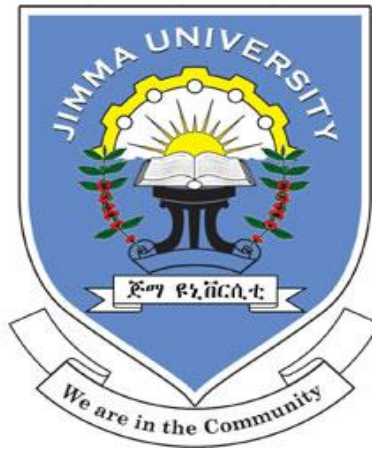


JIMMA UNIVERSITY
COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES
DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCES & TECHNOLOGY



ECOLOGICAL STATUS OF HOT SPRINGS IN EASTERN AMHARA REGION:
MACROINVERTEBRATES AND BIRDS DIVERSITY

BY:

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Ecological status of hot springs in Eastern Amhara Region: Macroinvertebrates
and birds diversity

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Declaration

I, the undersigned, declare that this research paper is my original work and has not been presented for a degree in any other university and that all sources of materials used for the research paper have been correctly acknowledged.

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Abstract

Springs are the places where ground water is discharged at specific locations. They vary dramatically as to the type of water they discharge. Hot springs are having the temperature of the water lies significantly above the mean of annual air temperature of that region. Temperature is one of the most important factors that govern species abundance and distribution. The objective of this study is to examine the relationship between biological parameters (macroinvertebrate and bird diversity) with physicochemical water and habitat quality of hot springs in Easter Amhara Region. A cross-sectional study of physical, chemical and biological components of the hot springs were carried out to assess their ecological status. Samples were collected from March to May 2013. Biological samples were collected to provide a qualitative description of the community composition at each sampling site. Water samples were collected for analysis of selected physicochemical parameters following water quality assessment protocols. A total of 1095 macroinvertebrates classified into 10 orders and 31 families of macroinvertebrates were collected from the 12 sampling sites. The most abundant orders were Diptera 49.90%, Odonata 15.53%, Coleopteran 12.97%, and Ephmeropetra 9.5% represented by 14 families. 2484 birds belonging to 56 species were recorded at the 12 sampling sites. Black headed oriole (*Oriolus Larvatus*), Spur-Winged Lapwing (*Vanellus spinosus*), Spectacled Weaver (*plouceous ocularis*) and Yellow Wagtail (*Motacilla flava*) were the most abundant bird species in the study area and accounts 35% of the total species. Macroinvertebrate taxa were absent at B1 and H1 sites with the temperature of 72 °C and 70 °C respectively. However, in this study, the macroinvertebrate taxa (Chironomidae and Hydrobiidae) were found within a temperature of 52 °C at S1 and H1 sites. The results are also revealed that as the temperature gradient declines, the macroinvertebrate diversity flourished. Due to this fact, both macroinvertebrate diversity and family biotic index were negatively correlated with temperature and the correlations were significant. Human disturbance and habitant conditions varied considerably among sites in the study area. Although human disturbance and water pollution are among the factors influencing ecological quality, the strong correlations between water temperature and species diversity suggest that temperature is the major environmental gradient affecting aquatic biodiversity in hot springs.

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‘HOW CAN I REPAY THE LORD FOR ALL HIS ACTS OF KINDNESS TO ME’ psalms 116:12

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Abbreviations

| | |
|------------------|---|
| APHA | America Public Health Association |
| ASPT | Average Scoring per Taxa |
| BC | Before Christ |
| BOD | Biochemical Oxygen Demand |
| BOD ₅ | Biochemical Oxygen Demand for five days |
| BSc | Bachelor of Science |
| CCA | Canonical Correspondence Analysis |
| CFU | Colony Forming Unit |
| COD | Chemical Oxygen Demand |
| DO | Dissolved Oxygen |
| FBI | Family Level Biotic Index |
| Ha | Hectare |
| ISO | International Standard Organization |
| NBI | Nutrient Biological Index |
| SASS | South Africa Scoring System |
| TDS | Total Dissolved Oxygen |
| TN | Total Nitrogen |
| TP | Total Phosphorus |

CHAPTER ONE: INTRODUCTION

1.1 background

Springs are the places where ground water is discharged at specific locations on the earth and they vary dramatically as to the type of water they discharge. It is described as a concentrated discharge of groundwater that appears at the surface as a current of flowing water (Oliver, venter, & Jonker, 2011). Many of the springs are the result of long cracks or joints in sedimentary rock. Springs that discharge water that has a temperature above that of the normal local groundwater are called thermal springs. Thermal springs are natural geological phenomena that occur on all continents. Hot springs are having the temperature of the water lies significantly above the mean of annual air temperature of that region (Bhusare & Wakte, 2011).

At rainy seasons, water descends behind it and forces the new heated water to ascend along the fault-line to surface as a hot or warm spring. If the water moves slowly from depth to the surface, it will cool back down before it bubbles out as a spring. However, many of these springs occur in limestone formations where the openings allow the water to the surface may create a virtual pipeline to the surface. Hot springs contained the life even long before they reach the surface, and the warm water of the springs allows an abundant of algae and bacteria to survive which are called as thermophilic microorganisms (Sen, Mohapata, Satpathy, & Rao, 2010).

Archaeological evidence shows that thermal springs have been in use for religious and/or medicinal purposes since before 2000 BC in India and for hundreds of years in Crete, Egypt, China, Japan, Turkey and many European and Middle-Eastern countries. Many thermal springs developed into flourishing centers of religion, culture and health, such as those at Bath in England, Vichy in France and Baden- Baden in Germany (Oliver, venter, & Jonker, 2011).

Active and fossil hot springs systems occur worldwide, and share many common characteristics that indicate common genetic histories. Active hot springs occur as surface expressions of geothermally and volcanically active areas, commonly associated with rhyolite-composition volcanic rocks. These thermal springs are also common in a variety of rocks in areas of geologically recent folding and faulting. Fossil hot springs are present as extinct portions of

modern, active systems, and preserved in the geologic record as epithermal mineral deposits (Kruse, 1997).

Microorganisms thriving in elevated temperature terrestrial and deep-sea hydrothermal systems have been observed and inspected by several authors. Temperature is one of the most important factors that govern species abundance and distribution. High temperatures in soil and/or water exert pressure on microbial species leading to the selection of specific flora capable of tolerating and surviving heat stress. Some species can survive at the elevated temperatures of hot springs, or in various other adverse environments. The defense mechanism cells utilize when confronted with high temperatures in their local environment is known as the heat shock response. This response has been described extensively in both eukaryotes and prokaryotes (Bhusare & Wakte, 2011).

Ethiopia possesses the fifth largest floral composition in tropical Africa. As many as 284 species of terrestrial mammals are known to occur in Ethiopia. Among these, 31 (11%) are endemic. There are about 926 bird species listed for the country, of which 21 are endemic and 19 are globally threatened. Nationally, 73 Important Bird Areas (IBAs) have been identified, 30 of these sites comprise wetlands, while the rest are representatives of other ecosystems (Aynalem & Bekele, 2008).

1.2 Statement of the problem

Thermal springs are the most under-researched and under-utilized of all natural resources. However, the increasing recognition of the value of geothermal resources suggests that there will be a rekindling of interest in thermal springs in the near future. The development around this resource should be eco-friendly and take into account its human and animal health impact. A number of studies have found that geothermal water may contain toxic elements such as arsenic and mercury (Oliver, Venter, & Jonker, 2011).

Many Ethiopians believe that water from hot springs can relieve from a number of diseases and is considered to be the cleanest of all. The physico-chemical properties of water from seven Ethiopian hyperthermal springs which were analyzed and revealed that the pH, turbidity, chlorine, sulphate, nitrate, nitrite and ammonia fell within the range stipulated for drinking water by WHO. Bicarbonate and sodium ions including conductivity values were high. As the practice is not hygienic the water may cause acute infectious diarrhea, repeat or chronic diarrhea

episodes, and other non-diarrheal disease, which can arise from the chemical species (Haki & Gezmu, 2012).

The ecological quality and safety of surface waters still today suffer strong degradation because of anthropogenic activities that directly impact the water-bed (e.g. fishing, water diversion, irrigation, and barrages), as well as those that alter the territory surrounding the watercourses (e.g. agriculture, livestock, industrial and urban complexes). In addition, rivers continue to be used as recipients for all kinds of waste materials, leading to eutrophication, organic pollution, acidification, and hydrological and hydromorphological alterations (Torrise, Scuri, Dell'Uomo, & Cocchioni, 2010).

Although water physicochemical analyses can provide a good indication of the pollution level in rivers and streams, these analyses do not consider the state of biological communities and, therefore, cannot properly reflect the condition of freshwater ecosystems. In consequence, over the last decades, the use of biological methods has been promoted and recommended as a useful and complementary technique for the assessment of freshwater pollution (Camargo, Gonzalo, & Alonso, 2011). Using the biological approaches to determine the ecological effects of pollution have more advantages than determining the pollution with just using physicochemical methods, because physicochemical variables give information about only the situation of water at the time of measuring (Rosenberg & Resh, 1993).

Assessment of river health using biological methods is currently commonplace in most temperate countries. Several of these methods have been standardized and included in national and regional monitoring programs ((De Pauw, Gabriels, & Goethals, 2006), (Hering, et al., 2003)), serving as a basis for policy decisions concerning surface water management. However, this is not the case in most tropical countries, where physical chemical methods, some of which require expensive laboratory analysis, are predominantly used to assess running water quality. Since most tropical regions consist of developing countries, their limited technical and financial resources for environmental issues constrain the establishment of national monitoring programs and therefore, cost-effective monitoring programs are needed. After a process of adaptation, testing, and standardization, biotic indices for macroinvertebrates can be reliable systems for application in river management of tropical regions (Dominguez-Granda, Lock, & Goethals, 2011). They are species-rich, respond to a broad range of environmental conditions, and are relatively immobile

and live in close contact with both bottom sediments and the water column, thereby having the potential for exposure to stresses via both sediment and aqueous pathways (Brazner, et al., 2007).

In order to fulfill the millennium development goals and to ensure environmental sustainability in Ethiopia, ecological indicator systems can support river managers to analyze the status of watercourses and to select critical restoration actions. In order to use macroinvertebrates as river water quality monitoring and assessment tools, Ethiopia needs data from reference as well as disturbed conditions of surface water ecosystems (Ambelu, Lock, & Goethals, 2010).

Site-specific factors such as local hydraulic conditions and substrate characteristics that influence the macroinvertebrate community structure may complicate assessment of impacts. Information needed to compare the capability of each habitat to indicate the impact of stressors is often limited due to the use of different sampling techniques in riffles and pools. Relatively shallow riffle areas, which are easily accessible by wading, are studied more frequently than deeper pools (Pace, et al., 2011).

Information is far from complete for most species of birds in different regions. Concentration of threatened avian species is greater in the tropics than elsewhere. Of the 1,029 threatened species, 884 occur in developing countries. Thus, the burden of conserving threatened species lies on the developing nations, where resources are scarce for effective conservation measures (Aynalem, Bekele, & Getahun, 2008).

Pollution has been one of the major anthropogenic disturbances imposed on river systems since the development of early civilizations though most of the early problems were in close proximity to centers of population. By the mid-20th century long reaches of streams and rivers were fishless within and downstream of the large industrial conurbations such as London, the industrial Midlands, and North of England. Many streams are polluted grossly to their sources with no clean, undisturbed reaches remaining (Langford, Shaw, Ferguson, & Howard, 2009).

CHAPTER TWO: LITERATURE REVIEW

2.1 Aquatic ecosystem

The Index of Biological Integrity (IBI) is an essential tool in the environmental assessment, restoration, and conservation of aquatic ecosystems. These systems are highly vulnerable to human impacts. The decline in biodiversity is far greater in freshwaters than in the most impacted terrestrial ecosystems. In the past decades the extinction rates of freshwater organisms in North America has been five times higher than other estimated for any terrestrial habitat (Ruaro & Gubiani, 2013).

The amount and types of aquatic vegetation found in aquatic ecosystem may be influenced by numerous physicochemical factors including light availability, water chemistry, wave exposure and substrate slope and type as well as by biological factors such as predation. Water transparency is one of the strongest influences on water body's plant communities. Submersed macrophyte abundance, growth, and distribution are regulated by light availability. Light absorption, shading, and competition with algae alter aquatic plant communities, and these interactions are confounded with turbidity, water clarity, and nutrient levels. The number of submerged aquatic macrophyte species often increases with increasing clarity as often measured by Secchi disk depth specifically found that native aquatic macrophyte species richness increased with water clarity. Productivity, or trophic status, is typically measured as total phosphorus. Species richness generally decreases with increasing nutrients (Radomski & Perleberg, 2012).

2.2 Components of Aquatic Ecosystem

Understanding how biodiversity patterns emerge from the distributions of rare and common species is a key concern of conservation biology. On one hand, rare species are regarded as having a high conservation priority, because local rarity may increase the likelihood that demographic and/or environmental stochasticity will eliminate populations. Indeed, a restricted spatial distribution (with individuals occurring with high or low densities) implies that populations will probably experience adverse conditions simultaneously. On the other hand, our understanding of the determinants of overall patterns of species richness may gain most from consideration of why common species occur in some areas and are absent from others, rather

than from consideration of the distributions of rare species (Cucherousset, Santoul, Figuerola, & Ce' re' ghino, 2008).

Biodiversity is often taken as a constellation of meanings, which can never be captured by a single number. This diversity of meanings encompasses a diversity of measures; each of them intended to represent some facet of total biodiversity. Examples include genetic and phenotypic variance, species numbers, ecosystem structural properties, and patterns of functional heterogeneity. This proliferation calls us to rationalization and synthesis: to identify which features of biodiversity are mathematically independent and thereby to find the irreducible set of metrics which must be included to encompass total biodiversity (Lyashevskaya & Farnsworth, 2012).

2.3 Ecological assessment

The increasing impact of human activities on fluvial ecosystems has forced the development of monitoring programs and bioassessment techniques in order to detect and account for a variety of effects in freshwater ecosystems. Nowadays, the "ecological status" assessment of aquatic ecosystems has become the cornerstone of water legislation worldwide (e.g. the U.S. Clean Water Act or the European Water Framework Directive). In this regard, the ecological status assessment of continental watercourses in Europe evaluates the integrity of every ecosystem component: hydrological, geomorphological, water and biological characteristics. Aquatic macroinvertebrates are one of the most important organism groups selected by the Water Framework Directive (WFD; European Commission, 2000) to evaluate the integrity of biological communities within the ecological status assessment process (Alvarez-Cabria, Barquin, & Juanes, 2010).

Biological as well as supporting hydromorphological and physico-chemical quality elements are used by Member States in ecological status evaluation. Ecological status of water bodies is defined by comparing the biological community composition with the reference condition. Ecological status must be classified into five quality classes (high, good, moderate, poor and bad). This classification is based on ecological quality ratios (EQRs: O/E scores) which are derived from biological quality values. The boundary between good and moderate status is especially important because it sets the targets for restoration plans within the programme of

measures of water bodies which fail the environmental objectives of achieving good ecological status (Sa´nchez-Montoya, Vidal-Abarca, & Sua´ rez, 2010)).

2.4 Indicators of Aquatic Ecosystem

An indicator group should at least be taxonomically and ecologically well understood, easily monitored, occur in various environmental conditions, and show strong relationships with other target groups in biodiversity value. Studies testing the utility of indicator groups have generally been based on the description of biodiversity across large-scale grids, countries, and regions (Heino, 2010). The emergent vegetation community in water body provides nesting habitat and food resources for economically important sport fish populations, wading birds, migratory waterfowl, alligators, and the federally listed endangered Everglade snail kite *Rostrhamus socialabilis* (Harwell & Sharfstein, 2009).

Most environmental management plans favour the adoption of an ecosystem-based approach. Ecosystem-based management postulates that effective management must (1) be integrated among components of the ecosystem and resource uses and users; (2) lead to sustainable outcomes; (3) take precaution in avoiding deleterious actions; and (4) be adaptive in seeking more effective approaches based on experience (Rombouts, et al., 2013). The Water Framework Directive (WFD) (2000/60/EC) gives great importance to biological indicators since they are more reliable than physico-chemical analysis for defining the ecological and quality status of the aquatic ecosystems. Therefore, the application of the Directive suggests the use of new bioindicators for the assessment of water quality, in addition to the already widely used benthic macroinvertebrate communities. Thus, the WFD has enlarged the use of possible bioindicators to fishes, diatoms, and macrophyte communities. Specifically, the study of macrophytes consists of an analysis of all aquatic plants visible to the naked eye, including phanerogams, pteridophytes, macroalgae, and bryophytes growing in water. Although there are different macrophyte components, plant research on the assessment of water quality is mainly carried out through the analysis of phanerogamic macrophytes (Ceschin, Aleffi, Bisceglie, Savo, & Zuccarello, 2012).

