Aquatic Phytodiversity and Macro-invertebrates in Water Bodies of Setikhola Watershed, Kaski, Nepal

A Dissertation submitted for Partial Fulfillment of the Requirement for the Master's Degree in Botany

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RECOMMENDATION

This is to certify that the M.Sc. dissertation work entitled "**Aquatic Phytodiversity and Macroinvertebrates in Water Bodies of Setikhola Watershed, Kaski, Nepal**" has been carried out by Kanchan Devi Upadhyay under my supervision. This work has been completed on the basis of candidate's original research based on field visit and lab work. The work has not been submitted for any other academic degree.I recommend this dissertation work to be accepted as a partial fulfilment for the Master's Degree in Botany (Plant Ecology and Resource Management Unit) at Institute of Science and Technology, Tribhuvan University.

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LETTER OF APPROVAL

The M.Sc. dissertation entitled "**Aquatic Phytodiversity and Macroinvertebrates in Water Bodies of Setikhola Watershed, Kaski, Nepal**" has carried out by Miss. Kanchan Devi Upadhyay submitted to the Central Department of Botany, Tribhuvan University has been accepted for the partial fulfillment of requirement for Master's of Science in Botany (Plant Ecology and Resource Management Unit).

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DECLARATION

I, the undersigned author of this work, declare that this dissertation of my own work and has not been submitted in any form either in whole or in part, for a degree at any other institution. All the views and opinions expressed here in remain the sole responsibility of the author and do not necessarily represent those of the other institute.

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ABBREVIATIONS AND ACRONYMS

°C	degree Celsius
µS/cm	micro Siemens per centimeter
ACAP	Annapurna Conservation Area
APHA	American Public Health Association
AWWA	American Water Works Association
BIS	Bureau of Indian Standards
CCA	Canonical Correspondence Analysis
CO_2	Carbon-dioxide
DCA	De-trended Correspondence Analysis
DO	Dissolved oxygen
E	Emergent
ENPHO	Environment and Public Health Organization
FAO	Food and Agriculture Organization
FDD	Fisheries Development Division
FF	Free Floating
HMGN	His Majesty's Government of Nepal,
ICMR	Indian Council of Medical Research
KATH	National Herbarium and Plant Laboratories
Km^2	square kilometer
MEA	Millennium Ecosystem Assessment
mg/L	milligram per liter
MoFSC	Ministry of Forest and Soil Conservation
NWP	Nepal Wetland Policy
QGIS	Quantum Geographic Information System
RFL	Rooted Floating Leaves
SM	Submerged
sp	species
SPSS	Statistical Package for Social Science
TDS	Total Dissolved Solids
UNESCO	United Nations Educational, Scientific and Cultural Organization
WPCF	Water Pollution Control Federation

ABSTRACT

Water quality and other physical environmental factors determine considerable variations in distribution and composition of aquatic species in freshwater ecosystems. This study determined the Aquatic Phytodiversity (Phytoplanktons and Macrophytes), Macroinvertebrates and Physico-chemical Characteristics of water in water bodies of Setikhola watershed, Kaski, Nepal. Among all studied water characteristics, conductivity was found most important influencing factor for causing variations in species composition of macrophyte, phytoplankton and macroinvertebrates while the influence of nitrate, phosphate and ammonia concentration was found to be more or less similar. Altogether 39 Macrophyte species belonging to 29 families were recorded with the dominancy of emergent species, 105 Phytoplankton species belonging to 5 classes with the dominancy of Bacillariophyceae and 9 order belonging to 35 families of Macroinvertebrates with the dominancy of order Diptera were recorded from the study area. *Navicula* sp, *Ghomphonema* sp and *Cymbella* sp of phytoplankton were dominant. The family Nepidae was commonly found in most of the water bodies while the family Hydropsychidae and Heptagenidae were found to be dominant in lotic water bodies. Most of the macrophytes showed strong but negative affinity with all studied water characteristics while phytoplankton showed positive affinity with all studied water characteristicss. Macro-invertebrates showed positive as well as negative affinity equally with ammonia and conductivity. These findings created a database for present status of different waterbodies of Setikhola watershed, Kaski, Nepal, which can be used for the management of lakes and river as well as to study the impact on water quality. This study provides useful information for decision makers aimed to the conservation and sustainable management of the watershed in Nepal.

Keywords: water quality, conductivity, diversity, lotic, lentic, macrophytes, phytoplanktons

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CHAPTER I
INTRODUCTION

1.1 Background

Watersheds are land areas that channel water to a particular location, such as river, lake, oceans, or other body of water. The history of watershed in Nepal Himalaya showed that 1980s decade to be the "warming up phase" and 1990s as the "expansion phase" of watershed management (SOWCAS, 2004). The second half of the 1990s saw the adoption of a participatory and integrated approach to watershed management (Sharma,1999) which involves the use and conservation of land water and forest resources at the farm household and community or watershed level in order to improve livelihood and human development (Sharma et al., 1997). This study is focused on water quality and aquatic biodiversity (Phytoplanktons, Macrophytes, and Macroinvertebrates) of water bodies of Setikhola Watershed. Setikhola watershed which comprises of Pokhara city and upstream region has numerous lakes, deep rivers, high mountains, and rich biodiversity. The lake system of Pokhara valley is recently listed as an important wetland site in the Ramsar List (Baral et al., 2016). Wetlands which are recognized as the'Kidneys of the landscape' or called as 'biological supermarkets' because of having rich food webs includes rivers, streams, lakes, reservoirs, villageponds, paddy fields, marshes and swamplands and can be divided as lotic(running) and lentic (stagnant) water environments. Wetlands in Nepal are exclusively freshwater in nature (Siwakoti, 2006) and occupy approximately 5% of the total area of Country mainly in the form of river, lakes, reservoirs, village ponds, paddy fields and marshes (HMG/N, 1992). But, in recent years, lakes and river arebeing polluted by discharge of agricultural waste, chemical and industrial effluent and municipal solid waste across the world (Milovanovic, 2007; Yadav et al, 2010; Shah and Shah, 2013).Increasing pollution not only deteriorate the water quality but also threatens on human health, aquatic ecosystem and biodiversity, impacts on economic development and social prosperity (Arsovski et al., 1991; Niraula, 2012).

Aquatic biodiversity provides a broad variety of valuable goods and services for human societies, some of them irreplaceable (Dudgeon et al., 2006), but these species are highly vulnerable to the degradation of water quality due to physical or chemical alterations (Alam et al., 2008). Loss of aquatic biodiversity reduces the efficiency of ecological communities to capturing essential resources, producing biomass, decomposing, and recycling essential nutrients (Cardinale et al., 2012).

As the identity of Pokhara valley is inherently linked with water, it is important to explore chemical, physical and biological characteristics of the water and understand whether they are appropriate to support the biodiversity and human population.

1.1.1 Physico-chemical Characteristics of Water

Water quality generally means the component of water which must be present for optimum growth of aquatic organisms (Verma and Khan, 2015). Water is known to contain large number of chemical elements, the interaction of both physical and chemical properties of water play a significant role in composition, distribution and abundance of aquatic community. Characteristics of water bodies influence the quality of water individually and in combination with various pollutants, thereby, influencing the biota. The Physico-chemical study could help in understanding the structure and function of particular water body in relation to its habitants. Abundance of particular element might suggest the type of organism that may be found as well as indication of ecologically unstable or unfavorable ecosystem which can have negative or positive impact on the population i.e. high concentration of nitrate or phosphate is indicative of eutrophication (Adedeji et al., 2019). Physical properties such as temperature, light penetration, water movement play important role in plankton's distribution and lake stratification. DO is also an important water quality parameter because of its significant biological and physicochemical property of surrounding water. Oxygen enters into the water by aerial diffusion and as a photosynthetic by-product of aquatic plants (Kotadiya and Acharya, 2014).The physico-chemical characteristic of lake can be significantly altered by human activities such as various agricultural practices and irrigation as well as natural dynamics which consequently affect the water quality and quantity, species distribution and diversity, production capacity and even disruption in the balance of ecological system operating in the lake.

The river water quality in South Asian (Hindu Kush Himalayan) countries is poor (Shrestha et al., 2008) and is deteriorated by the direct discharge of industrial, municipal, agricultural and solid wastes. The restoration and proper management of these 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 (Shah and Shah, 2013).

In most of the Asian Countries, commonly adopted methodology for determining the quality of river and lake water is mainly based on Physical and Chemical data. Chemical monitoring often does not account for human-caused habitat disturbance that can impair stream function but the effective monitoring programs include assessments of physical habitat and biotic integrity because the ability to sustain balanced biotic communities is a reliable indicator of stream health (Karr, 1981; Loeb and Spacie, 1994, Karr et al., 1999). Therefore, a sustainable water management and protection need to include the knowledge of bio-monitoring for sustainable management decision (Moog et al., 2008).

High water turbidity, low dissolved oxygen and an increase in nitrogen and phosphorus compounds (Kannel et al., 2007) have not only produced odour and aesthetic nuisances in the urban areas of Nepal but have also led to a decrease in aquatic life in urban rivers (Shah et al., 2008). Similarly, there are several potential sources of pollutant to water in this region. Sedimentation from the predominantly farming hills surrounding the valley, rapid urbanization of valley floor, and lack of proper management of waste are considered as potential reasons of habitat quality degradation of surface water in this region (Baral et al., 2017; Rimal et al., 2015; Ross, 1998).

1.1.2 Macrophytes

Aquatic macrophytes are macroscopic forms of aquatic vegetation, including macroalgae, mosses, ferns and angiosperms found in aquatic habitat (Dhanan and Elayaraj, 2015). Macrophytes living in aquatic environment make them different from terrestrial plants which don’t tolerate flooded environment. It is important to know that the evolution of angiosperms proceeded in terrestrial environments when some of them returned to aquatic environment had to evolve various adaptations. Hutchinson (1975) had classified the aquatic macrophytes on the basis of life forms (grouped by the relation of plant to water level and substratum) and growth form (grouped by structural similarity and relations to the physical environment) which was unified as “ecological classification”. Its main four categories that are most widely accepted until today are emergent, floating leaved, submerged, rooted floated leaves and freely floating macrophytes.These macrophytes can act as measurable indicators of the ecological conditions of surface water. Aquatic floating macrophytes take up inorganic nutrients, mainly nitrogen and phosphorus, by the roots; although uptake through the leaves may also be significant (Ferdoushi et al., 2008). Notably, the submerged species strongly dependent on water quality have proved to be vulnerable to changes in the aquatic environment (Robach et al., 1996; Dawson et al., 1999).While submerged macrophytes are considered to be suitable eutrophication indicators and are sensitive to local environmental conditions (Dennison et al., 1993; Lacoul and Freedman 2006; Sondergaard et al., 2010). Aquatic vegetation can also influence the abiotic conditions (Flessa, 1994; Moore et al., 1994; Barko et al., 1991) and influence wetland biota across multiple trophic levels (Norlin et al., 2005) by providing both habitat and food. Aquatic environment with low nutrient content usually have vegetation dominated by relatively small plants.With moderate nutrient loading, the biomass and proportion of aquatic macrophytes increases and plants can fill the entire water column (Zingel et al., 2006). However, with increasing eutrophication the species diversity of wetland macrophytes generally declines and the species communities are being replaced by monoculture forming strong competitors (Rejmankova, 2011).

Macrophytes serve as a link between the sediment, water, and (some times) atmosphere in wetlands, lakes, and rivers. The most notable function that plants serve is as primary producers. Moreover, macrophytes are also involved in ecosystem processes such as biomineralization, transpiration, sedimentation, elemental cycling, materials transformation, and release of biogenic trace gases into the atmosphere (Carpenter and Lodge, 1986). And they too influence the water quality changes and being used as bio-indicator of pollution (Tripathi and Shukla, 1991).

1.1.3 Phytoplankton

Phytoplanktonsare vital and important organisms which act as producer to the primary food supply in any aquatic ecosystem. They are the initial biological components from which the energy is transferred to higher organisms through food chain (Tiwari and Chauhan, 2006, Saifullah et al., 2014). The physico-chemical parameters are the major factors that control the dynamics and structure of the phytoplankton of aquatic ecosystem (Hulyal and Kaliwal, 2009). Changes in physico-chemical parameters of ecosystems have a substantial impact on the species that live within them. Seasonal variations in these parameters have an important role in the distribution, periodicity and quantitative and qualitative composition of freshwater biota.

Several recent studies on physico-chemical parameters and phytoplankton community of rivers are conducted on the Greater Zab River, Iraq (Ali, 2010), River Haraz, Iran (Jafari et al., 2011), Imo River, Nigeria (Ogbuagu and Ayoade, 2012), River Thames, UK (Waylett et al., 2013), and Kenti River, Republic of Karelia (Chekryzheva, 2014). In North India, many recent studies have been conducted. These were focussed on the Chandrabhaga River (Sharma et al., 2007), Yamuna River (Chopra et al., 2012), Ganga River and its tributaries (Negi et al., 2012), Sutlej River (Sharma et al., 2013) and Jhelum River (Hafiz et al., 2014).

Both the phytoplanktons and aquatic macroinvertebrates are sensitive to the changing environment and thus are used as bio-indicator for water quality as well as bio-marker for past climate of the lakes and glaciers. Their species composition in the water shows the level of water pollution and environmental condition of the habitats (Wojtal et al., 2009). Phytoplankton are also important as they are the major producer of aquatic ecosystem regulating flow of energy and biomass to maintain the ecological balance in the river, streams and ponds (Cantonati et al., 2006). Consumption of CO_2 by phytoplankton has been found many fold maximum than the total land vegetation of the earth which shows to play major role in minimizing the global warming.

1.1.4 Macro-Invertebrates

Freshwater macro-invertebrates are good indicators of the overall health of the ecosystem and have long been used as reliable indicators for bio-monitoring (Lucadamo et al., 2008). Invertebrates provide important ecosystem services in aquatic systems and their diversity and species composition can be used as indicators of changing environmental conditions (Harriman, 2016). Chironomidae has been used as indicadors of water quality since the beginning of the century after the introduction of the Saprobic System (Kolkwitz and Marsson, 1908, 1909, Chutter, 1972). Several biological indices concerning evaluation and monitoring of water quality rely heavily on them (Plafkin et al., 1990, Barbosa et al., 1995, 1997). Changes in species composition, dominance of pollution tolerant species, and frequency of occurrence of deformities on larval head capsules, are some of the commonly used features in these types of evaluations (Johnson et al., 1993).

Thus, aquatic macroinvertebrates helps in monitoring the environmental quality (Loeb and Spacie, 1994, Alam et al., 2008) that helps to evaluate the effect of various anthropogenic stressors at all level of biological organization from the molecular level to the ecosystem (Rosenberg and Resh, 1993, Carter et al., 2017).

1.2 Hypothesis

Aquatic Phytodiversity and diversity of Macro-invertebrates varies in different water bodies of watershed due to water quality status.

1.3 Objectives

General objective is to study an ecological status of different water bodies of Setikhola watershed, Kaski, Nepal.

Specific Objectives

I. To study the aquatic phytodiversity and macroinvertebrates of the study area.
II. To study the present water quality status of the study area.
III. To correlate the composition of aquatic phytodiversity and macroinvertebrates with physico-chemical properties of water of the study area.

1.4 Rationale of the Study

Although several physical, biological and chemical characteristics of the mountain environments in the Himalaya are well explored, but water quality has not gained enough attention (Ghezzi et al., 2017; Shah et al., 2011; Nakamura et al., 2007). Therefore, there is an increased need to understand different water quality parameters. The diversity and distribution of aquatic biota are affected by the changes in the water chemistry (Deshkar et al., 2010). But the exploration of aquatic biota especially the lower organisms i.e. macro-invertebrates and phytoplankton in the watershed have not been done yet. As these organisms are the best indicators to control water pollution and regulate the water bodies to curb further deteriorations. So, the study of the diversity and distribution pattern of those organism including macrophytes has been done. Correlating water quality with the abundance and diversity of macroinvertebrates and aquatic phytodiversity will be beneficial for the preparation of baseline information of Setikhola watershed for further monitoring and conservation of the ecosystem. Conserving the water bodies is to conserve the livelihood of the surrounding people as well as other. The study of the bio-physio-chemical characters of water is thus important to study the status of watershed. Moreover, the taxonomic documentation of these species is necessary to add and update the database of aquatic biodiversity. Hence, the studies of these aspects are essential.

