DIVERSITY AND DISTRIBUTION PATTERNS OF FRESHWATER MACROINVERTEBRATES IN MODI RIVER SYSTEM, GANDAKI PROVINCE, NEPAL

A Dissertation Submitted for

The partial fulfillment of the Requirement for the Degree of Masters of Science in Biodiversity and Environmental Management (BEM)

Submitted BY

BABITA GHIMIRE

TU Regd. No.:- 2-2-523-2-2009

Exam Roll No. BEM 203/073

Submitted to

Central Department of Botany

Tribhuvan University, Kirtipur, Kathmandu, Nepal

July 2022

DECLARATION

Date: July 15, 2022

I hereby declare that the work presented in this dissertation is a genuine work done originally by me and has not been submitted anywhere for the award of any degree. All the sources of information have been specifically acknowledged by reference to author(s) or institution(s).

………………

Babita Ghimire

TRIBHUVAN UNIVERSITY Tel No: 01-4332147

Central Department of Environmental Science

Kirtipur, Kathmandu, Nepal

Date: July 15, 2022

RECOMMENDATION

This is to certify that **Ms. Babita Ghimire** has completed this dissertation entitled **"Diversity and Distribution Patterns of Freshwater Macro-invertebrates in Modi River System, Gandaki Province, Nepal"** as a partial fulfillment of the requirements of M.Sc. in Biodiversity and Environmental Management under my supervision and guidance. To my knowledge, this research has not been submitted for any other degree, anywhere else.

I therefore, recommend the dissertation for acceptance and approval.

…………...............

Supervisor

Deep Narayan Shah, PhD

(Assistant Professor)

Central Department of Environmental Science

Tribhuvan University

Date: July 15, 2022

RECOMMENDATION

This is to certify that **Ms. Babita Ghimire** has completed this dissertation entitled **"Diversity and Distribution Patterns of Freshwater Macro-invertebrates in Modi River System, Gandaki Province, Nepal"** as a partial fulfillment of the requirements of M.Sc. in Biodiversity and Environmental Management under my co-supervision and guidance. To my knowledge, this research has not been submitted for any other degree, anywhere else.

I therefore, recommend the dissertation for acceptance and approval.

…………...............................

Co-supervisor

Narayan Ghimire, PhD

(Assistant Professor)

Central Department of Botany

Tribhuvan University

Date: July 15, 2022

LETTER OF APPROVAL

On the recommendation of supervisor Asst. Prof. Deep Narayan Shah (PhD), this dissertation submitted by **Ms. Babita Ghimire** entitled **"Diversity and Distribution Patterns of Freshwater Macro-invertebrates in Modi River System, Gandaki Province, Nepal"** has been approved for the examination and submitted to the Tribhuvan University in partial fulfillment of the requirements of M.Sc. in Biodiversity and Environmental Management

……………………………………

Prof. Dr. Suresh Kumar Ghimire

Acting Head of Department

Central Department of Botany

Tribhuvan University

Date: April 26, 2022

CERTIFICATE OF ACCEPTANCE

This dissertation entitled **"Diversity and Distribution Patterns of Freshwater Macro-invertebrates in Modi River System, Gandaki Province, Nepal"** has been examined and accepted as a partial fulfillment of the requirements of M.Sc. in Biodiversity and Environmental Management.

…………………………….

Deep Narayan Shah, PhD
Supervisor
Assistant Professor
Central Department of Environmental Science

……………………………..

Narayan Ghimire, PhD
Co-supervisor
Assistant Professor
Central Department of Botany

………………………..

Yadhav Upreti, PhD
Internal Examiner
Associate Professor
Central Department of Botany

………………………

Ramesh Raj Pant, PhD
External Examiner
Assistant Professor
Central Department of Environment Science

…………………………………….

Prof. Dr. Suresh Kumar Ghimire
Acting Head of Department
Central Department of Botany
Tribhuvan University
Kirtipur, Kathmandu, Nepal

ACKNOWLEDGEMENTS

I would like to acknowledge Central Department of Botany, Tribhuvan University for providing me an opportunity to carry out this dissertation. I would like to express sincere gratitude to my supervisor Dr. Deep Narayan Shah, Assistant Professor, Central Department of Environmental Science who constantly guided me during my entire dissertation. Similarly, I am thankful to my co-supervisor Dr. Narayan Ghimire, Assistant Professor, Central Department of Botany for his guidance during this research.

I am also thankful to Prof. Dr. Ram Kailash Prasad Yadav, Head of Central Department of Botany, Prof, Dr. Mohan Siwakoti, Former Head of Department, Prof. Dr. Sangeeta Rajbhandari, Former Coordinator of BEM and Prof. Dr. Suresh Kumar Ghimire, coordinator of BEM, Tribhuvan University for their academic and administrative support to conduct this research. I am also grateful to Dr. Ram Devi Tachamo Shah, Assistant Professor, Department of Life Science, Kathmandu University and Prof. Dr. Prem Bahadur Budha, Central Department of Zoology, Tribhuvan University for their help during proposal development and early stage of research. I am also grateful to Mr. Anil Chaudhari for supporting in GIS analysis.

Likewise, I would like to thank Ms. Kritika Shrestha for supporting me in field work, Mr. Ramesh Basnet in lab work, Ms. Bhumika Thapa and Sunita Thapa for their support and Mr. Mahesh Raj Bista and Mr. Basu Dev Poudel in data analysis.

Furthermore, I would like to thank Centre for Environment, Energy and Water Research (CREEW), WWF Nepal and University Grant Commission for the financial support to conduct this research. I would like to acknowledge Department of National Park and Wildlife Conservation (DNPWC) and National Trust for Nature Conservation/Annapurna Conservation Area Project (NTNC/ACAP) for providing research permit.

Finally, I am deeply indebted to my family members for their continuous support and inspiration for all the journeys of life.

Babita Ghimire

ABSTRACT

In the face of several stressors in Modi River system, this research was conducted with the aim of assessing diversity and distribution patterns of benthic macroinvertebrates along the altitudinal gradient including the ecological health of a River system. Benthic macroinvertebrates sampling was conducted in 20 different (13-river's mainstem and 7- tributaries) following a multi-habitat sampling approach based on Moog (2007). Macroinvertebrates were collected by kicking the substrate or jabbing with a D-frame dip net and transferred into labeled vials and preserved in 99.9% ethanol for sorting and identification in the lab. Threats were identified from direct observation and key informant interview. Taxa richness was assessed using Shannon Diversity Index, Simpson Diversity Index, and Evenness. Similarly, Shapiro-Wilk normality test, One way ANOVA, Turkey HSD test and Wilcoxon pair test were conducted to know the difference between groups. R studio (1.2.5 version) and CANOCO (4.5 version) was used for statistical analysis. In total, 40 families belonging to 10 orders were recorded in the study area. 29 families belonging to eight orders were recorded in river's mainstem while 39 families belonging to 9 orders were recorded in tributaries. Lowest numbers of taxa were recorded at higher elevations of both river (9 families at 1654m) and tributaries (14 families at 1719m). Among 40 families, order Diptera has the highest family richness. Turkey HSD test showed taxa richness between 1550-1800m and 1050-1300m (p=0.0004), and 1550-1800m and 1350-1500m (p=0.0389) differ significantly. Taxa richness is highest at the elevation of 1050-1300m and lowest at 1550-1800m. Similarly, Shannon Diversity index is highest in the sampling sites located at 1265m elevation and lowest at 1533m. Test Showed that the diversity index differs significantly (p=0.049) with altitude. Wilcoxon pair test did not show the difference in the Simpsons Diversity Index between river's mainstem and tributaries (p=0.195). Likewise, RDA diagram showed the temperature as the most important variable governing the BMI composition . Furthermore, river's mainstem are less polluted than the tributaries. Hydropower construction, sewage, sand and stone quarry, waste dumping, bathing and washing were the major threats for the river system.

Keywords: Benthic macroinvertebrates, freshwater, diversity, distribution, stressors

ABBREVIATIONS AND ACRONYMS

ACA: Annapurna Conservation Area

ACAP: Annapurna Conservation Area Project

ANOVA: Analysis of Variance

BMI: Benthic Macroinvertebrate

BOD: Biological Oxygen Demand

CO_2: Carbon Dioxide

COD: Chemical Oxygen Demand

DNPWC: Department of National Park and Wildlife Conservation

DO: Dissolved Oxygen

EPT: Ephemeroptera, Plecoptera and Trichoptera

GIS: Geographic Information System

NO_2: Nitrite

NO_3^-: Nitrate

NTNC: National Trust for Nature Conservation

O_2: Oxygen

pH: Potential of Hydrogen

PO_4^{3-}: Orthophophate

RDA: Redundancy Analysis

WWF: World Wide Fund for Nature

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I: INTRODUCTION

1.1 Background

Nepal is rich in freshwater resources with more than 6,000 rivers and rivulets (Shrestha 1990, Jha et al. 2010). The rivers range from glacial fed rivers originating from the Himalayan glaciers to rain-fed and spring originating from the Mahabharata and the Siwalik ranges (WECS 2011). Most of the rivers in Nepal are snow fed (Sharma et al. 2008). Rivers are the source of drinking water, irrigation, hydroelectricity generation and also add the natural beauty of the country. These water resources are the key strategic natural resources with the potential to act as the catalyst for the all-round development and economic growth of the country (WECS 2011).

River systems are the zone of earth's highest biological diversity (Tachamo Shah 2018). It harbors diverse aquatic communities like: invertebrates, fish, phyto planktons, periphytons, waterfowl, reptiles, mammals and amphibians. Despite various uses and benefits of river water, the integrity of water quality is being degraded due to various stressors such as: agricultural intensification, organic pollution, flow modification, overexploitation of resources, and invasion by exotic species, eutrophication and river bed extraction (Dudgeon et al. 2006). Thereafter, freshwater ecosystems are considered as most threatened ecosystems on earth. Climate change may further add to the severity of these threats (Allen et al. 2010). Ecosystem change through land use are considered as one of the major threat to freshwater diversity (Sala et al. 2000) and land use can have a wide variety of influence that directly or indirectly affect rivers. Loss of aquatic biodiversity reduces the efficiency of ecological communities to capturing essential resources, producing biomass, decomposing, and recycling essential nutrients. Similarly biodiversity loss reduces the ability to stabilize ecosystem functions throughout time (Cardinale et al. 2012).

Benthic macroinvertebrates are the bottom dwelling invertebrates which lacks backbone and can be seen with naked eyes. Macroinvertebrates represent a diverse group of long living sedentary species that react strongly and often predictable to human influences on aquatic systems (Cairns and Prall 1993, Rosenberg and race

1993, Prommi and Pakkaya 2015). These includes: Insects, Crustaceans, Annelids, Mollusca and Leeches. Macroinvertebrates spend their life in water for the most part, so their survival is directly related to the quality of water. The diversity and abundance of benthic maroinvertebrates alter with the changes in the quality of river ecosystem. Macroinvertebrates in rivers generally have life cycles of months up to more than one year and are therefore exposed to pollutants over long periods of time (Nieto et al. 2017). These are sensitive to changes in conditions such as precipitation, temperature and the associated flow regimes; hence they provide good indication of environmental change (Bunn and Arthington 2002, Lytle and Poff 2004, Tachamo Shah 2012, 2018). The tolerance level of macroinvertebrates to pollution/stressors varies according to classes. Some are sensitive to pollution level while others are tolerant to the pollution. Pollution sensitive organisms such as Ephemeroptera, Plecoptera, and Trichoptera are more susceptible to the effects of physical or chemical changes in a stream than other organisms. The dominance of these organisms act as indicator of the absence of pollutants. Pollution-tolerant organisms such as Diptera and Oligochaeta are less susceptible to the effects of physical or chemical changes in a stream than other organisms. The presence or absence of such organisms is an indirect measure of pollution (Sharma et al. 2009). Based on the tolerance of the benthic macroinvertebrate taxa to pollution gradients, tolerance scores (e.g. Ganga River System biotic score–GRS bios) are assigned to individual taxa, providing a biotic index for assessing the ecological river quality (Tachamo Shah and Shah, 2013).

Numerous researches have been conducted regarding the water quality assessment using physico-chemical parameters and biological indicators in different rivers of Nepal (Shah 2014, Sharma 1999, Rana and Chhetri 2015, Matangulu 2017). Water quality is important factor for drinking, beautification and understanding of spatio-temporal dynamics of aquatic organisms. The physico-chemical characteristics of water are of great significances for the survival of aquatic life. It is characterized by climatic, geochemical, geomorphologic and pollution level. Biological indicators are often considered advantageous over physic chemical approach as biota provides information on ecological status of the riverine ecosystem (Lie et al. 2010).

Among various biological indicator such as benthic macroinvertebrates, algae, periphyton, macrophytes and fish, benthic macroinvertebrates are considered to be highly suitable for assessing the quality of water. Hence, they are used in numerous bioassessment studies (Rosenberg and Resh 1993, Tachamo Shah and Shah 2013, Xu et al. 2014). It provides more reliable assessment of long term ecological changes in the condition of aquatic system than the physico-chemical measurement (Moog et al. 2008, Tachamo Shah 2013).

Within river systems, the distribution of biota largely is determined by the physical environment and by routes of dispersal (Downes and Keough 1998, Mac Nally et al. 2006). At larger geographic scales, biological assemblages may change along the length of a river in response to longitudinal changes in physical habitat variables, such as discharge, channel width and benthic sediments (Naiman et al. 1987, Rice et al. 2008). Mountain Rivers and their tributaries exhibit tremendous variation across spatio-temporal scales attributed to differences in their origin, tectonics, watershed geology and size, landuse, connectivity, hydrology etc. (Wohl 2010). These differences in turn result in a range of different river physical and chemical parameters and aquatic biota (Gurung 2021). Their community structures differ along the longitudinal gradient of lotic systems, reflecting the difference in abiotic variables such as temperature (Suren 1994), flow (LeCraw and Mackereth, 2010), and available food sources (Mantyka et al. 2014). Likewise, their abundance and distribution differs with the river's mainstem and stream size. In the river’s mainstem, macroinvertebrate abundances is evenly distributed among different functional feeding groups (Heino et al. 2005, Tachamo Shah, 2020). In general, the abundance of the macroinvertebrates increases with increasing stream size, and some species indicated restricted distribution only in the tributaries (Heino et al. 2005, Tachamo Shah, 2020).

Nepal is in the face of rapid development of infrastructure, hydroelectricity and urbanization. Urban areas are crowded with population day by day. Therefore, multiple threats to aquatic biodiversity have been increasing with development and population pressure. But, aquatic conservation science is still lacking in quality and quantity compared with terrestrial ecosystem for conservation and planning (Abell 2002).

This study was conducted in Modi River system, which is approximately 50 km in length. This river system has been facing threats from emerging developmental activities specially the hydropowers, road constructions and extraction of river bed materials. In addition, this river system has high altitudinal gradient within a very short span. I choose Modi River system because this river system is highly suitable to understand the diversity and distribution of benthic macroinvertebrates with several stressors and altitudinal gradient within limited time. The aim is to find out spatial variation in diversity and distribution of freshwater macroinvertebrates of Modi River systems and establish the relationship between macroinvertebrates and water quality and their response to different stressors. This study will generate very important information for long term bio-monitoring of the river.

1.2 Rationale of the study

Riverine ecosystem is one of the most productive ecosystems in the world which supports large proportion of biodiversity. Unfortunately, the decline in freshwater biodiversity is higher than that of terrestrial ecosystems (Sala et al. 2000, Dudgeon et al. 2006). Freshwater biodiversity has been threatened by a number of major impacts such as overexploitation, water pollution, and flow modification including water abstraction, destruction, or degradation of habitat as well as invasion by alien species (Craig et al. 2017). This indicates the severity of the threats possessing by the freshwater ecosystem globally. In Nepal, flow modifications (including hydropower development and surface and groundwater extraction), water pollution and urbanization/population growth, watershed and habitat alteration (including conversion of wetlands to agricultural lands), invasive species, overfishing and illegal fishing, and climate change has been threatening Nepal's freshwater biodiversity (Allen 2010).

Similarly, river systems in Chitwan-Annapurna Landscape have been facing several problems from construction of hydropower dams and rural roads, which has impacted the ecological integrity of riverine ecosystems. Such structure causes inundation of important habitats, reduce downstream water flows, alter nutrients dynamics, and act as barriers to migration of species (WWF 2013). Since, the river system has a pressure of hydropower projects, rural roads, collection of river bed materials (sand, stone and gravel), use of destructive methods for fishing and deforestation in the watershed have been threatening the freshwater biodiversity of the river system. Anthropogenic activities such as release of domestic sewage, runoff from agricultural lands and laundering into streams have resulted into increasing pollution loads and ultimately affecting the river health. These stressors alter physicochemical properties of water which might affect the distribution pattern of macroinvertebrates in water. In the context of all these stressors and threats, assessment of taxa diversity is very crucial for the long term monitoring of river health system and science based planning for the conservation of river ecosystem.

There are several hydropower projects in Modi River system therefore understanding of impact of hydropower on the freshwater ecosystem is very crucial. Previous studies in Modi River are only focused on the diversity and distribution of macroinvertibrates. These studies lack the assessment of impact of hydropower on the diversity and distribution of macroinvertebrates. Considering the research gap in previous studies and emerging threats this research will explore the distribution and diversity of macroinvertebrate both in river's mainstem and tributaries in the face of development pressure and other threats. This information will be beneficial for the long term monitoring of freshwater ecosystem and develop conservation strategies for the improvement of river health.

1.3 Research questions

- What is the diversity of benthic maroinvertebrates?
- What is the spatial distribution of benthic maroinvertebrates along the altitudinal gradient?
- What is the relationship between benthic maroinvertebrate and river quality?
- What are the major threats to the ecological health of Modi River?

1.4 Objectives

1.4.1 General objectives

The general objective of the research is to assess the diversity and distribution patterns of benthic macroinvertebrates and ecological health of Modi River system.

1.4.2 Specific objectives

The specific objectives are:

- To assess the diversity and spatial distribution of benthic macroinvertebrates along Modi River system in pre-monsoon season.
- To assess the relationship between benthic maroinvertebrates and river quality of Modi River.
- To identify the anthropogenic threats to ecological health of Modi River.

1.5 Limitations of the study

Limitations of this study are:

- The research was conducted only in pre-monsoon season.
- Maroinvertebrates were identified only up to family level.

CHAPTER II: LITERATURE REVIEW

Freshwater ecosystems, which occupy tiny fraction of earth surface, support remarkable biodiversity. It also contains high level of endangerment and endemism (Dudgeon, et al 2006, Li et al. 2012). It performs various environmental functions like nutrient cycling, water purification, recharge ground water, provide habitat for wildlife and many more. Freshwater habitats cover about 0.8% of the Earth′s surface but they support 9.5% of all animal species described (Turak et al. 2017). Among various components of freshwater ecosystems, benthic macroinvertebrate is an important component that explains the ecosystem function and biodiversity of running water (Wallace and Webster 1996). Aquatic macroinvertebrates are the diverse organisms with varying tolerances for pollution from chemicals, toxins, nutrients and sediments making them the best and well suited indicators for determining river and stream health and water quality. They are susceptible to degradation of water, sediment and habitat. Hence, they serve as a good indicator of localized environmental condition (Alam et al. 2008).

