified procedures prescribed by Barbour *et al*. (1996), Baptista *et al*. (2007) and Ferreira *et al*. (2011)

(iv) Spearman's rank correlation test

For more simplification of index, a Spearman's rank correlation was drawn with paired orders to eliminate any order if more than 75% of its values were identical. Orders with Spearman's correlation (r_s) > 0.75 were considered redundant in which the least abundant order was eliminated (Ferreira *et al*., 2011).

(v) More diverse orders (n>10 taxa) criterion

More diverse orders showing the highest representativeness of organisms distinguishing the reference sites from monitoring sites were chosen and used as potential candidates to develop the BMI. However, the orders were selected to establish the BMI if they had more than 10 families representing a wide range of occurrences and pollution sensitivity.

(e) Validation of macroinvertebrates sensitivity ratings or scores

Each of the identified family of selected macroinvertebrate orders was assigned a pollution sensitivity weighting after intensive literature review. The sensitivity scores of reported taxa were assigned based on:

(i) Reference scores of taxa obtained from closely related existing indices (Dickens and Grahm, 2002; Aschalew and Moog, 2015).

(ii) Autecological knowledge of macroinvertebrate taxa and;

(iii) Association of taxa occurrences or abundances with environmental variables and;

(iv) Simulated results for taxa with unknown sensitive scores to stressors using Canonical analysis of principal coordinates (CAP) predictive model.

The CAP model was first calibrated by simulating only abundances of taxa with known scores and their respective scores to facilitate the interpretation of unknown scores. The model was then re-simulated while including both all abundances of taxa with their known and unknown sensitivity scores as well as known scores. These known scores were extracted from closely related early African indices, with their capability and reliability been extensively tested and proven to be suitable for use in assessing the general aquatic conditions (Dallas, 1997).

6.2.3 Data Analysis

MS Excel, PRIMER version 7 (with PERMANOVA add-on), OriginPro version 8.5, Community Analysis Package version 4 (CAP IV), Species Richness and Diversity IV (SDR IV), and Instat® version 3 (GraphPad®) softwares were used to analyze the data. Prior to analysis all the data were transformed where appropriate and those of different S.I. unit were normalized into unit-less according to Barbour *et al*. (1996), and Baptista *et al*. (2007) in order to maintain uniformity among the values. Significance tests were performed by PRIMER7 after the biotic data being transformation (to either log (x+1), square root, or absent and present), with p set at 0.05 to determine the differences among basins and the site categories. Mann-Whitney U test and Non-Parametric Spearman's rank correlation were performed by Instat® version 3 (GraphPad®) and Box-and-Whisker plots by OrignPro 8.5 used to reveal the discrimination power of the order among the site categories. Canonical Analysis of Principal coordinates (CAP) predictive model was simulated using PERMANOVA+ software package, which is an add-on to PRIMER version 7 to calculate sensitivity weightings for taxa with unknown sensitivity ratings according to Anderson *et al*.

(2008). Moreover, CAP and non-parametric Spearman correlation analysis were used to validate the ability of EDOT taxa in discriminating reference sites from monitoring sites.

6.4 Results

Approximately 97 freshwater macroinvertebrate families belonging to 17 orders were identified collectively to summarize macroinvertebrate dataset for Tanzanian rivers (Appendix 2). Six validation criteria (shown in Figure 6.2) were used to select potential orders for use in the biomonitoring index (BMI). The selection criteria involved numerical and statistical tests that have been successfully applied in other regions to identify the potential candidates for inclusion during the development of their BMIs. Out of the 17 orders, Ephemeroptera, Diptera, Odonata and Trichoptera (EDOT) were found with significant discriminating power separating the reference from impaired sites according to truncate numerical test, Mann–Whitney U test ($p < 0.05$), Box-and Whisker plot test, RDA and more diverse orders ($n>10$ taxa) criterion. The rationale for the usefulness of each order is numerically and statistically tested in section 6.3.1 to 6.3.9.

6.4.1 Truncate test

To reduce unusual variability of the dataset (Gauch, 1982), orders with ≤ 0.5% of total macroinvertebrate abundance were numerically exempted for the next screening. Of the 17 macroinvertebrate orders, 10 had abundances ≥ 0.5% and thus, passed the truncate numerical test and consequently were retained for the next screening test, with Arhynchobdellida, Rhynchobdellida, Hydroida, Pelecypoda, Megaloptera, Lepidoptera and Turbellaria orders, considered redundant.

6.4.2 Mann-Whitney U test

Mann-Whitney U test was used to demonstrate the ability of orders to discern the difference between reference and monitoring sites of the river basins. Orders were considered strong discriminators of impairment if the difference between monitoring and reference sites were significant (Mann-Whitney U, with $p < 0.05$). All the tested orders were found to be non-redundant (with $p < 0.05$) and thus considered for the next test (Table 6.1).

Table 6.1: Results of Mann-Whitney tests for 10 Tanzanian orders of Tanzanian rivers (*indicates $p < 0.05$)

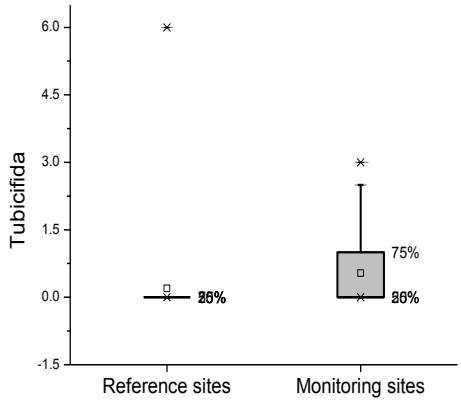
ORDER	Man-Whitney U, p-value	Test remarks	Meets the criteria test
Tubificida	0.0186	Significant*	Yes
Coleoptera	<0.0001	Extremely significant***	Yes
Decapoda	0.0049	Very significant**	Yes
Diptera	<0.0001	Extremely significant***	Yes
Ephemeroptera	<0.0001	Extremely significant***	Yes
Gastropoda	<0.0001	Extremely significant***	Yes
Hemiptera	0.0001	Extremely significant***	Yes
Odonata	0.0372	Significant*	Yes
Plecoptera	0.0049	Very significant**	Yes
Trichoptera	<0.0001	Extremely significant***	Yes

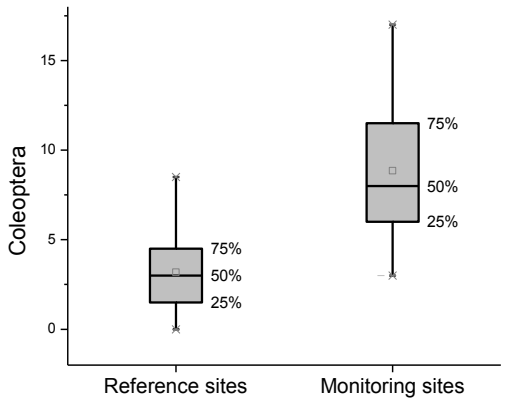
6.4.3 Box-and-Whisker plot test

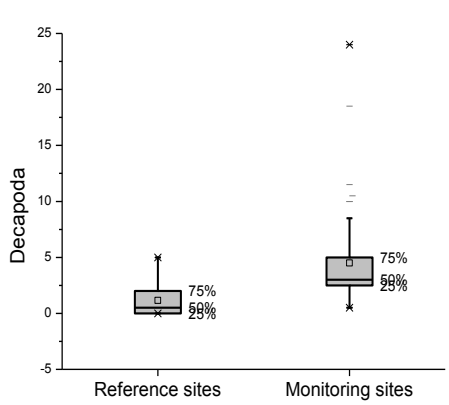
Box-and-Whisker plots were used to evaluate how well each order could discriminate between the sites categories, with a sensitivity score of 3 considered as a selection criterion (Barbour *et al*., 1996). The test showed that only six orders were highly sensitive (score = 3) and consequently retained for Non-parametric Spearman's rank correlation selection test. These include: Diptera, Decapoda, Odonata, Ephemeroptera, Coleoptera, and Trichoptera (Table 6.2).

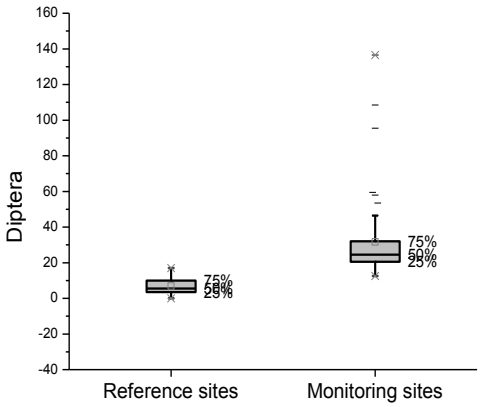
Table 6.2: Results of Box-and-Whisker Plot tests for 10 tested orders

ORDER	Response to pollution	Sensitivity score	Meets the criteria test
Tubicifida	Decrease	<2	No
Coleoptera	Decrease	3	Yes
Decapoda	Variable	3	Yes
Gastropoda	Decrease	<2	No
Diptera	Increase	3	Yes
Ephemeroptera	Decrease	3	Yes
Hemiptera	Decrease	2	No
Odonata	Increase	3	Yes
Plecoptera	Decrease	<2	No
Trichoptera	Decrease	3	Yes









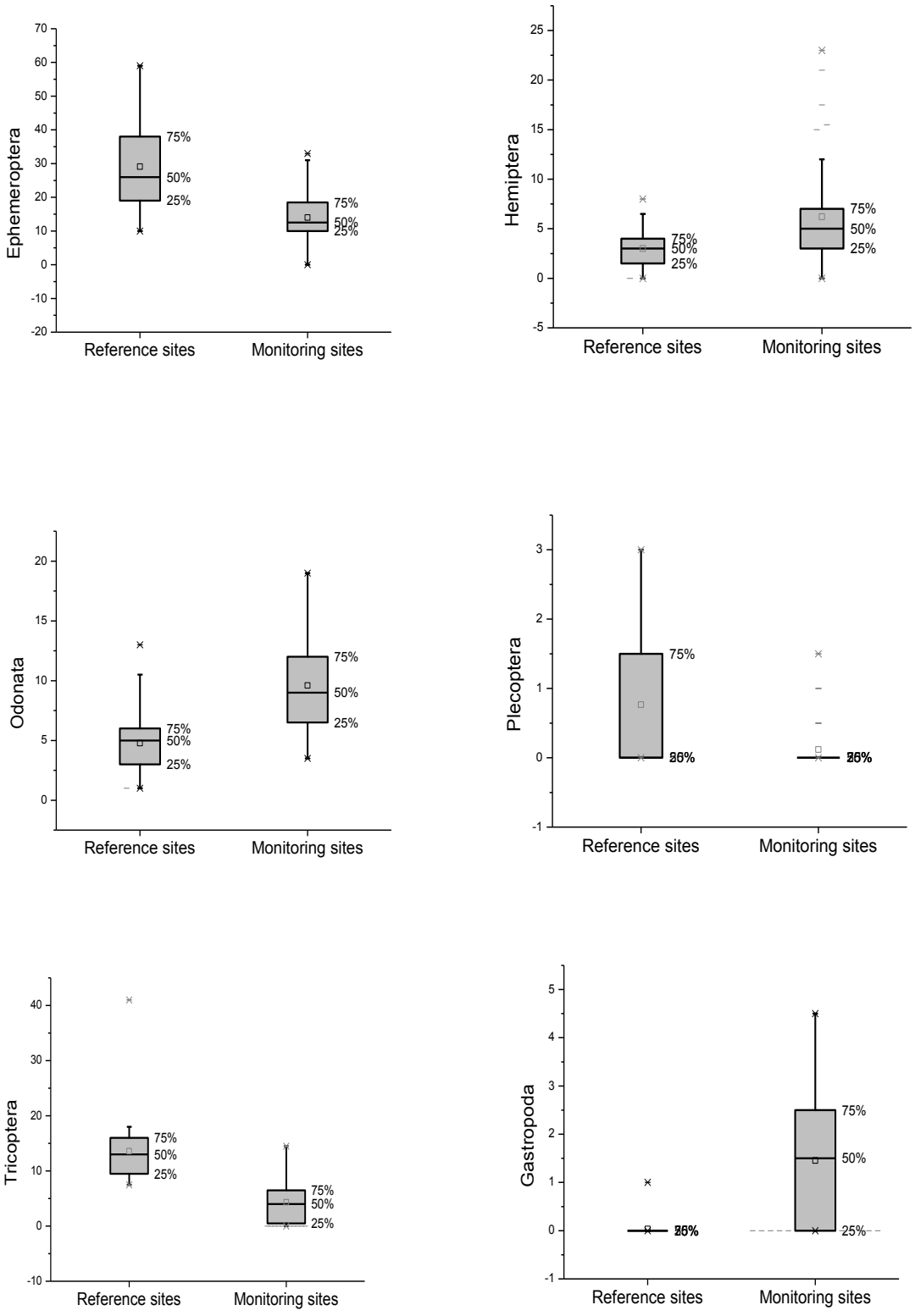


Figure 6.4: Box-and-Whisker plots for the orders distinguishing reference sites from monitoring sites of Tanzanian river basins

6.4.4 Spearman's rank correlation

Non-parametric Spearman's rank correlation was used to avoid repeating information already summarized by other orders and to ensure accurate depiction of patterns by separating reference sites from monitoring sites. Orders with poor range are unlikely to differentiate monitoring and reference sites because the response gradient is highly compressed. Six orders that passed Box-and-Whisker plots test were tested for redundancy amongst them using Spearman rank correlation analysis. Orders were considered redundant if the Spearman rank correlation coefficient (r_s) was higher than 0.75 with p-value < 0.05 (Whittier *et al*., 2007). However, all tested Diptera, Decapoda, Odonata, Ephemeroptera, Coleoptera, and Trichoptera orders were unique with $r_s < 0.75$ and $p < 0.05$ and thus, considered non-redundant and retained for further selection test.