From tropical to Polar Regions, aquatic ecosystems change in response to natural cycles and anthropogenic stressors. Seabirds are integral components of aquatic ecosystems. They forage over large geographic areas and feed at different trophic levels, and thus are often considered

effective monitors of the condition and health of aquatic systems. The use of seabirds as sentinels of the condition of aquatic ecosystems has been well established. Large environmental perturbations to aquatic food webs (e.g., chemical contamination, overfishing, particulate pollution) have all been detected or monitored by tracking seabirds at colonies. However, seabirds may elicit more subtle, sub lethal responses that can also be used to track ecosystem health, or the health of seabird populations (Mallory, Robinson, Hebert, & Forbes, 2010).

2.4.1 Macroinvertebrates

Streams, rivers, wetlands, and lakes are home for many small animals called macro-invertebrates. These animals generally include insects, crustaceans, mollusks, arachnids, and annelids. The term macro-invertebrate describes those animals that have no backbone and can be seen with the naked eye. Some aquatic macroinvertebrates can be quite large, such as freshwater crayfish; however, most are very small. Invertebrates that are retained on a 0.25mm mesh net are generally termed macro-invertebrates (Voshell & Reese, 2002).

Aquatic macroinvertebrate communities in temperate lotic systems are influenced by seasonal changes. Many aquatic insect life histories and development rates are influenced by temperature and other Physico-temporal factors, while thermal conditions temporally partition resources. Seasonal precipitation and discharge have been shown to be significant factors influencing community structure from year to year. Differences in disturbance rates can dictate the number and types of species (obligate vs. specialist) that may coexist within a habitat. Stream ‘patches’ change temporally and a snapshot of environmental conditions measured at the time of sampling may not reflect important events that could have affected the community prior to sampling. It is important to recognize that macro-invertebrate communities fluctuate and samples from one point in time may appear quite different from other points in time (E.Kosnicki and W.Sites,2010).

Macroinvertebrate species diversity and community composition are important themes in aquatic ecology, and are often used to evaluate environmental stress resulting from a variety of anthropogenic disturbances (Wolf, 1996).

Macroinvertebrates play significant roles in stream ecosystem. As a group, macro-invertebrates are the primary food source for most steam fishes. Their taxonomic, habitant, and life history

diversity insures that an array of food type available to many fish species over the entire annual life cycle. They also conduct the less apparent but not less important work of decomposing leaf litter and small particle of organic derbies on the stream bottom or in the water column, and of grazing stream algae, fungi, and bacteria. Considerable information is available on invertebrate responses to a variety of environmental conditions, and thus invertebrate may be used as indicator of stream condition (Sa´nchez-Montoya, Vidal-Abarca, & Sua´ rez, 2010).

These animals live in the water for all or part of their lives, so their survival is related to the water quality. They are significant within the food chain as larger animals such as fish and birds rely on them as a food source. Macroinvertebrates are sensitive to different chemical and physical conditions (Boyle & Fraleigh Jr., 2003). If there is a change in the water quality, perhaps because of a pollutant entering the water, or a change in the flow downstream of a dam, then the macro-invertebrate community may also change. Therefore, the richness of macro-invertebrate community composition in a water body can be used to provide an estimate of water body health. Macro-invertebrate communities vary across the State and different water bodies often have their own characteristic communities (Ivarez-Cabria, Barquin, & Juanes, 2010).

Life cycle

Most invertebrates follow a simple lifecycle. They hatch from eggs and spend some time developing. Once the larvae or nymphs have grown, they become adults, reproduce sexually and lay eggs from which young emerge to start the cycle again. The most common types of aquatic macro-invertebrates are insects. As insects grow from an egg to an adult, they change their body shape or metamorphoses. Insects show both complete and incomplete metamorphosis. Incomplete metamorphosis involves the egg hatching into a nymph. At every moulting, the nymph looks more and more like the adult form. Complete metamorphosis involves the egg hatching into a larva, which is very different to the adult. The final larval stage involves the animal developing into a pupa, which is very different from the larva. From this stage, the animal then develops into an adult. For most, the aquatic juvenile stage occupies by far the major proportion of the life cycle and is largely a feeding machine, leaving for the adult only a brief reproductive role. Some dragonfly larvae take three years to mature (Gooderham & Tsyrlin, 2002).

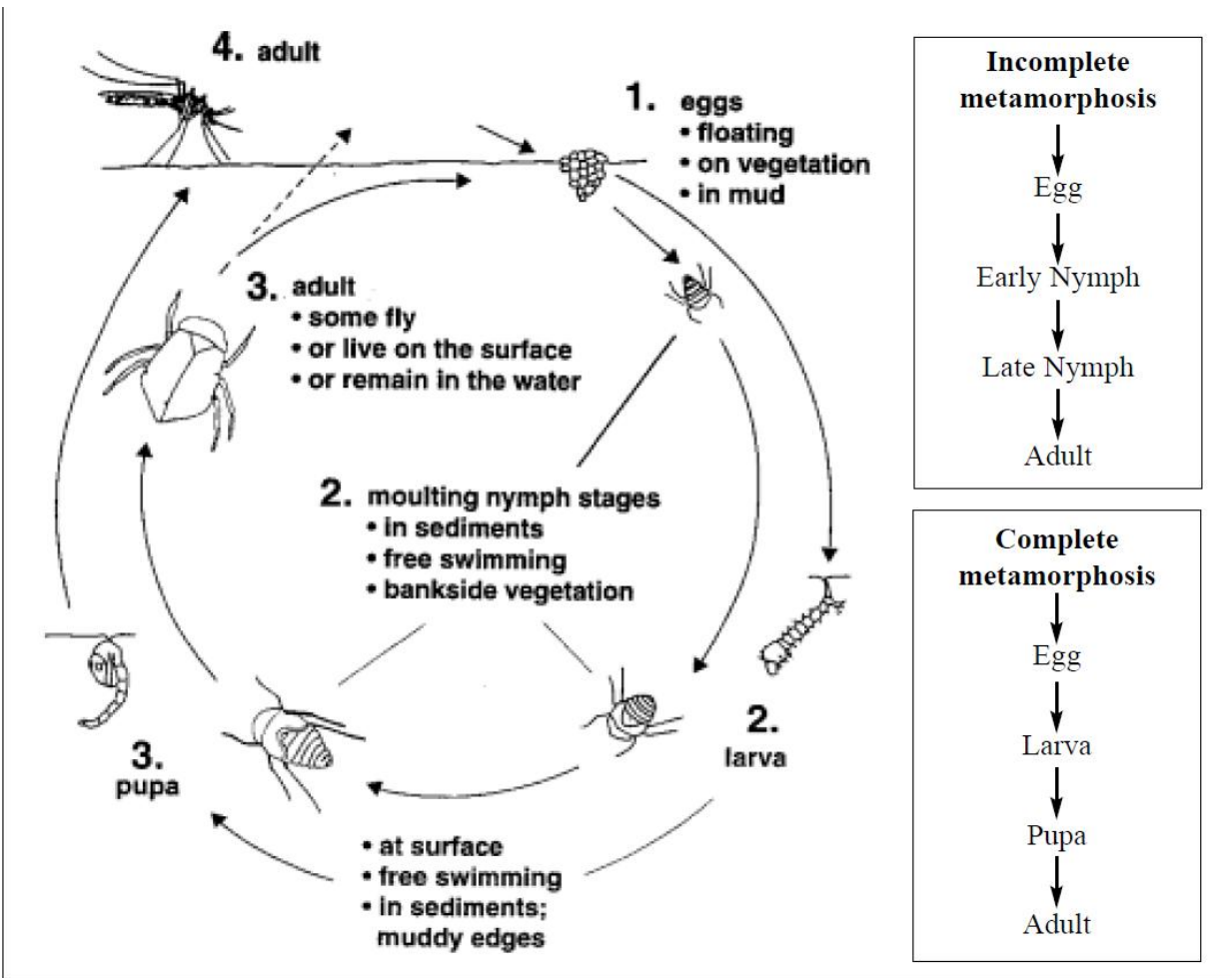


Figure. 1 Insect life cycle

What do macroinvertebrates eat?

Macroinvertebrates are an important part of the aquatic food chain and can be characterized by what the animal feeds on and how it acquires it. The categories are referred to as functional feeding groups and help describe the role each macro-invertebrate plays in an aquatic system. The study conducted in Ria Formosa of south Portugal, that data matrix a feeding group was assigned, among six groups: surface deposit feeders, subsurface deposit feeders, herbivores, suspension feeders and suspension/deposit feeders (species which have the two feeding modes depending on food availability). The carnivorous, parasites, omnivorous and scavengers were all grouped together, forming the sixth group. Most of the stations of Ria Formosa showed high feeding diversity, which could correspond to a good or high ecological status (ES) except at one

location, that occasionally showed low feeding diversity. This poor condition was essentially due to low water renewal and extreme environmental variation of some parameters, such as salinity. At some locations, an intermediate feeding diversity was observed mainly due to natural accumulation of organic matter. Other commonly used indices also point out to the same tendencies (Gamito & Furtado, 2009).

Shredders

Shredders feed on organic material, such as leaves and woody material, and help to convert this matter into finer particles. They require vegetation growing along a water body, so that plant material falls into the water and slow flowing water so that the plant material is not swept away. Such animals include amphipods, isopods, freshwater crayfish (marron, gilgies, koonacs) and some caddisfly larvae (Miserendino & Masi, 2010).

Collectors/filter feeders

Collectors/Filter feeders feed on fine organic particles that have been produced by shredders, microorganisms and by physical processes. Such animals include mayfly nymph, mussels, water fleas, some fly larvae, and worms (Miserendino & Masi, 2010).

Scrapers

Scrapers graze algae and other organic matter that is attached to rocks and plants. Such animals include snails, limpets and may fly larvae (Gamito, Patrício, Neto, Teixeira, & Marques, 2012).

Predators

Predators feed on live prey and are found where smaller collectors and shredders exist. Such animals include dragonfly and damselfly larvae, adult beetles and beetle larvae, some midge larvae and some stonefly larvae (Gamito, Patrício, Neto, Teixeira, & Marques, 2012).

Macroinvertebrates' habitat

Macroinvertebrates live in many different places in a water body. Some live on the water's surface, some in the water itself, others in the sediment, on the bottom, or on submerged rocks, logs, and leaf litter. Each type of habitat provides a surface or spaces on or within which macro-

invertebrates can live (Gamito & Furtado, 2009). In northern Portugal, separate macro-invertebrate samples from running-water and standing-water habitats and fish samples from a delineated reach were collected, inclusive of all habitat types. Macroinvertebrates from respective habitats differed in their relationship with habitat variable with running-water samples more strongly related to substrate and water quality and standing-water samples more strongly reflecting habitat characteristics at the reach scale. Running and standing-water samples from the same-site varied widely, indicating that substituting standing-water for running-water samples in macro-invertebrate-based bioassessment carries a high risk of misclassification. Overall, these data indicate how different ecological samples can be used to focus on different aspects of habitat quality and are suggestive of strategies for both the collection and interpretation of ecological data that would improve assessment performance (Monaghan & Soares, 2010).

The most important feature around a water body is vegetation. Aquatic plants, particularly rushes and sedges, provide a surface on which macro-invertebrates can live. In addition, they balance the water flow, light availability, and temperature around them. Shade by native trees and shrubs beside the water can reduce the extremes in temperature. Native trees, shrubs, rushes, and sedges protect banks from erosion, help to control the water flow, and act as nutrient filters. Logs, branches, bark and leaves that fall into the water provide habitat for aquatic organisms. Leaf litter forms an important part of a food web for macro-invertebrates, which feed, on this material, or on the bacteria and fungi that cause it to decay (Miserendino & Masi, 2010).

In fast flowing water (lotic) such as the upland streams, the bed consists of large rocks and stones and the stream is heavily shaded. The influence of vegetation is very high. This provides food supply for largely collectors and shredders. Macro-invertebrates are adapted to fast flowing water by having powerful suckers or gripping legs. In slow moving or still water (lentic) such as lowland rivers or wetlands, the bed may be sandy or muddy with increased light penetration. Nutrients are available and produce conditions for algal growth (Smith, Bode, & Kleppel, 2007). Collectors and scrapers dominate the macro-invertebrate community. Collectors will burrow into the sediment or filter their food directly from the water column. Grazers will be found on rocks, snags and woody debris or aquatic plants. In both lotic and lentic water bodies, predators are found where their preferred prey is located.

Water condition and macroinvertebrates

Environmental modifications or pollution can alter macro-invertebrate communities. Poor catchment management can exaggerate the turbidity of water. In highly turbid water, the light penetration is reduced affecting photosynthesis of plants and increases the temperature of the water. The suspended solids may clog respiratory surfaces or interfere with feeding appendages. The Filter feeders are receiving reducing nutritional value and expending more energy to collect food, otherwise, they will starve. High levels of suspended solids may begin to settle and change the composition of the bed of the water body as it coats rocks and vegetation. This can affect movement, feeding, habitat, and reproduction of some macro-invertebrates (Siggie, 2005).

The riparian vegetation balances the temperature in a healthy aquatic system. If this vegetation is cleared, it gives rise to more light penetration and an increase in turbidity from exposed soil. Industrial discharges or storm water runoff from hot surfaces (e.g. roads and car parks) could increase the temperature quickly and discharges from reservoirs could release cooler water. Some macro-invertebrates might be able to tolerate slight increases in temperature. Sensitive macro-invertebrates such as stoneflies, which are restricted to cool, fast flowing water bodies, cannot cope with such changes (Tran, Bode, Smith, & Kleppel, 2010).

High levels of nutrients in the form of nitrogen and phosphorus from fertilizers and wastewater can activate excessive algal growth (algal blooms). The death and decay of these algae can produce toxins and stagnant conditions. In these conditions, macro-invertebrate community diversity is usually reduced but there is generally an increase in the abundance of a few species (Paisley, Walley, & Trigg, 2011).

Toxic materials can enter water bodies from industrial and agricultural wastewater and can include such substances as pesticides and heavy metals (Greenberger, Desjarins, & Degagne, 2003). The effect to the macro-invertebrate communities may be short-term (acute) if the pollutant exists in the water at high enough concentrations. In most cases, however, toxicants concentrations and discharges vary considerably. Therefore, emphasis is placed on long-term effects (chronic) where toxins can accumulate and become concentrated in food chains. Macro-invertebrates communities could be affected by decreased reproduction, impaired behavioral responses, disease, or eventually death. The presence of such toxicants generally tends to reduce

the overall diversity of macro-invertebrates (Gerhadta, Janssens de Bisthoven, & Soares, 2004).

The response to pollutants can vary enormously. For example, most species of mayfly nymph do not respond well to sediment or organic pollutants, but some are quite tolerant. The larvae of dragonflies and damselflies can be quite tolerant of salinity, but are harmed by other pollutants. Some animals can act as pollution indicator species because they respond to specific changes in the water conditions (Pelletier, Gold, Heltshe, & Buffum, 2010).

Advantage of biomonitoring using macroinvertebrates

Macro-invertebrates are sampled in water bodies because they are useful biological indicators of change in the aquatic systems. The main advantages of using macro-invertebrates is that some have life span of up to a year and greater, they are relatively sedentary, have varying sensitivities to changes in water quality and they are easily collected and identified (Sánchez-Montoya, Vidal-Abarca, & Suárez, 2010). It is very important to note, however, that when assessing macro-invertebrates, other physical, chemical, and other biological data should be considered to support the water body assessment. Other biological measures could include riparian vegetation, fish, frogs, birds, algae, and faecal coliforms (Torrisoni, Scuri, Dell'Uomo, & Cocchioni, 2010). Common physical and chemical parameters assessments include temperature, turbidity, conductivity, pH, nutrients and dissolved oxygen (Quevauviller, Borchers, Thompson, & Simonart, 2008).

4.4.2 Birds

Currently there are 29 orders, 201 families, 2073 genera, and 10,010 species of the class Aves. Birds are grouped into a number of categories based on the regularity with which they occur such as resident species, summer visitors, winter visitors, transit passengers, and rare vagrants. Climate radically influences habitats and local movements of resident and migratory birds. Many species are characteristic of particular habitats or biomes. Resident and migrant bird distribution is strongly influenced by equatorial seasons (Aynalem & Bekele, 2008).