1.5 Limitations

I. One season data were taken for data collection

II. Only limited sampling site and samples were collected which cannot cover whole study area.

III. For the Physico-chemical parameters of the water, only the parameters such as temperature, DO, Nitrate, Phosphate and Ammonia were measured.

IV. Macro-invertebrates were identified up to family level only.

V. Anthropogenic effect and influences of invasive species on species diversity were not considered in the study.

CHAPTER II
LITERATURE REVIEW

Freshwater habitats cover about 0.8% of the Earth′s surface (Turak et al., 2017) but the undesirable consequence of having human activities close by freshwater ecosystems causes pollution, eutrophication, and erosion, not only in the rivers but also in the aquifer (Vorosmarty et al., 2010).Mountainous freshwater habitats are especially vulnerable ecosystems in the context of global warming (Ormerod, 2009, Shah et al., 2015). Though the fresh water ecosystems are of greater importance for biodiversity and human life they are the most threatened ecosystems worldwide (MEA, 2005), the decline in freshwater biodiversity is higher than that of terrestrial ecosystems (Shah et al., 2000; Dudgeon et al., 2006).

The study of different fields of freshwater environment (physico-chemical parameters of water, macro-invertebrates, plankton and fishes) of Nepal was carried out by various researchers only after 1950s, Brehm (1953) is considered as the pioneer scholar in the field of freshwater zooplankton of Nepal.

2.1 Aquatic Macrophyte

Studies on aquatic flora in Nepal were started with the work of Hamilton and Wallich who collected the plant species from different parts of the Country including some wetland species. Don (1852), Hooker (1872) and Burkill (1920) had undertaken the field study of aquatic macrophytes. There has been gradual increase in the number of works onwards 1970s (Shrestha, 1994).

Shrestha (1998) collected 65 species of aquatic macrophytes from seven lakes of Pokhara valley. Bastola (1999) analyzed ecology of Begnas Tal in Pokhara and recorded 29 aquatic species, Oli (1996) recorded 520 plant species from Begnas and Rupa watershed area. Oli (2002) recorded 6 species of invasive weeds from Rupa lake. Shrestha (2000) have analyzed spatial variation in species diversity of aquatic macrophytes in Phewa and Rupa lake and recorded 56 species with 5 different growth forms.

Ghimire (2007) found dominancy of emergent species followed by submerged rooted floating leaved and free-floating species in Pravas Lake, Palpa. He also determined dominant family as Poaceae and there is high aquatic macrophytes diversity during rainy season in Pravas lake. Similar result was observed by Burlakoti and Karmacharya (2004) in Beeshazar lake Chitwan.

Palit and Mukherjee (2012) studied on water quality and macrophyte composition of Bankura district, West Bengal and recorded 25 species of macrophyte belonging to 29 family.

Dhore et al. (2012) recorded 42 different species of macrophytes from five studied lakes in Maharastra which include three free floating macrophytes, seven submerged, two rooted floating and twenty-eight emergent species of macrophytes. The free-floating species *Eichhornia crassipes* occurs throughout the year. While the submerged species *Vallisneria spiralis*, *Ceratophyllum demersum*, *Hydrilla verticillata* occur throughout the year. In the rooted floating category *Ipomoea aquatica* occurs throughout the year.

Lamsal et al. (2014) studied aquatic and riparian biodiversity in an anthropogenically disturbed Ramsar site, the Ghodaghodi lake complex and found *Nelumbo nucifera*, *Trapa bispinosa*, *Nymphaea nouchali* and *Potamogeton natans* as the major anchored leaf floating species whereas *Hydrilla verticillata*, *Ceratophyllum demersum* and *Potamogeton* sp. as submerged and *Azolla imbricate*, *Lemna minor* and *Wolffia globosa* as free floating species.

Dhanam and Elayaraj (2015) studied aquatic macrophyte in Santhapettai Lake of Villupuram district in Tamil Nadu and found different growth forms which dominated the lake by submerged anchored (40%) followed by emergent anchored (33%), floating (21%), floating leaved anchored and submerged with 3% each.

Sharma and Singh (2017) conducted study on quantitative and diversity analysis of aquatic macrophytes dwelling littoral zone of sacred lake Doti Tal and found total of 45 macrophyte species belonging to 29 families with maximum number of species represented by emergent species followed by Submerged, rooted floating leaf type and free floating macrophytes.

Bhusal (2018) found altogether 115 vascular plant species of 45 families in lakes of Chitwan National park and found the dominancy of emergent species.

Roka (2019) recorded 42 macrophyte species with the dominancy of Asteraceae and Polygonaceae family and on the basis of growth form, emergent were dominant in Beeshazar lake.

Joshi Sharma (2019) reported 37 macrophyte species belonging to 22 families with the dominancy of emergent species in Ghodaghodi Lake, Kailali.

2.2 Phytoplanktons

Hirano (1955, 1963, 1965), made an extensive study on fresh water algae of Nepal. He collected algae from various water bodies of Gorkha, Tanahun and Syanjya districts of Nepal and identified 271 freshwater algal species. He reported 4 new Cosmarium species and a new genus, *Chaetomnion* (Ranjitkar, 2006).

Rai (2005) and Rai et al. (2010) made some contribution in Algae Flora with his work focused on Eastern Nepal. He reported 165 species new to Nepal.

Tiwari and Chauhan (2006) recorded Chlorophyceae as the most dominant group during winter followed by Bacillariophyceae and Cyanophyceae and Euglenophyceae asthe most dominant during summer at Kitham Lake, Agra. *Chlamydomonas conferat*,*Scendesmus bijuga*, *Zygnema* sp, *Spirogyra longata*, *Uronema* sp, *Rizoclonium hookeri*, *Ulothrix zonata*, *Drapernaldia* sp,*Closterium*, *Cynthia*, *Cyclotella glomerata*, *Nitzschia ciculari*s, *Fragilaria vaucheria*, *Frustulia* sp. were recorded only during winter season.

As per Mathivanan (2007) Phytoplankton from Cauvery River, India at station-I (Pannavadi), the species richness during January under different algal classes were observed as Chlorophyceae (10), Bacillariophyceae (8), Euglinae (6) and Myxophyceae (11) and during August were Chlorophyceae (18), Bacillariophyceae (7), Euglinae (9) and Myxophyceae (15). At Station II (SankalimuniappanKoil area), the species richness during January under different algal classes were observed as Chlorophyceae (6), Bacillariophyceae (4), Euglinae (7) and Myxophyceae (10) and during August were Chlorophyceae (10), Bacillariophyceae (9), Euglinae (5) and Myxophyceae (13).

Hassan et al. (2008) reported 97 species of the total belong to Bacillariophyceae, 37 species belong to Chlorophyceae, 13 species to Cyanophyceae, 5 species to Chrysophyceaeand 2 species to Euglenophyceae in Iraq. Five genus of phytoplankton were the highest number of species (*Nitzschia, Navicula, Gomphonema, Cymbella* and *Scenedesmus*). Some species was occurred continuously during study period such as, *Cyclotella ocellata, Cyclotella meneghiniana, Aulacoseira distans*, and *Gomphonema abbreviatum*.

Wu et al. (2011) explored the phytoplankton distribution in relation to environmental variables in Kielstau catchment, Lowland River in Germany. A total of 125 taxa were observed, among them *Tabellaria flocculosa*, *Euglena* sp, *Planothidium lanceolatum*, *Cocconeis placentula* and *Fragilaria biceps* were dominated species.

Fonseca and de Mattos Bicudo (2011) species richness of Phytoplankton was recorded highest during cool-dry winter than hot-rainy season in Ninfeias Pond, Brazil which is a tropical shallow reservoir with abundant macrophytes. They also reported the dominance of *Chlamydomonas* sp during hot-rainy season. *Monoraphidium griffithii, M. irregular, Chrysochromulina* cf. *breviturrita*, *Scenedesmus ecornis*, *Cryptomona serosa*, and *Chlorella vulgaris*as the most frequent taxa.

Pokharel (2012) recorded 28 genera belonging to 19 families and 5 classes in Seti Gandaki river.

Rai and Rai (2012) recorded 6 freshwater algae from Chimdi lake.*Oscillatoria splendida, Cylindrospermum stagnale, Gloeotrichia raciborskii, Melosira varians, Crucigenia crucifera and Euastrum spinulosum* was recorded which were new to Nepal.

Patil et al. (2012) observed altogether 19 species in which maximum number of sp observed were in the Rajaram lake i.e. 13 sp. There were 9 species observed from the class Chlorophyceae, 4 species of the class Cynophyceae, 3 of the class

Bacillariophyceae, 3 of the class Euglenophyceae. The *Microcystis* species was observed in Rajaram lake which indicates the signs of eutrophication in lake, while species like *Desmidium*, observed from Music Department and Bhshabhavan Lake were the indicator of better water quality. The physico-chemical parameters such as nitrates, phosphate, temperature and alkalinity are favorable for the growth of phytoplankton. Maximum species of the class chlorophyceae were observed during study period.

Shrestha et al. (2013) recorded 52 algal taxa belonging to 3 classes and 21 families with the dominancy of Chlorophyceae, followed by Cyanophyceae and Bacillariophyceae from Itahari and adjoining areas.

Ghimire et al. (2013) recorded 27 taxa of Cyanophyceae from Solukhumbu. 4 new records were identified for Nepal i.e *Euastrum oblongum*, *Panicum cylindrus, Scenedesmus quadricauda and Spirogyra amplectens.*

Hafiz et al. (2014) also recorded Bacillariophyceae as the dominant group over other groups in the Jhelum River in Kashmir Himalayas.

Mandal et al. (2016) from three different regions (Morang in Eastern, Chitwan in Central and Rupandehi district in western) of Nepal had reported the seasonal variation of phytoplankton under “Red bloom Non-Red bloom” condition. The seasonal variation of phytoplankton (x103cells/L) as per the division was concluded in the order of; Chlorophytes (2.04±0) > Cyanophytes (0.98±0) >Bascillariophytes (0.92±0) >Eulenophytes (0.29±0) during summer at non-red bloom fishponds of all three regions of country while the order was; Chlorophytes (1.25±0) >Eulenophytes (0.56±0) >Bascillariophytes (0.33±0) > Cyanophytes (0.32±0) during winter.

Choudhary (2016) reported water quality and phytoplankton in Gandak river, Bihar India. 75 species of fresh water algae were recorded having Bacillariophyceae as the largest group followed by the Cyanophyceae.

Gautam et al. (2014) recorded altogether 281 individuals of 14 taxa (family) belonging to 10 orders in dry season where as in rainy season 313 individuals of 18 taxa (family) belonging to 12 orders were found within the Ghol area of Rampur, Chitwan.

Similarly, Sharma et al. (2016) recorded 34 species of phytoplankton in the Baldi stream represented by three major groups, Bacillariophyceae (20 species), Chlorophyceae (10 species) and Cyanophyceae (4 species).

Rai and Khadka (2017) recorded 48 diatoms taxa from different sites of Bagmati river.Among which 20 diatoms were reported first time in Nepal.

Revathy and Krishnakumar (2018) studied about the abundance and population dynamics of phytoplankton community and its relationship with nutrients status of two tropical lakes, Vellayani and Sasthamkotta, Southern Kerala. Theyrecorded that there is positive relationship between plankton biomass with nutrients and found that planktonic biomass is regulated by nutrients status in freshwater bodies. Chlorophyceae show dominance in Vellayani Lake whereas Bacillariophyceae forms their dominance in Sasthamkotta Lake. The study reveals that there is positive relationship between plankton biomass with nutrients and found that planktonic biomass is regulated by nutrients status in freshwater bodies.

Roka (2019) recorded 45 phytoplankton genera with the dominancy of Bacillariophyceae, followed by Cyanophyceae and Euglenophyceae during winter season in Beeshazar lake, Chitwan.

2.3 Macro-Invertebrates

Understanding the importance of Macro-invertebrates, various studies on aquatic macro-invertebrate fauna have been performed by several biologists.Some works in this field have been conducted in Nepalese water bodies too (Sharma, 1975; Yadav and Rajbhandari, 1982; Vaidya, et al., 1988; Ormerod et al., 1994; Brewin et al., 2000; Sharma et al., 2006; Pokharel, 2011, 2013).

Shrestha (1980) studied the water quality of Bagmati biologically, using aquatic insects as bio-indicators. This was one of the first applied research work where aquatic insects were used to access the water quality of rivers of Nepal.

Yadav and Rajbhandari (1982) enquired the benthic macro fauna of Bansbarikhola and Dhobi khola in Kathmandu valley. They recorded 26 taxa at different sites of both streams. The major groups of bottom fauna recorded were*Tubifera* sp, Tipulidae, Dolichopodiae and Chironomidae.

These groups of fauna were found to be patchily distributed at the buttom of stream. The genus *Tubifera* was found to be most tolerant to the chemical pollution caused by discharges of Bansbari Leather and shoe factory in Bansbarikhola.

Nesseman et al. (2008) carried out detailed investigation on macroinvertebrates of Phewa Lake. They recorded altogether 37 taxa based on family level identification and 26 genera. According to him, Macroinvertebrates diversity was high due to the presence of numerous warm water species.

Shrestha et al. (2009) altogether 81 taxa based on family level identification were recorded from the Seti River basin in which 80.24 % was contributed by Arthropoda phyla only. While Diptera alone found to be contributed 22.22 % followed by 13.58 % Trichoptera and 12.35 % Heteroptera among recorded benthic invertebrates.

Dhonju (2010) have documented the macroinvertebrates from Jagadishpur reservoir and altogether 50, 53 and 42 macroinvertebrates taxa were recorded from inlet,outlets, and littoral zone of surrounding wetland respectively.

Zerlin and Henry (2014) recorded the taxa richness increases with the high water level in Camargo Lake.With the decrease in water level, substratum availability decreases and in turn decreases associated biota (invertebrate's communities)(Da Silva and Petrucio, 2018).

Shrestha and Adhikari (2016) a total of 2166 individual benthic macroinvertebrates from 10 families and 7 orders were recorded from Taudaha Lake, Kathmandu.

Bhandari et al. (2019) carried out research on benthic macroinvertebrates in five tributaries of Budhiganga River in western Nepal.

Pepa et al. (2018) reported the group EPT (E-Ephemeroptera, P-Plecoptera, T-Trichoptera) to be the highest number of organisms in the sampling stations.

According to him, these groups are more sensitive if there is presence of an organic pollution in an aquatic body, reflecting immediately on the number and diversity of organisms collected.

2.4 Physico-chemical characteristics of Water

Analysis of Nepal's surface water quality data collected in 1985 revealed that the ionic composition is significantly different from other parts of the world due to the dominance of bicarbonates, the absence of sulfates, with calcium being a dominant anion, and the N:P ratio being generally less than 10 (Jones et al., 1989). There is a general understanding of Nepal's water quality; organic matters are in higher abundance during the wet season (summer in Nepal) while nutrients like N and P are higher in the dry season due to low flow and reduction in dilution (Kannel et al., 2007; Pant et al., 2018). Similarly, urban river reaches are significantly more polluted than the forested and agriculture counterparts (Kannel et al., 2007, 2008).

Rai (2000) studied the limnological characteristics of subtropical Lakes Phewa, Begnas, and Rupa in Pokhara Valley. He found the annual water temperature ranged from 12° to 29°C in all lakes. Lake Phewa and Lake Begnas were monomictic and anoxic in the hypolimnion during thermal stratification from April to September. Dissolved oxygen was drastically depleted in April and/or May in shallow Lake Rupa when the macrophyte community began to decompose. NH4+-N accumulated below 5m during March–September when dissolved oxygen was depleted in Lakes Phewa and Begnas. Annual net primary production showed that the lakes were productive but will tend to become heterotrophic in the future.

Niroula et al. (2010) carried out research on topic "Seasonal Variations in Physico-Chemical Properties and Biodiversity in Betana Pond, Eastern Nepal", had found that water temperature of lake to be 30.8 ºC and 17.5 ºC during and rainy winter seasons respectively. The pH was 6.63 and 7.12 during rainy and winter season respectively. The conductivity of lake water was 411 µs/cm and 312 µs/cm during and rainy winter seasons respectively. Likewise, TDS was 201mg/l and 234 mg/l during and rainy winter seasons respectively. The DO was 5.41 mg/l and 3.19 mg/l during and rainy winter seasons respectively.

