INSTITUTE OF HEALTH, FACULTY OF PUBLIC HEALTH

DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCES & TECHNOLOGY

DISTRIBUTION AND COMPOSITION OF MACROINVERTEBRATES IN WALA HOT SPRING, SOUTHWESTERN ETHIOPIA

A THESIS SUBMITTED TO JIMMA UNIVERSITY, INTITUTE OF HEALTH, FACULTY OF PUBLIC HEALTH, DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCES AND TECHNOLOGY, FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ENVIRONMENTAL SCIENCE AND TECHNOLOGY.

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BY: ROBERA NEGASSA

August, 2022

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ADVISORS:

1. MAHMUD AHMEDNUR (MSc)

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Declaration

I declare that this thesis has been composed solely by myself and that it has not been submitted, in whole or in part, to any institution for the award of a master's degree, complies with the regulations of the university, and meets the accepted standard in terms of originality and quality.

Name: Robera Negasa ________________ _______________

Signature Date

Approval sheet

As thesis research advisors we hereby certify that we have read and evaluated this research paper under our guidance entitled as “Distribution and composition of macroinvertebrates in Wala hot spring, South-west Ethiopia” by Robera Negasa and we recommended that it could be submitted as fulfilling the thesis requirement.

Mr. Mahmud Ahmednur ____________________ __________________

Signature Date

Prof. Dr. Seid Tiku Mereta ____________________ ___________________

Signature Date

As member of board of examiners of the Msc. thesis defense examination, we certify that we have read and evaluated the thesis prepared by Robera Negasa and examined the candidate. We recommended that the thesis can be accepted as fulfilling the thesis requirement for the degree of masters of science in environmental science and technologmhjry.

Internal examiner ______________________________ __________ _______________

Name Signature Date

External examiner ________________________________ __________ _______________

Name Signature Date

Abstract

Background: *Macroinvertebrates can be used to indicate ecological status and effect of many stressors on water body. Hot spring water quality is getting affected by anthropogenic activities. Studying bio-indicators and physico-chemical parameters is important to predict the status and effect of stressors on hot springs water quality and use the information to maintain their ecosystem services*

Objective: *This study aimed to study the distribution and composition of macroinvertebrates across different sites of Wala hot spring, south western Ethiopia.*

Method and material: *This study was conducted in November, 11-17, 2021. A laboratory and field based cross-sectional study design was conducted**.** The macroinvertebrate sample was collected with physico-chemical parameters and human disturbance from 20 sampling sites of Wala hot spring. Beta diversity was used to investigate the distribution of macroinvertebrates across the sampling sites. Principal Component Analysis biplot was used to show the relation of sites with physico-chemical water quality. Canonical Correspondence Analysis was used to show correlation of macroinvertebrates with physico-chemical water quality. Analysis of variance was used to indicate the variation of macroinvertebrates richness and abundance between different sites and disturbance gradients. Pearson correlation was used to show the relation of macroinvertebrates with the water temperature variations.*

Result and discussion: *A total of 1027 macroinvertebrate individuals belonging to 7 orders and 21 families were collected. The total beta diversity* (β-sor) *of sampled macroinvertebrates was 0.857. The contribution of species turnover* (β-nes) *was 0.783 while, the contribution of species nestedness (*β-nes) *was only 0.074. Significant difference was found in richness (P = 0.015) and abundance (P =0.0183) of macroinvertebrates along disturbance gradients. CCA revealed that macroinvertebrates were significantly correlated with Dissolved Oxygen, pH, nitrate-nitrogen, total hardness, and Chemical Oxygen Demand. The findings of this study revealed that there is a strong negative correlation (r = -0.83) between macroinvertebrates richness and water temperature. Similarly, a significant negative correlation (***r*** = -0.75) was found between macroinvertebrates abundance and water temperature in different sites of Wala hot spring.*

Conclusion and recommendation: *Distribution of macroinvertebrates in Wala hot spring was structured by species replacement rather than difference in richness. Macroinvertebrates richness and abundance has changed along human disturbance gradients. The change in physico-chemical water quality in different sites has affected macroinvertebrates. Temperature variation in the water has strongly negatively affected macroinvertebrates abundance and richness. It was recommended to study physico-chemical parameters and bio-indicators of the water not used in this study and to reduce disturbance affecting the water quality.*

Key words: *Hot spring, Human disturbance, Macroinvertebrate, Physico-chemical parameters and Temperature variation*

Acknowledgment

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I dedicate this thesis to my mother Tabote Kebede, everything would be harder without your prayer, support, and love.

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List of Acronyms and Abbreviations

ANOVA……………………………….Analysis of variance

CCA …………………………………..Canonical correspondence analysis

COD …………………………………..Chemical oxygen demand

DO…………………………………….. Dissolved oxygen

EC……………………………………...Electrical conductivity

PCA…………………………………….Principal Component analysis

Turkey HSD Test……………………..Turkey Honest Significant Difference Test

EDTA……………………………………… Ethylenediamine Tetra-acetic Acid

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Chapter One

1. Introduction

1.1 Background of the study

Fresh water resources are vital in sustaining human health and life on earth (Arya, 2021). Freshwater resources have always played a major role in recreation and tourism, water supply, water quality control, habitat provision, climate regulation, drinking water, groundwater recharge, water quality control as well as cultural services, such as the existence of spiritual places (Vári et al., 2022). Despite their use fresh water sources are getting affected by anthropogenic activities and environmental factors in ways that compromise their value as a habitat for organisms, domestic use, and recreation (Khatri and Tyagi, 2015). Anthropogenic climate change and non- climatic drivers such as population increase, economic development, urbanization, and land use or natural geomorphic changes are some of the many stressors that may affect these important fresh water resources (Jiménez et al., 2015).

Natural hot springs are formed by the discharge of geothermally heated ground water to the earth's surface (Erfurt, 2011; Suryawanshi, 2019). The temperature of hot spring water lies above local ground water and the annual air temperature of that region (Olivier et al., 2011). If hot spring water percolates deeply enough into the earth's crust, it will get more heated as it comes into contact with hot rock which is why temperature varies from one hot spring to another (Pentecost, 2005; Rajapaksha et al., 2014, Suryawanshi 2019). Hot spring water is rich in dissolved minerals from underground rocks (Hamzah et al., 2013; Ta et al., 2020). Each hot spring has unique geophysical and biological characteristics which makes them an interesting area of research (Prieto-Barajas et al., 2017). Hot springs can be classified based on their different qualities. The main criteria to classify hot springs were temperature, mineral content, pH, and their usage (Simon et al., 2019; Subtavewung et al., 2005; Suryawanshi, 2019).

Hot spring waters have been utilized by man-kind for drinking, bathing, healing, therapy, and religious sites for many years (Porowski, 2019). Studies have proven that natural hot spring waters contain pharmacologically active compounds and minerals important for human body composition which made them suitable for therapeutic purposes. Hot springs are now playing a key role in the expansion of eco-tourism which is called the smokeless industry in many parts of the world (Erfurt-Cooper, 2010). Recently hot springs are being used for industrial processing, agriculture, aquaculture, bottled water, and the extraction of rare elements (Ghilamicael et al., 2017; Ma and Zhou, 2021).

Hot springs are found in many parts of Ethiopia. Many hot springs in Ethiopians believe that hot spring water can cure many diseases. There are also hot springs being used for recreation in some parts of the country, with hotels and lodges constructed around them. Many Ethiopians use and recommend hot spring waters to be cured of many diseases (Derso et al., 2015).

After being discharged to the earth's surface hot spring water quality gets affected by anthropogenic activities and environmental factors. Recently hot springs water quality and ecological integrity were negatively affected by the usage of hot springs for purposes like; industrial processing, agriculture, aquaculture, bottled water, the extraction of rare elements, and the development of hotels and resorts (Derso et al., 2015; Ghilamicael et al., 2017; Rajapaksha et al., 2014).

Biological indicators provides a more robust measure and precise understanding of changing aquatic conditions, and provide direct and indirect evidence of affectations occurring in the aquatic ecosystems supporting physico-chemical water analysis (Andem et al., 2013; Zhang et al., 2021;Armon and Hänninen, 2015). It has been proven that water physico-chemical analysis and biological indicators can be used for the assessment of springs (Abdelsalam and Tanida, 2013). Bio-indicator has high potential in predicting the cumulative impact of anthropogenic activities and predicting environmental changes promptly with minimum destruction of the biosphere (Samiyappan, 2019).

Many factors regulate the occurrence and community structure of benthic macroinvertebrates (Belal, 2019; Qazi, 2012). One of the factors that influence the aquatic macroinvertebrates community is human disturbance which affects the composition of macroinvertebrates in different sites of a water body, that is why macroinvertebrates are good indicators to predict effect of human disturbance on water body (Mykrä et al., 2008; Pinedo et al., 2015). Another main factor responsible for macroinvertebrates species distribution and species richness is water temperature, (Haidekker and Hering, 2008). Macroinvertebrate species' tolerance to temperature ranges is not the same, few can tolerate temperatures over their upper tolerance limit (Hussain and Pandit, 2012). It is indicated that organisms constantly exposed to high temperature stress including organisms inhabiting the hot-spring run-offs, might provide clues on how to cope with high-temperature stresses (Sasmita et al., 2014).

1.2 Problem statement

Despite many ecosystem services they provide, hot spring waters are threatened by anthropogenic activities and environmental factors (Rajapaksha et al., 2014). The usage of hot spring by human is not considering the water quality and ecological integrity(Ghilamicael et al., 2017; Lin et al., 2005). However, Studies have been widely conducted on hot spring water in different parts of the world (Dan Mainza, 2006; Miller et al., 2009; Naresh et al., 2013; Olivier et al., 2011), only few studies were conducted on assessment of hot springs ecological status using bio-indicators. Many previous studies conducted on hot spring rather focused on; physico-chemical investigation and bacteriological examination (Grab, 2014; Massello et al., 2020; Naresh et al., 2013; Purcell et al., 2007), therapeutic and touristic purposes of hot springs (Boekstein, 2014; Hamzah et al., 2013; Xinlei, 2018).

The physico-chemical properties of water from different Ethiopian hyperthermal springs were reported however, only a few studies were conducted on the ecological quality of hot springs in Ethiopia (Derso et al., 2015). More studies will be expected to be conducted on thermal springs in Ethiopia as they are being used for many purposes in many parts of the country including for bathing, drinking, recreation, and for therapeutic purposes by the local communities and some are used for the development of resorts around them. In Ethiopia, many considerably low temperature-thermal springs have the potential for recreation, health, and mineral water bottling which hot require further investigation of biological and physico-chemical to determine whether they are suitable for recreational development or mineral water bottling. Moreover, more studies are needed on hot springs to indicate how much they have been threatened by human activities. Using macroinvertebrates assemblage is one of the most effective and inexpensive ways to estimate the ecological quality of water resources in developing countries like Ethiopia and plays a key role in conservation, protection, and maintaining them for ecotourism. However, the composition and distribution of macroinvertebrates in hot spring waters in Ethiopia were less studied. Although it was proven that macroinvertebrates can be used to study hot spring ecological status and quality, the ecological condition of many hot springs in the country was unknown.