A number of environmental factors such as water temperature, water velocity, substrate composition, hydro median depth and turbidity are likely to influence the diversity, abundance and larger differences in faunal composition of aquatic benthic invertebrates (Ligeiro et al. 2010, Ward and Stanford 1979, Roy and Home chaudhuri 2017). Understanding the diversity and distribution of organisms is a fundamental goal of ecology, and a prerequisite for using species in monitoring programs or as bioindicators. This is especially relevant for freshwater systems, which are highly diverse, but also highly threatened (Vörösmarty et al. 2010, Altermatt 2013). Macroinvertebrate diversity and abundance are significant community attributes that are controlled by a variety of mechanisms at different spatial scales. A number of studies have documented how macroinvertebrate assemblages respond to environmental variables and which variables best explain their distribution and abundance (Buss et al. 2002). Studies on the spatial distribution patterns of macroinvertebrate assemblages based on their environmental relationships are crucial. The distribution of aquatic insects is influenced by several biotic and physico-chemical factors. Tributaries in a landscape determine the physical and ecological condition of a river's mainstem (Tachamo Shah et al. 2020). Their number and characteristics shape the river’s mainstem geometry, substrate types, hydraulics, nutrients, organic matters and water quality (Bruns et al. 1984, Rice et al. 2001). Based on the River Continuum Concept (RCC), tributaries receive allochthonous inputs, and thus the structure of the aquatic communities—the functional feeding groups—is different from that of the downstream river’s mainstem, in which the energy cycle depends on autochthonous inputs (Vannote et al. 1980, Tachamo Shah 2020). Consequently, understanding the drivers of diversity patterns of stream macroinvertebrates can improve our knowledge of community and ecosystem response to local and global change. Increasing such understanding is also a prerequisite for effective conservation planning in the near future (Dudgeon et al., 2006).

2.1 Species richness along altitudinal gradient

The Nepal Himalaya has the greatest altitudinal gradients on earth and indeed, Gandaki River systems flow through the world’s deepest valley (Suren 1994). However, few researchers have examined biological communities in rivers flowing down the pronounced altitudinal gradient in Nepal or elsewhere (Egglishaw 1980, Turcotte and Harper, 1982). Patterns in species richness along geographic and environmental gradients are a fascinating topic in ecology. Although taxon richness usually declines from low to high altitude, the exact relationship varies among systematic groups of organisms (Jacobsen 2004).

Benthic macroinvertebrate communities at different altitude respond differently. With the increase in altitude, the taxonomic richness decreases (Brewin 1995). The decline in atmospheric temperature with increasing altitude above sea level is usually identified as the prime factor governing the distribution of plants and animals along altitudinal gradients. The decrease in atmospheric oxygen pressure with increasing altitude is also a well known phenomenon. Low atmospheric oxygen pressure potentially affects life in water and on land (Jacobsen et al. 2003).

Multi-locality studies on faunal structures and richness in streams of similar size at different altitude are more appropriate for studying the effect of altitude. In addition, the effect of human disturbance through pollution and land use especially at lower altitudes also influence observed patterns. Hence, the specific influence of altitude on faunal composition and richness still is somewhat unclear (Jacobsen 2003). The patterns in taxon richness and community structure of macroinvertebrates in streams are also dependent on the spatial scale of the study (Downes et al. 1993, Carter et al. 1996). Rahbek (1995) reported that a unimodal pattern is the most common relationship between richness and altitude.

2.2 Benthic macroinvertebrates and water quality

Water is the most basic natural resource which is needed for the survival of all living organisms in the earth. It plays a very profound role for the maintenance of human health and aquatic ecosystems. Water quality of any specific area or specific source can be assessed using physical, chemical and biological parameters (Tyagi 2013). There is a need for comprehensive and accurate assessment of trend in water quality to address the consequences of present and future threat of contamination. Reliable monitoring data are indispensible basis for such assessment (Ballance and Bartram, 2002). Water quality monitoring is the foundation on which water quality management is based (Ballance and Bartram, 2002). Biomonitoring or biological monitoring has been proven to be necessary supplement to traditional monitoring techniques and is an important tool in assessing the condition of aquatic ecosystem. It involves the indicator species and communities. Biological indicators are more reliable than physico-chemical analysis for defining the ecological and quality status of the aquatic ecosystems (Ceschin et al. 2012).

Invertebrate communities are good indicator of water quality (Resh 1995) since freshwater macroinvertebrate species vary in their sensitivity to organic pollution (Rosenberg and Resh 1993). Benthic macroinvertebrates are highly suitable for monitoring the ecological condition and identifying the natural and human impacts to rivers (Barbour 2008, Korte et al. 2010). Benthic macroinvertebrate species respond differently to biotic and abiotic factors in their environment and consequently, macroinvertebrate community structure has been used as indicator of the status of water body.

Freshwater macroinvertebrates also requires physico- chemical condition in stream water as well as specific micro habitats to survive and to build sustainable population. Therefore, the assemblage of species (e.g. number and type of benthic invertebrate taxa) reflects the overall condition of a given site (Ofenböck et al. 2008). Benthic macro invertebrates are able to reflect ecological health of river ecosystem since they are sensitive to eutrophication, organic pollution, hydro morphological deterioration and human activities in the flood plain and catchment area respectively (Korte et al. 2008). Similarly, Benthic macroinvertebrates, as biological indicators of stream water quality, can be utilized to identify impaired river stretches, determine aquatic life stressors, set goals for reducing impairment, and indicate improvement (Kenny et al. 2009, Tachamo Shah and Shah 2013). As reliable bio-indicators, orders Ephemeroptera, Plecoptera and Trichoptera (EPT) are useful for ecological assessment of rivers to understand impacts of development of water resources (Holt et al. 2015, Tachamo Shah et al. 2020). In recent years, several studies have been carried out with the help of site-specific biotic scores. Score-based system for bio-indication using indicator taxa scored according to their sensitivity to stressors; facilitate the interpretation of large quantity of data resulting from the biological monitoring of rivers (Armitage et al. 1983). Score- based assessment method are mostly based on higher taxonomic bio-indication unit (genus, family, order), requires simple data processing facility and gives less expensive and quick judgments in river quality. In 1990's region specific score based method for Nepalese rivers (NEPBIOS) was developed which represents an adaptation of BMWP/ASPT system (Sharma 1996, Sharma and Moog 1996). Similarly, scoring system was developed for the Ganga River system (GRS-bios) with some modification following the application of NEPBIOS. It is the first specially adapted faunal list for a specific watershed (Nesemann et al. 2007).

2.3 Threats and conservation of aquatic ecosystems

Human pressure around freshwater systems and increasing human demands for water has led to high levels of degradation and threats to biodiversity in fresh waters. The stress on water resources is from multiple sources and the impact can take diverse forms. One estimate suggests that the 'human footprint' has significantly influenced more than 83% of the land surface surrounding freshwater systems (Vörösmarty et al. 2010). Effects of human activities are evident as widespread catchment disturbance, deforestation, riparian loss, water pollution, river corridor engineering, dams and water diversions, extensive wetland drainage, groundwater depletion, aquatic habitat loss and fragmentation, establishment of introduced alien species, and overfishing (Dudgeon et al. 2006). These threats have potentially harmful socio-economic effect on human welfare and well being. Similarly, the exposure of freshwater systems to different sequential stressors intensifies ecological impact and poses greatest challenge to freshwater biodiversity conservation and vastly complicates restoration and conservation planning. The stressors which are critical for one section may not be critical for other section of a river. Thus, different levels of mitigative and restoration processes need to be considered depending upon the respective human disturbance gradients. The classification of disturbance gradients into different zones using multivariate statistical methods allows river managers to develop and implement effective management programmes which optimize cost and time without reducing the outcome quality significantly (Tachamo Shah and Shah 2013).

The restoration and proper management of the degraded riverine ecosystems is essential for continuously providing ecosystem services including clear water, fresh air, recreational activities, mitigation of drought and floods, the cycling and transport of nutrients, the maintenance of biodiversity, detoxification, decomposition of waste, and so forth (Tachamo Shah and Shah 2013).

CHAPTER III: MATERIALS AND METHODS

3.1 Study area

This study was carried out in Modi River of Kaski and Parbat district. Modi River is located in Annapurna Rural Municipality of Kaski district and Modi Rural Municipality of Parbat in Gandaki Province of Nepal. This river is bordered in the east by Mardi and Seti river basins, in the west and south by Kaligandaki basin and in the north by Marshyangdi basin. Modi River is one of the major tributaries of Kaligandaki river system which lies in Chitwan-Annapurna Landscape and originates from the massif of Annapurna and flow through Annapurna Conservation Area. The catchment is of 675 km2 and altitude ranges from 748 to 8000 m in a very short span (JVS and GWP 2016). It is a snow fed river water system. Modi River is rich in water resources in terms of average annual flow. The upstream of the river system has dominancy of upper temperate broadleaf forest and downstream is with *Schima-castanopsis* mixed broadleaf forest. Since the Annapurna conservation area has very high potentiality of hydro-electricity (NTNC 2012), numbers of projects are under construction. Modi khola hydropower is already constructed and other several hydropower projects are under construction in Modi River.

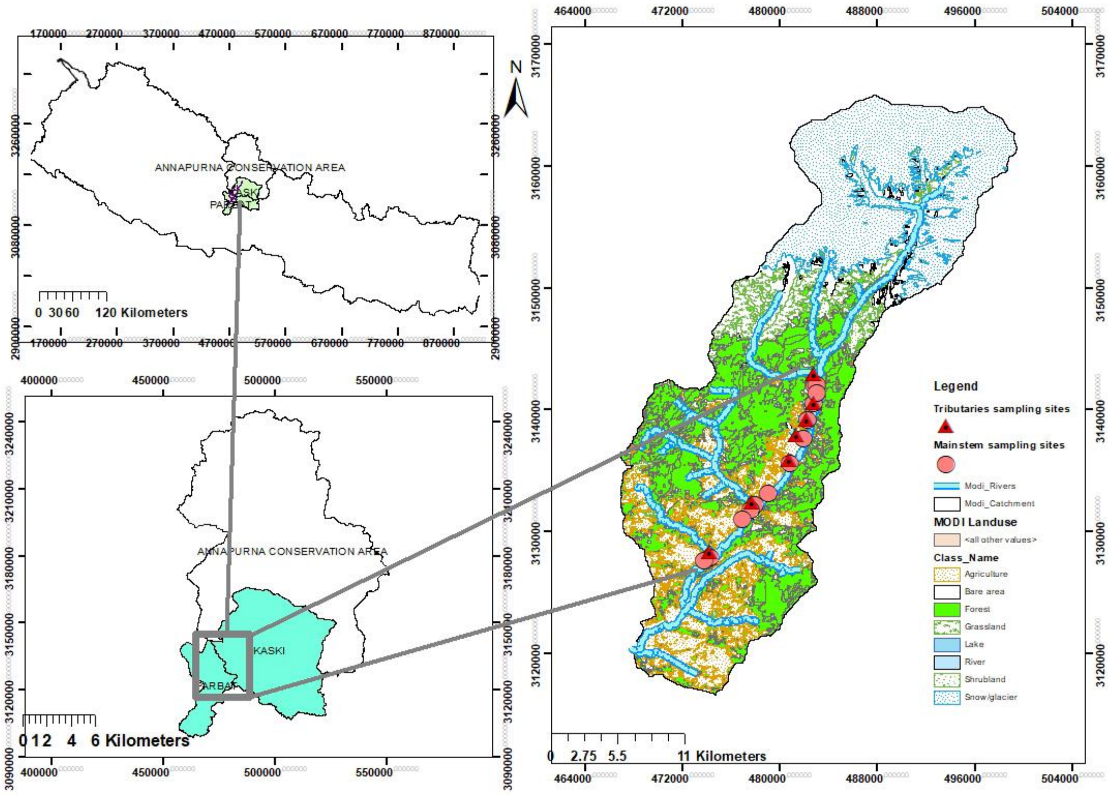


Figure 1: Study area with sampling sites of river's mainstem and tributaries of Modi River system

3.2 Site description

The study sites are located in river's mainstem and tributaries of the Modi River system. In total we selected 20 sampling sites. Out of them 13 were from river's mainstem and 7 were from tributaries. In river's mainstem, the highest sampling point was at 1654m and lowest sampling site was at 848m. Similarly, the highest sampling site of tributary was located at 1764m and lowest elevation site was at 931m. The sites were categorized into four groups based on altitudinal range (Group 1: 801m-1050m, Group 2: 1051m-1300m, Group 3: 1301m-1550m and Group 4: 1551m – 1800m). The sites were selected on the basis of land use pattern, human disturbance and substrate structure. MO indicates the sampling sites in river's mainstem and T represents the sampling sites in Tributaries. The summary of each site is described as below.

Table 1: Description of sampling sites of river's mainsteam and tributaries of Modi River system

Site	**Elevation**	**Habitat type**	**Surrounding and forest cover**	**Stressors**
MO01 (Group 4)	1654	Boulders and cobbles	Shaded with trees at the zenith. *Alnus nepalensis* (Uttis), *Schima castanopsis* (Chilaune-Katus) and *Bambusa nutans* (Bamboo) were major dominant species	Not observed
MO02 (group-4)	1611	Boulders and cobbles	Covered with trees and partly shaded with trees at the zenith *Alnus nepalensis* (Uttis), *Schima castanopsis* (Chilaune-Katus) and *Bambusa nutans* (Bamboo) were major dominant species	Fishing
MO03 (Group-4)	1563	Boulders and cobbles	Covered with scattered trees and mostly open. *Alnus nepalensis* (Uttis), *Schima castanopsis* (Chilaune-Katus) and *Bambusa nutans* (Bamboo) were major dominant species	Road construction and Fishing
MO04 (Group-3)	1418	Boulders and cobbles	The site is covered with trees and partly shaded with trees at the zenith *Alnus nepalensis* (Uttis),	Fishing

			Schima castanopsis (Katus-Chilaune), *Macaranga denticulate* (Mallato)	
MO05 (Group-3)	1378	Boulders and cobbles (algae observed in the rock of river	Partly covered with tree *Alnus nepalensis* (Uttis), *Schima castanopsis* (Katus-Chilaune), *Macaranga denticulate* (Mallato)	Sand quarry Fishing Industrial effluent
MO06 (group-3)	1326	substratum of river was dominated by boulders and cobbles	*Alnus nepalensis* (Uttis), *Schima castanopsis* (Katus-Chilaune), *Macaranga denticulate* (Mallato)	Fishing and sand quarry
MO07 (Group-2)	1216	Dominated by boulder, cobbles followed by coarse gravel	Fallow, pasture land and residential. Mainly *Schima castanopsis* (Katus-Chilaune), *Macaranga denticulate* (Mallato) and scattered *Alnus nepalensis* (Uttis)	Fishing and stone quarry
MO08 (group-2)	1123	Substratum of river was dominated by boulders, cobbles, bedrock and coarse gravel	Dominated by trees followed by pasture land, agriculture and residential area mainly *Schima castanopsis* (Katus-Chilaune), *Macaranga denticulate* (Mallato) and scattered *Alnus nepalensis*	River bank cutting, sand and stone quarry and fishing

			(Uttis)	
MO09 (Group-2)	1077	Substratum of river was dominated by boulders, cobbles, bedrock and coarse gravel	In the vicinity of human settlement hence influenced by human and agricultural activities. Dominated with *Schima castanopsis* (Katus-Chilaune).	Waste dumping, sewage and fishing
MO10 (Group-1)	1038	Substratum of river was dominated by boulders, cobbles, bedrock and coarse gravel	Human habitation and agriculture land in the surrounding. Dominated with *Schima castanopsis* (Katus-Chilaune).	Hydropower in the upstream, sewage, agricultural effluents, sand and stone quarry
MO11 (Group-4)	992	Substratum of river was dominated by boulders, cobbles, bedrock and coarse gravel	Human habitation and agriculture land in the surrounding. Dominated with *Schima castanopsis* (Katus-Chilaune).	Dumping, sewage, agricultural effluents, sand and stone quarry, and reservoir and dam of hydropower
MO12 (Group-4)	889	Substratum of river was dominated by boulders, cobbles, bedrock and coarse gravel	Human habitation and agriculture land in the surrounding. Dominated with *Schima castanopsis* (Katus-Chilaune). The site was partly open with trees at the zenith.	Sewage, embankment, channeling of water, sand and stone quarry, fishing are threatening the

				river ecology
MO13 (Group-4)	848	Substratum of river was dominated by boulders, cobbles, bedrock and coarse gravel	This site was located around settlement, agriculture and commercial area. Dominated with *Schima castanopsis* (Katus-Chilaune). The site was partly open with trees at the zenith.	Hhydropower. Waste dumping, sewage, agricultural effluents, sand and stone quarry, reservoir and dam construction
T01 (group 4)	1764	Dominated with boulders and cobbles	Shaded with trees at the zenith. The site is covered with trees and partly shaded with trees at the zenith *Alnus nepalensis* (Uttis), *Schima castanopsis* (Katus-Chilaune), *Macaranga denticulate* (Mallato)	Waste and sewage observed
T02 (group 4)	1719	Dominated with boulders and cobbles	The site is mostly covered by trees and was shaded with trees at the zenith *Alnus nepalensis* (Uttis), *Schima castanopsis* (Katus-Chilaune), *Macaranga denticulate* (Mallato).	Waste dumping and sewage observd
T03 (group 3)	1533	Habitat includes boulders and	The river is of glacial origin and mineral and covered by trees and was	Sewage and agricultural effluents were