Shah and Shah (2013) measured dissolved oxygen decreased from rural to urban areas while nitrate nitrogen, ortho-phosphate phosphorus, chloride and BOD greatly increased in Bagmati river. According to them, the continuous discharge of sewage and industrial input (Kannel et al., 2007) has led to increased levels of BOD and chloride and the higher level of dissolved oxygen in rural areas could be attributed to the water turbulence and fewer nutrients.

Niraula (2012) studied surface water quality and characterstics of underlying sediments of Beeshazari lake and found lake was less favourable to aquatic organisms, with low pH, transparency and DO and high with nitrogen and phosphorous.

Thapa and Soudh (2012), studied water quality of Jagdishpur reservoir and found high value of temperature, CO2 and alkalinity during summer, while found higher value of chloride,conductivity,DO,TDS, pH,nitrate, and phosphate during winter.

Kunwar and Devkota (2012), accessed the seasonal variation on physiochemical parameters of Rupa lake Kaski, Nepal. They concluded that the temperature during pre-monsoon was 23.24 ±1.20 °C and that of post-monsoon was 21.90 ±2.10°C. The pH value was 5.67 ± 0.25 and 6.29 ±0.16 during pre-monsoon and post-monsoon respectively. The DO was 4.16 ±1.10 mg/l and 7.68 ±1.50 mg/l respectively.

Bhusal (2018), analyzed limnological status of lakes of Chitwan National park and observed pH, DO, TSM, total alkalinity ,total hardness, conductivity, nitrate and inorganic phosphorous and found all studied lakes as hyper eutrophic for phosphorus concentration.

CHAPTER III
MATERIALS AND METHODS

3.1 Study Site

The Setikhola watershed includes Pokhara valley and adjoining hills and mountains. Pokhara Valley is a popular tourist destination and has moderately steep to very steep mountain terrain with many attractive lakes. Area of this watershed is about 990 km^2 with the total length of the river of 381 kilometers, while the lake area of three major lakes (Phewa, Begnas and Rupa lakes) is approximately 9km^2 (Baral et al., 2016).The city lies between 83^o 58' 30'' E to 80^o 02' 30'' E longitude and 28^{o}10' N to 28^o 16' N latitude. This city encompasses nine cluster lakes (Phewa, Begnas, Rupa, Dipang, Maidi, Khaste, Neurani, Kamalpokhari and Gunde) of ecological importance listed as 10th Ramsar Site/ Wetlands bearing an international gratitude and safeguarding the livelihoods and ecosystem. Our study area includes 6 important lakes i.e Begnas, Rupa, Dipang, Khaste, Gunde and Fewa and 9 rivers i.e. Bijayapur, Herpan, Kali, Seti, Dhunge sangu, Yamdi, Phusre, Kotre and Bhurjung. The climatic conditions of the central part of the basin ranges between sub-tropical to cool temperate, whereas the climatic deviations in the northern part ranges from temperate to alpine with high precipitation rate, around 3000 mm per year (Rimal et al, 2013; Tripathee et al., 2016). The valley floor is a growing metropolis while hills are dominated by subsistence agriculture based land use. The high elevation regions are mostly pristine wilderness with forests, prairies, and snow-covered mountains and are protected as a part of Annapurna Conservation Area (ACAP, 2017). The valley at its lower limit (especially 900 - 1700m) embraces natural belt of *Shorea robusta* mixed with *Pinus* spp., *Schima wallichii, Castanopsis indica* and patches of *Alnus nepalensis*. Among the aquatic species, species of *Lemna*, *Eichhornia*, *Hydrillla*, *Utricularia, Ceratophyllum*, *Nymphaea, Nelumbo and Sagittaria* are mainly distributed in the water bodies. Pokhara valley gets the highest amounts of rainfall in Nepal. The valley and lakes play an important role in the hydrological cycle in the area by recharging groundwater, controlling floods and trapping sediments. Pokhara is one of the most rapidly urbanizing city of Nepal residing mixture of climatic conditions within the Seti river watershed.Thousands of people depend on the lakes for income from tourism, fishing, irrigation, electricity generation, and water supply.

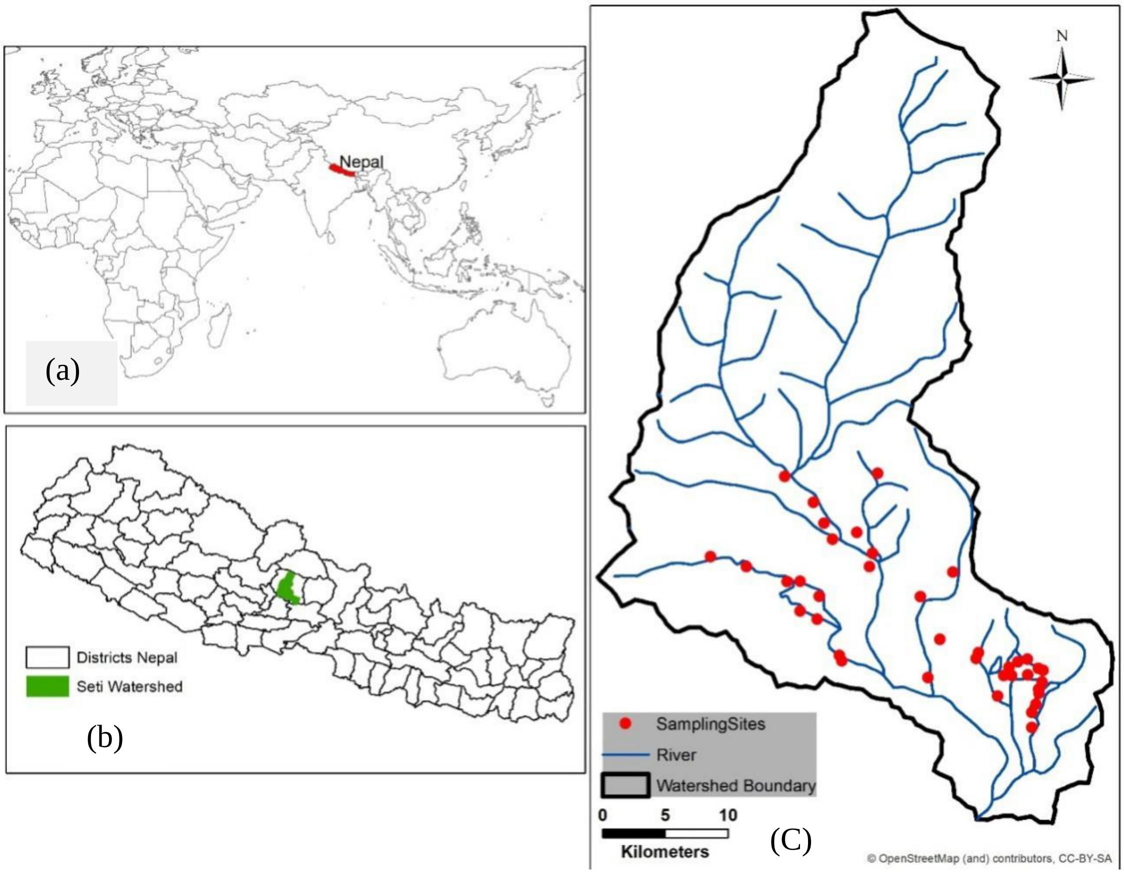

Figure 1. Study area a. Showing Nepal from google map b.Showing district in map of Nepal c. District boundary with study watershed showing sampling sites through QGIS.

3.2 Research Design and Methodology

Systematic and integrated methodology was followed. Field studies and collection of primary and secondary data were applied in the research. Primary data were collected through field survey (Janauray 1 to Janauray 15, 2019) and laboratory analysis. Secondary data regarding the research were collected from different published and unpublished literature journals, articles, and internet. The research design for this research was descriptive and explanatory.

Table 1: Objective wise Materials and method

S.No	Objectives (specific)	Materials and Methods
1	To study the aquatic Phytodiversity (macrophytes, phytoplanktons) and macroinvertebrates of the study area.	i. Literature Review ii. Field Sampling, Preservation and Taxonomic identification
2	To study the present water quality status	i. Physio-chemical parameters (Conductivity, DO, Temperature) using Field kit for water analysis in the field. ii. Water sample collection for Lab in the field Sample analysis for Nitrate (NO_3), phosphates (PO_4) and Ammonia (NH_3) based on (Trivedy and Goel, 1986) method using Spectrophotometer Statistical analysis: Bivariate
3	To correlate the composition of aquatic phytodiversity and macroinvertebrates with physico-chemical properties of water of the study area.	i. CCA ordination

Table 2 : List of instruments

S.No.	Instruments	Company	Model Number
1.	GPS	GARMIN	ETRAX 10
2.	Air/water temperature	CENTER	305 DATA LOGER
3.	Pro Plus Polarographic DO/pH/Conductivity Quatro Kit,4meter	YSI	603190 Professional plus model
4.	Spectro-Photometer	Hanna Instruments	HI 96715
5.	Microscope for Macroinvertebrate	High resolution Microscope	
6.	Microscope for Phytoplankton	Compound Microscope	G016004265

3.2.1 Sampling, Preservation and Identification of Macrophytes

Standard quadrate method was applied (Ghos and Biswas, 2015). Macrophytes were collected from 41 sampling unit by using quadrat (1m×1m). The Collected species from the study area were tagged, pressed and dried by standard herbarium specimen preparation technique. Appropriate field notes were also prepared in the field (Annnex XII). Herbarium of collected Macrophyte specimens were identified and labelled in herbarium sheet. Identification was done by consulting relevant literatures (Annotated Checklist of the Flowering Plants of Nepal (Press et al., 2000). Besides, taxonomists were also consulted and cross checking of specimens with the herbarium specimen's house in the National Herbarium and Plant Laboratories (KATH) was also done.

3.2.2 Sampling, Preservation and Identification of Phytoplanktons

About 41 sampling unit from the water bodies of Setikhola watershed were taken. From which 41 Phytoplankton samples were collected following quadrate (1m×1m) method by squeezing roots of macrophytes and distinct large filamentous forms by free hand picking.Then the materials collected were transfer to collection bottle. Collected samples were tagged and labelled with collection no., date of collection and habitat. Then the Sample was preserved in 4% formalin for later sorting and taxonomic analysis in the laboratory.

After bringing the sample in the lab from the field the screening of phytoplankton sample was done by preparing temporary slides of each samples and examined under magnification of 40 mega pixel in compound microscope. Microphotographs of the best algae were taken by Olympus trinocular microscope attached with Canon Power-shoot digital camera. Common and rare species were observed. Identification was done by consulting relevant literatures, Photographs and consulting with Phytoplankton specialist.

3.2.3 Sampling, Preservation and Identification of Macro-invertebrates

About 41 sampling unit from the water bodies of Setikhola watershed were taken. From which 41 Macro-invertebrate samples were collected in the collection bottle using the standard hand net of 500mm. Moreover, by turning over the stones, cobbles and plant parts within the sampling area in order to dislodge and collect macro-invertebrates that are hidden underneath or attached to the bottom. The collected samples were preserved in 70% ethanol for later identification. Coordinates of the sampling points and composition as well as diversity of species was noted in the data sheet. Collected samples were sorted group wise in the lab and taxonomic identification was done at environment lab TU, Kirtipur by consulting Literatures and macro-invertebrate's specialist.

3.2.4 Water Sampling, Preservation and Laboratory analysis

Water samples were collected from the depth of 0.5m in water bodies for the lab analysis. The samples were collected in cleaned one-liter plastic containers, which were rinsed with and lake water before collection. Water samples were collected avoiding floating materials. The stoppers of the sample bottle were closed properly to prevent outside contamination. The bottle was labelled describing the name of the sampling site, sampling point, date and time under which it was sampled.The water temperature, dissolved oxygen (DO) and conductivity were measured on the spot using a portable water analysis kit. Nitrate as total nitrogen, inorganic phosphorus and ammonia were determined in the laboratory of Central Department of Botany.

The nitrate as total nitrogen in water was determined by Phenol DisulphonicAcid method, Phosphate as Inorganic Phosphorus of water was determined by Ammonium Molybdate-Stannous chloride solution method and Ammonia of water was determined by Phenate method (Trivedy and Goel,1986) in the lab in order to provide the physico-chemical context in which the phytoplankton, macrophyte and macro-invertebrate species assemblages. Aquatic phytodiversity and macro-invertebrates sampling and water sampling were performed during the same period.

3.3 Data Analysis

3.3.1 Descriptive Analysis

The normality in data distribution of different Physico-chemical parameters and aquatic vegetation were tested by SPSS Statistics version 23. Shapiro-Wilk significant value was used to test the normality of the data.If $p \geq 0.05$ the data distribution would be considered as normal but if $p<0.05$ the data distribution is significantly different from the normal distribution. Parametric Statistical tests i.e. coefficient of correlation using Karl Pearson method was performed between Physico-chemical parameters of water and species composition to analyze the data.

Co-relation charts between Physico-chemical characterstics of water and species composition were drawn by using SPSS Statistics version 23.

3.3.2 Multivariate Analysis

The relationship of Macrophyte, Phytoplanktonas well as Macroinvertebrate with Physico-chemical variables was evaluated using ordination. According to (Peck, 2010), prior to analysis rare species (species that occurred only in one lake or river) were deleted from species matrices to reduce dataset sparsity (Kisoon et al., 2013). Detrended Correspondence Analysis (DCA), an unconstrained (indirect) gradient analysis, revealed a gradient length of first axis as4.206,3.381,and 6.583 in terms of SD in Macrophytes,Phytoplanktons and Macro-invertebrates respectively thatindicate the linear relationship among species along the main gradient.

Since the length of the gradient of first DCA axis was greater (>2.00), Canonical Correspondence Analysis (CCA) was performed to access the species environment relationship. CANOCO version 4.5 (Ter Braak, 1995) was used for DCA and CCA ordination. The length of arrow is relative to the importance of the explanatory variable in the ordination, and arrow direction indicates positive and negative correlations (Jasprica et al., 2012, Laskar and Gupta, 2013). Percentages of variance and Eigen values of each site in axis 1 were found to be higher than axis 2 (Annex 5, 6 and 7). Similar findings were reported by Liu et al., (2010).

CHAPTER IV

RESULTS

4.1 Macrophyte Composition

Altogether 39 plant species belonging to 21 families were recorded from 41 different sampling sites of Setikhola watershed (Annex I). By growth form, 4 species were rooted with floating leaves,5 species were free floating, 13 species were submerged and 16 species were emergent which is highest in number in growth form during study period (fig. 3) (Annex I).

The study found Potamogetonaceae to be the largest family having 5 species followed by Cyperaceae, Hydrocharitaceae, Poaceae and Polygonaceae with 3 species, likewise Araceae, Menyanthaceae, Najadaceae, Onagraceae and Pontederiaceae with 2 species and other remaining family with single species (Annex I).

Rupa lake has the highest diversity with 32 Macrophyte species which is followed by Fewa lake with 30 species, Begnas lake with 19 species, Dipang and Gunde with 15 species and Khaste has 14 species. Similarly, Bijayapur and Seti river has macrophyte with 7 species, followed by Herpan river with 6 species, Kali, Dhungesangu and Phusre with 4 species. Kotre 3 and Yamdi have only 1 species which is the lowest number of macrophyte species among studied water bodies (fig. 2).

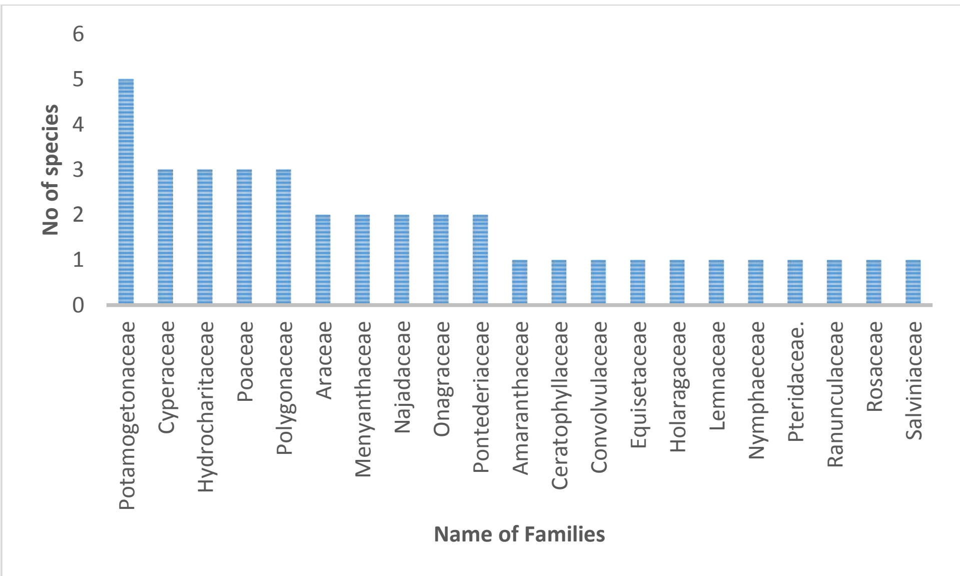

Figure 2: Macrophyte species belonging to different families of Setikhola watershed.