South western Ethiopia is one of the areas where ecotourism will be expected to expand in the near future. Wala hot spring which is found in the Gera district, southwestern Ethiopia is one of the potential natural resources for ecotourism attraction and research area for many studies. Previous studies conducted in southwestern Ethiopia mainly focused on the Ecological condition of wetlands, rivers, and other fresh water bodies (Chawaka et al., 2018; Mereta et al., 2012). Although the ecological condition of hot springs was studied in the northern part of the country (Derso et al., 2015), there is no research conducted on the distribution and composition macroinvertebrates in hot springs in southwestern Ethiopia. Therefore, assessment of the distribution and composition of macroinvertebrates in Wala hot spring is important to indicate the status and maintain the ecosystem service the hot spring is providing. This study was conducted on the distribution and composition of macroinvertebrates along physico-chemical and disturbance gradients in Wala hot spring, south western Ethiopia.

1.3 Significance of the study

Investigating the ecological status of Wala hot spring using macroinvertebrates is important to indicate the potential factors affecting the hot spring water quality which can be used to generate information for the conservation, protection and restoration to maintain the ecosystem services of the hot spring. Investigating the effect of water temperature gradient on the macroinvertebrate community can indicate the effect of high temperature stress on aquatic ecology. Studying species response to environmental gradients is important to separate the effects of pollution from the effects of natural variables on community structures. This study can contribute in development of bio-assessment tools for diagnosing hot spring ecosystem using macroinvertebrate community structure. Moreover, this study can be used to classify Wala hot spring based on their different qualities.

Chapter Two

2. Literature review

2.1. Hot spring

Hot springs are natural resources found almost in all parts of the world. It was agreed that there is no universally accepted definition of a hot spring (Pentecost et al., 2003). Robin and Brain defined hot spring as ‘a type of water characterized by discharge of hot water from a vent (Renaut and Jones, 2003). A hot spring or thermal spring was also defined as, water with a higher temperature than ground water, mean air temperature, and human body temperature (Pentecost et al., 2003). The widely used definition of hot springs was, hot springs are natural thermal springs produced by geothermally heated groundwater (Olivier et al., 2011). Each hot spring has unique geophysical and biological characteristics (Prieto et al., 2017).

2.2. Water temperature of hot sprinbgs

Hot spring water is characterized by higher water temperature compared to other fresh water resources (Sahu et al., 2020). As many studies indicated the temperature of hot spring water emerges from underground as they get heated geothermally by contact with hot rocks (Suryawanshi, 2019). The temperature of underground hot rocks increases with depth which is called geothermal gradient. That is why hot spring water heat depends on how deep it permeates into the earth's crust and the water gets more heated as it percolates deep into the earth's crust and then, comes in contact with hot rocks (Kato and Kraml, 2010). The temperature of hot spring water varies from one hot spring to another because it depends on the depth and distance from hot rocks (Olivier et al., 2011).

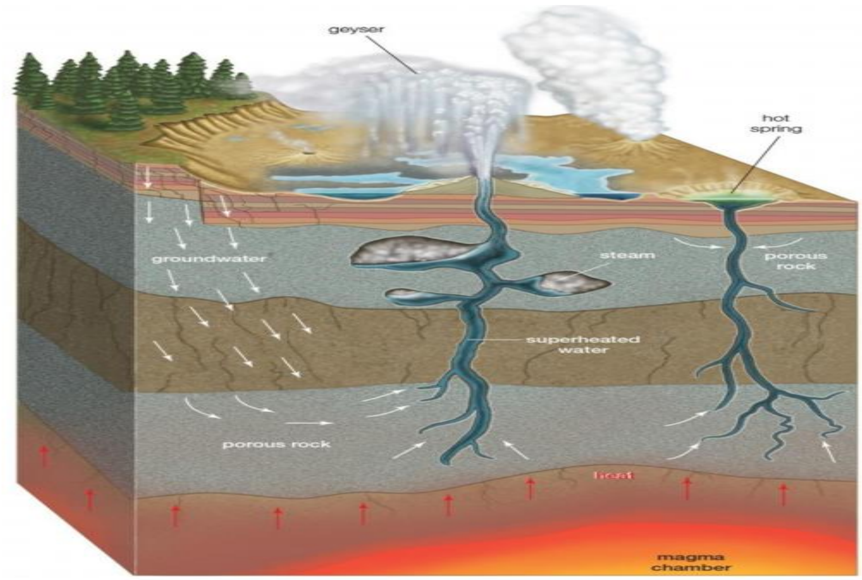


Figure 1 Source of hot springs water temperature (Sahu et al., 2020)

The influence of hot spring water temperature on hot spring organisms especially on Microbial community composition and diversity has been extensively studied (Massello et al., 2020; Naresh et al., 2013; Purcell et al., 2007). The studies conducted on the ecology of hot spring waters have proven that temperature is one of the main factors that govern the composition, distribution, and diversity of organisms in hot spring water (Belal, 2019). The higher temperature of hot spring waters is an interesting area of research for researchers interested in studying extreme environments (Prieto et al., 2017).

2.3. Mineral content of hot springs

It was proven that hot spring water mineral content is higher compared to other fresh water resources (Ghilamicael et al., 2017). The minerals in hot springs gets dissolved into the water from soluble underground rocks and emerge with the hot spring water to the earth's surface (Mainza, 2006). Hot spring waters are known for their richness in minerals beneficial for human body composition (Boekstein, 2014). As a result of interaction with geological material hot spring is rich in minerals like calcium ($Ca2+$), bicarbonate ($CO3$ H-), silicates, iron compounds, sodium, magnesium, sulfur, metal, and selenium (Simon et al., 2019). Water containing those minerals in addition to warm temperature is believed to cure many diseases, suitable for skin care and relaxation (Farhat et al., 2021).

2.4. Ecosystem services of hot spring

It has been assumed that human life depends on the existence of a finite natural resource base. There is a strong relationship between nature and humans because nature plays a key role in the fulfillment of human needs. Ecosystem services were defined as ‘the benefits that people obtain from ecosystems’ (Julia et al., 2015). Fresh water resources including hot springs are vital in sustaining human health and life. Man-kind have used hot springs for many purposes including; drinking, bathing, healing, therapy, and religious sites for many years ((Porowski, 2019).

Thermal waters are extracted primarily for their heat content and secondarily for their mineral content. Thermal spring water comes from underground with a warm temperature and minerals beneficial to human body composition (Porowski, 2019). Hot spring water is rich in pharmacologically active compounds like F-, Br-, I-, HBO2, CO2, Fe, and H2SiO3 (Mainza, 2006). Their warm temperature and high content of beneficial minerals make them suitable for therapeutic purposes(Farhat et al., 2021; Hamzah et al., 2013).

Moreover, hot springs are important geothermal resources playing a key role in the expansion of eco-tourism which is called the smokeless industry (Simon et al., 2019). The recreational and therapeutic values of hot springs made them attractive to tourists (Olivier et al., 2011). Hot spring tourism is one of the most popular types of tourism, which often attracts many domestic and international tourists, in many countries (Boekstein, 2014). The economic value of hot spring is arising as many resorts and lodges are being constructed around hot springs in many countries. Ecotourism is seen as the solution combining the recreational use of nature with its protection. In addition, hot springs are being utilized for industrial processing, agriculture, aquaculture, bottled water, and the extraction of rare elements recently (Ghilamicael et al., 2017). The usage of hot springs for ecotourism or other purposes should consider the protection of the natural resources by monitoring their quality (Strecker et al., 2014). There are many hot spring water resources in Ethiopia and most of them are widely used by local communities for curing and religious sites in many parts of the country. Recently hotels and lodges are being constructed around hot springs in some parts of the country.

2.5. Classification of hot springs

Hot spring has been classified depending on their different qualities including, their mineral content, temperature, pH, and their usage (Subtavewung et al., 2005). The temperature of hot springs was the commonly used characteristic of hot springs to classify them. The rate of temperature increases with the depth. If water percolates deeply enough into the crust, it will get more heated as it comes into contact with hot rocks(Suryawanshi, 2019).

Hot springs can be classified variously based on their temperature. The most common classification of hot springs based on their temperature was, Cold hot spring, if the temperature was less than 20°C, Hypothermal if the temperature of the hot spring was between 20-30°C, Mesothermal hot spring if the temperature of the hot spring between 30- 40 and Hyperthermal if hot spring water temperature exceeds 40°C (Simon et al., 2019). In another temperature-based classification of hot spring, hot spring can be classified under Heterothermal hot spring if a variation of temperature changes daily and seasonally, and if the variation of temperature in a hot spring is negligible both daily and seasonally it is called Homothermal (Suryawanshi, 2019)

pH is one of the criteria to classify hot springs into several classes. Based on its pH hot spring can be a Strong acid hot spring at a pH of below two, an acid hot spring if the pH of the hot spring is between 2 and 4, a weak acid hot spring with a PH between 4 and 6, neutral hot spring if the PH of the water is, above 6 and below 7.5, weak alkaline spring at 7.5 -9 and alkaline hot spring if the pH of the hot spring is above 9 (Simon et al., 2019). Depending on their usage those hot springs can be classified as, power plants, Tourism hot springs, consumption, and unutilized hot springs (Subtavewung et al., 2005).

2.6. Threats to hot springs

Human activities are negatively affecting freshwater biodiversity by reducing species richness, distribution patterns, food web interactions, and aesthetical values. Human land-use changes, such as the expansion of urban and crop cover, are threats to freshwater biodiversity in many parts of the world, increasing human population and development pressures, are creating squeezes on freshwater ecosystems (Jiménez et al., 2015). Hot spring water resources are getting affected by human activities and environmental factors after being discharged onto to earth's surface (Rajapaksha et al., 2014).

Recently hot spring water is being utilized by man for various purposes in ways that affect their water quality and ecological integrity. The usage of hot springs for industrial processing, agriculture, aquaculture, bottled water, the extraction of rare elements, bathing, and the non-eco-friendly development of hotels and resorts around them are some of the anthropogenic activities threatening hot spring water (Derso et al., 2015; Ghilamicael et al., 2017). Bathing in hot spring water is common in many countries since hot springs are mainly used for therapeutic purposes. Studies indicated that bathing in hot spring water can affect the water quality and ecological integrity of the water (Lin et al., 2005).

In Ethiopia, many thermal springs are being used for bathing, drinking, recreation, and for therapeutic purposes by the local communities and some are used for the development of resorts around them. It was suggested that the usage and human development around hot springs in Ethiopia should be ecofriendly. More studies are needed on hot springs to indicate how much they have been threatened by human activities. However; most of the hot springs in Ethiopia are potential resources for ecotourism development, there are only a few studies conducted on the status of water quality of hot springs in Ethiopia (Derso et al., 2015).

2.7. Physico-chemical water quality of hot springs

Studying the physico-chemical characteristics of water samples is important to indicate the status of water quality by providing current information about the concentration of various solutes at a given place and time (Shukla, 2013). In ecological studies, the physico-chemical parameters of water like temperature, pH, electric conductivity, dissolved oxygen, biological oxygen demand, chemical oxygen demand, hardness, chlorides, nutrients like nitrates and phosphates, turbidity and total dissolved solids are important to know the quality and trophic nature of water bodies (Mohapatra et al., 2014; Pal et al., 2016). Physico-chemical water quality will be affected by various water quality stressors and the main causes of water quality deterioration are anthropogenic and natural agents (El Morhit and Mouhir, 2014). Some of the natural and human-induced factors which affect the quality of water for various purposes are, natural hazards, sedimentation/erosion, agricultural activities, industrial usage, mining, fishing, sewage discharging/disposal, deforestation, and other commercial activities, these activities aggravate the pollution of the water body and greatly influence the physico-chemical quality of water (Worako, 2015).