Birds have proved to be excellent indicators of biodiversity or productivity and are vital for ecological functioning of our environment such as indicators of pollution, seed dispersal,

scavenging offal and as predators of numerous insect and other pests. Apart from their beauty, birds are excellent indicators of water quality. Wetlands provide suitable habitats for innumerable organisms including birds. The presence or absence of shelter may influence whether birds will inhabit a wetland or a nearby upland area. Water resource development is a major cause for the decline of wetlands throughout the world (Aynalem, Bekele, & Getahun, 2008).

Study conducted in Boye wetland, southwestern, Ethiopia revealed that A total of 36 bird species were recorded during the surveys. Among these, two species; *Poicephalus flavifrons* and *Macronyx flavicollis* are endemic to Ethiopia. Some of the species limited only to Ethiopia and Eritrea were also inhabited in Boye wetland such as; *Bostrychia carunculata*, *Dioptrornis chocolatinus* and *Corvus crassirostris*. Among the recorded species *Balearica pavonina* and *Balearica regulorum* were vulnerable while *M. flavicollis* was near threatened. These species will be endangered within a short period unless the necessary actions are taken (Mekonnen & Aticho, 2011).

4.5 Water quality assessment

Stream biodiversity is tightly linked to physico-chemical factors and it used as a measure of stream health representing the cumulative physical and chemical conditions. Biological condition is also used to provide an indication of aquatic ecosystem conditions (Stranko, Hiderbrand, & Palmer, 2011).

The physicochemical and biological study conducted in Pakistan of Lakki hills Eu-thermal and chiliaro-thermal springs revealed that the Eu-thermal (40-42 °c) with significant evolution of hydrogen sulfide (135-152 mg/l) with high electric conductivity 13000-14300 µS/cm and the pH 6.35-6.85. On the other hand, the chiliaro-thermal having water temperature of 18-30 °c and is a fresh water spring and forms seepage pool with pH 7.15, total dissolved solid/TDS/ 1088 mg/l and electrical conductivity 1700 µS/cm. the result showed that zooplankton, insects, mollusca and fishes were found at the end of hot springs (Leghari, Jahangir, Khuhawar, & Leghari, 2001).

The study conducted in England examined the long-term chemical and biological changes in historically polluted rivers to elucidate the responses of macroinvertebrate biota to improvements in chemical water quality. For three historically polluted sites in the English Midlands, data from

surveys over a period of Ca. 50 years were analyzed. Ammonia (NH₃) and 5-day biochemical oxygen demand (BOD₅) were used as chemical water quality indicators. Variations in the ecological recovery of the study sites were assessed using an average pollution sensitivity score (Average Score per Taxon) and the number of taxa present (usually to family level) present in hand-net samples. Ecological recovery varied widely and was influenced by the intensity and spatial extent of the pollution and the proximity of available sources of potential colonizers.. Where clean water colonizers were more readily available, significant improvements in ecological quality followed within 2–5 years of the improvements in chemical quality. Macroinvertebrate communities and hence monitoring data may thus be indicative of long past conditions or of biological isolation rather than contemporaneous chemical conditions. Combined chemical and biological data were used to explore a generic model for predicting recovery rates and success. Long-term relationships between macroinvertebrate variables and chemical water quality variables, however, were non-linear, suggesting that water quality thresholds may have to be exceeded before biological recovery can occur. Even when chemical water quality has been improved substantially, the apparent ecological status of macroinvertebrate communities may not reflect reduced pollution levels attained until adequate time to allow for re-colonization (possibly decades) has elapsed (Langford, Shaw, Ferguson, & Howard, 2009).

Chemical characteristics

Thermal springs are usually mineralized to a greater or lesser extent depending on the characteristics of the geological formations associated with the circulating groundwater. The study results of the chemical analyses of water samples collected from springs in South Africa were varied from Water quality standards provided by the South African Bureau of Standards (Olivier, Venter, & Jonker, 2011).

The intensive study on water quality and benthic macroinvertebrate fauna of Behzat Stream in Turkey showed that, the upper section supported a more diverse community than the lower section. The low macroinvertebrate abundance was observed during summer in the lower section, this was due to high values of phosphate and nitrogen ions as well as under threat of anthropogenic disturbances, in the lower section (M.Duran, 2006).

Aquatic macroinvertebrates have been among the principal biological communities that used for freshwater monitoring and assessment as well as can use for nutrient assessment strategies. Two nutrient biotic indices were developed for benthic macroinvertebrate communities, one for total phosphorus (NBI-P), and one for nitrate (NBI-N). Weighted averaging was used to assess the distributions of macroinvertebrate taxa across TP and NO_3^- gradients and to establish nutrient optima and subsequent nutrient tolerance values. A three tiered scale of eutrophication for TP and NO_3^- (oligotrophic: 0.0175 mg/l TP, 0.24 mg/l NO_3^- , mesotrophic: >0.0175 to 0.065 mg/l TP, >0.24 to 0.98 mg/l NO_3^- , eutrophic: >0.065 mg/l TP, >0.98 mg/l NO_3^-) was established through cluster analysis of invertebrate communities using Bray–Curtis (quantitative) similarity. Therefore, the nutrient biotic indices (NBIs) appear to reflect accurately changes in stream trophic state. Therefore, the suggested threshold for nutrient impairment is the boundary between mesotrophic and eutrophic (0.065 mg/l TP and 0.98 mg/l NO_3^-). The NBI and index score thresholds of impairment will provide monitoring programs with a robust measure of stream nutrient status and serve as a useful tool in enforcing regional nutrient criteria (Smith, Bode, & Kleppel, 2007).

2.6 Significant of the study

Macroinvertebrate diversity is known for their potential indicators of water quality in different parts of the world. This present study was conducted for first time to determine Macroinvertebrate diversity in hot water springs in Ethiopia particularly in Eastern Amhara Region:-

- provide information on the physicochemical characteristics and quality of hot spring
- delivering information on diversity of macroinvertebrate & bird in hot springs of East Amhara
- the study can generate baseline data that may give an insight for future study
- evaluate the potential pollution of hot springs from human disturbance
- the finding of this study can be used by local to state level concerning bodies to regulate, manage and protecting the hot springs

CHAPTER THREE: OBJECTIVES

3.1 General Objective

The main objective of this study is to examine the relationship between biological parameters (macroinvertebrate and bird diversity) with physicochemical water quality, habitat quality and human disturbance of Hot springs in Easter Amhara Region.

3.2 Specific Objectives

- ✓ To determine macro-invertebrate diversity in hot springs
- ✓ To determine bird diversity near the hot springs
- ✓ To examine physicochemical properties in relation to macroinvertebrate and bird diversity of hot springs
- ✓ To determine ecological status of hot spring

3.3 Research Questions

What is the diversity of macroinvertebrates and birds in hot springs and their relationship with the water and habitat quality?

CHAPTER FOUR: METHODS AND MATERIALS

4.1 Study area

The study was conducted in the eastern Amhara region namely North Showa (Shewa Robit Aregawi hot springs in Kewet district), Oromia zone(Shekla and Borkena hot springs in Cheffie Dolana district) and South Wello(Harbu hot springs in Kalu district). These areas located in rift valley regions which are known for several hot springs.

Cheffa Wetland is located 300 km northeast of Addis Ababa, the capital of Ethiopia. The wetland is located within $10^{\circ}32'$ – $10^{\circ}58'$ and latitudes and $39^{\circ}46'$ – $39^{\circ}56'$ E longitudes in the Borkena and Jara River Basins. Its total area is estimated to be 82,000 ha (Tamene, Bekele, & Kelbessa, 2000). The altitude of the wetlands ranges from 1402 m to 1520 m above sea level but altitudes exceed 2000 m and even 3000 m in the surrounding Ethiopian Highlands. This wetland contains many hot springs, which are used for local community as means of traditional healing and as source of drinking water for domestic purpose and their cattle. Two main sites were selected Shekla and Borkena based on their importance in this wetland. Shekla wetland is located at the entry of the main Cheffa wetland and Borkena site is located at the exit of Cheffa wetland. The Shewarobit Aregawi wetland is located 220 km away from Addis Ababa the capital of Ethiopia to Northeast direction in Kewet district of north Shewa. The three hot spring sampling sites of this wetland is located within $09^{\circ}59'37$ N, $09^{\circ}53'06$ N and $09^{\circ}59'35$ N latitude and $39^{\circ}52'54$ E, $39^{\circ}53'13$ E and $39^{\circ}53'13$ E longitude with altitude of 1301m, 1293m and 1288m respectively. The Harbu hot spring sampling sites are located 370 km distance from the capital city of Ethiopia to the northeast direction in Kalu district of South Wello. The hot springs sampling sites are located within $10^{\circ}55'53$ N, $10^{\circ}55'26$ N and $10^{\circ}48'14$ N latitude and $39^{\circ}48'32$ E, $39^{\circ}48'31$ E and $39^{\circ}49'25$ E longitude with altitude of 1566m, 1561m and 1517m respectively. Totally twelve sampling points were selected from the four main hot spring sites i.e. three from each sampling site. Namely Borkena hot spring(B1,B2 and B3), Shekla(S1,S2 and S3), Harbu(H1,H2 and H3) and Shewarobit Aregawi(A1,A2 and A3) were selected based on distance and temperature gradient. The Oromo ethnic group constitutes the majority of the people living in the Cheffa Riverine plain and Amhara dominated the Shewarobit Aregawi and Harbu areas in Eastern Amhara region. Subsistence mixed agriculture (crop production and livestock rearing) is the mainstay of the permanent wetland population (Tamene, Bekele, & Kelbessa, 2000). The

population of the nearby Woredas (districts) of Dewa Cheffa, Artuma Fursi, Kemise Town, Antsokiya Gemza, Efratagidim and Kalu was 614,476 during the 2007 census. In the absence of census data, we estimate that fewer than 10,000 people live in about two dozen villages in Cheffa Wetland, 2000 in Harbu, and about 3000 in Shewarobit Aregawi sites. The major town near the periphery of the Cheffa wetland is Kemise, with about 20,000 populations (Getachew, et al., 2012) for Aregawi wetland Shewa Robit and for Harbu hot spring site is Harbu town.

During the dry season, Afar, Oromo, Argoba and Amhara pastoralists move with their herds to the Cheffa Wetland, a practice that has been associated with environmental degradation elsewhere in Ethiopia (MCKEE, 2007); (Getachew, et al., 2012). In 2002, the United Nations Emergencies Unit for Ethiopia reported that about 50,000 pastoralists together with 200,000 livestock, mostly cattle, used the Cheffa Wetlands for watering and grazing (Piguet, 2002). The same scenario in Shewarobit and Harbu sites seen as it noticed Cheffa wetland. The Shewarobit wetland is converted to farmland in alarming rate than any of other sites that studied. The site was offensive in smell due to washing, open defecation, and urination of pilgrims from two churches. The site was used for harvesting of many vegetables, fruit, cereal crops, and tobacco plant. The site also degraded by hundreds of cattle grazing. Firing of wetland part is practiced daily. This site comprised two churches which being used as holy water sites and in daily basis, hundreds of people were gotten services there. The people practiced open defecation and no any means of waste management practices there especially Aregawei church is located at the entry of wetland and it constitutes about four hot springs which feed the wetland. In Harbu site, people who got services form temporary residence up to months and practicing open defecation. The site is degraded due to farming in the nearby and over grazing by local community too.

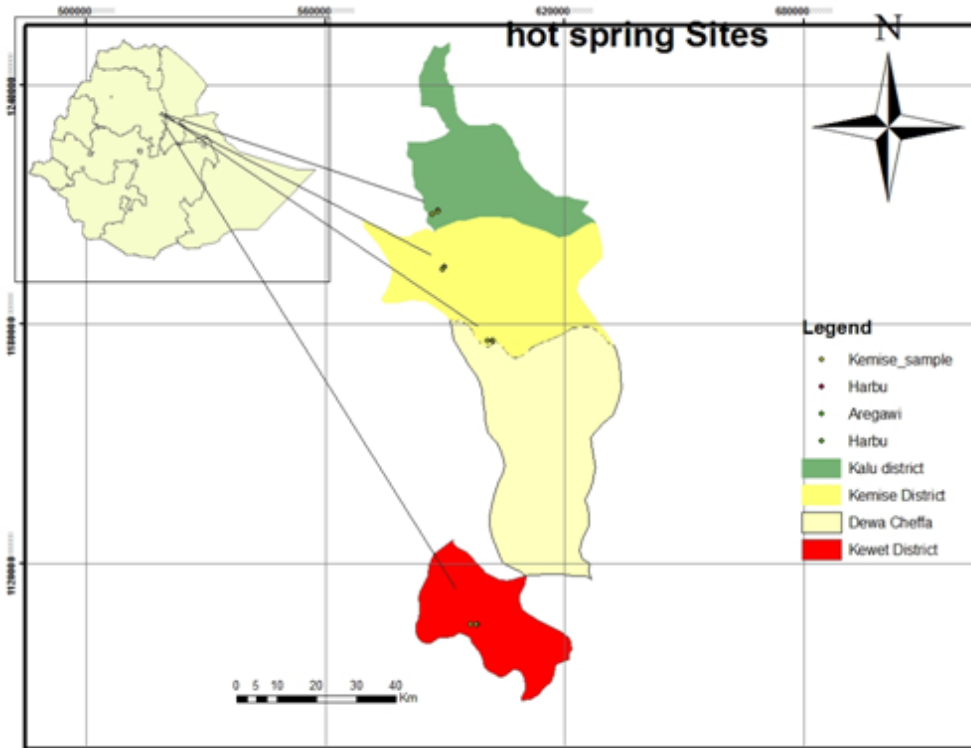


Figure. 2 Hot spring sampling sites in East Amhara Region, Ethiopia

4.2 Sampling sites and sampling frequency

Aquatic macroinvertebrates and water samples were collected at 12 sites selected to represent the water quality in hot springs. The sampling criteria was the distance between sampling points and difference of water temperature and the basis of factors such as: Ease of access, Variety of habitats, Proximity to a local point-source of pollution e.g. a factory, a drainage canal or Proximity to a non-point source of pollution e.g. a farm. The selected sites were representative of the local catchment characteristics (UN-HABITAT, 2005). Water samples were taken simultaneously with the macroinvertebrates samples. Samples were collected from February to April 2013 in mid and near the end of the dry season period in the study area. At each site two water samples were taken (two replicates per site per sampling date) and were analyzed for physicochemical parameters during the sampling period. To obtain a visual record of sampling sites, digital photographs of the water body upstream and downstream of the sampler locations were taken during sampling periods. furthermore, for integration into a GIS (Geographic Information System) database, longitude and latitude and elevation of each sampling site was recorded using a GPS (global positioning system) unit.

4.3 Study design

A cross-sectional study of physical, chemical and biological components of the hot springs were carried out to assess its ecological status.

4.3.1 Macroinvertebrates sampling and identification

Benthic macroinvertebrates were collected to provide a qualitative description of the community composition at each sampling site. Macroinvertebrates were sampled using a D-shaped sweep-net specified by the International Standards Organization (ISO); with mesh size of 250 µm. Sweeping was done in a vigorous action for 5 min for a distance of 10 m with multi-habitat approach at each site to dislodge macroinvertebrates attached to any substrates present (Baldwin, Nielsen, Bowen, & Williams, 2005). Collected organisms were removed from the sweep-net and the net's content was washed into a sieve to collect organisms attached to the net. The kick samples of all members of a group in one site was composited in a single bottle and preserved with 70% ethanol with a label identifying the location, date and time and was sorted from the detritus and was transported to the Jimma University Environmental Health Laboratory. The samples were transferred to white enamel or plastic tray and a small amount of the sample was randomly placed in a Petri dish and was identified using a dissecting microscope and identification keys aquatic insects were identified to family level and finally placed in vials containing 70% ethyl alcohol for future use. Aquatic taxonomic keys developed by (Deliz Quinones, 2005) and (Cummins, 1973) were used for identifying specimens at family level using a dissecting microscope.

Only the organisms from the sweep were used to estimate the index, based on relative abundances of macroinvertebrates. All sweeps were used to calculate the index based on taxon diversity.

4.3.2 Bird count and identification

The method of total count (also called “direct counts”) was employed to census the bird population (U.S.EPA, 2002). In this method, representative sites were identified and the birds at these sites were counted using field binoculars. Observations were carried out for 5 h; 6:30 to 10:00 and 16:30 to 18:00 h, during these lapses, the activities of birds became prominent. Birds

were identified using physical features with the help of field guides and reference books on the bird fauna of East Africa (Perlo, 2009). Photographs and videos were taken to justify the species type for those species, which were difficult to identify. Some inconspicuous bird species were also identified based on their calls (Aynalem, Bekele, & Getahun, 2008).