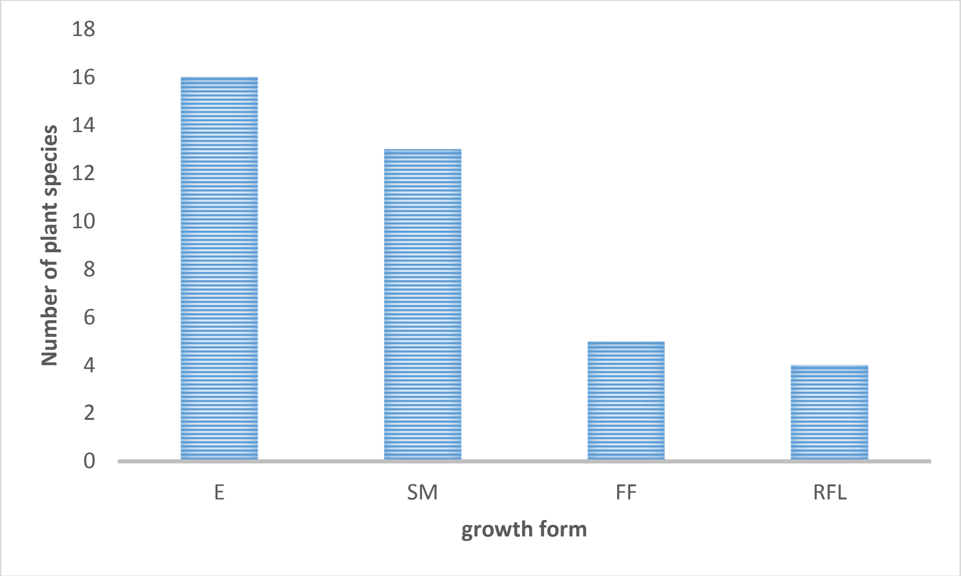

Figure 3: Macrophyte species with growth form.

(E= Emergent, SM= Submerged, FF= Free floating, RFL= Roots of free floating leaves)

4.2 Phytoplankton composition

Altogether 105 Phytoplankton species were recorded from 41 different sites of Setikhola watershed. Among which 68 species were recorded from lakes, 77 species were recorded from river while 41 species were found in both lake and river and 2 species were unidentified (fig. 5). Bacillariophyceae showed the highest number with 52 species, followed by the Cyanophyceae with 30 species, Chlorophyceae with 21 species. Charophyceae and Euglenophyceae with only 2 species which is lowest among the recorded family (fig. 4) (Annex II).

Fewa lake showed the highest diversity with 29 phytoplankton species which is followed by Begnas lake and Depang lake with 28 species, Kotre river with 26 species, Bhurjung with 24 species, Bijayapur with 23 species, Dhungesangu with 21 species,Rupa and Khaste with 19 species, Phusre and Kalikhola with 18 species , Seti river with 16 species,Herpan with 14 species and Mardikhola with 13 species. Gunde with 11 speciesand Yamdi river showed lowest diversity with 6 species of Phytoplanktons among the studied Water Bodies. *Navicula* sp, *Gomphonema* sp, *Nitzchia* sp and *Aulacoseira* sp are dominant in most of the lentic Water Bodies.

Ulothrix sp, *Mougeota* sp, *Crucigenia* sp, *Oscillatoria* sp, *Tetraedron* sp, *Cymbella* sp and *Gomphonema* sp are common in lotic Water Bodies. *Navicula* sp, *Ghomphonema* sp and *Cymbella* sp are dominant in most of all Water Bodies.

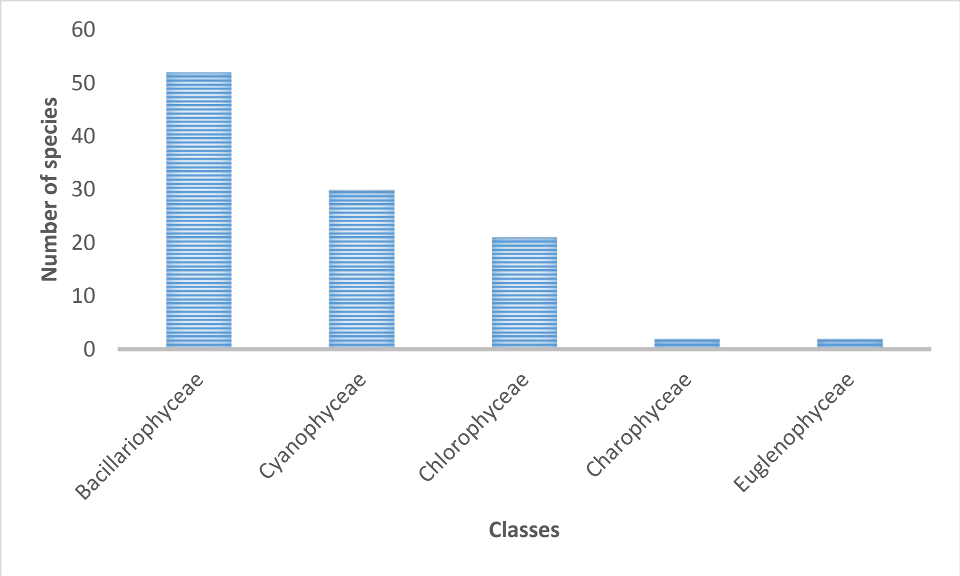

Figure 4: Phytoplankton species belonging to different Classes of Setikhola watershed

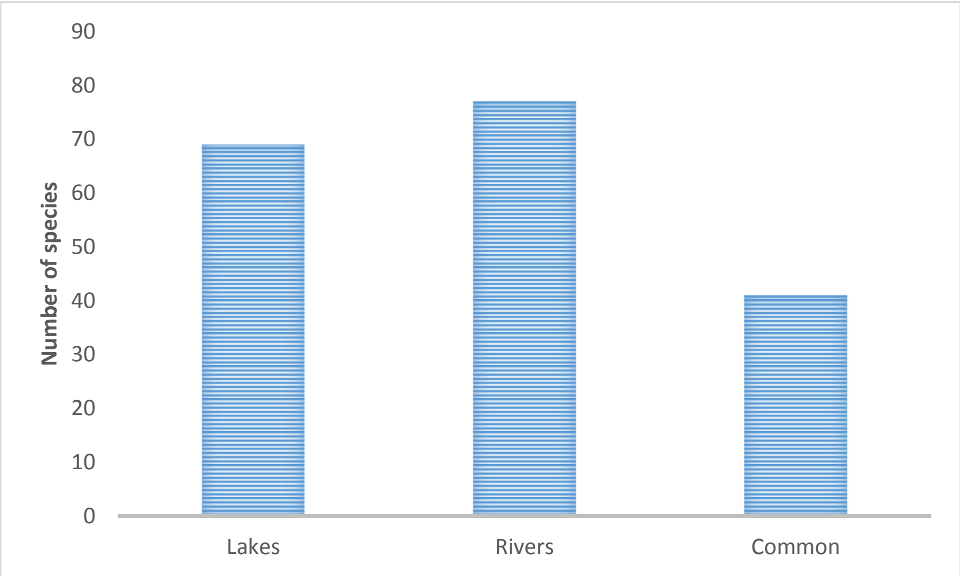

Figure 5: Phytoplankton Species composition in Water Bodies of Setikhola watershed.

4.3 Macro-invertebrate Composition

Altogether 9 orders with 35 families of Macro-invertebrates were recorded from different 41 sampling sites in different Water Bodies of Setikhola watershed. Among which the diversity of order Diptera was found larger in most of the water bodies.

Seti river with 10 family and 8 order with the dominancy of Plecoptera and Kali river with10 family and 5 order with the dominancy of Diptera which showed the highest diversity among the studied Water Bodies. Begnas lake with 9 family and 4 order with the dominancy of Hemiptera which is followed by Rupa lake with 8 family and 4 order with the dominancy of Gastropoda. Fewa lake with 6 family and 4 order with the dominancy of Diptera, Khaste with 5 family and 3 order with the dominancy of Hemiptera. These are followed by Dhungesangu with 4 family and 4 order, Gunde ,Yamdi and Herpan with 4 family and 3 order and Kotre with 3 family and 3order,Bijayapur river with 4 family and 2 order, Dipang with 3 family and 2 order which showed the lowest diversity (fig. 6).

The family Nepidae was commonly found in most of the Water Bodies while the family Hydropsychidae and Heptagenidae was found to be dominant in most of the rivers and the family Culicidae was found to be dominant in most of the lakes.

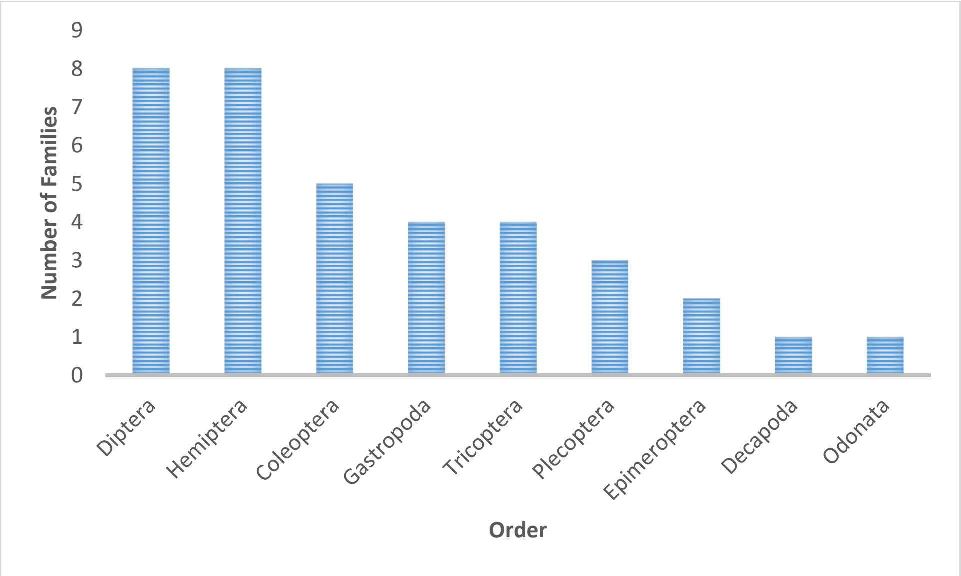

Figure 6: Macro-invertebrate Family belonging to different Order

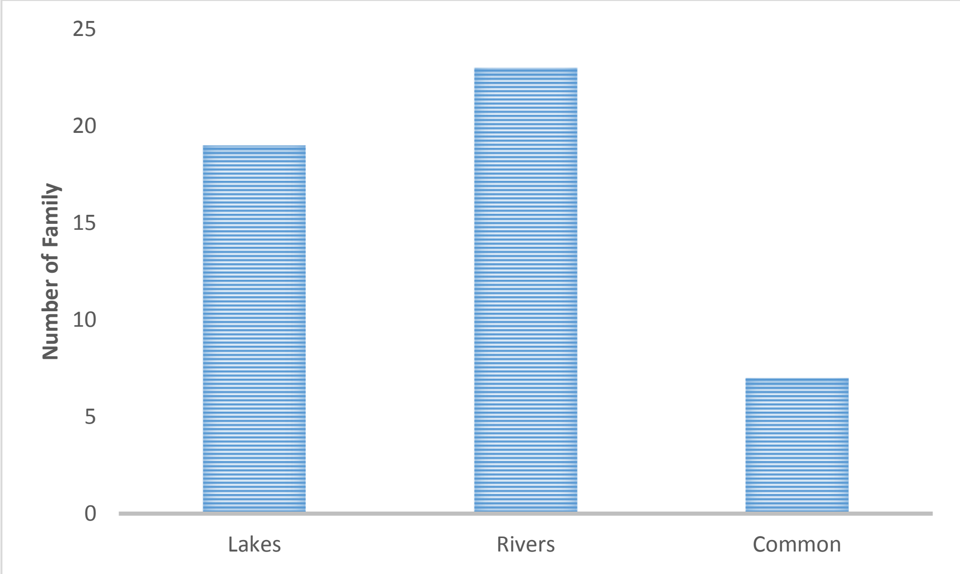

Figure 7: Macro-invertebrate composition in Water Bodies of Setikhola watershed.

4.4 Physico-chemical Characteristics of Water

Present study involved in the analysis of water quality in terms of physico-chemical parameters in different water bodies of Setikhola watershed. The measured parameters, unit wise average value noted in the water sample collected from different locations. Following figures are showing individual waterbodies variation in physico-chemical parameters of Setikhola watershed. The results of water quality in Water Bodies were obtained as follows.

4.4.1 Water Temperature

Phusre has highest water temperature 20.7℃ followed by Yamdi river 18. 1℃, Herpan 17.5℃, Begnas lake 17.46℃, Kali khola 17.3℃ , Fewa lake 16.8℃,Bijayapur 16.1℃, Dipang lake 16.1℃, Khaste 14.8℃ andKotre 14.6℃ . The study showed lowest water temperature at Gunde Lake as 11.7℃. and Seti River 11.3℃ (fig. 8).

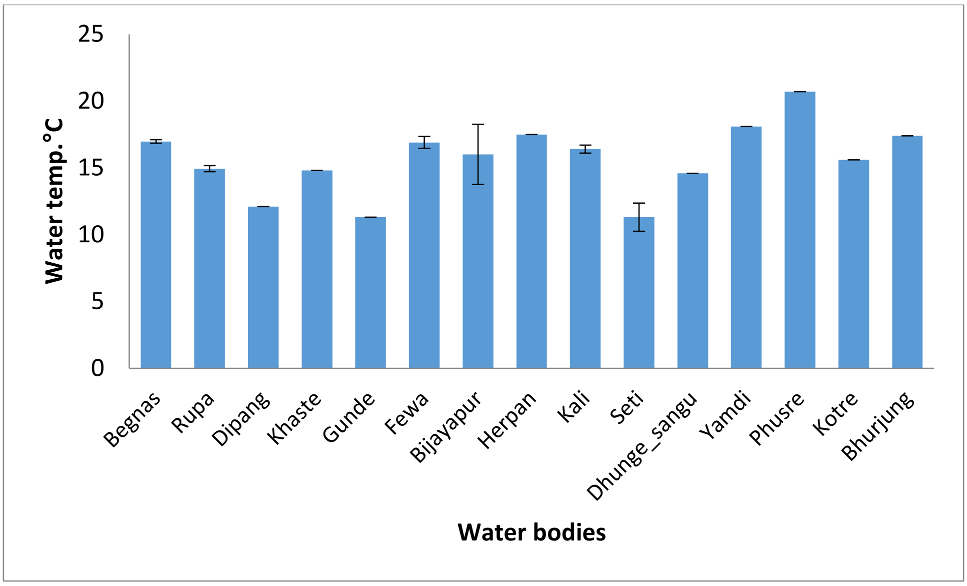

Figure 8: Water temperature variation among studied Water Bodies of Setikhola watershed

4.4.2 Dissolved Oxygen (DO)

Setikhola has 8.42 mg/LDissolved oxygen which is highest and is followed by Dhungesangu 7.89mg/L, Bijayapurkhola 7.27 mg/L, Kotre 7.14 mg/L, Begnas lake 7.04mg/L, Rupa lake has 6.73mg/L, Fewa 6.12mg/L,Phusre 6.06mg/L,Bhurjung 5.84mg/L, Dipang 5.5 mg/L, Khaste 5.19 mg/Land Herpankhola 5.56 mg/L . Yamdi 4.9 mg/Land Gunde lake has 4.7mg/L Dissolved oxygen which is lowest among studied water bodies (fig. 9).