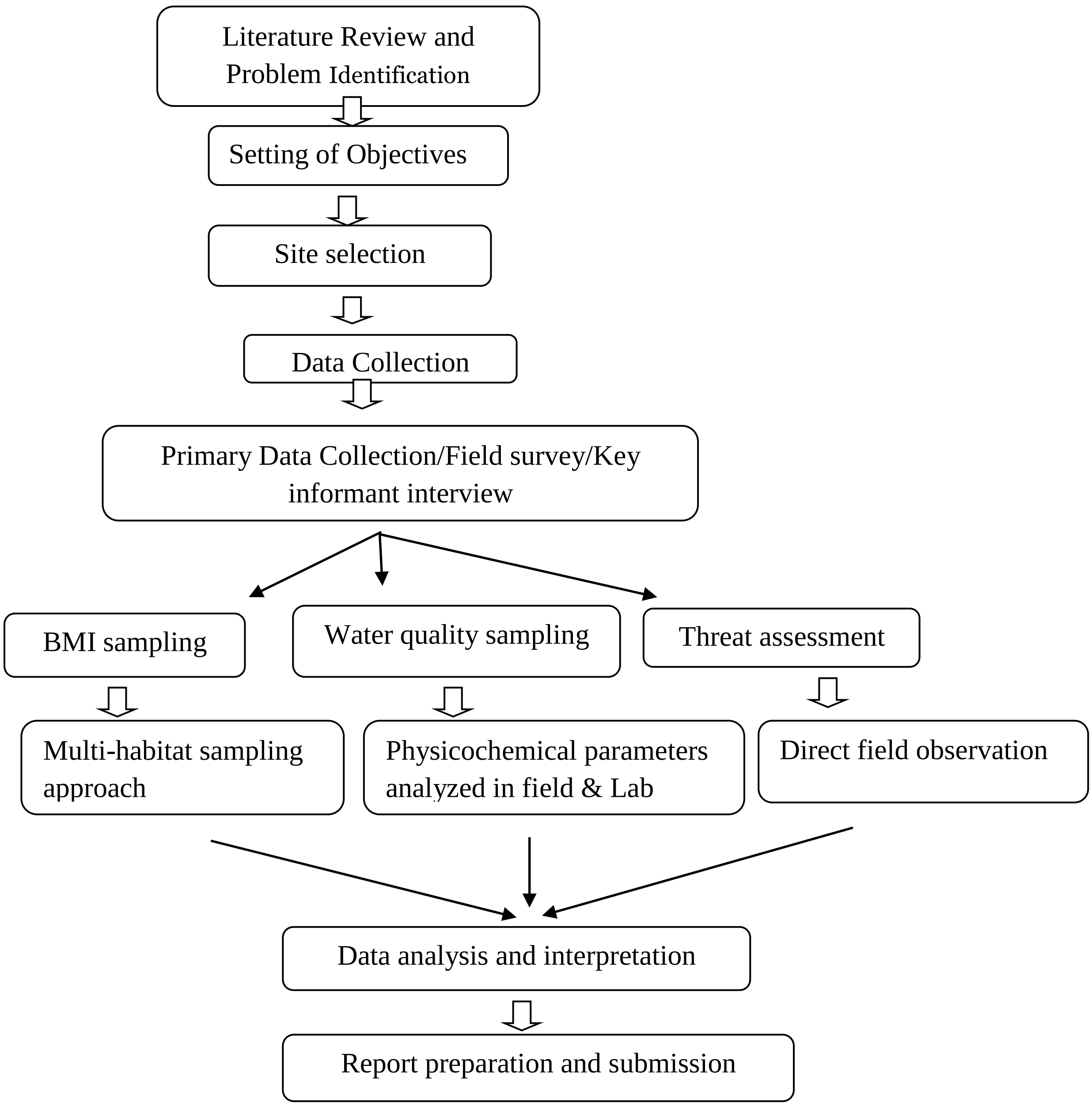
		cobbles	shaded with trees at the zenith. Settlement around and dominated by *Alnus nepalensis* (Uttis), *Schima castanopsis* (Katus-Chilaune), *Macaranga denticulate* (Mallato)	observed in the river.
T04 (group 3)	1476	Habitat includes boulders and cobbles	The site was mostly covered by trees and was shaded with trees at the zenith. Settlement around and dominated by *Alnus nepalensis* (Uttis), *Schima castanopsis* (Katus-Chilaune), *Macaranga denticulate* (Mallato)	Sewage and fishing were observed in the river.
T05 (group 2)	1265	Habitat includes boulders and cobbles	The site was mostly covered by trees and was shaded with trees at the zenith. Site was near the settlement area. *Alnus nepalensis* (Uttis), *Schima castanopsis* (Katus-Chilaune), *Macaranga denticulate* (Mallato)	Sewage, waste dumping, agricultural effluents
T06 (group 2)	1189	Habitat includes boulders and cobbles	The site was mostly covered by trees and was shaded with trees at the zenith. Site was near the settlement area. *Schima castanopsis* (Katus-Chilaune), *Macaranga*	Waste dumping, sewage, agricultural effluents, washing clothes, open

			denticulate (Mallato)	defecation, road construction, sand and stone quarry, fishing, littering by picnic goers
T07	931	Habitat includes boulders and cobbles	The site was nearby settlement and commercial area. The site was mostly covered by trees and was shaded with trees at the zenith. *Schima castanopsis* (Katus-Chilaune), *Macaranga denticulate* (Mallato)	Waste dumping, sewage, agricultural effluents, washing clothes, open defecation.

3.3 Methods

3.3.1 Research process

The overall process is as follows:



3.3.2 Water sampling and physicochemical parameter test

Water sample was collected from run, riffle and pool section of river at 10 cm depth and mixed for each site before carrying out macroinvertebrate sampling. Physico-chemical parameters such as water temperature, pH, dissolved oxygen, free CO_2, alkalinity, electrical conductivity, chloride, total hardness, calcium hardness, magnesium hardness were measured in-situ while sample for total dissolved solids, turbidity, nitrate, phosphate, ammonia, bio-chemical oxygen demand (BOD) and Chemical oxygen Demand (COD) were stored in labeled polyethylene jars and stored in ice box until the arrival at CDES/TU laboratory for the analysis. The physicochemical parameters of the water samples were evaluated by methods specified in 'Standard Methods for Analysis of Water and Wastewater' (APHA AWWA, 2005). The width and depth of river was measured using measuring tape. The river velocity was measured using float method.

Table 2: Standard methods and instruments used for water quality analysis

SN.	Parameter	Method/Instruments	Location/Source
1	pH	Hanna Calibrated Electrode Probe pH Meter	Insitu
2	DO & Temperature	Hanna Calibrated Electrode Probe DO Meter	Insitu
3	EC	Hanna Calibrated Electrode Probe Conductivity Meter	Insitu
4	Free CO_2, Chloride, Alkalinity (mg/L)	Titration method	Insitu
5	Total Hardness, Calcium hardness (mg/L,CaCO3)	Titration method	Insitu
8	Nitrate (mg/L)	Phenol-disulphonic acid method, Spectrophotometer	Laboratory
9	Phosphate (mg/L)	Calorimetric method, Spectrophotometer	Laboratory
10	Ammonia	Nessler reagent method, Spectrophotometer	Laboratory

11	BOD (mg/L)	Titration Method (5 days Incubation)	Laboratory
12	COD (mg/L)	Dichromate reflux method	Laboratory
13	Turbidity	HACH 2100 AN Turbidimeter	Laboratory

3.3.2.1 Laboratory analysis for physico-chemical parameters Ammonia

The ammonia concentration was determined by the Nessler reagent method. 100 mL of sample was taken in a conical flask and 2 mL Nessler reagent was added. The mixture was left for 10 minutes to develop an orange-brown color. After 10 minutes reading was taken at 420nm using a spectrophotometer. The concentration of the ammonia was determined with the help of standard value obtained from plotting absorbance value against concentration value.

Nitrate

Phenol disulphonic method was applied for the determination of nitrate concenteration. 50 mL of the sample was taken in a porcelain basin and equivalent quantity of Ag_2SO_4 was added to remove chloride from the water sample. The mixture was evaporated to the dryness and residue was allowed to cool down. The residue was dissolved in 2 mL phenol disulphonic acid and diluted to 50 mL. Then, 6 mL of liquid ammonia was added to develop yellow color. The reading was taken at 410 nm using a spectrophotometer. By plotting the value against absorbance from the standard curve the concentration of Nitrate-N was determined.

Phosphate

For the determination of phosphate in the given water sample, a calorimetric method was used. 50 mL of water sample was taken in a conical flask and 2 mL of ammonium molybdate followed by 5 drops of stannous chloride solution was added. The solution was left for 10 minutes after which blue color appeared. The reading was taken at 690nm using a spectrophotometer. The concentration of phosphate was determined with the help of standard value obtained from plotting absorbance value against concentration value.

Free CO_2

For free CO_2, 100 mL of sample water was taken in a conical flask and a few drops of phenolphthalein indicator were added. If the solution turned pink after the addition of phenolphthalein, it indicates the absence of free carbon-dioxide. The contents were titrated against (0.05 N) NaOH until pink was obtained at the endpoint and reading was noted down.

$$\text{Free } CO_2 \text{ (mg/L)} = \frac{\text{(Volume} \times \text{Strength(N)of NaOH} \times 1000 \times 44}{\text{Volume of sample taken (mL)}} \times 1000$$

Total Alkalinity

100 mL of sample was taken in a conical flask and 2 drops of phenolphthalein indicator was added. If color changed to pink it was titrated with 0.01 HCl until pink color disappeared at the end point. This gave phenolphthalein end point. Then 2 drop of methyl orange indicator was added to the same sample and titrated till the color changes from yellow to orange.

$$\text{Total alkalinity } \left(\frac{\text{mg}}{\text{L}} \text{ as CaCO3}\right) = \frac{\text{mL} \times \text{N of HCl}}{\text{Volume of sample taken}} \times 1000 \times 50$$

Turbidity

The turbidity was measured with the help of turbidity meter (HACH 2100). For this the turbidity tube was washed properly with distilled water. The tube was ringed with the adequate amount of shake sample and sample was poured into it. Then the displayed number was recorded from turbidity meter in NTU unit.

Chloride

Chloride is determined by argentometric titration method. For this, 50 mL water sample was taken in a conical flask. 3 drops of potassium chromate (K2CrO4) solution indicator was added in it. The contents were titrated against 0.02 N silver nitrate solution until reddish brown color appeared. The titration was repeated until the concurrent reading was obtained. A blank titration was also conducted by placing 50 mL chloride free distilled water instead of sample, (APHA, 1998).

$$\text{Chloride (mg/L)} = \frac{(\text{mL} \times \text{N})\text{ofAgNO3} - \times 35.5}{\text{Volume of sample taken}} \times 1000$$

Where, m = Volume of $AgNO_3^-$ consumed during titration

N= Normality of Ag NO_3^- (0.02)

Total Hardness

At the beginning, 50 mL of sample water was taken in a conical flask. 1mL buffer solution and 100-200mg of Erichrome Black T indicator was added, and then solution turned wine red. Then the contents were titrated against EDTA solution at the end point color changes from wine red to blue. Then by calculation total hardness was determined.

$$\text{Total hardness (mg/L CaCO3)} = \frac{\text{V olume of EDTA used}}{\text{Volume of sample taken (mL)}} \times 1000$$

Calcium Hardness

The calcium hardness was measured with the help of a microprocessor flame photometer (ESICO, Model 1382). For this calibration curve of the standard solution was made. The sample was poured in the test tube and kept in the flame photometer. Then, reading was noted down.

Magnesium Hardness

Magnesium hardness is calculated as the difference between total hardness and calcium hardness.

Magnesium Hardness (mg/L) = Total hardness-Calcium hardness

Biological Oxygen Demand (BOD)

BOD of water sample was determined using 5-days test titration method. BOD bottles of 300 ml were taken. Then sample was filled in it without incorporating any air bubbles. First bottle: It was wrapped with carbon paper and DO5 was calculated after incubating in an incubator for 20°C for 5 days using Wrinkler‘s Iodometric method. Second bottle: DO0 (initial) was determined using Wrinkler‘s Iodometric method.

BOD (mg/Lt.) = (D0 – D5) x Dilution Factor

Where, D0 = Initial DO content in the sample

D5 = DO after 5 days

Chemical Oxygen Demand (COD)

COD of the water sample was determined by using the dichromate reflux method. 20 mL of water sample was taken in the reflux flask. Then, 10 mL of 0.25N of potassium dichromate followed by a pinch of silver and mercuric sulphate was added. 30 mL of conc. sulphuric acid was added in the mixture and allowed to reflux for one and half hour. The reflux flask was allowed to cool and the final volume was made to about 140 mL. 2-3 drops of ferroin indicator were added and titrated with 0.1 N ferrous ammonium sulphate solution. At the endpoint, the color changes from blue-green to reddish-brown.

$$\text{COD (mg/L)} = \frac{(b-a)\times \text{normality of ferrous ammonium sulphate} \times 8}{\text{Volume of sample}} \times 1000$$

Where, b = Blank titre value

a = Sample titre value

3.3.3 Benthic macroinvertebrates (BMI) sampling

Benthic macroinvertebrates sampling was conducted following a multi-habitat sampling approach (Moog 2007). At each site, benthic maroinvertebrates were collected systematically from 20 microhabitats available in-stream by kicking the substrate or jabbing with a D-frame dip net. The substrate in front of the opening of net was disturbed for a minute to capture macroinvertebrate. The net was placed against the river flow. The stones, cobbles and plant parts within the sampling area were turned over, washed on mouth of net in order to dislodge and collect specimens that are hidden underneath or attached to the bottom. Maroinvertebrates were collected using a standard hand net with a mesh size 500µm. Samples were transferred into labeled vials and preserved in 99.9% ethanol. The samples were brought to the laboratory for sorting and identification purpose. Identification of specimens was done using stereomicroscope up to family level using different identification keys (Dudgeon 1999, Nesemann et al. 2007, Nesemann et al. 2011).

3.3.4 Threat assessment

For the assessment of threats, anthropogenic pressure like road construction, hydropower construction, bridge construction, use of destructive fishing methods, extraction of sand, gravel and stones, waste dumping, sewage were directly observed during field survey and properly recorded. Site protocol was filled to document the sampling site information. Besides these, key informants interview was conducted representing the key stakeholders (Conservation Area Management Committee members, Annapurna Conservation Area Project Officials and Hydropower Project site Managers).

3.3.5 Taxa richness and diversity measure

Taxa richness is the total number of families present in the study area. It measures the variety of taxa. Shannon's diversity index (H) and Simpson's diversity index (1-D) (Magurrun 2004) was used for the calculation of taxa diversity of macroinvertebrates. Evenness index (e) (Magurrun 2004) was used for the calculation of taxa evenness of macroinvertebrates. Evenness index is also known as Pielou's Evenness Index. Following formulae were used to calculate these indices:

Shannon-Wiener's Diversity Index (H) $= -\sum_{i}^{n} = 1(pi \times lnpi)$

Evenness Index (e) = $\frac{(H)}{LnS}$

Simpson's Diversity Index $(1 - \text{D}) = 1 - \frac{\sum n(n-1)}{N(N-1)}$

Where, H= Shannon Weiner Diversity Index

S= Species Richness

pi= (n/N)

n = Total number of individuals of each species

N = Total number of individuals of all species

Ln = Natural logarithm values

3.3.6 River water quality class (RWQC) by using biological index

Benthic Macroinvertebrate was scored using the Ganga River System Biotic Score (GRS-BIOS) which includes 420 taxa of family, genus and species level (Nesemann et al. 2007). GRS-BIOS/ASPT is calculated by dividing the sum of total score by the number of scored taxa. The obtained numerical value is then compared to its transformation table (Sharma and Moog, 2005) that gives the river quality class of studied river stretch. The transformation table is shown below:

Table 3: GRS-BIOS/ASPT transformation table for Midland

GRSBIOS/ASPT for Midland	River Quality Class	Description
7.50-10.00	I	Not polluted
6.51-7.49	I-II	Slightly polluted
5.51-6.50	II	Moderately polluted
4.51-5.50	II-III	Critically polluted
3.51-4.50	III	Heavily polluted
2.01-3.50	III-IV	Very heavily polluted
1.00-2.00	IV	Extremely polluted

3.4 Statistical Analysis

To test the significance of family richness along the altitudinal gradient of the River's mainstem and its tributaries, all the sampling sites were categorized in four groups with 250m intervals (group 1 – 801m-1050m, group 2-1051m-1300m, group 3-1301m-1550m and group 4- 1551m – 1800m). Out of four groups each group has 5 replications. To assess the taxa richness, Shannon Diversity Index, Simpson Diversity Index, and Evenness were calculated. To know about the family richness between the different clusters, box plot was made in R studio (1.2.5 version).

Similarly, Shapiro-Wilk normality test was used to check the normality of data. The data were normally distributed, so parametric test was performed. Software R (1.2.5 version) and CANOCO (4.5 version) was used for statistical analysis. One way ANOVA was done to test the significance of family richness along altitudinal gradient. Turkey HSD test was done to know the difference between groups. To assess the significance of Shannon Diversity Index in River and tributaries, Wilcoxon pair test was performed.

Box and whisker plot was made to see species/taxa richness along altitudinal gradient. The relationship between environmental variable and macroinvertebrates was performed using Redundancy Analysis (RDA). All statistical tests were performed at 95% confidence level.

CHAPTER IV: RESULTS

4.1 Diversity and distribution of macroinvertebrates

4.1.1 Taxa richness

Altogether 40 families belonging to 10 orders were recorded in 20 sampling sites (13 sites in river's mainstem and 7 in tributaries) of Modi River system (figure 2 and 3). Twenty nine families belonging to eight orders were recorded in river's mainstem while thirty nine families belonging to nine orders were recorded in tributaries. Haplotaxida and Hemiptera were not recorded in River's mainstem whereas Lumbriculida were not found in tributaries. Among the 40 families, order Diptera has the highest family richness i.e. 11. Order Tricoptera has second family richness with a record of 9 different families. Only one family belonging to each order Megaloptera, Haplotaxida and Lumbriculida were recorded. The details of taxa observed in different sampling sites in given in annex 1 and 2.

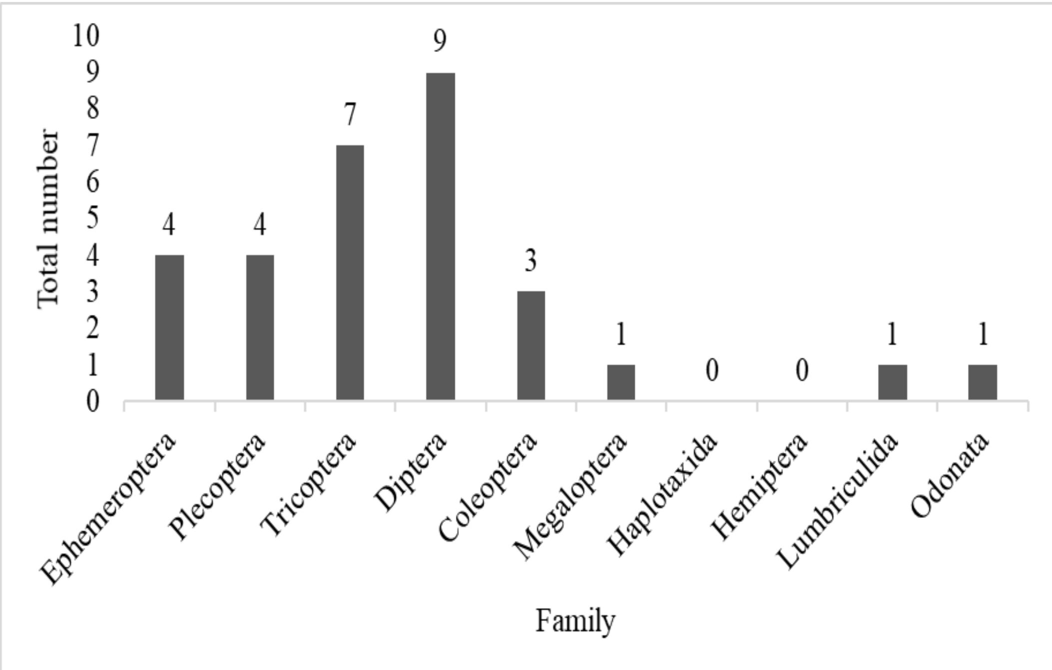


Figure 2: Taxa richness in the sampling sites of river's mainstem of Modi River system

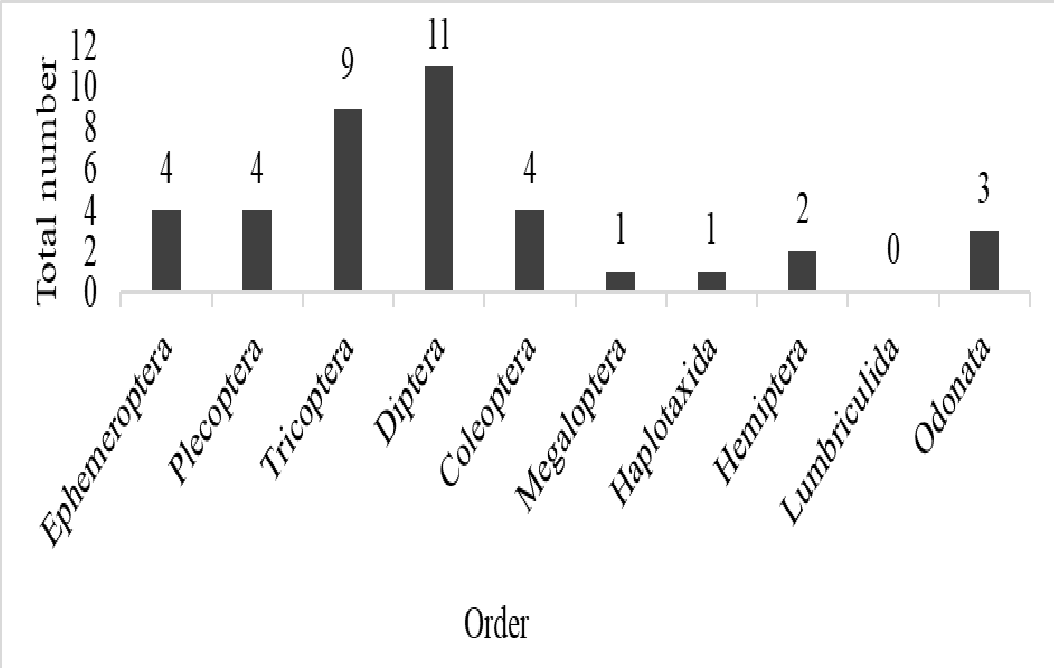


Figure 3: Taxa richness of sampling sites of tributaries of Modi River system

4.1.2 Taxa richness in different sampling sites

Out of 13 sampling sites in River's mainstem, 29 families of 8 orders were recorded while 39 families of nine orders were found in 7 sampling sites of tributaries. Only 9 families were found at the sampling sites of highest altitude located at 1654m of a river's mainstem. That was the minimum number of the family recorded among all the sampling sites. Similarly, 23 families were recorded in the Lamkhet located at 1077m. MO11 and MO13 are the sampling sites located in the downstream of the Hydropower construction. Both the sampling sites contain low number of the family in comparison to the nearest upstream sampling sites. Likewise, 14 is the minimum number of families recorded at the elevation of 1719m and 26 is the highest number of family recorded at the elevation of 1189m in the tributaries. The total number of families observed in each sampling sites of both River's mainstem and tributaries has been shown in the bar diagram as:

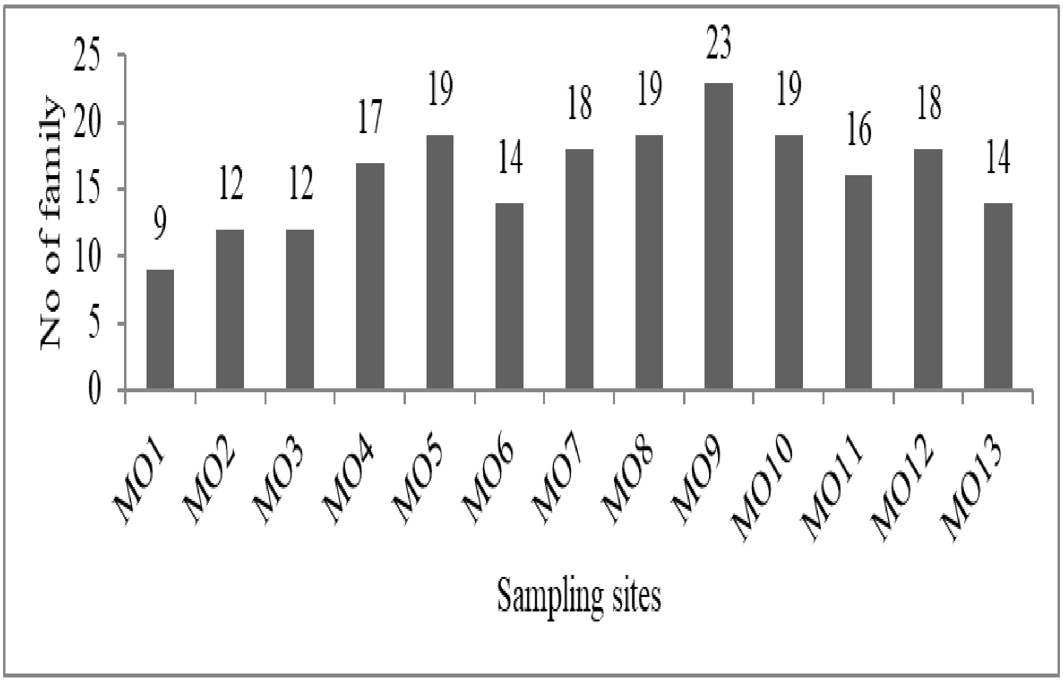


Figure 4: Taxa richness (family level) of sampling sites of river's mainstem of Modi river system

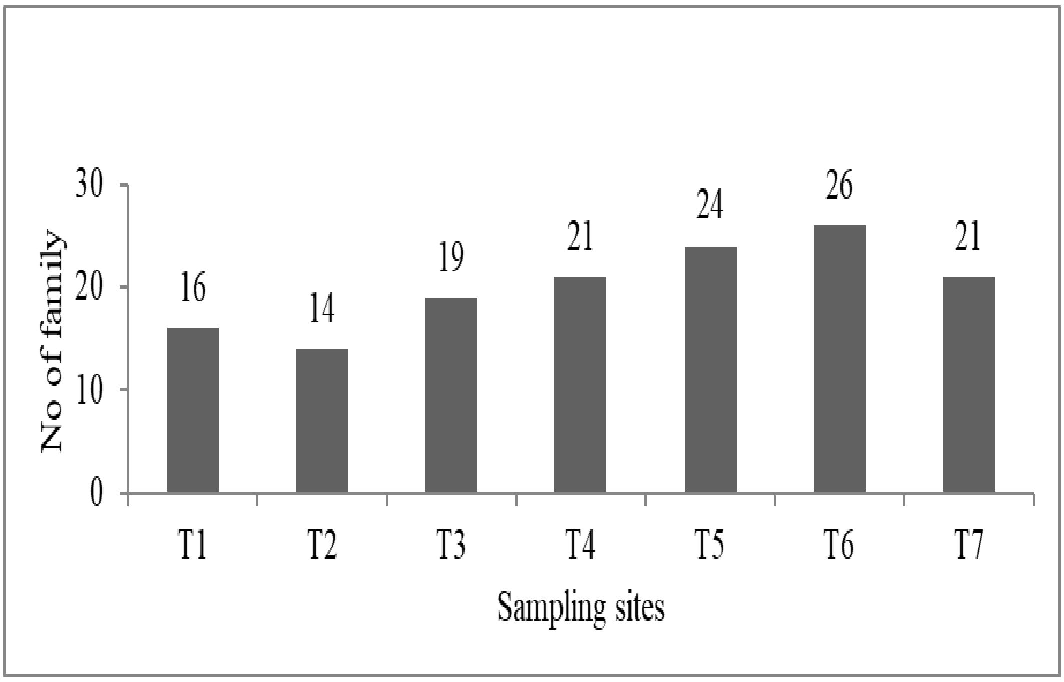


Figure 5: Taxa richness (family level) of sampling sites of tributaries of Modi River system

4.1.3 Taxa richness along altitudinal gradient

Shapiro-Wilk normality test showed that the P value higher than α value (p= 0.969, H_0 is accepted) so the data were found to be normally distributed. ANOVA table showed the difference of the family richness among different groups along the altitudinal gradient (P=0.000949).

Table 4: ANOVA table showing difference of family richness among different groups

	Sum of squares	df	Mean Squares	F	Sig.
Between groups	222.6	3	74.18	9.102	**0.000949**
Within groups	130.4	16	8.15		
Total	153.0	19			

After the ANOVA, Turkey HSD test was performed to know the difference between the groups. Group 4 and group 2 (p=0.0004) and group 4 and group 3 (p=0.0389) differs significantly. The box plot showed that group 2 has highest diversity and group 4 has lowest diversity. Box plot shows species richness increases with the decreasing altitude from 1800m to 1050m and decreases in the group 1050m to 800m. Though all the sampling sites in group 1 were at lower elevations than group 2 it showed the lower diversity of taxa. Most of the sampling sites in group 1 were taken at the downstream of hydropower. So that low diversity in group 1 may be due to disturbance from hydropower construction. Box plot of taxa richness in four different groups has shown in the figure as:

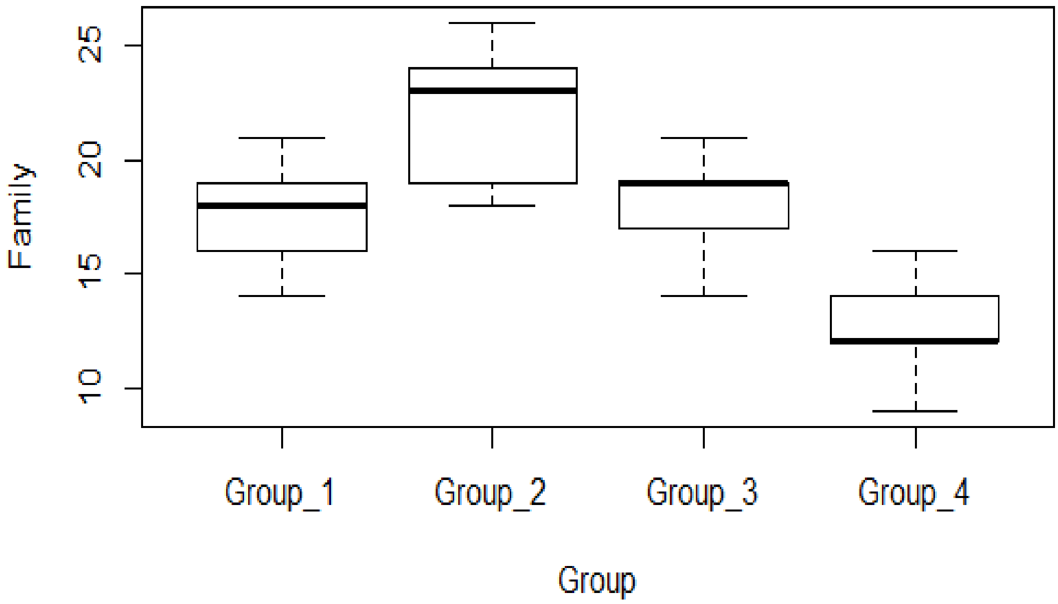


Figure 6: Box plot to show the taxa richness of different groups

4.1.4 Diversity index

The Shannon Diversity Index value of a river sampling sites ranges from 1.23 to 2.64 (figure 7). This Index value of tributaries ranges from 1.19 to 2.55 (figure 8). Shannon Diversity Index of a river is highest in a sampling sites located an elevation of 1326m and lowest index value is in the sampling sites of 848m, just in the downstream of hydropower construction sites. Similarly, Shannon Diversity index is highest in the sampling sites located at 1265m elevation and lowest at 1533m. Test Showed that the diversity index differs significantly (p=0.049).

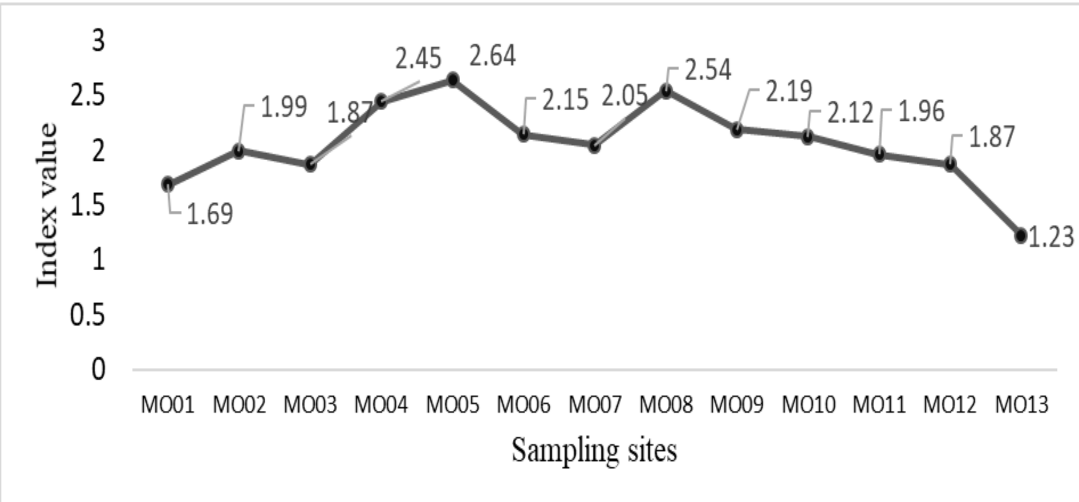


Figure 7: Shannon Diversity Index of river's mainstem

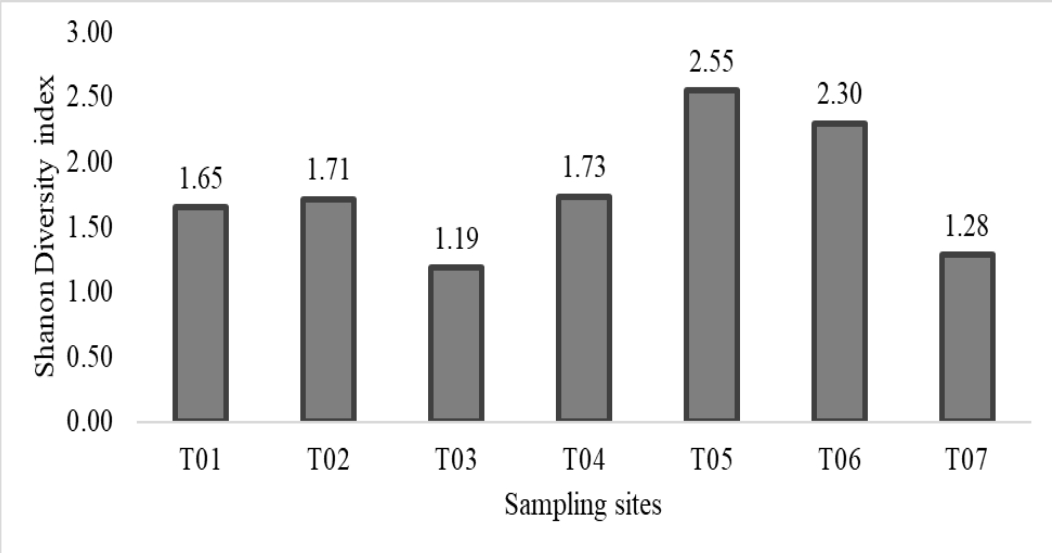


Figure 8: Shannon Diversity Index of Tributaries

Simpsons Diversity Indexes of river sampling sites ranges from 0.91 to 1.00 and 0.99 to 1.00. The lowest Simpsons Diversity Index value was observed at the elevation of 1611m. The highest value 1.00 was observed in five different sampling sites located at an elevation of 1378m, 1216m, 1123m, 1038m and 889m elevation. Similarly, lowest index value of tributaries was observed at an elevation 1189m whereas the highest index value was observed at an elevation of 1764m, 1719m, 1533m, 1476m and 1265m. To assess the significance of the Simpsons Diversity Index between the river and tributaries, Wilcoxon pair test was conducted but there was no difference in the diversity of river and tributaries (p=0.195).

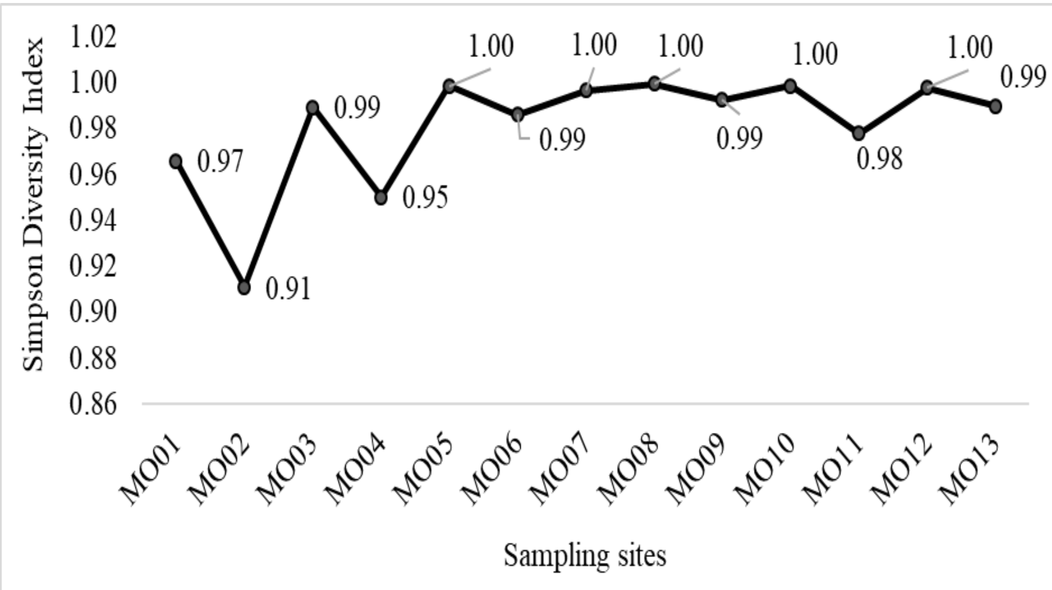


Figure 9: Simpson diversity index of river's mainstem

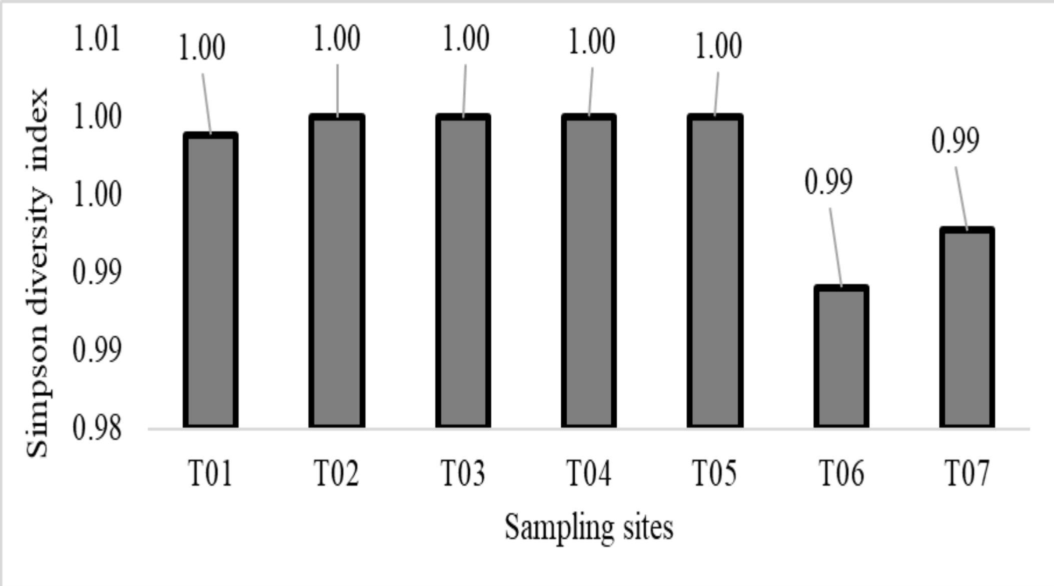


Figure 10: Simpson diversity index of tributaries

Species Evenness Index value of the River's mainstem varies from 0.48 to 0.90 and Evenness Index value of tributaries ranges from 0.44 to 0.80. In the River system the evenness value is higher in the sampling sites located at 1378m and minimum at sampling sites located at 848m. Similarly, the sampling site located at 1265m has higher eveness value and sampling sites located at 1533m elevation showed a lower eveness value. Assessment of evenness of a taxa showed a significant difference between sampling sites of river's mainstem (p=0.004).

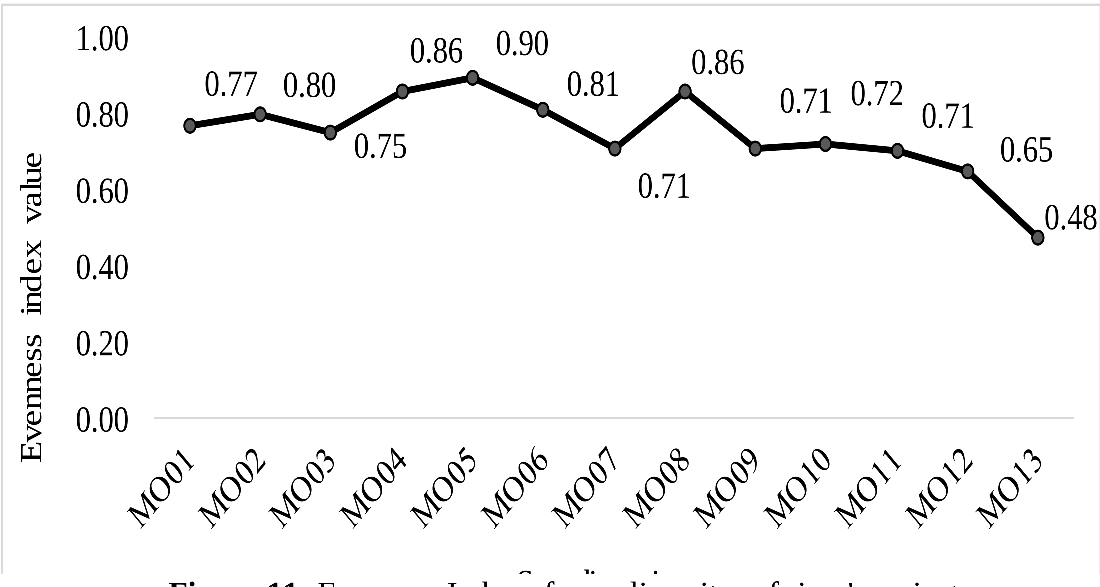


Figure 11: Evenness Index of sampling sites of river's mainstem

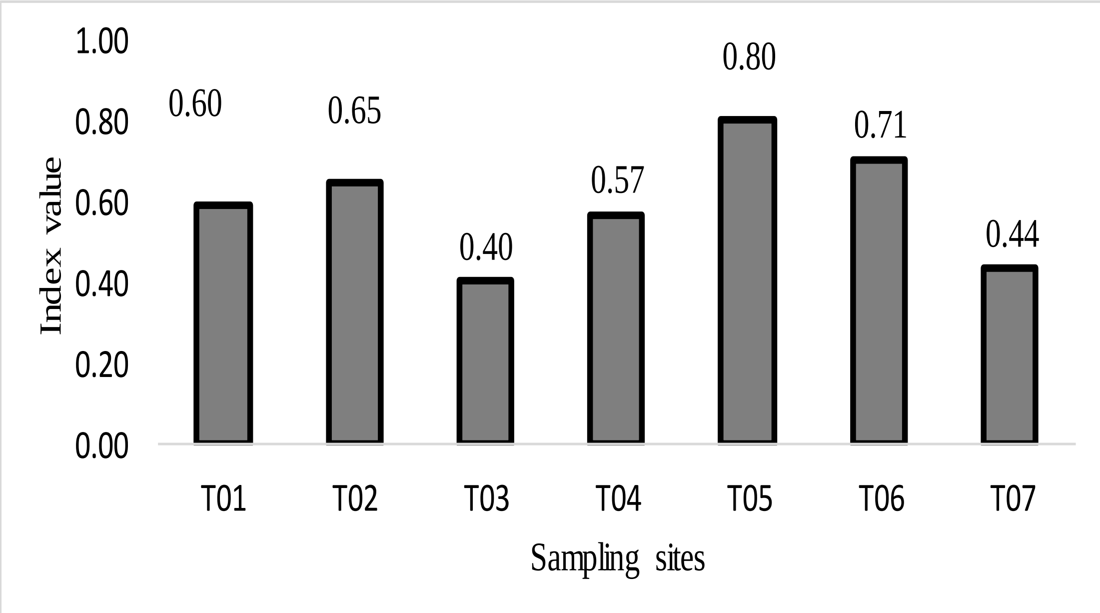


Figure 12: Evenness index of sampling sites of tributaries

4.2 Relationship between benthic macroinvertebrates and water quality

4.2.1 Water quality parameter

In total, 19 physcochemical parameters were measured at each site in of mainstem and its tributaries. The difference between the parameters of river and tributaries were tested by using Wilcoxon Rank-Sum Test in R. Depth (25.384±7.76 and 16.43±8.02,), velocity (0.5±0.21 and 0.13±0.05) and Dissolved Oxygen (8.26±2.67 and 7.23±0.71) of a river and tributaries differs significantly and the sample mean of a river is higher than the tributaries. Temperature in tributaries is higher (19.21±2.96 and 13.27±1.89) than the rivers and differs significantly. Similarly, other measured parameters EC, TDS, Turbidity, Nitrate, Phosphate, Ammonia, Free CO_2, Chloride, Alkalinity, Total Hardness, Calcium Hardness, Magnesium Hardness, BOD and COD from the rivers and tributaries do not differs significantly. The details of Min-Max observations, mean, standard deviation and the p-value is mentioned in the table below. The details of physic-chemical parameters of river's mainstem and tributaries is given in annex 3 and 4 respectively.

Table 5: Mean and SD of physico-chemical parameters and p value of Wilcoxon Rank Test to see the differences in river's mainstem and tributaries

Parameter	River		Tributaries		P-value
	Min-Max	Mean ±SD	Min-Max	Mean±SD	
Depth	15.00-40.00	25.38±7.76	10.00-30.00	16.43±8.02	0.0263*
Velocity	0.300-0.90	0.50±0.21	0.1-0.2	0.13±0.05	0.0002*
Temperature	9.10-16.20	13.27±1.89	15.1-24.1	19.21±2.96	0.0001*
DO	7.00-9.80	8.26±2.67	6.1-8.38	7.23±0.71	0.023*
pH	8.10-8.90	8.50±0.20	8.3-8.8	8.56±0.21	0.616
EC	155.00-190.00	165.61±10.70	65-368	183.86±102.55	0.475
TDS	77.50-95.00	82.80±5.35	32.5-184	91.93±51.28	0.475
Turbidity	64.00-301.00	182.44±71.35	3.62-10.50	6.74±2.58	0.00*
Nitrate	0.05-0.06	0.052±0.00	0.05-0.48	0.11±0.16	0.056
Phosphate	0.12-0.45	0.21±0.10	0.13-2.41	0.50±0.84	0.874
Ammonia	0.01-0.55	0.16±0.16	0.001-059	0.11±0.21	0.131
Free CO2	0.26-13.34	7.04±5.80	1.06-26.67	9.87±8.65	0.629
Chloride	0.00-3.79	1.78±1.49	0.19-4.73	1.88±1.73	0.903
Alkalinity	86.00-266.67	122.51±46.43	66.67-266.67	135.62±74.22	0.935
Total hardness	46.66-133.33	94.35±29.67	26.67-213.33	92.95±60.79	0.473

Calcium hardness	40.00-88.00	66.35±14.08	26.67-93.33	59.05±24.47	0.523
Magnesium hardness	1.62-13.01	6.83±4.32	0.00-29.28	8.27±10.08	0.661
BOD	1.14-3.54	2.21±0.74	1.09-3.54	2.56±0.80	0.526
COD	2.11-5.10	3.37±0.89	1.54-4.18	3.59±0.92	0.426

4.2.2 River Quality Class (RQC) based on Biological Index

The ecological status of Modi River basin ranged from Class I to Class II i.e not polluted to moderately polluted. The water quality of river's mainstem from 1038m-1654m elevation was not polluted while from 848m-992m elevation was moderately polluted. In the tributaries, water quality from 1533m -1764m and 931m-1265m elevation was moderately polluted while at the elevation of 1476 m, the water was not polluted. GRSBIOS assigned in given in annex 5.

Table 6: River quality Class of sampling sites calculated by using GRSBIOS

Mainstem/Tributaries	Site code	GRSBIOS/ ASPT	RQC	Description
River	MO01	7.00	I	Not polluted
	MO02	7.00	I	Not polluted
	MO03	6.66	I	Not polluted
	MO04	6.66	I	Not polluted
	MO05	6.88	I	Not polluted
	MO06	6.92	I	Not polluted
	MO07	7.56	I	Not polluted

	MO08	6.82	I	Not polluted
	MO09	7.09	I	Not polluted
	MO10	6.64	I	Not polluted
	MO11	6.35	II	Moderately polluted
	MO12	6.31	II	Moderately polluted
	MO13	5.91	II	Moderately polluted
Tributaries	T01	6.47	II	Moderately polluted
	T02	6.07	II	Moderately polluted
	T03	5.82	II	Moderately polluted
	T04	6.80	I	Not polluted
	T05	6.50	I	Moderately polluted
	T06	6.18	I	Moderately polluted
	T07	5.60	II	Moderately polluted

4.2.3 River quality map

The river quality of Modi River basin (River's mainstem and tributaries) are presented in the form of a water quality map, which is prepared based on GRSBIOS/ASPT

(Figure 2). The river quality is shown in the map using indicative colors and sign such as green for RWQC I (Not polluted) and Red for RWQC II (Moderately polluted).

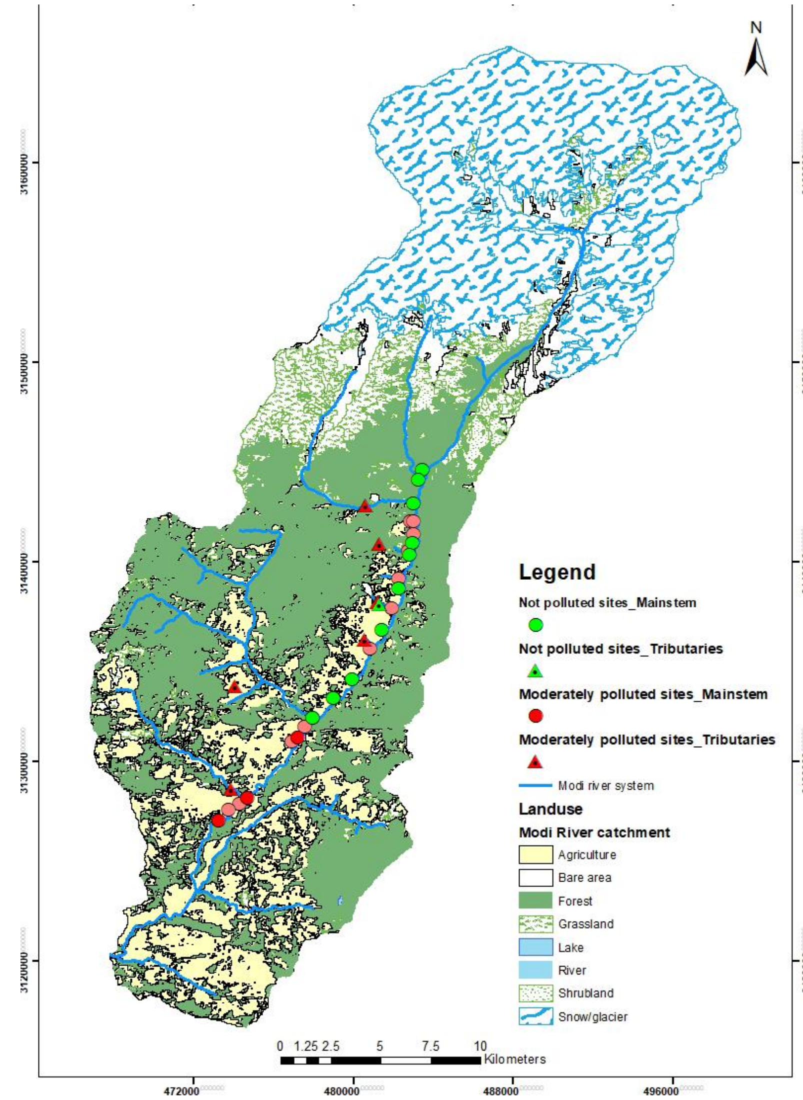


Figure 13: River quality map of sampling sites of Modi River system based on GRSBIOS

4.2.4 Relationship between benthic BMI and water quality

The RDA ordination explained the relationship between BMI present and environment variables. The collinearity test was conducted to assess the association between these environmental variables. RDA ordination was made with 11 environmental variables out of 19 variables (pH, temperature, ammonia, turbidity, nitrate, total hardness, EC, velocity, depth, TDS, Magnesium hardness) and BMI assemblages observed.

It showed that temperature was the most important variable governing the BMI composition which was followed by ammonia. First axis of RDA ordination explained 39.5 % variance in species composition data and 51.0 % variance in species and environmental relation. The length of arrow is proportion to the magnitude of change in RDA plot. RDA ordination shows total inertia: 1.00, sum of all eigen value: 1.00, and sum of all canonical eien value: 0.775. DCA ordination summary and relative importance of water characteristics on BMI composition analyzed based on RDA analysis is given in annex 7 and 8 respectively.

Abbreviations represent concatenated form of first four letters of family of BMI as presented in annex. Caenidae, Hydropsychidae showed positive relationship with pH. Scirtidae, Leptoceridae, showed positive relationship with ammonia while Empididae, Gerridae, Euphaeidae showed negative relationship. Nemouridae showed positive relationship with magnesium hardness and total hardness. Baetidae showed positive relation with temperature. Families such as Empididae, lepidostomatidae, polycentropodidae, Corydalidae, Simulidae, Tubificidae etc are confined towards Nitrate and showed strongly positive relationship. Blephariceridae showed positive relationship with depth. Perlodidae showed positive relationship with Electrical conductivity, TDS, Turbidity and velocity. (Figure 13).

Table 7: RDA ordination summary table

Axes		1	2	3	4	Total variance
Eigen values :		0.395	0.247	0.066	0.038	1.00
Species-environment correlations:	0.960	0.861	0.989	0.772		
Cumulative percentage variance						
of species data :		39.5	64.1	70.7	74.5	
of species-environment	51.0	82.8	91.2	96.1		

relation:						

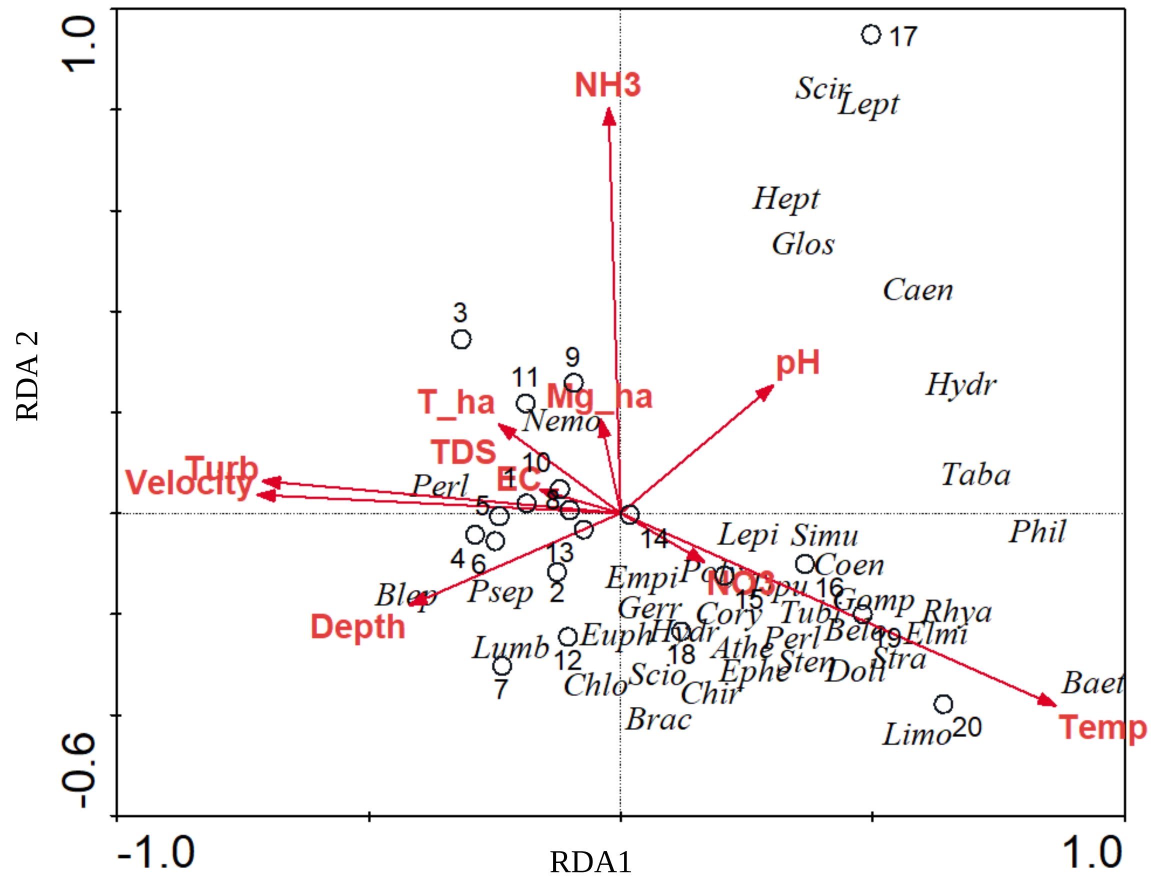


Figure 14: RDA biplot showing the relationship between BMI and environmental variables in Modi River system

4.3 Threats to aquatic ecosystems

The major stressors observed in Modi River basin are Sewage, sand and stone quarry, waste dumping, bathing and washing. The score were given based on the frequency (1-5) of occurrence of each stressor in each section of river. Score one was given to the value containing low frequency of stressor while five score were given having a high frequency of stressor. No such stressors were observed at site study 1 of river's mainstem and tributaries. The intensity and frequency of stressors increased with increase in human settlements. The stressors recorded in the study sites are shown in table 8.

Table 8: Stressors recorded in the site

Green: Very low	Yellow: Low	Orange:Medium	Red:High

Stressor group	Stressors	MO 01	MO 02	MO 03	MO 04	MO 05	MO 06	MO 07	MO 08	MO 09	MO 10	MO 11	MO 12	MO 13	T0 1	T0 2	T0 3	T0 4	T0 5	T0 6	T0 7
Solid waste	Waste dumping	0	0	0	0	0	0	0	0	1	0	3	0	4	0	0	0	0	2	4	4
	Cremation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Effluents	Sewage	0	0	0	0	0	0	0	0	1	4	3	2	3	0	0	1	1	2	2	4
	Agricultural effluents	0	0	0	0	0	0	0	1	0	1	3	0	0	0	0	0	0	2	1	4
	Industrial effluent	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Landfill leachate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Activities and facilities	Squatter settlements	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	Picnic spots close to river	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0
	Vehicle crossing along river	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Littering by picnic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0

	goers																				
Hydromorphological degradation and ecological degradation	Channel, embankment and weir	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	2
	Bank cutting	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2
	Reservoir, dam and impoundment	0	0	0	0	0	0	0	0	0	3	0	0	4	0	0	0	0	0	0	0
	Irrigation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Fishing	0	1	1	1	1	1	1	1	1	0	0	1	0	0	0	0	1	1	4	1
	Stone quarry and crushing	0	0	0	0	0	0	2	2	0	4	4	3	3	0	0	0	0	0	0	2
	Sand quarry	0	0	0	0	1	1	2	2	0	4	4	3	3	0	0	0	0	0	0	2
Personal Hygiene and Sanitation	Bathing and Washing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4
	Open Defecation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3
Others	Road construction	0	0	2	0	0	0	0	0	0	0	2	0	0	0	0	0		0	4	3
	Total	**0**	**1**	**3**	**1**	**3**	**2**	**5**	**8**	**3**	**16**	**19**	**11**	**17**	**0**	**0**	**2**	**2**	**7**	**29**	**35**

CHAPTER V: DISCUSSION

5.1 Taxa richness and water quality

In our study we found higher taxa richness and abundance in tributaries than in river's mainsteam. This result is similar to the recent study conducted in the river's mainstem and tributaries of Karnali River Basin (Tachamo Shah et al. 2020). The main reason is the high water discharge and volume in the river’s mainstem where mineral habitats are tightly bounded making colonization of macroinvertebrates unfavorable. In this study we found Ephemerellidae, Heptageniidae, Baetidae and Caenidae family belonging to order Ephemeroptera and Perlidae, Perlodidae, Nemouridae and Chloroperlidae family belonging to order Plecoptera and Leptoceridae, Lepidostomatidae, Glossomatidae, Hydropsychidae, Philopotamidae, Polycentropodidae, Rhyacophilidae, Stenopsychidae and Brachycentridae belonging to order Trichoptera. These are the very common fresh water taxa observed in former studies (Nautiyal et al. 2004). In Modi River system we observed Ephemeroptera and Plecoptera at higher elevations both in river's mainstem and tributaries at high water quality sites. Both of these taxa are considered as the pollution sensitive taxa and are indicators of clean water (Alam et al. 2008). Whereas Diptera is considered as pollution tolerant group (Dhakal 2006). As per (Hamid et al. 2017), presence of EPT denotes that water quality parameters in the habitat are within the tolerance limit. In many instances, active anthropogenic activities nearby river can have an effect on abundance and diversity of EPT (Ghani et al. 2016). Overall, the finding of this research matched with previous studies conducted in different places.

Though, GRSBIOS result showed that, water quality ranges from not polluted to moderately polluted, all three high water quality taxa were also observed in moderately polluted sites. Their abundance is higher at upstream of river's mainstem and tributaries which was similar to the previous study conducted by (Gabriels et al. 2010).

The abundance of families belonging to Ephemeroptera in moderately polluted sites is relatively higher then Plecoptera and Tricoptera. Only few families of later two taxa were recorded in few moderately polluted sites. The reason might be the lower level of pollution even if included in moderately polluted sites. Also, the local site specific pollution might be generalized or taxa might be observed in less polluted microhabitats. 9 families belonging to order Tricoptera were observed in all the sampling sites. This finding is similar to previous study conducted in Nepal streams (Rundle et al. 1993, Ormerod et al. 1994, Suren 1994, Jiang et al. 2014).

Our study depicted 11 families of Diptera in both river's main stem and tributaries but they are highly abundant in tributaries than in river. Among the sampling sites within the tributaries this taxa is more abundant in moderately polluted sites. The presence of Diptera indicated the water pollution in comparison to other sites. In comparison to river, tributaries are found to be moderately polluted. These sites contain low DO, high BOD and COD so these sites should be polluted. Similar result was observed by Alavaisha et al. 2019 in Tanzania. In addition, (Sharma et al. 2008) observed a high distribution of Diptera in polluted water in the Ninglad stream of Uttarkhanda, India. Therefore, the distribution of Diptera seems logical. Furthermore, three families Gomphidae, Coenagrionidae and Euphaeidae of order Odonota was observed which are more abundant in tributaries and moderately polluted sites. Previous study showed that Odonota is moderate pollution tolerant (Ganguly et al. 2018). Therefore, its distribution is similar to the finding of earlier research conducted by (Ganguly et al. 2018) in Mahanadi river of India. Though Hemiptera can exist in a wide range of water quality conditions or moderate water quality (Bagalwa et al. 2019), Belostomatidae and Gerridae families belonging to order Hemiptera was recorded only in one sampling sites of tributaries.

GRSBIOS indicators showed the water quality in both river and tributaries ranges from not polluted to moderately polluted and most of the pollution observed are site specific. Therefore, most of the taxa was observed in both river and tributaries except Hemiptera (Belostomatidae and Germidae) which was found to be absent in the river whereas Lumbriculida (Lumbriculidae) was found to be absent in the tributaries. Belostomatidae and Germidae families were observed only in one sampling sites of tributaries and Lumbriculidae was observed in only two sampling sites. Both taxa are the inhabitant of the stagnant water therefore they are recorded only in few sampling sites.

5.2 Taxa richness along altitudinal gradient

This research showed that taxonomic richness decreased with the increasing altitudinal gradient. We observed only 9 families of BMI at the sampling sites of highest elevation of river's mainstem. This was the lowest number of taxa among all the sampling sites. The sampling sites were grouped into four different categories of 250m interval. Tukey HSD test showed the difference between the groups. Group 4 and group 2 and group 4 and group 3 differed significantly. Group 1 and 4, group 1 and 2 and group 1 and group 3 did not differ significantly because of the low diversity than expected in group 1. Sampling sites in group 4 showed the minimum average number of families followed by the group 3 and 2 located at lower elevations. However, lower number of average families in group 1 was observed at lowest elevations. The main reason of observation of lowest number of average families even in the lowest elevation is due to the high anthropogenic pressure. Most of the sampling sites in group 1 were either in the downstream of hydropower or the sites of extraction of sand, gravel and stones from river.

Similar studies were conducted in several locations across the world. Tonkin et al. (2017) conducted community structuring along the large altitudinal gradient in the central and eastern Himalayas of Nepal and they also observed attitudinal effect on the community structure of macroinvertebrate community. Jun et al. (2016) also observed a similar result in South Korean rivers. They found the variability among the macroinvertebrate-based stream groups was more prominently explained by the altitudinal gradients together with streambed composition and water velocity than chemical variables. Therefore, finding from this research is similar to other studies conducted in Nepal and other parts of Asia. Though altitude shows effect on taxa richness, it may not only be the driver. Various other chemical, physical and biotic parameters may be the factor that changes with elevation, including dissolved oxygen, temperature (Jacobsen 2008) and anthropogenic pressure. Our study also showed low taxa richness at the sites of high anthropogenic pressure.

Elevation is a major organizational gradient of biodiversity in the Himalayas (Vetaas and Grytnes 2002, Baniya et al. 2010, Tonkin et al. 2017) and elsewhere (Rahbek 1995, Lomolino 2001, Tonkin et al. 2017). Wang et al. (2014) found invertebrates, diatoms and bacteria were structured through a combination of environmental and spatial (including elevation and geographical distance) factors along a large elevational stream gradient. The clear importance of elevation in streams of this region has recently been supported at both population, genetic (Hoppeler et al., 2016) and community (Tachamo Shah et al., 2015) levels. Considering the finding of a previous study, we also hypothesized that the taxa richness of freshwater macroinvertebrate differs with elevation gradient. We observed significant difference in taxa richness with elevation.

5.3 Diversity measure

In Modi River system, Shannon diversity index ranges from 1.19 to 2.64. As the Shannon diversity index value normally ranges from 1.5-3.5 (Magurrun 2004) and a threshold value of 2 is a minimum value above which an ecosystem can be regarded as medium to highly diverse (Mwakalukwa et al. 2014). This indicates the sites of Modi River system are low to medium diversity. Among the sampling sites the average Shannon diversity indexes decreases with the altitude, this shows that lower altitudes are more diverse than the higher one. From the altitudinal range of 1800 to 1050, Shannon diversity index increases with decreasing altitude. Among four groups, groups 2 and 3 have higher average diversity than the minimum threshold but group 4 and group 1 has less then minimum threshold value. In group 4 the Shannon diversity index value is less than the minimum threshold limit. The reason might be the effect of altitude at higher elevations. Similarly, most of the sampling sites in group 1 were taken downstream to hydropower and other stressors such as road construction, bathing, washing, sewage disposal etc. Therefore, the minimum average Shannon diversity in group 1 might be the impact of stressors.

This finding contradicts with the previous result from (Sharma et al. 2005). They reported dam building had significant impacts on the macroinvertebrate composition just above the dam site, probably as a result of deposition of inorganic material within the small reservoir and changes in water speed. Damming of the Tinau River thus seems only to have a relatively minor impact on the river biota downstream of the dam site. Our result also showed a low family richness downstream to the hydropower construction sites. This might be the effects of dams with high sediment loads and low competent flood events resulting in fine sediment accumulation, these aggradations reduces taxa richness, diversity and macroinvertebrates density, and only high sediment-tolerant species may increase. Our finding match with former study conducted by (Takao et al. 2008) and (Bona et al. 2008) who observed less taxon richness and reduction in diversity assemblage towards the downstream of the hydro project dam.

We observed Ephemeroptera, Trichoptera, Diptera, and Plecoptera as dominant taxa in the research sites. These taxa were found to be distributed up to higher elevations of Modi River system. As these taxa are the major components of the benthic macro invertebrate community (Hynes 1970). They are also known as the components of high mountain stream fauna and have been reported in glacial head waters of the Akbura River of the Tien shan Mountains in middle Asia at 3670 m asl despite water temperature (Ward 1994). Therefore, our finding matches with the former studies.

5.4 Water quality parameters

The present study showed that temperature is most important and limiting factor for the aquatic environment. The average temperature of tributaries is observed higher than the river's mainstem. The variation of temperature in river's mainstem and tributaries could have been attributed as it has been measured at different time of the day. Muller et al. 2015 has stated that temperature fluctuation in river water depends mainly on the longitudinal change, season and time of sampling. It has been found that temperature increases with decreasing elevation and velocity decreases with decreasing elevation. This result is similar to the previous research finding conducted by (Jiang et al. 2014) in Tibetan rivers. They also found that the river temperature increases with decreasing elevation and water velocity decreases with decreasing elevation. Conductivity in Modi River basin decreased from high altitude to low altitude in both river and tributaries. This is similar to the result of previous study conducted in Niyang River in China (Lu et al. 2011, Jiang et al. 2014). However, as per (Kefford 1998), increase in temperature and dissolved ions, increases the conductivity i.e. the warmer the water, the higher the conductivity. Contradiction of this finding could be due to sampling in different time period or effect of microclimate.

The pH of both river and tributaries were found to be alkaline (8.1-8.9) and the pH do not differs significantly between the rivers and tributaries. The alkaline pH could be contributed by the presence of limestone and carbonates in the upstream of the river (English et al. 2000). DO is another important indicator of river's health. Running waters are known to contain higher DO values than stagnant water bodies. The DO concentration was higher in rivers than in the tributaries of Modi River system. DO is also affected by temperature and there exists a reciprocal relationship between DO and temperature (Wetzel 2001). High water velocity was observed in the river than in the tributaries. Therefore, the higher DO in the river seems logical. In addition, the temperature of tributaries is higher than that of the river so the comparatively low DO in tributaries is justifiable. Therefore, DO differs significantly between the tributaries and the rivers ($p=0.002$).

In this study total alkalinity was found to be 86-266 mg/L. The total alkalinity greater than 90 mg/L in water bodies is considered as highly productive (Jhingran 1991). So, it shows that the site is suitable of aquatic life. Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) are two common measures of water quality used to gauge the degree of organic matter pollution of an aquatic system (Zaghloul et al. 2019). BOD is a measure of the amount of oxygen removed from aquatic environments by aerobic micro-organisms during the breakdown of organic substances while COD is a measure of the oxygen equivalent of the organic matter in a water sample that is susceptible to oxidation by a strong chemical oxidant (Radojevic and Bashkin, 1999). High BOD and COD depict low Dissolved Oxygen (DO) in the water. Though the BOD and COD both do not differ between the river and tributaries the average value of BOD is slightly higher than that of river. Similarly, the average value of COD in both rives and tributaries are higher than average BOD. This shows the BOD has more influence in the tributaries to reduce DO then COD.

Patrick et al. 2015 reported that physico-chemical parameters like conductivity, turbidity, pH, NO_2- and temperature increased from upstream to downstream. These parameters of water were most associated with the distribution of benthic macro invertebrate taxa (Lewin et al. 2014). A great number of studies: for conductivity and pH (Lewin et al. 2014), for temperature and turbidity (Meutter et al. 2005) revealed the role of these parameters in structuring of macro invertebrate assemblages. Patric et al. (2015) also explained that PO_4^{2-}, Ca^{2+} and Mg^{2+} displayed an opposite evolution. This could be resulted probably from changing activities and nature of materials that are dumped along the stream. Subsistence agriculture and organic material were observed in the upper stream and those could be explained by highest values of NO_3^-, PO_4^{2-} and Mg^{2+} to those sites.

5.5 Relationship between benthic macroinvertebrates and water quality

In Modi River basin, different family showed a relationship with different physio-chemical parameter. Out of 19 different parameters assessed, temperature is the most influential parameter to determine macroinvertebrate community assemblage. Highest number of a family showed a strong relationship with temperature. This result is similar to the previous study conducted by Yazdian et al. (2014). Clarke (1997) examined the relationship between chemical and physical parameters and density of macroinvertebrates and found a significant relationship between invertebrate bio-mass and temperature in the studied streams. Therefore, the influence of temperature resembles with other several studies. According to (Vannote et al. 1980), temperature influences the distribution, abundance and richness of aquatic organism along the gradients in latitude and altitude. In addition, some family showed a positive relationship with pH, BOD, Free CO_2, alkalinity, Phosphate and turbidity. Furthermore, (Graca et al. 2004) observed the streams with lower pH, conductivity and alkalinity had the higher species diversity. Thus, the influence of different physico-chemical parameters on macroinvertebrate assemblage is site specific and influenced by microclimate and human disturbance.

CHAPTER VI: CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Altogether 40 families belonging to 10 different orders were recorded in 20 sampling sites. Out of 13 sampling sites in River's mainstem, 29 families of 8 orders were recorded while 39 families of 9 orders were observed in 7 sampling sites of tributaries. Only 9 families were found at the sampling sites of highest altitude located at 1654m of a river's mainstem where as 16 families were observed at the highest altitude of tributaries located at 1764m elevation. Wilcoxon Rank-Sum Test showed that the Shannon diversity index is higher in river than in tributaries and differed significantly but Simpshon diversity and species evenness did not differ significantly between the river and tributaries. Water quality parameters like depth, velocity, temperature, DO and turbidity differed significantly between the river and tributaries. One way ANOVA showed that family richness along the altitudinal gradient differs significantly. Increase in elevation decreases the family richness. The sampling sites located downstream to the hydropower construction and high stressors showed a low family richness than other sampling sites. Multivariate analysis (RDA) showed that temperature has the strong relationship on the distribution of BMI family.

GRSBIOS showed the samplings sites were in I and II categories i.e. not polluted to moderately polluted. Sewage disposal, sand mining and bathing and washing were the major threats observed in the river and tributaries. This study showed that water quality of Modi River basin ranges from not polluted to moderately polluted. It shows that diversity of BMI increases with good water quality. Taxa richness was observed higher in tributaries than in River's mainstem and taxa richness decreases with increasing altitude. Sites with high anthropogenic stressor have lower taxa richness.

6.2 Recommendation

As Modi river system has been facing the impact of hydro-powers, collection of river bed materials, road constructions in its catchment, human waste and pesticides in agriculture land, it unique site to study the impact of multiple stressors on the diversity and distribution of freshwater macroinvertebrates. Based on this study following points are recommended for the improvement and get the wider prospective on diversity and distribution of freshwater macroinvertebrate in Modi river system.

- Study should be conducted in both pre-monsoon and post-monsoon season to get diversity of year by increasing the sampling intensity and distribution.
- Expanding the sampling intensity across habitats and land use types has a high probability to record additional taxa from the study area.
- Coverage on altitudinal gradient up to the origin of river and tributaries gives clearer picture of taxa distribution along the altitudinal gradient.
- Long term study is required for better understanding of eco-hydrological relationship and its effect on macroinvertebrate distribution.

REFERENCES

Ab Hamid, S., & Rawi, C. S. M. (2017). Application of aquatic insects (Ephemeroptera, Plecoptera and Trichoptera) in water quality assessment of Malaysian headwater. *Tropical life sciences research*, *28*(2), 143.

Abell, R. (2002). Conservation biology for the biodiversity crisis: a freshwater follow-up. *Conservation Biology*, *16*(5), 1435-1437.

Alam, M. S., Hoque, M. M., Bari, M. F., Badruzzaman, A. B. M., Huber, T., & Fliedl, B. (2008). Aquatic macro-invertebrates as bio-indicators: A new approach for river water quality assessment in Bangladesh. In *ASSESS-HKH Proceedings of the Scientific Conference on the Ecology and Environmental Assessment of Rivers in the Hindu Kush-Himalaya* (pp. 65-76).

Alavaisha, E., Lyon, S. W., & Lindborg, R. (2019). Assessment of water quality across irrigation schemes: A case study of wetland agriculture impacts in Kilombero Valley, Tanzania. *Water, 11*(4), 671.

Allen, D. J. (2010). *The status and distribution of freshwater biodiversity in the Eastern Himalaya*. IUCN.

Altermatt, F. (2013). Diversity in riverine metacommunities: a network perspective. *Aquatic Ecology*, *47*(3), 365-377.

APHA. (2005). Standard methods for the examination of water and wastewater. *American Public Health Association (APHA): Washington, DC, USA*, *21*.

Armitage, P. D., Moss, D., Wright, J. F., & Furse, M. T. (1983). The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-water sites. *Water research*, *17*(3), 333-347.

Bagalwa, M. & Mukumba, I. & Ndahama, N. & Zirirane, Dieudonné & Kalala, A.O. (2019). Assessment of River Water Quality using Macroinvertebrate Organisms as Pollution Indicators of Cirhanyobowa River, Lake Kivu, DR Congo. *International Journal of Current Microbiology and Applied Sciences*. 8. 2668-2680. 10.20546/ijcmas.2019.804.310.

Baniya, C. B., Solhøy, T., Gauslaa, Y., & Palmer, M. W. (2010). The elevation gradient of lichen species richness in Nepal. *The Lichenologist*, *42*(1), 83-96.

Barbour, M. T. (2008). The societal benefit of biological assessment and monitoring in rivers. In *ASSESS-HKH: Proceedings of the scientific conference "Rivers in the Hindu Kush–Himalaya—Ecology & Environmental Assessment* (pp. 5-7).

Barrantes, G., & Sandoval, L. (2009). Conceptual and statistical problems associated with the use of diversity indices in ecology. *Revista de biología tropical*, *57*(3), 451-460.

Bona, F., Falasco, E., Fenoglio, S., Iorio, L., & Badino, G. (2008). Response of macroinvertebrate and diatom communities to human- induced physical alteration in mountain streams. *River Research and applications*, *24*(8), 1068-1081.

Brewin, P. A., Newman, T. M., & Ormerod, S. J. (1995). Patterns of macroinvertebrate distribution in relation to altitude, habitat structure and land use in streams of the Nepalese Himalaya. *Archiv für Hydrobiologie*, 79-100.

Bruns, D. A., Minshall, G. W., Cushing, C. E., Cummins, K. W., & Brock, J. T. (1984). Tributaries as modifiers of the river continuum concept: analysis by poplar ordination and regression models. *Archiv fur hydrobiology*.

Bunn, S. E., & Arthington, A. H. (2002). Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environmental management*, *30*(4), 492-507.

Buss, D. F., Baptista, D. F., Silveira, M. P., Nessimian, J. L., & Dorvillé, L. F. (2002). Influence of water chemistry and environmental degradation on macroinvertebrate assemblages in a river basin in south-east Brazil. *Hydrobiologia*, *481*(1), 125-136.

Cairns, J., & Pratt, J. R. (1993). A history of biological monitoring using benthic macroinvertebrates. *Freshwater biomonitoring and benthic macroinvertebrates*, *10*, 27.

Carter, J. L., Fend, S. V., & Kennelly, S. S. (1996). The relationships among three habitat scales and stream benthic invertebrate community structure. *Freshwater Biology*, *35*(1), 109-124.

Ceschin, S., Aleffi, M., Bisceglie, S., Savo, V., & Zuccarello, V. (2012). Aquatic bryophytes as ecological indicators of the water quality status in the Tiber River basin (Italy). *Ecological indicators*, *14*(1), 74-81.

Clarke, K. D., & Scruton, D. A. (1997). The benthic community of stream riffles in Newfoundland, Canada and its relationship to selected physical and chemical parameters. *Journal of Freshwater Ecology*, *12*(1), 113-121.

Craig, L. S., Olden, J. D., Arthington, A. H., Entrekin, S., Hawkins, C. P., Kelly, J. J., ... & Wooten, M. S. (2017). Meeting the challenge of interacting threats in freshwater ecosystems: A call to scientists and managers. *Elementa: Science of the Anthropocene*, *5*.

de Freitas Nunes-Neto, N., do Carmo, R. S., & El-Hani, C. N. (2016). Biodiversity and ecosystem functioning: An analysis of the functional discourse in contemporary ecology. *Filosofia e História da Biologia*, *11*(2), 289-321.

Dhakal, S. (2006). Study on Physiochemical Parameters and Benthic Macroinvertibrates of Balkhu Khola in Kathmandu Valley, Central Nepal. *Management of Water, Wastewater and Environment: Challenges for the Developing Countries, Kathmandu*.

Downes, B. J., & Keough, M. J. (1998). Scaling of colonization processes in streams: parallels and lessons from marine hard substrata. *Australian Journal of Ecology*, *23*(1), 8-26.

Downes, B. J., Lake, P. S., & Schreiber, E. S. G. (1993). Spatial variation in the distribution of stream invertebrates: implications of patchiness for models of community organization. *Freshwater Biology*, *30*(1), 119-132.

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z. I., Knowler, D. J., Lévêque, C., ... & Sullivan, C. A. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological reviews*, *81*(2), 163-182.

Egglishaw, H. J. (1980). Benthic invertebrates of streams on the Alburz Mountain Range near Tehran, Iran. *Hydrobiologia*, *69*(1), 49-55.

English, N. B., Quade, J., DeCelles, P. G., & Garzione, C. N. (2000). Geologic control of Sr and major element chemistry in Himalayan Rivers, Nepal. *Geochimica et Cosmochimica Acta*, *64*(15), 2549-2566.

Gabriels, W., Lock, K., De Pauw, N., & Goethals, P. L. (2010). Multimetric Macroinvertebrate Index Flanders (MMIF) for biological assessment of rivers and lakes in Flanders (Belgium). *Limnologica-Ecology and Management of Inland Waters*, *40*(3), 199-207.

Ganguly, I., Patnaik, L., & Nayak, S. (2018). Macroinvertebrates and its impact in assessing water quality of riverine system: A case study of Mahanadi river, Cuttack, India. *Journal of Applied and Natural Science*, *10*(3), 958-963.

Ghani, W. M. H. W. A., Rawi, C. S. M., Abd Hamid, S., & Al-Shami, S. A. (2016). Efficiency of different sampling tools for aquatic macroinvertebrate collections in Malaysian streams. *Tropical Life Sciences Research*, *27*(1), 115.

Gurung, S., Singh, R., Wagle, B., Jha, B. R., Khatri, K., & Jacobsen, D. (2021). Macroinvertebrate assemblages in mountain tributaries of glacial-fed and rain-fed rivers in eastern Nepal. *Nepal Journal of Environmental Science*, *9*(2), 45-55.

Heino, J. (2005). Functional biodiversity of macroinvertebrate assemblages along major ecological gradients of boreal headwater streams. *Freshwater Biology*, *50*(9), 1578-1587.

Heino, J., Parviainen, J., Paavola, R., Jehle, M., Louhi, P., & Muotka, T. (2005). Characterizing macroinvertebrate assemblage structure in relation to stream size and tributary position. *Hydrobiologia*, *539*(1), 121-130.

Holt, C. R., Pfitzer, D., Scalley, C., Caldwell, B. A., & Batzer, D. P. (2015). Macroinvertebrate community responses to annual flow variation from river regulation: An 11- year study. *River Research and Applications*, *31*(7), 798-807.

Hoppeler, F., Tachamo Shah, R. D., Shah, D. N., Jähnig, S. C., Tonkin, J. D., Sharma, S., & Pauls, S. U. (2016). Environmental and spatial characterisation of an unknown fauna using DNA sequencing–an example with Himalayan Hydropsychidae (Insecta: Trichoptera). *Freshwater Biology*, *61*(11), 1905-1920.

Hynes, H. B. N. (1970). *The ecology of running waters* (Vol. 543). Liverpool: Liverpool University Press.

Jacobsen, D. (2003). Altitudinal changes in diversity of macroinvertebrates from small streams in the Ecuadorian Andes. *Archiv fur Hydrobiologie*, *158*(2), 145-168.

Jacobsen, D. (2004). Contrasting patterns in local and zonal family richness of stream invertebrates along an Andean altitudinal gradient. *Freshwater Biology*, *49*(10), 1293-1305.

Jacobsen, D. (2008). Low oxygen pressure as a driving factor for the altitudinal decline in taxon richness of stream macroinvertebrates. *Oecologia*, *154*(4), 795-807.

Jacobsen, D., Rostgaard, S., & Vásconez, J. J. (2003). Are macroinvertebrates in high altitude streams affected by oxygen deficiency?. *Freshwater Biology*, *48*(11), 2025-2032.

Jha, B. R., Waidbacher, H., Sharma, S., & Straif, M. (2010). Study of substrate and physico-chemical base classification of the rivers of Nepal. *Geo-Spatial Information Science*, *13*(1), 70-76.

Jhingran, V. G. (1991). Fish and Fisheries of India, Hindustan Pub. *Co.*, *New Delhi*, *727*.

Jiang, X., Xie, Z., & Chen, Y. (2013). Longitudinal patterns of macroinvertebrate communities in relation to environmental factors in a Tibetan-Plateau river system. *Quaternary International*, *304*, 107-114.

Jun, Y. C., Kim, N. Y., Kim, S. H., Park, Y. S., Kong, D. S., & Hwang, S. J. (2016). Spatial distribution of benthic macroinvertebrate assemblages in relation to environmental variables in Korean nationwide streams. *Water*, *8*(1), 27.

JVS, J. V. S., & Nepal, G. W. P (2016). Assessment of the Environmental Flow in the Gandaki River Basin: A Case of Modi Khola.

Kefford, B. J. (1998). The relationship between electrical conductivity and selected macroinvertebrate communities in four river systems of south-west Victoria, Australia. *International Journal of Salt Lake Research*, *7*(2), 153-170.

Kenney, M. A., Sutton-Grier, A. E., Smith, R. F., & Gresens, S. E. (2009). Benthic macroinvertebrates as indicators of water quality: The intersection of science and policy. *Terrestrial Arthropod Reviews*, *2*(2), 99.

Korte, T., Baki, A. B. M., Ofenböck, T., Moog, O., Sharma, S., & Hering, D. (2010). Assessing river ecological quality using benthic macroinvertebrates in the Hindu Kush-Himalayan region. *Hydrobiologia*, *651*(1), 59-76.

LeCRAW, R. O. B. I. N., & Mackereth, R. (2010). Sources of small- scale variation in the invertebrate communities of headwater streams. *Freshwater Biology*, *55*(6), 1219-1233.

Lewin, I., Jusik, S., Szoszkiewicz, K., Czerniawska-Kusza, I., & Ławniczak, A. E. (2014). Application of the new multimetric MMI_PL index for biological water quality assessment in reference and human-impacted streams (Poland, the Slovak Republic). *Limnologica*, *49*, 42-51.

Li, F., Chung, N., BAE, M. J., KWON, Y. S., & PARK, Y. S. (2012). Relationships between stream macroinvertebrates and environmental variables at multiple spatial scales. *Freshwater Biology*, *57*(10), 2107-2124.

Li, L., Zheng, B., & Liu, L. (2010). Biomonitoring and bioindicators used for river ecosystems: definitions, approaches and trends. *Procedia environmental sciences*, *2*, 1510-1524.

Ligeiro, R., Melo, A. S., & Callisto, M. (2010). Spatial scale and the diversity of macroinvertebrates in a Neotropical catchment. *Freshwater Biology*, *55*(2), 424-435.

Linli, L., Ciren, N., & Weichen, W. (2011). Analysis on runoff spatiotemporal distribution of Niyang River. *Water Power*, *37*(2), 5-7.

Lomolino, M. V. (2001). Elevation gradients of species- density: historical and prospective views. *Global Ecology and biogeography*, *10*(1), 3-13.

Lytle, D. A., & Poff, N. L. (2004). Adaptation to natural flow regimes. *Trends in ecology & evolution*, *19*(2), 94-100.

Magurran, A. E. (1988). *Ecological diversity and its measurement*. Princeton university press.

Magurran, A. E. (2004). Measuring Biological Diversity. Oxford: Blackwell Publishing. 256 p.

Mantyka- Pringle, C. S., Martin, T. G., Moffatt, D. B., Linke, S., & Rhodes, J. R. (2014). Understanding and predicting the combined effects of climate change and land- use change on freshwater macroinvertebrates and fish. *Journal of Applied Ecology*, *51*(3), 572-581.

Matangulu, M., Gurung, S., Prajapati, M., & Jyakhwo, R. (2017). Macroinvetebrate assemblages as indicators of water quality of the West Seti River, Bajhang, Nepal. *International Journal of Environment*, *6*(3), 25-45.

Moog, O. (2007). Manual on pro-rata multi-habitat-sampling of benthic invertebrates from wadeable rivers in the HKH-region. *Deliverable 8, Part, 1*.

Moog, O., Hering, D., Sharma, S., Stubauer, I., & Korte, T. (2008). Water quality assessment of River Satluj using benthic macroinvertebrates. *Rivers in the Hindu Kush-Himalaya–Ecology & Environmental Assessment*, 77.

Moog, O., Hering, D., Sharma, S., Stubauer, I., & Korte, T. (2008). Results and consequences of the ASSESS-HKH Research Project in Nepal. *Rivers in the Hindu Kush-Himalaya–Ecology & Environmental Assessment*, 55.

Müller, D., Warneke, T., Rixen, T., Müller, M., Jamahari, S., Denis, N., ... & Notholt, J. (2015). Lateral carbon fluxes and CO 2 outgassing from a tropical peat-draining river. *Biogeosciences*, *12*(20), 5967-5979.

Mwakalukwa, E. E., Meilby, H., & Treue, T. (2014). Floristic composition, structure, and species associations of dry miombo woodland in Tanzania. *International Scholarly research notices*, *2014*.

Naiman, R. J., Melillo, J. M., Lock, M. A., Ford, T. E., & Reice, S. R. (1987). Longitudinal patterns of ecosystem processes and community structure in a subarctic river continuum. *Ecology*, *68*(5), 1139-1156.

Nally, R. M., Lloyd, N. J., & Lake, P. S. (2006). Comparing patterns of spatial autocorrelation of assemblages of benthic invertebrates in upland rivers in south-eastern Australia. *Hydrobiologia*, *571*(1), 147-156.

National Trust for Nature Conservation (NTNC). (2009). *Management plan of Annapurna Conservation Area (2009-2012).*

Nautiyal, P., Kala, K. & Nautiyal, R. 2004. A preliminary study of the diversity of diatoms in streams of Mandakini basin (Garhwal Him alaya). 235-269. In: Poulin, M. (ed.) Proceedings of 17th International Diatom Sym posium 2002 Ottawa. Biopress Ltd, Bristol.

Nepal, W.W.F. (2013). Chitwan annapurna landscape (CHAL): a rapid assessment. *World Wildlife Fund Nepal: Kathmandu, Nepal*.

Nesemann, H., Shah, R. D. T., & Shah, D. N. (2011). Key to the larval stages of common Odonata of Hindu Kush Himalaya, with short notes on habitats and ecology. *Journal of threatened Taxa*, 2045-2060.

Nesemann, H., Sharma, S., Sharma, G., Khanal, S. N., Pradhan, B., Shah, D. N., & Tachamo,R. D. (2007). Aquatic Invertebrates of the Ganga River System, *Mollusca*, *Annelida, Crustacea* , *Volume 1*. Hardcover, 263 pp. Kathmandu, Nepal.

Nieto, C., Ovando, X. M., Loyola, R., Izquierdo, A., Romero, F., Molineri, C., & Miranda, M. J. (2017). The role of macroinvertebrates for conservation of freshwater systems. *Ecology and evolution*, *7*(14), 5502-5513.

Ofenböck, T., Moog, O., & Sharma, S. (2008). Development and application of the HKH Biotic Score to assess the river quality in the Hindu Kush-Himalaya region. In *Proceedings of the Scientific Conference Rivers in the Hindu Kush-Himalaya-The Ecology & Environmental Assessment* (pp. 17-32).

Omayio, D., & Mzungu, E. (2019). Modification of shannon-wiener diversity index towards quantitative estimation of environmental wellness and biodiversity levels under a non-comparative Scenario. *Journal of Environment and Earth Science*, *9*(9), 46-57.

Ormerod, S. J., Rundle, S. D., Wilkinson, S. M., Daly, G. P., Dale, K. M., & Juttner, I. (1994). Altitudinal trends in the diatoms, bryophytes, macroinvertebrates and fish of a Nepalese river system. *Freshwater biology*, *32*(2), 309-322.

Patrick, M. S., Jean-Marie, T. M., & Lulendo, M. (2015). Benthic macroinvertebrates as indicators of water quality: a case-study of urban Funa Stream (in Kinshasa, Democratic Republic of Congo). *Open Journal of Water Pollution and Treatment*, *1*, 8-24.

Prommi, T., & Payakka, A. (2015). Aquatic insect biodiversity and water quality parameters of streams in Northern Thailand. *Sains Malaysiana*, *44*(5), 707-717.

Radojevic, M., Bashkin, V., & Bashkin, V. N. (1999). *Practical environmental analysis*. Royal society of chemistry.

Rahbek, C. (1995). The elevational gradient of species richness: a uniform pattern?. *Ecography*, 200-205.

Rana, A., & Chhetri, J. (2015). Assessment of river water quality using macro-invertebrates as indicators: a case study of Bhalu Khola tributary, Budhigandaki river, Gorkha, Nepal. *International Journal of Environment*, *4*(3), 55-68.

Resh, V. H. (1995). Freshwater benthic macroinvertebrates and rapid assessment procedures for water quality monitoring in developing and newly industrialized countries. *Biological assessment and criteria*, 167-177.

Rice, S. P., Greenwood, M. T., & Joyce, C. B. (2001). Tributaries, sediment sources, and the longitudinal organisation of macroinvertebrate fauna along river systems. *Canadian Journal of Fisheries and Aquatic Sciences*, *58*(4), 824-840.

Rice, S. P., Kiffney, P., Greene, C., & Pess, G. R. (2008). The ecological importance of tributaries and confluences. *River confluences, tributaries and the fluvial network*, 209-242.

Rosenberg, D. M. (1993). Introduction to freshwater biomonitoring and benthic macroinvertebrates. *Freshwater biomonitoring and benthic macroinvertebrates*, 1-9.

Roy, A., & Homechaudhuri, S. (2017). Comparing diversity of freshwater macroinvertebrate community along habitat gradients within a riverine system in North Bengal, India. *J Entomol Zool Stud*, *5*(4), 86-93.

Rundle, S. D., Jenkins, A., & Ormerod S. J. (1993). Macroinvertebrate communities in streams in the Himalaya, Nepal. *Freshwater Biology*, *30*(1), 169-180.

Sala, O. E., Stuart Chapin, F. I. I. I., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., & Wall, D. H. (2000). Global biodiversity scenarios for the year 2100. *science*, *287*(5459), 1770-1774.

Sharma, C. M., Sharma, S., Borgstrom, R., & Bryceson, I. (2005). Impacts of a small dam on macroinvertebrates: A case study in the Tinau River, Nepal. *Aquatic Ecosystem Health & Management*, *8*(3), 267-275.

Sharma, M. P., Sharma, S., Goel, V., Sharma, P., & Kumar, A. (2008). Water quality assessment of Ninglad stream using benthic macroinvertebrates. *Life Science Journal*, *5*(3), 67-72.

Sharma, S. (1996). *Biological Assessment of Water Quality in the Rivers of Nepal PHD* Doctoral dissertation, Thesis, University of Agriculture, Forestry and Renewable Natural Resources at Vienna (Austria).

Sharma, S. (1999). Water Quality Status of the Saptakosi River and Its tributaries in Nepal: A Biological Approach. *Nepal Journal of Science and Technology*, *1*(1).

Sharma, S., & Moog, O. (1996, March). The applicability of biotic indices and scores in water quality assessment of Nepalese rivers. In *Proceedings of the Ecohydrology Conference on High Mountain Areas* (pp. 641-657).

Sharma, S., & Moog, O. (2005). A reference based Nepalese Biotic Score and its application in the midland Hills and Lowland plains for river water quality assessment and management. *Proceedings of Plant Response to Environmental Stress. IBD CO. Publisher, Lucknow*, 105-112.

Sharma, S., Moog, O., Nesemann, H., & Pradhan, B. (2009). Application of Nepalese Biotic Score and its extension for river water quality management in the central Himalaya. In *International Symposium on Environment, Energy and Water in Nepal: Resent Researches and Direction for Future, Kathmandu, Nepal*.

Shrestha, T. K. (1990). Resource ecology of the Himalayan waters. curriculum development centre. *Tribhuvan University, Kathmandu*, *645*.

Suren, A. M. (1994). Macroinvertebrate communities of streams in western Nepal: effects of altitude and land use. *Freshwater Biology*, *32*(2), 323-336.

Tachamo Shah, R. D. (2018). *Monitoring river degradation and climate change impacts in the central Himalaya: Assessment tools and management approaches* (Doctoral dissertation).

Tachamo Shah, R. D. (2018). *Monitoring river degradation and climate change impacts in the central Himalaya: Assessment tools and management approaches* (Doctoral dissertation).