4.3.3 Water quality assessment

Two liters of water samples were collected for analysis of nitrate-nitrogen(NO_3^- -N), Orthophosphate(PO_4^{3-}), total Nitrogen(TN) and chloride concentration as chemical variables, temperature, pH, and conductivity, Dissolved Oxygen(DO), turbidity included as environmental variables were measured following water quality assessment protocols. Dissolved oxygen, electrical conductivity, water temperature, turbidity, and pH were measured on site using HACH multimeter handheld probe, model HQ40D. Water samples were collected with a 2 L plastic container from each site; samples were stored in a refrigerator at 4 °C. Then all samples were transported to Jimma University, Environmental Health Science, and Technology department Laboratory in an insulated box containing ice packs. A spectrophotometer, model HACH DR 5000, and a digester, model HACH LT200, were used to determine total nitrogen. The kits were used for determination of total nitrogen was LCK 138 following the procedures set for the parameter. Ortho-phosphate concentration was determined by stannous chloride method and Nitrate-N concentration was measured with ultra violet spectrophotometer screening method as well Chloride concentrations of water samples was determined by the argentometric method (APHA, 1995).

4.3.4 Habitat Quality Assessment/HQA/

Physical habitat information was collected at each site with visual estimate measurement technique. At each of six evenly spaced channel cross sections, wetted width, bankfull width, bankfull and incised heights, and bank angles were estimated. Canopy cover was measured on the left and right bank, and in four directions (upstream, downstream, left, and right) in the center of the channel cross section as partly open, partly shaded or shaded. Stream water depth was measured at five equally spaced locations along each cross section. Substrate composition was determined by size tallies, performed by placing a finger into the water and classifying the size of the particle first touched as bedrock (> 4000 mm), boulder (250–4000 mm), cobble (64–250

mm), coarse gravel (16–64 mm), fine gravel (2–16 mm), sand (0.06–2.00 mm), fines (<0.06 mm), wood, hardpan (firm, consolidated fines), or other. Embeddedness percentage was visually estimated from the area immediately surrounding each sampled particle. Immediately following cross section surveys, large wood (>six in diameter) was tallied and organic layer accumulation in depositional zones was measured. Visual estimates or classifications were then made of dominant bank material, percent stable bank, percent undercut bank, dominant erosional bed material and dominant depositional bed material, erosional habitat embeddedness (%), and depositional habitat embeddedness. On each bank, the riparian zone buffer width (defined for this study as the area within which natural mature vegetative communities occurred) and the dominant adjacent land uses outside the riparian buffer area were recorded. The reach also was classified using the Rosgen Level 2 stream morphology classification system (Rosgen, 1996). This system classifies stream reaches based on channel slope, dominant channel materials, channel entrenchment, the width-to-depth ratio, and sinuosity. Streams were classified using this system to more precisely characterize high and low-gradient reaches in relation to morphological features. The habitat conditions of the hot springs were evaluated based on the method developed by (Barbour, Gerritsen, Snyder, & Stribling, 1999) and human and animal impact assessment was made following the methods of the Maine Department of Environmental Protection (MDEP, 2009).

Biotic indices

Family level biotic index

Family level biotic index (Hilsenhoff's) index was calculated based on the scores given to each taxon (Table 1)

Table 1: Family level biotic index scoring value

| S/N | Order | Family | Score | Reference | | |
|----------------|---------------|-------------------------|-----------|--------------------------|----|--------------------|
| 1 | Ephemeroptera | Baetidae | 4 | Barbour et al,1999 | | |
| | | Heptagenidae | 7 | Barbour et al,1999 | | |
| | | Caenidae | 4 | Barbour et al,1999 | | |
| 2 | Diptera | Tipulidae | 3 | Barbour et al,1999 | | |
| | | Culicidae | 8 | Barbour et al,1999 | | |
| | | Tabanidae | 6 | Hauer & Lamberti 1996 | | |
| | | Dolichopodidae | 4 | Barbour et al,1999 | | |
| | | Chironomidae | 6 | Bode et al,1996 | | |
| | | Psychodidae | 10 | Hauer & Lamberti 1996 | | |
| | | | | | | |
| 3 | Odonata | Gomphidae | 1 | Hauer & Lamberti 1996 | | |
| | | Coenagrionidae | 9 | Hauer & Lamberti 1996 | | |
| 4 | Coleopteran | Haliplidae | 5 | Bode et al,1996 | | |
| | | Hydrophilidae | 5 | Bode et al,1996 | | |
| | | Dytiscidae | 5 | Bode et al,1996 | | |
| 5 | Gastropod | Corbiculidae | 6 | Bode et al,1996 | | |
| | | Physidae | 8 | Barbour et al,1999 | | |
| | | Planorbidae | 7 | Barbour et al,1999 | | |
| | | Hydrobiidae | 7 | Barbour et al,1999 | | |
| | | Sphaeriidae | 8 | Bode et al,1996 | | |
| | | Valvatidae | 7 | Barbour et al,1999 | | |
| | | Viviparidae | 6 | Barbour et al,1999 | | |
| | | Lymnaeidae | 8 | Barbour et al,1999 | | |
| | | 6 | Hemiptera | Mesovelidae | 10 | Barbour et al,1999 |
| | | | | Naucoridae | 5 | Barbour et al,1999 |
| Corixidae | 5 | | | Barbour et al,1999 | | |
| Belostomatidae | 10 | | | Bode et al,1996 | | |
| 7 | Trichoptera | Hydropsychidae | 4 | Barbour et al,1999 | | |
| 8 | Araneae | Agelenidae/Water spider | | | | |
| 9 | Amphipoda | Gammaridae | 4 | Hauer & Lamberti | | |

| | | | | |
|----|-------------|-------------|---|-------------------------|
| 10 | Oligochaeta | Oligochaeta | 8 | 1996 Bode et al,1996 |
|----|-------------|-------------|---|-------------------------|

Table 2: Evaluation of water quality using the family-level biotic index (**Hilsenhoff, 1988**)

| Family Biotic Index | Water Quality | Degree of Organic Pollution |
|---------------------|---------------|-----------------------------------|
| 0.00-3.75 | Excellent | Organic pollution unlikely |
| 3.76-4.25 | Very good | Possible slight organic pollution |
| 4.26-5.00 | Good | Some organic pollution probable |
| 5.01-5.75 | Fair | substantial pollution likely |
| 5.76-6.50 | Poor | substantial pollution likely |
| 7.26-10.00 | Very poor | Severe organic pollution likely |

South Africa Scoring System

Table 3: Evaluation of water quality using South Africa Scoring System/SASS/ (Dallas & Day, 2006)

| SASS | ASPT | ecological category | category name | Description |
|---------|---------|---------------------|---------------|-------------------------------|
| 137-166 | 8.2-9 | A | Natural | unmodified natural |
| 108-137 | 7.4-8.2 | B | Good | natural with few modification |
| 79-108 | 6.6-8.2 | C | Fair | moderately modified |
| <79 | <6.6 | D | Poor | largely modified |

South African Scoring System (SASS) index was calculated based on the scores given to each taxon (Table 4)

Table 4: South Africa Scoring System value

| S/N | Order | Family | score | Reference |
|------------|---------------|-------------------------|--------------|--------------------------|
| 1 | Ephemeroptera | Baetidae | 4 | (Dickens & Graham, 2002) |
| | | Heptageniidae | 13 | (Dickens & Graham, 2002) |
| | | Caenidae | 6 | (Dickens & Graham, 2002) |
| 2 | Dipteral | Tipulidae | 5 | (Dickens & Graham, 2002) |
| | | Culicidae | 1 | (Dickens & Graham, 2002) |
| | | Tabanidae | 5 | (Dickens & Graham, 2002) |
| | | Dolichopodidae | 4 | (Dickens & Graham, 2002) |
| | | Chironomidae | 2 | (Dickens & Graham, 2002) |
| | | Psychodidae | 1 | (Dickens & Graham, 2002) |
| | | 3 | Odonata | Gomphidae |
| | | Coenagrionidae | 4 | (Dickens & Graham, 2002) |
| 4 | Coleopteran | Haliplidae | 5 | (Dickens & Graham, 2002) |
| | | Hydrophilidae | 5 | (Dickens & Graham, 2002) |
| 5 | Gastropoda | Dytiscidae | 5 | (Dickens & Graham, 2002) |
| | | Corbiculidae | 5 | (Dickens & Graham, 2002) |
| | | Physidae | 3 | (Dickens & Graham, 2002) |
| | | Planorbidae | 3 | (Dickens & Graham, 2002) |
| | | Hydrobiidae | 3 | (Dickens & Graham, 2002) |
| | | Sphariidae | 3 | (Dickens & Graham, 2002) |
| | | Valvatidae | 3 | (Dickens & Graham, 2002) |
| | | Viviparidae | 6 | (Dickens & Graham, 2002) |
| 6 | Hemiptera | Lymnaeidae | 3 | (Dickens & Graham, 2002) |
| | | Mesovelidae | 5 | (Dickens & Graham, 2002) |
| | | Naucoridae | 7 | (Dickens & Graham, 2002) |
| | | Corixidae | 3 | (Dickens & Graham, 2002) |
| 7 | Tricoptera | Belostomatidae | 3 | (Dickens & Graham, 2002) |
| | | Hydropsychidae | 4 | (Dickens & Graham, 2002) |
| 8 | Araneae | Agelenidae/Water spider | | |
| 9 | Amphipoda | Gammaridae | 13 | (Dickens & Graham, 2002) |
| 10 | Oligochaeta | Oligochaeta | 1 | (Dickens & Graham, 2002) |

4.5 Quality control

Quality control was conducted on field procedures to ensure a high level of consistency and accuracy in all operations i.e. in situ field measurements; sample collection and field processing, human disturbance and habitat assessment. A standard procedure method and protocol was followed.

4.6 Quality assurance

For the sake of quality, assurance data was assessed carefully using standard operating procedures and Double entry of data was performed to assure quality of data.

4.7. Data analysis

The Shannon diversity index (Turkmen & Kazanci, 2010) ,Simpson diversity index (Smith & Wilson, 1996) and Margalef diversity index (Gamito, 2010) were used to measure diversity of macroinvertebrate and bird which were recorded at the 12 sampling sites. Bray-Curtis cluster analysis and Shannon diversity index were calculated from family level macroinvertebrate taxa of each site using Bio-Diversity Professional software. The physicochemical and macroinvertebrate taxa, as well as other environmental variables of hot water and bird were analyzed by Past software to identify influencing parameters on macroinvertebrates and birds of the Eastern Amhara hot springs. Before running past, the biological and environmental data were transformed using square root and $\log(x + 1)$, respectively.

Multiple regression analysis were performed to analyze the existence of linear relationship between biological data represented by Shannon diversity indexes, Simpson diversity index and other biotic indices (macroinvertebrate communities and bird community) and the environmental variables by stepwise forward selection method to select the best environmental predictors using STATISTICA® software package version 7.1. Prior to the analysis, the environmental data were transformed to $\log(x + 1)$, where x is the value of an environmental variable.

4.8 Ethical consideration

The study was conducted after getting permission from ethical committee of Jimma University, college of public health and medical sciences.

4.9 Dissemination plan

the final result of this study was presented to Jimma University public health and medical science, department of Environmental health science and technology and was disseminated to concerning ministers, Amhara regional state, Oromia special zone and other governmental and non-governmental organizations which are concerned with the study findings. Publication in national and international journals will also be considered.

CHAPTER FIVE: RESULT

5.1 Physicochemical characteristics of water samples

The values of the physicochemical examination of samples from the different sites are shown in Table 5. Values vary considerably among the 12 sites. Water temperature levels were particularly high at the B1, H1, S1, and the A1 sites where the hot springs emerged. The turbidity level was ranging from 4.4 to 33.8 in all sites except high turbidity level at site S2 that was 185. The pH values of all water samples were within the range of 7.09–8.63. Dissolved oxygen was generally low at emerging sites of hot springs even null at H1 and B1 sites. The electric conductivity was high particularly at the sites of S2, S1, and A2. On the other hand, EC is very low at A1 and S3 sites where the water was submerged into sands before the sampling sites. The rest sites were at similar pattern in EC value fallen in the range of 974 to 1398. Generally, the water depth of all sampling sites was shallow and had low flow rate. The nutrient values were distributed in similar pattern except ortho phosphorus and nitrate nitrogen exceed in S2 and H3 sites than the rest sites. The chloride concentration of H3 site was greater than the other sites of hot springs.

Table 5 physicochemical parameters of water samples and summary statistics (N=12) of the 12 sampling sites of hot springs in Eastern Amhara region, Ethiopia, 2013

| Environmental variables | | | | | | | | | | | | | Min | Max | Mean | StDv |
|-------------------------|-------|-------|-------|-------|-------|------|------|-------|-------|------|-------|-------|-------|--------|---------|--------|
| | A1 | A2 | A3 | B1 | B2 | B3 | H1 | H2 | H3 | S1 | S2 | S3 | | | | |
| Altitude | 1301 | 1293 | 1288 | 1403 | 1399 | 1392 | 1566 | 1561 | 1517 | 1437 | 1435 | 1433 | 1288 | 1566 | 1418.75 | 95.583 |
| Ambient Temperature(0c) | 28 | 30 | 28 | 25 | 21 | 21 | 27 | 28 | 32 | 22 | 24 | 28 | 21 | 32 | 26.17 | 3.563 |
| Water Temperature(0c) | 51.6 | 32.5 | 29.9 | 72 | 42 | 38.8 | 70 | 40 | 34 | 52 | 33.4 | 27.3 | 27 | 72 | 43.58 | 15.018 |
| DO(mg/l) | 5.41 | 5.72 | 5.28 | 0.46 | 4.23 | 5.84 | 0 | 9.05 | 8.4 | 2.23 | 5.58 | 7.87 | 0 | 9 | 4.92 | 2.968 |
| EC(μS/cm) | 2.68 | 1508 | 1204 | 1394 | 1240 | 1247 | 1211 | 974 | 1077 | 1798 | 2181 | 3.34 | 3 | 2181 | 1153.33 | 629.73 |
| Ph | 7.99 | 8.07 | 7.95 | 7.84 | 8.32 | 8.63 | 7.14 | 8.12 | 8.13 | 7.09 | 8.39 | 8.42 | 7 | 9 | 7.92 | 0.51 |
| Velocity | 0.1 | 0.4 | 0.5 | 0.1 | 0.5 | 0.5 | 0.2 | 0.36 | 0.5 | 0.2 | 0.3 | 0.3 | 0.10 | 0.50 | 0.33 | 0.15 |
| Water depth | 0.7 | 0.4 | 0.5 | 1.2 | 0.5 | 1 | 0.4 | 0.3 | 0.15 | 0.2 | 0.2 | 0.4 | 0.15 | 1.20 | 0.49 | 0.32 |
| Discharge | 0.05 | 0.28 | 0.5 | 0.01 | 1.5 | 1.5 | 0.3 | 0.36 | 0.5 | 0.1 | 0.3 | 0.5 | 0.01 | 1.50 | 0.49 | 0.49 |
| Turbidity | 10.27 | 13.3 | 30.9 | 33.8 | 9.44 | 11.5 | 4.4 | 17.1 | 31.5 | 8.87 | 185 | 13.5 | 4.00 | 185.00 | 30.83 | 49.57 |
| Orto-phosphate | 0.1 | 0.08 | 0.09 | 0.06 | 0.14 | 0.2 | 0.08 | 0.1 | 0.18 | 0.11 | 0.91 | 0.11 | 0.06 | 0.91 | 0.18 | 0.23 |
| Nitrate -N | 0.16 | 1.18 | 0.8 | 0.75 | 0.42 | 0.45 | 0.28 | 0.52 | 2.48 | 0.66 | 1.2 | 0.89 | 0.16 | 2.48 | 0.82 | 0.61 |
| Total-N | 2.41 | 2.46 | 3.18 | 3.36 | 31.7 | 1.33 | 1.71 | 2.28 | 3.26 | 3.8 | 4.5 | 3.7 | 1.33 | 31.70 | 5.31 | 8.36 |
| Chloride | 36.84 | 35.49 | 41.84 | 37.98 | 29.99 | 53 | 39.7 | 52.98 | 53.67 | 43.9 | 50.63 | 52.98 | 30.00 | 54.00 | 44.16 | 8.38 |
| Valid N (listwise) | | | | | | | | | | | | | | | | |

Principal Component Analysis of environmental variables

A bi-plot of the sampling sites and environmental variables showed that there was a clear distinction between sampling sites (Figure 3). Conductivity, ortho-phosphate, altitude, Nitrate-N and chloride were strongly negatively correlated with H2, S2 and H3, whereas TN, discharge, pH, velocity and DO were more correlated with A2, A3, B3 and B2 sampling sites. Water depth solely was correlated with A1 and sites of B1. H1 and S1 were not correlated with any of these environmental variables. S3 site association was relay at the x-axis between component 1 and component 2.