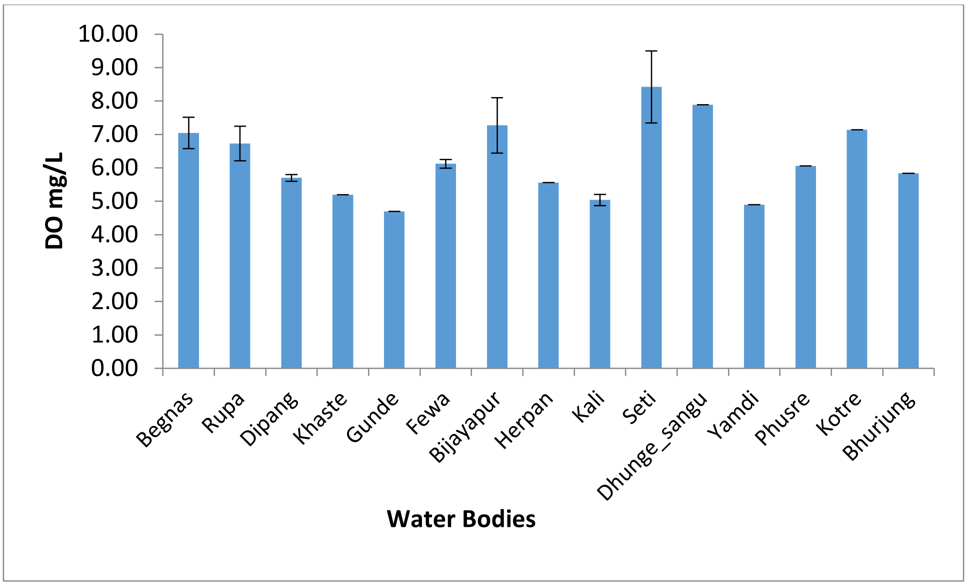

Figure 9: DO variation among studied Water Bodies of Setikhola watershed

4.4.3 Conductivity

Bhurjung has 340µs/cm conductivity, which is highest and is followed by Dhunge sangu 334.5 µs/cm, Kotre 314µs/cm, Kali river 304.3µs/cm and Yamdi river 285.8µs/cm, Bijayapur 246.69 µs/cm, Setikhola 223.92 µs/cm, Fewa lake 83.4 µs/cm, Begnas 73.47 µs/cm, Rupa 42.56 µs/cm, Khaste 41.56 µs/cm, Herpan river has 37.8µs/cm. Dipang 33 µs/cm and Gunde has 29. 6µs/cm conductivity which is lowest among studied water bodies (fig. 10).

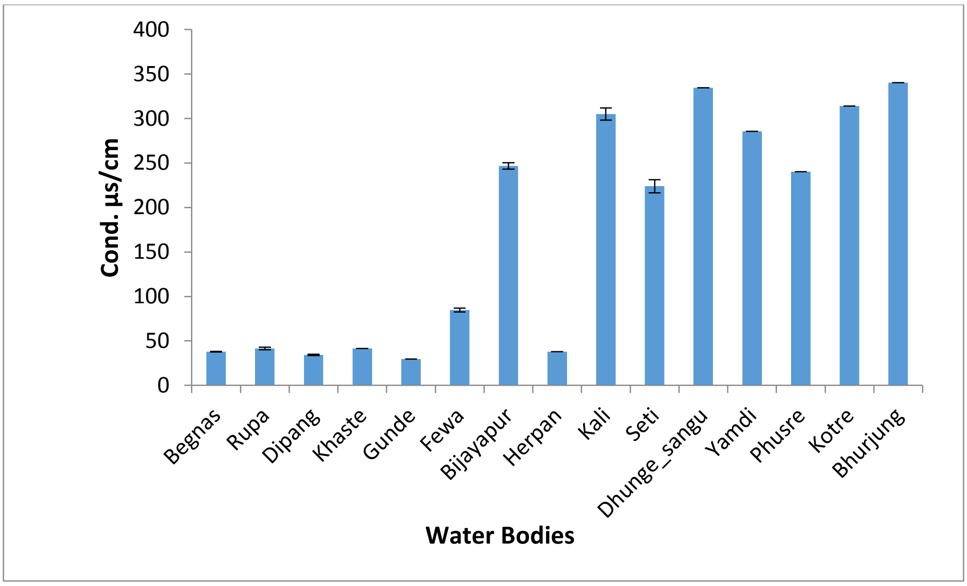

Figure 10: Conductivity variation among studied Water Bodies of Setikhola watershed

4.4.4 Phosphate as Inorganic Phosphorus

Seti river has 0.29mg/L phosphate concentration which is highest among studied Water Bodies, followed by Dipang lake 0.226 mg/L, Kotre 0.22 mg/L, Kalikhola 0.20 mg/L, Phusre 0.19mg/L, Bhurjung, Bijayapur and Rupa 0.18µg/L. Similarly, Fewa, Yamdi has 0.17 mg/L phosphate concentration, Begnas, Khaste, Gunde lake has 0.16 mg/L Concentration (fig. 11).

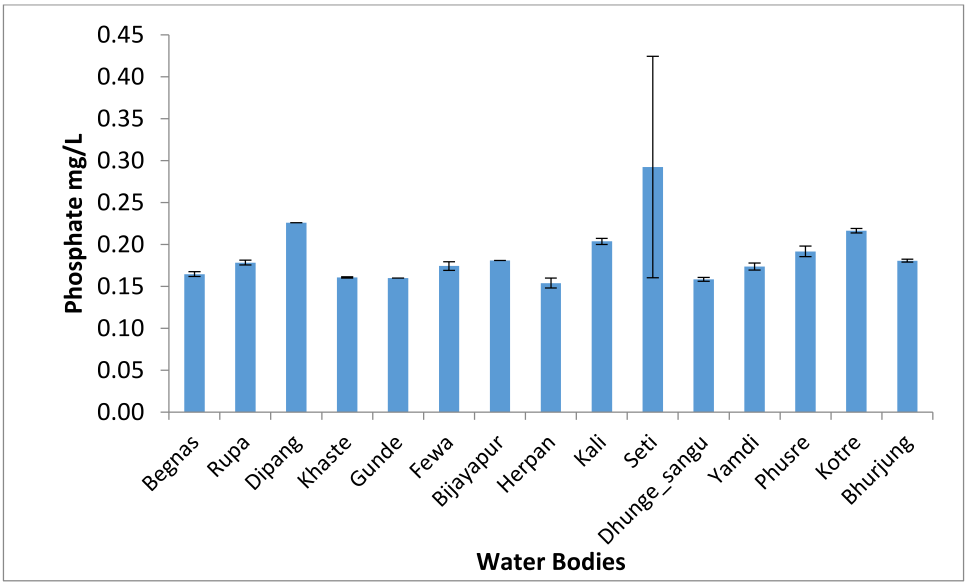

Figure 11: Phosphate variation among studied Water Bodies of Setikhola watershed

4.4.5 Nitrate as Total Nitrogen

Herpankhola has highest Nitrate concentration as 0.05 mg/L, followed by Yamdi 0.049 mg/L, Kali 0.048 mg/L. Fewa lake 0.047 mg/L followed by Dipang, Khaste, Gunde,Bijayapur and Seti has 0.046 mg/L and Begnas,Dhungesangu 0.045 mg/L and lowest in Kotre as 0.043 mg/L nitrate concentration (fig. 12).

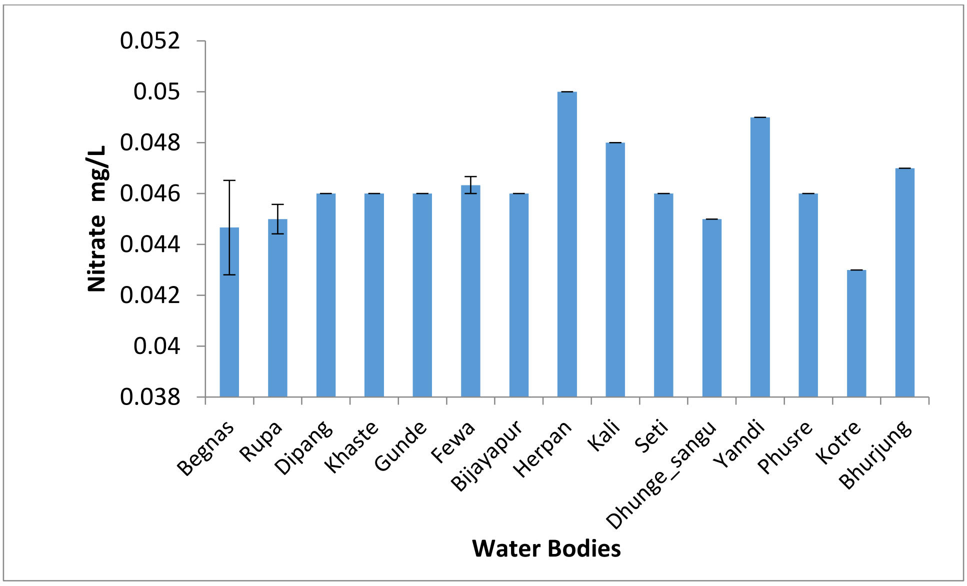

Figure 12: Nitrate variation among studied Water Bodies of Setikhola watershed

4.4.6 Ammonia

Yamdi has 1.451mg/L ammonia concentrationwhich is highest among studied Water Bodies, followed by Phusre 1.124 mg/L, Kotre 0.859 mg/L, Bhurjung 0.701mg/L and then Dhungesangu 0.681 mg/L, Herpan 0.517 mg/L. Similarly Fewa lake has 0.511 mg/Lammonia concentration followed by Begnas lake 0.503 mg/L, Gunde 0.459 mg/L, Rupa lake 0.499 mg/L ,Kalikhola 0.423 mg/L, Dipang 407 mg/L, Seti 0.368 mg/Land Khaste lake has 0.337 mg/L.Bijayapurkhola has the 0.283 mg/L ammonia concentration which is lowest among studied water bodies (fig. 13).

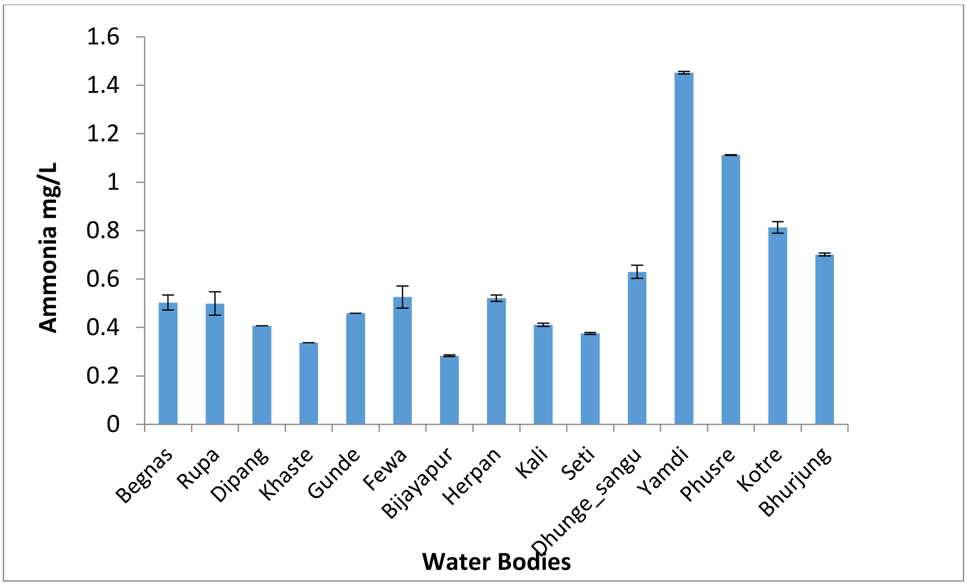

Figure 13: Ammonia variation among studied Water Bodies of Setikhola watershed

Table 3 : Pearson Correlation Coefficient values among Physico-chemical parameter of water

		Elev	DO_mg/L	Cond	Nitrate	Ammonia	Phosphorus	Water_temp
Elev	Pearson Correlation	1						
DO_mg/L	Pearson Correlation	.609*	1					
Cond	Pearson Correlation	0.359	0.473	1				
Nitrate	Pearson Correlation	.671*	0.153	-0.034	1			
Ammonia	Pearson Correlation	0.177	-0.378	0.433	0.188	1		
Phosphorus	Pearson Correlation	-0.031	0.185	0.279	-0.122	0.066	1	
Water_temp	Pearson Correlation	-0.240	-0.302	0.040	0.046	0.391	-0.251	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Elev= elevation, DO= dissolved oxygen, Pi= inorganic phosphorus, Cond = conductivity, Water_temp= water temperature)

Dissolved oxygen showed strong negative correlation with water temperature (r = -537) (Table 3).

4.5 Relationship between Physico-chemical Characteristics of Water and Aquatic Macrophytes

The CCA ordination shows the relationship between macrophytes and different Physico-chemical parameter of water (fig. 14). Forward selection and Monte Carlo Permutation test revealed that Conductivity was the most important variable (p=0.001) governing the macrophyte composition (Annex V).

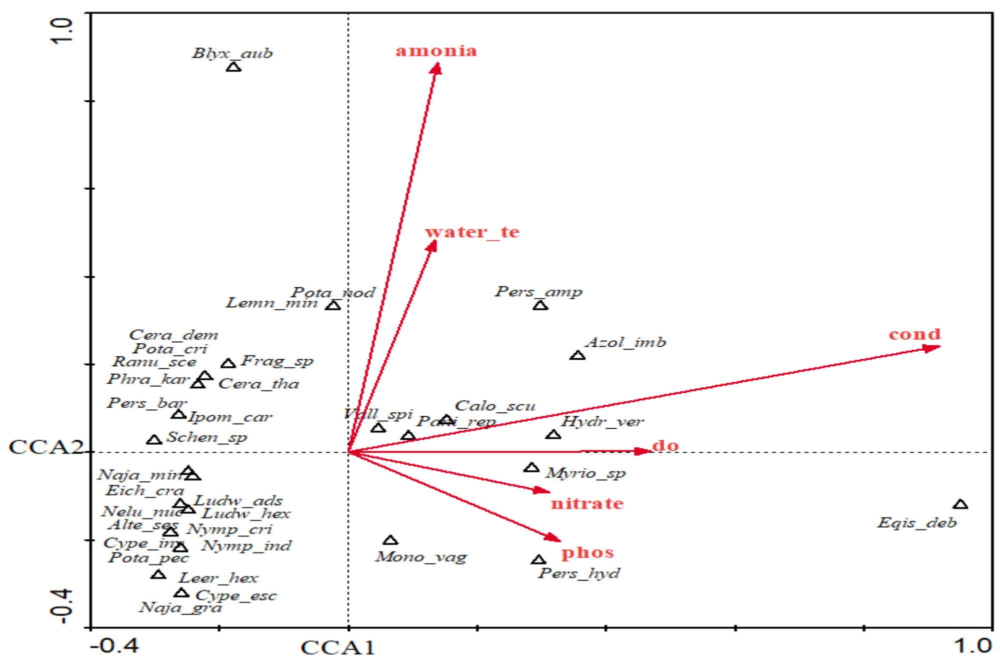

Figure 14: CCA biplot for Macrophyte Species along with Physico-chemical Characteristics of water

Species abbreviations represent concatenated forms of first three letters of generic name and specific epithet,as presented in (Annex I) and concatenated forms for Physico-chemical parametersare (water_te = water temperature, cond = Conductivity, do = Dissolved oxygen, nitrate = Nitrate as total nitrogen, phos = Phosphate as inorganic phosphorus, amonia = Ammonia).

First axis of CCA ordination between Macrophyte species and Physico-chemical characteristics of water explained 0.884% variance of species data and 26.2 % of relation between species and Physico-chemical characteristics of water (Annex V).

Most of the Macrophyte species showed negatively strong affinity towards studied Physico-chemical parameters. The species like *Ceratophyllum demersum, Potamogeton nodosus, Potamogeton crispus, Frageria sp, Persicaria barata, Ipomoea carnia, Schoenoplectus* sp, *Ranunculus sceleratus, Phragmites karka, Ceratopteris thalictroides and Blyxa aubertii* showed negatively strong affinity with nitrate and inorganic phosphate. *Eichhornia crassipes, Nelumbo nucifera, Ludwigia adscendens, Ludwigia hexapetala, Najas graminea, Najas minor, Alternenthera sessilis, Cyperus involucratus, Leersia hexandra, Ranunculus sceleratus, Nymphoides cristata, Nymphoides indica and Cyperus esculentus* species showed negatively strong affinity with conductivity, ammonia and water temperature.