Studying the physico-chemical property of hot spring water could signify their scientific significance which is an important element in geo-tourism development to develop them for a specific purpose and classify them into different classes (Simon et al., 2019). Physico-chemical analysis of hot spring waters has been reported extensively from different parts of the world. Temperature is one of the most important physical parameters in hot spring waters since, it governs the water quality and biological characteristics of the water (Naresh et al., 2013). The increase in temperature of hot springs at the point of discharge to the earth's surface affects other physico-chemical properties of the water on the surface. Dissolved oxygen is low, especially in upstream of the waters where the water temperature is highest.

2.8. Bio-indicators for hot springs

Biomonitoring is the use of biological variables to assess the environment (Gerhardt, 2002). Bio-indicators are organisms or communities of organisms, which has the potential to be used representatively to evaluate a situation and give clues for the condition of the whole ecosystem. Since they are adapted to specific environmental conditions, using biological indicators in water quality assessment is adequate to indicate long-term changes to water quality (Holt and Miller, 2011). Bio-indicator has high potential in predicting the impact of anthropogenic activities, particularly pollutants, and predicting environmental changes promptly with minimum destruction of the biosphere (Samiyappan, 2019).

Bio-indicator species effectively indicate the condition of the environment because of their difference in tolerance to environmental variability. Bio-indicator species can be too sensitive to environmental changes, there are also moderate tolerant species to environmental variables and other species may have very broad tolerances and are less sensitive to environmental changes which otherwise disturb the rest of the community (Holt and Miller, 2011). Benthic macroinvertebrates are one of the bio-indicators which are, considered good indicators of local-scale conditions(Kwakye et al., 2021).

2.9. Macroinvertebrates as bio-indicators in hot springs

Benthic macroinvertebrates are animals that are visible to be seen by the naked eye and they are mainly consist of aquatic insects, mites, mollusks, crustaceans, and annelids (Carter et al., 2017). Benthic macroinvertebrates are commonly used as indicators of the biological condition of waterbodies. Aquatic macroinvertebrates spend a part of or their entire life cycle in the aquatic environment. The structure and composition of macroinvertebrate communities may get changed along changing water quality and anthropogenic activities related to extensive urbanization, agricultural practices, industrialization, population expansion, and other different stressors of water quality can led to water quality deterioration which has a major influence on the macroinvertebrates community (Zhang et al., 2021). We can predict the effects of water quality stressors on the ecological integrity of the water body by using an assemblage of macroinvertebrates (Carter et al., 2017).

Macroinvertebrates are regarded as good bio-indicators because; their community is highly diverse and represented by several species categorized into a different tropic groups, different species have different tolerance to water quality stressors, macroinvertebrates are easy to sample, their long life span is important in an indication of stream quality over a period of time, and they can be found in any aquatic habitat. Abundance and richness of macroinvertebrates are simple measures and are often used in assessments; species-poor systems are generally assumed to have degraded water quality. At family level certain taxa can be more sensitive to pollutants or other stressors and their presence is often considered an indicator of a healthy stream whereas other taxas might be tolerant to pollution and their presence indicate degraded water quality(Joao et al., 2012; Magbanua et al., 2010)

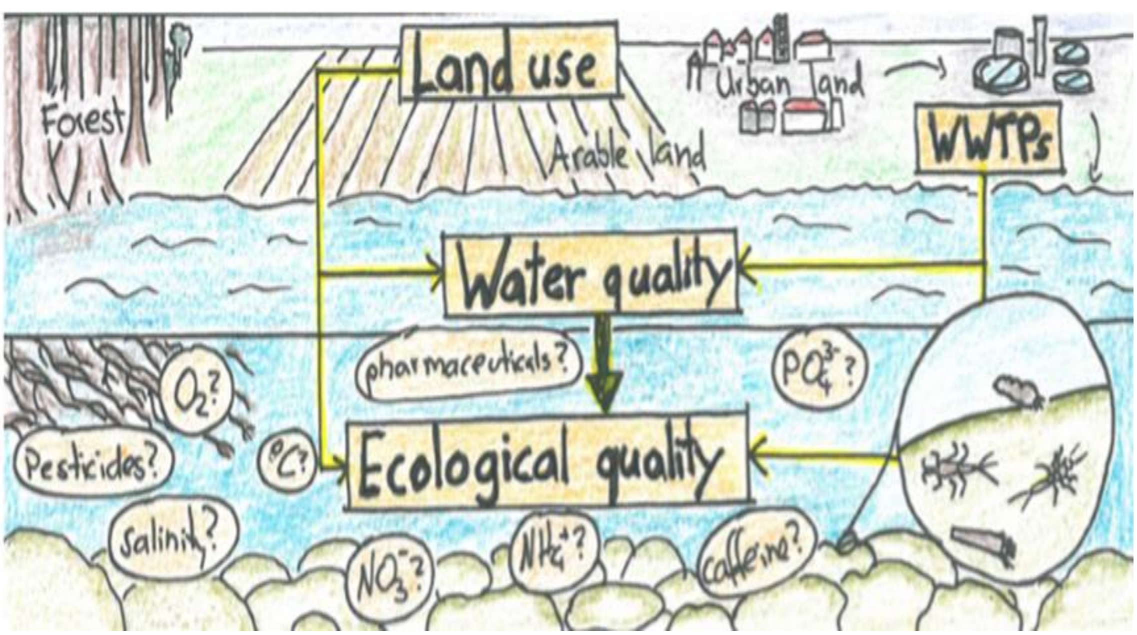


Figure 2 Effects of water quality stressors on the ecological integrity of macroinvertebrates in the water body (Berger et al., 2017)

As studies indicated water temperature gradient was identified as one of the main factors responsible for macroinvertebrate species distribution and species richness (Li et al., 2013). Water temperature affects the distribution and changes the community structure of macroinvertebrates in a water body. Macroinvertebrates tolerance to high temperature is different from one species to another. Some macroinvertebrates are tolerant to high temperatures and others are sensitive to high temperature (Campbell, 2020). Macroinvertebrates distribution in hot spring water in relation to temperature gradient can be used to predict effect of high temperature on aquatic organisms and the effect of a climate-driven rise of temperaturse in water bodies. It is proven that macroinvertebrates can be used to assess hot springs (Derso et al., 2015).

2.10. Conceptual frame work of the study

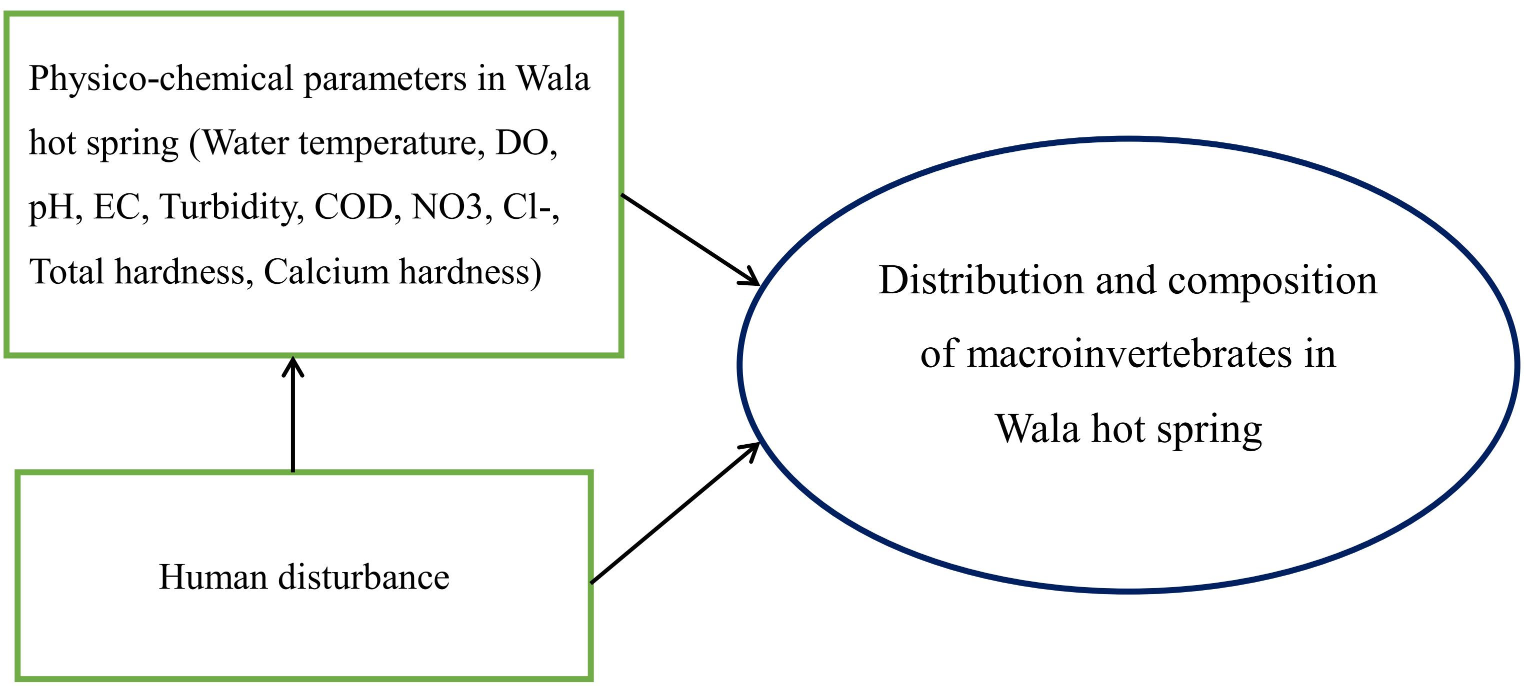


Figure 3 Conceptual framework of the study showing relation between study variables

Chapter Three

3. Objectives

3.1. General objective

- To assess the distribution and composition of macroinvertebrates of Wala hot spring in Gera district, Jimma zone, southwest Ethiopia, 2021.

3.2. Specific objectives

- To investigate the distribution of macroinvertebrates in Wala hot spring
- To assess the composition of macroinvertebrates along human disturbance gradients in the Wala hot spring
- To assess the physico-chemical parameters of Wala hot spring.
- To investigate the relation of macroinvertebrates with the physico-chemical parameters of Wala hot spring.

3.3. Research questions

1. What is the distribution of macroinvertebrates in Wala hot spring?

2. What is composition of the macroinvertebrates along human disturbance in Wala hot spring?

3. What is the average physico-chemical parameters influencing macroinvertebrates composition in Wala hot spring?

4. What is the relation of macroinvertebrates with physico-chemical parameters in Wala hot spring?

3.4. Hypothesis

1. The distribution of macroinvertebrate in Wala hot spring is mainly structured by turnover along sites

2. Human disturbance significantly affects the richness and abundance of macroinvertebrates in Wala hot spring

3. Physico-chemical water quality parameters of Wala hot spring is related with macroinvertebrates composition

Chapter Four

4. Methods and materials

4.1. Study area

This study was conducted at Wala hot spring situated in Wala kebele, Gera district, South western Ethiopia. The study area is located about 480 km southwest of Addis Ababa. Wala hot spring is found in the Gera district which has many potential natural resources in southwestern Ethiopia (Gebrehiwot and Hundera, 2014). The study area has a drastic nitosol soil type while Orthic acrisol and drastic nitosol are the major soil types. The mean annual temperature of the Wala is about 18.40_^{0}C and the mean minimum and maximum temperatures are 11.7_^{0}C and 26.50_^{0}C respectively (Ayele, 2014).

Figure 4

4.2. Study Period and Design

This study was conducted in November, 11-17, 2021. Field and laboratory-based cross-sectional study design was employed in this study.