6.4.5 More diverse orders (n>10 taxa) criterion

More diverse orders showing the wide representativeness of families in all sites were chosen and used as potential candidates in developing BMI. Ephemeroptera (E), Diptera (D), Odonata (O) and Trichoptera (T) were the only four orders containing the large numbers of different taxa (n > 10) at all levels of pollution tolerance. Odonata was represented by 12 instances, ephemeropterans by 13 instances whereas, dipterans and trichopterans contain 14 instances each, making a total of 53 instances, representing about 55% (N = 97) of all Tanzanian taxa.

6.4.6 Validation of EDOT taxa

A constrained CAP discrimination analysis was performed to analyse macroinvertebrate assemblages for their ability to discern the reference sites from monitoring sites along Tanzanian river basins (Figure 6.5).

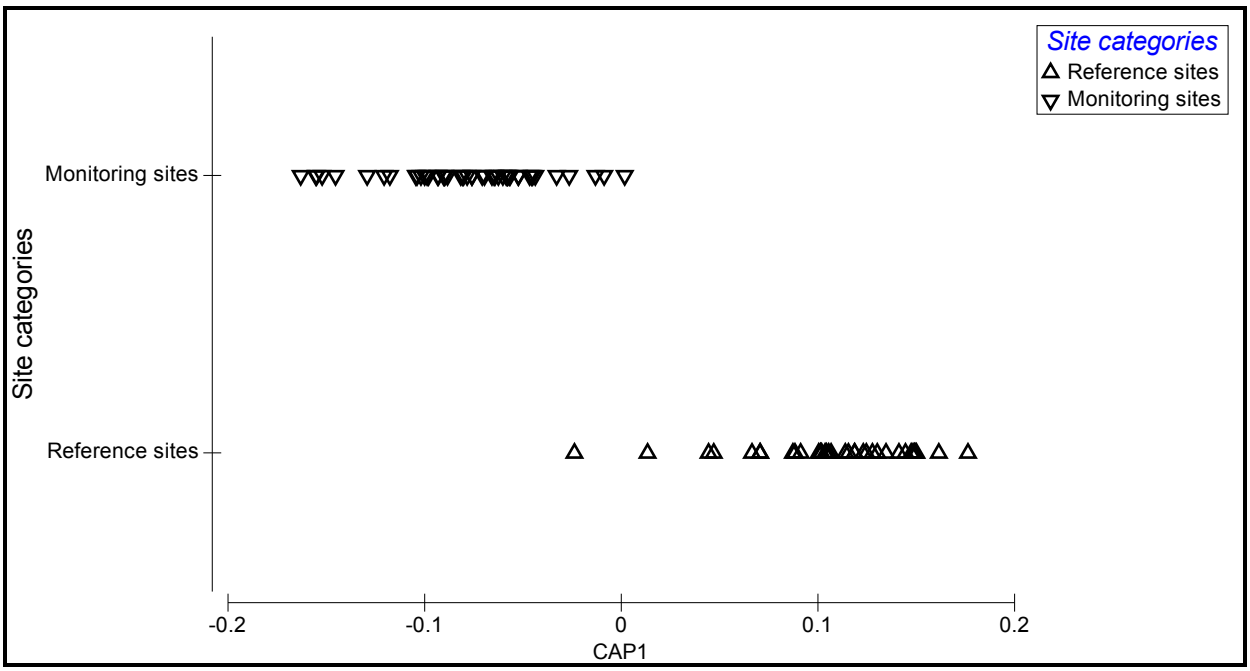


Figure 6.5: Macroinvertebrate taxa of four selected orders showing their discriminatory power separating reference from monitoring sites using canonical discrimination analysis in Tanzanian river basins ($\delta^2 = 0.8479$, $p < 0.001$): r = reference sites; m = monitoring sites.

EDOT taxa were also assessed together with various factors that may influence the scores. The results from non-parametric Spearman's rank correlation analysis showed strong significant correlation (with $p < 0.0001$) between the four orders and most environmental variables structuring macroinvertebrate assemblages. Ephemeroptera abundances showed strong correlation with conductivity (R = -0.4330) and temperature (R = -0.4235); dipterans with conductivity (R = -0.4117), temperature (R= 0.5023), NH_4^+ -N (R= 0.6544), BOD (R= 0.5434), COD (R = 0.6005), NO_3^- -N (R = 0.7399), SO_4^{2-} (R = 0.4914) and potassium (R = 0.5734); Odonata with conductivity (R= 0.4098) and pH (R= 4152), and trichopterans with BOD (R = -0.5229), COD (R = -0.5492), NO_3^- -N (R = 0.6278), NH_4^+ -N (R = -0.5324) and potassium (R = -0.4530). Since EDOT taxa have demonstrated their ability to discern the reference sites from monitoring sites via CAP and Spearman's rank correlation analysis, they can therefore be used as potential bio-indicators in developing EDOT index.

6.4.7 Scoring of selected bioindicator taxa

Generally, the sensitivity score ranged from 0 to 15 representing three categories of macroinvertebrate groups. Sensitivity scores for highly tolerant taxa to stressors ranged from 11 to15, whereas, 6 to 10 is for moderately tolerant taxa and 1 to 10 for least tolerant taxa (Gerber and Gabriel, 2002). Of the 53 taxa, 50 were assigned scores based on related scoring

systems (Table 6.3) while the sensitivity scores for the remaining three taxa were simulated by CAP predictive model. The CAP predictive model with correlation of 0.8543 and/or correlation square (δ^2 = 0.7299) calculated sensitivity scores for Dicercomyzidae, Ephemerythidae and Macromiidae as 9.7246 ≈ 10; 8.8258 ≈ 9 and 3.1 ≈ 3 respectively (Figure 6.6).

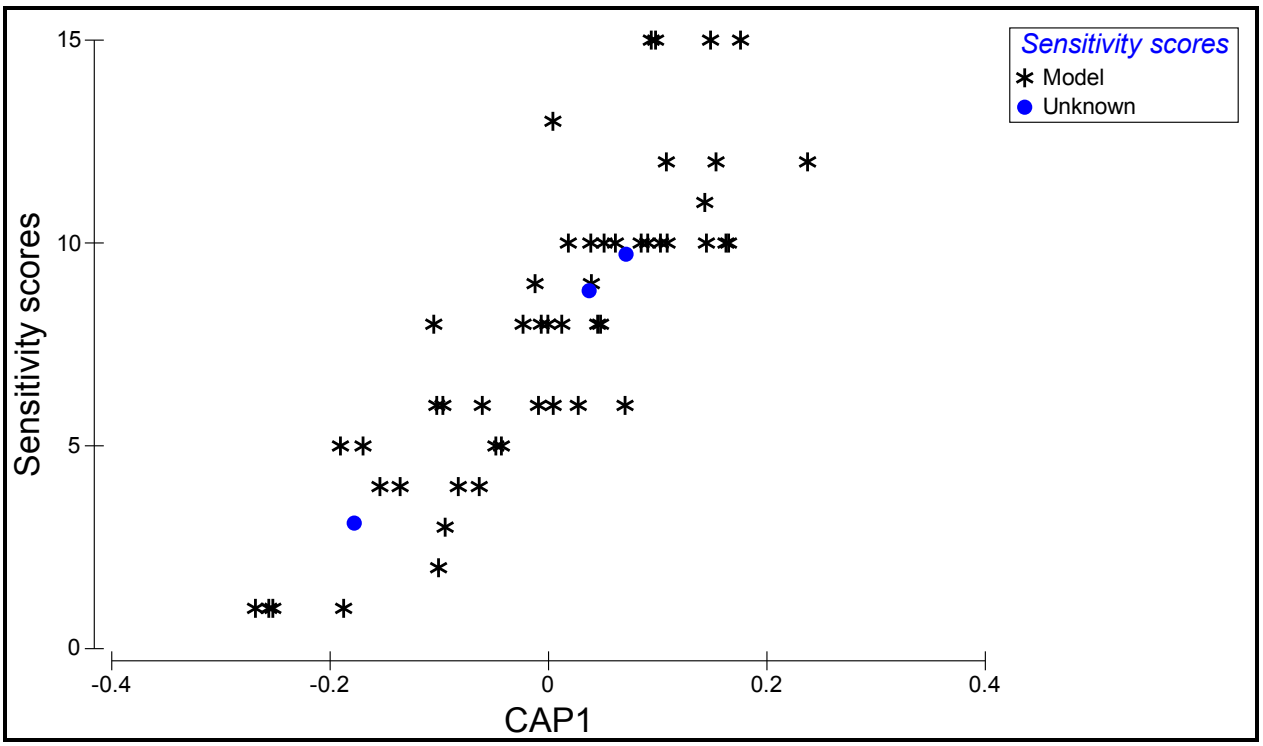


Figure 6.6: Canonical Analysis of Principal coordinates (CAP) predictive model showing the position of taxa in relation to their sensitivity scores

6.4.8 The EDOT(f) Index

Table 6.3: The new EDOT(f) index developed under Tanzanian riverine conditions

DESCRIPTION OF PHYSICAL ENVIRONMENT	EDOT(f) INDEX						
EDOT Index Version 1 Score Sheet @2015	Order	Family	Scores	S	MV	GSM	TOT
Date:...../...../20.....; Time:.....	Ephemeroptera	Baetidae 1 sp.	4				
Operator:.....		Baetidae 2 spp.	6				
Tittle:.....		Baetidae > 2spp	12				
Ecoregion:.....		Caenidae	6				
River:.....		Dicercormyzidae	10				
Site Code:.....		Ephemeridae	15				
Latitudes: S:.....°.....',.....''		Ephemerythidae	9				
Longitudes: E:.....°,.....',.....''		Heptageniidae	13				
Altitude:.....m a.s.l		Leptophlebiidae	9				
Slope @ Left bank:.....%; Right bank:.....%		Oligoneuridae	15				
Landform:.....		Polymitarcyidae	10				
Flow:.....m/s		Potomanthidae	10				
Temp:.....°C		Prosopistomatidae	15				
pH:.....		Tricorythidae	9				
DO:.....mg/l	Diptera	Athericidae	10				
Conductivity:.....mS/m		Blephariceridae	15				
Turbidity:.....NTU		Ceratopogonidae	5				
Site Description:		Chironomidae	2				
		Culicidae	1				
		Dixidae	10				
		Empididae	6				
Instream Disturbance:		Ephydridae	3				
		Muscidae	1				
		Psychodidae	1				
		Simuliidae	5				
Riparian Land Use:		Syrphidae	1				
		Tabanidae	5				
		Tipulidae	5				
	Odonata	Aeshnidae	8				
		Calopterygidae	10				
		Chlorocyphidae	10				
Stone In Currenct (SIC) sampling time (min):.....		Chlorolestidae	8				
Stone Out Of Current (SOOC) sampling time (min):....		Coenagrionidae	4				
Aquatic vegetation dominant sp.:.....		Corduliidae	8				
Marginal Vegetation In Current Dominant sp:.....		Gomphidae	6				
Marginal Vegetation Out Of Current Dominant sp:.....		Lestidae	8				
Gravel:.....		Libellulidae	4				
Sand:.....		Macroiidae	3				
Mud:.....		Platycnemidae	10				
Average size of Stones:.....cm		Protoneuridae	8				
Average size of Bedrock:.....cm	Trichoptera	Calamoceratidae	11				
Hand Picking/Vissual Observation:		Ecnomidae	8				
		Dipseudopsidae	10				
		Hydroptilidae	6				
Other Observations:		Hydropsychidae 1 sp.	4				
		Hydropsychidae 2 spp.	6				
		Hydropsychidae >2 spp	12				
		Lepidostomatidae	10				
		Leptoceridae	6				
		Philopotamidae	10				
		Phryganeidae	10				
		Polycentropodidae	12				
		Psychomyiidae	8				
		EDO(f) SCORE					
		NUMBER OF TAXA					
		ASPT					

6.4.9 Application of EDOT Index (Calculating EDOT Index)

EDOT index is a field based rapid scoring system entailing *insitu* observation (with the help of 10 × 45 magnified stereo microscope where deemed necessary), in which taxa are identified up to family level. Regardless of its abundance, each observed taxon is estimated upon observation and tallied in the respective biotope (stone, vegetation and GSM) and the combined column (C) of EDOT scoring sheet. Single macroinvertebrate is estimated as 1 organism whereas, >1 to 10 organisms as 2, >10 to 100 as 3, >100 to 1 000 as 4 and >1 000 as 5 in order to minimize scoring time. Time less than 15 minutes per site is recommended to end the field work but if there is not any observed taxon in duration of 5 minutes. Samples observed with rarely specific taxa groups with number of types i.e., Baetidae (with 1 sp., 2 spp. and > 2spp.), and Hydropsychidae (1 sp., 2 spp. and > 2 spp.) were not scored unless one of these taxa showed clear preference for a given river quality class, the score was adopted from Kaaya (2015). For example, if Baetidae 1 sp. or Hydropsychidae 1 sp. appeared in the SIC and Veg biotopes it should only be considered as a single taxon in the total column assessment. Similarly, a single Baetidae 1 sp. or Hydropsychidae 1 sp. in the SIC and a single Baetidae 2 spp. or Hydropsychidae 2 spp. in the marginal vegetation must be considered as two species in the total column assessment. Ticked sensitivity score of each taxon in the combined column is summed up to provide EDOT(f) score whereas, total number of taxa is obtained by counting the recorded taxa. ASPT, on the other hand, is calculated by dividing the EDOT (f) scores by the Number of Taxa. EDOT Index can be calculated as EDOT (f) Score, Number of Taxa (No. Taxa) and Average Score per Taxa (ASPT) but only the result calculated from the combined column will represent the EDOT result for that particular site. Mathematically, EDOT(f) is calculated as:

$$EDOT(f) = \sum_{i=1}^{n} Score_i$$

The Average Score Per Taxon (ASPT) is calculated by dividing EDOT $_{(f)}$ scores with total number of taxa found as follows.

$$ASPT = \frac{(\sum_{i=1}^{n} Score_i)}{n}$$

where: $Score_i$ stands for the score of taxon *i* and *n* for number of taxa.