Likewise, *Persicaria hydropiper* and *Monochoria vaginalis* showed positive affinity with inorganic phosphate while *Myriophyllum* sp showed positive affinity with nitrate. *Colacasia sculenta, Vallisneria spiralis, Panicum repens, Azolla imbricate, Persicaria* sp, *Hydrilla verticillata* showed positive affinity with Conductivity and water temperature (fig. 14).

4.6 Relationship between between Physico-chemical Characteristics of water with Phytoplanktons (Multivariate analysis)

The CCA ordination shows the relationship between plot, Phytoplanktons and different Physico-chemical parameters (fig. 15). Forward selection and Monte Carlo Permutation test revealed that Condictivity was the most important variable ($p=0.002$) governing the Phytoplanktons composition (Annex IX).

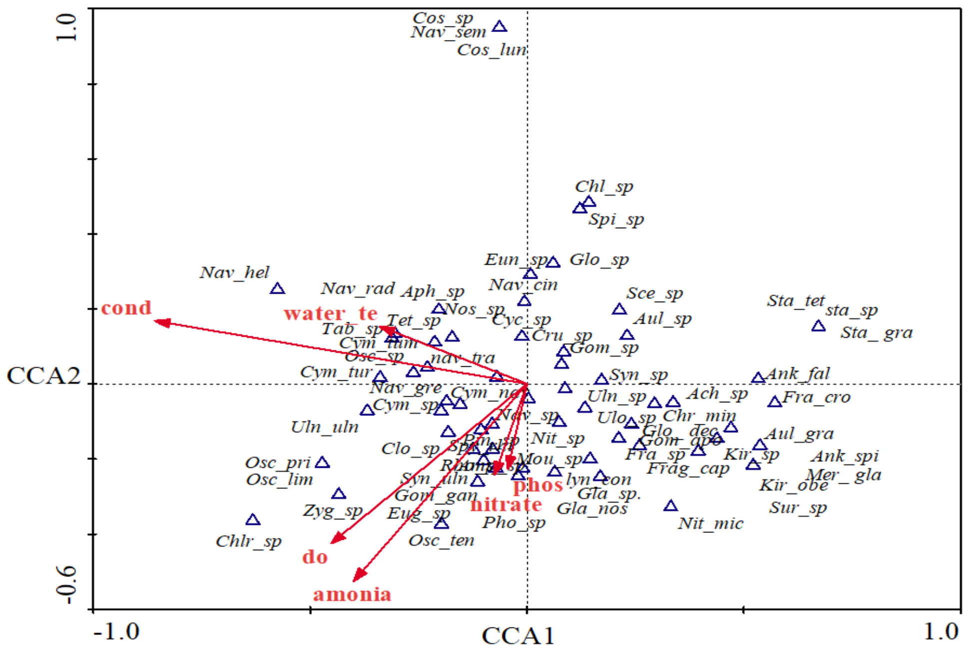

Figure 15: CCA biplot for Phytoplankton Species along with Physico-chemical Characterstics of water

Many species are found towards CCA1 plot and showed negative correlation with all physico-chemical parameter that are studied. The species like *Ulnaria*, *Ulothrix, Aulocoseira*, *Mougeota*, *Gomphonema*, *Fragillaria*, *Kirchneriella obesa*, *Kirchneriella* sp etc showed strong negative correlation with conductivity. But *Oscillatoria*, *Cymbella*, *Tabellaria*, *Navicula transitans*, *Navicula radiosa*, *Navicula helophila*, *Nostoc* showed positive affinity with conductivity and water temperature. The species like *Oscillatoria tenuis*, *Euglena*, *Zygnema*, *Chlorella*, etc are concentrated with high dissolved oxygen and ammonia. The strength of nitrate and phosphate towards species growth is similar where *Pinnularia* sp, *Synedra ulna*, *Gomphonema gandhi*, *Rhoicosphenia* sp, etc are concentrated (fig. 15).

4.7 Relationship between Physico-chemical Characteristics of Water and Macro-invertebrates (Multivariate analysis)

The CCA ordination shows the relationship between plots, Macro-invertebrates and different Physico-chemical characteristics of water (Figure 16). Forward selection and Monte Carlo Permutation test revealed that Conductivity was the most important variable (p=0020) governing the Macro-invertebrates composition (Annex X).Nitrate as total nitrogen was the second most significant variable (p=0.0034) (Annex X).

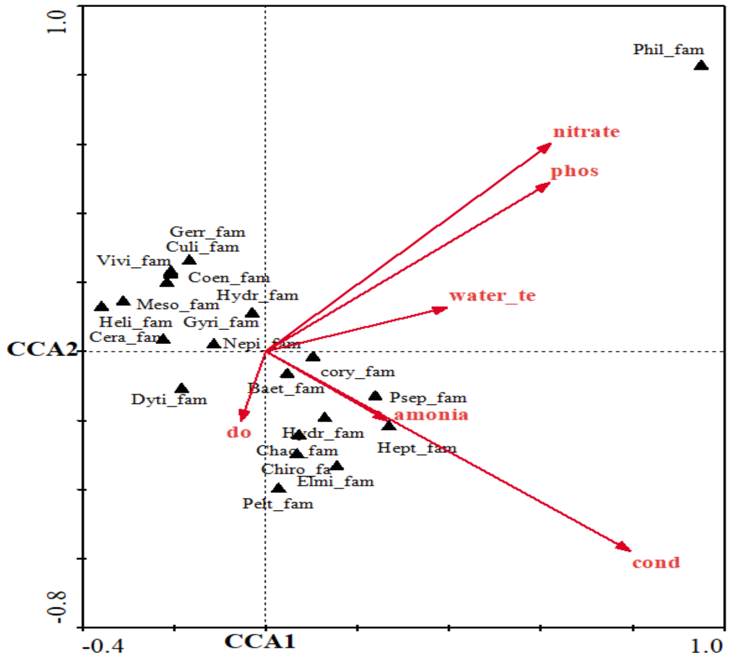

Figure 16: CCA biplot for Macro-invertebrate Species along and Physico-chemical Characteristics of water

Species abbreviations represent concatenated forms of first four letters of family name as presented in (Annex III) and concatenated forms for Physico-chemical parametersare (water_te = water temperature, cond = Conductivity, do = Dissolved oxygen, nitrate = Nitrate as total nitrogen, phos = Phosphate as inorganic phosphorus, ammonia = Ammonia).

First axis of CCA ordination between Macro-invertebrate species and Physico-chemical parameters explained 15.7% variance of species data and 21.8% of relation between Species and Physico-chemical characteristics (Annex X).

Study showed that Dytiscidae family has positive co-relation with dissolved oxygen (DO) but strong negative correlation with Nitrate, inorganic phosphorus and water temperature. That is; the dissolved oxygen in lakes and river water increases with decrease in nitrate, inorganic phosphorus and water temperature. Only Philopotamidae family showed strong positive affinity with nitrate, inorganic phosphorus and water temperature. The ammonia and conductivity are correlated with each other i.e the ammonia concentration increases with increase in conductivity. The family like Nepidae, Gerridae, Culicidae, Viviparidae, Mesoveliidae, Hydropsychidae, and Coenagrionidae showed strong but negative affinity with conductivity.While the growth of the family like Elmidae, Psephenidae, Chironomidae, Heptagenidae, Hydrometridia showed positive correlation with ammonia and conductivity (fig. 16).

CHAPTER V
DISCUSSION

5.1 Macrophyte Composition

Potamogetonaceae was found to be dominant family and are followed by Hydrocharitaceae, Poaceae and Polygonaceae family in the study period. The dominancy of this family might be due to the presence of different physical and physiological nature of the plant species belonging to this family. Such as ability of efficient long-distance dispersal, successful establishment biology, ecological flexibility, resilience to disturbance and the capacity to modify environments by changing nature of fire and mammalian herbivory (Linder et al., 2018).

Among growth form, highest number of emergent macrophytes (Fig. 3) were recorded which indicates the disturbance caused by the various anthropogenic activities of the tourists in the riparian zone of the wetland. The fact of getting highest species diversity of emergent and lowest free-floating species signifies the increasing richness in species with decreasing water level. (Sharma and Singh, 2017). Similar result was supported by Roka (2019), Dongol et al. (2014) and Burlakoti and Karmacharya (2004).

Highest species composition in Rupa and then in Fewa and Begnas may be due to the presence of moderate erosion riparian and anthropogenic disturbance. It may also be due to high water level and large area of the lakes. And lowest species composition in Dipang, Khaste and Gunde may be due to low water level and small area of Dipang, Khaste and Gunde lakes which was noted in the field. Similar result was supported by Zerlin and Henry (2014).

The mechanical stress induced by the river water current may also cause reduced primary production of plants (Ohle, 1955), which leads to low macrophytes species in rivers than in lakes. The velocity of the river flow and its discharge regime prevents the development of diversified aquatic plants in between the river channel (Rui et al. 2013). The stable substrate is another important factor that allows for rooting and supports on establishment of macrophyte communities (Haslam, 2006). Bijayapur and Seti river has highest diversity of macrophyte while Yamdi has lowest number of macrophyte species among lentic water body. The high river velocity might be one reason for the presence of lowest number of macrophyte species in Yamdi river than Bijayapur and Seti. Bijayapur river is a slow flowing river consisting of gravel substrate in some sites than fast flowing Yamdi (Grinberga, 2010).

The distribution of macrophytes composition may also depends upon morphometric aspect like depth, slope, degree of connectivity and the distance of a site in a lake from the river channel, are particular to floodplain lakes. Depth affects the distribution of macrophytes and creates plant zonation along littoral zones (Sculthorpe, 1967; Santos and Thomaz, 2007). Similarly, the slope of the littoral zone may affect macrophyte establishment because in steeper habitats, few species are able to settle and anchor (Duarte and Kalff, 1986).

5.2 Phytoplankton Composition

Bacillariophyceae showed the most dominant group in the study. Similar result has been shown by many types of result (Kohler and Hoeg, 2007; Wu et al., 2011; Hafiz et al., 2014; Sharma et al., 2016). Bacillariophyceae was also recorded as the dominant group in the Imo River, Nigeria (Ogbuagu and Ayoade, 2012), Kenti River, Republic of Karelia (Chekryzheva, 2014) and Greater Zab River, Iraq (Ali, 2010). Due to short regeneration time and sensitive behavior towards ecological characteristics, Bacillariophyceae can be used as bio-indicators for water quality evaluation as well (Stevenson and Pan 1999, Goma et al., 2005). Besides Bacillariophyceae other dominant groups were Cyanophyceae and Chlorophyceae. Tiwari and Chauhan (2006) also reported maximum abundance of Chlorophyceae during winter season in Kitham Lake, Agra, India. Patil et al. (2012) also shows similar result in Shivaji University lakes, India. The Cyanophyceae and Chlorophyceae presence in the Water Bodies indicates the presence of domestic sewage, municipal waste and effluents of organic waste of animals and human beings (Tas and Gonulol, 2007; Jafari and Alavi, 2010).

Charophyceae and Euglenophyceae with only 2 species which is lowest among the recorded family (fig. 4) (Annex 2).

Moreover, the common ways of reproduction in chlorophyceae are vegetative, asexual and sexual but also gives out several perennating bodies which can increase individual species even in adverse environmental conditions. In Cyanophyceae, only vegetative and asexual type of reproduction is present. In Euglenophyceae, only asexual reproduction occurs (Sahoo and Seckbach, 2015).

Fewa lake showed the highest diversity with 29 phytoplankton species which is followed by Begnas lake and Depang lake with 28 species and Kotre river 26. Gundelake and Yamdi river showed the lowest phytoplankton species among the studied lakes (fig. 4). *Navicula* sp, *Gomphonema* sp, *Nitzchia* sp and *Aulacoseira* sp are dominant in most of the lotic Water Bodies. This result was supported by Holmes and Whitten, (1981).

As Phytoplankton is very sensitive to the river velocity and turbulence of flow as the rapid current and mechanical stresses inhibit the development of new plankton (Alhassan and Ofori-Danson, 2016) which might be the reason for the low phytoplankton diversity in Yamdi river. *Ulothrix* sp, *Mougeota* sp, *Crucigenia* sp, *Oscillatoria* sp, *Tetraedron* sp, *Cymbella* sp and *Gomphonema* sp are common in lentic Water Bodies. *Navicula* sp, *Ghomphonema* sp and *Cymbella* sp are dominant in lentic as well as in lotic ecosystem. It might provide the evidence of their ability of resilience and tolerance to both harsh and suitable environment for their growth and reproduction (Hassan et al., 2008).

5.3 Macro-invertebrate Composition

Biological monitoring using macroinvertebrates has been found accurate and advantageous compared with using other organisms because macroinvertebrates are extremely sensitive to organic pollutants, widely distributed, and easy and economical to sample (Patang et al., 2018). Dense riparian vegetation found on the banks of the river may provide high nutrient sources for macroinvertebrates (Patang et al., 2018). The diversity of order Diptera was found larger in most of the Water Bodies. Similar result was obtained by (Olomucoro and Ezemonye, 2015). Begnas lake showed highest diversity with 9 family and 4 order with the dominancy of Hemiptera which is followed by Rupa lake with 8 family and 4 order with the dominancy of Gastropoda, because they can tolerate intermediate level of pollution and found mostly in lowlands and pond (Nasemann, 2007). While Dipang with 3 families and 2 orders showed the lowest diversity among lakes. It may be due to low water level (Choi et al., 2014). During low water level, macrophyte diversity is lower which generally decreases the physical complexity of aquatic environment and provide stressful habitat for algae and aquatic macroinvertebrates (Choi et al., 2014) and hence it may have low diversity.

Seti river with 10 family and 8 order with the dominancy of Plecoptera and Kali river with 10 family and 5 order with the dominancy of Diptera which showed the highest diversity and Kotre with 3 family and 3 order with the lowest diversity among the studied rivers .The family Nepidae is commonly found in most of the Water Bodies while the family Hydropsychidae, Heptagenidae and Baetidae is found to be dominant in most of the rivers. This result were also supported by (Pepa et al., 2018; Shrestha et al., 2008) and the family Culicidae is found to be dominant in most of the studied lakes because stagnant water is found in the lakes which may provide favourable condition for the growth of Culicidae.

According to (Dévai, 1990), the larvae of Diptera, Hemiptera, Plecoptera are most essential to the circulation of nutrients in lakes and reservoirs and may in fact change the speed of the eutrophication process in the course of their feeding. Furthermore, their quick regeneration turnover and rapid growth rate guarantee an availability of biomass to aquatic ecosystem dynamics (Menzie, 1981). They are also considered as the common and most tolerant species in wide array of all environmental condition and thus these species are dominant.

5.4 The influence of Physico-chemical Characteristics on Phytodiversity and Macroinvertebrates

Water quality and other physical environmental factors determine the considerable variations in distribution, diversity and abundance of aquatic species in freshwater ecosystems. The availability of light and temperature appears to be the most important factors in determining the species distribution (Dar et al., 2014). Temperature is one of the controlling factors, which alter the functions of the aquatic ecosystem, and it influences the growth and distribution of flora and fauna (Dwivedi and Pandey, 2002; Singh and Mathur, 2005; Jalal and Sanalkumar, 2012; Tank and Chippa, 2013). A rise in temperature of the water leads to the speeding up of the chemical reactions in water, reduces the solubility of gases and amplifies the odors (Trivedi & Goel, 1986).Aquatic organism has both an upper and lower temperature limit for optimal growth. This growth is varies from species to species. Temperature has been determined using the pattern of distribution of macrophytes, thereby influencing the productivity and species composition, and this is varied with depth, season and geographical location (Singh et al., 2019).Even though,it is not easy to discriminate the difference between the effects of environmental variables on the distribution of aquatic macrophytes. Human effect on the environment and natural activities switches the structure of aquatic macrophytes. Eutrophication affects the concentration of distribution, diversity and density and productivity of macrophytes. When the altitude increases, the temperature gradually decreases and vice versa. Here, Phusre khola lies at low altitude of 660.8m from the sea level so, it might have recorded highest water temperature (20.7℃) and while Gunde lake and Seti river lies at the altitude of 747.9 m asl and 961.9 m asl respectively. Thus, lowest water temperature might be recorded at Gunde lake and Seti river as 11.7 C and 11.3℃ respectively. Low water temperature in Gunde lake which might be the cause for lowest phytoplankton composition (Ghimire, 2013).