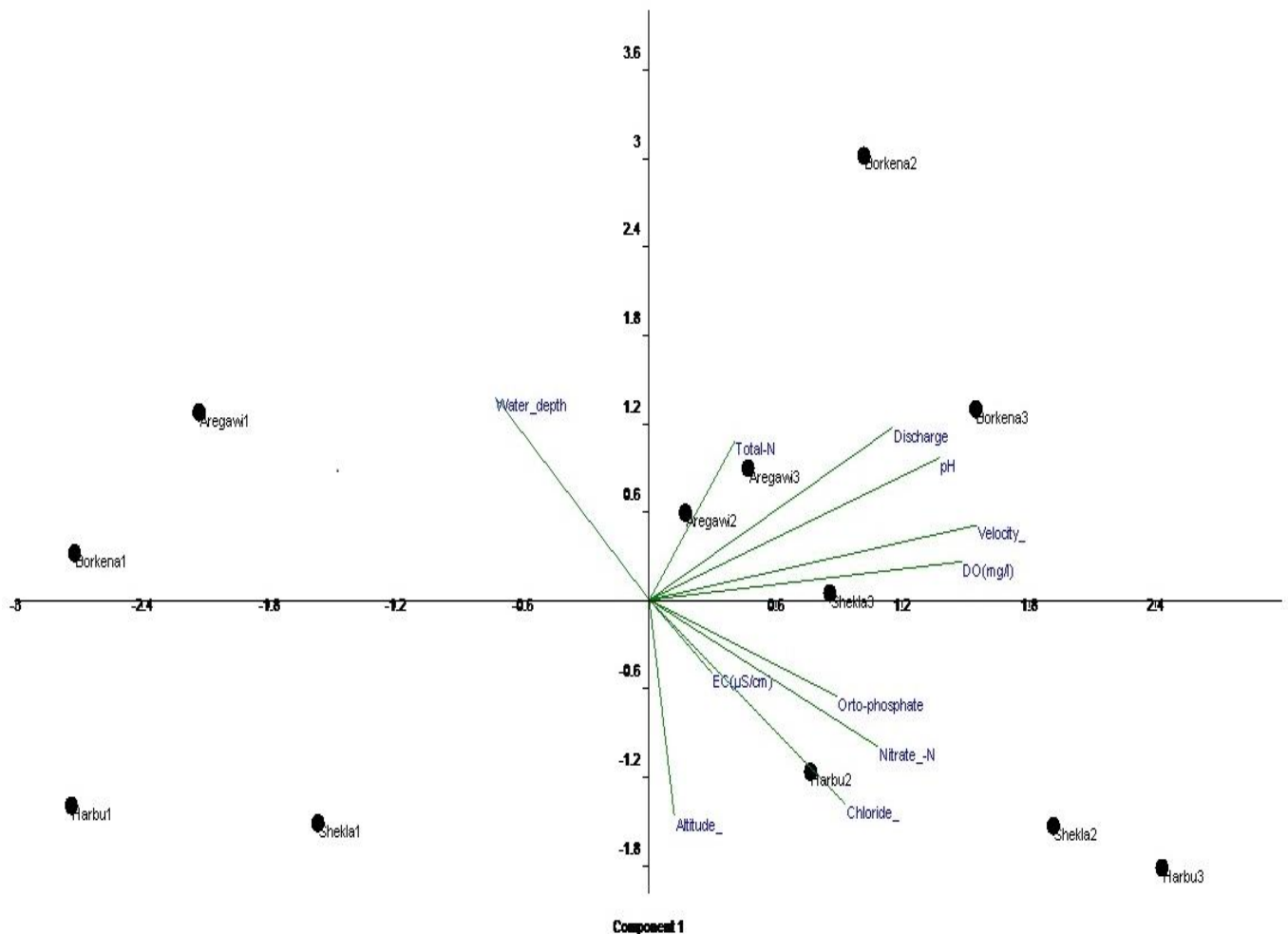


Figure 3: Principal Component Analysis biplot of environmental variables in 12 sampling sites of hot spring in Eastern Amhara Region, Ethiopia, 2013

5.2 Macroinvertebrate community

A total of 1095 macroinvertebrates in which belongs to 10 orders and 31 families of macroinvertebrates were collected from 12 sampling sites of the four. The most abundant orders were Diptera 548(49.90%), Odonata 170(15.53%), Coleopteran 142(12.97%), and Ephemeroptera 104(9.5%) represented by 14 families. These families were accounted more than 88% of the overall macroinvertebrate samples.

Table 6: Percentage of macroinvertebrates order in 12 sampling sites of hot springs in Easter Amhara region, Ethiopia, 2013

| Order | Number | % |
|---------------|--------|-------|
| Ephemeroptera | 104 | 9.50 |
| Diptera | 547 | 49.90 |
| Odonata | 170 | 15.5 |
| Coleopteran | 142 | 12.96 |
| Gastropoda | 75 | 6.85 |
| Hemiptera | 39 | 3.56 |
| Trichoptera | 1 | 0.09 |
| Araneae | 1 | 0.09 |
| Amphipoda | 7 | 0.64 |
| Oligochaeta | 9 | 0.82 |
| Total | 1095 | 100% |

Macroinvertebrates were not found in two sites of H1 and B1. In the rest 10 sites, only chironomidae was found. Most macroinvertebrate taxa were found at five sites, namely B3 (13 families), S2 (13 families), A3 (12 families), B2 (9 families), and A2 (8 families) (Table 7).

Table 7: number of macroinvertebrate family in 12 sampling sites of hot springs in Eastern Amhara Region, Ethiopia, 2013

| Site code | Richness |
|-----------|----------|
| A1 | 2 |
| A2 | 8 |
| A3 | 12 |
| B1 | 0 |
| B2 | 9 |
| B3 | 13 |
| H1 | 0 |
| H2 | 6 |
| H3 | 2 |
| S1 | 1 |
| S2 | 13 |
| S3 | 7 |

5.2.1 Macroinvertebrate indices

Simpson Index

The Shannon diversity index of macroinvertebrate communities was significantly lower at all 10 sites where macroinvertebrate was found with range from 0.075-0.837(Table 8).

Simpson diversity index

The Simpson diversity index of macroinvertebrates communities were also significantly lower at all 10 sites where macroinvertebrate were found ranging from 0.14 to 0.917(Table 8).

Margaleff diversity index

The values of Margaleff Diversity Index of macroinvertebrate as shown in Table 8 was between 10.842 – 26.034. The lowest value for macroinvertebrate was for S2 site and the highest value was for H2 site.

Table 8: The Shannon, Simpson and Margaleff diversity index for macroinvertebrate community in 12 sampling sites of hot springs in Eastern Amhara , Ethiopia 2013. H' = Shannon H' Log base 10, H_{max} = Shannon H_{max} Lof Base 10, J' = Shannon J' , D = Simpson diversity and M = margalef M Base 10

| site name | H' | H_{max} | J' | D | M |
|-----------|-------|-----------|-------|-------|--------|
| A1 | .148 | 0.301 | 0.493 | 0.805 | 15.996 |
| A2 | 0.821 | 0.903 | 0.909 | 0.14 | 19.633 |
| A3 | 0.837 | 1.079 | 0.775 | 0.178 | 15.071 |
| B1 | | | | | |
| B2 | 0.479 | 0.954 | 0.502 | 0.405 | 13.513 |
| B3 | 0.761 | 1.114 | 0.683 | 0.255 | 15.238 |
| H1 | | | | | |
| H2 | 0.726 | 0.778 | 0.933 | 0.141 | 26.034 |
| H3 | 0.075 | 0.301 | 0.25 | 0.917 | 21.011 |
| S1 | 0.217 | 0.301 | 0.722 | 0.6 | 41.49 |
| S2 | 0.805 | 1.079 | 0.745 | 0.214 | 10.842 |
| S3 | 0.406 | 0.845 | 0.48 | 0.598 | 12.845 |

Family level Biotic index

The family level biotic index showed significant variation among the studied sites. Four sites (A1, B3, H3, and S1) were categorized as poor water quality. B2 categorized as excellent water quality as opposed to A2 which was under severe organic pollution likely. The rest four sites fall under fair water quality status as shown Table 4.

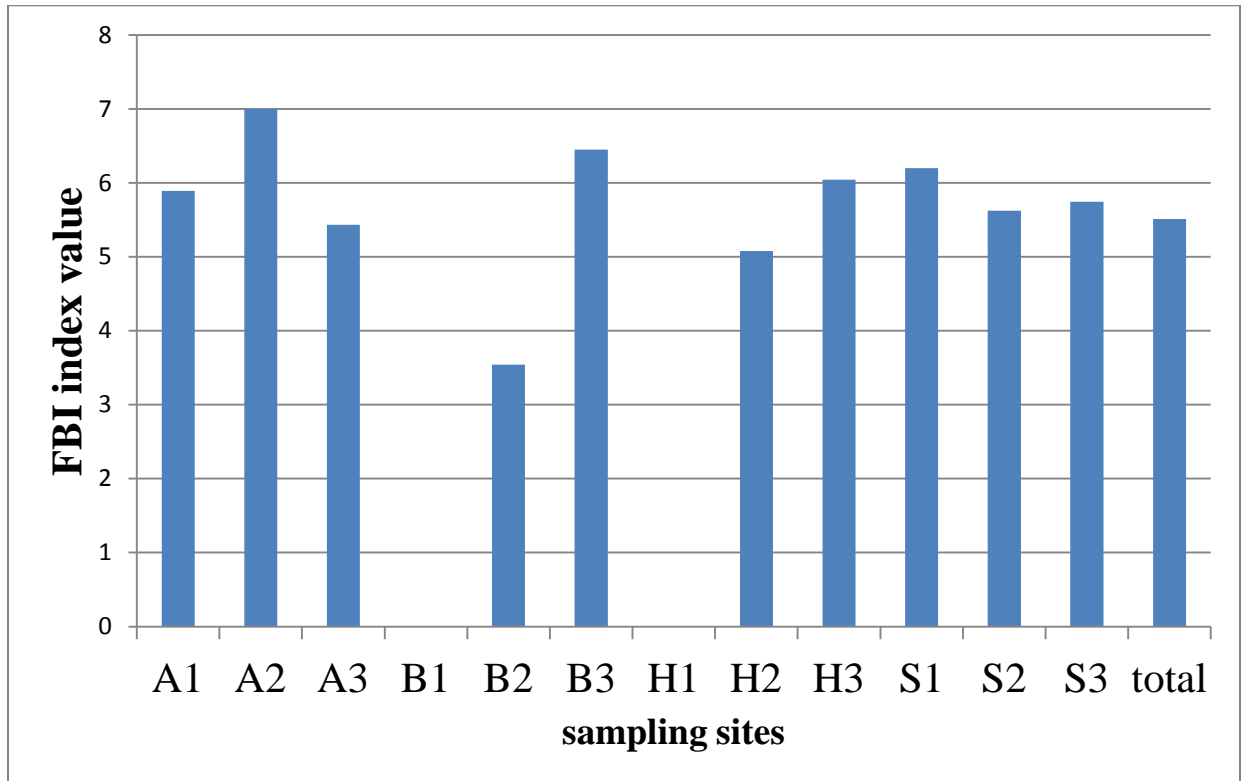


Figure .4 Family level biotic index category of macroinvertebrates in 12 sampling sites of hot springs in Eastern Amhara Region, Ethiopia, 2013

Dominant Taxa

Chironomidae was dominated more than 50% of the studied sites and the rest four sites was dominated by oligochaeta(A2),Hydrobiidae(A3), Gomphidae(B2) and Haliplidae(H2). In B1 and H1, macroinvertebrates were not found (Table 9).

Table 9 dominant taxa of macroinvertebrate in 12 sampling sites of hot spring in Eastern Amhara Region, Ethiopia, 2013.

| Sampling site name | Dominant Taxa | % value |
|--------------------|---------------|---------|
| A1 | Chironomidae | 89.23 |
| A2 | Oligochaeta | 23.33 |
| A3 | Hydrobiidae | 27.38 |
| B1 | | |
| B2 | Gomphidae | 47.86 |
| B3 | Chironomidae | 41.25 |
| H1 | | |
| H2 | Haliplidae | 30.77 |
| H3 | Chironomidae | 95.83 |
| S1 | Chironomidae | 80 |
| S2 | Chironomidae | 35.3 |
| S3 | Chironomidae | 76.8 |

South Africa Scoring System

South African Scoring System (SASS) and Average Score per Taxa (ASPT) value were varied significantly among the twelve sites of hot springs. The SASS and ASPT scored maximum at B3 site and as a minimum at S1 as shown from Table 5.

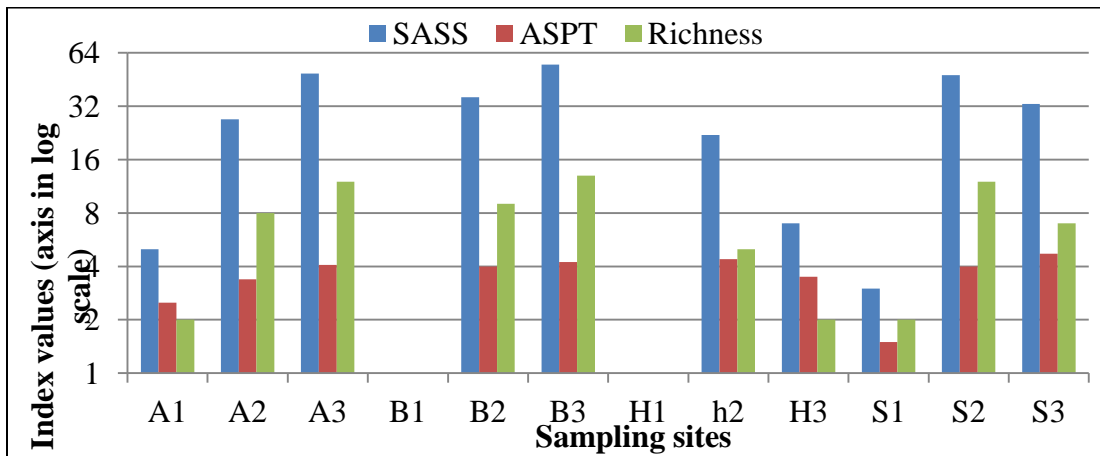


Figure 5: South Africa Scoring System/SASS/, Average Scoring Per Taxa/ASPT/ and Richness of macroinvertebrates in 12 sampling sites of hot springs in Eastern Amhara Region, Ethiopia, 2013.

5.2.2. Multivariate analyses of macroinvertebrate data

Human Disturbance Score and Habitat Quality Index

Human disturbance score in the habitats studied varied considerably among sites. Ten of the twelve sites had total human disturbance scores greater than B2 and B3, which had moderate disturbance class (Table 10).

Habitant quality index of A3, B2 and B3 were classified as sub-optimum and B1, H1 and S1 were categorized as poor conditions and the rest six sites were categorized as marginal condition class and none of the sites was characterized by optimum conditions (Table 10).

Table 10: Habitat and human impact score in 12 sampling sites of hot springs in Eastern Amhara Region, Ethiopia, 2013

| Sampling site | Human impact | | Habitat condition | |
|---------------|--------------|----------|-------------------|-------------|
| | Score | Class | Score | Class |
| A 1 | 100 | Severe | 83 | Marginal |
| A2 | 104 | Severe | 106 | Marginal |
| A3 | 100 | Severe | 124 | Sub-optimum |
| B 1 | 105 | Severe | 34 | Poor |
| B 2 | 74 | Moderate | 142 | Sub-optimum |
| B3 | 67 | Moderate | 146 | Sub-optimum |
| H 1 | 105 | Severe | 59 | Poor |
| H 2 | 87 | Severe | 91 | Marginal |
| H3 | 93 | Severe | 79 | Marginal |
| S1 | 105 | Severe | 50 | Poor |
| S2 | 97 | Severe | 93 | Marginal |
| S3 | 95 | Severe | 92 | Marginal |

Habitat condition score poor < 60, marginal 60-109, sub-optimum 110-159 and optimum 160-200 (Barbour, Gerritsen, Snyder, & Stribling, 1999), and low disturbance < 25, moderate disturbance > 25-75 and severe disturbance > 75-125 (MDEP, 2009).