Moreover, separate results may be achieved for each biotope and used in various investigations, only the result calculated from the Total column will represent the EDOT(f) result for a site. Since this new index is designed to describe the degree at which tropical African riverine systems are impacted by human induced pollution, the scores towards 0 represent stressed river while towards 100 refers to unstressed river. However, Dickens and Grahm (2002) have cautioned on the implication of combining the scores from the three biotopes by adding the Score of any index, Number of Taxa and ASPT and dividing the total by three. The resulting EDOT(f) score and ASPT score are then interpreted using modified threshold values in Table 6.4.

Table 6.4: The suggested EDOT(f) threshold limits for assessing river health status

EDOT Score	ASPT-Score	Water quality	Impairment level
< 20	<2	Very poor water quality	Severe ecological impairment
20 to 40	2.1 to 3	Poor water quality	Slight ecological impairment
41 to 60	3.1 to 4	Moderate water quality	Moderate ecological impairment
61 to 100	4.1 to 5	Good water quality	Slight ecological impairment
>100	>5	High water quality	Little ecological impairment

6.5 Discussion

Towards EDOT(f) development, the tighter validation of sampling protocol used previously by Elias *et al*. (2014b); Chapter four and Kaaya (2014) respectively in Tanzania was imposed and in return yielded a positive result. The validation process has increased number of sampling seasons while twisting the sampling time from the mid of dry, long rain and short rain sampling seasons to the end of each respective season. This was done purposely in order to capture the effects of each respective season including recovery of the riverine ecosystem while extracting all available macroinvertebrate organisms. The positivity of the adjustment is supported by the newly observed Macromiidae family which was not reported by the four studies previously done in the same area, some of which, involved even more sampling points compared to this study. Likewise, the similar study done in the same sampling points while targeting only two (dry and wet) seasons (Chapter four), has collected fewer number of taxa (N = 79) compared to what was collected during this study (N = 97) in three sampling seasons. Similarly, number of taxa (N = 79) collected only in two (dry and wet) seasons at the same sampling points (Chapter four) had increased from N = 79 to N = 97 in this study, after

three seasons have been involved. However, the former study regarded each of Baetidae and Hydropsychidae families as a single taxon despite the two being reported by early scoring systems as six families (Baetidae 1 sp., 2 spp., and > 2spp. and Hydropsychidae1 sp., 2 spp., and > 2spp.). Moreover, two of the three families (Nepidae and Notonectidae) found missing previously along Wami-Ruvu sampling stations for a study that considered only two (mid dry and rain) seasons, were observed in the same stations by this study and thus might suggest the influence of short rains on the occurrence of these taxa.

A 15 sensitivity scoring range following SASS5 (Dickens and Grahm, 2002) and TARISS (Kaaya, 2015) was used for all identified families of selected orders. Out of the total 97 taxa recorded from all sites, scores were assigned only to 53 taxa that showed clear water quality preferences, using either closely related earlier indices (50 taxa) or CAP predictive model (3 taxa). However, a flexible consideration was applied to assign sensitivity scores for specific taxa groups with number of types i.e., Baetidae (with 1 sp., 2 spp. and > 2spp.), and Hydropsychidae (1 sp., 2 spp. and > 2 spp.) that cover wide pollution gradients (Aschalew and Moog, 2015) in order to increase the discrimination efficiency of these taxa among site categories.

The sensitivity scores for taxa obtained from earlier indices strongly support the simulated CAP predictive model results with some families of the same order found matching the scores. For instance, the calculated score of 10 for Dicercomyzidae concurs with that of Polymitarcyidae whereas the score of 9 awarded to Leptophlebiidae and Tricorythidae by earlier studies was at par with that simulated for Ephemerythidae (9). Contrary to Dicercomyzidae and Ephemerythidae, Macromiidae was the least sensitive taxon (with a score of 3) compared to the other Odonata families but close to sensitive score of 4 which was reported for Coenagrionidae and Libellulidae by early indices. According to Gerber and Gabriel (2002), the simulated sensitivity scores for Dicercomyzidae (10) and Ephemerythidae (9) fall well within the range of moderately sensitive taxa while the Macromiidae (3) is grouped with the least sensitive taxa along the y-axis in Figure 6. The varied sensitivity levels to human stressors allow families of the EDOT orders to function as bio-indicators for assessing freshwater health status with strong relevance on conservation and management aspects (Hornung and Rice, 2003).

Validation criteria (that included six selection criteria) were also set during the selection of orders to be involved in developing the index for the sake of simplifying taxonomic complications and improving the accuracy and efficiency of the index while minimizing data collection time and cost. Indeed, EDOT are well known as more diverse and abundant orders in freshwater ecosystems (Harrison and Hynes, 1988) with the large number of taxa and species (Hofmann and Mason, 2005; Hughes, 2006; Mereta *et al*., 2013), varied degrees of sensitivity to a wide range of anthropogenic stressors (Verdonschot *et al*., 2012) and a recognizable contribution in the biomonitoring programmes (Kashian and Burton, 2000). In the presence of various environmental stress types i.e., organic pollution (Zamora-Munoz *et al*., 1996), heavy metals (Smolders *et al*., 2003), hydro-morphological degradation (Lorenz *et al*., 2004), nutrient enrichment (Johnson *et al*., 2006), acidification (Sandin, 2000) and general stressors (Barbour *et al*., 1999), their families can collectively reflect short and long term health status of aquatic ecosystems (Baptista *et al*., 2007). However, other 13 orders were eliminated because they either failed to reflect the different features of freshwater macroinvertebrates communities or discriminating reference sites from monitoring sites according to truncate numerical test, the Mann-Whitney U test (with $p < 0.05$), Box-and-Whisker plot test, Non-parametric Spearman's rank correlation test and more diverse taxa ($n > 10$) criteria. Similarly, the presence of cryptic species (e.g., chironomids) with varied response towards pollution (Li *et al*., 2010), and some being rarely identified to species level (Mereta *et al*., 2013) has restricted the development of species level EDOT index. Moreover, lowest taxonomical unit identification has cost and time bounded implications and also require more specialized knowledge and expertise (Schmidt-Kloiber and Nijboer, 2004; Aschalew and Moog, 2015). However, sensitivity variation for some families of the same order might contradict the biomonitoring efforts. For example, the Odonata family Gomphidae, has been classified among the most sensitive taxa whereas Coenagrionidae is far less sensitive to pollution (Foote and Hornung, 2005).

Ephemeropterans are considered as ecologically important order in biomonitoring programmes all over the world due to their least tolerant character against low dissolved oxygen, higher levels of nutrients, and toxicant chemical elements and compounds (Arimoro and Muller, 2010). The order is abundantly found in sites with good water quality at interstitial spaces between rocks, rock surfaces, sediment, sub-merged underwater vegetation and in the littoral water zone, with high amount of dissolved oxygen (Arimoro and Muller, 2010).

Contrary to Ephemeroptera, Trichoptera are somewhat more tolerant to pollution, but they do not persist as a diverse community in the presence of significant impairment (Houghton, 2004). Trichopterans on the other hand inhabit a wide variety of habitats, ranging from fast flowing riffles to slow moving water type of sparsely vegetated pools. Being diverse, abundant and able to thrive in lentic conditions of both slow and fast moving rivers makes them excellent indicators of habitat quality (Houghton, 2004). Regardless of their reported inconsistent nature in detecting impacts (Kashian and Burton, 2000), the inclusion of trichopterans in biomonitoring programmes is not only virtual in evaluating the long-term interaction of several environmental conditions, but also in detecting short-term impact.

The strong significant correlation (with $p < 0.0001$) showed between the EDOT orders and most of the environmental variables structuring macroinvertebrate assemblages indicate better performance of the orders to organic pollution. In polluted rivers, abundance and diversity of more sensitive orders (ephemeropterans and trichopterans) are strongly reduced due to direct and indirect impact of pollutants where dipterans commonly possess the dominant status. Ability of dipterans to survive well on highly polluted freshwater environment and in slow moving water than most of the ephemeropterans, trichopterans and Odonata, render them good indicators for assessing of aquatic health status (Shelly *et al*., 2011). EDOT has ensured response of overall ecological status in river basins by segregating reference sites from monitoring sites and thus, conforming with other studies in the U.S. (Barbour *et al*., 1996), Europe (Pinto *et al*., 2004), Brazil (Baptista *et al*., 2007), and Tanzania (Chapter four). The EDOT index is in line with the interest shown by African and non-African environmental and water quality monitoring institutions in the application of biomonitoring methods, which tend to be lower cost and more effective than physical-chemical methods (Mereta *et al*., 2013; Aschalew, 2014), with emphasis on regionally or a country based water quality biomonitoring programmes.

6.6 Conclusion

EDOT orders are comprised of many taxa with a wide range of occurrence, trophic levels and pollution tolerances that provide strong information facilitating the interpretation of cumulative human induced effects in freshwater ecosystems. Numerically and statistically, these orders have proved their potentiality for inclusion in developing EDOT index as a tool of assessing water pollution in Tanzanian river basins according to truncate numerical test, the Mann-Whitney U test (with $p < 0.05$), Box-and-Whisker plot test, Spearman's rank

correlation test and more diverse orders (with taxa > 10) criteria. Being developed using only few (four) and more diverse orders (with > 10 taxa), minimizes data variability, needs for great expertise and time in the field and thus makes it less sophisticated method than existing multimetric indices. High EDOT index score describes an ecosystem containing diversified physical habitats, good water quality with conducive physicochemical conditions and adequate food resources for sustaining the lives of many species. Upon validation, the resulting EDOT index can therefore be regarded as simple, quick, robust and cost-effective tool for assessing the ecological condition in Tanzanian rivers and other related watersheds in tropical African regions, where freshwater resources are under high pressure as a result of anthropogenic activities.

CHAPTER SEVEN

7.0 GENERAL DISCUSSION

7.1 Introduction

This chapter presents general discussion on significant findings of the study reported in this dissertation. It covers discussion on the developed biomonitoring tool, research hypotheses and provides concluding remarks and recommendations.

7.2 Discussion

Generally, freshwater habitats are amongst the most threatened ecosystems on earth and subject to the most pressing requirements for improved attention to sustainable use due to the rapidly increasing demand for water. Similarly, all areas related to freshwater ecosystems require more data and information, from water availability and quality to the status and population trends of macroinvertebrate species. In that sense, the use of local macroinvertebrate communities as indicators in tropical African biomonitoring programmes as opposed to the adoption and use of non-tropical indices in tropical rivers would hold great promise in providing accurate data which is hitherto not available through the use of adopted indices. However, the presence of conflicting aspects regarding the adoption of or relying on existing non-tropical indices in tropical biomonitoring programmes has necessitated the design of this study in order to develop a non-sophisticated and cost-effective tropical biomonitoring method, for initial application in Tanzanian rivers (Chapters one, two and six). In achieving index development, a number of field, laboratory and review (desk) studies were done to characterize macroinvertebrate communities and examine physico-chemical variables as a way of laying down the needed groundwork.

In the development of any biomonitoring programme, various aspects need to be considered due to the fact that within aquatic ecosystems, complex interactions of physical and biological cycles exist. Indeed, a strong relationship observed in this study between the families found and the degradation of the watercourses appears to support such contention. Presence of human practices of different magnitude within the two basins of varying hydrological patterns, explained the main variability in taxonomy, ecology and macroinvertebrate composition; and thus, conform to the first hypothesis. Macroinvertebrate communities at degraded sites, for example, are characterized by either absence of any

sensitive taxa or presence of few if any, greater dominance of only few taxa, and larger numbers of macroinvertebrates that are tolerant to pollution (Chapter four). Similarly, families of orders Ephemeroptera (Dicercormyzidae, Ephemerythidae, Heptageniidae, Oligoneuridae, and Polymitarcyidae) and Trichoptera (Lepidostomatidae, Leptoceridae, Philopotamidae and Phryganeidae) disappeared or their numbers reduced drastically in impacted sites as opposed to some Diptera and Odonata taxa which were observed in all sites (Chapter four). Therefore, the complete absence of these taxa from impacted sites is probably related to the differences of in-stream environmental degradation along rivers as a result of human activities

Macroinvertebrates and environmental variables showed significant differences between the studied basins as a result of differences in hydrological patterns, experiencing different levels of habitat modification (Chapters four and six). Differences in hydrological patterns are the local and/or ecoregion-scale factor that might affect macroinvertebrates occurrence and distribution before other more local factors. Similarly, human induced pollution and habitat degradation (i.e., habitat modification and/or stability) of different magnitudes played a second role on defining the dynamics of macroinvertebrate communities along the studied basins (Chapter four). The absence of Nepidae, Notonectidae and Lumnichidae families and the observed least abundant taxa at Wami-Ruvu compared to Pangani, for example, might be associated with differences in geo-hydrological patterns and levels of impairment between the basins. Regarding hydrological patterns and improper land use practices, the study results have also conformed to the second hypothesis.