Dissolve oxygen (DO) regulates the health of the ecosystems that refers to the volume of oxygen present in the water body. Its presence is essential to maintain the higher forms of biological life in the water (Trivedi and Goel, 1986). Concentration of DO indicates water quality and its relation to the distribution and abundance of various algal species (Singh et al., 2010). The DO in the studied water bodies ranged from 4.7 mg/L to 8.42 mg/L which depends upon the temperature, salinity and pressure of the water. A minimum DO of 5 mg/L is recommended (BIS/ICMR).In the present study, Dissolved oxygen was found negatively correlated with water temperature. Similar result was obtained by Gautam and Bhattarai, (2008). Setikhola has 8.42 mg/L dissolved oxygen which was highest among all studied Water Bodies; it might be due to low water temperature. Gunde lake and Yamdi river has 4.7mg/L and 4.9 mg/L dissolved oxygen respectively which was lowest among all studied Water Bodies. Dissolved oxygen levels that drop below 5.0 mg/L cause stress to aquatic life. Thus, Gunde lake and Yamdi river is not suitable for aquatic life, as a result species composition were was also recorded less as compare to other studied Water Bodies.

Water Bodies with high dissolved oxygen shows positive correlation with Dytiscidae family of macroinvertebrates (fig. 16). Dissolved oxygen was found to be negatively correlated with nitrate and phosphate concentration. Nitrate, phosphate and water temperature were found to be positively correlated with each other (fig. 15), which might be the reason for the high abundances of phytoplankton sp in the presence of their concentration. The species like *Synedra ulna, Gomphonema ghandhi, Mougeota, Pinnularia, Rhoicosphenia , Oscillatoria ,Euglena* , etc were concentrated (fig. 15) . Likewise *Persicaria hydropiper, Monochoria vaginalis* and *Myriophyllum* sp of macrophyte also showed positive affinity with nitrate and phosphate (fig. 14).

Electrical conductivity is the measure of the ability of an aqueous solution to transmit an electric current in the aquatic environments (Lodh et al., 2014). Lowest conductivity denotes lowest organic water whereas highest conductivity denotes highly organic water (Singh et al., 2010).Moreover, the higher value of Conductivity is attributed to the high degree of anthropogenic activities like waste disposal, household waste, and chemicals runoff from agricultural and apiculture activities (Kangabam et al., 2017). In the present study, Kotre has 314 µs/cm which is highest and Begnas has 29.6 µs/cm conductivity which is lowest among the studied water bodies.Conductivity was found to be must influencing factor for all studied species composition (fig. 13, 14, 15). Macrophyte species like *Panicum repens, Hydrilla verticillata, Colacasia sculenta* showed high abundances with Conductivity. While *Ludwingia adscendens, Ludwingia hexapetala, Eichhornia crassipes, Najas minor, Cyperus involucratus, Nymphoides sp* showed strong negative affinity with conductivity i.e. the species composition highly decreases with increase in Conductivity. Similar result was reported by (Dhote, 2007) showed in Shahapura Lake in Bhupal.

Similarly, the phytoplankton species like *Ulnaria, Ulothrix, Aulocoseira, Fragillaria, Kirchneriella,* etc also showed negative correlation with conductivity. But *Oscillatoria, Cymbella, Tabellaria, Navicula, Nostoc* showed positive affinity with conductivity (fig. 14).

Conductivity also showed strong impact on Macroinvertebrates species. Nepidae, Gerridae, Culicidae, Viviparidae, Mesoveliidae, Hydropsychidae, Coenagrionidae showed strong negative affinity with conductivity. While the growths of the family like Elmidae, Psephenidae, Chironomidae, Heptagenidae, Hydrometridia showed positive correlation with conductivity as well as ammonia.

In the present study, Dipang lake has highest concentration of Phosphate 0.226 mg/L and Kotre has 0.218 mg/L.In most of the natural water, phosphate concentration ranges from 0.005 to 0.020 mg/L (GoN/FDD, 1998) and 0.02 mg/L is considered to be factor for accelerating eutrophication in lake (WHO, 1993). Here, phosphate content may be high due to release of phosphate from bottom sediment and organic load of the water, this helps in growth of the phytoplankton and weeds in the lake. Household detergents, domestic sewage leaching of phosphate fertilizer may be reason for phosphate levels increase (Das, 2017).

According to UNESCO (1996), unpolluted water contains small amounts of ammonia usually less than 0.1 mg/L. But in the present study, ammonia concentration ranges from 0.283 mg/L to 1.451 mg/L.Yamdi has the highest ammonia concentration as 1.451 mg/L. High levels of ammonia and inorganic phosphate showed the effect of high anthropogenic activities and agricultural runoffs (Kannel et al., 2007). In natural conditions, the nitrate concentration seldom exceeds 0.1 mg/L (UNESCO, 1996). In the investigation period, Nitrate value ranges from 0.05 mg/L to 0.045mg/L which was found within the range of natural condition. There was no significant change in nitrate values in all studied Water Bodies during study period.

CHAPTER VI
CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Altogether 39 Macrophyte species, 105 Phytoplankton species and 9 order with 35 families of Macroinvertebrates were recorded from the study area. Emergent and Potamogetonaceae family of macrophyte was found to be dominant. Among all studied Water Characteristics, conductivity was found most important influencing factor for all studied species community. The parameters of water like Conductivity and ammonia plays significant role in the growth of most of the macrophyte species. The species like *Eichhornia crassipes, Nelumbo nucifera, Ludwigia adscendens, Ludwigia hexapetala, Najas graminea, Najas minor*, *Alternanthera sessilis, Cyperus involucratus, Leersia hexandra, Ranunculus sceleratus, Nymphoides cristata, Nymphoides indica* and *Cyperus esculentus* species showed negatively strong affinity with conductivity and ammonia. The phytoplankton species like *Ulnaria, Ulothrix, Aulocoseira, Mougeota, Gomphonema* etc showed strong negative correlation with conductivity.

Bacillariophyceae showed the highest numbers of phytoplankton with 52 species were recorded, followed by the Cyanophyceae with 30 species, Chlorophyceae with 21 species. Charophyceae and Euglenophyceae with only 2 species which is lowest among the recorded family. Among the different studied water bodies, Fewa lake showed the highest diversity with 29 phytoplankton species. *Navicula* sp, *Gomphonema* sp, *Nitzchia* sp and *Aulacoseira* sp are dominant in most of the lakes. Likewise among river, Kotre river showed the highest phytoplankton diversity with 26 species. *Ulothrix* sp, *Mougeota* sp, *Crucigenia* sp, *Oscillatoria* sp, *Tetraedron* sp, *Cymbella* sp and *Gomphonema* sp are common in rivers. *Navicula* sp, *Ghomphonema* sp and *Cymbella* sp are dominant in lakes as well as in rivers.

Similarly, altogether 9 orders with 35 families of Macroinvertebrates were recorded, among which the diversity of order Diptera is found larger in both the lakes and river.Begnas lake shows highest diversity of macroinvertebrate with 9 family and 4 orders with the dominancy of Hemiptera among lentic water boies while Seti river with 10 families and 8 order with the dominancy of Plecoptera and Kali river with10 families and 5 order with the dominancy of Diptera which shows the highest diversity among the studied lotic Water Bodies. The family Nepidae is commonly found in most of the Water Bodies while the family Hydropsychidae and Heptagenidae is found to be dominant in most of the river and the family Culicidae is found to be dominant in most of the lakes of the studied water bodies.

6.2 Recommendations

I. The study of macrophytes, phytoplanktons and macro-invertebrates as well as Water Characteristicss in other season is strongly recommended.
II. The study of other physico-chemical parameters like pH, alkalinity, acidity, transparency, turbidity, biological oxygen demand, chemical oxygen demand is recommended.
III. Research related to ecosystem services should be conducted which will be beneficial for conservation of aquatic species.
IV. Public awareness about the importance of aquatic biodiversity to the people is necessary.

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ANNEXES

ANNEX I : Macrophyte composition in Water bodies of Setikhola watershed.

S.N.	Name of Macrophytes	Family	Growth form	Abbrebrations
1.	*Azolla imbricata* R.Br.	Salviniaceae	FF	Azo_imb
2.	*Blyxa aubertii*	Hydrocharitaceae	SM	Bly_aub
3.	*Ceratophyllum demersum* L.	Ceratophyllaceae	SM	Cer_dem
4.	*Eichhornia crassipes* (Mart.) Solms	Pontederiaceae	FF	Eic_cra
5.	*Frageria* sp	Rosaceae	SM	Fra_sp
6.	*Hydrilla verticellata* (L.F.) Royle	Hydrocharitaceae	SM	Hyd_ver
7.	*Lemna minor* L.	Lemnaceae	FF	Lem_min
8.	*Ludwigia adscendens* (L.) H. Hara	Onagraceae	RFL	Lud_ads
9.	*Ludwigia hexapetala*	Onagraceae	RFL	Lud_hex
10.	*Myriophyllum aquaticum*	Holaragaceae	SM	Myr_aqu
11.	*Najas graminea*	Najadaceae	SM	Naj_gra
12.	*Najas minor* All.	Najadaceae	SM	Naj_min
13.	*Nelumbo nucifera* Gaertn.	Nymphaeceae	FF	Nel_nuc
14.	*Nymphoides cristata* (Roxb.) Kuntze	Menyanthaceae	RFL	Nym_cri
15.	*Nymphoides indica*	Menyanthaceae	RFL	Nym_ind
16.	*Pistia stratoides* L.	Araceae	FF	Pis_str
17.	*Potamogeton crispus*	Potamogetonaceae	SM	Pot_cri
18.	*Potamogeton nodosus*	Potamogetonaceae	SM	Pot_ nod
19.	*Potamogeton pectinatus*	Potamogetonaceae	SM	Pot_pec
20.	*Potamogeton octandrus*	Potamogetonaceae	SM	Pot_oct
21.	*Vallisneria spiralis*	Hydrocharitaceae	SM	Val_spi
22.	*Zannichellia palustris*	Potamogetonaceae	SM	Zan_pal
23.	*Alternenthera sessilis*	Amaranthaceae	E	Alt_ses
24.	*Colacasia sculenta* (L.) Schott	Araceae	E	Cal_scu
25.	*Ceratopteris thalictroides*	Pteridaceae.	E	Cer_tha
26.	*Cyperus involucratus*	Cyperaceae	E	Cyp_inv
27.	*Equisetum debile*	Equisetaceae	E	Eqi_deb

28.	*Cyperus esculentus*	Cyperaceae	E	Cyp_esc
29.	*Ipomea carnea* ssp fistula (Mart.ex Choisy) D.F.Austin	Convolvulaceae	E	Ipo_car
30.	*Leersia hexandra* Swartz.	Poaceae	E	Lee_hex
31.	*Monochoria vaginalis*	Pontederiaceae	E	Mon_vag
32.	*Panicum repens*	Poaceae	E	Pan_rep
33.	*Persicaria* sp	Polygonaceae	E	Per_amp
34.	*Persicaria barbata* (L.) H. Hara	Polygonaceae	E	Per_bar
35.	*Persicaria hydropiper* (L.) Spach	Polygonaceae	E	Per_hyd
36.	*Phragmites karka* (Burm.f.)	Poaceae	E	Phr_kar
37.	*Ranunculus sceleratus*	Ranunculaceae	E	Ran_sce
38.	*Schenoplectus* sp	Cyperaceae	E	Sch_sp

ANNEX II: Phytoplankton composition in water bodies of Setikhola watershed.

S.N.	Name of Phytoplankton species	Class	Abbreviation
1.	*Achnanthes* sp	Bacillariophyceae	*Ach*_sp
2.	*Amphora* sp	Bacillariophyceae	*Amp*_sp
3.	*Anabaena* sp	Cyanophyceae	*Ana*_sp
4.	*Ankistrodesmus falcatus* (Corda) Ralfs	Chlorophyceae	*Ank_fal*
5.	*Ankistrodesmus spiralis* (Turn.) Lemna.var. fasciculatus Smith	Chlorophyceae	*Ank_spi*
6.	*Aphanocapsa* sp	Cyanophyceae	*Aph*_sp
7.	*Arthrodesmus* sp	Charophyceae	*Art*_sp
8.	*Aulacoseira granulate* (Ehrenberg) Simonsen	Bacillariophyceae	*Aul_ gra*
9.	*Aulacoseira* sp	Bacillariophyceae	*Aul_* sp
10.	*Calothrix sp*	Cyanophyceae	*Cal_sp*
11.	*Chlorella* sp	Chlorophyceae	*Chl*_sp
12.	*Chlorogonium* sp	Chlorophyceae	*Chl_* sp
13.	*Chroococcus minutes* (Kützing) Nägeli.	Cyanophyceae	*Chro_min*
14.	*Chroococcus* sp	Cyanophyceae	*Chr*_sp
15.	*Closterium* sp	Chlorophyceae	*Clo*_sp
16.	*Cosmorium lundelii* var. ellipticum West	Chlorophyceae	*Cos_lun*
17.	*Cosmorium* sp	Chlorophyceae	*Cos*_sp
18.	*Crucigenia fenestrate* (Schmidle) Schmidle	Chlorophyceae	*Cru_fen*
19.	*Crucigenia* sp	Chlorophyceae	*Cru*_sp
20.	*Cyclotella* sp	Bacillariophyceae	*Cyc*_sp
21.	*Cymbella herbidica*	Bacillariophyceae	*Cym_her*
22.	*Cymbella naviculiformis* Auersw. ex Heib.	Bacillariophyceae	*Cym_nav*
23.	*Cymbella* sp	Bacillariophyceae	*Cym*_sp
24.	*Cymbella tumida*	Bacillariophyceae	*Cym_tum*
25.	*Cymbella turgid*	Bacillariophyceae	*Cym_ tur*
26.	*Diatom frustules*	Bacillariophyceae	*Dia_ fru*
27.	*Diploneis* sp	Bacillariophyceae	*Dip*_sp

28.	*Encyonema* sp	Bacillariophyceae	*Enc*_sp
29.	*Eudorina elegans*	Chlorophyceae	*Eud_ele*
30.	*Euglena* sp	Euglenophyceae	*Eug*_sp
31.	*Eunotia binularis*	Bacillariophyceae	*Eun_bin*
32.	*Eunotia* sp	Bacillariophyceae	*Eun*_sp
33.	*Fragilaria capuncina* var. mesolepta (Rabenh.)	Bacillariophyceae	*Frag_cap*
34.	*Fragilaria crotonensis*	Bacillariophyceae	*Fra_cro*
35.	*Fragilaria* sp	Bacillariophyceae	*Fra*_sp
36.	*Frustulia frenguelli*	Bacillariophyceae	*Fru_ fre*
37.	*Frustulia rhomboids*	Bacillariophyceae	*Fru_rho*
38.	*Glaucocystis nostochinearum*	Cyanophyceae	*Gla_nos*
39.	*Glaucocystis* sp.	Cyanophyceae	*Gla*_sp.
40.	*Glenodinium borgei (*Lemm.) Schiller	Chlorophyceae	*Gle_bor*
41.	*Gloeocapsa decorticans*	Cyanophyceae	*Glo_dec*
42.	*Gloeocapsa* sp	Cyanophyceae	*Glo*_sp
43.	*Gomphonema augur* var. augur	Bacillariophyceae	*Gom_aug*
44.	*Gomphonema Gandhi*	Bacillariophyceae	*Gom_gan*
45.	*Gyrosigma sp*	Chlorophyceae	*Gom_pse*
46.	*Gomphonema* sp	Bacillariophyceae	*Gom*_sp
47.	*Gomphosphaeria aponina*	Cyanophyceae	*Gom_apo*
48.	*Kirchneriella lunaris*	Chlorophyceae	*Kir_lun*
49.	*Kirchneriella obese*	Chlorophyceae	*Kir_obe*
50.	*Kirchneriella* sp	Chlorophyceae	*Kir*_sp
51.	*Lyngbya contorta*	Cyanophyceae	*Lyn_con*
52.	*Meridion* sp	Bacillariophyceae	*Mer*_sp
53.	*Merismopedia glauca*	Cyanophyceae	*Mer_gla*
54.	*Mougeotia* sp	Chlorophyceae	*Mou*_sp
55.	*Navicula cincta*	Bacillariophyceae	*Nav_cin*
56.	*Navicula gregaria*	Bacillariophyceae	*Nav_gre*
57.	*Navicula helophila*	Bacillariophyceae	*Nav_hel*
58.	*Navicula radiosa*	Bacillariophyceae	*Nav_ rad*
59.	*Navicula rostellata*	Bacillariophyceae	*Nav_ ros*
60.	*Navicula seminulum*	Bacillariophyceae	*Nav_sem*
61.	*Navicula* sp	Bacillariophyceae	*Nav_* sp