Cluster Analysis of environmental Variables

The hierarchical cluster analysis from environmental variables (Figure 6) showed that the sampling sites possibly classified in to three main categories. The first categories samples from low electrical conductivity values and the second group with samples characterized by similar turbidity and velocity parameters. The last possible group was established based on average pH and velocity value

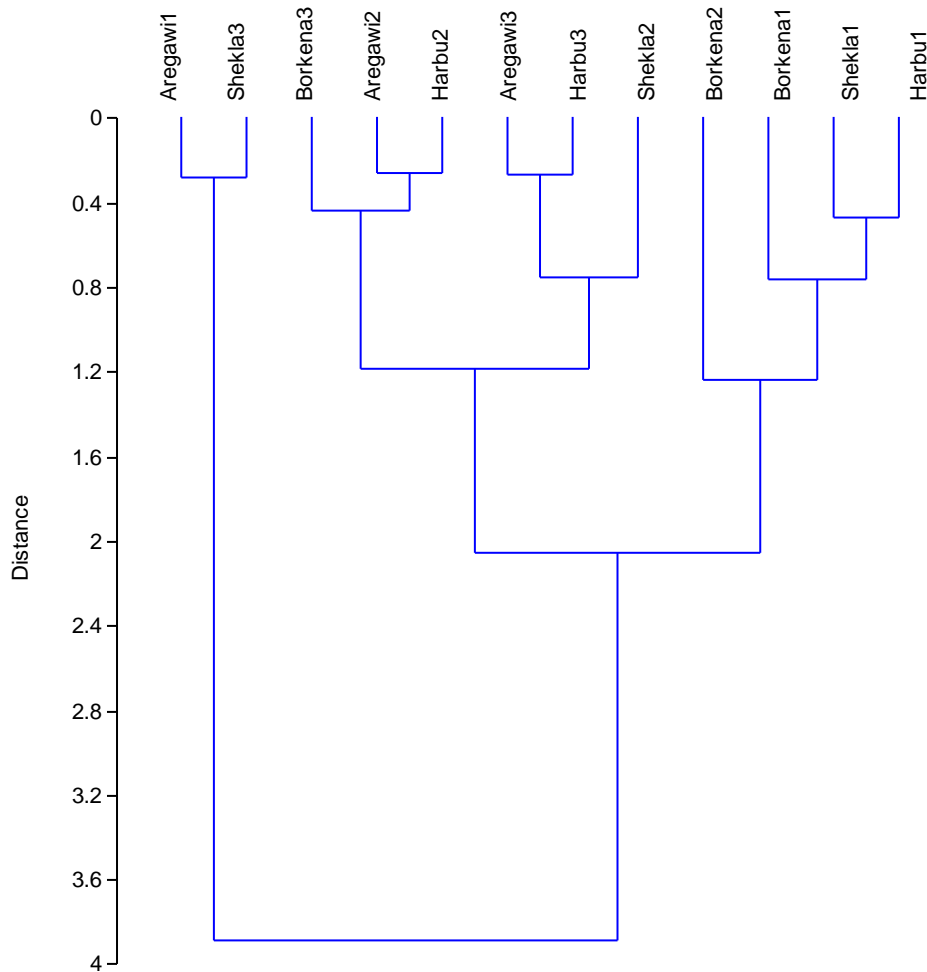


Figure. 6 cluster analysis (single link) based on environmental data in 12 sampling sites of hot springs in Eastern Amhara Region, Ethiopia, 2013

Influence of Water temperature on Biotic indices

Water temperature has strong correlation with family level biotic index and Shannon diversity index but has weak correlation with Shannon diversity of birds (Figure. 7).

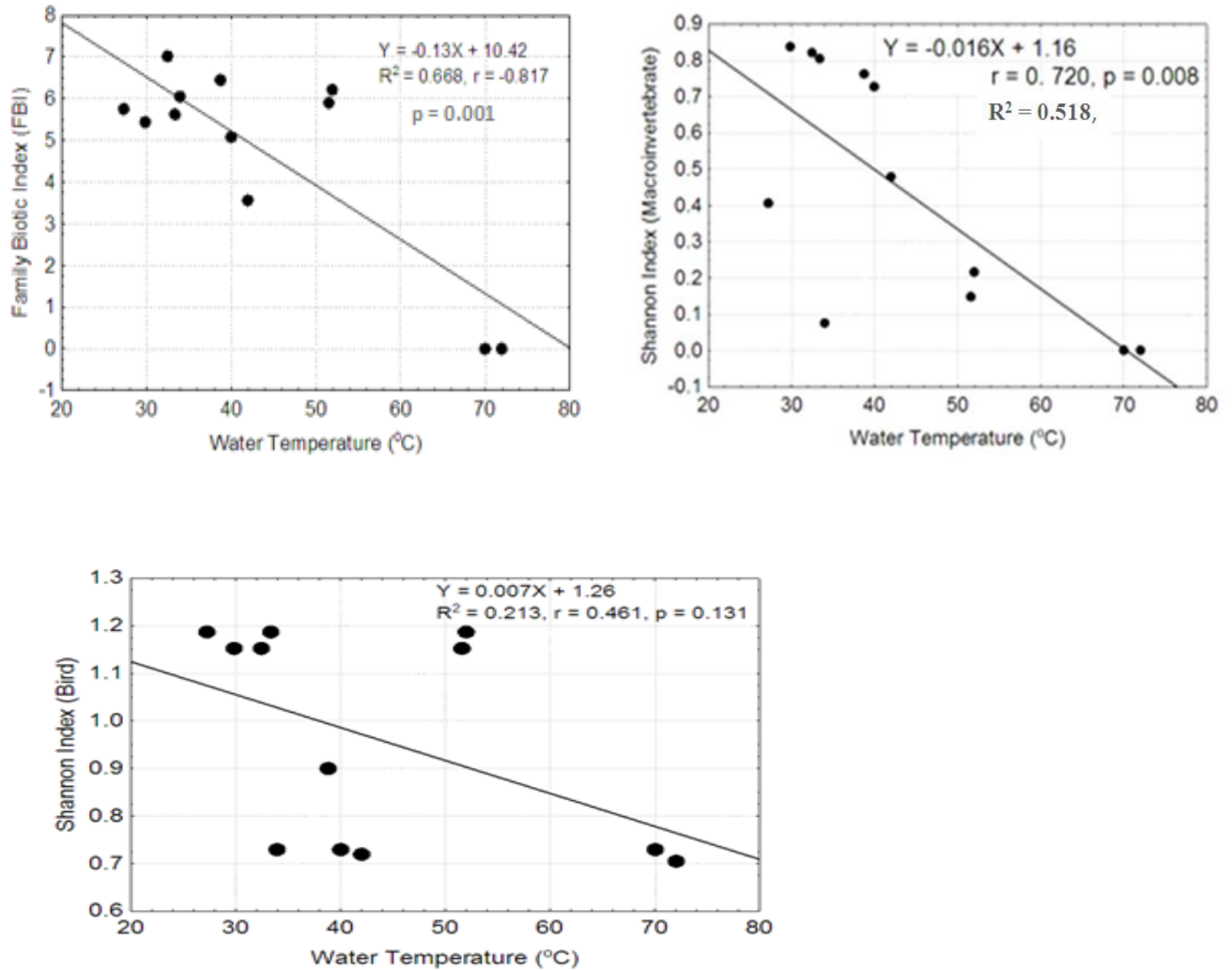


Figure 7: The influence of water temperature on macroinvertebrate and bird biotic indices in 12 Hot spring sites in East Amhara Region, Ethiopia, 2013

5.3 Bird diversity

A total of 2484 birds belonging to 56 species were recorded at the 12 sampling sites. Black headed oriole(*Oriolus Larvatus*), Spur-Winged Lapwing(*Vanellus spinosus*), Spectacled Weaver(*placeous ocularis*) and Yellow Wagtail(*Motacilla flava*) were the most abundant bird species in the study area, accounting 10.5%, 8.75%, 8.4% and 7.8% of all species recorded, respectively. The largest number of species (23) was recorded at the Shekla(S1,S2 and S3) sites and the largest number of birds (441) was observed at the B1 site.

5.3.1 Bird diversity

Shannon diversity Index

The Shannon diversity index of birds were lower at six sites (H1, H2, H3, B1, B2 and B3) showed the index between 0.72 and 0.9. However, the index calculated based on bird communities at six sites (S1, S2, S3, A1, A2 and A3) showed above 1 and the range from 1.153-1.187(Table 11).

Simpson Diversity Index

The Simpson diversity index of bird communities were also significantly lower at all 12 sites where birds studied which was ranging from 0.08 to 0.319 as shown Table 11.

Margaleff Diversity Index

The values of Margaleff Diversity Index of bird were between 20.798-25.015 (Table 11). The lowest value was for B1 and the highest was for H1, H2 and H3(Table 10). This index shows variation depending on number of species, so that the number of individuals is less important for calculation.

Table 11: The Shannon, Simpson and Margaleff diversity index of bird community in 12 sampling sites of hot springs in Eastern Amhara , Ethiopia, 2013. H' = Shannon H' Log base 10, H_{max} = Shannon H_{max} Lof Base 10, J' = Shannon J' , D = Simpson diversity and M = margaleff M Base 10

| site name | H' | H_{max} | J' | D | M |
|-----------|-------|-----------|-------|-------|--------|
| A 1 | 1.153 | 1.322 | 0.872 | 0.088 | 24.832 |
| A2 | 1.153 | 1.322 | 0.872 | 0.088 | 24.832 |
| A3 | 1.153 | 1.322 | 0.872 | 0.088 | 24.832 |
| B 1 | 0.705 | 1.255 | 0.562 | 0.295 | 20.798 |
| B 2 | 0.72 | 0.954 | 0.754 | 0.269 | 24.185 |
| B3 | 0.9 | 1.23 | 0.731 | 0.184 | 23.727 |
| H 1 | 0.729 | 1.146 | 0.636 | 0.319 | 25.015 |
| H 2 | 0.729 | 1.146 | 0.636 | 0.319 | 25.015 |
| H3 | 0.729 | 1.146 | 0.636 | 0.319 | 25.015 |
| S1 | 1.187 | 1.362 | 0.872 | 0.08 | 23.344 |
| S2 | 1.187 | 1.362 | 0.872 | 0.08 | 23.344 |
| S3 | 1.187 | 1.362 | 0.872 | 0.08 | 23.344 |

5.3.2 Multivariate analyses of bird data

The hierarchical cluster analysis from bird (Figure 8) indicated that the sampling sites could be grouped in to three major classes. The first category included samples from stream sites without any wetlands. The second group incorporated samples from both wetland and streams and the third class consists sample from typically wetlands.

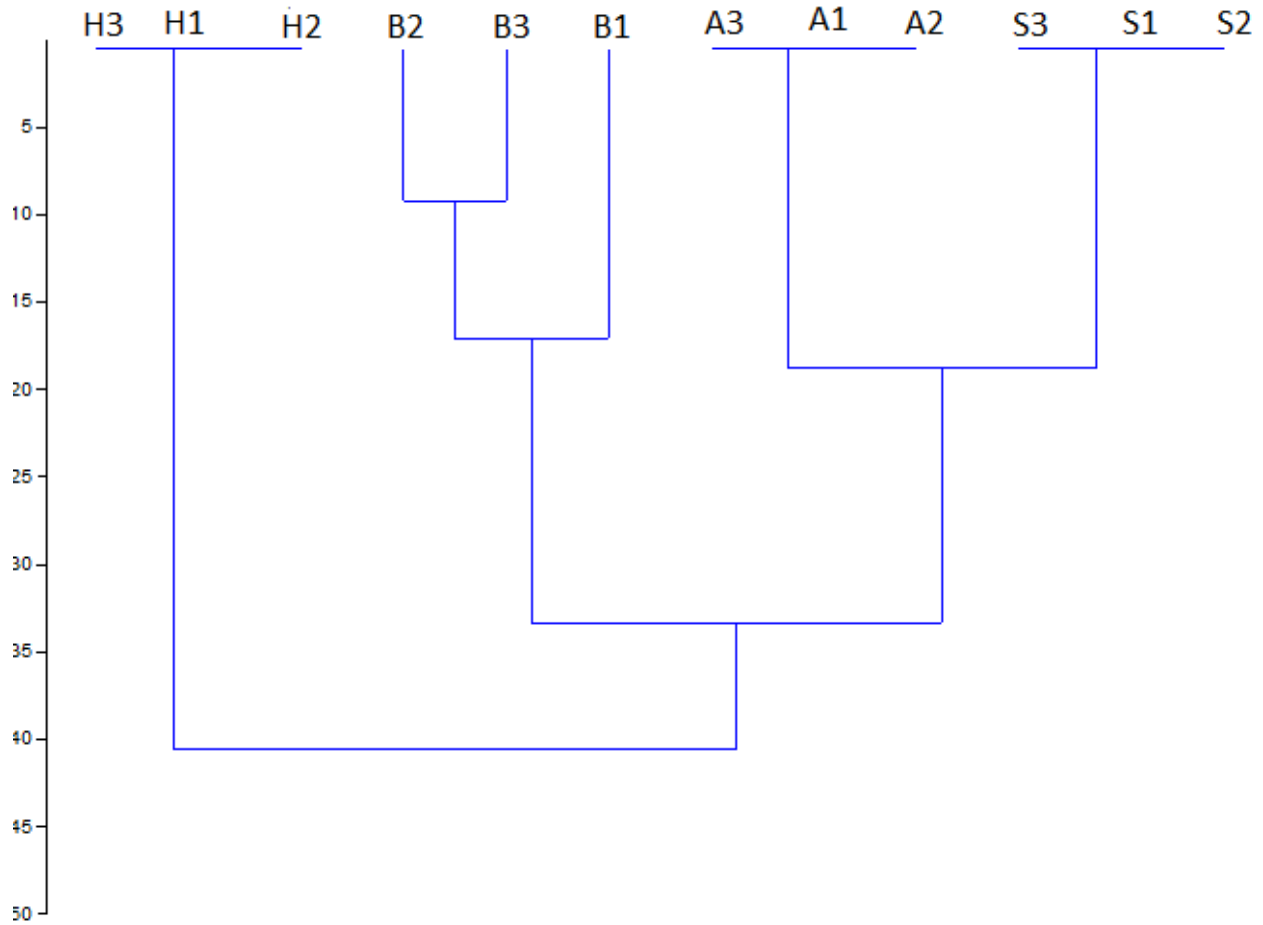


Figure. 8 cluster analysis (single link) based on bird data in 12 sampling sites of hot springs in Eastern Amhara Region, Ethiopia, 2013

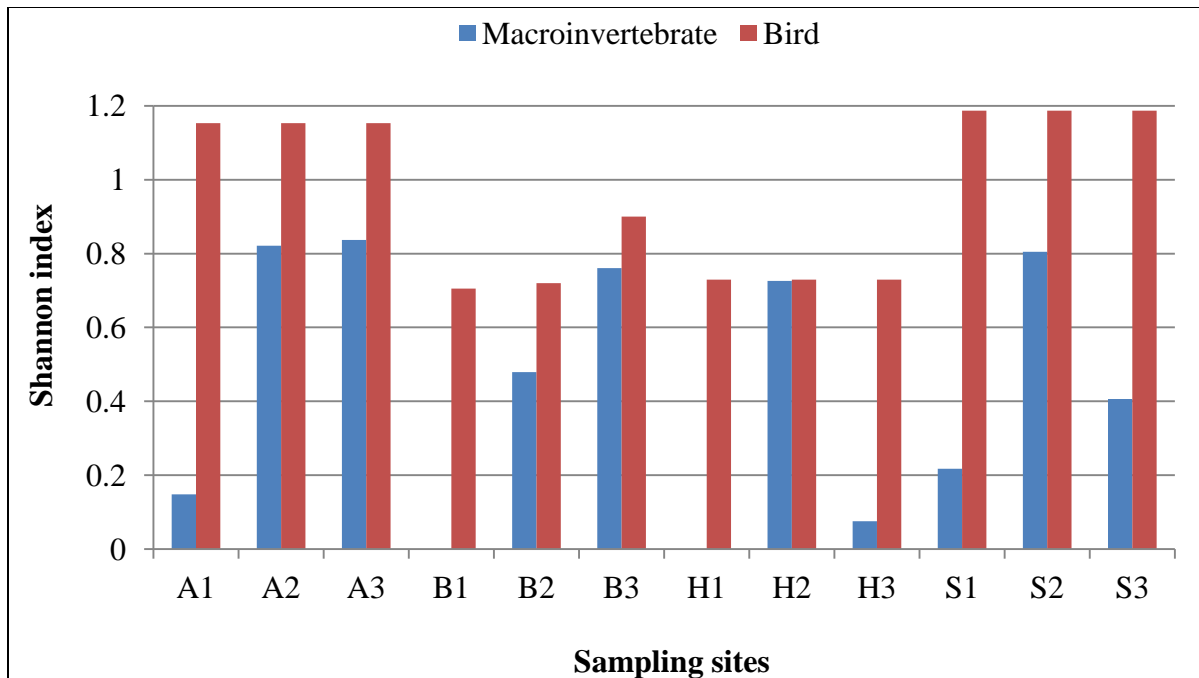


Figure 9: Average Shannon diversity index calculated from macroinvertebrates and birds in 12 sampling sites of hot springs in Eastern Amhara region, Ethiopia, 2013.