4.3. Study variables

4.3.1. Explanatory variables

Physico-chemical water quality (water temperature, dissolved oxygen, electrical conductivity, pH, turbidity, nitrate-nitrogen, ortho-phosphate, chemical oxygen demand, chloride, total hardness, and calcium hardness) and human disturbance (Grazing, vegetation removal, tree plantation, farming, waste dumping, washing, bathing, and swimming) in Wala hot spring.

4.3.2. Response variables

- Spatial distribution of macroinvertebrates in Wala hot spring
- Composition of macroinvertebrates in Wala hot spring.

4.4. Data Collection and site selection

The macroinvertebrates, water samples, and human disturbance data were collected from 20 sampling stations of Wala hot spring, in a 100 meters interval (Figure 5). The data were collected from downstream to upstream. The main criteria to select the sampling locations were the distance from the source and the distance between sampling sites. Criteria like accessibility, if the site was not accessible in the 100 meters intervals, disturbances near the sampling point and habitat variations were also considered. (Arnórsson et al., 2006). The sampling points were classified in to upstream, mid-stream and downstream based on a direction of the water flow, position on the hot spring and distance from the source. Based on critarias listed above, the upper seven sites (1-7) were upstream, Seven sites in the mddle (8-14) were mid-stream and the six sites found to the end of the samling points (15-20) were downstream.

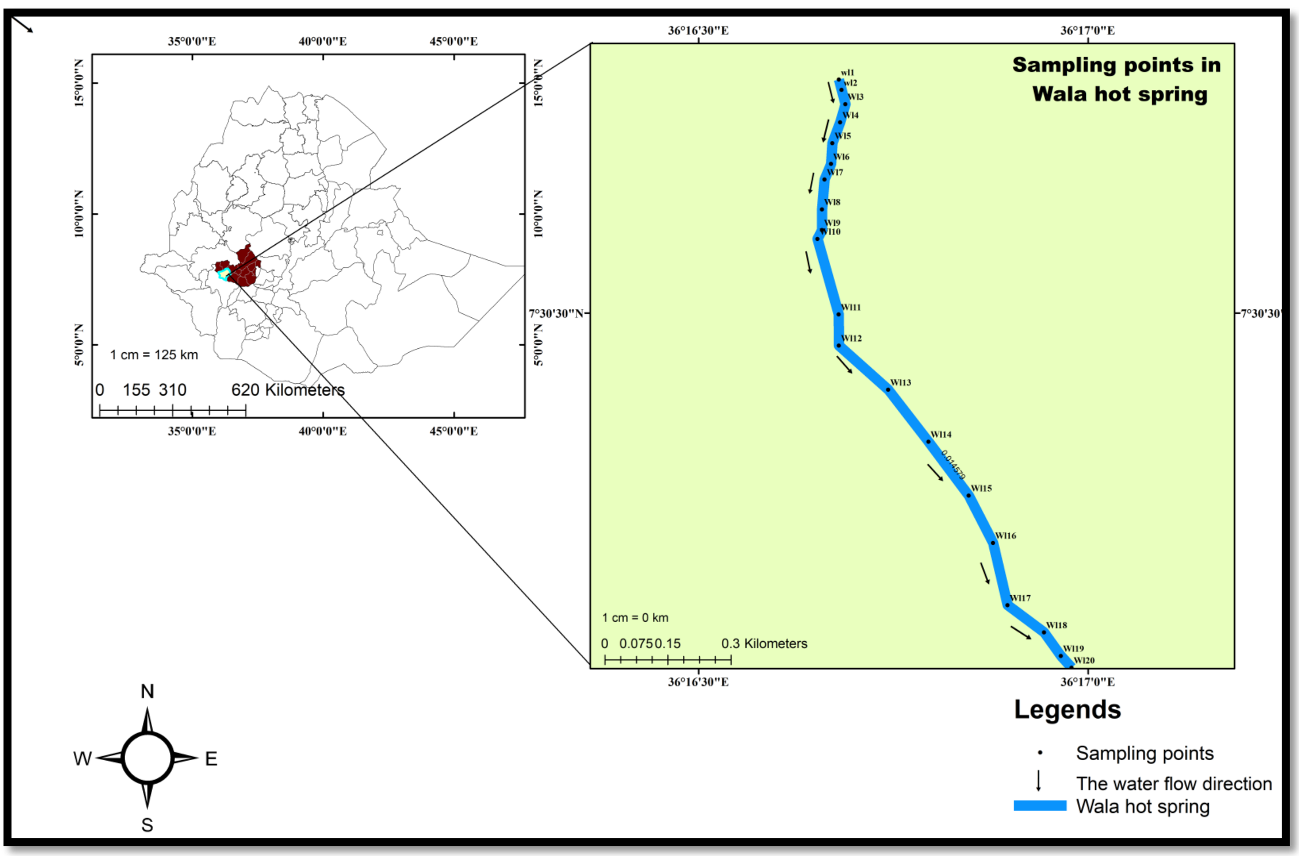


Figure 5 Study area map showing the sampling locations in Wala hot spring

4.5. Macro invertebrate sampling and identification

Macro-invertebrates were collected from the 20 sampling points of Wala hot spring starting from downstream to upstream. The collection of macroinvertebrates was carried out using a D-shaped kick-net with a mesh size of 250 µm for 10 minutes, for a distance of 10 meters by disturbing the water by foot (Boothroyd et al., 2001). The macroinvertebrates collected by the kick-net were removed into a sieve by washing the net's content from the sweep-net. Forceps were used to separate and collect macroinvertebrates into vials. The specimen was preserved with 70% ethanol. Finally, the vials with collected macroinvertebrates were labeled with a location and date and then transported to a laboratory for identification.

The identification of collected macroinvertebrates was carried out in Jimma University, environmental biology laboratory. For the identification, a microscope with a magnification of 10x was used to magnify the macroinvertebrates. Bouchard (2004) identification key was used for morphological identification of the macroinvertebrates at a family level.

Figure 6 Macroinvertebrates sampling and identification Gera district, Jimma zone Southwest Ethiopia, 2021 a) Macroinvertebrates sampling; b) Macroinvertebrates identification

4.6. Water sampling and analysis

Onsite measurements were conducted for the water temperature, pH, electrical conductivity and dissolved oxygen using HACH HQ40d Portable multiparameter-probe, while turbidity was measured using HI-83749-02 portable turbidity meter for each sampling site. From each sampling site, two litters of two water samples were taken and transported to Jimma University, environmental biology laboratory for chemical analysis. The water samples were stored in a cold box while transported to the laboratory. The samples were stored in a refrigerator until the laboratory analysis was conducted. Chemical analysis of the collected water samples were carried out in Jimma University, laboratory of environmental biology to determine the concentration of nitrate-nitrogen, ortho-phosphate, COD, chloride ion, total hardness, and calcium hardness in the waters.

The analysis of the physicochemical parameters was carried out as per the method described in APHA (1995). Nitrate nitrogen concentration was determined using the standard Phenoldisulfonic Acid Method. The stannous Chloride Method was used to indicate the Ortho-phosphate concentration of the water samples. The chemical oxygen demand of the water samples was determined following the procedures of the open reflux method, which is a titrimetric method for COD analysis. For chloride concentration, an argentometric method was conducted using potassium dichromate indicator solution and silver nitrate titrant following the standard procedure for chloride ion identification. The total and calcium hardness of the water was measured by Ethylenediamine Tetra-acetic Acid titrimetric method (APHA, 1995).

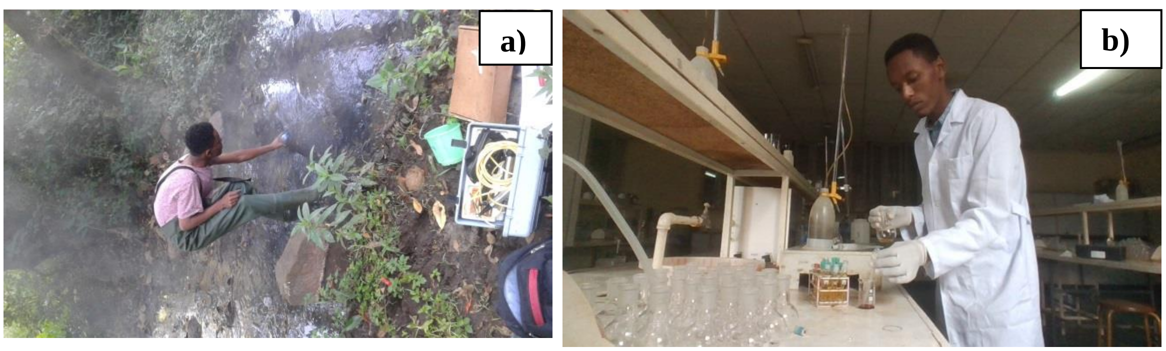


Figure 7 Water sampling and laboratory analysis Wala hot spring, Gera district, Jimma zone Southwest Ethiopia, 2021 a) Water sampling b) Sampled waters laboratory analysis

4.7. Human disturbance

The human disturbance was measured by assessing different activities, habitat alteration, and land-use practices in the study area. In total the eight different activities used to measure human disturbance in the study area included washing, bathing, swimming, grazing, vegetation removal, tree plantation, farming, and waste dumping. The score given to each disturbance were 1, 2, or 3. The disturbance score was given based on the distance of disturbances from the sampling points and the severity of the disturbance. A score of 1 was given to sampling sites with very minimal or no indicated disturbance. A score of 2 (moderate) was given to disturbances taking place in some distance from the water. A score of 3 (high) was given several disturbances taking place in the water and very close to the water following procedures indicated in Alemneh et al., (2017) and Mereta et al., (2013).

4.8. Data Analysis

The richness of macroinvertebrates was calculated by counting the number of families at each site using the COUNTIF function in Excel. The abundance of macroinvertebrates for each site was obtained by summing the number of each macroinvertebrate family present in the site using the SUM function in Excel. The most abundant families of macroinvertebrates were identified by calculating the relative abundance of each family in excel. The Shannon Diversity Index and Simpson Diversity Index were calculated for each sampling site using an excel.

Shapiro Wilk Normality test was used to check the normality of richness and abundance of macroinvertebrates. Since the data was normally distributed a parametric One-way ANOVA was performed using R-software to indicate the variation in richness and abundance of macroinvertebrates between upstream, mid-stream and downstream and disturbance gradients. Tukey HSD test was used for multiple comparisons of macroinvertebrates richness and abundance between different site groups and and disturbance gradients.

Beta diversity was calculated using betapart package on R-software. The function beta.multi(x, index.family) was used to indicate the total dissimilarity across all sites in Wala hot spring and the contribution of turnover and nestedness to that dissimilarity following Baselga and Orme, (2012). In the analysis: β-sor, total beta diversity, β-sim, the Simpson dissimilarity representing dissimilarity due to turnover, and β-nes, the dissimilarity due to the occurrence of species nested were calculated.

To get the overall human disturbance for each site, all the individual disturbance score given to the eight human activities were summed, which makes the minimum disturbance score 8 and maximum disturbance score 24. Based on the overall sum of all disturbance scores at each site the disturbance was categorized into very low, low, moderate, and high. Based on the overall human disturbance score sum, sites with overall sum 8 to 11 were categorized to very low, 12 to 15 were low, 16 to 19 were moderate and 20 to 24 were assigned to high disturbance adopting from Alemneh et al., (2017) and Mereta et al., (2013).