Significant differences in macroinvertebrate assemblages were also observed based on seasonality, number of sampling seasons, site categories and the types of biotopes (Chapters three, four and six). Taxonomic richness, for example, was significantly higher in reference than monitoring sites regardless of seasonality. Likewise, the relative and absolute richness and abundance of many sensitive stream insect orders decline with increasing nutrients loading (Chapter five). Tolerant taxa tend to have higher abundances at monitoring sites, due to their dominant character as opposed to intolerant taxa (Chapters three and four).

Increases in nutrient loads from agrochemical inputs and discharge of waste into watershed exacerbate the impact by altering the presence and distribution of biota (Chapter five) and consequently, favouring pollution tolerant species (Chapter four). For instance, pollution

tolerant organisms e.g., Chironomidae and *Tubifex* worms have a remarkable capability of resisting against pollution or to recover from disturbances in comparison with moderate (*Baetis* spp.) and least tolerant species e.g., plecopterans (Chapter five). This finding concurs with the third hypothesis that, mortality tallies increase with increased toxicants concentrations. Moreover, the responses of test organisms on different levels of nitrates and phosphates concentration were clear indications that macroinvertebrate communities were good candidates for assessing overall ecosystem integrity.

Occurrence and distribution of more diverse orders like ephemeropterans and trichopterans in reference sites and dipterans and Odonata along monitoring sites, point to the ability of these orders in detecting disturbance in riverine ecosystems (Chapter six). However, general response among macroinvertebrate families toward pollution levels necessitates some of families to be studied in detail and where possible, to their lowest taxonomic levels so as to strengthen biomonitoring programmes. Sensitivity variation for some families of the same order might contradict the biomonitoring efforts. For example, the Odonata family Gomphidae, has been classified among the most sensitive taxa whereas Coenagrionidae is far less sensitive to pollution (Chapters four and six). Likewise, the families Hydropsychidae and Caenidae have also been classified among the most sensitive taxa of Trichoptera and Ephemeroptera orders respectively while they are far less sensitive taxa to pollution (Chapters four and six). Similarly, the presence of cryptic species (e.g., chironomids) with varied response towards pollution and some being rarely identified to species level has restricted the development of species level EDOT index (Chapter six). Moreover, lowest taxonomical unit identification has cost and time bound implications and also requires more specialized knowledge and expertise (Chapters four and six). Therefore, all EDOT's families can also be subjected to the same stepwise criteria (used by this study for selection potential orders) to identify the ones with much potential of discriminating reference from monitoring sites for the sake of simplifying taxonomic complications and improving the accuracy and efficiency of the index while minimizing data collection time and cost.

This EDOT index sets a good basis for developing management strategies for the tropical rivers. The varied sensitivity levels to human stressors allow families of the EDOT orders to function as bio-indicators for assessing freshwater health status with strong relevance on conservation and management aspects (Chapters three and six). High EDOT index score describes an ecosystem containing diversified physical habitats, good water quality with

conducive physicochemical conditions and adequate food resources for sustaining the lives of many species. Being developed using only four and most diverse local orders, EDOT minimizes data variability, needs for great expertise and time in the field, the credibility that is not hitherto possessed by existing indices. Upon further validation with an independent data set, this index will present non-sophisticated and cost- effective scoring tool for evaluating the ecological condition of Tanzanian rivers and other related tropical African riverine environment, where aquatic resources are under high pressure as a result of improper land uses.

7.3 Conclusion and Recommendations

7.3.1 Conclusion

The EDOT index has been developed sufficiently to be used nationally as a bioassessment method in Tanzania but that it would be worthwhile to establish reference conditions for sites in ecoregions not covered by this study. EDOT should also work for other tropical countries after examining and developing reference conditions for the respective areas. In addition, limited freshwater macroinvertebrate taxonomists, identification keys and guides in Tanzania and in the region may limit quality assurance of EDOT samples and compromise the data accuracy. For accurate and reliable use of EDOT, there is a need to train taxonomists and EDOT practitioners in freshwater macroinvertebrates for purposes of quality assurance and developing identification keys and guides for macroinvertebrates at family level for Tanzanian rivers.

7.3.2 Recommendations

Apart from ecological and taxonomical knowledge on macroinvertebrates and the developed EDOT index, the following recommendations are considered useful for the betterment of bioassessment programmes in tropical African regions.

- Sampling protocol should be more integrative and not restricted only to site categories criteria (i.e., biotic and abiotic aspects) but also by incorporating ecoregion slopes and landforms;
- Further consideration should be directed to development of appropriate identification keys for tropical African freshwater organisms that can enable identification to the species level and hence increase the sensitivity of metrics. Also an attempt should be made in reviewing the primary taxonomic literature;

- Tolerance limits should also be verified for more local macroinvertebrates by toxicity tests to identify the various environmental minimal and optimal levels for the taxa, and;
- Lastly, efforts must be made towards community education on the safe use and disposal of agrochemicals, alternative energy use and waste disposal methods with a view to making improvements in freshwater quality.

REFERENCES

Abrehet, K. M., Wondie, A., Mingist, M. and Vijverberg, J. (2014). Spatial and seasonal variation in the macroinvertebrates and physico-chemical parameters of the Enfranz River, Lake Tana Sub-Basin in Ethiopia. *Journal of Ecohydrology and Hydrobiology*. 60: 1-9. DOI: 10.1016/j.ecohyd.2014.07.004.

Akasaka, M., Takamura, N., Mitsuhashi, H. and Kadono, Y. (2010). Effects of land use on aquatic macrophyte diversity and water quality of ponds. *Freshwater Biology*. 55: 909-922.

Ak'habuhaya, J. and Lodenius, M. (1988). Metal pollution of River Msimbazi, Tanzania. *Environment International*. 14:511-514.

Alonso, A. and Camargo, J. A. (2004). Toxic effects of unionized ammonia on survival and feeding activity of the freshwater amphipod *Eulimnogammarus toletanus* (Gammaridae, Crustacea). *Bulletin of Environmental Contamination and Toxicology*. 72:1052-1058.

Andersen, M. M., Riget, F. F. and Sparholt, H. (1984). A modification of the Trent Index for use in Denmark. *Water Research*. 18: 141-151.

Anderson, M. J., Gorley, R. N. and Clarke, K. R. (2008). PERMANOVA + for PRIMER: Guide to software and statistical methods. The University of Auckland, Plymouth, UK. 214 pp.

Andersen, T., Carstensen, J., Hernandez-Garcia, E. and Duarte, C. M. (2009). Ecological thresholds and regime shifts: approaches to identification. *Trends in Ecology and Evolution*. 24(1): 49-57.

Angelier, E. (2003). Ecology of streams and rivers. Science Publishers Inc., Enfield, USA. 215 pp.

APHA (1998). *Standard methods for the examination of water and wastewater*, 20th edition. American Public Health Association, Washington, D.C., USA. 541 pp.

APHA (2000). *Standard methods for the analysis of water and wastewater*, 15th edition. American public health association and water pollution control federation. Washington, DC, USA. pp. 12-56.

Arimoro, F. O. and Muller, W. J. (2010). Mayfly (Insecta: Ephemeroptera) community structure as an indicator of the ecological status of a stream in the Niger Delta area of Nigeria. *Environmental Monitoring and Assessment*. 166: 581-594.

Aschalew, L. (2014). Development of biological monitoring systems using benthic invertebrates to assess the ecological status of central and south-east highland

rivers of Ethiopia. Unpublished Thesis for Award of PhD Degree at University of Natural Resources and Life Sciences. Vienna, Austria, 163 pp.

Aschalew, L. and Moog, O. (2015). A multimetric index based on benthic macroinvertebrates for assessing the ecological status of streams and rivers in central and southeast highlands of Ethiopia. *Hydrobiologia*, (in press).

Ashton, P. J. (2002). Avoiding conflicts over Africa's water resources. *Ambio*. 31 (3): 236-242.

ASTM (1978). Estimating the hazard of chemical substances to aquatic life. In: *Special Technical Publication No. 657* (Edited by Cairns, J., Dickson, K. L. and Maki, A. W)..Philadelphia, USA. 264 pp. DOI: 10.1520/STP 657. EB.

ASTM E729-96 (2002). *Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians*. ASTM International, West Conshohocken, PA. 22 pp. DOI: 10.1520/E0729-96R02.

ASTM E729-96 (2007). *Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians*. ASTM International, West Conshohocken, PA, DOI: 10.1520/E0729-96R07.

Aura, C. M., Raburu, P. O. and Herman, J. (2010). A preliminary macroinvertebrate index of biotic integrity for bioassessment of the Kipkaren and Sosiani Rivers, Nzoia River basin, Kenya. Lakes and Reservoirs. *Research and Management*. 15: 119-128.

Bahar, M. D. M., Ohmori, H. and Yamamuro, M. (2008). Relationship between river water quality and land use in a small river basin running through the urbanizing area of Central Japan. *Limnology*. 9: 19-26.

Barnard, P. C. and Biggs, J. (1988). Macroinvertebrates in the catchment streams of Lake Naivasha, Kenya. *Reviews of Tropical Hydrobiology.* 21:12713.

Baptista, D. F., Buss, D. F., Egler, M., Giovanelli, A., Silveira, M. P., and Nessimian, J. L. (2007). A multimetric index based on benthic macroinvertebrates for evaluation of Atlantic Forest streams at Rio de Janeiro State, Brazil. *Hydrobiologia*. 575(1): 83-94.

Barbour, M. T. and Gerritsen, J. (1996). Sub sampling of benthic samples: a defense of the fixed-count method. *Journal of the North American Benthological Society*. 15(3): 386-391.

Barbour C. D. M. T., Gerritsen, J., Snyder, B. D. and Stribling, J. B. (1999). *Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish; 2nd edition*. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water, Washington D.C.

Barbour, M. T., Swietlik, W. F., Jackson, S. K., Courtemanch, D. L., Davies S. P. and Yoder. C. O. (2000). Measuring the attainment of biological integrity in the USA: a critical element of ecological integrity. *Hydrobiologia.* 422/423: 453-464.

Benfield, E. F. and Buikema, A. L. (1980). Synthesis of miscellaneous invertebrate toxicity tests. In: *Aquatic invertebrate bioassays. ASTM STP 715.* (Edited by Buikema, A. L., Cairns, J.). American Society for Testing and Materials. Philadelphia. pp. 174-187.

Bird, M. S. (2012). *Effects of habitat transformation on temporary wetlands in the South-Western Cape*. Unpublished Thesis for Award of PhD Degree at University of Cape Town, South Africa. 234 pp.

Blakely, T. J., Harding, J. S., Clews, E. and Winterbourn, M. J. (2010). *An illustrated guide to the freshwater macroinvertebrates of Singapore*. School of Biological Sciences, University of Canterbury, Christchurch. 74 pp.

Böhmer, J., Rawer-Jost, C. and Zenker, A. (2004). Multimetric assessment of data provided by water managers from Germany: assessment of several different types of stressors with macrozoobenthos communities. *Hydrobiologia.* 516: 215-228.

Bolnick, D. I. and Preisser, E. L. (2005). Resource competition modifies the strength of trait-mediated predator-prey interactions: a meta-analysis. *Ecology*. 86: 2771-2779.

Bonada, N. and Resh, V. H. (2013). Mediterranean-climate streams and rivers: geographically separated but ecologically comparable freshwater systems. *Hydrobiologia*. 719: 1-29.

Bonada, N., Dolédec, S. and Statzner, B. (2007). Taxonomic and biological trait differences of stream macroinvertebrate communities between Mediterranean and temperate regions: implications for future climatic scenarios. *Global Change Biology.* 13: 1658-1671.

Bonada, N., Rieradevall, M., Prat, N. and Resh, V. H. (2006). Benthic macroinvertebrates assemblages and macrohabitat connectivity in Mediterranean-climate streams of northern California. *Journal of the North American Benthological Society.* 25: 32-43.

Bonada, N., Dallas, H., Rieradevall, M., Prat, N. and Day, J. (2006). A comparison of rapid bio-assessment protocols used in two Mediterranean regions, Iberian Peninsula and South Africa. *Journal of the North American Benthological Society*. 25(2): 487-500.

Boulton, A. J., Boyero, L., Covich, A. P., Dobson, M., Lake, S. and Pearson, R. (2008). Are tropical streams ecologically different from temperate streams? In: *Tropical Stream Ecology*. (Edited by Dudgeon, D.). Elsevier Inc., UK, London. pp. 257-284.

Boyle. T. P. and Fraleigh Jr. H. D. (2003). Natural and anthropogenic factors affecting the structure of the benthic macroinvertebrate community in effluent-dominated reach of Santa Cruz river, AZ. *Ecological Indicators*. 3:93-117.

Buikema, A. L. Jr., Niederlehner, B. R. and Cairns, J. Jr. (1982). Biological monitoring. Toxicity testing. *Water Research*. 16: 239-262.

Camargo, J. A and Alonso, A. (2006). Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment International*. 32: 831-849.

Camargo, J. A., Alonso, A. and Salamanca, A. (2005). Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. *Chemosphere*. 58: 1255-1267.

Canadian Council of Ministers of the Environment (2003). Canadian water quality guidelines for the protection of aquatic life: nitrate ion. Winnipeg, Canada: CCMI. 146 pp.

Carpenter, S. R, Caraco, N. F, Correll, D. L, Howarth, R. W, Sharpley, A. N. and Smith, V. H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications*. 8: 559-68.

Cereghino, R., Park, Y., Compin, A. and Lek, S. (2003). Predicting the species richness of aquatic insects in streams using a limited number of environmental variables. *Journal of the North American Benthological Society*. 22(3): 442-456.

Chapman, L. J., Schneider, K. R., Apodaca, C. and Chapman, C. A. (1996). Respiratory ecology of macroinvertebrates in a swamp river system of East Africa, *Biotropica*. 36: (4) 572-585.

Chaves, M. L. (2008). Spatio-temporal dynamics of undisturbed macroinvertebrate communities in the Mondego River basin - Contribution to the ecological assessment of streams. Unpublished Thesis for Award of PhD Degree at University of Lisboa, Portugal. 220 pp.

Chutter, F. M. (1994). The rapid biological assessment of streams and river water quality by means of macroinvertebrate communities in South Africa. In: *Classification of Rivers and Environmental Health Indicators. Water Research Commission Report No. TT 63/94*. (Edited by Uys, M. C. South Africa. pp. 217-234.

Chutter, F. M. (1998). Research on the rapid biological assessment of water quality impacts in streams and rivers. Water Research Commission Report No 422/1/98.Water Research Commission, Pretoria, South Africa.

Clarke, R. T. and Hering, D. (2006). Errors and uncertainty in bioassessment methods–major results and conclusions from the STAR project and their application using STARBUGS. *Hydrobiologia*. 566: 433-439.

Constable, M., Charlton, M., Jensen, F., McDonald, K., Craig, G. and Taylor, K. W. (2003). An ecological risk assessment of ammonia in the aquatic environment. *Human and Ecological Risk Assessment*. 9: 527-48.

Cormier, S. M., Paul, J. F., Spehar, R. L., Shaw-Allen, P., Berry, W. J. and Suter, G. W. (2008). Using field data and weight of evidence to develop water quality criteria. *Integrated Environmental Assessment and Management*. 4(4): 490-504.

Cushing, C. E. and Allan. J. D. (2001). *Streams: Their Ecology and Life*, *Abiotic Factors*. Academic Press, San Diego, California. 366 pp.

Dallas, H. F. (1997). A preliminary evaluation of aspects of South African Scoring System (SASS) for the rapid bioassessment of water quality in rivers, with particular reference to the incorporation of SASS in a national biomonitoring programme. *African Journal of Aquatic Science*. 23(1): 79-94.

Dallas, H. F. (2004a). Spatial variability in macroinvertebrate assemblages: comparing regional and multivariate approaches for classifying reference sites in South Africa. *African Journal of Aquatic Science*. 29(2): 161-171.

Dallas, H. F. (2004b). Seasonal variability of macroinvertebrate assemblages in two regions of South Africa: implications for aquatic bioassessment. *African Journal of Aquatic Science.* 29(2): 173-184.

Dallas, H. F. (2009). Wetland monitoring using aquatic macroinvertebrates. Technical Report. Report 5/2009 prepared for the Biokavango Project, Harry Oppenheimer Okavango Research Centre, University of Botswana. The freshwater Consulting Group, University of Cape Town, Cape Town, South Africa. 27 pp.

Dallas, H. F., Kennedy, M., Taylar, J., Lowe, S., and Murphy, S. (2010). SAFRASS. South African Rivers Assessment Scheme, WP4. Review Paper. 39 pp.

Daniel, F. B. and Erika, L. B. (2008). Application of rapid bioassessment protocols (RBP) for benthic macroinvertebrates in Brazil: comparison between sampling techniques and mesh sizes. *Neotropical Entomology*. 37 (3): 288-295.

Day, J. A. (2000). Biomonitoring: appropriate technology for the 21st century. 1st WARFSA/WaterNet Symposium: Sustainable Use of Water Resources, Maputo, Mozambique. 7 pp.

Day, J. A., Stewart, B. A., De Moor, I. J. and Louw, A. E. (Editors) (1999). *Guides to the freshwater invertebrates of Southern Africa: Volume 2 Crustacea I - Notostraca, Anostraca, Conchostraca and Cladocera*. WRC Report no. TT 121/00. Water Research Commission, Pretoria. 126 pp.

Day, J. A., de Moor, I. J., Stewart, B. A. and Louw, A. E. (2001a). *Guides to the freshwater invertebrates of Southern Africa: Volume 3 Crustacea II - Ostracoda, Copepoda and Branchiura*. WRC Report no. TT 148/01. Water Research Commission, Pretoria. 177 pp.

Day, J. A., de Moor, I. J., Stewart, B. A. and Louw, A. E. (2001b). *Guides to the freshwater invertebrates of Southern Africa: Volume 4 Crustacea III - Bathynellacea, Amphipoda, Isopoda, Spelaeogriphea, Tanaidacea and Decapoda*. WRC Report no. TT 141/01. Water Research Commission, Pretoria. 126 pp.

Day, J. A. and de Moor, I. J. (2002a). *Guides to the freshwater invertebrates of Southern Africa: Volume 6 Arachnida and Mollusca - Araneae, water mites and Mollusca*. WRC Report no. TT 182/02. Water Research Commission, Pretoria. 141 pp.

Day, J. A. and de Moor I. J. (2002b). *Guides to the freshwater invertebrates of Southern Africa: Volume 5 Non-Arthropods - The Protozoans, Porifera, Cnidaria, Platyhelminthes, Nemertea, Rotifera, Nematoda, Nematomorpha, Gastrotrichia, Bryozoa, Tardigrada, Polychaeta, Oligochaeta and Hirudinea*. WRC Report no. TT 167/02. Water Research Commission, Pretoria. 293 pp.

Day, J. A., Harrison A. D. and de Moor I. J. (2003). *Guides to the Freshwater Invertebrates of Southern Africa: Volume 9 Diptera*. WRC Report no. TT 201/02. Water Research Commission, Pretoria. 288 pp.

Day, J. A., De Moor, I. J., Stewart B. A. and A. E. Louw, (editors), 2001a. *Guides to the freshwater invertebrates of Southern Africa: Volume 3 Crustacea II - Ostracoda, Copepoda and Branchiura*. WRC Report no. TT 148/01. Water Research Commission, Pretoria. 177 pp.

Day, J. A., Stewart, B. A., De Moor I. J. and Louw, A. E. (Editors) (2001b). *Guides to the freshwater invertebrates of Southern Africa: Volume 4 Crustacea III-Bathynellacea, Amphipoda, Isopoda, Spelaeogriphea, Tanaidacea and Decapoda*. WRC Report no. TT 141/01. Water Research Commission, Pretoria. 126 pp.

Day, J. A. and De Moor, I. J. (editors) (2002a). *Guides to the freshwater invertebrates of Southern Africa: Volume 6 Arachnida and Mollusca - Araneae, water mites and Mollusca*. WRC Report no. TT 182/02. Water Research Commission, Pretoria. 141 pp.

Day, J. A. and De Moor, I. J. (Editors) (2002b). *Guides to the freshwater invertebrates of Southern Africa: Volume 5 Non-Arthropods - The Protozoans, Porifera, Cnidaria, Platyhelminthes, Nemertea, Rotifera, Nematoda, Nematomorpha, Gastrotrichia, Bryozoa, Tardigrada, Polychaeta, Oligochaeta and Hirudinea*. WRC Report no. TT 167/02. Water Research Commission, Pretoria. 293 pp.

Day, J. A., Harrison A. D. and De Moor, I. J. (Editors) (2003). *Guides to the Freshwater Invertebrates of Southern Africa: Volume 9 Diptera*. WRC Report no. TT 201/02. Water Research Commission, Pretoria. 288 pp.

De Moor, I. J., Day J. A. and de Moor F. C. (2003a). *Guides to the freshwater invertebrates of Southern Africa: Volume 7 Insecta I - Ephemeroptera, Odonata and Plecoptera*. WRC Report no. TT 207/03. Water Research Commission, Pretoria. 288 pp.

De Moor, I. J., Day J. A. and de Moor F. C. (2003b). *Guides to the freshwater invertebrates of Southern Africa: Volume 8 Insecta II - Hemiptera, Megaloptera, Neuroptera, Trichoptera and Lepidoptera*. WRC Report no. TT 214/03. Water Research Commission, Pretoria. 209 pp.

De Moor, I. J., Day, J. A. and De Moor, F. C. (editors) (2003a). *Guides to the freshwater invertebrates of Southern Africa: Volume 8 Insecta II - Hemiptera, Megaloptera, Neuroptera, Trichoptera and Lepidoptera*. WRC Report no. TT 214/03. Water Research Commission, Pretoria. 209 pp.

De Moor, I. J., Day, J. A. and De Moor, F. C. (editors) (2003b). *Guides to the freshwater invertebrates of Southern Africa: Volume 7 Insecta I - Ephemeroptera, Odonata and Plecoptera*. WRC Report no. TT 207/03. Water Research Commission, Pretoria. 288 pp.

De Pauw, N. and Vanhooren, G. (1983). Method for biological quality assessment of watercourses in Belgium. *Hydrobiologia*. 100: 153-168.

Dickens, C. W. S. and Grahm, P. M. (2002). The South African Scoring System (SASS) Version 5 Rapid bioassessment method for rivers. *African Journal of Aquatic Science*. 27: 1-10.

Dodds, W. K., Clements, W. H., Gido, K., Hilderbrand, R. H. and King, R. S. (2010). Thresholds, breakpoints, and nonlinearity in freshwaters as related to management. *Journal of the North American Benthological Society*. 29 (3): 988-997.

Dudgeon, D. (1992). Endangered ecosystems: a review of the conservation status of tropical Asian rivers. *Hydrobiologia.* 248: 167-191.

Eggermont, H. and Verschuren, D. (2003). Impact of soil erosion in disturbed tributary drainages on the benthic macroinvertebrate fauna of Lake Tanganyika, East Africa. *Biological Conservation*. 113: 99-109.

Elias, J. (2009). The assessment of benthic macroinvertebrate structures in some Tanzanian rivers as an indicator for water quality biomonitoring programme. Unpublished report submitted to IFS, Stockholm, Sweden. 81 pp.

Elias, J. D., Ijumba, J. N., Mamboya, F. A. (2014a). Effectiveness and compatibility of non-tropical biomonitoring indices for assessing pollution in tropical rivers – a review. *International Journal of Ecosystem*. 4(3): 128-134.

Elias, J. D., Ijumba, J. N., Mgaya, Y. D. and Mamboya, F. (2014b). Study on freshwater macroinvertebrates of some Tanzanian rivers as a basis for developing biomonitoring index for assessing pollution in tropical African regions. *Journal of Ecosystems*. http://dx.doi.org/10.1155/2014/985389. 8 pp.

Ellison, A.M. (2004). Wetlands of Central America. *Wetlands Ecology Management.* 12:3-55.

Ferreira, W. R., Paiva, L. T. and Callisto, M. (2011). Development of a benthic multimetric index for biomonitoring of a neotropical watershed. *Brazil Journal of Biology.* 71(1): 15-25.

Foote, A. L. and Hornung, C. L. R. (2005). Odonates as biological indicators of grazing effects on Canadian prairie wetlands. *Ecological Entomology*. 30: 273-283.

Foto Menbohan, S. (2012). Bioecology of epilithic diatoms in the Mfoundi River: Diversity, spatial distribution and influence of organic pollution; Yaoundé, Cameroon. *Journal of Water Science*. 25(3): 203-218.

Fuji, T. (2007). Spatial patterns of benthic macrofauna in relation to environmental variables in an intertidal habitat in the Humber Estuary, UK: developing a tool for estuarine shoreline management. *Estuarine, Coastal and Shelf Science*. 75: 101-119.

Galdean, N., Callisto, M. and Barbosa, F. A. R. (2000). Lotic ecosystem of Serra do Cipó, South east Brazil: water quality and a tentative classification based on the benthic macroinvertebrate community. *Aquatic Ecosystem Health and Management.* 3: 545-552.

Gauch, H. G. Jr. (1982). Multivariate analysis in community ecology. Cambridge University Press, Cambridge. 307 pp.

Gerber, A. and Gabriel, M. J. M. (2002). *Aquatic invertebrates of South African rivers. Field Guide*. Institute for Water Quality Studies, Vol. I and II. 150 pp.

Gruber, N. and Galloway, J. (2008). An Earth-system perspective of the global nitrogen cycle, *Nature*. 451: 293-296.

Hall, R. K., Wolinsky, G. A., Husby, P., Harrington, J., Spindler, P., Vargas, K. and Smith, G. (2000). Status of aquatic bioassessment in US EPA region IX. *Environmental Monitoring and Assessment*. 64: 17-30.

Harrison, A. D. and Hynes, H. B. N. (1988). Benthic fauna of Ethiopian mountain streams and rivers. *Hydrobiologia*. 1:1-36

Hart, D. D. (1994). Building a stronger partnership between ecological research and biological monitoring. *Journal of the North American Benthological Society.* 13(1): 110-116.

Hawks, H. A. (1997). Origin and Development of the Biological Monitoring Working Party system. *Water Research.* 32(3): 964-968.

Hawkins, C. P, Olson, J. R, and Hill, R. A. (2010). The reference condition: predicting benchmarks for ecological and water quality assessments. *Journal of the North American Benthological Society*. 29(1): 312-343.

Hayford, B. L. and Ferrington, L. C., Jr. (2005). Biological assessment of Cannon Creek, Missouri by use of emerging Chironomidae (Insecta: Diptera). *Journal of the Kansas Entomological Society*. 78(2): 89-99.

Heiskary, S., and Markus, H. (2003). *Establishing relationships among in-stream nutrient concentrations, phytoplankton and periphyton abundance and composition, fish and macroinvertebrate indices, and biochemical oxygen demand in Minnesota USA rivers*. Minnesota Pollution Control Agency, Environmental Outcomes Division. St. Paul, MN. 106 pp.

Henne, L. J, Schneider, D. W and Martinez, L. M. (2002). Rapid Assessment of Organic Pollution in a West-central Mexican River Using a Family-level Biotic Index. *Journal of Environmental Planning and Management*. 45(5): 613-632.

Hilsenhoff, W. L., 1988. Rapid field assessment of organic pollution with a family level biotic index. *Journal of the North American Benthological Society*. 7: 65-68.

Hofmann, T. A. and Mason, C. F. (2005). Habitat characteristics and the distribution of Odonata in a lowland river catchment in eastern England. *Hydrobiologia*. 539: 137-147.

Hornung, J. P. and Rice, C. L. (2003). Odonata and wetland quality in Southern Alberta, Canada: A Preliminary study. *Odonata*. 32: 119-129.

Houghton, D. C. (2004). Biodiversity of Minnesota caddisflies (Insecta: Trichoptera): delineation and characterization of regions. *Environmental Monitoring and Assessment*. 95: 153-181.

Hughes, S. J. (2006). Temporal and spatial distribution patterns of larval Trichoptera in Madeiran streams. *Hydrobiologia*. 553: 27-41.

Ikonomov. T. R., 1963. Mayflies of Lake Hovsgol Region, Mongolia. Pp. 103 – 115.

International Union for Conservation of Nature and Water and Nature Initiative Panganiu Basin Water Board (PBWB/IUCN) (2010). Basin delineation report. Pangani Basin Water Board, Moshi and IUCN Eastern and Southern Africa Regional Programme, Nairobi. 76 pp.

Jacobsen, D. and Marın, R. (2007). Bolivian Altiplano streams with low richness of macroinvertebrates and large diel fluctuations in temperature and dissolved oxygen. *Aquatic Ecology*. 42: 643-656.

Jacobsen, D., Cressa, C., Mathooko, J. M. and Dudgeon, D. (2008). Macroinvertebrates: composition,lLife histories and production. (Ed. Dudgeon) Academic Press, USA. pp. 66-96.

Jamil, K. (2001). *Bioindicators and biomarkers of environmental pollution and risk assessment*. Science Publisher Inc., Enfield, USA.

Jensen, F. B. (2003). Nitrite disrupts multiple physiological functions in aquatic animals. *Comparative Biochemistry and Physiology*, 135A: 9-24.

Johnson, R. K., Hering, D., Furse, M. T., Verdonschot, P. F. M. (2006). Indicators of ecological change: comparison of the early response of four organism groups to stress gradients. *Hydrobiologia*. 566: 139-152.

Karr, J. R. and Chu, E. W. (1999). Restoring life in running waters: Better biological monitoring. Island Press, Washington, D.C. 206 pp.

Karr, J. R., and Chu. E. W. (2000). Sustaining living rivers. *Hydrobiologia.* 422/423:1-14.

Kaaya, L. (2014). Biological assessment of tropical riverine systems using aquatic macroinvertebrates in Tanzania, East Africa. Unpublished Thesis for Award of PhD Degree at University of Cape Town, South Africa. 141 pp.

Kaaya, L. T. (2015).Towards a classification of Tanzanian rivers: a bioassessment and ecological management tool. A case study of the Pangani, Rufiji and Wami-Ruvu river basins. *African Journal of Aquatic Science*. 40(1): 37-45.

Kasangaki, A., Chapman, L. J. and Balirwa, J. (2008). Landuse and the ecology of benthic macroinvertebrate assemblages of high-altitude rainforest streams in Uganda. *Freshwater Biology*. 53: 681-97.

Kasangaki A, Babaasa D, Efitre J, McNeilage A and Bitariho, R. (2006). Links between anthropogenic perturbation and benthic macroinvertebrate assemblages in Afromontane forest streams in Uganda. *Hydrobiologia*. 563: 231-245.

Kashian, D. R. and Burton, T. M. (2000). A Comparison of macroinvertebrates of two great Lakes Coastal Wetlands: testing potential metrics for an index of ecological integrity. *Journal of Great Lakes Research*. 26: 460-548.

Kenega, E. E. (1978). Test organisms and methods useful for early assessment of acute toxicity of chemicals. *Environmental Science and Technology*. 12: 1322-1329.

Kibichii, S., Shivoga, W. A., Muchiri, M. and Miller, S. N. (2007). Macroinvertebrate assemblages along a land-use gradient in the upper River Njoro watershed of Lake Nakuru drainage basin, Kenya. Lakes and Reservoirs: *Research and Management*. 12: 107-117.

Kidan, W. G. (2010). Biodiversity assessment: Major vegetation and land use impacts of Enfranz Riverine wetland in Lake Tana watershed, Northern Bahir Dar, Ethiopia. Unpublished Thesis for Award of MSc Degree at Bahir Dar University, Ethiopia. 104 pp.

Kimmel, W. G. (1983). The Impact of Acid Mine Drainage on the Stream Ecosystem. In: *Pennsylvania Coal: Resources, Technology and Utilization*., (Edited by Majumdar, S. K. and Miller, W. W.). The Pennsylvania Academy of Science Publication. pp. 424-437

Kinyua, A. M. and Pacini, N. (1991). The impact of pollution on the ecology of the Nairobi-Athi River system in Kenya. *Journal of Biochemical and Physics*. 1: 5 - 7.

Koel, T. M. and Stevenson, K. E. (2002). Effects of dredged material placement on benthic macroinvertebrates of the Illinois River. *Hydrobiologia*. 474(13): 229-238.

Lake, P. S. (2003). Ecological effects of perturbation by drought in flowing waters. *Freshwater Biology*. 48: 1161-1172.

Lang, C. and Reymond, O. (1995). An improved index of environmental quality of Swiss rivers based on benthic invertebrates. *Aquatic Sciences*. 57: 172-180.

Lenat, D. R., and Penrose D. L. (1996). History of the EPT taxa richness metric. *Bulletin of the North American Benthological Society*. 13: 305-307.

Leveque, C. and Balian, E. V. (2005). Conservation of freshwater biodiversity: does the real world meet scientific dreams? *Hydrobiologia*. 542:23-26.

Li, L., Zheng, B. and Liu, L. (2010). Biomonitoring and bioindicators used for river ecosystems: Definitions, approaches and trends. International Society for Environmental Information Sciences. Annual Conference (ISEIS); *Beijing, 100012, PR China*. *Procedia Environmental Sciences.* 2: 1510-1524.

Liess, M. and Schulz, R. (1999). Linking insecticide contamination and population response in an agricultural stream. *Environmental Toxicology and Chemistry*. 18(9):1948-1955.

Liess, M., Schäfer, R. B. and Schriever, C. A. (2008). The footprint of pesticide stress in communities. Species traits reveal community effects of toxicants. *Science of the Total Environment*. 406(3): 484-490.

Liess, A., Drakare, S. and Kahlert, M. (2009). Atmospheric nitrogen-deposition may intensify phosphorus limitation of shallow epilithic periphyton in unproductive lakes. *Freshwater Biology*. 54:1759-1773.

Likens, G. E. (2010). *Lake Ecosystem Ecology*. Academic Press, San Diego, California. 463 pp.

Lowe, S., Dallas, H., Kennedy, M., Taylor, J. C., Gibbins, C., Lang, P., Day, J., Sichingabula, H., Saili, K., Willems, F., Briggs, J. A. and Murphy, K. (2013). The SAFRASS biomonitoring scheme: general aspects, macrophytes (ZMTR) and benthic macroinvertebrates (ZISS) protocols. Produced for the ACP Science and Technology Programme. 16 pp. DOI: 10.13140/2.1.2112.2883.

Lorenz, A., Hering, D., Feld, C. K. and Rolauffs, P. (2004). A new method for assessing the impact of hydromorphological degradation on the macroinvertebrate fauna of five German stream types. *Hydrobiologia*. 516: 107-127.

Lyimo, E. (2012). Amphibian and Benthic Macroinvertebrate Response to Physical and Chemical Properties of Themi River, Arusha, Tanzania. Unpublished Thesis for Award of MSc Degree at Faculty of Applied Ecology and Agricultural Sciences, Hedmark University College, Norway. 34 pp.

Malmqvist, B. and Rundle, S. (2002). Threats to the running water ecosystems of the world. *Environmental Conservation*. 29: 134-153.

Masese, F. O, Muchiri, M. and Raburu, P. O. (2010). A preliminary benthic macroinvertebrate index of biotic integrity (B-IBI) for monitoring the Moiben River, Lake Victoria, Kenya. *African Journal of Aquatic Science*. 34: 1-14.

Masese, F. O., Omukoto, J. O. and Nyakeya, K. (2013). Biomonitoring as a prerequisite for sustainable water resources: a review of status, opportunities and challenges to scaling up in East Africa. *Ecohydrology and Hydrobiology*. 13: 173-191.

Marshall, J. C., Steward, A. L. and Harch, B. D. (2006). Taxonomic resolution and quantification of freshwater macroinvertebrate samples from an Australian dryland river: Benefits and costs of using species abundance data. *Hydrobiologia.* 572 (1): 171-194.

Mason, C. F. and Parr, B. L. (2003). Long-term trends in water quality and their impact on macroinvertebrate assemblages in eutrophic lowland rivers. *Water Research*. 37: 2969-2979.

Mathooko, J. M. (2002). The sizes, maturity stages and biomass of mayfly assemblages colonizing disturbed streambed patches in Central Kenya. *African Journal of Ecology*. 40: 84-93.

Merritt, R. W., Cummins, K. W. and M. B. Berg, M. B. (2008). *An Introduction to the Aquatic Insects of North America*. 4th Edition. Kendall/Hunt Publishing Company, Dubuque, Iowa. 1158 pp.

Mereta, S., Boetsa, P., De Meesterc, L. and Goethalsa, P. L. M. (2013). Development of amultimetric index based on benthic macroinvertebrates for the assessment ofnatural wetlands in Southwest Ethiopia. *Ecological Indicators*. 29: 510-521.

McCaffrey, S. C. and Weber, G. S. (2005). Guideline for policy and legislative development on conservation and sustainable use of freshwater resources. United Nation Environmental Programme. Institute for Sustainable Development at Pacific/McGeorge University of the Pacific, McGeorge School of Law Sacramento, California, ISBN: 92-807-2566-1. 136 pp.

Mkuula, S. (1993). Pollution of wetlands in Tanzania. Unpublished report for National Environment Management, Dar es Salaam. 85 pp.

Mtetwa, S., Chipfunde, L., and Makwanise, R. (2002). Establishment of biomonitoring reference sites for Zimbabwe. A tool for effective integrated catchment management. Proceding of the 3rd WaterNet/WARFSA Symposium "Water Demand Management for Sustainable Development. Dar es Salam. 30-31 October, 2002. 8 pp.

Morris, C. C., Jawyer, J. A., Bennet, H. H. and Robinson, C. D. (2003). Effects of sediment quality on the health of aquatic ecosystem: A case study of depth of fines in coastal plain streams in Alabama. In: *Biological response signatures: indicator patterns using aquatic communities* (Edited by Simon, T. P.). Boca Raton CRC Press. pp. 113-120.

Morse, J. C., Bae, Y. J., Munkhjarkal, G., Sangpradub, N., Tanda, K., Tatyana, S. V., Wang, B., Yang, L. and Yule, C. M. (2007). Freshwater biomonitoring with macroinvertebrates in East Asia Frontiers. *Ecology and the Environment*.1: 33-42.

Mustow, S. E. (2002). Biological monitoring of rivers in Thailand: use and adaptation of the

BMWP score. *Hydrobiologia*. 479: 191-229.

Ndaruga, A. M, Ndiritu, G. G, Gichuki, N. N. and Wamich, W. N. (2004). Impact of water quality on macroinvertebrate assemblages along tropical stream in Kenya. *African Journal of Ecology*. 42: 208–216.

Ngana, J. O. (2001). Integrated water resources management. The case of the Pangani river basin. In: *Water resources management in the Pangani river basin: challenges and ppportunities*. (Edited by Ngana, J. O.). Dar es Salaam University Press, Dar es Salaam. pp. 1-8.

Ngupula, G. W. and Kayanda, R. (2010). Benthic macrofauna community composition, abundance and distribution in the Tanzania and Uganda inshore and offshore waters of Lake Victoria. *African Journal of Aquatic Science*. 35:185-192.

Ollis, D. J, Boucher, C., Dallas, H. F. and Esler, K. (2006). Preliminary testing of the Integrated Habitat Assessment System (IHAS) for aquatic macroinvertebrates. *African Journal of Aquatic Science.* 31(1): 1-14.

Palmer, C. G., Goetsch, P. A. and O'Keeffe, J. H. (1996). *Development of a recirculating artificial stream system to investigate the use of macroinvertebrates as water quality indicators*. WRC Report No. 475/1/96, Water Research Commission, Pretoria. 185 pp.

Palmer, R. W. and Taylor, E. D. (2004). The Namibian Scoring System (NASS) Version 2 rapid bioassessment method for rivers. *African Journal of Aquatic Science*. 29(2): 229-234.