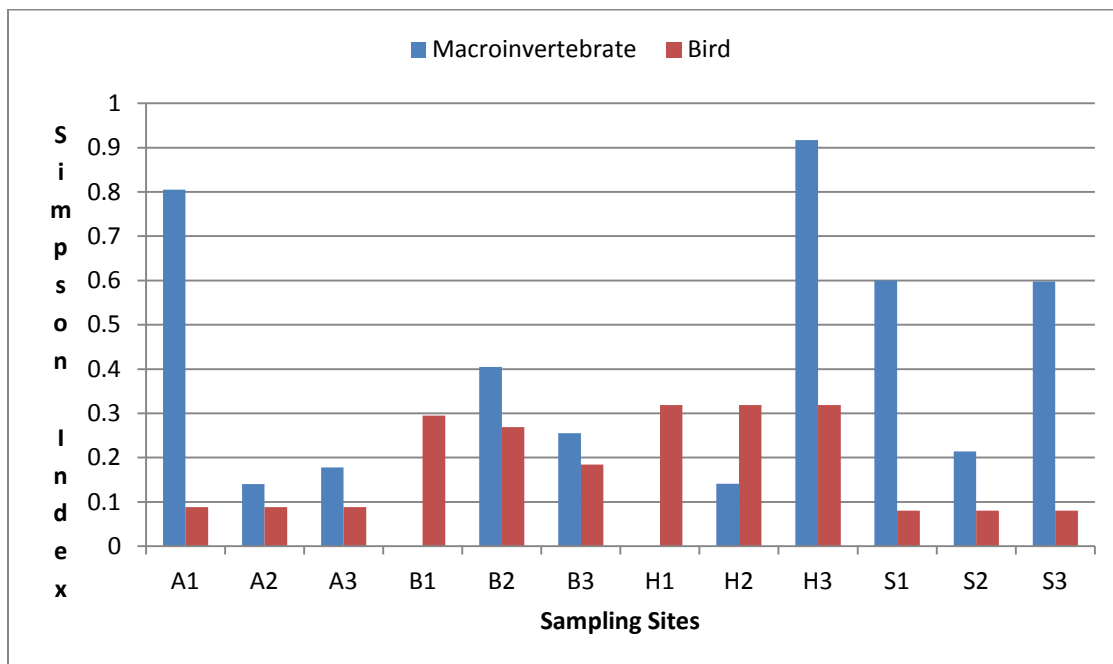


Figure 10: Average Simpson diversity index (D) calculated from macroinvertebrates and birds in 12 sampling sites of hot springs in East Amhara region, Ethiopia, 2013.

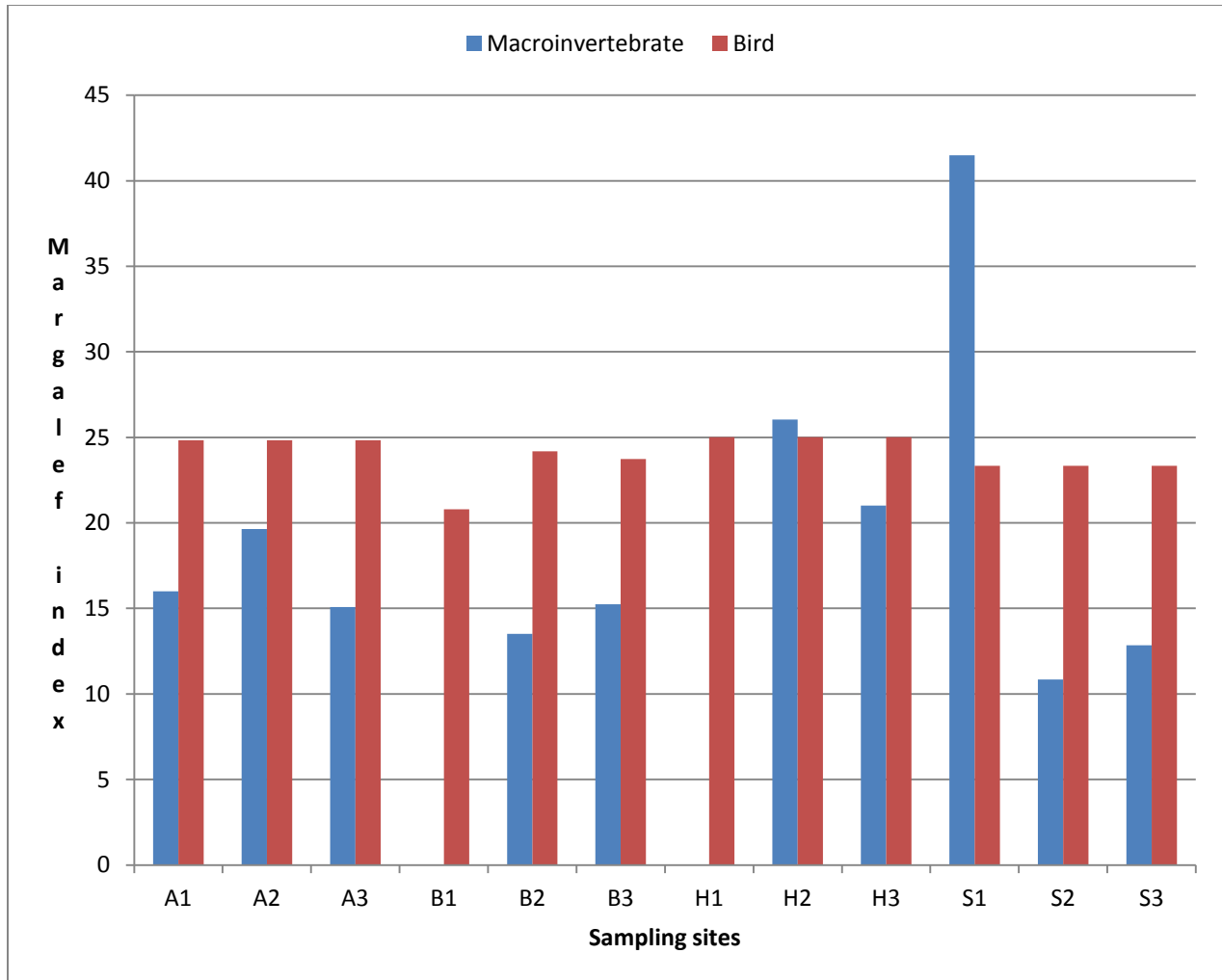


Figure 11: Average Margaleff calculated from macroinvertebrates and birds in 12 sampling sites of hot springs in East Amhara region, Ethiopia, 2013.

CHAPTER SIX: DISCUSSION

The diversity of birds and macroinvertebrate, habitat condition and water quality were heavily affected by anthropogenic activities, which carried out on the water body as well as in the surrounding area of hot springs in Eastern Amhara Region. The high turbidity and chloride concentration, low dissolved oxygen values (Table 5) might be mainly due to organic pollution from animal excrements and sewage discharges from towns, villages, and hot springs temporary residence tents, which practiced open defecation around the springs. Other study conducted in the Borkena valley also found that the main cause of water quality deterioration and biodiversity decline in wetlands were activities associated with agriculture, overgrazing and deforestation (Getachew, et al., 2012). The other study in Kenya also revealed that there were clear effects of catchment on some physicochemical measures like conductivity, turbidity were significantly higher at sites with high compared to low disturbance and agriculture use around the stream (Minaya, McClain, Moog, Omengo, & Singer, 2013).

Temperature is known to influence the physicochemical process of species, possibly leading to changes in the timing of life history events and trophic interactions (Burgmer, Hillebrand, & Pfenninger, 2009). This may alter diversity and community composition. Thus, dissimilarity of the communities' diversity between the twelve sites of hot springs may be related to temperature difference. Virtually all facets of life history and distribution of aquatic insects are influenced by temperature. Aquatic insects occur at temperatures ranging from zero to about 50 °C. Due to this fact, macroinvertebrate was not found during the survey from B1 and H1 sites (Table 7) where the temperature was 72 °C and 70 °C respectively (Table 5). However, in this study, the macroinvertebrate found at temperature of 52°C that were chironomidae and Hydrobiidae at S1 and 51.8°C at H1 site, which was chironomidae, and following the temperature gradient, the macroinvertebrate diversity improved. Metabolism, growth, emergence, and reproduction are directly related to temperature, whereas food availability, both quantity and quality, may be indirectly related (Wallace & Anderson, 2002). The study conducted in Iceland revealed that five species of Diptera, one Coleopteran, and two species of semi-aquatic mites were collected from the algae and detritus in the outflow channel of hot springs. Chironomidae larvae were dominant below 41 °C. Species such as *Scatella nitidifrons* pupae and unidentified *Stratiomyinae* larvae

were found at 47 °C. (Stark, Fordyce, & Witerbourn, 1976). This is in agreement with the scenario of Eastern Amhara hot springs that have developed considerable thermal acclimation.

All the hot spring sites had high electrical conductivity value (Table 5) this may be due to the texture of mineral soil and the degree of humification of organic soil. Similar finding was obtained in the same study area in Borkena valley for non hot spring sites (Getachew, et al., 2012) as well in Kenya showed that electrical Conductivity was identified as an indicator of anthropogenic activities (Minaya, McClain, Moog, Omengo, & Singer, 2013)..

In all four upstream catchment areas the water flow was very low (Table 5) compared to its cascaded down stream sites. As we witnessed during the survey all this upstream, sampling sites using for community as the holy water sites, as sources of drinking water and other domestic purposes. Even they constructed local reservoir, installed piping system and conduit at the source of the springs to distribute to their pilgrims in A1 and H1 sites. Other study conducted in South Africa revealed that Extent of different land-use and magnitude of impact of each land-use in reducing water quantity and quality (Kotze, Ellery, Macfarlane, & Jewitt, 2012).

Water temperature, Dissolved oxygen (DO), total Nitrogen, and turbidity varied among sampling sites. Among the physicochemical variables, pH remained within acceptable ranges of surface water standards in each site but not the other variables (Table 5). The Shannon diversity index (Figure 9) revealed that the macroinvertebrate and bird communities had higher diversities at A2, A3, B3, and S2 than the remaining eight sampling sites. Nevertheless, their diversity of Shannon index was lower than the previous study conducted in non hot spring areas (Getachew, et al., 2012), in the wetlands of Cheffa in the North-East Ethiopian.

Most values measured using the Shannon diversity index (Turkmen & Kazanci, 2010) range from 1.5 to 3.5, rarely exceeding 4.5. Values above 3.0 indicate that habitat structure is stable and balanced and values under 1.0 indicate the presence of pollution and degradation of habitat structure. Based on these criteria, none of the sites of hot springs in Eastern Amhara region exceeded the 1.5 level of the Shannon diversity index, either for birds (Table 11) or for macroinvertebrates (Table 8). Especially macroinvertebrate Shannon diversity index in all sites was below 1 and for bird community was below 1 at 50% of the studied sites. Similarly, the Shannon diversity index for similar study conducted in Cheffa Wetland for normal streams was

below one, further indicating the presence of elevated levels of pollution and degradation of habitat structure in the studied area (Getachew, et al., 2012).

According to (Smith & Wilson, 1996), values measuring using Simpson diversity index range between zero and one. Zero represents minimum evenness and one for the maximum. Based on this fact, all the sites fallen nearly zero and indicated the presence of severe pollution in all sites of the hot springs(Figure 10).

The family biotic index showed a strong organic pollution level in all sites of the hot springs. Although this biotic index was originally formulated to provide a single ‘tolerance value’ which is the average of the tolerance values of all species within the benthic arthropod community (Hilsenhoff, 1988), these results showed that the index responded well to loading of organic pollutants. In unpolluted streams, the FBI was higher than the BI, suggesting lower water quality was, and in polluted streams, it was lower, suggesting higher water quality. These results occurred because the more intolerant genera and species in each family predominate in clean streams, whereas the more tolerant genera and species predominate in polluted streams (Mandaville, 2002). On the basis of these criteria, all sites macroinvertebrate family scored high family biotic index value (Figure 5) and all the sites were severely deteriorated by anthropogenic activities.

SASS is a biomonitoring system adapting from South Africa River to give an indication of water quality. This done by looking at the macroinvertebrates present in the system adding a value derived from the species tolerance to pollution, with the most sensitive species having high score while the most tolerant providing low score (Dickens & Graham, 2002). Based on this criteria, most of macroinvertebrate species were most tolerance and having low scoring value and it indicated that all hot spring sites water quality were severely deteriorated by anthropogenic activities since all sites were categorized under category D bellow <79 scoring value as shown from Figurer 5.

Multivariate analysis of Most environmental variables with biotic indices were not significant except water temperature. A linear relationship was found between water temperature and macroinvertebrate based biotic indices. The rest environmental variables did not show strong association with biological indices of either for macroinvertebrate or bird community. In

addition, macroinvertebrate and bird diversity was not showing strong correlation each other too. The Shannon diversity index and family level biotic index calculated based on macroinvertebrate communities show significant negative correlation with p-value <0.05 by water temperature but bird diversity did not show strong association with water temperature (Figure. 7).

The habitat classes of Eastern Amhara region hot springs could be generalized into three (marginal, sub-optimal, and optimal) as shown from Table 9. Although hot springs support a diverse and abundant invertebrate community consisting of aquatic, semi-aquatic species as depending on the human disturbance and habitants score level. The most abundant orders were Diptera, Odonata, Coleopteran, and Ephemeroptera represented by 14 families (Table 6). These families were accounted more than 88% of the overall macroinvertebrate samples, all of them belonging to families called generalists. This group uses a variety of food resources, including detritus, plants, epiphytic algae and other organisms (Barbour, Gerritsen, Snyder, & Stribling, 1999) and is able to resist disturbance when food resources change. In addition, Invertebrate assemblages were relatively poor taxon and had low densities in those locations with high fine sediment, detritus, and mud content. Similar scenario were notified in Spain when macroinvertebrate Assemblages showed significantly nested patterns, with those in sediment rich locations consisting of a subset of those in locations with little fine sediment (Buendia, Gibbins, Vericat, Batalla, & Douglas, 2013). Moreover, the diversity of wetland birds was lower than in most other studies conducted previously in current study area where conducted (Getachew, et al., 2012) and other studies in Lake Tana (Aynalem & Bekele, 2008). This might be linked to habitat destruction resulting from human activities. As we notified during data collection farmers were firing, cutting, and plowing the wet land parts to convert to farmland and currently cultivation were practiced in majority of Aregawi wetland in Shewa Robit in Kewet district of North Shewa site. The study conducted by (Mekonnen & Aticho, 2011) in Jimma, Ethiopia, indicated shortage of agricultural land and decrease of agricultural land productivity, forced the surrounding communities to drain the wetland for crop cultivation, in order to meet the increasing food demand of household. Generally, wetlands around the hot springs, which used for the production of birds, were severely deteriorated by human activities like over grazing, intensive farming, and open burning to convert to farmlands as notified during the survey.

Communities with a high abundance of generalists, including the studied hot springs in Eastern Amhara, were representative of a disturbed environment. Most of the invertebrate taxa at all sampling sites, including Baetidae, the pollution tolerant family in the order Ephemeroptera (9.5% of the total abundance), were pollution-tolerant. More-over, the large populations belonging to the families in the order Diptera (49.9%), Odonata (15.53%), and Coleopteran (12.97%)(Table 6), do not depend entirely on water quality to survive as previously mentioned by (Getachew, et al., 2012).