The average physico-chemical water quality was analyzed and presented in a table. The mean and standard deviation were calculated for each variable in excel. A principal Component

Analysis biplot was performed using R-software to indicate the correlation between environmental variables and different site groups. Detrended Correspondence Analysis (DCA) was performed to choose between CCA and RDA since, Eigenvalue was greater than 50% and axis length is greater than 3, Canonical Correspondence Analysis was performed to show the correlation between macroinvertebrates and physico-chemical water quality.

The correlation between water temperature and macroinvertebrates richness and abundance in Wala hot spring was analyzed using Pearson correlation analysis to indicate the strength of correlation between them and presented using a scatter plot. The Pearson correlation and scatter plot analysis were also conducted to show the correlation of Shannon Diversity Index, Simpson Diversity Index and dominant species of macroinvertebrates, with water temperature in Wala hot spring.

4.9. Data quality assurance

Different techniques were used to ensure data quality. The macroinvertebrates and water samples were collected, transported and analyzed following standard methods and procedures. Calibration of all materials used in this study was conducted using standard measurement methods. After collection of macroinvertebrates, both the inside of the net and the sieve bucket were inspected to make sure no organisms adhered to these surfaces and were not transferred to the sample container. This was done to ensure that a representative sample is collected and to make sure there is no cross contamination between sampling sites. Inspection of the net and sieve bucket was done when sampling at a site is completed.

For the sample collection for chemical analysis the SESD Operating Procedure for Surface Water Sampling was followed. The collected water samples were preserved in cold box with ice packs while transporting to the laboratory. It was ensured that sample labels are properly completed, including the station identification code, date, collectors' initials and number of containers comprising the sample.

4.10. Ethical issue

This study was conducted after getting ethical clearance from the ethical committee of Jimma University, Institute of health, faculty of public health. The ethical clearance from Jimma University was given to Wala Kebele administration and the study was conducted after getting permission. This study was conducted in a way that it does not impose irreversible change that threatens the environment.

4.11. Dissemination plan

The final result of this study will be submitted to Jimma University, Institute of health, Faculty of public health and all the concerned bodies about the study. Publishing this study in national or international journal will be the major concern. The findings of the study will help stakeholders to get information in order to take the necessary engagement and actions in the study area.

Chapter five

5. Result

5.1. Macroinvertebrate community

A total of 1027 macroinvertebrate individuals belonging to 7 orders and 21 families were collected. The most dominant macroinvertebrate families were Hydropsychidae and Heptagenidae followed by Caenidae with a relative abundance of 33.98%, 17.33%, and 17.04% respectively.

Table 1 Composition of macroinvertebrates in Wala hot spring, Gera district, Jimma zone Southwest Ethiopia, 2021

Order	**Family**	**Relative abundance (%)**	**Total abundance**
Diptera	Chironomidae	8.96	92
	Tabanidae	1.17	12
	Tipulidae	0.29	3
Coleoptera	Hydrophilidae	4.87	50
	Hydraenidae	0.39	4
	Psephenidae	1.66	17
	Dytiscidae	0.19	2
Hemiptera	Mesoveliidae	0.19	2
	Naucoridae	4.77	46
	Belostomatidae	1.07	11
	Heptagenidae	17.33	178
	Gerridae	0.10	1
	Nephidae	0.19	2
Odonata	Gomphidae	1.07	11
	Aeshnidae	1.46	15
	Coenagrionidae	3.31	34
	Libellulidae	1.27	13
	Letsidae	0.58	9
Trichoptera	Hydropsychidae	33.98	349
Ephemeroptera	Caenidae	17.04	175
Rchynchobdelia	Hemopidae	0.10	1
Total			1027

The result revealed that there is a statistically significant difference (df = 2, P = 0.001) in the richness of macroinvertebrates between upstream, mid-stream, and downstream. The richness of macroinvertebrates was significantly higher in the downstream (P = <0.001) followed by mid-stream (P = 0.022). The macroinvertebrates richness was lower in upstream (Figure 9). Macroinvertebrate abundance also showed significant variation (df = 2, P =0.018) between upstream midstream and downstream. Abundance was significantly higher in downstream (P = 0.0473) followed by mid-stream (P = 0.0014) similar to richness abundance was also lower in upstream (figure 8). Hydrophilidae is the most dominant family in the upstream while Hydropsychidae is the most dominant family both in mid-stream and downstream

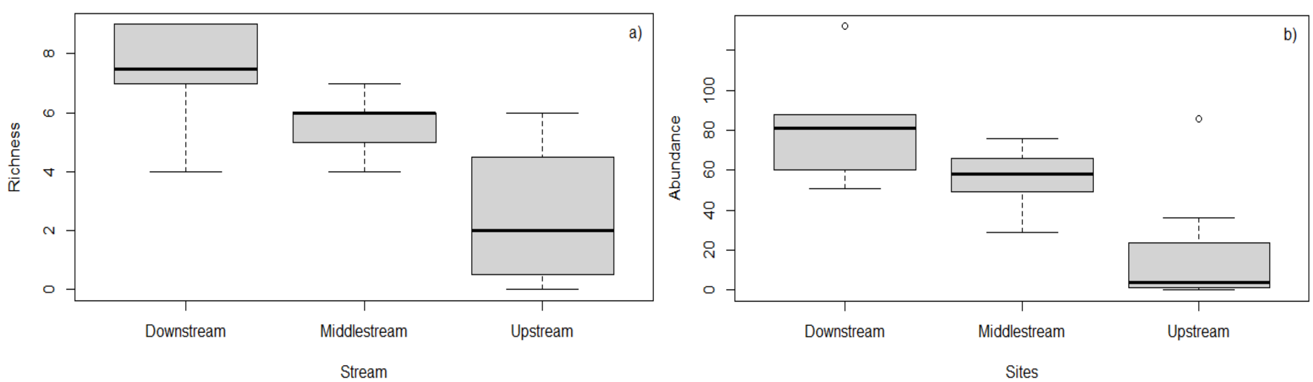


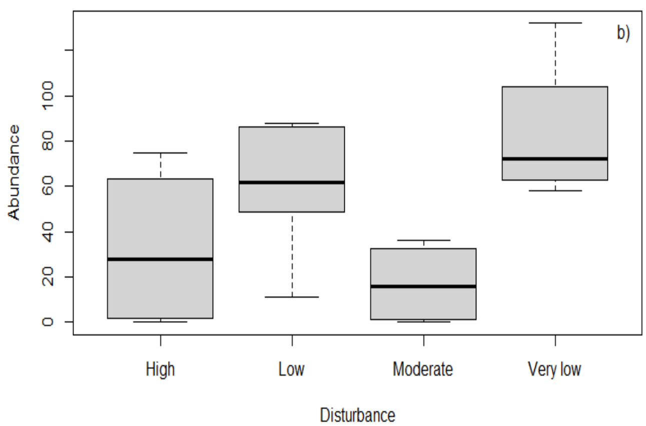
Figure 8 Boxplots showing richness and abundance of macroinvertebrates along different site groups a) Abundance of macroinvertebrates in the upstream, mid-stream and downstream; b) Richness of macroinvertebrates along upstream, mid-stream and downstream

5.2. Distribution of macroinvertebrates

The distribution of macroinvertebrates was structured by replacement of species between different sampling sites rather than, the difference in richness. The total beta diversity(β-sor) of sampled macroinvertebrates from different sites of Wala hot spring was 0.857. The contribution of species turnover (β-sim) to the total beta diversity was 0.783 while, the contribution of species nestedness (β-nes) was only. The contribution of species replacement to the total dissimilarity of macroinvertebrates across all the sampling sites was 91.37% while, the contribution of species difference was only 8.63%

5.4. Macroinvertebrates composition along disturbance gradients

Macroinvertebrates composition has changed along the disturbance gradients in Wala hot spring. The richness of macroinvertebrates showed significant variation (df = 3, P = 0.015) among human disturbance gradients of Wala hot spring. The richness of macroinvertebrates significantly higher in sites with low disturbance (P = 0.0401). Similar to the richness, the abundance of macroinvertebrates also significantly varied (df = 3, P =0.0183) across the disturbance gradients. An abundance of macroinvertebrates was significantly higher only in sites with very-low human disturbance (P = 0.02). Both richness and abundance of macroinvertebrates were higher in sites with very low and low disturbance while relatively lower macroinvertebrates richness and abundance was found in moderately and highly disturbed sites.



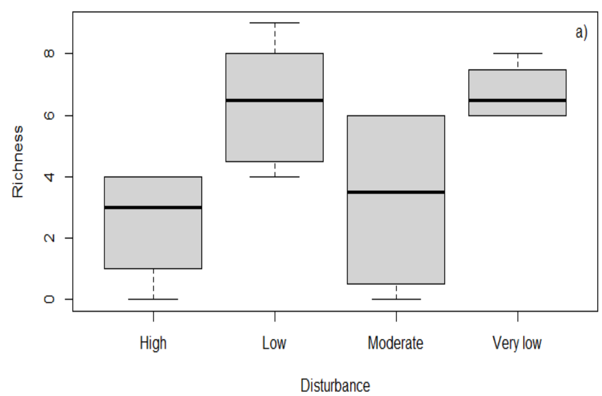


Figure 9 Boxplots showing richness and abundance of macroinvertebrates along disturbance gradients in Wala hot spring a) Abundance of macroinvertebrates in different disturbance gradients; b) Richness of macroinvertebrates across the disturbance gradients

Table 2 Human disturbances, macroinvertebrates abundance, and richness in Wala hot spring Jimma zone, Southwestern Ethiopia, Gera district, 2021

Sampling sites	**Disturbances**								**Total disturbance Score**	**Disturbance Category**	**Abundance**	**Richness**
	Grazing	**Vegetation removal**	**Tree Plantation**	**Farming**	**Waste Dumping**	**Washing**	**Bathing**	**Swimming**				
WL1	1	3	1	1	3	3	3	3	18	Moderate	0	0
WL2	2	3	1	2	3	3	3	3	20	High	0	0
WL3	2	2	2	2	2	2	2	2	16	Moderate	3	1
WL4	3	3	2	3	3	3	3	2	22	High	4	2
WL5	2	3	1	1	2	2	1	1	13	Low	86	5
WL6	2	2	2	2	1	2	1	1	13	Low	11	4
WL7	3	2	1	3	3	3	2	1	18	Moderate	36	6
WL8	2	2	2	2	2	2	2	2	16	Moderate	29	6
WL9	1	1	2	2	2	2	2	1	13	Low	64	6
WL10	3	3	3	3	2	3	2	1	20	High	52	4
WL11	2	2	2	2	1	2	1	1	13	Low	46	4
WL12	2	1	2	1	1	1	1	1	10	Very-low	58	6
WL13	1	2	2	2	1	1	1	1	11	Very-low	68	7
WL14	1	2	2	2	1	1	1	1	11	Very-low	76	6
WL15	3	2	3	3	3	2	1	1	17	Moderate	60	7
WL16	2	2	2	1	1	1	1	1	11	Very-low	132	8
WL17	1	2	2	3	1	2	1	1	13	Low	51	7
WL18	1	3	3	3	2	1	1	1	15	Low	87	9
WL19	1	3	2	2	2	2	1	1	14	Low	88	9
WL20	3	3	2	3	3	3	2	1	20	High	76	4

5.5. Physico-chemical water quality

The average physico-chemical water quality parameters of the Wala hot spring were presented in Table 1. The highest water temperature (59.5°**C**) was found at the first site of the upstream, while the lowest temperature (18.7°**C**) was obtained in downstream of the water. The pH of the Wala hot spring was found to range from 8.14-8.9. The highest conductivity with 1492 was recorded at upstream whereas the lowest value of conductivity value with 946 was found in mid-stream. The highest value of dissolved oxygen with 7.59 was obtained at downstream while; the lowest DO value of 2.94 was recorded at upstream. Turbidity was highest at upstream with a value of 63.4NTU and lowest at midstream with a 0.28NTU value.