Paunovic, M., Simic, V., Simonovic, P., Cvijan, M., Subakov, G., Simic, S., Stojanovic, B., Petrovic, A. and Gacic, Z. (2007). Biological quality elements in WFD implementation in Serbia: typology, reference conditions and ecological status class boundaries. Technical report, Institute for Biological Research, Beograd. pp. 1 - 209.

Persoone, G. D. and Jannsen, C. R. (1993). Freshwater invertebrate toxicity tests. In: *Handbook of Ecotoxicology*. (Edited by Calow P.) Blackwell Scientific Publications, Oxford. pp. 51 – 65.Pearson, R. G. and Boyero, L. (2009). Gradients in regional diversity of freshwater taxa. *Journal of the North American Benthological Society*. 28: 504-514.

Peckarsky, B. L., Fraissinet, P. R., Penton, M. A. and Conklin, Jr., D. J. (1990). Freshwater macroinvertebrates of Northeastern North America. Cornell University Press. ISBN: 0-8014-9688-8. 12442 pp.

Philips, S., Laanbroek, H. J. and Verstraete, W. (2001). Origin causes and effects of increased nitrite concentrations in aquatic environments. *Reviews in Environmental Science and Biotechnology*. 1: 115-41.

Pinto, P., Rosado, J., Morais, M. and Antunes, I. (2004). Assessment methodology for southern siliceous basins in Portugal. *Hydrobiologia*. 516(13): 191-214.

Primbas, A. (2005). *Save Our Stream.* http://www.saveourstream.org/ Accessed 30 May, 2015.

Pond, G. J., Call, S. C., Brumley, J. F. and Compton, M. C. (2003). The Kentucky macroinvertebrate bioassessment index: derivation of regional narrative criteria for headwater and wadeable streams. Kentucky Department for Environmental Protection, Division of Water, Frankfort. 53 pp.

Rae, J. G. (1989). Chironomid midges as indicators of organic pollution in the Scioto river basin, Ohio. *The Ohio Journal of Science*. 89(1): 5-9.

Ramírez, A., Pringle, C. M. and Wantzen, K. M. (2008). Tropical stream conservation. (Ed. D. Dudgeon) Academic Press, USA. pp. 285-300.

Rand, G. M. and Petrocelli, S. R. (1985). *Fundamentals of Aquatic Toxicology*. (New York: Hemisphere). 42 pp.

Richards, C., Havo, R. J., Johnson, L. B. and Host, G. E. (1997). Catchment and reach scale properties as indicator of macroinvertebrates species traits. *Freshwater Biology*. 37: 219-230.

Revenga, C. and Kura, Y. (2003). *Technical Series no. 11.* Secretariat of the Convention on Biological Diversity; Montreal, Canada: 2003. Status and trends of biodiversity of inland water ecosystems. 126 pp.

Rosenberg, D. M. and Resh, V. H. (editors) (1993). *Freshwater biomonitoring and benthic macroinvertebrates*. New York: Chapman and Hall. 488 pp.

Rosenberg, D. M, Reynoldson, T. B, and Resh, V. H. (1999). Establishing reference conditions for benthic invertebrate monitoring in the Fraser River catchment, British Columbia, Canada. Fraser River Action Plan, Environment Canada, Vancouver, B.C. FRAP Report No. DOE-FRAP 1998-32.

Rouse, J. D., Bishop, C. A. and Struger, J. (1999). Nitrogen pollution. An assessment of its threat to amphibian survival. *Environmental Health Perspectives*. 107: 799-803.

Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, R., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L. F., Jackson, R. B., Kinzig, A., Leemans, R., Lodge, D., Mooney, H. A., Oesterheld, M., Poff, N. L., Sykes, M. T., Walker, B. H., Walker, M. and Wall, D. H. (2000). Global biodiversity scenarios for the year 2100. *Science*. 287: 1770-1774.

Salomoni, S. E., Ocha, O. R. and Eite, E. H. L. (2007). Limnological characterization of Gravataí river, Rio Grande do Sul. Acta: *Limnologica Brasiliensia*. 19(1): 1-14.

Sandin, L. and Johnson, R. K. (2000). The statistical power of selected indicator metrics using macroinvertebrates for assessing acidification and eutrophication of running waters. *Hydrobiologia*. 422/423: 233-243.

Seaby, R. M. and Henderson, P. A. (2006). Species diversity and Richness Package 4. Limington, England: Pisces Conservation Ltd. 123 pp.

Scheffer, M. and Carpenter, S. R. (2003). Catastrophic regime shifts in ecosystems: linking theory to observation. *Trends in Ecology and Evolution*. 18(12): 648-656.

Schmidt-Kloiber, A. and Nijboer, R. C. (2004). The effect of taxonomic resolution on the assessment of ecological water quality classes. *Hydrobiologia*. 516: 269-283.

Sharma, K. K. and Chowdhury, S. (2011). Macroinvertebrate assemblages as biological indicators of pollution in a Central Himalaya River, Tawi. *International Journal of Biodiversity Conservation.* 3: 167-174.

Shelly, S. Y., Mirza, Z. B. and Bashir, S. (2011). Comparative ecological study of aquatic macroinvertebrates of Mangla dam and Chashma barrage wetland areas. *Journal of Animal and Plant Science*. 21: 340-350.

Simic, V. (1999). Use of River macroinvertebrates of a Serbia River to formulate a biotic index. *Hydrobiologia*. 16: 43-51.

Simpson, J. C. and Norris, R. H. (2000). Biological assessment of river quality: development of AUSRIVAS models and outputs. In: *Assessing the biological quality of freshwaters: RIVPACS and other techniques* (Edited by Wright, J. F, Sutcliffe, D. W. and Furse, M. T.). Freshwater Biological Association, United Kingdom. pp.125 – 142.

Sitotaw, B. (2006). Assessment of benthic macroinvertebrate structures in relation to environmental degradation in some Ethiopian rivers. Unpublished Thesis for Award of MSc Degree at University of Addis Ababa, Ethiopia. 127 pp.

Song, M. Y., Leprieur, F., Thomas, A., Lek-Ang, S., Chon, T. S. and Lek, S. (2009). Impact of agricultural land use on aquatic insect assemblages in the Garonne river catchment (SW France). *Aquatic Ecology*. 43: 999-1009.

Smolders, A. J. P., Lock, R. A. C, Van der Velde, G., Medina Hoyos, R. I. and Roelofs. (2003). Effects of mining activities on heavy metal concentrations in water, sediment and macroinvertebrates in different reaches of the Pilcomayo River, South America. *Archives of Environmental Contamination and Toxicology*. 44(3): 314-323.

Stals, R. and De Moor, I. J. (editors) (2007). *Guides to the freshwater invertebrates of Southern Africa: Volume 7 Insecta I - Ephemeroptera, Odonata and Plecoptera*. WRC Report no. TT 320/07, Water Research Commission, Pretoria. 263 pp.

Standard Methods (1992). *Standard methods for the examination of water and wastewater* (18th Edition). American Public Health Association, Washington, DC.

Stone, M. K. and Wallace, J. B. (1998). Long-term recovery of a mountain stream from clear-cut logging: the effects of forest succession on benthic community structure. *Freshwater Biology*. 39: 151-169.

Stone, M. L., Whiles, M. R. and Reeve, J. D. (2005). Macroinvertebrate communities in agriculturally impacted southern Illinois streams: patterns with riparian vegetation, water quality, and in-stream habitat quality. *Journal of Environmental Quality*. 34 (3): 907 - 917.

Swarthout, R. (2003). "Impact of deforestation on benthic macroinvertebrate communities in tributaries of Lake Tanganyika, East Africa." *The Nyanza Project, 2003 Annual Report*. University of Arizona. pp. 63-67.

Suleiman, K and Abdullahi, I. L. (2011). Biological assessment of water quality: A study of Challawa river water in Kano, Nigeria. *Bayero Journal of Pure and Applied Sciences*. 4(2): 121-127.

Tesfaye Berhe. (1988). The Degradation of the Abo- Kebena River in Addis Ababa. Ethiopia. Unpublished Thesis for Award of MSc. Degree at Addis Ababa University. 102 pp.

Tiller, D., and Metzeling, L. (2002). Australia wide assessment of river health: Victorian AusRivAS sampling and processing manual. Monitoring river heath initiative technical report no. 15. Commonwealth of Australia and VIC Environment Protection Authority, Canberra and Melbourne. 26 pp.

Tolkamp, H. H. and Gardiener, J. J. P. (1977). Hydro-biological survey of lowland streams in the Achterhoek (The Netherlands) by means of a system for the assessment of water quality and stream character based on macroinvertebrates. *Hannover*. 41: 215-235.

Thieme, M. L, Abe, R., Stianssny, M. L. J, Skelton, P., Lehner, B., Teugels, G. G., Dinerstein, E., Toham, A. K., Burgess, N. and Olson, D. (2005). Freshwater ecoregions of Africa and Madagascar. A conservation assessment. Island Press. 483 pp.

Thorn, J. H. and Covich, A. P. (1991). *Ecology and Classification of North American Freshwater Invertebrates*, Academic Press, San Diego, California, USA. 1056 pp.

Tumiwesigye, C.; Yusuf, S.K. and Makanga, B. (2000). Structure and composition of benthic macroinvertebrate of a tropical forest stream, River Nyamweru, western Uganda. *African Journal of Ecology*. 38: 72-77.

Umar, D. M., Harding J. S. and Winterbourn, M. J. (2013). *Freshwater Invertebrates of the Mambilla Plateau, Nigeria*. Gombe State University and University of Canterbury. 88 pp.

USEPA (2002). Methods for evaluating wetland condition: Developing an invertebrate index of biological integrity for wetlands. United States Environmental Protection Agency, Office of Water, Washington, DC, USA. EPA-822-R-02-019.

Verdonschot, P. F. M. (2006). Data Composition and Taxonomic Resolution in Macroinvertebrate Stream Typology. *Hydrobiologia.* 566: 59-74.

Verhoeven, J. T. A., Arheimer, A., Yin, C. and Hefting, M. M. (2006). Regional and global concerns over wetlands and water quality. *Trends in Ecology and Evolution*, 21: 96-203.

Verdonschot, R. C. M., Keizer-Vlek, H. E. and Verdonschot, P. F. M. (2012). Development of a multimetric index based on macroinvertebrates for drainage ditch networks in agricultural areas. *Ecological Indicators*. 13: 232-242.

Wente S. P. (2000). Proximity-based measure of land use impacts to aquatic ecosystem integrity. *Environmental Toxicology and Chemistry.* 19: 1148-1152.

Wetzel, R. G. (2001). Limnology. 3rd Edition. New York: Academic Press. 1006 pp.

Wetzel, R. G. and Linkens, G. (2000). Limnological analyses. Springer (India) publisher private limited, New Delhi, India. 426 pp.

Whittier, T. R., Stoddard, J. L., Larsen, D. P. and Herlihy, A. T. (2007). Selecting reference sites for stream biological assessments: best professional judgment or objective criteria. *Journal of the North American Benthological Society*. 26: 349-360.

WHO (2000). Guidelines for drinking water quality. World Health Publication. NY, USA.

Wright, J. F. (1994). Development of RIVPACS in the UK and the value of the underlying data base. *Limnética*. 10(1): 15-31.

Wright, J. F, Moss, D., Armitage, P. D. and Furse, M. T. (1984). A preliminary classification of running-water sites in Great Britain based on macroinvertebrate species and prediction of community type using environmental data. *Freshwater Biology*. 14:

221-256.

Wente S. P. (2000). Proximity-based measure of land use impacts to aquatic ecosystem integrity. *Environmental Toxicology and Chemistry.* 19:1148-1152.

Yuan, L. L. (2004). Assigning macroinvertebrate tolerance classifications using generalized additive models. *Freshwater Biology*. 49: 662-677.

Zedler, J. B. and Kercher, S. (2005). Wetland resources: status, trends, ecosystem services, and restorability. *Annual Review of Environment and Resources*. 30: 39-74.

Zamora-Muñoz, C. and Alba-Tercedor, J. (1996). Bioassessment of organically polluted Spanish rivers, using a biotic index and multivariate methods. *Journal of the North American Benthological Society*. 15(3): 332-352.