62.	*Navicula transitans*	Bacillariophyceae	*Nav_tra*
63.	*Nitzchia microcephala*	Bacillariophyceae	*Nit_mic*
64.	*Nitzchia palea*	Bacillariophyceae	*Nit_pal*
65.	*Nitzchia* sp	Bacillariophyceae	*Nit_* sp
66.	*Nitzschia amphibian*	Bacillariophyceae	*Nit_amp*
67.	*Nostoc* sp	Cyanophyceae	*Nos*_sp
68.	*Oedogonium* sp	Chlorophyceae	*Oed_* sp
69.	*Oscillatoria limosa*	Cyanophyceae	*Osc_lim*
70.	*Oscillatoria princeps*	Cyanophyceae	*Osc_pri*
71.	*Oscillatoria* sp	Cyanophyceae	*Osc*_sp
72.	*Oscillatoria* sp	Cyanophyceae	*Osc*_sp
73.	*Oscillatoria tenuis*	Cyanophyceae	*Osc_ten*
74.	*Phormidium* sp	Cyanophyceae	*Phor*_sp
75.	*Pinnulaia subcapitata*	Bacillariophyceae	*Pin_sub*
76.	*Pinnularia divergens*	Bacillariophyceae	*Pin_div*
77.	*Pinnularia interrupta*	Bacillariophyceae	*Pin_int*
78.	*Pinnularia* sp	Bacillariophyceae	*Pin*_sp
79.	*Planothidium sp*	Bacillariophyceae	*Pla_sp*
80.	*Rhoicosphenia* sp	Bacillariophyceae	*Rho*_sp
81.	*Scenedesmus opoliensis*	Chlorophyceae	*Sce_opo*
82.	*Scenedesmus quadricaudata*	Chlorophyceae	*Sce_ qua*
83.	*Scenedesmus* sp	Chlorophyceae	*Sce*_sp
84.	*Scytonema* sp	Cyanophyceae	*Scy*_sp
85.	*Sphaerocystis* sp	Bacillariophyceae	*Sph*_sp
86.	*Spirogyra pratensis*	Chlorophyceae	*Spi_pra*
87.	*Spirulina princeps*	Cyanophyceae	*Spi_ pri*
88.	*Spirulina* sp	Cyanophyceae	*Spi*_sp
89.	*Spyrogyra rhizophus*	Chlorophyceae	*Spy_rhi*
90.	*Staurastrum gracile*	Cyanophyceae	*Sta_gra*
91.	*Staurastrum* sp.	Cyanophyceae	*Sta*_sp.
92.	*Staurastrum tetracerum* var. trigonum P. Lundell	Cyanophyceae	*Sta_tet*
93.	*Staurodesmus* sp	Chlorophyceae	*Sta*_sp
94.	*Surirella* sp	Bacillariophyceae	*Sur*_sp
95.	*Synedra* sp	Bacillariophyceae	*Syn*_sp

96.	*Synedra ulna*	Bacillariophyceae	*Syn_ uln*
97.	*Tabellaria* sp	Bacillariophyceae	*Tab*_sp
98.	*Tetracyclus rupestris* (A. Braun)	Bacillariophyceae	*Tet_ rup*
99.	*Tetraedron* sp	Chlorophyceae	*Tet*_sp
100.	*Trachelomonas* sp	Euglenophyceae	*Tra*_sp
101.	*Ulnaria* sp	Bacillariophyceae	*Uln*_sp
102.	*Ulnaria ulna* (Nitzsch)	Bacillariophyceae	*Uln_uln*
103.	*Ulothrix* sp	Chlorophyceae	*Ulo*_sp
104.	*Zoochlorella parasitica* K.Brandt.	Chlorophyceae	*Zoo_par*
105.	*Zygnema* sp	Charophyceae	*Zyg*_sp

ANNEX III: Macro-invertebrates composition in Water bodies of Setikhola watershed.

Order	Family	Abbrebrations
Coleoptera	Gyrinidae Heliplidae Elmidae Psephenidae Dytiscidae	Gyri_fam Heli_fam Elmi_fam Psep_fam Dyti_fam
Diptera	Baetidae Ceratopogonidae Culicidae Limoniidae Simuliidae Chaoboridae Chironomidae Tabanidae	Baet_fam Cera_fam Culi_fam Limo_fam Simu_fam Chao_fam Chir_fam Taba_fam
Epimeroptera	Heptagenidae Viviparidae	Hept_fam Vivi_fam
Gastropoda	Physidae Thiaridae Lymneridae Gerridae	Phys_fam Thia_fam Lymn_fam Gerri_fam
Hemiptera	Hydrometridia Mesoveliidae	Hydr_fam Meso_fam

	Valiidae Corixidae Nepidae Belostomatidae Pleidae Corydalidae	Valii_fam Cori_fam Nepi_fam Belo_fam Plei_fam cory_fam
Plecoptera	Perlidae Peltoperlidae Potamidae	Perl_fam Pelt_fam Pota_fam
Decapoda	Hydropsychidae	Hydr_fam
Tricoptera	Caenidae Coenagrionidae Philopotamidae	Caen_fam Coen_fam Phil_fam
Odonata	Euphaeidae	Euph_fam

Annex IV: Tests of Normality

	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistic	Df	Sig.	Statistic	Df	Sig.
Total_Macrophyt	0.205	13	0.138	0.916	13	0.219
Total_Phytoplnk	0.144	13	.200*	0.940	13	0.455
Total_MacrInv	0.195	13	0.187	0.958	13	0.723
Elev	0.186	13	.200*	0.904	13	0.151
DO	0.132	13	.200*	0.950	13	0.603
DO_mg/L	0.188	13	.200*	0.906	13	0.164
Cond	0.259	13	0.017	0.794	13	0.006
Nitra	0.215	13	0.101	0.938	13	0.429
Ammon	0.253	13	0.022	0.910	13	0.186
Phos	0.245	13	0.032	0.812	13	0.009
water_temp	0.151	13	.200*	0.909	13	0.175
*. This is a lower bound of the true significance.						
Lilliefors Significance Correction						

Annex V: CCA ordination summary (Total inertia= 2.484, sum of all canonical Eigen values 0.988) of Water Characteristics with Macrophyte.

Axes	1	2	3	4	Total inertia
Eigenvalues :	0.652	0.215	0.128	0.081	2.484
Lengths of gradient :	4.206	7.628	2.048	1.94	
Species-environment correlations :	0.884	0.652	0.643	0.527	
Cumulative percentage variance					
of species data :	26.2	34.9	40	43.3	
Of species-environment relation:	44.6	54.2	0	0	

Annex VI. CCA ordination summary (total inertia= 3.228, sum of all canonical Eigen values 1.216) of Water Characteristics with Phytoplanktons.

Axes	1	2	3	4	Total inertia
Eigenvalues :	0.486	0.231	0.142	0.075	3.228
Lengths of gradient :	3.381	2.286	1.767	2.162	
Species-environment correlations :	0.771	0.68	0.415	0.646	
Cumulative percentage variance					
of species data :	15.1	22.2	26.6	29	
of species-environment relation:	22.4	31.8	0	0	

Annex VII. CCA ordination summary (total inertia= 5.331, sum of all canonical Eigen values 2.047) of Water Characteristics with Macroinvertebrate.

Axes	1	2	3	4	Total inertia
Eigenvalues :	0.839	0.53	0.279	0.113	5.331
Lengths of gradient :	6.583	5.263	4.117	2.142	
Species-environment correlations:	0.708	0.638	0.693	0.177	
Cumulative percentage variance					
of species data :	15.7	25.7	30.9	33	
of species-environment relation:	21.8	33.1	0	0	

Annex VIII: Relative importance of Water Characteristics on Macrophyte composition analyzed based on CCA analysis.

Environmental variables	Abbreviation	F	P
Conductivity (µS/cm)	Cond.	3.950	**0.0001**
Dissolved oxygen(mg/L)	DO	0.819	0.6121
Phosphate as inorganic Phosphorus(mg/L)	Phosphate	0.551	0.8286
Nitrate as total nitrogen(mg/L)	Nitrate	0.588	0.7993
Ammonia (mg/L)	Amonia	1.756	0.0550
Water temperature (°C)	Water_temp	1.232	0.2339

Annex IX: Relative importance of Water Characteristics on Phytoplanktoncomposition analyzed based on CCA analysis.

Environmental variables	Abbreviation	F	P
Conductivity (µS/cm)	Cond	2.174	**0.0002**
Dissolved oxygen(mg/L)	DO	1.411	0.0659
Phosphate as inorganic Phosphorus(mg/L)	Phosphate	1.244	0.2121
Nitrate as total nitrogen (mg/L)	Nitrate	1.152	0.3065
Ammonia (mg/L)	Amonia	1.333	0.0968
Water temperature (°C)	Water_temp	0.914	0.5970

Annex X: Relative importance of Water Characteristics on Macro-invertebrate's composition analyzed based on CCA analysis.

Environmental variables	Abbreviation	F	P
Conductivity (µS/cm)	Cond	2.019	**0.0020**
Dissolved oxygen(mg/L)	DO	1.252	0.3002
Phosphate as inorganic Phosphorus(mg/L)	Phosphate	1.144	0.3206
Nitrate as total nitrogen(mg/L)	Nitrate	1.792	0.0034
Ammonia (mg/L)	Amonia	0.726	0.7523
Water temperature °C	Water_temp	0.652	0.8215

Annex XII: Data sheet

Field Data sheet

SN : Station:

Water quality

Sampling point: Date:

Sample code: Time:

Temp. : Longitude:

PH: Latitude:

Conductivity: Land use:

DO: Vegetation:

Water depth:

Macrophytes			
SN.	Scientific name	Common name	Habitat

- Habitat: Submerged **(S)** (below surface)
  Floating **(F)** (at the surface)
  Emergent **(E)** (projecting above the surface / mainly at the shoreline)
- Coverage : None = no plant
  Sparse=1 to 25% cover

Algae				
SN.	Algae type			
	Filamentous	Periphyton	Phytoplankton	Unknown

Algae type :

1. Filamentous algae (i.e., algae in long strands that can be picked up by hand);
2. Periphyton (i.e., algae growing on rock, soil, or sand); and
3. Phytoplankton (i.e., algae growing in the water column).

Macroinvertebrates

Habitat Codes:

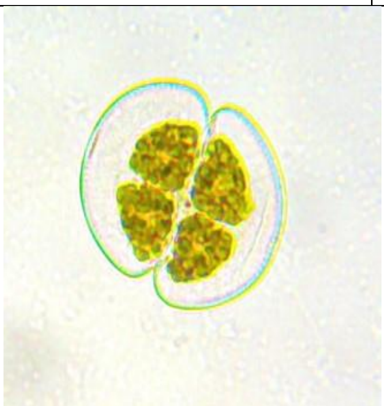
1. Open water – standing (ponds, marshes)
2. Open water – flowing (river/stream channels)
3. Aquatic macrophyte bed (floating/submerged vegetation dominant
4. Emergent - non-persistent vegetation dominant (non-woody species not visible at certain seasons, such as pickerelweed)
5. Emergent - persistent vegetation dominant (non-woody species that remain standing until the beginning of

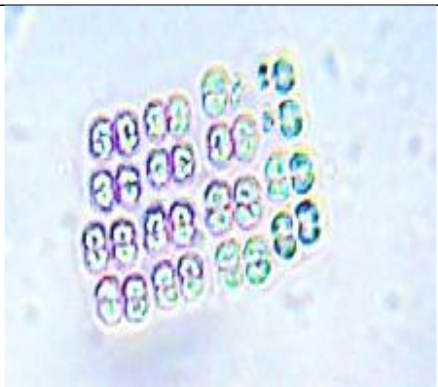
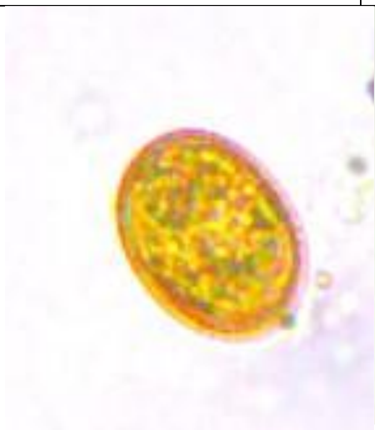
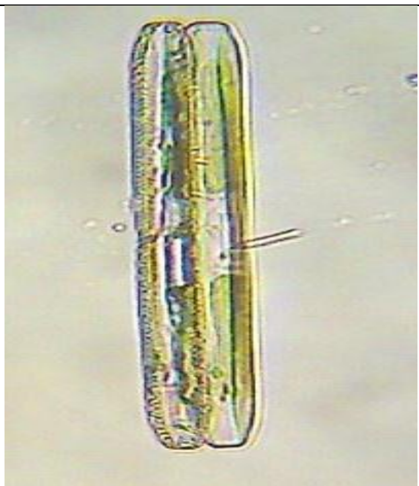
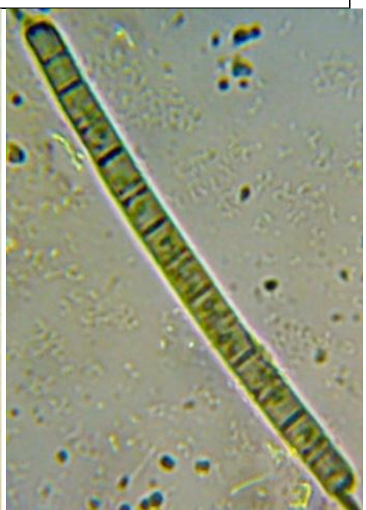
Substrate code

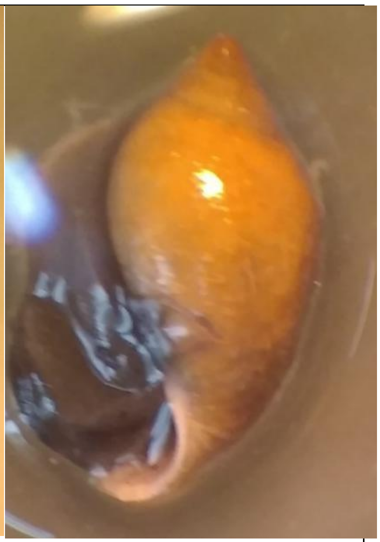
1. sand (<1/8”)
2. gravel (1/8” – 3”)
3. rubble (3” – 10”)
4. silt/muck
5. clay
6. organic soil (well decomposed)
7. peat
8. boulders (>10”)
9. bedrock
10. detritus

SN.	Habitat code	Substrate code	Scientific name	Common name

PHOTOPLATES

 1. *Navicula rostellata*	 2. *Navicula radiosa*	 3.*Spirogyra* sp
 4. Fragilaria sp	 5. *Synedra ulna*	 6.*Gomphonema augur* var.augur
 17. *Navicula seminulum*	 8. *Cosmerium lundelli*	 9.*Gomphosphaeria aponina*

		
18. *Merismopedia glauca*	11.*Closterium ehrenbergii*	12. *Navicula gregaria*
		
13. *Staurastrum gracile*	14.*Trachelomonas hispida*	15. *Kirchneriella* sp
		
16. *Pinnularia* sp	17. *Cymbella turgida*	18.*Aulocoisera granulate*

 19. Psephenidae	 20. Dytiscidae	 21. Physidae
 22. Thiaridae	 23. Belostomatidae	 24.Gyrinidae
 25.Mesoveliidae	 26.Baetidae	 27. Culicidae larvae

28. Free floating macrophytes

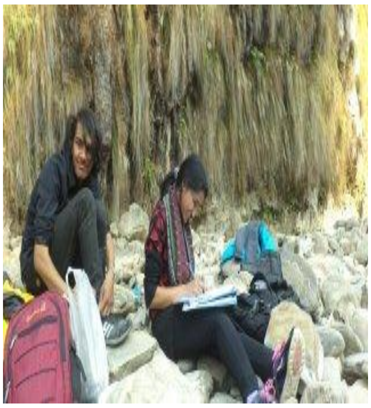
29. Fish dying with eutrophication

30.Collecting phytoplankton

31.Collecting macroinvertebrates

32. Our research team at Rupa lake

33. Women washing clothes in Kali khola



34. Collecting primary data on field data sheet.

35. Working at dry lab of CDES

36. Working at physiology lab of CDB