The value of ortho-phosphate was highest at downstream and lowest at upstream. Nitrate-nitrogen value ranged from 0.125-0.075 in Wala hot spring with a little variation. The COD result ranged from 32-64, and BOD from 17.00- 29.51 with a very little variation through sampling sites. The highest value of total hardness which is 26 was found at downstream whereas the lowest value of 13 was found at upstream. The highest value of calcium hardness (22) was recorded at upstream while, the lowest calcium hardness (12) has recorded at the mid-stream. The chloride concentration was highest (37.99) in upstream whereas; the lowest (24.992) in the mid-stream.

Table 1 Physico-chemical water qualities of Wala hot spring, Jimma zone, South western Ethiopia, Gera district 2021

Phsico-chemical water quality	Upstream n=7		Mid-stream n=7		Downstream n=6	
	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
T (°C)	40.11	9.79	27.21	3.02	20.57	1.61
DO (mg/L)	4.22	0.81	6.18	0.59	7.19	0.34
pH	8.45	0.21	8.64	0.26	8.30	0.15
EC (µmhos/cm)	1240.14	172.85	971.00	24.70	993.00	22.79
Turbidity (NTU)	19.62	20.83	25.54	7.08	28.60	3.36
PO_4^3 (mg/L)	0.25	0.07	0.36	0.04	0.43	0.02
NO_3-N (mg/L)	0.09	0.02	0.10	0.01	0.10	0.01
COD (mg/L)	36.57	12.09	36.57	12.09	48.00	17.53
Total hardness(mg/L)	23.57	3.51	17.71	2.56	21.17	2.71
Calcium hardness (mg/L)	20	2.83	15.71	2.43	19.00	1.41
Cl- (mg/L)	32.70	4.23	26.85	1.07	30.16	3.65

5.5.1. Relation of sites with physico-chemical parameters

The PCA biplot displayed that most of sites in the upstream were positively correlated with temperature and chloride. Most sites found in the mid-stream showed positive correlation with pH whereas, negatively correlated with calcium hardness and COD. Most sites found in the downstream were positively correlated with calcium hardness and COD whereas negatively correlated with pH (Figure 12). The variation in the sites explained by the two pirincipal components used for the PCA biplot was 62.52%.

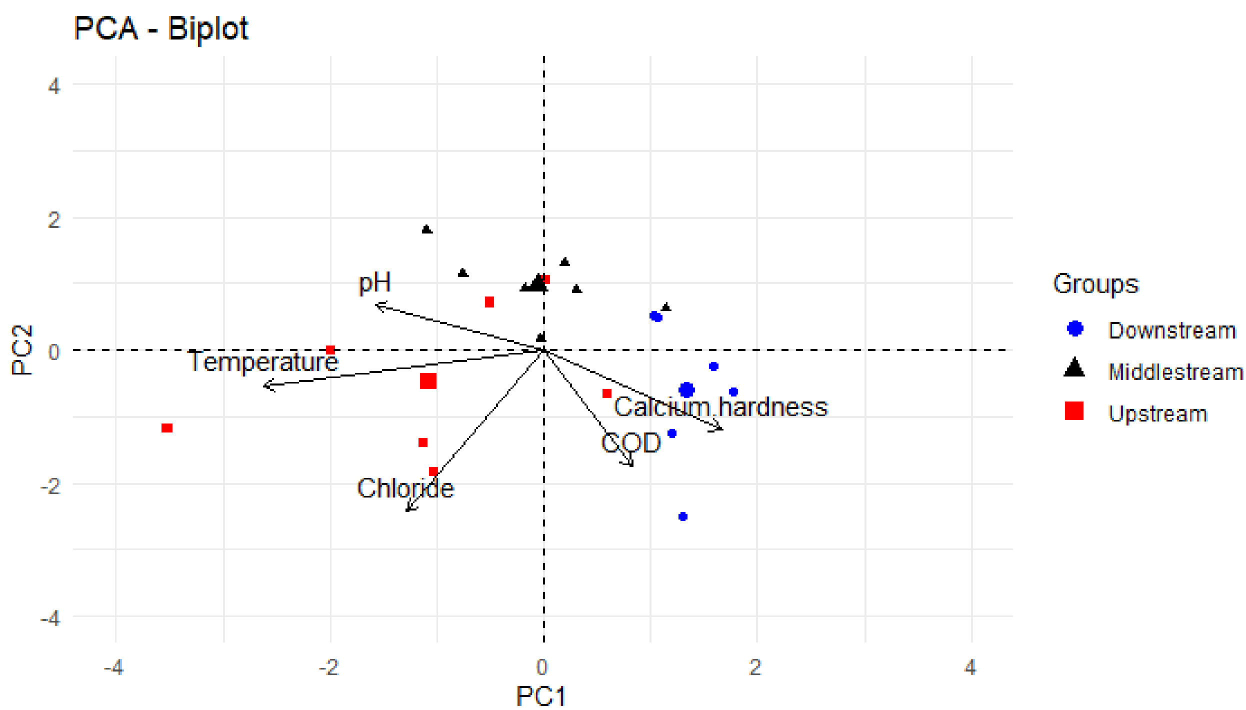


Figure 10 PCA biplot for the correlation between environmental variables and different site groups in Wala hot spring.

5.7. Relation of macroinvertebrates with physico-chemical water quality of Wala hot spring

The Canonical Correspondence Analysis (CCA) has visualized the correlation of macroinvertebrates with different physico-chemical gradients (Figure 13)**.** The CCA found that Psephenidae, Hydropsychidae, Heptagenidae, and Tabanidae were positively correlated with DO, nitrate nitrogen, COD, and total hardness. Chironomidae, Aeshnidae, and Caenidae were positively correlated to pH. Species Naucoridae and Gomphidae showed a negative correlation with DO, nitrate nitrogen, COD, and total hardness. Coenagrionidae, Hydrophilidae, and Belostomatidae were negatively correlated with pH. The two constrained CCA axis explained 66.07% of variation in the species data.

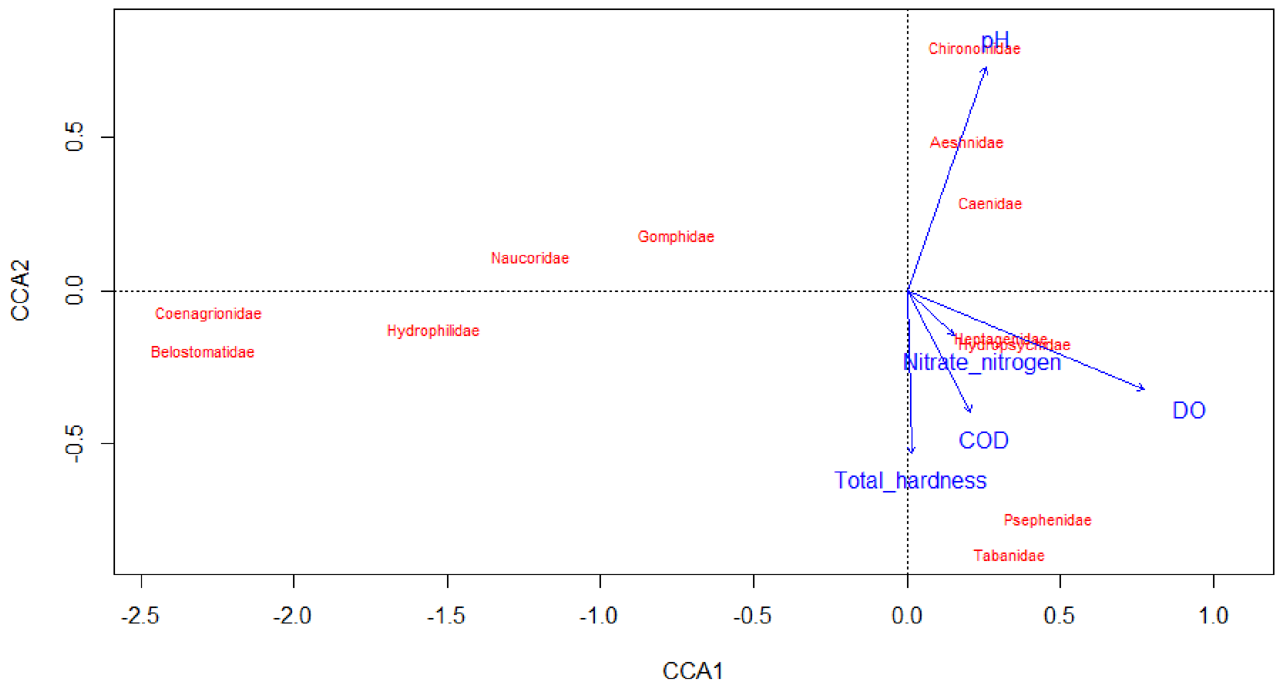


Figure 11 CCA for the relation between macroinvertebrates and significant environmental variables in Wala hot spring.

Macroinvertebrate richness and abundance were varied along temperature gradients in the hot spring. The Pearson correlation analysis found that macroinvertebrates richness has strongly negatively correlated (r = -0.83) with the water temperature. The richness of macroinvertebrates highly decreased with an increase in water temperature (Figure 10). The abundance of macroinvertebrates in different sites of Wala hot spring also showed a strong negative correlation (**r** = -0.75) with water temperature. The abundance of macroinvertebrates decreased as the temperature increased across the study site (figure 10). No macroinvertebrates were found at a temperature of 45.7°C and above while, the highest temperature recorded in upstream was 59.5 °C. Only one species (Hydropliidae) was found at a water temperature of 40.5°C, the richness and abundance of macroinvertebrates increased with the decline in the water temperature as the water flowed downstream (Figure 14).