APPENDICES

Appendix 1: Macroinvertebrate communities collected based on major site categories

TAXA	PANGANI SITES				WAMI-RUVU SITES				TOTAL	
	REFERENCE		MONITORING		REFERENCE		MONITORING		ALL SITES	
	Abundance	%	Abundance	%	Abundance	%	Abundance	%	Abundance	%
Hirudinidae	**0**	**0.0**	**8**	**0.15**	**0**	**0.0**	**6**	**0.17**	14	0.11
Glossiphoniidae	**0**	**0.0**	**14**	**0.27**	**0**	**0.0**	**5**	**0.14**	19	0.15
Naididae/Tubificidae	0	0.0	50	0.96	**0**	**0.0**	**9**	**0.25**	59	0.47
Dytiscidae	31	1.26	82	1.57	17	1.23	99	2.41	229**	1.81
Dryopidae/Elmidae	9	0.36	115	2.20	12	0.87	96	2.71	232**	1.84
Gyrinidae	64	2.59	50	0.96	0	0.0	47	1.33	161*	1.27
Haliplidae	2	0.08	30	0.57	0	0.0	52	1.47	84	0.67
Hydraenidae	11	0.45	28	0.54	0	0.0	30	0.85	69	0.55
Hydrophilidae	30	1.2	43	0.82	0	0.0	58	1.64	131*	1.04
Limnichidae	12	0.49	22	0.42	**0**	**0.0**	**0**	**0.0**	34	0.27
Psephenidae	28	1.13	55	1.05	26	1.88	45	1.27	154*	1.22
Scirtidae	5	0.20	34	0.65	0	0.0	22	0.62	61	0.48
Amphipoda	**0**	**0.0**	**5**	**0.10**	2	0.14	22	0.62	29	0.23
Atyidae	0	0.0	17	0.33	0	0.0	16	0.45	33	0.26
Palaemonidae	**0**	**0.0**	**15**	**0.29**	2	0.14	29	0.82	46	0.36
Potamonautidae	59	2.39	209	4.00	19	1.37	45	1.27	332**	2.63

Athericidae	55	2.23	132	2.53	31	2.24	22	0.62	240**	1.90
Ceratopogonidae	14	0.57	183	3.50	0	0.0	43	1.21	240**	1.90
Chironomidae	68	2.75	1455	27.84	45	3.25	639	18.02	2207***	17.48
Culicidae	0	0.0	57	1.09	0	0.0	52	1.47	109*	0.86
Dixidae	62	2.51	31	0.59	7	0.51	45	1.27	145*	1.15
Ephydridae	**3**	**0.12**	**14**	**0.27**	**0**	**0.0**	**5**	**0.14**	22	0.17
Muscidae	0	0.0	31	0.59	0	0.0	11	0.31	42	0.33
Simuliidae	17	0.69	384	7.35	0	0.0	137	3.86	538**	4.26
Tabanidae	34	1.38	84	1.61	17	1.23	50	1.41	185*	1.46
Tipulidae	38	1.54	51	0.98	22	1.59	59	1.66	170*	1.35
Baetidae	751	30.40	470	8.99	235	16.96	227	6.40	1683***	13.33
Caenidae	115	4.66	266	5.09	26	1.88	128	3.61	535**	4.24
Dicercormyzidae	19	0.77	28	0.54	36	2.60	30	0.85	113*	0.89
Ephemerythidae	8	0.32	15	0.29	22	0.62	52	1.47	97	0.77
Heptageniidae	41	1.66	45	0.86	29	2.09	79	2.23	194*	1.54
Leptophlebiidae	29	1.17	64	1.22	34	2.45	66	1.86	193*	1.53
Oligoneuridae	62	2.51	18	0.34	82	5.92	12	0.34	174*	1.38
Polymitarcyidae	38	1.54	6	0.11	26	1.88	0	0.0	70	0.55
Prosopistomatidae	57	2.31	16	0.31	45	3.25	41	1.16	159*	1.26
Tricorythidae	10	0.40	5	0.10	23	1.66	32	0.90	70	0.55
Lymnaeidae	**0**	**0.0**	**12**	**0.23**	0	0.0	25	0.71	37	0.29
Physidae	0	0.0	22	0.42	0	0.0	19	0.54	41	0.32
Planorbidae	**0**	**0.0**	**11**	**0.21**	0	0.0	18	0.51	29	0.23
Thiaridae	**0**	**0.0**	**9**	**0.17**	0	0.0	28	0.79	37	0.29
Belastomatidae	17	0.69	0	0.0	24	1.73	8	0.23	49	0.39

Corixidae	6	0.24	152	2.39	10	0.72	0	0.0	168*	1.33
Gerridae	9	0.36	43	0.82	0	0.0	19	0.54	71	0.56
Hydrometridae	6	0.24	14	0.27	**0**	**0.0**	**10**	**0.28**	30	0.24
Naucoridae	14	0.57	65	1.24	22	1.59	90	2.54	191*	1.51
Nepidae	9	0.36	13	0.25	**0**	**0.0**	**0**	**0.0**	22	0.17
Notonectidae	13	0.53	113	2.16	**0**	**0.0**	**0**	**0.0**	126*	1.0
Pleidae	**5**	**0.20**	**5**	**0.10**	**0**	**0.0**	**4**	**0.11**	14	0.11
Veliidae	20	0.81	64	1.22	12	0.87	71	2.00	167*	1.32
Hydridae	**0**	**0.0**	**4**	**0.08**	**0**	**0.0**	**8**	**0.23**	12	0.10
Pyralidae	**5**	**0.20**	**1**	**0.02**	**2**	**0.14**	**3**	**0.08**	11	0.09
Aeshnidae	43	1.74	102	1.95	33	2.38	80	2.26	258**	2.04
Calopterygidae	57	2.31	31	0.59	37	2.67	83	2.34	208**	1.65
Chlorocyphidae	28	1.13	13	0.25	22	1.59	71	2.00	134*	1.06
Chlorolestidae	**0**	**0.0**	**7**	**0.13**	6	0.43	11	0.31	24	0.19
Coenagrionidae	32	1.30	92	1.76	20	1.44	86	2.43	230**	1.82
Corduliidae	5	0.20	38	0.73	13	0.94	56	1.58	112*	0.89
Gomphidae	33	1.34	68	1.30	12	0.87	42	1.18	155*	1.23
Lestidae	**0**	**0.0**	**12**	**0.23**	15	1.08	47	1.33	74	0.59
Libellulidae	0	0.0	28	0.54	29	2.09	52	1.47	109*	0.86
Macromiidae	0	0.0	18	0.34	**0**	**0.0**	**8**	**0.23**	26	0.21
Perlidae	19	0.77	0	0.0	27	1.95	13	0.37	59	0.47
Turbellaria	**0**	**0.0**	**13**	**0.25**	0	0.0	14	0.39	27	0.21

Corbiculidae	**0**	**0.0**	**3**	**0.04**	**0**	**0.0**	**1**	**0.03**	4	0.03
Sphaeriidae	**0**	**0.0**	**4**	**0.08**	**0**	**0.0**	**1**	**0.03**	5	0.04
Unionidae	**0**	**0.0**	**1**	**0.02**	**0**	**0.0**	**1**	**0.03**	2	0.02
Corydalidae	**4**	**0.16**	**2**	**0.04**	0	0.0	6	0.17	12	0.10
Sialidae	**3**	**0.12**	**1**	**0.02**	6	0.43	3	0.08	13	0.10
Calamoceratidae	112	4.53	5	0.01	58	4.18	38	1.07	213**	1.74
Dipseudopsidae	16	0.65	4	0.08	34	2.45	32	0.90	86	0.68
Ecnomidae	21	0.85	11	0.21	6	0.43	32	0.90	70	0.55
Hydroptilidae	13	0.53	9	0.17	3	0.22	23	0.65	48	0.38
Hydropsychidae	55	0.22	32	0.61	56	0.40	31	0.87	174*	1.38
Lepidostomatidae	20	0.81	0	0.0	40	2.89	35	0.99	95	0.75
Leptoceridae	59	2.39	19	0.36	6	0.43	27	0.76	111*	0.88
Philopotamidae	69	2.79	19	0.36	52	3.75	32	0.90	172*	1.36
Phryganeidae	25	1.01	4	0.08	20	1.44	45	1.27	94	0.74
Polycentropodidae	58	2.35	4	0.08	54	3.90	34	0.96	150*	1.19
Psychomyiidae	22	0.89	27	0.52	9	0.65	28	0.79	86	0.68
TOTAL	**2470**		**5227**		**1386**		**3546**		**12629**	**100.0**

Appendix 2: List of 53 EDOT taxa observed in Tanzanian river basins, 2014-2015

S/N.	ORDER	TAXA NAME	NUMBER OF TAXA	
			Pangani	Wami-Ruvu
1.	Arhynchobdellida	Hirudinidae	1	1
2.	Coleoptera	Dytiscidae, Dryopidae/Elmidae, Gyrinidae, Haliplidae, Hydraenidae, Hydrophilidae, Limnichidae, Psephenidae & Scirtidae	9	9
3.	Decapoda	Amphipoda, Atyidae, Palaemonidae & Potamonautidae	4	4
4.	Diptera	Athericidae, Blephariceridae, Ceratopogonidae, Chironomidae, Culicidae, Dixidae, Empididae, Ephydridae, Muscidae, Psychodidae, Simuliidae, Syrphidae, Tabanidae, Tipulidae	14	14
5.	Ephemeroptera	Baetidae 1sp, Baetidae 2sp, Baetidae >2sp, Caenidae, Dicercormyzidae, Ephemeridae, Ephemerythidae, Heptageniidae, Leptophlebiidae, Oligoneuridae, Polymitarcyidae, Prosopistomatidae & Tricorythidae	13	13
6.	Gastropoda	Ancylidae, Bulininae, Hydrobiidae, Lymnaeidae, Neritidae, Physidae, Planorbidae, Thiaridae & Viviparidae	9	8
7.	Hemiptera	Belastomatidae, Corixidae, Gerridae, Hydrometridae, Naucoridae, Nepidae, Notonectidae, Pleidae & Veliidae	9	9
8.	Hydroida (Hydra)	Hydridae	1	1
9.	Lepidoptera	Phyralidae	1	1
10.	Megaloptera	Corydalidae & Sialidae	2	2
11	Odonata	Aeshnidae, Calopterygidae, Chlorocyphidae,	12	12

		Chlorolestidae, Coenagrionidae, Corduliidae, Gomphidae, Lestidae, Libellulidae, Macromiidae, Platycnemidae & Protoneuridae		
12.	Pelecypoda	Corbiculidae, Sphaeriidae & Unionidae	3	3
13.	Plecoptera	Perlidae & Notonemouridae	2	2
14.	Rhynchobdellida	Glossiphoniidae	1	1
15.	Tubificida	Tubificidae	1	1
16.	Turbellaria	Turbellaria	1	1
17.	Trichoptera	Calamoceratidae, Dipseudopsidae, Ecnomidae, Hydroptilidae, Hydropsychidae 1 sp, Hydropsychidae 2 spp, Hydropsychidae > 2 spp, Lepidostomatidae, Leptoceridae, Philopotamidae, Phryganeidae, Polycentropodidae, Pisuliidae & Psychomyiidae	14	14
	TOTAL		**53**	**52**

Appendix 3: Relative frequency of occurrence (%) of EDOT taxon in Tanzanian rivers

MACRO-INVERTEBRATE COMMUNITIES		PANGANI RIVER BASIN		WAMI-RUVU RIVER BASIN	
ORDER	TAXA	REFERENCE SITES	MONITORING SITES	REFERENCE SITES	MONITORING SITES
EPHEMEROPTERA	Baetidae 1 sp.	2.84	39.80	2.61	18.04
	Baetidae 2 spp.	15.53	9.98	2.90	21.00
	Baetidae > 2 spp	48.27	2.55	35.94	1.60
	Caenidae	10.20	29.49	10.72	18.26
	Dicercormyzidae	0.53	0.33	2.46	0.46
	Ephemeridae	0.71	0.33	0.72	0.68
	Ephemerythidae	0.89	0.44	1.74	0.46
	Heptageniidae	3.64	4.99	6.23	14.84
	Leptophlebiidae	2.57	7.10	7.54	10.96
	Oligoneuridae	5.50	2.00	12.32	2.05
	Polymitarcyidae	3.37	0.67	3.77	0.00
	Prosopistomatidae	5.06	1.77	7.97	7.08
	Tricorythidae	0.89	0.55	5.07	4.57
DIPTERA	Athericidae	18.27	5.40	9.84	1.41
	Blephariceridae	3.32	0.00	2.34	0.00
	Ceratopogonidae	4.65	7.49	4.45	3.08
	Chironomidae	22.59	59.53	48.71	61.18
	Culicidae	0.00	2.33	4.22	4.37
	Dixidae	20.60	1.27	3.75	4.63
	Empididae	0.00	0.29	0.00	0.64
	Ephydridae	1.00	0.57	1.17	0.00
	Muscidae	0.00	1.27	1.41	0.64
	Psychodidae	0.00	0.33	0.23	0.26
	Simuliidae	5.65	15.71	8.90	12.72
	Syrphidae	0.00	0.29	0.23	0.13
	Tabanidae	11.30	3.44	7.73	4.37
	Tipulidae	12.62	2.09	7.03	6.56
ODONATA	Aeshnidae	20.67	24.46	15.36	15.34
	Calopterygidae	27.40	7.43	21.88	10.23
	Chlorocyphidae	13.46	3.12	13.54	11.65
	Chlorolestidae	0.00	1.68	3.13	1.42
	Coenagrionidae	15.38	22.06	8.59	20.74
	Corduliidae	2.40	9.11	8.85	9.94
	Gomphidae	15.87	16.31	3.65	11.36
	Lestidae	0.00	2.88	9.11	7.67
	Libellulidae	0.00	6.71	11.98	9.94
	Macromiidae	0.00	4.32	0.78	0.57
	Platycnemidae	2.88	0.48	1.56	0.00
	Protoneuridae	1.92	1.44	1.56	1.14
TRICHOPTERA	Calamoceratidae	23.24	4.13	16.41	8.70
	Dipseudopsidae	3.32	3.31	9.98	8.30
	Ecnomidae	4.36	9.09	3.10	9.49
	Hydroptilidae	2.70	7.44	2.88	5.14
	Hydropsychidae 1 sp	0.41	15.70	1.11	7.51
	Hydropsychidae 2 spp	3.53	9.92	4.43	2.37
	Hydropsychidae > 2 spp	7.47	0.83	8.20	0.40
	Lepidostomatidae	4.15	0.00	10.20	11.46
	Leptoceridae	12.24	15.70	3.33	7.11
	Philopotamidae	14.32	15.70	13.30	9.49
	Phryganeidae	6.22	4.13	8.20	13.04
	Polycentropodidae	12.03	3.31	14.41	9.09
	Pisuliidae	1.45	5.79	0.44	0.40
	Psychomyiidae	4.56	4.96	3.99	7.51