Tachamo Shah, R. D., Sharma, S., & Bharati, L. (2020). Water diversion induced changes in aquatic biodiversity in monsoon-dominated rivers of Western Himalayas in Nepal: Implications for environmental flows. *Ecological Indicators*, *108*, 105735.

Tachamo Shah, R. D., Sharma, S., Haase, P., Jähnig, S. C., & Pauls, S. U. (2015). The climate sensitive zone along an altitudinal gradient in central Himalayan rivers: a useful concept to monitor climate change impacts in mountain regions. *Climatic Change*, *132*(2), 265-278.

Tachamo Shah, R. D., Sharma, S., Narayan Shah, D., & Rijal, D. (2020). Structure of benthic macroinvertebrate communities in the rivers of Western Himalaya, Nepal. *Geosciences*, *10*(4), 150.

Takao, A., Kawaguchi, Y., Minagawa, T., Kayaba, Y., & Morimoto, Y. (2008). The relationships between benthic macroinvertebrates and biotic and abiotic environmental characteristics downstream of the Yahagi dam, central Japan, and the state change caused by inflow from a tributary. *River Research and Applications*, *24*(5), 580-597.

Tonkin, J. D., Tachamo Shah, R. D., Shah, D. N., Hoppeler, F., Jähnig, S. C., & Pauls, S. U. (2017). Metacommunity structuring in Himalayan streams over large elevational gradients: The role of dispersal routes and niche characteristics. *Journal of biogeography*, *44*(1), 62-74.

Turak, E., Dudgeon, D., Harrison, I. J., Freyhof, J., Wever, A. D., Revenga, C., ... & Flink, S. (2017). Observations of inland water biodiversity: Progress, needs and priorities. In *The GEO handbook on biodiversity observation networks* (pp. 165-186). Springer, Cham.

Turcotte, P., & Harper, P. P. (1982). The macro- invertebrate fauna of a small Andean stream. *Freshwater Biology*, *12*(5), 411-419.

Tyagi, S., Sharma, B., Singh, P., & Dobhal, R. (2013). Water quality assessment in terms of water quality index. *American Journal of water resources*, *1*(3), 34-38.

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Cushing, C. E. (1980). The river continuum concept. *Canadian journal of fisheries and aquatic sciences*, *37*(1), 130-137.

Vetaas, O. R., & Grytnes, J. A. (2002). Distribution of vascular plant species richness and endemic richness along the Himalayan elevation gradient in Nepal. *Global Ecology and Biogeography*, *11*(4), 291-301.

Vörösmarty, C. J., McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, A., Green, P., ... & Davies, P. M. (2010). Global threats to human water security and river biodiversity. *nature*, *467*(7315), 555-561.

Ward, J. V., & Stanford, J. A. (1979). Ecological factors controlling stream zoobenthos with emphasis on thermal modification of regulated streams. In *The ecology of regulated streams* (pp. 35-55). Springer, Boston, MA.

Warne, M. S. J., Batley, G. E., Braga, O., Chapman, J. C., Fox, D. R., Hickey, C. W., ... & Van Dam, R. (2014). Revisions to the derivation of the Australian and New Zealand guidelines for toxicants in fresh and marine waters. *Environmental Science and Pollution Research*, *21*(1), 51-60.

Water and Energy Commission Secretariat (WECS). 2011. Water resources of Nepal in the context of climate change.Government of Nepal (GoN), pp2, 67.

Wetzel, R. (2001). Limnology 3rd Edition Lake and River Ecosystems.

Wheeler, D. (1996). The role of nourishment in oogenesis. *Annual review of entomology*, *41*(1), 407-431.

Wohl, E. (2010). Mountain Rivers Revisited. American Geophysical Union: Washington, DC.

WWF (2013). Chitwan annapurna landscape (CHAL): a rapid assessment. *World Wildlife Fund Nepal: Kathmandu, Nepal*.

Xu, M., Wang, Z., Duan, X., & Pan, B. (2014). Effects of pollution on macroinvertebrates and water quality bio-assessment. *Hydrobiologia*, *729*(1), 247-259.

Yazdian, H., Jaafarzadeh, N., & Zahraie, B. (2014). Relationship between benthic macroinvertebrate bio-indices and physicochemical parameters of water: a tool for water resources managers. *Journal of Environmental Health Science and Engineering*, *12*(1), 1-9.

Zaghloul, A., Saber, M., & El-Dewany, C. (2019). Chemical indicators for pollution detection in terrestrial and aquatic ecosystems. *Bulletin of the National Research Centre*, *43*(1), 1-7.

Annex 1: Identified taxa of BMI in Modi River

Order	Family	MO01	MO02	MO03	MO04	MO05	MO06	MO07	MO08	MO09	MO10	MO11	MO12	MO13
Ephemeroptera	Ephemerellidae	22	46	9	20	10	11	15	2	20	5	27	5	0
	Heptageniidae	39	16	27	11	3	20	58	7	82	44	50	4	0
	Baetidae	29	20	24	5	10	19	74	16	32	27	44	40	16
	Caenidae	0	0	1	2	1	0	1	2	5	2	2	2	0
Plecoptera	Perlidae	13	34	6	9	1	6	9	9	10	4	26	3	1
	Perlodidae	4	0	0	7	1	0	0	0	1	0	1	0	0
	Nemouridae	1	10	3	0	1	1	17	3	1	0	0	0	0
	Chloroperlidae	0	7	1	0	1	4	7	0	1	2	0	0	0
Tricoptera	Leptoceridae	1	0	0	2	1	15	9	4	20	1	5	1	5
	Lepidostomatidae	0	0	0	2	4	0	0	1	1	2	1	1	0
	Glossosomatidae	0	6	0	2	1	3	3	2	1	2	3	0	2
	Hydropsychidae	0	0	0	2	5	0	0	1	11	3	1	1	0
	Philopotamidae	0	0	0	0	0	0	0	0	0	0	0	0	0

	Polycentropodidae	0	0	0	0	0	1	0	0	0	0	0	0	0
	Rhyacophilidae	0	0	0	0	0	0	0	0	0	0	0	0	0
	Stenopsychidae	0	0	0	0	0	0	0	0	0	0	0	0	0
	Brachycentridae	0	0	0	3	5	0	0	2	2	2	0	1	0
Diptera	Chironomidae	2	5	1	1	4	2	0	1	3	3	3	10	108
	Simuliidae	0	4	3	3	2	1	10	5	10	5	2	1	5
	Tabanidae	0	0	0	0	0	0	0	0	0	0	0	0	0
	Tipulidae	0	0	4	2	5	1	1	1	1	2	0	1	2
	Limonidae	0	0	0	0	0	0	1	1	2	2	0	2	11
	Blephariceridae	6	3	3	13	4	5	45	2	17	18	10	1	2
	Athericidae	0	0	1	0	0	0	1	1	2	0	0	0	0
	Dolichopodidae	0	0	0	0	0	0	0	0	0	0	0	0	0
	Empididae	0	0	0	0	0	0	0	0	0	0	1	1	1
	Stratiomyidae	0	0	0	0	0	0	0	0	0	0	0	0	1
	Sciomyzidae	0	0	0	0	0	0	0	0	0	0	0	0	1

Coleoptera	Elmidae	0	0	0	2	1	1	2	2	2	0	2	1	0
	Psephenidae	0	0	0	0	0	0	1	0	1	1	0	0	1
	Hydrophilidae	0	1	0	0	0	0	0	0	0	0	0	0	0
	Scirtidae	0	0	0	0	0	0	0	0	0	0	0	0	0
Megaloptera	Corydalidae	0	0	0	0	0	0	0	0	1	1	0	1	0
Haplotaxida	Tubificidae	0	0	0	0	0	0	0	0	0	0	0	0	0
Hemiptera	Belostomatidae	0	0	0	0	0	0	0	0	0	0	0	0	0
	Gerridae	0	0	0	0	0	0	0	0	0	0	0	0	0
Lumbriculida	Lumbriculidae	0	1	0	0	0	0	1	0	0	0	0	0	0
Odonata	Gomphidae	0	0	0	2	1	0	1	8	2	1	1	1	1
	Coenagrionidae	0	0	0	0	0	0	0	0	0	0	0	0	0
	Euphaeidae	0	0	0	0	0	0	0	0	0	0	0	0	0

Annex 2: Identified taxa of BMI in Tributaries

Order	Family	T01	T02	T03	T04	T05	T06	T07
Ephemeroptera	Ephemerellidae	6	3	2	1	1	74	40
	Heptageniidae	11	10	20	114	26	78	20
	Baetidae	95	132	263	97	79	211	322
	Caenidae	3	0	2	9	0	12	1
Plecoptera	Perlidae	5	14	19	15	86	81	6
	Perlodidae	0	0	0	0	0	0	1
	Nemouridae	0	0	0	9	0	0	0
	Chloroperlidae	0	2	0	0	0	7	0
Tricoptera	Leptoceridae	1	21	5	357	14	10	3
	Lepidostomatidae	1	0	0	2	7	3	2
	Glossosomatidae	6	38	0	38	0	0	0
	Hydropsychidae	11	8	1	11	8	5	11

	Philopotamidae	1	0	0	1	0	2	2
	Polycentropodidae	0	4	0	0	0	0	0
	Rhyacophilidae	0	0	7	0	0	4	0
	Stenopsychidae	0	0	0	0	0	3	0
	Brachycentridae	1	0	0	0	36	3	1
Diptera	Chironomidae	11	10	6	7	86	20	21
	Simuliidae	6	163	12	19	8	41	5
	Tabanidae	0	2	11	7	11	6	1
	Tipulidae	1	0	1	2	12	8	1
	Limonidae	1	6	0	3	19	13	22
	Blephariceridae	2	0	1	4	17	0	0
	Athericidae	0	5	1	1	89	2	0
	Dolichopodidae	0	0	0	0	1	2	0
	Empididae	0	0	1	0	1	0	0
	Stratiomyidae	0	0	1	0	0	3	0

	Sciomyzidae	0	0	0	0	16	1	0
Coleoptera	Elmidae	0	0	2	13	59	96	3
	Psephenidae	0	0	0	0	1	0	0
	Hydrophilidae	0	0	0	0	5	0	0
	Scirtidae	0	0	0	1	0	0	0
Megaloptera	Corydalidae	0	0	0	0	0	0	1
Haplotaxida	Tubificidae	0	0	0	0	0	1	0
Hemiptera	Belostomatidae	0	0	0	0	0	2	0
	Gerridae	0	0	0	0	1	0	0
Lumbriculida	Lumbriculidae	0	0	0	0	0	0	0
Odonata	Gomphidae	0	0	1	4	15	16	1
	Coenagrionidae	0	0	1	0	0	0	0
	Euphaeidae	0	0	0	0	1	0	0

Annex 3: Water quality parameters of mainstem

Parameter	MO01	MO02	MO03	MO04	MO05	MO06	MO07	MO08	MO09	MO10	MO11	MO12	MO13
Depth	30.00	25.00	20.00	20.00	20.00	15.00	40.00	35.00	20.00	20.00	35.00	20.00	30.00
Velocity	0.90	0.50	0.50	0.90	0.70	0.40	0.40	0.40	0.50	0.40	0.30	0.30	0.30
Temp	14.30	13.10	9.10	12.20	13.80	11.00	14.70	12.10	14.50	14.10	12.50	15.00	16.20
DO	8.12	7.88	9.80	8.20	7.33	7.80	7.00	7.92	9.50	8.80	8.33	9.50	7.26
pH	8.50	8.30	8.50	8.70	8.10	8.50	8.50	8.90	8.50	8.30	8.50	8.50	8.70
EC	190.00	168.00	155.00	171.00	174.00	160.00	163.00	156.00	180.00	155.00	156.00	162.00	163.00
TDS	95.00	84.00	77.50	85.50	87.00	80.00	81.50	78.00	90.00	77.50	78.00	81.00	81.50
Turbidity	64.00	70.80	177.00	172.00	199.00	148.00	250.00	193.00	301.00	102.00	240.00	213.00	242.00
Nitrate	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.06	0.05	0.05	0.05	0.05
Phosphate	0.12	0.14	0.18	0.15	0.15	0.33	0.14	0.25	0.29	0.12	0.45	0.21	0.27
Ammonia	0.01	0.01	0.33	0.03	0.11	0.04	0.17	0.17	0.55	0.13	0.30	0.02	0.31

Free CO_2	13.34	13.33	13.33	8.00	2.53	0.27	13.33	0.27	13.33	6.67	0.27	6.67	0.27
Chloride	3.79	3.79	0.66	0.00	0.28	1.89	0.47	3.79	3.79	0.95	0.95	0.95	1.89
Alkalinity	133.33	106.67	133.33	133.33	133.33	86.00	100.00	100.00	266.67	100.00	100.00	100.00	100.00
Total hardness	133.33	133.33	106.67	106.67	106.67	80.00	93.33	93.33	133.33	80.00	53.33	46.67	60.00
Calcium hardness	80.00	88.00	80.00	64.00	66.67	64.00	73.33	60.00	80.00	66.67	46.67	40.00	53.33
Magnesium hardness	13.01	11.06	6.51	10.41	9.76	3.90	4.88	8.13	13.01	3.25	1.63	1.63	1.63
BOD	2.1	3.54	1.88	2.11	2.8	2.47	2.73	1.14	2.14	3.32	1.33	1.82	1.33
COD	2.11	4.31	3.26	2.97	4.22	3.57	2.97	2.97	4.17	5.1	2.11	2.54	3.54

Annex 4: Water quality parameters of tributaries

Parameter	T01	T02	T03	T04	T05	T06	T07
Depth (cm)	25.00	15.00	10.00	10.00	10.00	30.00	15.00
Velocity	0.20	0.10	0.10	0.10	0.10	0.20	0.10
Temp	15.10	19.80	20.40	16.10	19.40	19.60	24.10
DO	8.38	7.00	6.10	7.56	7.60	7.00	7.00
pH	8.50	8.30	8.70	8.80	8.30	8.80	8.50
EC	232.00	368.00	205.00	158.00	183.00	76.00	65.00
TDS	116.00	184.00	102.50	79.00	91.50	38.00	32.50
Turbidity	6.48	10.00	10.50	4.97	3.62	6.23	5.38
Nitrate	0.05	0.48	0.05	0.05	0.05	0.05	0.05
Phosphate	2.41	0.21	0.13	0.18	0.29	0.17	0.13
Ammonia	0.00	0.11	0.04	0.59	0.04	0.00	0.00

Free CO_2	26.67	12.00	2.67	1.07	13.33	6.67	6.67
Chloride	4.73	3.79	0.19	0.95	0.95	0.66	1.89
Alkalinity	266.67	200.00	126.67	89.33	133.33	66.67	66.67
Total hardness	117.33	213.33	80.00	93.33	73.33	46.67	26.67
Calcium hardness	80.00	93.33	53.33	53.33	73.33	33.33	26.67
Magnesium hardness	9.11	29.28	6.51	9.76	0.00	3.25	0.00
BOD	1.09	2.94	3.1	3.54	2.68	2	2.56
COD	1.54	3.8	3.97	4.18	3.64	3.91	2.54

Annex 5: Taxa list with GRS BIOS score assigned

Families Name	**Value**
Ephemerellidae	6
Heptageniidae	7
Baetidae	6
Caenidae	3
Perlidae	10
Perlodidae	9
Nemouridae	8
Chloroperlidae	9
Leptoceridae	N.A.
Lepidostomatidae	7
Glossosomatidae	9
Hydropsychidae	3
Philopotamidae	8

Polycentropodidae	3
Rhyacophilidae	6
Stenopsychidae	7
Brachycentridae	8
Chironomidae	N.A.
Simuliidae	5
Tabanidae	4
Tipulidae	7
Limonidae	8
Blephariceridae	10
Athericidae	9
Dolichopodidae	N.A.
Empididae	4
Stratiomyidae	4
Sciomyzidae	N.A.

Elmidae	10
Psephenidae	8
Hydrophilidae	N.A.
Scirtidae	10
Corydalidae	7
Tubificidae	2
Belostomatidae	7
Gerridae	4
Lumbriculidae	7
Gomphidae	N.A.
Coenagrionidae	5
Euphaeidae	8

Annex 6: Benthic Macroinvertebrates Composition

Order	Family	Abbreviation
Ephemeroptera	Ephemerellidae	Ephe_Fam
	Heptageniidae	Hept_Fam
	Baetidae	Baet_Fam
	Caenidae	Caen_Fam
Plecoptera	Perlidae	Prli_Fam
	Perlodidae	Prlo_Fam
	Nemouridae	Nemo_Fam
	Chloroperlidae	Chlor_Fam
Tricoptera	Leptoceridae	Lept_Fam
	Lepidostomatidae	Lepi_Fam
	Glossosomatidae	Glos_Fam
	Hydropsychidae	Hydr_fam
	Philopotamidae	Phil_Fam

	Polycentropodidae	Poly_Fam
	Rhyacophilidae	Rhya_Fam
	Stenopsychidae	Sten_Fam
	Brachycentridae	Brac_Fam
Diptera	Chironomidae	Chir_Fam
	Simuliidae	Simu_Fam
	Tabanidae	Taba_Fam
	Tipulidae	Tipu_Fam
	Limonidae	Limo_Fam
	Blephariceridae	Blep_Fam
	Athericidae	Athe_Fam
	Dolichopodidae	Doli_Fam
	Empididae	Empi_Fam
	Stratiomyidae	Stra_Fam
	Sciomyzidae	Scio_Fam

Coleoptera	Elmidae	Elmi_Fam
	Psephenidae	Psep_Fam
	Hydrophilidae	Hydr_Fam
	Scirtidae	Scir_Fam
Megaloptera	Corydalidae	Cory_Fam
Haplotaxida	Tubificidae	Tubi_Fam
Hemiptera	Belostomatidae	Belo_Fam
	Gerridae	Gerr_Fam
Lumbriculida	Lumbriculidae	Lumb_Fam
Odonata	Gomphidae	Gomp_Fam
	Coenagrionidae	Coen_Fam
	Euphaeidae	Euph_Fam

Annex 7: DCA ordination summary

RDA Summary

Axes	1	2	3	4	Total variance
Eigenvalues :	0.395	0.247	0.066	0.038	1.000
Species-environment correlations :	0.960	0.861	0.989	0.772	
Cumulative percentage variance					
of species data :	39.5	64.1	70.7	74.5	
of species-environment relation:	51.0	82.8	91.2	96.1	
Sum of all eigenvalues	1.000				
Sum of all canonical eigenvalues	0.775				

Annex 8: BMI composition analyzed based on RDA analysis

Environmental variable	**F**	**P**
Temperature	**8.943**	**0.0001**
Ammonia	**3.023**	**0.005**
Nitrate	**1.888**	**0.0955**
Electrical Conductivity	**1.636**	**0.1695**
TDS	**1.636**	**0.161**
Magnesium hardness	**1.575**	**0.1146**
PH	**1.492**	**0.1925**
Total Hardness	**1.268**	**0.1952**
Depth	**1.182**	**0.32**
Velocity	**1.121**	**0.26**
Turbidity	**1.062**	**0.36**

Calcium hardness	0.956	0.4268
BOD	0.811	0.5592
COD	0.53	0.66
CO_2	0.553	0.73
Alkalinity	0.48	0.67
Chloride	0.441	0.87
DO	0.127	0.992
PO_4	0.116	0.87

Annex 9: Photo plates

Modi River

Sample collection

Sample collection

Lab work

Key informant interview

Wastes