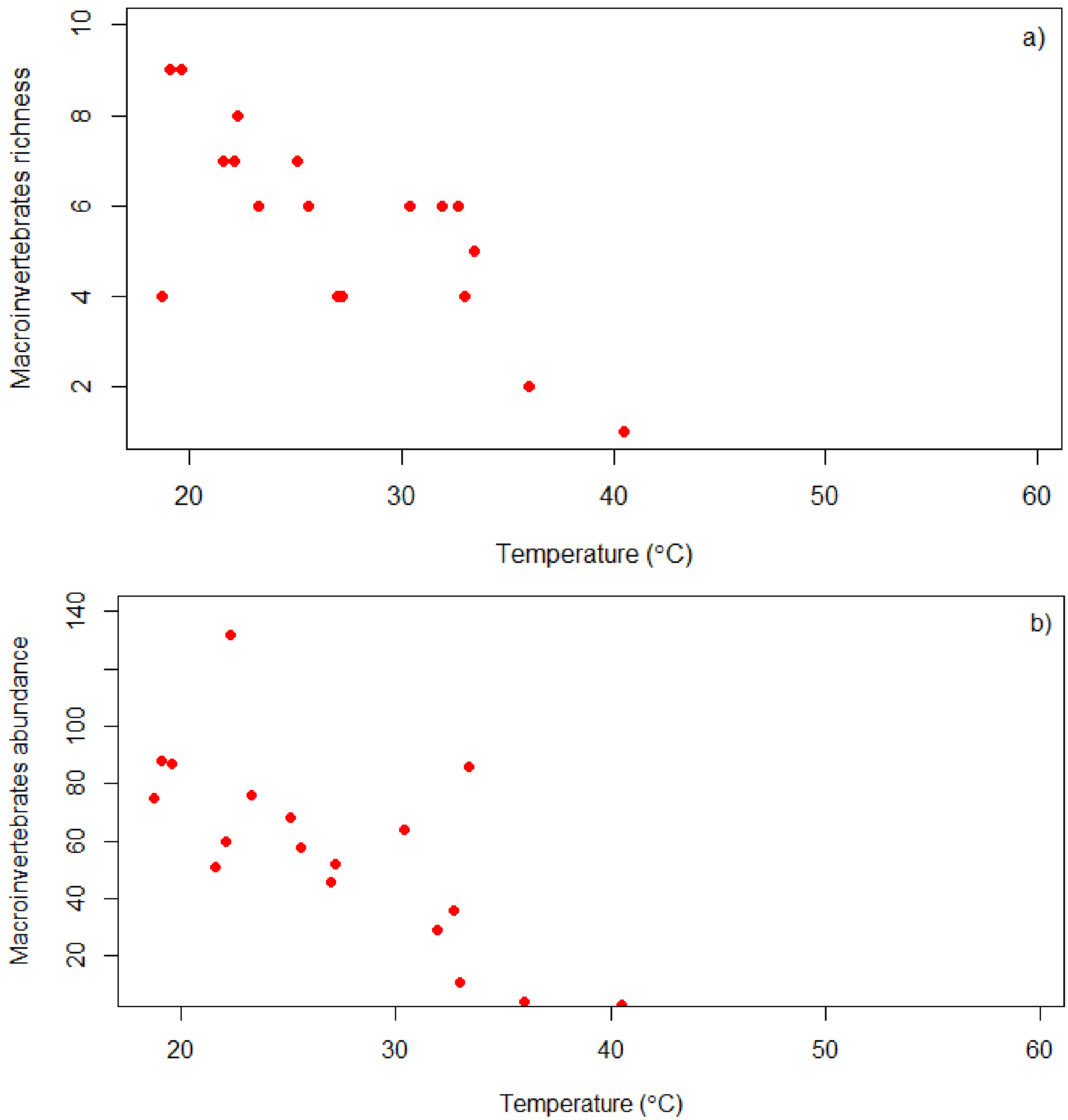


Figure 12 Scatter plot showing abundance and richness of macroinvertebrates along temperature (°C) variations in Wala hot spring a) Richness of macroinvertebrates ; b) Abundance of macroinvertebrates.

The Simpson and Shannon diversity indexes of macroinvertebrates were affected by temperature variation in Wala hot spring. The temperature variation highly negatively affected the Simpson Diversity Index (r = -0.76) as well as, Shannon Diversity Index (r = -0.74). The diversity indexes thoroughly decreased as the water temperature increased (Figure 15)

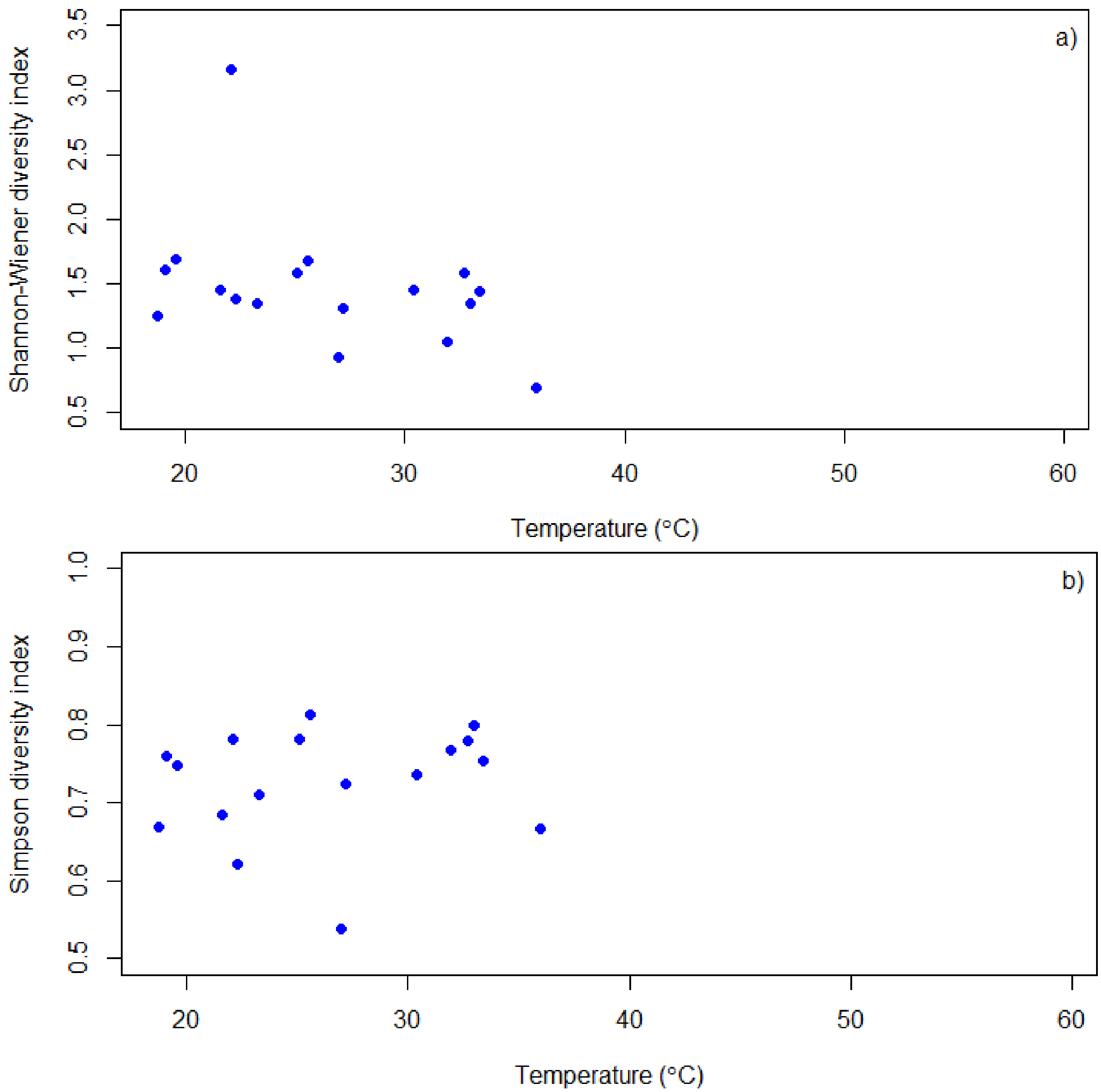


Figure 13 Scatter plot showing Shannon Diversity Index and Simpson Diversity Index of macroinvertebrates along temperature variations in Wala hot spring a) Shannon Wiener Diversity Index of macroinvertebrates; b) Simpson Diversity Index of macroinvertebrates

The most dominant families of macroinvertebrates were showed stronger correlation with water temperature rather than other physico-chemical parameters. Negative correlation was found between water temperature and most dominant macroinvertebrates. The temperature was strongly negatively correlated with Heptageniidae (r = -0.67), Caenidae (r = -0.67) and Hydropsychidae (r = -0.60) whereas, weakly negatively correlated with Chironomidae (r = -0.16) (figure 16)

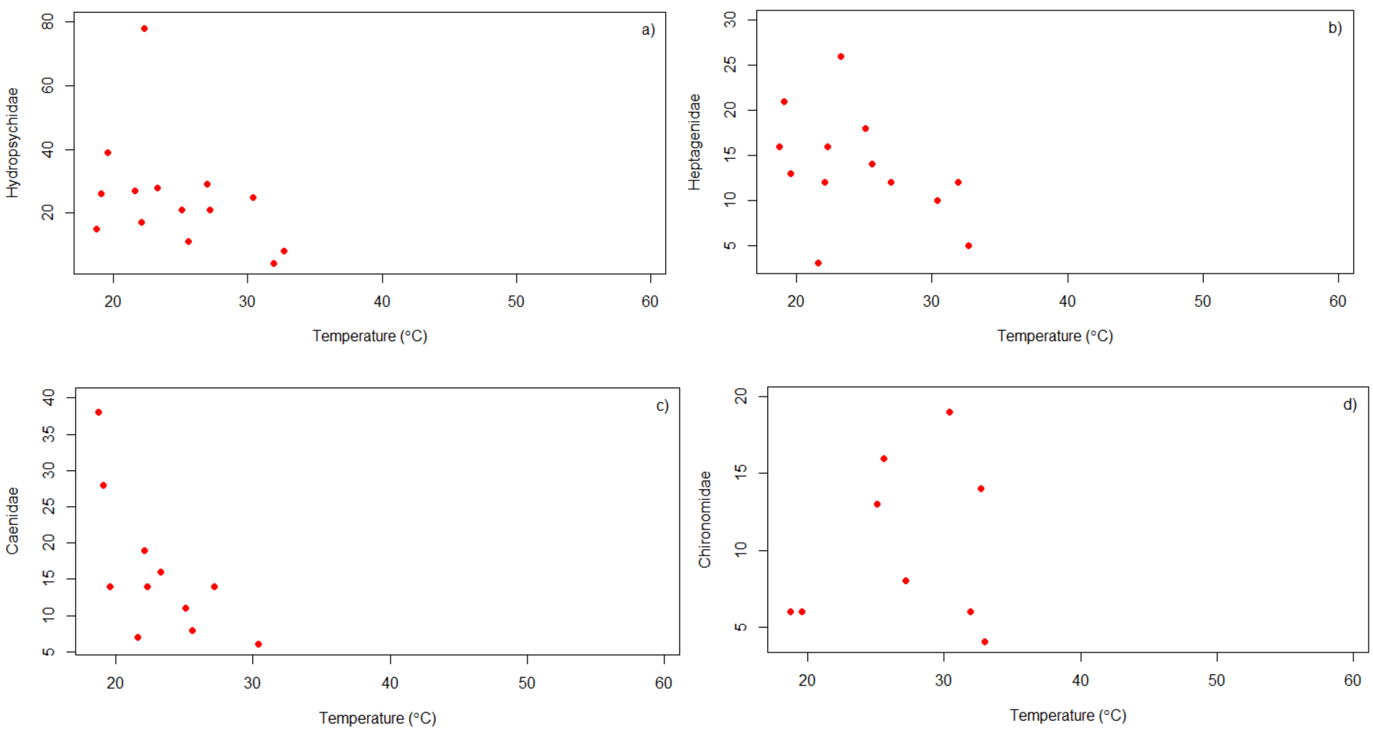


Figure 14 showing dominant macroinvertebrates Distribution patterns along temperature; a) Hydropsychidae b) Heptagenidae c) Caenidae d) Chironomidae

Chapter six

6. Discussion

Response of macroinvertebrates to changes in water quality through a change in their community distribution and composition is of great importance to indicate the status and environmental health of fresh water resources including hot springs (Bonada et al., 2008; Derso et al., 2015; Hanh et al., 2017). This study result showed that the distribution of macroinvertebrates in different sites of Wala hot spring is structured by species replacement rather than deference in richness. This might be resulted due to high variation in temperature and dissolved oxygen across the sampling sites. The studies Wang et al., (2019) and Connolly et al., (2004) indicated that temperature and dissolved oxygen are major drivers for species replacement in different sites. The possible reason for this might be difference in tolerance of macroinvertebrates to variation of those variables.

Results of this study showed that disturbance gradients across different sites of Wala hot spring have affected macroinvertebrates richness and abundance. Studies revealed that human disturbance has major influences on aquatic macroinvertebrates community composition (Ligeiro et al., 2013; Mykrä et al., 2008; Pinedo et al., 2015). In this study, high richness and abundance of macroinvertebrates were found in sites with very low and low human disturbance whereas, decreased in moderately and highly disturbed sites. The result is in line with the findings of Alemneh et al., (2017) which, found low macroinvertebrate richness and poor water quality in areas with high disturbance and indicated that sites with low human impact are characterized by high biological diversity than highly impacted areas.

In this study the physico-chemical water qualities have changed in different sites. Dissolved oxygen has highly fluctuated between different sampling points. The lowest dissolved oxygen value was found in upstream of the water whereas the highest was recorded in downstream of the water. This result is in line with the finding of Chimeddorj et al., (2021) which illustrated an increase in DO as water flowed to downstream in hot springs. The study conducted by Febiyanto (2020) on the effect of temperature and aeration on dissolved oxygen reviled that increase in water temperature and decrease in aeration highly affect dissolved oxygen in a water. Since Wala hot spring is discharged from underground with higher temperature the lower DO in the upstream might be due to the high temperature and lower aeration. The improvement in dissolved oxygen content as water flowed to the downstream of the hot spring might be due to decrease in water temperature and increase in aeration.

The pH in Wala hot spring is found to be alkaline with values ranging between 8.14 - 8.9. According to Subtavewung et al., (2005) hot spring with pH 7.5-9 is classified as a weakly alkaline hot spring. Both the highest and lowest pH was found in the mid-stream of the water. This fluctuation of pH might happen due to different disturbances between different sites. The study (Stets et al., 2014) found that the increase in pH of water results from runoffs that enter the water. The highest electrical conductivity and chloride ion concentration was recorded in the first point of upstream which has also the highest water temperature and chloride concentration. The result is in agreement with the findings of the study by Shrestha et al., (2017) which reviled that electrical conductivity increases with an increase in water temperature and chloride. Electrical conductivity increases with an increase in water temperature and concentration of ions since, an increase in temperature cause a decrease in viscosity which increase the mobility of the ions and increase in ions, increase charges of ion that facilitates the flow of electrical current in water (Shrestha et al., 2017).

Ortho-phosphate and nitrate nitrogen are important parameters to indicate the nutrient level of water (Schliemann et al., 2021; Tappin et al., 2016). The lowest orthophosphate and nitrate nitrogen concentration was found in the upper sites of Wala hot spring whereas the highest concentration of those variables was recorded at downstream. This might be resulted predominantly due to exposure of the water to the disturbance from poor agricultural practices as the water flowed to the downstream. More farming practices were observed in the midstream and downstream of Wala hot spring. This result supports the study of Khatri and Tyagi, (2015) which indicated that nitrogen nitrates concentration is high in areas with runoff from agricultural land and grasslands.

The PCA analysis for the correlation of physico-chemical water quality in different site groups (upstream, midstream, and downstream) showed that temperature and chloride were high at most of the sites in upstream. This result is in agreement with studies Sahu et al., (2020) who indicated that upper sites of hot springs are characterized by higher water temperature compared to other fresh water resources. The studies Salah et al., (2020) and Thakur (2013) found increase in chloride ions in the upstream sites of hot springs and indicated that it was resulted due to the presence of saline rocks in the upper sites of hot springs.

This study result revealed that macroinvertebrates in Wala hot spring were strongly correlated with DO, pH, nitrate-nitrogen, total hardness, and COD. Heptagenidae, Hydropsychidae, Psephenidae showed positive correlation with DO. This result is in line with the result of Degefe et al., (2017) which indicated that those species are an indicator of higher DO which, also implied that they are indicators of good water quality. Naucoridae and Gomphidae were negatively correlated with DO. This result supported the findings of Adu and Oyeniyi, (2019) who found that Gomphidae and Naucoridae can thrive in polluted water that has lower DO. Species Aeshnidae, Caenidae and Chironomidae positively responded to the increase of pH in the Wala hot spring. This result supported the study Tamiru, (2021) to some extent who found that the increase in pH values from the standard can increase the abundance of those species. The increase in pH of Wala hot spring has negatively affected Hydrophilidae Coenagrionidae and Balestomatidae. This result is in agreement with the result of Odountan et al., (2019) who indicated that pH has a negative correlation with those species.

The correlation and scatter plot analysis showed the significant negative effect of water temperature on macroinvertebrates richness and abundance in Wala hot spring. No macroinvertebrates were found at the first two sites of upstream with a temperature of 59.5°C and 45.7°C. This result is in line with those of Derso et al., (2015) who found the absence of macroinvertebrates in hot springs at a temperature above 52°C however, it contradicted the same study in the absence of macroinvertebrates in 45.7°C since the study found macroinvertebrates in a water temperature of 52°C. This might happen due to the difference in macroinvertebrates that inhabited the hot springs. Only Hydrophilidae was found at a temperature of 40.5°C. This is in agreement with the finding of the study Akünal and Aslan, (2017) which indicated that Hydrophilidaes can tolerate a temperature up to 44.5°C. Macroinvertebrate richness and abundance were increased with a decrease in temperature and relatively the highest macroinvertebrate richness and abundance was recorded in downstream of Wala hot spring with a relatively lowest temperature between 18.7°C -22.1°C. This result is in line with the studies Roux et al., (1992) and Stark et al., (2014) who found that most macroinvertebrates metabolism works best at temperatures between 5−20 °C.

Chapter seven

7. Conclusion and recommendation

7.1. Conclusion

This study investigated the distribution and composition of macroinvertebrates in the Wala hot spring southwestern Ethiopia. The distribution of macroinvertebrates in Wala hot spring was structured by species replacement rather than difference in richness. The macroinvertebrates richness and abundance has changed along human disturbance gradients. Both richness and abundance were high in sites with very low and low human disturbance compared to sites with moderate and high human disturbance. Wala hot spring can be classified as Hyperthermal based on its water temperature (>40°C) and alkaline hot spring based on is pH (7.5-9). Macroinvertebrates has responded to changing physico-chemical parameters in different sites of Wala hot spring. Macroinvertebrates richness and abundance in Wala hot spring has affected by temperature variation in the water. Macroinvertebrates richness, abundance, Simpson diversity index, Shannon diversity index and dominant families were strongly negatively correlated with water temperature in Wala hot spring.

Recommendations

Investigating contribution of species replacement (β-sim) and difference in species (β-nes) to total beta diversity is important for planning conservation. Finding of this study indicated that distribution of macroinvertebrates in Wala hot spring is structured by species replacement rather than difference in species, which implied that conservation of Wala hot spring has to target all sites. Therefore it is recommended that all sites of Wala hot spring has to be conserved

Other bio-indicators in the water have to be assessed. In the habitat assessment the presence of fish were also assessed and many fish were observed. Fish are economically important organisms and since hot spring can also be used for aquaculture, their structure in Wala hot spring has many implications.

The mineral content of Wala hot spring and its relation with the distribution and composition of macroinvertebrates were not studied because it is beyond scope and budget of this study. Other important physicochemical parameters including BOD and Fluoride were also not measured therefore future studies have to consider those parameters.

Disturbances affecting the hot spring macroinvertebrates and physico-chemical water quality have to be monitored. The potential human activities affecting the water quality and ecological integrity of Wala hot spring were identified and assessed in this study. Concerning stake holders, governmental institutions and the community have to integrated and work together to reduce human disturbance affecting the water quality which is important to improve water quality of Wala hot spring and facilitate ecotourism in the study area.

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Appendixes

Appendix 1: Wala hot spring assessment form.

General Information

1. DD/MM/YYY--Time------------------ --------
2. Name of the spring ----------------------------Sampling station -------------------- -----------
3. Altitude (m)---------------------Coordinates ------------------------,---------------- --------
4. Weather condition -- --------
5. Previous day rain history --- ---------
6. Photo number -- --------

Notes and/or sketch of the site

physico-chemical parameters (Field)

7. pH --
8. Water temperature (°C) --------------DO (mg/l)---------%--------------
   EC(μS/cm)---
9. Turbidity(NTU)----------------------------

physico-chemical parameters (laboratory)

10. nitrate-nitrogen-------------------------------Ortho-phosphate-------------------------
    -----
11. chemical oxygen demand---------------------------------
12. Chloride---
13. Total hardness------------------------------- Calcium hardness-------------------

Land use

14. Adjacent land use pattern

a. Agriculture ------------------------------ e. Road ------------------------------

b. Pasture-- f. Commercial-----------------------

c. Native vegetation------------------------------- g. Industrial -------------------------

d. Residential area--------------------------------- h. Recreational ----------------------

Habitat Assessment

15. Fauna

a. Birds(ducks)----------------------------------- c. Invertebrates----------------------

b. Fish --d. Others -------------------------

16. Anthropogenic activities

Disturbance type	Score = 1	Score = 2	Score = 3
Grazing			
Vegetation removal			
Tree plantation			
Farming/cultivation			
Waste dumping			
Washing			
Bathing			
Swimming			

17. Other Potential threats

a. Agricultural biocides---

b. Point source pollution --

18. spring ecological state

a. Unmodified, natural---

b. Largely natural with few modifications--

c. Moderately modified---

d. Largely modified---

e. Seriously modified---

f. Critically / Extremely modified---

19. Any additional comments

Appendix 2: Materials needed for the sampling.

A. Biological survey

1. Standard D-frame net 300μm mesh size
2. Sieve and bucket
3. Ethanol 80%
4. Forceps
5. Vials
6. Labelling material
7. Wading suit
8. Permanent marker
9. Sorting tray
10. Pencil
11. Clipboard
12. Pipettes

B. Water quality sampling

1. Bottle for water sample
2. Multi-parameter probe
3. Deionized water
4. Spare batteries for meters
5. Thermometer
6. Fluorometer for chlorophyll a
7. Cool box

C. Habitat survey

1. Tape measure
2. Field protocol
3. Portable GPS
4. Camera

Appendix 3: Water temperature, disturbance level, macroinvertebrates richness and abundance

Site code	Water temperature	Disturbance level	Macroinvertebrates richness	Macroinvertebrates abundance
wl1	59.5	Moderate	0	0
wl2	45.7	High	0	0
Wl3	40.5	Moderate	1	3
Wl4	36	High	2	4
Wl5	33.4	Low	5	86
Wl6	33	Low	4	11
Wl7	32.7	Moderate	6	36
Wl8	31.9	Moderate	6	29
Wl9	30.4	Low	6	64
Wl10	27.2	High	4	52
Wl11	27	Low	4	46
Wl12	25.6	Very-low	6	58
Wl13	25.1	Very-low	7	68
Wl14	23.3	Very-low	6	76
Wl15	22.1	Low	7	60
Wl16	22.3	Very-low	8	132
Wl17	21.6	Low	7	51
Wl18	19.6	Low	9	87
Wl19	19.1	Low	9	88
Wl20	18.7	High	4	76

Appendix 4: Sampled macroinvertebrates with their relative abundance

Macroinvertebrate family	Relative abundance
Hydrophilidae	4.87
Mesoveliidae	0.19
Naucoridae	4.77
Gomphidae	1.07
Caenagronidae	3.31
Belostomatidae	1.07
Ashnidae	1.46
Chironomidae	8.96
Heptagenidae	17.33
Hydropsychidae	33.98
Hydraenidae	0.39
Caenidae	17.04
Lebeliludae	1.27
Hemophidae	0.10
Geridae	0.10
Psephinidae	1.66
Tabanidae	1.17
Nephidae	0.19
Dyticidae	0.19
Tipulidade	0.29
Lestidae	0.58