These results indicated that the water quality at all 12 sites has been degraded to varying degree because of human activities(Table 10). The observed low diversity of birds(Table 10) in the Wetlands feeding by hot springs were in agreement with other studies in the same wetland (Getachew, et al., 2012) and the other study in Ethiopia (Aynalem & Bekele, 2008). Those studies revealed that in natural habitats where human interference is relatively small, the diversity and abundance of species is greater than in fragmented habitats and where intensive farming is carried out. The changes in this bird assemblage reflect the hydrological modifications induced by agriculture at the watershed scale, which have significant effects on the relative representation of wetland habitats (Robledano, Esteve, Farinos, Carreno, & Martinez-Fernandez, 2010). Papyrus vegetation, indispensable for many wetland bird species, was degraded because of its heavily use by the local community, for contribution involves like as local hunt building material, selling to nearby community as daily base needs, serving as grazing). The loss of this vegetation also reduces the wetland's anti-pollution services because of the effectiveness of this species in reducing nitrogen and phosphorus levels in water (Abe, Ozaki, & Mizuta, 1999) considered vegetation-based indicators to be a promising tool for wetland nutrient conditions in areas where landscape disturbance is slight to moderate.

According to (Chiputwa, Morardet, & Mano, 2005) Water from the wetland was harnessed for a variety of purposes within the households, which include drinking, washing, bathing, irrigation, and building among others. Increasing drainage and cultivation of hot springs catchment and related Wetlands when the water level recedes after the rains, has greatly affected the wetland ecosystem. The study conducted in Mediterranean showed that human activities in wetlands were threaten the existence of many birds by destroying their habitat or directly affecting their survival and on their reproductive success and the most important family Alaudidae (and

particularly species like *Melanocorypha calandra*) has lost due to degradation of wetlands, which were ideal habitats for roosting and thermoregulation (Robledano, Esteve, Farinos, Carreno, & Martinez-Fernandez, 2010). This situation prevailing also in the studied hot springs' wetland.

CHAPTER SEVEN: CONCLUSION AND RECOMMENDATION

The study of hot springs in Eastern Amhara region provides a preliminary assessment of what happens to be predominantly on temperature gradient, anthropogenic impacts, physicochemical parameters and other environmental variables on macroinvertebrate and bird communities. The generally low bird and macroinvertebrate diversity indicates an overall high water temperature, water quality degradation and vegetation disturbance effect throughout the hot springs, although variable correlations between water temperature and species diversity suggest temperature gradient affects the overall sites. Longitudinal studies covering both wet and dry seasons are required to examine the hydrological influence on macroinvertebrates and birds communities by considering the origin, movement, soil profile, and minerals in surface and groundwater, as well as soil degradation and vegetation diversity, to better assess the relative contribution of anthropogenic and natural impacts. These studies can also validate and update the local macroinvertebrate and bird index of hot springs initiated by the investigators. This broadly based biophysical information, together with detailed land use studies of the agricultural, pastoralist and urban communities may form the basis for a hot springs ecotourism framework that can inform managers and other decision makers at the local and state levels on taking integrated planning and preventive measures for further protection and sustainable use of the beauty of nature.

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ANNEX

Annex 1. Macro-invertebrates collection protocol

1. River assessment form

1. DD/MM/YYYY-----
2. Site code-----Name of stream-----
3. Altitude(m)-----coordinates-----
4. Previous day rainfall history-----
Physico-Chemical parameters
5. Ambient temperature(⁰C)-----water temperature(⁰C)-----
6. DO(mg/l)-----%-----EC(μ S/cm)-----pH-----
7. Velocity(m/s)-----water depth(m)-----discharge(m³/s)-----
8. Turbidity(NTU)-----color-----smell-----
Habitat assessment
9. River bank width(m)-----Bank height(m)-----
10. River bed(%)
 - a. Bed rock-----
 - b. Boulder-----
 - c. Coble-----
 - d. Pebble-----
 - e. Gravel-----
 - f. sand-----
 - g. silt-----
 - h. detritus NLSME
 - i. sticks NLSME
 - j. branches NLSME
 - k. loges NLSME
11. Riparian vegetation
 - a. Trees>10m-----
 - b. Trees<10m-----
 - c. Shrubs-----
 - d. grass-----
 - e. bare land-----
12. Width riparian vegetation Right----- Left-----
13. Canopy cover-----
14. Protection riparian vegetation Right----- Left-----
15. %pool-----
16. % riffle -----
17. Sinuosity -----

- 18. Slope-----
- 19. List the available anthropogenic disturbance-----

- 20. Upstream land use-----
- 21. Adjacent land use Right----- Left-----
- 22. Farming distance from the river bank-----
- 23. Take picture(picture number)-----

2. Wetland assessment

General information

- 1. DD/MM/YYYY-----Time-----
- 2. Name of wetland-----Sampling station-----
- 3. Altitude(M)-----coordinates-----
- 4. Weather condition-----
- 5. Previous day rain history-----
- 6. Photo number-----
- 7. Size of site under assessment(ha)-----
- 8. Size of total wetland(ha)-----

Notes and or sketch of the site

Physico-Chemical parameters (Field)

- 9. Ambient Temperatures(⁰c) -----pH-----
- 10. Water temperature(⁰c)-----DO(mg/l)-----EC(μ S/cm)-----
- 11. Turbidity (NTU)-----Transparency(cm)-----
- 12. Chlorophyll a(ABS)----- $(0.1309*ABS+11.274)$ -----(μ g/l)
- 13. Color-----odor-----

Physico-Chemical parameters (laboratory)

- 14. COD-----NO₂-----
- 15. Chloride-----NH₄-----
- 16. TSS-----TN-----
- 17. BOD₅-----TP-----
- 18. NO₃-----PO₄³⁻-----

Hydrological assessment

- 19. Wetland geographic setting -----
 - a. Reverine-----
 - b. Depressional -----
 - c. Meandering flood plain-----
 - d. Other-----
- 20. Site setting/degree of isolation from other wetland
 - a. The site is connected upstream and downstream with other wetland
 - b. The site is only connected upstream with other wetlands
 - c. The site is only connected downstream with other wetlands
 - d. Other wetlands are nearby (within 0.25 mile) but not connected
 - e. The wetland site is isolated
- 21. Free water depth(cm)
 - a. Minimum ----- b. maximum----- Average-----
- 22. Sludge depth
 - a. Minimum-----b. maximum-----Average-----
- 23. Soil type
 - a. Organic-----
 - b. Mineral-----
 - c. Both organic and mineral-----
- 24. Apparent hydroperiod
 - a. Permanently flooded
 - b. Seasonally flooded
 - c. Saturated(surface water seldom present)

- d. Artificially flooded
- e. Artificially drained

25. Hydrological modified

- a. Ditch inlet and outlet----- d. culverts-----
- b. Drainage----- e. filling or bulldozing-----
- c. Storm water input----- f. others specify-----

Land use

26. Adjacent land use pattern

- a. Agriculture tilled----- e. road-----
- b. Pasture----- f. commercial-----
- c. Native vegetation----- g. industrial-----
- d. Residential area----- h. recreational-----

Habitant assessment

27. Hydrophytic vegetation coverage (%)

- a. Woody plants----- e. floating macrophytes-----
- b. Water grass----- f. periphyton-----
- c. Emerged macrophytes----- g. filamentous algae-----
- d. Submerged macrophytes----- h. other specify-----

28. Wetland fauna

- a. Birds(ducks)----- c. invertebrates-----
- b. Fish----- d. others-----

29. Anthropogenic activities

wetland

upland

- | | | |
|----------------------|-------|-------|
| a. Cultivation | ----- | ----- |
| b. Tree removal | ----- | ----- |
| c. Shrub removal | ----- | ----- |
| d. Tree plantation | ----- | ----- |
| e. Grazing | ----- | ----- |
| f. Grass cutting | ----- | ----- |
| g. Brick manufacture | ----- | ----- |

- h. Car washing -----
- i. Clay mining/pottery -----
- j. Waste dumping -----
- k. Fishing -----
- l. Swimming -----

30. Other potential threats

- a. Agricultural biocides-----
- b. Point source pollution-----

31. Wetland ecological state

- a. Unmodified, natural-----
- b. Largely natural with few modification-----
- c. Moderately modified-----
- d. Largely modified-----
- e. Seriously modified-----
- f. Critical/extremely modified-----

32. Any additional comments

Annex II: Laboratory procedures

1. CHLORIDE (APHA, 1995)

Argentometric Method

1. Measure the appropriate sample volume for the indicated chloride range using the Following Table and transfer to a 250 ml Erlenmeyer flask or porcelain casserole.

| Sample volume mL. | Alkalinity range mg/L as CaCO ₃ |
|-------------------|--|
| 100 | 1-50 |
| 50 | 51-100 |
| 25 | 101-200 |
| 10 | 201-500 |

2. Bring the total volume to 100 mL with distilled water if the sample size is less than 100 mL
3. Prepare a color comparison blank by placing distilled water in a similar flask and the
Volume must be equal to that of the sample
4. Add 1 mL potassium dichromate indicator solution to the blank and the sample; and Mix
5. To the color comparison blank carefully add from a burette drop by drop silver nitrate titrant until the yellow color changes to a brownish tinge.
6. Record the mL silver nitrate titrant consumed.
7. If the sample turns yellow, gradually add silver nitrate titrate from a burette. Shake the Flask continuously and continue adding the titrant until the sample turns the same Orange- red color as in the color comparison blank.
8. Record mL silver nitrate titrant consumed.

9. Calculation:

$$\text{mg Cl/L} = \frac{(A-B) \times N \times 35,450}{\text{Ml of sample}}$$

Where

A= mL titration for sample

B= mL titration for blank, and

N= normality of silver nitrate

$$\text{Mg NaCl/L} = (\text{mg Cl/L}) \times 1.65$$

Note:

1. Directly titrate sample in the P H range 7 to 10. Adjust sample P H to 7 to 10 with H₂SO₄ or NaOH if not in this range.
2. For highly colored samples clarification with aluminum hydroxide suspension is necessary
3. If sulfide, sulfite thiosulphate is present, add 1 ml hydrogen peroxide and stir for 1 minute.
2. **PHOSPHATE** (APHA, 1995)

Stannous Chloride Method

Determination of Orthophosphate

1. Prepare the following series of phosphate standards by measuring the indicated volume of standard phosphate solution into separate 100 mL volumetric flasks (Or graduated cylinders).

| Standard | Phosphate Solution. mL | Phosphate (PO ₄ ³⁻) μg/100 mL |
|----------|------------------------|--|
| 0 | | 0 |
| 1 | | 5 |
| 2 | | 10 |
| 3 | | 15 |
| 4 | | 20 |
| 5 | | 25 |
| 6 | | 30 |

2. To the sample, add 0.05 ml (1 drop) of phenolphthalein indicator solution. If the sample turns pink, add strong acid solution drop wise until the color is discharged
3. With a measuring pipette, add 4 mL acid- molybdate solution to each of the standards and sample
4. Mix thoroughly by inverting each flask four to six times.
5. With medicine dropper, add 0.5 mL (10 drops) of stannous chloride solution to each of the standards and sample.
6. Stopper and mix by inverting each flask four to six times
7. After 10 minutes, but before 12 minutes, measure the color photo metrically at 690 nm using distilled water as blank.
8. Construct a calibration curve using the standards and determine the amount of phosphate in μg present in the sample.
9. Calculation

Calculation

$$\text{a) mg/L PO}_4^{3-} = \frac{\mu\text{g phosphate}}{\text{Ml of sample}}$$

Ml of sample

$$\text{b) mg/L P} = \frac{\mu\text{g PO}_4^{3-} \times 0.32614}{\text{Ml of sample}}$$

Ml of sample

$$\text{C) mg/L P}_2\text{O}_5 = \frac{\mu\text{g PO}_4^{3-} \times 1.4946}{\text{Ml of sample}}$$

Ml of sample

3. Ultraviolet Spectrophotometric Screening Method for Nitrate Determination

General Discussion

Principle

Use this technique only for screening samples that have low organic matter contents, i.e., uncontaminated natural waters and potable water supplies. The NO_3^- calibration curve follows Beer's law up to 11 mg N/L.

Measurement of UV absorption at 220 nm enables rapid determination of NO_3^- . Because dissolved organic matter also may absorb at 220 nm and NO_3^- does not absorb at 275 nm, a second measurement made at 275 nm may be used to correct the NO_3^- value. The extent of the empirical correction is related to the nature and concentration of organic matter and may vary from one water to another. Consequently, this method is not recommended if a significant correction for organic matter absorbance is required, although it may be useful in monitoring NO_3^- levels within a water body with a constant type of organic matter. Correction factors for organic matter absorbance can be established by the method of additions in combination with analysis of the original NO_3^- content by another method. Sample filtration is intended to remove possible interference from suspended particles. Acidification with 1 N HCl is designed to prevent

interference from hydroxide or carbonate concentrations up to 1000 mg CaCO₃/L. Chloride has no effect on the determination.

Interference

Dissolved organic matter, surfactants, NO₂⁻ and Cr⁶⁺ interfere. Various inorganic ions not normally found in natural water, such as chlorite and chlorate may interfere. Inorganic substances can be compensated for by independent analysis of their concentration and preparation of individual correction curves.

Apparatus

Spectrophotometer with cuvette that transmits UV light which was use quartz.

Reagents

Nitrate-free water: Use redistilled or distilled deionized water of highest purity to prepare all solutions and dilutions.

Stock nitrate solution: Dry potassium nitrate (KNO₃) in an oven at 105 °C for 24 h. Dissolve 0.7218 g in water and dilute to 1000 mL; 1.00 mL = 100 µg NO₃⁻-N.

Intermediate nitrate solution: Dilute 100 mL stock nitrate solution to 1000 mL with water, 1.00 mL = 10.0 µg NO₃⁻-N.

Hydrochloric acid solution, HCl, 1 N.

Procedure

Treatment of sample

To 50 mL clear sample, filtered if necessary, add 1 mL HCl solution and mix thoroughly.

Preparation of standard curve

Prepare NO₃⁻ calibration standards in the range of 0 to 7 mg NO₃⁻-N/L by diluting to 50 mL the following volumes of intermediate nitrate solution: 0, 1.00, 2.00, 4.00, 7.00... 35.0 mL. Treat NO₃⁻ standards in same manner as samples.

Spectrophotometric measurement

Read absorbance using distilled deionized water as the reference. Use a wavelength of 220 nm to obtain NO_3^- reading and a wavelength of 275 nm to determine interference due to dissolved organic matter.

Calculation

For samples and standards, subtract two times the absorbance reading at 275 nm from the reading at 220 nm to obtain absorbance due to NO_3^- . Construct a standard curve by plotting absorbance due to NO_3^- against NO_3^- -N concentration of standard. Using corrected sample absorbance, obtain sample concentrations directly from standard curve. Note: If correction value is more than 10% of the reading at 220 nm, do not use this method (APHA, 1995).

Table. Macroinvertebrate Taxa in 12 sampling sites of Hot springs in Eastern Amhara Region, 2013

| site code | A1 | A2 | A3 | B1 | B2 | B3 | H1 | H2 | H3 | S1 | S2 | S3 |
|----------------|----|----|----|----|----|----|----|----|----|----|-----|-----|
| Baetidae | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 2 | 0 | 0 | 42 | 10 |
| Belostomatidae | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 5 |
| Caenidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 38 | 7 |
| Chironomidae | 58 | 4 | 7 | 0 | 59 | 33 | 0 | 3 | 23 | 4 | 167 | 139 |
| Coenagrionidae | 0 | 3 | 7 | 0 | 7 | 23 | 0 | 0 | 0 | 0 | 18 | 0 |
| Corbiculidae | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Corixidae | 0 | 0 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 18 | 0 |
| Culicidae | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| Dolichopodidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 |
| Dytiscidae | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gammaridae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 |
| Gomphidae | 0 | 0 | 22 | 0 | 67 | 5 | 0 | 2 | 0 | 0 | 13 | 3 |
| Haliplidae | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 4 | 1 | 0 | 122 | 12 |
| Heptagenidae | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hydrobiidae | 7 | 6 | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hydrophilidae | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hydropsychidae | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lymnaeidae | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| Mesovelidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Naucoridae | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 5 |
| Oligochaeta | 0 | 7 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Physidae | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Planorbidae | 0 | 0 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Psychodidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 37 | 0 |
| Sphariidae | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tabanidae | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tipulidae | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Valvatidae | 0 | 6 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Viviparidae | 0 | 1 | 3 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Water spider | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |