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Masters of Science Thesis on

Impact of Wet Coffee Processing Plant Effluent on Water Quality and Macro-Invertebrate of Ketalla and Ajacho Rivers in Kecha-Birra Woreda, SNNPR, Ethiopia

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Dedication

This postgraduate thesis is heartily dedicated to those professionals and goodhearted individuals who strive and contributed to the improvement of polluted water bodies in making it suitable and utilized by aquatic animals and people at large. Above all, I would like to dedicate this thesis work to my lovely brother Teshome Haile who passed away before the completion of this work and really deserved my dedication. Brother may God rest your soul in peace.

APPROVAL SHEET FOR A RESEARCH THESIS DEFENSE JIMMA UNIVERSITY INSTITUTE OF HEALTH SCIENCES FACULTY OF PUBLIC HEALTH DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCE AND TECHNOLOGY

This is to certify that the thesis prepared by Gizachew Haile, entitled with "Impact of Wet Coffee Processing Plant Effluent on Water Quality and Macro-Invertebrate" and Submitted to: Jimma University Institute of Health Sciences School of Health Science & Technology for partial fulfillment of the requirement the degree of Masters of Science and technology complies with the regulation of the university and meets the accepted standards with respect to originality and quality Signed by the Examining Committee.

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Declaration

I the researcher Gizachew Haile wants to let you know that this thesis work which is entitled "**Impact of Wet Coffee Processing Plant Effluent on Water Quality and Macroinvertebrate of Ketalla and Ajacho Rivers in Kecha-Birra Woreda, SNNPR, and Ethiopia**" is my original contribution in the scientific research endeavor. And, those literature materials used in the background, statement of the problem, literature review and discussion parts of this paper are given due acknowledgment by the researcher both in in-text citation and references. Thus, I dare to give you my assurance to check it with plagiarism inspection instruments to confirm whether it is accurate or not.

Biographical sketch

The author Gizachew Haile was born in 1991 in Kecha-Birra district, Shinishicho Town. He attended his primary education in Methoma Zararo, Primary School from 1998 to 2006. Then he continued his secondary education at Shinishicho preparatory and Secondary School from 2007 to 2012. After passing the university entrance exam, he joined Haramaya University and studied his first-degree education from 2013 to 2015. He graduated with BSc degree in environmental science, he employed in Shinishicho Town administration in office. Then after two years' service he gets chance of government sponsorship to study. He joined directly to Jimma University Institute of health Sciences faculty of public health department of Environmental health sciences and technology to follow his MSc degree in environmental science and technology in 2017.

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May the Almighty God richly bless you all!!

ACRONYMS AND ABBREVIATIONS

ANOVA	**A**nalysis of Variance
APHA	**A**merican Public Health Association
AWWA	**A**merican Water Work Association
AusRivAS	**A**ustralian Rivers Assessment System
BOD_5	**5** Day Biochemical Oxygen Demand
CCA	**C**anonical Correspondence Analysis
COD	**C**hemical Oxygen Demand
CSA	**C**entral Statistical Authority
ECTA	**E**thiopian coffee and tea development and marketing authority
ENP	**E**ntry point
EEPA	**E**thiopian Environmental Protection Agency
EPT	**E**phemeropter, Plecoptera and Trichoptera
FDRE	**F**ederal Democratic Republic of Ethiopia
GPS	**G**lobal Positioning System
HFBI	**H**ilsenhoff Family Biotic Index
QHEI	**Q**ualitative Habitat Evaluation Index
TDS	**T**otal Dissolved Solids
TSS	**T**otal Suspended Solids
RIVPACS	**R**iver Invertebrate Prediction and Classification System
SPSS	**S**tatistical Package for Social Studies
UNECD	**U**nited Nations Conference on Environment and Development
USEPA	**U**nited States Environmental Protection Agency
WHO	**W**orld Health Organization
RBP	**R**apid Bio-assessment Protocols
K-DS	**K**etalla Downstream
K-ENP	**K**etalla Entry Point
K-UPS	**K**etalla Upstream

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Abstract

Freshwater resources are increasingly subject to pollution because of escalating human population growth, accompanied by urbanization, industrialization, and the increased demand for food. Consequently, freshwater quality and aquatic ecosystem structure and function have been severely impaired. The use of benthic macro-invertebrates together with physicochemical parameters was considered as the reliable method of water quality monitoring. The present study was conducted at Kecha-Birra district, SNNPR to assess the impact of wet coffee processing plant effluent on benthic macro-invertebrates and water quality of rivers that receiving coffee wastewater. In this study, environmental impact assessment was conducted by comparing UPS (reference) site with DS locations during peak coffee processing period. Physicochemical, biological and QHEI surveys were applied in data collection. Cross-Sectional study design was used in this research. Three composite replicates were taken in each refinery and on 7 sampling sites. In current study, physicochemical parameters of coffee wastewater on average consist of a low amount of (DO=5.7), (pH=6.7) and DO (>1) at the site of row influent sampling sites. The study revealed slightly increased level of organic pollution at location at ENP and below coffee effluent discharge points. One way analysis of variance (ANOVA) showed insignificant variation ($P<0.05$) for all measured physicochemical parameters except BOD5 and COD. In present study, results of some of physicochemical parameters were out of Ethiopian and WHO environmental protection agency water quality standards. Furthermore, for biological analysis 450 macro-invertebrate individuals were identified belonging 30 different taxa. In this study, (%EPT), EPT and Chironomidae ratio, Simpson, Shannon, Evenness, FBI and Odonata matrices were used to understand water quality. Except, FBI and %Odonata all selected index was lower at DS site. Besides, QHEI score result displays that except the Ajacho DS site all values were at the range of good water quality condition (55 % to 69%). Generally, the study depicted that the Study Rivers was not affected by physical habitat degradation but, somewhat affected by wet coffee processing effluent. The government (stockholders) not only put rules and regulations for wet coffee processing plant owners and crews. However, prior to punishment they should create awareness and support how to design & construct modern wastewater stabilization ponds or other waste water trearment technologies. .

Key Words: Macro-invertebrates, Taxa, Effluent, Influent, Water Quality, Biotic Index, Physicochemical Parame

1. CHAPTER ONE: GENERAL INTRODUCTION

1.1 Background of the study

Water is one of the keys to both natural ecosystems and human development. It is essential for various activities such as drinking, cooking, industrial, agricultural and recreational purposes. Rivers are vital component of the biosphere that contains less than one percent of the world's fresh water with their higher ecological and social significance which are being polluted by indiscriminate disposal of sewerage, industrial waste and by excess of human activities affecting their physicochemical characteristics and leads to various deleterious effects on aquatic organisms (Annalakshmi & Amsath, 2012).

Two-thirds of about 70% of the earth's surface is covered by water. From the 70% of water coverage only less than 1% of the water on the planet is readily available for drinking or for most agriculture. About 97% water on the earth is saltwater stored in the oceans; only 3% of water on the earth is freshwater. From all of the fresh water on earth, 68% is in the icecaps of Antarctica and Greenland, 30% of it is freshwater in the ground, and only 0.3% covers surface waters, such as lakes and rivers (Tenalem, 2009).

Ethiopia is known for its high tableland waters and is often called 'the water tower of East Africa' because of the existence of many out-flowing rivers to the neighboring countries. There are nine major international rivers, 12 large lakes, and at least eight artificial reservoirs distributed throughout the country. The total surface area of inland waters is about 8,800 km^2; representing 0.72 % of the total surface area of the country (Greboval, Bellemans, & Fryd, 1994). River systems are among the ecosystems more sensitive to pollution. Rivers have always been the focus of human settlement and recreation. The water is used for transportation, irrigation, recreation, and development of port and boating facilities. Water pollution happens when the water becomes overloaded with too much of one thing and the aquatic organisms cannot keep up with their cleaning responsibilities. Some organisms may die and others may grow too fast.

There are two major water pollution sources; they are the point of water pollution and non-point water pollution sources. Point source pollution is a single identify localized source. Point sources are relatively easy to identify, quantify and control. Point sources of water pollution include discharge from municipal sewage treatment plants and industrial plants (Peavy, Rowe, & Tchobanoglous, 1985).

Coffee is cultivated in many parts of Ethiopia, but based on the ecological requirements of the crop and volume of the production, Southern Ethiopia is the most important region of the country producing Arabica coffee and an original home of coffee along with the highest diversity in its genetic resource (ITC, 2002) (Rani, Vijender, & Ashok, 2008). In Ethiopia, most commonly Coffee is processed by two well-known methods - dry (30%) and wet (70%) coffee processing. Its superior quality and cheap human power to carry out the process make to an enormous number of wet coffee processing plants will exist in the vicinity of water in the country. The number of wet coffee processing stations in Southern Ethiopia has increased tremendously in recent years which in turn has resulted in the generation of processing byproducts mainly coffee pulp and effluents. It is one of the major agro-based industries which are responsible for fluctuating freshwater ecosystems (Selvamuruga, 2010).

The sewage produced during wet coffee processing results foul-smelling in the downstream of the river and intensifies the growth of disease-causing microbes in the surface water bodies (Mburu J. J., 1994). Coffee effluents are the main source of organic pollution in environment where intensive coffee processing is practiced without appropriate by-product management systems. Environments that are affected by coffee processing plants display change in terms of their physical, biological and chemical behavior (Asrat, 2014).

The most widely used bio-assessment system for running waters is to analyze the community composition of benthic macroinvertebrates. Benthic “macroinvertebrates” are bottom-dwelling invertebrates large enough to be seen with the naked eye and when their communities are impaired by human activities are presumed to show predictable changes in composition in response to changes in water quality (Klemm, et al., 2002). Predictable responses of benthic macroinvertebrates to reduced water quality include decreased in taxonomic diversity and an increase in the relative abundance of taxa resistant to perturbation at the expense of sensitive taxa (Karr & Chu, 1999).

Aquatic macroinvertebrates are ubiquitous, and their sensitivity to environmental changes makes them good indicators of water condition. Therefore, the use of benthic macroinvertebrate community metrics and/or a multi-metric method for evaluating biological impairment in streams has become a familiar approach in many developed countries.

Nevertheless, the utilization of macro-invertebrates for routine surface water quality monitoring was very limited in Ethiopia. Recently, attempts have been made to use invertebrates as indicators of water pollution (Beyene & Worku, 2005); (Baye, 2006); (Birenesh, 2007). Numerous taxa play to biodiversity in surface water ecosystems. Aquatic macro-invertebrates contribute a central ecological role in many running water ecosystems (Boulton, 2003) and are among the most omnipresent (Voelz & Mcarthur, 2000) and diverse organisms in freshwaters. Despite the essential roles played by macroinvertebrates in aquatic ecosystems, it is apparent that macro-invertebrate research (their diversity and anthropogenic impacts on them) in Ethiopia is limited (Strayer, 2006).

People around the area use the water for domestic consumption and for other activities like irrigation of garden areas (Ademe & Alemayehu , 2014). In general there was water pollution problem from coffee processing plant effluent on the water quality of discharge water body. In addition to water quality, the richness of benthic macro invertebrate's community composition was used to monitoring water pollution. Moreover, in the study area (Ketalla and Ajacho Rivers) has wet coffee processing plants which discharge their wastes directly into the rivers or stored the waste around rivers. So this study was done to assess the macroinvertebrate community and the impact of the effluent on the water quality.

1.2 Statement of the problem

The main problem associated with the quality of water is the bad/poor quality of the river water which has its impact on the domestic uses. Different studies indicate that wet coffee processing plants discharge considerable amounts of physical and chemical pollutants in their wastewaters. Some of these chemical compounds resist normal wastewater treatment and may end up in surface water where they may influence the aquatic ecosystem and affect with the food chain (Pauwels & Verstraete, 2006). Untreated liquid waste from traditional and modern industries is treating surface waters Worldwide and it is severed in developing countries. Ethiopia is one of the most known countries that produce coffee in the world.

There are various dry and wet coffee processing plants in the country and they discharge their byproducts into the nearby environment especially, into watercourses. The wastewater produced by wet coffee processing plants is one of the common pollutant sources of water which is discharged into the rivers in many cases without any treatment or in some cases disposed into unprotected wells lagoon. Thus causing many severe health problems, these are spinning sensation, eye, ear and skin irritation, stomach pain, nausea, and breathing problems among the residents of nearby areas (Asrat, 2014). In such conditions the coffee wastewater emanated from wet coffee processing plants in Kecha-Birra Woreda, SNNPR is the valuable resource, it is disposed into the nearby watercourses without any treatment. Consequently, it becomes a severe threat to the aquatic ecosystem and downstream users.

To limit this problem, understanding the nature and characteristic of wet coffee processing wastewater composition is fundamental by comparing its quality with raw river water quality on the bases of standards put by legislative bodies. Thus, the main aim of this study was to assess the quality of wet coffee processing plant byproduct i.e. coffee effluent and its effects on the quality of (Ketalla and Ajacho) downstream river water through characterization of some physical, chemical parameters and observations in the field to determine physical nature of the receiving environment.

1.3 Research Questions

- What is the current health status of water quality and benthic macroinvertebrates of Katella and Ajacho rivers rivers exposed to wet coffee processing plant effluent?
- What is the current water chemistry of study rivers?
- What are the factors that influence the existence of benthic microinvertebrates in the study rivers?
- What is the current physical habitat health status of study rivers?

1.4 Significance of the study

River systems are among the ecosystems more sensitive to pollution. Rivers have always been the focus of human settlement and recreation. The water is used for transportation, irrigation, recreation, and development of port and boating facilities. Human activities can influence the distribution, quantity and chemical quality of water sources. Human activities are the main contributor to the pollution loading in aquatic systems. Industrial, commercial and domestic effluents that are directly released in aquatic bodies are the major pollutants in freshwater systems.

However, river pollution is a common problem in developing country, there is no well-developed water quality monitoring tool in Ethiopia. As known our country has no well-organized water quality monitoring tool. Consequently, the results of the study will be used by environmental authorities, regulatory agencies & the scientific community on how to work together to establish sustainable coffee production that is economically viable, environmentally amendable and maintain ecologically and develop management options to regulatory agencies.

Furthermore, the study will formally show direction to the concerned government agencies to take control measures for generating coffee waste from the plant. The public at large will be also benefited from the application of law and regulation by concerned authorities; they get clean water for their intended activities.

1.5 Objectives of the study

1.5.1 General objective

❖ To assess the impact of wet coffee processing plant effluent on water quality and benthic macroinvertebrates of Katella and Ajacho rivers in Kecha-Birra Woreda Southwest, Ethiopia.

1.5.2 Specific objectives

- To determine physicochemical parameters of study rivers
- To evaluate the downstream water quality and benthic macroinvertebrate assemblages compared to the upstream (reference) site in the study rivers
- To determine the habitat integrity of the rivers using visual-based physical habitat assessment in the study rivers
- To determine the factors that affecting water quality parameter and benthic macroinvertebrate assemblage

1.6 Limitations of the study

- On top of this, scientific communities have been arguing on the ontological and epistemological stance they have about the nature of reality and its comprehension.
- Hence, it was challenging for me to compromise the subjective and objective reality.
- Because integrating qualitative data for the purpose of triangulation and enhancing the quality findings/results.
- Even though it is advisable to use a mixed research approach in the current scientific endeavor, it is tiresome to implement it as integrated use.

2. CHAPTER TWO: LITERATURE REVIEW

2.1.1 Water resource in Ethiopia

Ethiopia is known for its high tableland waters and is often called ‘the water tower of East Africa’ because of the existence of many out-flowing rivers to the bordering countries. There are 12 major international rivers, over 12 major swamps, 12 large lakes, and at least eight artificial reservoirs distributed throughout the country. The 123.25 billion m^3 of water flow annual from these 12 rivers and can say ‘Ethiopia is the water tower of East Africa’. This potential is not fully utilized and translated into development because of many factors including limited finacial resources, technical challenges, and lack of good governance in the water sector (Belete, 2014). The water tower of East Africa said in terms of receiving and donating the sufficient water with adjacent countries (Tenalem, 2009). The total surface area of surface waters is about 8,800 km^2, representing 0.72 % of the total surface area of the country (Greboval, 1994). While immense surface water and groundwater availability is recorded in the country, Ethiopia has one of Africa’s lowest rates of access to fresh water supply, sanitation, and hygiene service (CSA, 2007).

Rivers are the most important freshwater resources. They support and maintain different aquatic ecosystems. They carry water from the mountains to the sea, fueling the water cycle coupling land, ocean and the atmosphere (Karr & Chu, 1999). The social, economic and political development of mankind has, in the past, been largely related to the availability and distribution of freshwater contained in riverside systems. The Egyptian, Mesopotamian, Chinese and Indian civilizations were centered along the rivers of Nile, Euphrates and Tigris, Yellow and Yangtze Rivers and the Ganges River, respectively.

Among the most critical challenges facing global society is the failure to maintain and improve environmental quality to achieve sustainable development. Although developing countries have established policies, laws, and formal governmental structures to monitor and control environmental pollution, they fail to implement and enforce them to protect the environment (Bell RG, 2002). Most of these environmental policies and laws of developing countries are based on those developed in North America and Europe with slight modification or as a direct copy without considering the availability of local technologies and resources.

This might be one of the impediments to their implementation (Tedla & Lemma, 1998). Due to the financial and employment problem water resource of Ethiopia densely reflected as cause for environmental degradation (Mekiso, 2004).

2.2 Major Sources of Water Pollution

In the last five decades; because of the increased natural and man-made impacts, pollution of the human environment has been highly increased (Beasley & Kneale, 2004) (Welch, 2000). Untreated wastewater discharge was one of those factors but, the issue has given less attention (Dahl, Johnson, & Sandin, 2004). Pollution of the aquatic environment is defined as introduction of substances (wastes) into the system which can result in such deleterious effects as harm to living resources, hazards to human health, hindrance to aquatic activities including fishing, impairment of water quality with respect to its use in agricultural, industrial and often economic activities and reduction of amenities (Meybeck & Helmer, 1996).

According to the World Health Organization (WHO), wastes are usually referred to as "something" which the owner no longer wants at a given time and space and which has no current or perceived market value. Pollution may result from point sources or diffuse sources (non-point sources). An important difference between a point and a diffuse source is that a point source may be collected and treated or controlled (diffuse sources consisting of many point sources may also be controlled if all point sources can be identified). The major point source pollutions to freshwaters originate from the collection and discharge of domestic wastewaters and industrial wastes (Meybeck & Helmer, 1996). Human activities such as agriculture, housing and recreation contribute to the degradation of water quality of the lake. Excessive loadings of pollution into river, lakes, reservoir, and estuaries have become contribution to the major cause of water pollution. Source of pollution can be categorized into two types, point source pollution, and non-point source pollution. Point source pollution is a single identify localized source.

Point sources are relatively easy to identify, quantify and control. Point sources of water pollution include discharge from municipal sewage treatment plants and industrial plants (Peavy, Rowe, & Tchobanoglous, 1985).In coffee producing countries, coffee wastes and by-products constitute a source of severe contamination and a serious environmental problem (Rajkumar & Giorgio, 2005).Degradation of water resources has long been a concern of human society.

The earliest anthropogenic threats to water resources were often associated with human health, especially disease-causing organisms and oxygen-demanding wastes (Meybeck & Helmer, 1996).

2.3 Industrial wastewater

Industrial effluents are the major source of pollution of water and air in the environment. Based on the types of industry, various levels of a pollutant can be discharged into the environment directly or indirectly through public sewer lines. The operation and processing of many industries generate Wastewater. Depending on the industry and their water use, the wastewater contains suspended solids, both degradable and non-degradable organic matter, heavy metal ion, dissolved inorganic acids and coloring compounds (Kanu, Ljeoma, & Ach, 2011).The industrial wastewater is significantly different in both movement and contamination strength. Also the wastewaters from industrial discharge may contain suspended, colloidal and dissolved organic mineral. Industrial wastewater is excessively acidic or basic and also comprises high or low concentrations of bleached matter. It also affected by inert, organic, toxic materials and perhaps by pathogenic bacteria. These wastes discharged into the drain system without effective treatment and have undesirable effects on the drain system.

The solid content, color, odor, and temperature are physical characteristics of industrial wastewater. The solid in wastewater contains the insoluble, suspended solids and soluble compounds dissolved in water. Color is a qualitative characteristic used to assess the general condition of industrial wastewater (Alturkmani, 2014). Wastewater with light brown color is less decomposition, light-to-medium grey color is characterized by some degree of decomposition and when the color is dark grey or black, the wastewater is characteristically septic. The odor of fresh wastewater is usually not offensive, but a variety of odorous compounds are released when wastewater is decomposed biologically under anaerobic conditions (Alturkmani, 2014). The biochemical oxygen demand (BOD), chemical oxygen demand (COD) and entire organic carbon (TOC) are the components of industrial wastewater quality determination. Specific organic compounds are determined to assess the presence of priority pollutants". The BOD, COD, and TOC are gross measures of organic content and do not reflect the response of the industrial wastewater to various types of biological treatment technologies (Metcalf & Eddy, 1991).

2.4 The Coffee Tree

The coffee tree is a shrub with a straight trunk that can survive for about 70 years (Mutua, 2000). Botanists classify Coffee as a member of the Rubiaceous family. However, commercial coffee production relies on two species of coffee Arabica (Coffea Arabica) and Robusta (Coffea Canephora). Coffee Arabica is considered as a superior quality coffee, and contributes over 65 percent of the world's coffee production, while Robusta currently makes up around 35 percent (Scholer, 2004).

2.5 History of Coffee Production in Ethiopia

Ethiopia is the homeland of Arabica coffee and one potentially producing countries in Africa. According to (ITC, 2002).Likely Ethiopia is very high coffee producing country because of the country's suitable altitude, rainfall, temperatures, and fertile soil. It grows well under the large indigenous trees such as the *CordiaA byssinica* and the Acacia species, in two regions of the country Oromiya and Southern nation nationality and people regional state (SNNPR). Ethiopia is the birthplace of coffee Arabica and has the best inherent potential for production (FDRE, 2006). Coffee farming systems in Ethiopia are conventionally divided into four categories: forest coffee, semi-forest coffee, garden coffee, and semi-modem plantation. Yields are considered to be very low compared to other countries, with estimates of less than 200 kg per ha for forest coffee and around 450-750 kg per ha for semi-modem coffee plantations (FDRE, 2003).

2.5.1 Coffee processing

After harvesting coffee berries are taken to where coffee processing plants are found. There are two ways in which coffee can be processed. These are wet and dry coffee processing methods. In most cases wet coffee processing is a higher quality product, but some areas prefer dry-processed coffee for its fuller flavor, however, waste products are generated from both coffee processing methods (Bressani, 2003).

2.5.2 Wet coffee processing method

Wet coffee processing is widely accepted for the selection of ripe coffee fruit which is essential for producing good quality coffee beans.

Approximately half of the world coffee harvest is processed by the wet method in which the coffee berry is subjected to mechanical and biological operation in order to separate the bean seed from the outer red skin, white flesh pulp, and the endocar (Gieljam, 2010).
In the fermentation reservoirs the grains remain between 12 and 36 hours, depending on the temperature, the thickness of the mucilage layer and the concentration of enzymes. Then, mucilage layer is fermented through a combination of microbial activity and the work of endogenous enzymes contained within the mucilage (Adams, 2006). The process is finished after the grains are washed to eliminate the last remnants of decomposed mucilage. Finally, coffee brought to this mill is to be sun-dried or by using electrical dryer. When it is dried the parchment is manually or mechanically removed then, green coffee is stored in silos and is ready to be used and exported (Gieljam, 2010).

2.5.3 Dry coffee processing method

In the dry method, the coffee cherries are dried immediately after harvest by letting in the open field to be sun-dried. After the cherries have lost almost all their water content, they are ground or hulled to eliminate the dehydrated mucilage, the pectin, the pericarp, and the parchment. This can be done by hand using a pestle and mortar or in a mechanical huller. The mechanical hullers usually consist of a steel screw, the pitch, of which increases as it approaches outlet so removing the pericarp (Chellamuthu, 2000). Finally, the green coffee is stored in silos and is ready to be exported or used.

2.5.4 Nature and characteristics of coffee wastewater

Wet coffee processing effluents are complex mixtures of chemicals, varying in composition over time and from system to system as well as on coffee diversity. (Rani, Vijender, & Ashok, 2008) noted that, effluent from wet coffee processing plants are highly colored, acidic and contain non-biodegradable compounds, and are high in biological and chemical oxygen demand. Coffee wastewater had high concentrations of suspended solids, dissolved solids, and elevated nutrients. Moreover, it usually has high amount of conductivity, lower dissolved oxygen and elevated amount of turbidity to nearby water bodies (JARC & EIARC, 2007).

The wastewater from wet coffee processing can be basically divided into two parts. Firstly the pulping water with a high content of quickly fermenting sugars using enzymes from the bacteria on the coffee cherries.

Secondly, depending on the processing method applied the water from 9 fermentation/washing or the thick effluents from the mechanical mucilage removers. The main components of coffee pulp are ether extract, crude fiber, crude protein, ash, nitrogen fiber extract, tannin, reducing sugars, and caffeine. On the other hand, the second effluents released from fermentation/washing of mucilage are mainly composed of water, protein, sugar, pectic acid and ash (Vossen, 2005).

In general, the characteristic features of coffee wastewater are may be classified as physical-chemical and biological characteristics. Physical parameters include color, odor, temperature, electrical conductivity and turbidity, suspended and dissolved solids. Chemical parameters are associated with the organic content of wastewater include the Chemical Oxygen Demand and Biochemical Oxygen Demand, Total Organic Carbon, Total Nitrogen, Total Phosphorus, Orthophosphate, (Manahan, 2001).

2.5.5 Water Quality

Depending on the processing technology applied, quantities of coffee wastewater are varying. Modern mechanical mucilage removal machines producing semi-washed coffee use only about 1 m^3 per ton fresh cherry (without finish fermentation and washing) whereas the traditional fully washed technique without recycling uses up to 20 m^3 per tonne cherry (Mburu & Mwaura, 1996). In order to treat wastewater properly and at reasonable costs, the amounts of wastewater must be minimized.

2.5.6 Organic Components

The main pollution in coffee wastewater stems from the organic matter set free during pulping when the mesocarp is removed and the mucilage texture surrounding the parchment is partly disintegrated (Mburu & Mwaura, 1996). Pulping water consists of quickly fermenting sugars from both pulp and mucilage components.

2.6 Physicochemical parameters of water

2.6.1 Water temperature

Temperature of water is a very important physical parameter to assess thermal pollution and associated effects on aquatic biota. This is because abnormal water temperature alters chemical reactions, reaction rates and solubility of gases. Temperature affects the growth and reproduction of aquatic organisms. A sudden change in the temperature of river water can lead to a higher rate of mortality of aquatic biota (Fakayode, 2005).

Temperature affects the speed of chemical reactions, the rate at which algae and aquatic plants photosynthesis, the metabolic rate of other organisms, as well as how pollutants, parasites, and other pathogens interact with aquatic residents. It is important in aquatic systems because it can cause mortality and it can influence the solubility of dissolved oxygen and other materials in the water column (e.g., ammonia) (Buren & Marsalek., 2000).

2.6.2 Electrical conductivity

Conductivity is the ability of a substance to conduct electricity. The conductivity of water is a more-or-less linear function of the concentration of dissolved ions. The electrical conductivity of the water is also be related to total concentration, concentrations of the dissolved substances, their ions in the water, their valence charge and mobility. If the conductivity of a river or a stream suddenly increases, it indicates there is a source of dissolved ions in the neighborhood. Therefore, conductance can be used to detect pollution sources (Stoddard, 1999). According to AWWA (2000) conductivity measurements can be used as a quick way to locate potential water quality problems. Thus changes in conductivity of water samples may show signal changes in mineral composition of water, seasonal variation in reservoirs, and pollution of water from industrial wastes.

2.6.3 Turbidity

Turbidity refers to water clarity and is used to indicate water quality and filtration effectiveness. It is related to the scattering of light by fine and suspended particles that cause water to have a cloudy appearance.

Turbidity is mainly caused by suspended matter or impurities and the major source in the open water zone of most rivers are typically clays and silts from soil erosion, suspended bottom sediments, building and road construction, urban runoff, decaying plants, industrial wastes, and organic detritus from the stream and/or water discharges. Elevated concentrations of solids affect the clarity of the water (USEPA, 2011).

2.6.4 Total suspended solids

Total suspended solids- include all particles suspended in water, which will not pass through a filter. Suspended solids are present in sanitary wastewater and in many types of industrial wastewater. There are also nonpoint sources of suspended solids, such as soil erosion from agricultural and construction sites (WHO, 2004). Suspended particulate matters in water systems reduce clarity and contribute to a decrease in photosynthesis, act as binding sites for toxic substances and lead to increase water temperature through the absorption of sunlight.

2.6.5 Total dissolved solids

Total dissolved solids are differentiated from total suspended solids (TSS), in that the latter cannot pass through a sieve of two micrometers and yet are indefinitely suspended in solution. Total Dissolved Solids (TDS) in water originate from natural sources, sewage, urban runoff, and industrial wastewater. These solids include inorganic salts, principally calcium, magnesium, potassium, sodium, bicarbonate, chlorides, sulphates, and small amounts of organic matter that are dissolved in water (WHO, 2004). Discharge of wastewater with a high TDS level would have an adverse impact on aquatic life, render the receiving water unfit for drinking and domestic purposes, reduce crop yield if used for irrigation, increase conductivity, and exacerbate corrosion in water networks. The presence of high levels of TDS in drinking water may be objectionable (WHO, 2004).

2.6.6 pH

pH is generally lower in the effluent. It is a measure of the acidity or alkalinity balance of a solution and is defined as the negative of the logarithm to the base 10 of the hydrogen ion concentration.

The relative proportion of hydrogen and hydroxyl ions is measured on a negative logarithmic scale from 1 (acidic) to 14 (alkaline): 7 being neutral (Friedl, Teodoru, & Wehrli, 2004). Various factors bring about changes in the pH of water. The high values of pH are associated with water points that receive wastes from human activities. As acidity increases, most metals become more water-soluble and more toxic. Measurement of pH is one of the most important and frequently used tests, as every phase of water and wastewater treatment and, waste quality management is pH-dependent.

2.6.7 Oxygen

Oxygen that is dissolved in the water column is one of the most important components of aquatic systems. Oxygen is often used as an indicator of water quality, such that high concentrations of oxygen usually indicate good water quality (Carr & Neary, 2009). DO concentrations below 5mg/l may adversely affect the functioning and survival of biological communities (Chapman, 1996).

Increased organic pollutants reduce oxygen levels due to increased biological Oxygen demand (BOD); a generic term for the oxygen requiring activities of aquatic organisms. Oxygen is removed from the water, as organic materials are oxidized by biological processes (Meyrick, 2005). During aerobic decomposition, carbohydrates are converted to carbon dioxide with a demand of 1.07gm of oxygen per gram of carbon dioxide produced (Evangelou, 1998). Ammonia which is produced by decaying organic waste is also oxidized to less toxic nitrates by Nitrosomonas and Nitrobacter bacteria and this process also consumes a high amount of oxygen (Evangelou, 1998). Consequently, low O_2 levels reduce the capacity of a body of water to clean it (Vousta, 2000).

2.6.8 BOD5 and COD

According to (Woldesenbet, 2014), the COD:BOD5 ratio can be used as an indicator of biological degradability, with ratios below 5:1 indicating a high digestibility. Pulp and mucilage consume the oxygen in water, resulting in the death of plants and animals due to the lack of oxygen or the increased acidity (Pandey, 2000a). Biological Oxygen Demand (BOD) is the quantity of Oxygen required for the metabolic activities of microorganism for the biological degradation of organic matter present in water. Whereas Chemical Oxygen Demand (COD) is a method to determine the organic load of water body i.e, susceptible to oxidation.

2.6.9 Nutrient enrichment and eutrophication

Eutrophication is one of the most serious problem to the natural environment resulting from human activity and impact (Chmiel, 2009). The nitrogen and phosphorus content in the waters is a commonly used hydro chemical index for the assessment of the eutrophic potential of a river or lake (Chmiel, 2009).

2.7 Assessment of Biological Condition

The most direct and effective measure of the water body's integrity is the status of life in the water (Karr & Chu, 2000). Living communities reflect watershed conditions better than any chemical and physical measure because they respond to the entire range of biogeochemical factors in the environment. When water is no longer support living things, it will no longer support human affairs (Karr & Chu, 2000). The water quality of a stream can be measured with physical, chemical, and biological information. Surface water information (e.g., temperature, pH, dissolved oxygen) is commonly used in water quality surveys but can miss past events that would have resulted in criteria violations.

The long-term residence of macroinvertebrates in streams makes biological descriptions an effective complement to water quality characterization. Stream macroinvertebrates respond to physical changes that can be related to impacts from logging in watersheds. Stream biology is usually the most sensitive indication of stream degradation. Changes to the chemical and physical characteristics of a stream are significant if the aquatic life is affected. Processes and functions in streams that are altered by human intervention can be reflected in the biological community (Karr J. , 1997). The consequences of change in a community influence its biological integrity. In order to describe the biological integrity of freshwater streams, a standard definition was adopted. (Karr & Dudley, 1981) suggested the following to describe a system that has biological integrity:

- "A balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of the natural habitat of the region."

- To evaluate possible influences on the biological condition of sites, relationships may indicate the influence of particular categories of stressors on the biological condition of individual sites. Considerations relevant to assessment and diagnostics of biological condition are as follows:
- Evaluate the relationship of biological response signatures such as functional attributes to specific stressors.
- Hold physical habitat relationships constant and look for associations with other physical stressors (e.g., hydrologic modification, streambed stability), chemical stressors (e.g., point-source discharges or pesticide application to cropland), and biological stressors (i.e., exotics).
- Explore the relationship between historical change in biota and change in landscape (e.g., use available historical data from the state or region).

2.8 The integrity of the Aquatic Ecosystem

Freshwaters are finite resources, essential for agriculture, industry and human existence. Without freshwater of adequate quantity and acceptable quality, sustainable development will not be possible (Bartram & Balance, 1996). Aquatic ecosystems including freshwaters are threatened on a world-wide scale by a variety of pollutants as well as destructive land-use or water-management practices that affect their ecological integrity. The integrity of the river ecosystem refers to its biotic integrity (also called biological integrity). Biotic integrity according to (Karr & Dudley, 1981) is defined as “the ability of an aquatic ecosystem to support and maintain a balanced, adaptive community of organisms having species composition, diversity and functional organization comparable to that of natural habitats within a region”.

Therefore, a system has ecological integrity when its drivers (energy sources, physical habitat, flow regime, water quality, and biotic interactions) and responding biotic attributes (species distribution and abundance) have not been altered by human activities (Karr & Dudley, 1981). Conversely, a system’s integrity is compromised to the extent that its drivers and responding biotic attributes deviate from natural reference conditions. This notion of integrity is widely used as a conceptual foundation for assessing local and regional stream conditions and comparing impacts across watersheds (Karr & Chu, 2000).

2.9 Habitat characteristics

Habitat refers to all aspects of the physical and chemical environment and the biotic interactions within an ecosystem (Flotemersch, 2006). Stream habitat forms an essential component of river 'health' (Maddock, 1999) that can be used to evaluate the overall ecological integrity of a river system. Habitat assessment approach provides an independent way of matching new sites with reference sites, enabling predictions to be made that can be used to determine the potential of the stream to support and maintain biota that is comparable to those found in natural habitats (Plafkin, 1989). However, most of the rapid assessment approaches have limited ability to determine whether biological impairment results from poor water quality or from poor habitat. Therefore, the ability to predict local stream habitat features may be useful for distinguishing between the effects of water quality and the effects of habitat on biological conditions.

2.10 Quantitative measures to the habitat assessment

An evaluation of habitat quality is critical to any assessment of ecological integrity and should be performed at each site at the time of the biological sampling. In general, habitat and biological diversity in rivers are closely linked (Raven, 1998). In the truest sense, "habitat" incorporates all aspects of physical and chemical constituents along with the biotic interactions. The presence of an altered habitat structure is considered one of the major stressors of aquatic systems (Karr J. R., 1986).

The presence of a degraded habitat can sometimes obscure investigations on the effects of toxicity and/or pollution. The assessments performed by many water resource agencies include a general description of the site, physical characterization, and water quality assessment, and a visual assessment of in-stream and riparian habitat quality. The habitat quality evaluation can be accomplished by characterizing selected physicochemical parameters in conjunction with a systematic assessment of physical structure. Through this approach, key features can be rated or scored to provide a useful assessment of habitat quality (Barbour M. a., 1991). For communicating general habitat quality to the public, general narrative categories have been assigned to QHEI scores.

The habitat assessment rates the following habitat features along a continuum from excellent to very poor: bottom substrate availability, embeddedness, velocity-depth, channel alteration, bottom scouring and deposition, pool- riffle: run-bend ratio, bank stability, and streamside cover (Plafkin, 1989) (Barbour M. a., 1991). The combination of this score (physical characterization and water quality) will provide insight as to the ability of the stream to support a healthy aquatic community, and to the presence of chemical and non-chemical stressors to the stream ecosystem (Barbour M. a., 1991).

2.11 Biological Integrity Assessment of Rivers Using Macroinvertebrates

Benthic macroinvertebrates are the most common group of organisms used to assess water quality (Rosenberg & Resh, 1993). They are attractive as indicators because they represent a diverse group of long-lived, sedentary species that react strongly to human influences on aquatic systems (Cairns & Pratt., 1993). They inhabit the sediment or live on the bottom substrates of lakes, streams, and rivers and reflect the biological integrity of the aquatic ecosystem (Rosenberg & Resh, 1993). Furthermore, some groups are tolerant and are found in polluted environments, while other groups are intolerant and respond to either specific stressors or a wide array of stressor and anthropogenic disturbances (Klemm D. J., 1990) (Meyer, 1997) (Rosenberg & Resh, 1993) (Karr & Chu, 1999).

2.12 Advantages of Using Benthic Macroinvertebrates for biological integrity

The use of benthic macroinvertebrates is widespread and constitutes the basis for most aquatic biomonitoring programs currently in use (Rosenberg & Resh, 1993).

- Macroinvertebrate Diversity (Ecology sensitive) measure of environmental change and stress.
- Second, their limited mobility and relatively long life spans (a few months to year or more) make the presence or conspicuous absence of macroinvertebrate species at a site a meaningful record of environmental quality during the recent past, including shorter infrequent events that might be missed by periodic water sampling or avoided by more mobile/migratory fish.
- Third, aquatic macroinvertebrates are an important link in the food web, functioning as primary consumers (herbivores and detritivores) of plant and microbial matter that are then available to secondary consumers such as fish.

- Fourth, their abundance lends itself to statistical analysis, which can play an integral role in water quality assessment programs.
- Finally, aquatic macroinvertebrates have proven to be effective tools for communicating water and watershed issues to students, decision-makers, and the public.

2.13 Diversity Indices

There are different types of indices used to change biological information (macroinvertebrate) into water quality status of the river under investigation. Diversity indices (such as Shannon index and Simpson index) and equitability index are the most widely used indices. Diversity indices usually require a count of the total number of individuals and a total count for each of the taxa (Huges, 1978).The use of diversity indices in biological monitoring is based on the assumption that more species occur at un-impacted habitats and the total number of individuals is distributed more evenly among species than in impacted habitats (Cao, 1996). Similarly, the combination of abundance and richness in a diversity index supposedly indicates the state of the community. It is generally accepted that values of most indices decrease with decreasing water quality and low diversity supposedly indicates a stressed community that tends to be unstable (Goodman, 1975). Although these indices have been regionally developed, they are typically appropriate over wide geographic areas with minor modifications (Barbour M. J., 1999).

2.14 Biotic Indices

Benthic macroinvertebrate species are differentially sensitive to many biotic and abiotic factors in their environment. Consequently, macroinvertebrate community structure has commonly been used as an indicator of the condition of an aquatic system (Rosenberg & Resh, 1993). Biotic index systems have been developed which give numerical scores to specific “indicator” organisms at a particular taxonomic level (Armitage, 1983).

Such organisms have specific requirements in terms of physical and chemical conditions. Changes in presence/absence, numbers, morphology, physiology or behavior of these organisms can indicate that the physical and/or chemical conditions are outside their preferred limits (Rosenberg & Resh, 1993). The presence of numerous families of highly tolerant organisms usually indicates poor water quality (Hynes, 1998).

According to (Barbour M. J., 1999), Metrics (or indices) allow the investigator to use meaningful indicator attributes in assessing the status of assemblages and communities in response to perturbation. For a metric to be useful, it must have the following technical attributes:

- Ecologically relevant to the biological assemblage or community under study and to the specified program objectives;
- Sensitive to stressors and provides a response that can be discriminated from natural variation.

2.15 Multivariate statistical approaches

Pearson correlation analysis was used to relate benthic macro invertebrate’s metrics to physicochemical parameters and macroinvertebrate. The correlation coefficient (r) between parameter and benthic macro invertebrate’s indices calculated by using their index values. The correlation coefficient (r) was the degree of line association between two of the water quality parameters. All statistical analysis was performed using the SPSS statistical software Excel spreadsheet and PAST version 3.08 (Ryan, 1995).

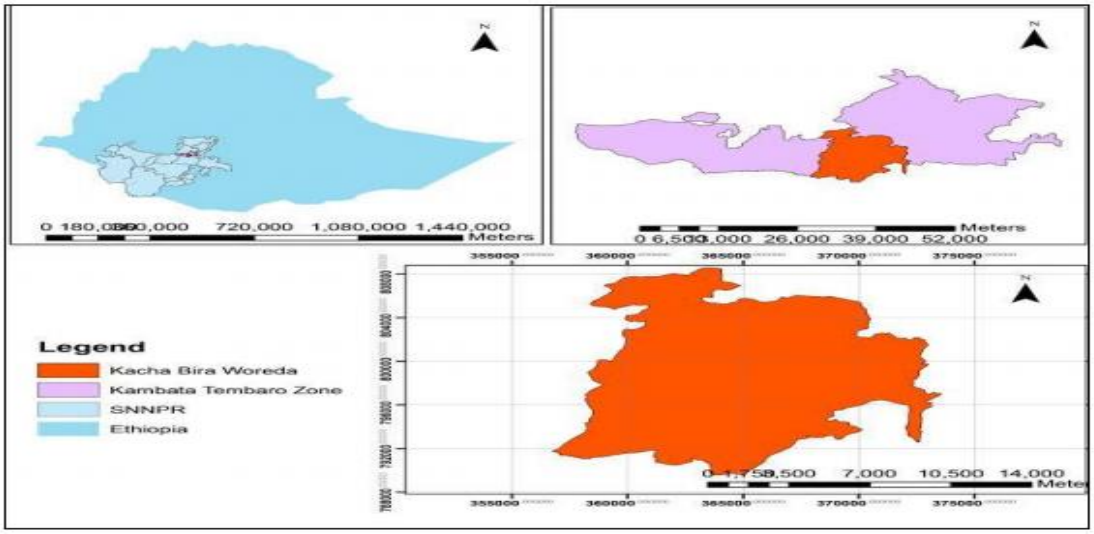
3. CHAPTER FOUR: RESEARCH METHODS AND MATERIALS

3.1 Description of Study area and period

The study was conducted in the Kecha-Birra district found in Kembata Tembaro Zone, SNNPR. Kecha-Birra is located at 136 Km away from Hawassa, the capital city of SNNPR and 297 Km away from Addis Ababa, Ethiopia. Kecha-Birra Woreda is lies between 7.1^0 - 7.34^0 latitude, 37.58^0 - 37.86^0 longitudes, and at an altitude of 1001-3000 meters above sea level. The total surface area of Woreda is about 367.9 square kilometers. The total population of Woreda is about 150,449 based on projection made from 1999 national census report (Kecha-Birra district Finance and Economy Development Office, 2009). The average temperature (^{0}c) and annual rainfall of the area are range between 20^0c to 16^0c and 800mm to 1200mm rainfall (CDP, 2012 and KBWAO, 2013).

The main economic activity of the people living in this area is agriculture. Some crops and grains cultivated include corn, bean, wheat, barley, and ginger. The coffee plant was one of the most important agricultural plants cultivated because of its high economic value in many areas of the Woreda. The major income sources for households in the Woreda are Zinjibel (ginger) and coffee (CSA, 2007). Coffee cherries are mainly processed by wet fermentation to obtain the parchment coffee (dried beans covered by paper-like coating). Subsequently wet coffee processing is a seasonal activity, the study was conducted during the harvesting period for ripe coffee cherries, which varies from year to year and usually falls between October and February, Consequently, these are the critical months for wet coffee processing. In this study to assess the impact of wet coffee processing effluent on water quality and benthic macroinvertebrates was conducted in (October, 2019) within two consecutive days.

Figure 1 Location map of the study area, Kecha-Birra district



3.1.1 Study design

In this work, laboratory-based cross-sectional study design was utilized in order to assess the impact of wet coffee processing effluent being discharged; on benthic macro-invertebrate community and water quality of receiving rivers. Water samples, benthic macroinvertebrates and physical habitat assessment data were conducted during data collection. In order to have representative samples, 14 samples for the analysis of each physical parameter were collected from all chosen sample sites at the times of peak coffee processing period, based on (APHA, 1998) guidelines of water and wastewater sampling techniques.

In addition to water samples ten (10) benthic macroinvertebrate samples were collected systematically from all selected sampling sites by kicking the substrate or jabbing with a D-frame dip net (Barbour M. J., 1999); guidelines of wastewater and macroinvertebrates sampling techniques. Furthermore, a visual-based habitat assessment was conducted concurrently with the measurement of the water quality parameters and recorded for each monitoring reach during sampling (Karr J. R., 1986). Evaluation of habitat quality is critical to any assessment of ecological integrity and should be performed at each site at the time of the biological sampling. Physicochemical biological and physical habitat measures taken in impacted sites were compared to reference site. The work further investigated the ability of the river water for different purposes based on the quality parameter indicators.

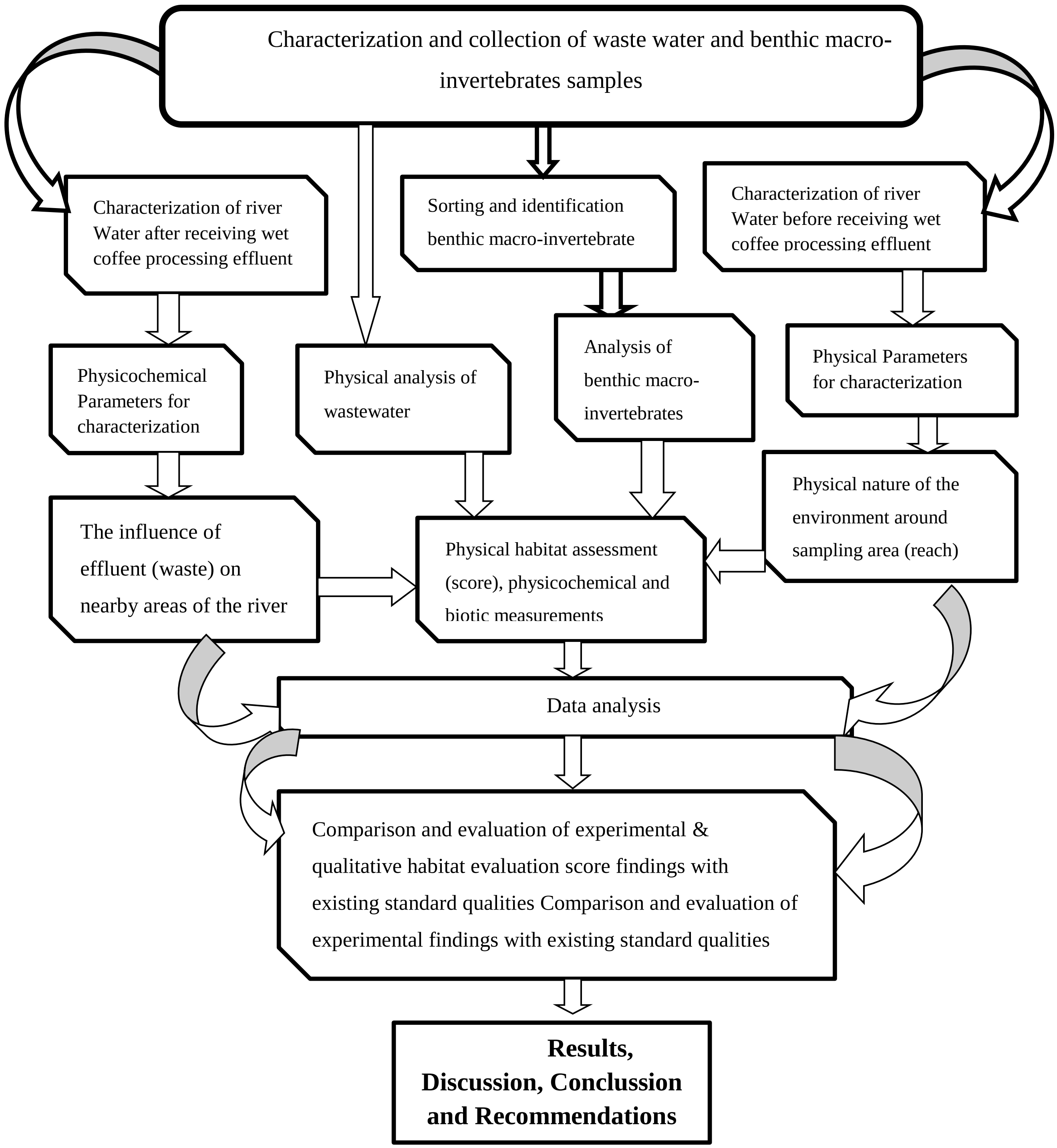


Figure 2 Schematic overview of the study

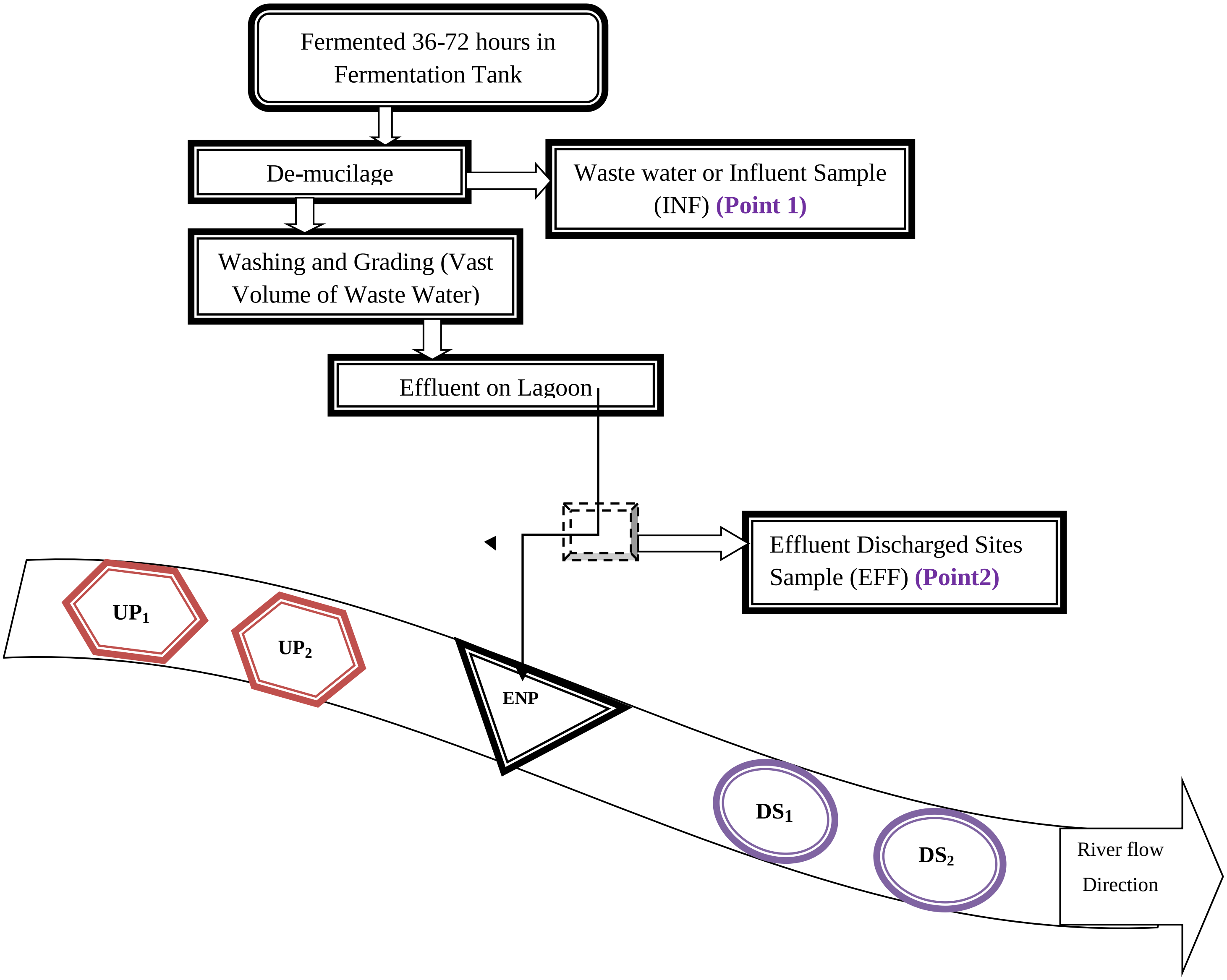


Figure 3 Map indicating general flow diagram of wet coffee processing plant and effluent sampling points (Ejeta & Haddis, 2016)

3.1.2 Sampling Sites

Sampling sites were selected based on purposely sampling methods; these were (UPS_1, UPS_2, ENP, ENF, IFU, DS_1 and DS_2). To address levels of impact to any given river, impacted site and reference approach are very essential to compare the level of impact. The reference approach is based on minimal human impact (Huges, 1978).

Finding reference sites in streams is a difficult task because no regions are entire without areas of human disturbance.

Therefore, the reference site has to be selected based on minimally disturbed attributes (Amanu, 2015). Physicochemical and biological measures taken in impacted sites were compared to reference site. The reference site was chosen because it was found in the upper stream of the river with good habitat quality and had minimal anthropogenic interventions.

The sites selected for physicochemical parameters along the course of the river.

- **(UPS_1 and UPS_2)** are located at the upstream of the river. The area was covered by distinctive vegetation and grasses. This portion was clear, clean and was chosen as the control point on the assumption that it was unpolluted.
- **(ENP of the effluent)** is located at the point of effluent from the coffee processing plant. It is outlet of the effluent and contact point of the effluent from the plant before the flow to downstream is highly impacted; it is located after the EFF and the point at which lagoon effluent enters the river.
- **(DS)** It is approximately 250m far from outlet and mainly covered by polluted water. Thus a composite sampling technique was employed to take water samples at 3 points across the width of rivers for chemical analysis (Bartram & Balance, 1996).
- **(INF)** was the point at which wastewater enters the treatment plants; in this case, lagoon (treatment plants).
- **(EFU)** is wastewater leaving the lagoon before it enters the river water?
- **($DS_1$and DS_2)** is located below ENP. Each sampling points where situated 100m blow one another respectively.

3.1.3 Inclusion and Exclusion Criterion

In this study, the researcher included samples from influent, effluent, up-stream, entry point, outlet, and dawn-stream of water samples. Benthic micro-invertebrates samples have been taken from all sampling sites except from influent and effluent sampling sites. Hence, these sampling sites are not the part of the rivers where the researcher planned to find benthic micro-invertebrates which are important for this study purpose.

3.1.4 Sampling Techniques and sample size

In this study, a total of fourteen (14) samples collected for selected sampling sites for physicochemical analysis. Samples used for the determination of all selected parameters were collected by using plastic bottles.

Prior to sampling bottles were soaked in nitric acid (HNO_3) solution, cleaned by hot water bath and then washed and rinsed repeatedly with de-ionized water. One liter of water sample was taken from each site using a one-liter plastic bottle and stored in icebox prior to analysis.

In addition to physicochemical variables ten (10), benthic macroinvertebrate samples were collected systematically from all selected sampling sites by kicking the substrate or jabbing with a D-frame dip net (Barbour M. J., 1999).

3.1.5 Selected macroinvertebrate traits

Macroinvertebrate traits selected for this study are in two broad categories: biological traits and ecological traits. Biological traits are innate characteristics of an organism, reflecting its physiological requirement, morphology, and life history attributes (Vieira, 2006). Ecological traits/characteristics are derived from an understanding of the relationship between the organism and its environment, reflecting its habitat preference, behavioral response and other aspects it employs to adapt to its environment (Vieira, 2006). Both sets of traits are capable of providing a mechanistic explanation between a species and its environment.

3.1.6 Habitat survey

The habitat conditions of the sampling sites were assessed using a qualitative habitat evaluation index (Annex 10). Six habitat parameters were used for evaluating QHEI, based on references commonly used (USEPA, 1983a) (Plafkin, 1989). Only locations where benthic data were present were considered for QHEI field investigations. The habitat attributes are summarized in (Table 2). Each principal parameter was divided into metric components and summed to provide the total QHEI site score of 100.

The health status of the physical habitat was assessed by sum of scores obtained from the six parameters and was categorized as very poor, poor, fair, good and excellent narrative range displayed in (Table 3).

QHEI Metric	Metric Component	Component Scoring Range	Metric Max. Score
1) Substrate	a) Type	0 to 20	20
	b) Origin	-2 to 1	
	c) silt cover	-2 to 1	
	d) embeddedness	-2 to 1	
2) Instream Cover	a) Type	0 to 11	20
	b) Amount	1 to 11	
3) Channel Morphology	a) Sinuosity	1 to 4	20
	b) Development	1 to 7	
	c) Channelization	1 to 6	
	d) Stability	1 to 3	
4) Bank Erosion and riparian zone	a) Bank Erosion	1 to 3	10
	B Riparian Width	0 to 4	
	c) floodplain Quality	0 to 3	
5a) Pool/Glide Quality	a) Maximum Depth	0 to 6	12
	b) Channel Width	0 to 2	
	c) Current Velocity	-2 to 5	
5b) Riffle/Run Quality	a) Riffle Depth	0 to 2	8
	b) Run Depth	1 to 2	
	c) Substrate Stability	0 to 2	
	d) Substrate	-1 to 2	
	Embeddedness		
6) Gradient		2 to 10	10
Total Maximum Score 100			**100**

Table 1 Qualitative Habitat Evaluation Index Scoring system

In addition to the physical habitat quality assessments for each entire reach, additional measures of habitat characteristics within each sampling point were recorded. Altitude, longitude, and latitude were measured using global positioning system (GPS). Proper interpretation of the significance of water quality variables in a sample taken from a river requires knowledge of the velocity of the flow of the river at the time and place of sampling (Jamie & Richard, 1996).

Thus, the river flow rate was calculated using the formula:

Flow Rate =ALC/T…………… equation 1

Where A = average cross-sectional area of the river (mean width x mean depth)

L = length of river stretch examined

C = coefficient factor for substrate type (0.9 for smooth substrate, 0.8 for rocky substrate)

T = time it took the ball to travel from the beginning point to endpoint

3.1.7 Study Variables

In this study, independent (explanatory) variables were the environmental variables or all physicochemical parameters and while; dependent (response) variables were biological variables or macroinvertebrates. Environmental variables were (WT, pH, EC, TURB, TSS, TDS, TN, NH_4^+-N, NO_3^-- N, TP, PO_4^{3-}-P, DO, COD, BOD, and QHEI score) whereas, biological variables were macroinvertebrate taxa.

3.1.8 Operational definition

- **Water quality** is a term used to express the suitability of water to sustain various uses or processes (Chapman, 1996).
- **Monitoring** is defined by the International Organization for Standardization (ISO) as "the programmed process of sampling, measurement, and subsequent recording or signaling, or both, of various water characteristics, often with the aim of assessing conformity to specified objectives" (Chapman, 1996)
- **Water quality monitoring** is the process needed to verify whether the observed water quality is suitable for intended uses, to determine trends in the quality of the aquatic environment and how the environment is affected by the release of contaminants, by other human activities, and/or by waste treatment operations (Chapman, 1996).
- **Macroinvertebrates** are invertebrates that are visible to the naked eye (larger than 0.5 mm) but smaller than 50mm.

- **Habitat** is the quality of the in-stream and riparian habitat that influences the structure and function of the aquatic community in a stream (Barbour M. J., 1999).
- **Habitat assessment** is the evaluation of the structure of the surrounding physical habitat that influences the quality of the water resource and the condition of the resident aquatic community (Barbour M. J., 1999).
- **A metric** is a characteristic of the biota that changes in some predictable way with increased human influence (Barbour M. J., 1999).
- **Eigenvalues** are values that represent the amount of the original variance explained by each of new derived variables (Quinn & Keough, 2002).
- **Eigenvectors** are lists of the coefficients or weights showing how much each original variable contributes to each new derived variable in the linear combination (Quinn & Keough, 2002).
- **Ordination** is the operation of arranging observations along in a defined order either by measurements on some pre-known environmental data (direct ordination) or along an abstract mathematical axis (indirect ordination) (Shaw, 2003).
- **A dendrogram** is a tree-like branching diagram showing relationships between data points (Shaw, 2003).

3.2 Methods of Data Collection

3.2.1 Onsite and Laboratory Analysis

Analyses for some important physicochemical variables can be carried out in the field using apparatus made specifically for field use. The on-site measurement was carried for (EC, pH, Water WT, TURB and DO. A significant advantage of field analysis is that tests are carried out on fresh samples whose characteristics have not been contaminated or otherwise changed as a result of storage in a container (Bartram & Balance, 1996). A composite sampling technique was employed to take water samples for the analyses of physicochemical parameters. All the water samples were collected in one-liter plastic BOD bottles except phosphate which requires glass bottle containers to avoid the effect of adsorption to plastic bottles (Bartram & Balance, 1996). The bottles were thoroughly washed with distilled water and rinsed repeatedly with the water to be sampled.

The water samples were collected by inserting the bottles to a 30 cm depth to the opposite direction of the course of the river flow and capped tightly immediately after filling to the tip of the mouth of the bottles (Bartram & Balance, 1996).

The measurements of EC, pH, water temperature and dissolved oxygen (DO) were carried out using a Hach multi-meter probe (P/N HQ40d multimeter). The samples were kept in ice boxes in order to arrest microbial activities and were transported to Environmental Health Laboratory of Jimma University for further analysis.

In the laboratory, (TSS) was done by gravimetric method (by using glass microfiber GF/AWhatman filter paper having 4.7 cm diameter and with a pore size of 1.6 micron) and BOD_5 was determined by the Azide modification of the Winkler's titrimetric method by determining DO contents of the samples before and after 5 days' incubation at 20 °C, the Azidemodification of the Winkler method (by using aerator TRITON 2000cc, China) were used, respectively. For the remaining parameters (COD, NO_3^--N, NH_4^+-N and phosphorous as Ortho-phosphate), LCK test kits (Hach Lange, Germany) were used and TN test was done by Kjeldahl method. The analysis of water samples was made following the standard methods for the examination of water and wastewater (APHA, 1998). The analysis methods for each parameter were as displayed in (Table 4).

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(a) Lab. analysis of physicochemical parameters ***(b) Lab. analysis of macroinvertebrates***

Figure 4 (a) and (b) Laboratory analyses of physicochemical parameters and benthic-macro invertebrates

Table 2 Physicochemical parameters selected for the study site and techniques used for a sample of analysis

S.S	Physicochemical parameters	SYM	Methods of sample analysis	Unit
1	Water temperature	WT	Probes multi-parameter methods	o C
2	Turbidity	TURB	Turbidity meter	NTU
3	Electrical conductivity	EC	Probes multi parameter methods (EC meter)	µS/cm
4	pH	pH	Probes multi-parameter methods (pH meter)	-
5	Total Dissolved Solids	TDS	Gravimetric Method, dried at 180°C	mg/L
6	Total Suspended solid	TSS	Gravimetric Method, dried at 103-105°C	mg/L
7	Dissolved Oxygen	DO	Probes multi parameter methods (DO meter)	mg/L
8	5 Day, Biological Oxygen Demand	BOD_5	The Azide Modification of the Winkler Method	mg/L
9	Chemical oxygen demand	COD	Kit (Hachlange cuvette test, LCk 614 &114)	mg/L
10	Total Phosphorus	TP	LCK test kits (Hach Lange, Germany)	mg/L
11	Nitrate-Nitrogen	NO_3-N	Phenoldisulfonic Acid Method	mg/L
12	Ammonia-Nitrogen	NH_4^+- N	Direct Nesslerization Method	mg/L
13	Total nitrogen	TN	Kjeldhal method	mg/L
14	Ortho-phosphate	PO_4^{3-}P	Stannous Chloride Method	mg/L

Abbreviation: *(SS) Sampling sites; (SYM) Symbols; (TSS) Total suspended solids; (TDS) Total dissolved solids; (NO_3^- -N) nitrate-nitrogen; (TN) Total Nitrogen; (NH_4^--N) ammonia-nitrogen; (PO_4^{3-}-P) Ortho-phosphate; (TP) Total Phosphorus; (BOD5) 5day, Biological Oxygen Demand; (COD) Chemical Oxygen Demand;(WT) Water Temperature; (EC) Electrical conductivity; (pH); (TURB) Turbidity; (DO) Dissolved Oxygen; All physicochemical parameters are measured in (mg/L) except, WT, ^{O}C; TURB, NTU; EC, µs/cm and pH no unit respectively.*

3.2.2 A sampling of benthic macroinvertebrate (Kick Sampling)

The sample was collected from (10) selected sites to make sample representative. The samples were selected using a triangular D-net (net with 500 µm mesh size) with simple stream-dwelling macroinvertebrates. During sampling, the river bed is thoroughly disturbed by feet to dislodge the macroinvertebrates from the substrate (Figures '3a' and '3b'). It was used in the riffle site by disturbing the river bed using kicking action over a distance of 10 meters for 3 minutes as described by (Beyene & Legesse, 2009), a 100-m stretch was representative of the rivers sampling in riffle area. The method was quite effective in determining relative stream health (Typical riffle) locate shallow, faster moving mud-free section of stream with a stream bed composed of material ranging in size from one-quarter inch gravel or sand to ten-inch cobbles.

Macroinvertebrate samples were conducted only once from each sampling site. After three minutes all the contents of the net were transferred into a five-liter collecting jar. To each jar 70% ethanol was added and transported to Jimma University Environmental Health laboratory for sorting and identification each group have followed family level of macroinvertebrate identification because of genus and species level of identification does not offer significant advantage over family level identification and it appeared to be unnecessary for monitoring programs (Beyene & Legesse, 2009). The number of taxa identified was listed in the (Annex 2). These samples were properly and carefully labeled, sealed and transported to the laboratory of the Department of Environmental Health Sciences and Technology, Jimma University. Cold storage was maintained throughout the process until analysis.

Ajacho river *(b) On-site sorting of macro-invertebrate* *(c) Ketalla river*

Figure 5 On-site identification and sorting of macro-invertebrate at sampling site of study rivers

In the laboratory, the collected samples were transferred into a bowl containing a sufficient amount of water, agitated and sieved with 500 pm mesh to discard the mud and retain the macroinvertebrate. This was repeated until all the macroinvertebrates were washed from the mud. The samples were then transferred to wheel trays to easily pick them up using forceps. All macroinvertebrates were sorted and identified using binocular dissecting microscope and using.

3.2.3 Analysis of samples for biological parameters macroinvertebrate metrics)

Macroinvertebrate metrics are attributes of the biological assemblage that respond in predictable and measurable ways to human disturbance (Karr J. R., 1986) (Barbour M. P., 1989). Biotic indices and multivariate analytical tools were used for data analysis. Correlation analysis was also used to assess the relationship between physicochemical and macroinvertebrates parameters. Metrics describe a sampled macroinvertebrate population in terms of its taxonomic composition, community structure, and presence of tolerant and intolerant taxa (Mandaville, 2002).

3.2.4 Biotic Indices

The biotic approach combines the relative abundance on the basis of certain taxonomic groups with their sensitivities or tolerances into a single index or score. The sensitivity and tolerance of indicator assemblages to a number of environmental characteristics, such as organic pollution, heavy metals, pesticides, eutrophication, and pH, are known to differ among species. Therefore, these species-specific pollution indications can be used to infer environmental conditions in a habitat (Tolkamp, 1985).

3.2.5 Diversity Indices

As traditional bio-monitoring approaches, many diversity indices have been developed to describe responses of a community to environment variation, combining the three components of community structure, namely richness (number of species present), evenness (uniformity in the distribution of individuals among the species) and abundance (total number of individuals present) (e.g., Shannon-Wiener Index and Simpson Index. The assumption is that un-disturbed environments are characterized by high diversity or richness, an even distribution of individuals among the species, and moderate to high counts of individuals.

The best use of diversity-related indices in the river and stream monitoring is probably as an indicator of changes in species composition when comparing impacted and reference assemblages (Stevenson, 1984).

3.2.6 Measurement of Diversity

Simpson's Diversity Index (D)-the Standard Expression Of Simpson's Index Is To Calculate The Probability That Two Consecutive Samples Will Be Of Different Species, Which Is 1- (The Probability That The Samples Will Be Of The Same Species), So Is Calculated As Follows;

$$D = -\sum_{i=0}^{s}(Pi)$$ …………………… Equation2

Where "pi" is the proportion of individuals in the "i^{th}" taxon of the community and "s" is the total number of taxa in the community.

This index places relatively little weight on rare species and more weight on common species. Being a probability, this version of Simpson's index ranges from zero (meaning zero diversity, i.e. the sample only contains a single species) to unity (meaning infinite diversity).

Shannon-Wiener Diversity Index (H)- is commonly used to calculate aquatic and terrestrial biodiversity (heterogeneity). Shannon index is probably the most widely used diversity index, although its interpretation is less simple than the Simpson index (Shaw, 2003). It is calculated from the proportional abundances of each species (abundance of the species / total abundances)

This index was calculated as;

$$H = \sum_{i=0}^{S}(pi)\ (\log 2pi)$$ ………………… (Equation 3)

Where H' is standerd symbol for Shannon-Wiener Diversity Index log(pi) is the logarithum of pi, the proportion of species i to s.

Evenness (Equitability) - as diversity is at a maximum when all species within a community are equally abundant, a measure of evenness is the ratio of the observed diversity to the maximum possible for the observed species number.

Equitability (£) or evenness is given as:

$$\mathbf{E} = \frac{\mathbf{H}'}{\mathbf{H}\,\max} = \frac{-\sum \mathrm{pi}\ \mathrm{X}\ \mathrm{Log(p)}}{\mathbf{Log(s)}} \quad \text{…………equation 4}$$

Family Biotic Index (FBI)-This Biotic Index was originally developed by (Hilsenhoff, 1987) to provide a single 'tolerance value' which is the average of the tolerance values of all species within the benthic community. The Biotic Index was subsequently modified to the family-level with tolerance values ranging from 0 (very intolerant) to 10 (highly tolerant) based on their tolerance to organic pollution. FBI was further developed by the State of New York to include other macroinvertebrates for the use of the U.S. EPA Rapid Bioassessment Protocol (Plafkin, 1989) (Bode, 1991).

FBI was calculated as:

$$\mathrm{FBI} = \sum \frac{\mathrm{xi} * \mathrm{ti}}{\mathrm{n}} \quad \text{……………………….. (Equation.3)}$$

Where "x_i" is the number of individuals in the "I^{th}" taxon, "t_i" is the tolerance value of the "i^{th}" taxon, and "n" is the total number of organisms in the sample.

Table 3 Physicochemical parameters selected for the study site and techniques used for a sample of analysis

Biotic Index (FBI)	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	Fairly substantial pollution likely
5.76 – 6.50	Fairly poor	Substantial pollution likely
6.51 – 7.25	Poor	Very substantial pollution likely
7.26 – 10.00	Very poor	Severe organic pollution likely

Source, *Mandeville 2002)*

EPT Index or EPT richness-EPT index displays the taxa richness within the insect groups which are considered to be sensitive to pollution and therefore should increase with increasing water quality. The EPT index is equal to the total number of families represented within these three orders in the sample. The ratio of EPT to Chironomidae-abundance of EPT and Chironomidae indicates the balance of the community since EPT is considered to be more sensitive and Chironomidae less sensitive to environmental stress (Plafkin, 1989). A community considered to be in good biotic condition will display an even distribution among these four groups, while communities with disproportionately high numbers of Chironomidae may indicate environmental stress (Plafkin, 1989). The EPT/Chironomidae index is calculated by dividing the sum of the total number of individuals classified as Ephemeroptera, Plecoptera, and Trichoptera by the total number of individuals classified as Chironomidae.

$$\text{Ratio of EPT} = \frac{\sum \text{EPT}}{\sum \text{Chiro}}$$ ……………….. (Equation. 4)

The Ratio of EPT to Chironomidae abundance shows the number of individuals from sensitive orders compared to pollution tolerant or (Chironomidae). A high ratio indicates low levels of water pollution.

3.2.7 Data quality control

To assure the quality of data to minimize the errors during on-site physicochemical parameters (water sample) sampling and physical habitat assessment the following measure was taken:

- All physical habitat assessment measures were conducted with well-trained personnel (professional),
- The judgment criteria for each habitat parameters (variables) was calibrated for the stream classes under study and the assessment results were completed using pictures of the sampling reach with discussion with qualified co-workers professional and,
- For physicochemical parameters, the apparatus calibrated wall, expiry date of all chemicals was checked before starting the real analysis.

Further, to control macro invertebrate quality the tray was used for sorting and to look the organism’s missed by the sorter.

3.2.8 Ethical consideration

A formal letter was taken from Jimma University, Institute of health science, department of environmental science and technology and delivered to the Kecha-Birra Woreda agricultural, animal and fish resource offices.

3.2.9 Dissemination plan

The result of this study work (thesis) will be disseminated into scientific society by presenting papers at scientific conferences of seminars and publishing in journals and books.

4. CHAPTER FOUR: RESULTAS

4.1 Pollutant sources for sampling sites and physical habitat features

Sampling points were identified based on proximity to sources of pollutants and from upstream to downstream in order to compare the response of macro-invertebrates taxa composition in relation to pollution load. The major sources of pollution for sampling sites were wet coffee processing effluent from coffee refiners.

4.1.1 Water Physicochemical Parameters

The samples taken from Kecha-Birra wereda showed variations in Physico-chemical parameters along the course of the rivers and among the rivers (Table 3). BOD_5 levels extend from 7.6mg/L at upstream site of Ketalla River to 990mg/L at Entry point (ENP) sites of Ajacho River. Although the upstream sites on average contained 12.08mg/L of BOD_5, the upstream site of K-UP_1 were found with high BOD_5 values, while it was increased to 344mg/L at the downstream sites. Nevertheless, the BOD5 values at the downstream side of Ajacho River reached to 436mg/L and 454mg/L values from 8 mg/L and 7.75 mg/L level at upstream (reference) sites. These BOD_5 values were close to the BOD_5 content recorded for the raw effluent (1,090mg/L) at Ketalla rive effluent sampling site (before it entered into the river body. Chemical oxygen demand- the maximum and minimum value of the COD level was recorded at (A-ENP, 1,238mg/L, and K-UP_2, 11mg/L) respectively. The value of COD shows drastic increment in effluent entry point and downstream watercourses. The COD values were close to the COD content recorded for raw effluent (K-ENP, 1,562) (Table 3).

Dissolved oxygen (DO) concentration values at the entry point (ENP) of wastewater and downstream (DS) sites of the rivers showed decrement compared to the upstream/reference sites at Study Rivers. The upstream (DO) values of all sampling sites were found to be more than 5.42mg/L (Table 3). There was a change/decrement in DO at the downstream stations of all sampling sites. The DO levels extended 6.9mg/L at Ajacho (A-$UP_{1,}$ 6.9mg/L and Ketalla (K-UP_2, 6.8mg/L to the (A-$DS_{2,}$ 4.47mg/L and Ketalla (K-DS_2, 5.8mg/L) respectively.

In general, the minimum DO level recorded was 4.47mg/L at the downstream side of Ajacho River also the maximum value was 6.9mg/L recorded it's at upstream sampling site River. There was a slight difference between DO values of the study site.

The minimum value of the Total dissolved solid was found at the Ajacho river upstream sampling sites (50.5mg/L). While the maximum value was recorded at (ENP) Ajacho river wastewater inlet site (197mg/L). Also the lowest amount of TSS found was 48mg/L at downstream site of Ketalla River. While highest was 360mg/L at wastewater releasing station (ENP) of Ajacho River. The average TDS value at upstream, entry point and downstream of study sites were (55.7mg/L, 151mg/L, and 122.25mg/L) respectively. Also the average TSS value at UPS, ENP, and DS sampling stations were (67.5mg/L, 217.5mg/L and 80.5mg/L) respectively. In general, the recorded value displays the level of TSS and TDS were higher in upstream than effluent receiving (ENP) and downstream sampling sites (Table 3).

A slight reduction in average pH values before (6.9) and after (6.65) the entrance of effluent was observed at sampling sites. The average pH value was 6.9mg/L, 6.4mg/L and 6.65mg/L for the upstream, inlet of the effluent (ENP) and downstream sites respectively. The mean value of the pH was high at upstream and show slightly decrease ENP and downstream sites. The minimum value of pH was recorded at the Ketalla river downstream site ($K\text{-}DS_1$, 6.1mg/L). While the maximum value was recorded at Ajacho upstream site ($A\text{-}UP_1$, 7.1mg/L). In general the average pH value of upstream sampling sites shows similar manner to Study Rivers (6.9 and 6.9) at Ketalla River and (6.5 and 6.9) at Ajacho respectively (Table 3).

The Turbidity of the water samples among the upstream, ENP and downstream was 29.73NTU, 36.95 NTU and 29.5 NTU, respectively. The turbidity level relatively shows similar manner at most of sampling sites. The average recorded value of TURB shows no difference at upstream and downstream sampling stations. While it displays only slight change at ENP site (Table 3). The average value of the water temperature recorded at upstream, entry point and downstream of sampling stations was (21.85°C, 22.1°C and 22.4 °C) respectively. The highest and lowest water temperature was recorded at downstream sites of Ajacho river ($A\text{-}DS_1$, 22.9 °C and $A\text{-}DS_2$, 22.9°C) respectively. The entire water temperature value displays a similar manner at all study sites (Table 3). The electrical conductivity level extends from 92mg/L at upstream site to 305mg/L at A-ENP of Ajacho River.

Although the upstream sites on average contained 101.75mg/L of EC and its average value were increased to 171.25mg/L at downstream sampling sites. Generally all recorded values at their respective sampling stations show increments at their downstream and entry points (Table 3). In this study the highest nutrient level of Nitrate- Nitrogen (No_3^--N), (Total Nitrogen (TN), Ammonia-Nitrogen ($NH4^+$-N), Ortho-phosphate (PO_4^{3-}-P) and Total Phosphorus (TP) was recorded at (1.17mg/L, 2.48mg/L, 0.046mg/L, 0.35mg/L and1.24mg/L at the downstream and wastewater inlet sampling sites of Ajacho river (Table). From all 4 nutrient concentrations tested the maximum was total nitrogen (2.69mg/L) which recorded at Ajacho river downstream one sampling site. While the lowest nutrient recorded was (0.027mg/L) which recorded at in Ketalla River upstream /reference sampling sites.

The average value of Nitrate- Nitrogen (No_3^- -N), (Total Nitrogen (TN), Ammonia-Nitrogen ($NH4^+$-N), Ortho-phosphate (PO_4^{3-}-P) and Total Phosphorus (TP) recorded at upstream, entry point and downstream of sampling stations was (0.62mg/L, 1.222mg/L, 0.037mg/L, 0.1575mg/L and 0.21mg/L) at upstream, (0.85mg/L, 1.96mg/L, 0.042mg/L, 0.265mg/L and 0.85mg/L) at inlet of wastewater (ENP) and (1.19mg/L, 2.269mg/L, 0.041mg/L, 0.26mg/L and 0.27mg/L) at downstream sampling sites of study rivers. Generally, there was change/increments in all nutrient enrichment values at the ENP and downstream sampling sites (Table 3).

Table 4 Physicochemical parameters characteristics of water samples of Ketalla and Ajacho rivers at Kecha-Birra Woreda at selected sites of coffee effluent discharge and reference sites.

S.S	TSS	TDS	No_3^{-}N	TN	$NH4^{+}$-N	PO_4^{3-}-P	TP	BOD_5	COD	W T	EC	pH	TURB	DO
K-UP_1	64	59.9	0.43	0.85	0.027	0.14	0.22	25	26	22.1	109	7	41.2	6.29
K-UP_2	55	60.4	0.51	1.01	0.031	0.16	0.2	7.6	11	22.1	112	6.8	46.7	6.8
K-DS_1	87	106	1.1	2.5	0.032	0.1	0.18	344	430	22.5	163	6.1	40.1	5.12
K-DS2	48	104	0.99	2.25	0.032	0.17	0.17	364	485	22.3	159	6.9	28.4	5.8
K-ENP	75	105	0.7	1.72	0.038	0.18	1.24	728	910	21.8	170	6.1	24.6	5.23
K-EFU	484	208	1.07	2.8	0.129	0.22	0.32	1,090	1,562	21.6	294	5.7	52.2	1.36
K-INF	790	350	1.24	3.54	0.594	0.18	0.33	1,626	2,732	22.8	479	5.6	138	0.71
A-UP_1	87	50.5	0.66	1.29	0.052	0.16	0.23	8	17	21.1	92	7.1	21.9	6.99
A-UP_2	64	52	0.88	1.74	0.038	0.17	0.19	7.75	25	22.1	94	6.7	9.12	5.42
A-DS_1	76	130	1.19	2.69	0.032	0.24	0.26	436	545	22.9	199	6.8	29.1	5.43
A-DS_2	111	109	1.15	2.5	0.041	0.26	0.27	454	567	21.9	164	6.8	20.5	4.47
A-ENP	360	197	1	2.2	0.046	0.35	0.46	990	1,238	22.4	305	6.7	49.3	5.73
A-EFU	1,326	759	2.01	5.25	0.043	0.81	1.08	1,575	1,978	21.8	1085	5.5	113	0.84
A-INF	1,540	1,609	2.3	6.4	0.056	0.9	1.14	3,282	4,152	22.4	2300	4.7	301	0.24

Abbrevations *K-UPS_1, K-UPS_2 A-UPS_1 and A-UPS_2 (Ketalla and Ajacho rivers) sampling points above effluent discharge (references sites); K-DS_1, K,-DS_2, A-DS_1 and A-DS_2= sampling points below effluent discharge; K-ENP and A-ENP inlet/entry points of wastewater and EFU and INFU sampling points of raw effluent and influent of both rivers; all units except pH and Temperature and Turbidity are in mg/L*

4.2 Biological assemblage

4.2.1 Descriptive Analysis of Macroinvertebrate Data

From selected sampling sites of the different two rivers in Kecha-Birra Woreda Southern, Ethiopia benthic macro-invertebrates were collected to assess and evaluate the quality of upstream, downstream and entry point/outlet point of river water that receive discharge from wet coffee processing stations. A total number of 271 individual taxa from Ketalla and 179 individuals from Ajacho River totally 450 taxa which belong, 30 families, were collected from 10 selected sampling sites.

All macro-invertebrates taxa collected and identified samples from the two rivers are indicated in table (Annex 3). Out of 450 recorded individual macro-invertebrates highest number of pollution resistant taxa was found at ENP and DS of effluent receiving sites. The highest number of individuals was found at upstream sites and in contrast lowest individuals were recorded at downstream sampling sites.

4.2.2 The Benthic Macroinvertebrate Metrics

In order to understand the effect of wet coffee processing discharge on the biotic environment of the rivers, different diversity indices were tested (Table 4). These indices would indicate the environmental impact of wet coffee processing effluent discharge on the receiving water bodies. The diversity and distribution of the benthic macro-invertebrate species were estimated by calculating the benthic macro-invertebrate index such as Shannon Weaver diversity index, Simpson's Diversity Index (D), Family biotic index, evenness of species, (%Odon) present of Odonata family, (% EPT) present of Ephemeroptera, Plecoptera and Trichoptera (EPT/Chiro) Ratio of Ephemeroptera, Plecoptera and Trichoptera to Chirnomidea (Table 4).

	UPS	ENP	DS
Taxa	8	6	2.73
Individuals	50	43.5	12.67
Dominance.	0.18	0.36	0.18
Simpson index (D)	0.81	0.63	0.81
Shannon index (H')	1.8	1.29	1.83
Equitability/Evenness.	0.84	0.66	0.84
Family biotic index (FBI)	4.3	4.69	4.39
% Odonata	0.024	0.165	0.024
% EPT	0.69	0.0704	0.691
EPT/Chirnomidea	16.61	0.142	0

Abbrivation: UPS, upstream; ENP, Entry Point; DS, Downstream

Table 5 Average biotic indices at the upstream, entry point and downstream sites of study siver

According to the Shannon-Wiener Diversity Index, the result obtained in upstream, at the outlet point and downstream shows difference in water quality (Table 4). The Shannon Wiener index recorded at the coffee processing wastewater receiving sites (ENP and DS) of study site displays lower (H') value than all upstream sites (reference sites).

The H' obtained at the upstream was higher value than the other. The result obtained was (1.8) at upstream (1.29) at the point of outlet and (1.83) at the downstream (Table 4)

The standard expression of Simpson's index is to calculate the probability that two consecutive
samples will be of different species and this version of Simpson's index ranges from zero (no diversity) to unity (infinite diversity). The highest Simpson's diversity index of the macroinvertebrate community of the study sites was found at the upstream one and upstream two sampling sites of K-UP_1 (0.878) and K-UP_2 (0.821) respectively. While the lowest diversity index was found at the Ketalla river effluent outlet point K-ENP (0.603) and Ajacho downstream one (K-DS1 (0.632) sampling sites respectively (Table 4).

A similar pattern between Simpson's and Shannon's indices was observed. Thus, the highest Shannon index was found at the upstream side of Ketalla (2.241) and upstream site of Ajacho rivers (2.083) while the lowest Shannon index was found at the A-ENP sites of Ajacho river (1.208 and A-UP_2 Ajacho river (1.224). Similarly, Shannon index decreased from 1.8 at the upstream sites to1.29 at the outlet/ENP sites and displays similar manner with downstream sites. But the Equitability index which describes the evenness of species distribution within the site showed completely similar pattern. On average the Equitability indices found at Upstream, ENP/outlet and Downstream site were (0.84, 0.66 and 0.84) respectively (Table 4).

The highest number of %EPT individuals was found at the upper course of Ajacho river A-UP_1 sampling site (0.849) followed by Ketalla river (K-UP2) sampling site (0.8392), while the lowest %EPT taxa were found in almost all of the downstream sites of study rivers. The average % EPT taxa at the upstream, ENP and downstream sites were 0.69, 0.07 and 0.691 respectively. The average recorded value displays that the value of %EPT drastically decreased at a waste water outlet/ENP (Table 4).

With regard to the FBI evaluation, the upper course of Ajacho river sampling site one (3.64) and Ketalla river sampling site one (4.26) had the lowest FBI score; whereas Ketalla river downstream two sampling station (7.205) and Ajacho downstream one (7.142) had the highest FBI value. On average the recorded FBI value at upstream, outlet/entry point and downstream site were (4.3, 4.69 and 4.39) respectively. In general the FBI value of all sampling sites slightly increased at downstream and outlet/entry points of coffee wastewater (Table 4).

Domination of similar taxa was high at the point of release (outlet) of effluent and downstream. The dominant taxa can survive with the stress the other taxa which were intolerant to the stress cannot survive and their number was decreased. The highest dominant taxa were recorded at sampling sites of K-ENP (0.3972) and A-DS_1 (0.3682) sites. Therefore the accumulation of similar species at the outlet and downstream indicates the water of the river was with low-quality (Table 4).

The richness of taxa also indicates water quality. The average species richness (H_{max}) of study Rivers was 8, 6 and 2.71 at upstream, point releasing (outlet) of the effluent and downstream respectively. The Ratio of EPT to Chironomidae at the point of effluent release and downstream sites was lower than the Ratio of EPT to Chironomidae at the upstream. The Ratio of EPT/Chironomidae at downstream sites of study shows complete reduction from upstream sampling stations. The average found value of Ratio of EPT/Chiro at upstream, outlet/entry point and downstream was (16.61, 0.142 and 0) respectively. The percent composition of order Odonata was 0.024% at the upstream, 0.165% at the point of releasing effluent (ENP) and 0.024% at the downstream. The highest %odonata value was recorded at downstream sites of Ajacho River respectively. While the lowest recorded value was (0) at Ajacho upstream sampling site and (0.13) at downstream two sampling points of that river (Table 4).

S.S	Taxa	Indi	Dom.	D	H’	Even.	FBI	% Odon	% EPT	EPT/Chiro
K-UP_1	12	71	0.1216	0.878	2.241	0.7833	4.2631	0.02816	0.67605	48
K-UP_2	7	56	0.1786	0.821	1.796	0.8608	4.7049	0.01785	0.8392	5.875
K-DS_1	8	43	0.2288	0.771	1.734	0.708	5.1818	0.37209	0	0
K-DS_2	9	49	0.2178	0.782	1.794	0.6684	7.2051	0.20408	0	0
K-ENP	8	51	0.3972	0.603	1.377	0.4956	4.4927	0.1923	0.0576	0.09677
A-UP_1	9	53	0.1378	0.862	2.083	0.8919	3.6415	0	0.849	11.25
A-UP_2	4	20	0.315	0.685	1.224	0.8502	4.9545	0.05	0.4	1.333333
A-DS_1	7	35	0.3682	0.632	1.368	0.5612	7.1428	0.2	0	0
A-DS_2	5	35	0.2424	0.758	1.492	0.8893	6.0909	0.05714	0	0
A-ENP	4	36	0.3349	0.665	1.208	0.8366	4.8888	0.13888	0.08333	0.1875

Table 6 Summary of Macroinvertebrate indices along sampling sites of Study Rivers

Abbreviation: (*S.S) sampling site; (Indi) individuals; (Dom) Dominance; (D) Simpson's Diversity Index (H') Shannon-Wiener Diversity Index;* (*Even) Evenness; (FBI) Family biotic index; (%Odon) present of Odonata family; (% EPT) present of Ephemeroptera, Plecoptera and Trichoptera; (EPT/Chiro) Ratio of Ephemeroptera, Plecoptera and Trichoptera to Chirnomidea.*

4.3 Multivariate analysis results

4.3.1 Cluster Analysis

Bray-Curtis Cluster Analysis (Single-Link)

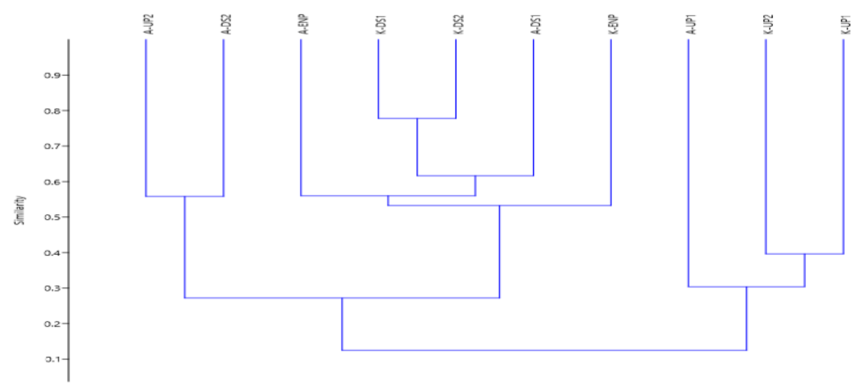

Figure 6 The hierarchical clustering "Dendrogram" of sampling sites using macro-invertebrate taxa composition.

In order to show the relationship of each cluster to all others and dissimilarity levels of each cluster, dissimilarity analysis of the macroinvertebrate composition was undertaken in both river sampling sites. This analysis (Figure 14) revealed that the highest similarity was found between sites K-DS$_1$ and K-DS$_2$ of Ketalla River and while the lowest similarity was found between sites (K-UP$_1$ and K-UP$_1$). In general 3 types of clustering can be seen from the diagram. The first extends from site (K-UP$_1$) to (A-UP$_1$), the second from site (K-ENP) to (A-ENP) and the third one from (A-DS$_1$) to (A-UP$_2$). The cluster analysis was effective in clustering the impacted points of (K-DS$_1$, K-DS$_2$, A-DS$_1$, and A-ENP).

4.3.2 Canonical Correspondence Analysis (CCA)

The first and the second canonical axes explained 59.39% (eigenvalue of 0.18) and 37.14% (eigenvalue of 0.11) of the variation in the species data, respectively. The first axis was positively correlated with TN (r = 0.83), NO_3^--N (r = 0.74), %Odonata (r = 0.73), TDS (r = 0.65), FBI (r = 0.63), Dominance (r=0.6), COD (r=0.39) TSS (r=0.14) and BOD (0.09). %EPT (r=-0.95), NH4+-N (-0.89), WT (r=-0.86), PO_4^{3-}-P (r=-0.73) and Evenness (r=-0.64) respectively were negatively correlated with CCA axis 1. CCA axis 2 was strongly positively correlated with %EPT (r=0.63), Shannon (r=0.54), Simpson (r=0.52) DO (r=0.48) and pH (0.44).

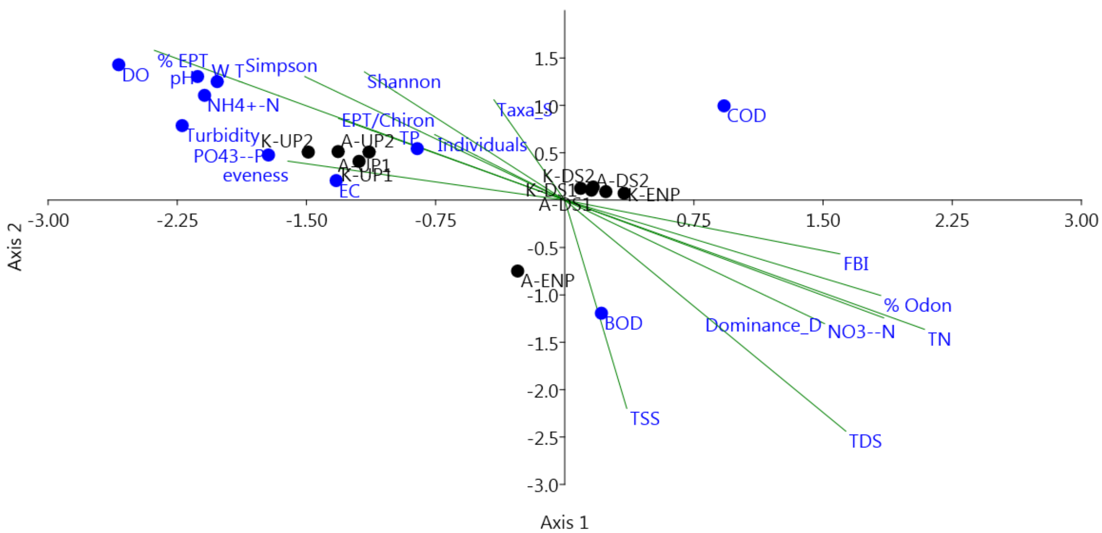

Figure 7 Canonical Correspondence Analysis (CCA) of macro-invertebrate indices and environmental variables of the rivers in Kecha-birra SNNPR, Ethiopia

The first synthetic gradient is positively correlated with (TSS, TDS, NO_3^- -N, TN and negatively with PO_4^{3-}-P, DO, TURB, pH, and NH_4^+-N). The negative side of second axis is strongly correlated with TSS. The variablesNO$_3^-$ -N and TSS does not change much. As a result variation in macroinvertebrate indices was strongly correlated with TDS and TN.

4.3.3 Principal Component Analysis (PCA)

If most of the variance is accounted for by the first one or two components, you have scored a success, but if the variance is spread more or less evenly among the components, the PCA has in a sense not been very successful.

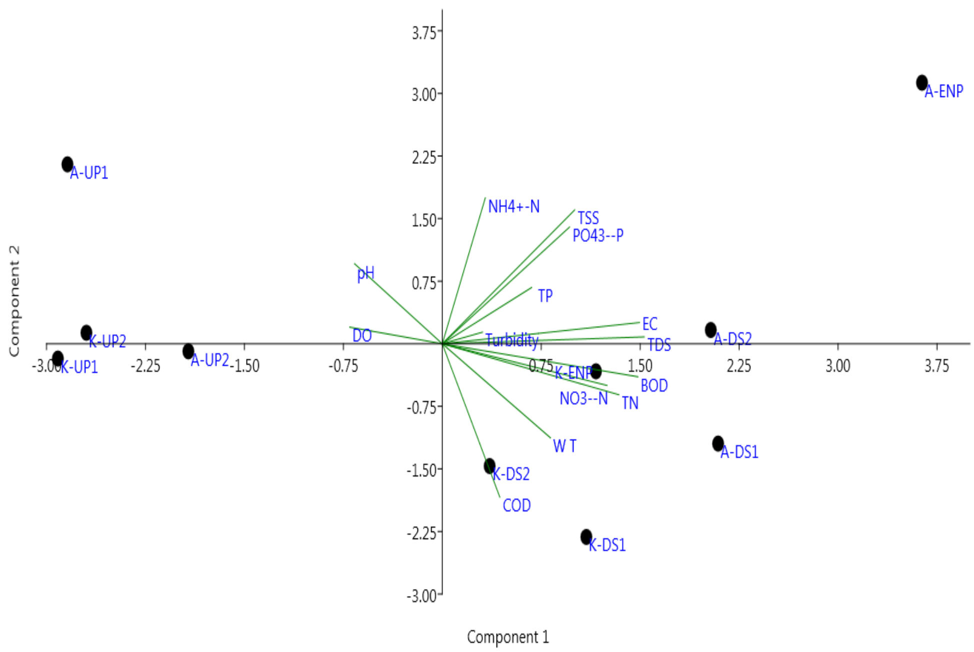

Figure 8 Canonical Correspondence Analysis (CCA) of macro-invertebrate indices and environmental variables of the rivers in Kecha-birra SNNPR, Ethiopia

PCA is a rigid rotation of original data matrix and can be defined as projection of samples onto a new set of axes such that the maximum variance is projected or "extracted" along the first axes, the maximum variation uncorrelated with the first and second axes is projected on the third axis.

4.3.4 Correlation Analysis between physicochemical Parameters and benthic macroinvertebrates Indices

The overall Pearson correlation coefficients (r) calculated for the quantification between various pairs of the physicochemical parameters of surface water samples from Ketalla and Ajacho rivers were provided in the (Table 6). Results of correlation analysis (Table 7) shows that there was positive correlation with a strong relationship between TDS with WT, TURB and %odonata shows “r” in the ranges between 0.4 to 0.7 and No_3^--N with PO_4^{3-}-P, BOD, COD, WT, EC, and &odonata shows week correlation in the ranges of 0.4 to 0.7 similar to TDS. And also, NH_4^+-N with PO_4^{3-}-P, TN with PO_4^{3-}-P, BOD, COD, WT, EC, and TP with BOD and COD shows similar trend of those mentioned above. TSS with TURB, DO with Simpson and Shannon with EPT were also fitted in the range of 0.4 to 0.7. But in this linear correlation COD and BOD show strong relationship r = 0.97.

	TSS	TDS	NO_3^--N	TN	NH_4^+-N	PO_4^{3-}-P	TP	BOD5	COD	W T	EC	pH	TURB	DO	D	H’	Even	FBI	% Od	% EPT	$\frac{EPT}{Chiro}$
TSS	1																				
TDS	.797**	1																			
NO_3^--N	0.262	0.61	1																		
TN	0.25	.664*	.986**	1																	
NH_4^+-N	0.501	0.18	0.116	0.06	1																
PO_4^{3-}-P	.804**	.780**	0.425	0.406	0.447	1															
TP	0.141	0.26	-0.144	-0.02	0.176	0.169	1														
BOD5	.712*	.927**	0.497	0.583	0.252	.710*	0.582	1													
COD	.709*	.926**	0.503	0.588	0.254	.708*	0.578	0.97**	1												
W T	0.154	0.53	0.53	0.56	-0.615	0.215	-0.19	0.325	0.325	1											
EC	.832**	.995**	0.544	0.596	0.189	.793**	0.283	.924**	.923**	0.513	1										
Ph	-0.03	-0.27	-0.246	-0.34	0.157	0.173	-0.59	-0.455	-0.451	-0.25	-0.262	1									
TURB	0.44	0.4	-0.215	-0.16	-0.292	0.135	-0.08	0.252	0.243	0.365	0.447	-0.061	1								
DO	-0.07	-0.28	-0.606	-0.6	-0.067	-0.342	-0.08	-0.337	-0.334	-0.1	-0.218	0.214	0.343	1							
D	-0.31	-0.58	-0.513	-0.57	-0.099	-0.494	-0.61	-.694*	-.695*	-0.42	-0.579	0.551	0.223	0.275	1						
H’	-0.44	-0.58	-0.598	-0.6	-0.217	-0.617	-0.36	-0.622	-0.625	-0.4	-0.576	0.426	0.242	0.4	.907**	1					
Even	0.239	-0.27	-0.201	-0.34	0.396	0.118	-0.59	-0.407	-0.409	-0.42	-0.247	0.543	0.032	-0.05	0.59	0.23	1				
FBI	-0.12	0.39	.726*	.743*	-0.373	0.247	-0.24	0.254	0.265	.671*	0.324	0.045	-0.126	-0.41	-0.3	-0.3	-0.39	1			
% Od	0.048	0.48	0.588	.680*	-0.297	-0.132	0.173	0.476	0.479	0.587	0.439	-.728*	0.187	-0.13	-0.4	-0.3	-.648*	0.432	1		
% EPT	-0.25	-.709*	-.836**	-.902**	0.096	-0.374	-0.28	-.736*	-.742*	-0.57	-.651*	0.528	0.106	0.608	.673*	0.61	0.549	-.688*	-.747*	1	
$\frac{EPT}{Chiro}$	-0.19	-0.43	-.684*	-.689*	-0.324	-0.322	-0.18	-0.458	-0.468	-0.2	-0.388	0.424	0.257	0.325	0.62	.722*	0.193	-0.43	-0.43	0.568	1
** Correlation is significant at the 0.01 level (2-tailed).																					
* Correlation is significant at the 0.05 level (2-tailed).																					

Table 7 Table Pearson’s Correlation (r -values) between the physicochemical parameters and benthic macroinvertebrates’ assemblages of seven metrics ‘biotic index’ fourteen physicochemical variables of study sites

Abbreviation: *(TSS) Total suspended solids; (TDS) Total dissolved solids; (NO_3^- -N) nitrate-nitrogen; (TN) Total Nitrogen; (NH_4^--N) ammonia-nitrogen; (PO_4^{3-}-P) Ortho-phosphate; (TP) Total Phosphorus; (BOD5) 5day, Biological Oxygen Demand; (COD) Chemical Oxygen Demand;(WT) Water Temperature; (EC) Electrical conductivity; (pH); (TURB) Turbidity; (DO) Dissolved Oxygen; All physicochemical parameters are measured in (mg/L) except, WT, OC; TURB, NTU; EC, µs/cm and pH no unit respectively. (D) Simpson’s Diversity Index; (H’) Shannon-Wiener Diversity Index; (Even) Evenness; (FBI) Family biotic index; (%Od) Present of*

Odonata Family; (EPT) Present of Ephemeroptera, Plecoptera and Trichoptera; (EPT/Chiro) Ratio of Ephemeroptera, Plecoptera, and Trichoptera to Chirnomidea.

4.3.5 Qualitative Habitat Evaluation Index (QHEI)

Upstream sites				Downstream site			
Rivers		Ketalla	Ajacho	**Rivers**		Ketalla	Ajacho
	Type	12	13		Type	16	7
Substrate	Quality	2	0	**Substrate**	Quality	2	0
Instream cover	Type	8	4	**Instream cover**	Type	4	6
	Amount	7	3		Amount	7	1
Channel morphology	Sinuosity	4	4	**Channel morphology**	Sinuosity	3	1
	Development	3	3		Development	5	3
	Channelizetion	6	6		Channelizetion	6	5
	Stability	2	2		Stability	2	2
Riparian zone	Width	3	1	**Riparian zone**	Width	3	4
	Quality	3	0		Quality	2	0
	Bank Erosion	3	3		Bank Erosion	3	3
Pool quality	Maximum depth	1	0	**Pool quality**	Maximum depth	0	0
	Currrent	1	1		Currrent	2	1
	Morophology	1	1		Morophology	1	1
Riffle quality	Development	1	4	**Riffle quality**	Development	2	4
	Stability	2	2		Stability	3	2
	Embededness	2	2		Embededness	2	2
	Gradient	5	7		Gradient	6	5
Total		66	56	**Total**		69	47

Table 8 Qualitative habitat evaluation index score for each (QHEI) metric for the upstream and downstream sampling sites.

The physical habitat conditions assessed using the integrated habitat assessment nt system (IHAS) revealed that the physical habitat did not vary much between the sampling sites (Table 8). On the basis of QHEI score, the habitat score was in the range (55% and 66%) at upstream samplings site and (47% and 69%) at downstream sampling sites were indicated in the (Table 8).

Table 9 General narrative ranges assigned to the QHEI score

Narrative Rating	QHEI Range Narrative	
	Headwaters	Large streams
Excellent	>70	>75
Good	55 to 69	60 to 74
Fair	43 to 54	45 to 59
Poor	30 to 42	30 t0 44
Very poor	<30	<30

5. CHAPTER FIVE: DISCUSSION

5.1 Physicochemical Parameters characteristics of Coffee Wastewater

The physicochemical water quality parameters that increase with the increase in pollution load such as WT, EC, TSS, TDS, No_3^- -N, TN, BOD_5, COD, PO_4^{3-} -P and TP at all sampling sites along the course of the rivers were higher than at the reference station, K-UP_1, K-UP_2, A-UP_1 and A-UP_2 (Table 3). Discharging of untreated industrial liquid waste into the rivers were the observed environmental stressors that affected the water quality of the rivers. Water physicochemical variables are important factors capable of exerting influences on the species diversity and composition of freshwater ecosystems (Sundermann, 2013). The polluting potential of the wet coffee processing plants at distant locations at the outlet or effluent entry points and below effluent discharge points (downstream sites) in the present study indicates deterioration of physicochemical water quality parameters in some sampled sites, where coffee effluent is steeped into the watercourses.

The high nutrient, TDS, and BOD_5, COD and contents in some sampling sites (Table 3) in coffee wastewater were well above the organic effluent limit that could be allowed to enter into natural surface water bodies in most developed countries (Tomar, 1999). Although the highest recorded value of BOD_5 content (990mg/L) of coffee wastewater of the sampling rivers, it was much lower than the previous work undertaken (Haddis & Rani., 2007) reported BOD values up to 7,800mg/l in a river at area of Jimma zone. The pollution loads were generally increasing outlet and downstream along the course of the rivers. This indicated that a load of pollution along the course of the rivers were beyond the natural self-purification capacity to recover in some sampling stations.

5.1.1 Physicochemical Parameters

The main ecological effect of organic pollution in a watercourse is the reduction in oxygen content (Von Enden & Calvert, 2002); (Murthy, 2004). In the present work, there was a steady decline of DO at the downstream sampling sites. However, the downstream sites of Ajacho River displayed a relatively high decrease in DO level to Ketalla river sampling stations.

Although the depletion of oxygen of Ajacho river may be associated the high BOD (990mg/L and 454mg/L) measurement, high DO values were recorded at the downstream sites of Ajacho river that contain high BOD_5 values despite the opposite relationship between DO and BOD (Meyrick, 2005).

These high DO values might be related to the discharge of the rivers that have high potential to dilute the organic effluent entered into the river and continuous aeration along its way to downstream sites. Similarly, (Giller & Malmqvist.B, 1998) stated that flow velocity directly affects the amount of dissolved oxygen in river water. Fast flowing waters favor diffusion of atmospheric oxygen to the river water, thereby raises the amount of dissolved oxygen than slow backwaters.

Nevertheless, the very high BOD content was mainly related to organic enrichment from the coffee processing effluent (Von Enden & Calvert, 2002), (Haddis & Rani., 2007) and its effect was much more pronounced at the downstream wastewater outlet sites of Ajacho River.

The BOD result at the upstream was lower than that of the point of outlet and downstream. The outlet point of effluent had higher BOD than that of downstream this was happening because of the concentrated effluent discharge into the water. Very high BOD value recorded was recorded at the raw effluent and influent sampling sites before outlet of wastewater into watercourses (K-EFU, 1,090, K-INF, 1,626, A-EFU, 1,575 and A-INF, 3,228) mg/L respectively.

The BOD contents of upstream sampling stations of Study Rivers were a very low amount of BOD concentration. While the high BOD content at the downstream sites of Ajacho River might be related to other pollution sources such as erosion from the nearby agricultural fields. Additionally, in both study rivers the recorded results depicted that the pollution status of the rivers much-increased blow discharge or outlet point of wet coffee processing effluent into the river which had increased the organic load of the river further. In general, most of the downstream sites were impacted by coffee processing effluent so that the average BOD value was 394mg/l which according to (Von Enden & Calvert, 2002) should be reduced to less than 200mg/l before its entrance to natural waterways.

In general sense the BOD content value obtained in this study compared to similar studies conducted in different regions (Dessalegn, 2018)(Haddis & Rani., 2007) (Beyene A, 2011)shows high decrement.

Decrement in BOD values in the downstream water bodies may be due to a reduction of chemical and biological oxygen demanding wastes as the effluents pass through the disposal pits and due to the dilution of river water. For surface water, a BOD_5 above 10 mg/L usually indicates the presence of gross pollution (Nathanson, 2000). In this study all the effluents and downstream water bodies show values exceeding this limit during the wet coffee processing season. Thus, it was evident that the downstream water bodies were substantially polluted with organic matter. This finding is consistent with similar studies conducted (Endaris, 2008).

Chemical Oxygen Demand is the total measurement of all chemicals in the water that can be oxidized. BOD is supposed to measure the amount of food (or organic carbons) that bacteria can oxidize. In the present study, all samples taken from ENP and DS sites displayed high amount of COD concentration. In this study COD value recorded pronounced high increment in outlet/ENP of wastewater and downstream sites. This could be an indication of organic pollution due to the discharge of wastewater from coffee processing mill. This value is very low compared with similar study conducted in Jimma, (Haddis & Rani., 2007) (Dejen, 2015) Nevertheless, the value recorded in this study was higher than recommended (WHO, 2004). Thus, it was evident that the entry and downstream water bodies were substantially polluted with organic matter from wet coffee processing.

The relatively higher amount of TDS at the outlet and downstream site of Study Rivers might be attributed to the high mucilage coming out from coffee processing stations. Moreover, (Murthy, 2004) described the high suspended material (especially the digested mucilage) when precipitated out of the solution builds a crust on the surface, clogging up waterways and further contributing to anaerobic condition. In natural water dissolved solids are consists of inorganic salts, small amounts of organic matter and dissolved materials. TDS concentrations in natural waters often result from industrial effluent, changes to the water balance (by limiting

In this study, the average pH value of upstream sampling sites shows similar manner. A slight reduction in pH values on average might be attributed to the assimilation capacity of water. However, the pH value obtained at the lower course of study rivers. The average value of the pH was high at upstream, decrease at outlet point and slightly increases downstream. The comparable study was done by Solomon (Endaris, 2008) in Jimma with In 11 river sampling sites and has got similar results. The recorded pH value at entry/outlet site of Ketalla River was lower and it was above the WHO standard range of (6.5-8.5) for human use.

In this work water temperature measured at all sampling sites was relatively similar. Water temperature is an important element affecting the abundance and richness of freshwater stream aquatic macro-invertebrate communities. It controls the rate of all chemical reactions and affects growth and reproduction of aquatic ecosystem. (WHO, 2010) has set a standard limit for the river water temperature (25^{o}C). Similarly, according to (EFEPA, 2003) water quality standards (surface waters discharge must not result in variation of more than 1.5 °C - 3 °C temperature downstream of thermal discharge the result obtained was not above the recommended standards.

Electrical conductivity- is regarded as a crude indicator of water quality. Conductivity is the ability of water to carry an electrical current. It indicates the physical presence of dissolved chemicals in the water. The movement of these ions conducts electricity through the water. The electrical conductivity of the water depends on the water temperature, the higher the temperature, the higher the electrical conductivity. According to (Sawyer, 2003)conductivity is directly related to total TDS with proportionality constant ranging between (0.55 to 0.9), when TDS and EC are expressed in mg/L and μS /cm, respectively.

The trend of EC is not uniform, but generally, the EC values in the effluent are higher than at all sampling sites of rivers. The relatively higher amount of EC at the outlet and downstream site of Study Rivers might be attributed be more ions that are present, the higher the conductivity of water. In this study all the recorded values were found at the range interval of (92μS/cm-305μS/cm) for all sampling site of river water samples and it ranges (294 μS/cm-2300 μS/cm) in the raw effluent and influent sampling sites

The raise in the EC value of ENP and DS sites was maybe because of the solubility or decomposition of compounds during de-pulping and fermentation of coffee pulp. The relative decrements of EC value at certain downstream sampling sites in the sampling sites bodies are lower due to dilution with water; however, this dilution may not always be sufficient. This finding is consistent with similar studies done by (Dejen, 2015). Generally, it was found that wet coffee processing wastewater has impacted the EC of downstream water bodies. When we compare the values at above and below points of coffee wastewater discharging point all the values evidently indicate higher values at points below coffee effluent receiving sites. In general sense, EC in streams and rivers are affected primarily by the geology of the area through which the water flows.

The Turbidity of the water samples among the upstream, outlet point and downstream sites showed slight change at their respective sampling sites. Turbidity in water is caused by suspended particles or colloidal matter that obstructs light transmission through the water (WHO, 2004). The increase in the amount of turbidity may be due to the increase in the number of suspended solids. Generally Coffee processing effluent solid causes the DO problem by sedimentation and forming oxygen demanding sludge deposit which causes turbidity in the receiving water and may alter biological life. The turbidity values of the current study show similar trend in all upstream sites and little change in downstream and outlet of waste receiving site. In this study, almost all sites of study site turbidity concentrations were higher than 25 NTU WHO permissible levels. The result obtained in this study was consistent with (Dejen, 2015) (Tsigereda, 2011).

Total Suspended Solids-give measures of the turbidity of the water. TSS values were indeed observed to follow approximately the trend of EC. This increment in EC and TSS might be due to the presence of inorganic compounds and floating particles having larger sizes, respectively. This might be due to the difference in soaking time, washing frequency, de-pulping and duration of fermentation of coffee beans. In addition, this may be due to the difference in the type of pulping machine used by the plant (Dessalegn, 2018). It is obvious that these values surpass by far the Ethiopian surface water quality standards (50 mg/L), and also the US-EPA standards for discharge of pollutants to inland surface waters (100 mg/L) (EFEPA, 2003) (WHO, 2010).

World Health Organization (WHO) has recommended that water with TSS less than 30 mg/L to be clear and potable for drinking and less than 150 mg/L was suitable for the survival of aquatic life. In this study (except) the higher TSS values obtained at (A-ENP, 360) all sampling sites were found in the range of environmentally prescribed water for drinking (30 mg/L) and aquaculture (150 mg/L). So, water from such sites was suitable for the indicated beneficial purposes such as washing and aquaculture.

Generally, restriction of light penetration into the water column by high concentrations of inorganic suspended solids can potentially limit the growth of both benthic and suspended algae in rivers. Furthermore, the hydraulic flow regime can greatly alter periphytons standing crops in flowing waters, and herbivore grazing often is noted as an additional biological constraint on periphytons growth and productivity.

For many years, flowing waters thus were frequently perceived as nutrient saturated, because factors such as light limitation and short hydraulic residence times should restrict or prevent any potential algal responses to nutrient enrichment.

Nutrient pollution is a growing problem for water bodies around the world. Cultural eutrophication related to nutrient pollution can lead to unhealthy ecosystems with a lack of oxygen and biodiversity. Nutrient enrichment, or eutrophication, can lead to highly undesirable changes in ecosystem structure and function, however (Rathore.P, 2016). It is a vital nutrient for all living things but, introduction of excessive phosphorus in form of phosphates in aquatic environment can cause eutrophication.

The nitrogen and phosphorus content in the waters is a commonly used hydro chemical index for the assessment of the eutrophic potential of a river or lake. Nutrient concentrations have such a straightforward effect on phytoplankton populations because they generally exist in low concentrations in natural waters. Therefore, the growth of phytoplankton populations is limited by their access to phosphorus and nitrogen for metabolic processes.

As indicated in (Table 3) most of the sampling sites, the concentrations of NO_3^--N, $NH4^+$-N, Po_4^{3-}-P and TN increased from upstream of the coffee processing plant site to downstream of the effluent disposal site. Orthophosphate mean values recorded for in the river water ranges (0.029 and 0.135) mg/L for upstream and downstream sampling sites of Ketalla river and (0.165 and 0.25) mg/L for Ajacho river respectively. The maximum value of PO_4^{3-}-P recorded at the A-INF (0.9mg/L) and (the value is very reduced at similar sampling site of Ketalla River (0.18mg/L).

This may be due to differences in pulping machines used by the plants, fermentation time and the amount of water used by these plants (Dessalegn, 2018). With regard to No_3^--N content of the sampling sites, there is a general pattern of its increment at downstream sites compared to the upstream sites. Although in Ajacho sampling stations raw coffee wastewater (effluent) contains 2.1mg/L and 2.3mg/L at Ajacho River effluent and influent sampling sites.

The lowest and highest No_3^--N content of the downstream samples was within the range of (0.43mg/L to 0.99mg/L) at Ketalla river and (0.66mg/L to 1.19mg/L) at Ajacho river respectively (Table 3). A decrease in No_3^--N and other nitrogenous pollutants may be associated with flow rate and dilution effects of the river water.

A large volume of water has the capacity to reduce BOD and other organic pollutants. Similarly, NH_4^+-N followed an increasing trend after the effluent discharge.

The concentrations observed in this study are much less than in the findings of (Endaris, 2008) (Dessalegn, 2018) who reported a maximum PO_4^3-P concentration of 9.9 mg/L, 110.75 mg/L, and 18.5 mg/L, respectively. In general, a comparison of the NO_3^--N, NH_4^+-N and TN, TP and PO_4^{3-}P values found for upstream outlet and downstream waters clearly show that the NO_3^--N, NH_4^+-N and TN, TP and PO_4^{3-}P values increased in the downstream sites of wet coffee processing plants (effluent receiving) (Table 7).

Thus, it can be decided that wet coffee processing plant wastewater impacted the NO_3^--N, NH_4^+-N, and TN, TP and PO_4^{3-} P concentration of downstream water bodies.

In general, the physicochemical data of the upstream/reference sites (most sites) revealed that their biological integrity was un-impacted by human interference, and hence served as the reference sites (most of them are forested areas) to evaluate the impact of coffee processing effluent on the receiving water bodies. Therefore, the comparison between upstream outlet/ENP and downstream sites clearly demonstrated the deterioration of water quality in certain outlet/ENP and downstream sites. However, the construction of lagoon/pit for the containment of coffee pulp and coffee wastewater around both Study Rivers and the placement of processing station at a considerable distance from both rivers made the rivers relatively un-impacted (minimal impact compared to other similar studies conducted at different area

5.1.2 Analysis of Macroinvertebrate Data

Benthic macro-invertebrate species are differentially sensitive to many biotic and abiotic factors in their environment. Consequently, macro-invertebrate community structure has commonly been used as an indicator of the condition of an aquatic system (Armitage, 1983) (Rosenberg & Resh, 1993).

Changes in presence/absence, numbers, morphology, physiology or behavior of these organisms can indicate that the physical and/or chemical conditions are outside their preferred limits (Rosenberg & Resh, 1993)..

The presence of numerous families of highly tolerant organisms usually indicates poor water quality (Hynes, 1998).

Species abundance is generally assumed to be a useful measure of the severity of pollution (Sheehan, 1984). The general increment in total number of individuals at the downstream sites might be attributed to the enrichment of downstream sites from coffee processing wastewater, which provides physiologically adapted organisms to exploit the excess nutrients available despite low oxygen tension and this response of macro-invertebrates to organic pollution has been well documented (Cao, 1996).

However, the general increment in species richness at the downstream sites was not supported with species diversity. Hence, the outlet and downstream site of Ajacho River which had 71 individuals were found to contain 9 species (Table 4). While the upstream site one and upstream site two of Ketalla River which had 127 individuals were belonging to 19 species. This finding is consistent with (Plafkin, 1989) in that pollution tolerant species like Chironomidae species dominate the impacted sites. Likewise, many reports have documented a significant decrease in species richness in responses to toxic exposure (Peitz, 2003).

5.1.3 The Benthic Macroinvertebrate Metrics

High values of diversity indices and equitability at the downstream sites of Ketalla River despite their presence below coffee discharge points was un-impacted by coffee processing effluent. This finding of biological data is also supported by physicochemical data. Their good river water quality might be related to their efficient coffee effluent control mechanism (lagoon) and the relative placement of the stations at a higher distance from the rivers i.e. large area possessed by the station enabled the construction of efficient pits to the containment of both coffee wastewater and pulp.

Furthermore, the evaluation of the physical characteristics of the stream reach includes water quantity, channel morphology, and substrate composition. Riparian canopy shading the stream surface is also measured. The variables measured are physical characteristics of a stream reach likely to be influenced by changes to the riparian corridor and watershed land use such as water temperature or dominant substrate size.

Besides, a good habitat index of 64 (good) in all sampling stations of study rivers except, Ajacho river downstream sampling stations (47%) fair presented the intact nature of the habitat and absence of other pollution sources to these rivers (Table 7).

The low diversity at the upstream sites of Ajacho Rivers was mainly related to the the dominance of these sites by the Chironomidae family. Many reports have shown a significant decrease in species diversity indices associated with pollution (Sheehan, 1984) (Norris & Georges A., 1993). A better balance/stability between all the species was found at the upstream and downstream sites of Ajacho River. On the contrary the outlet/entry point of wastewater site of Ketalla River had equitability of 0.49. This low equitability index for these sites is also consistent with physicochemical data. Therefore biological indices confirmed the impact of wet coffee processing effluent on macro-invertebrate communities in most of the downstream sites. (Meyrick, 2005) also reported the reduction of aquatic macro-invertebrate diversity below urban effluent discharge streams.

The abundance of EPT and Chironomidae indicates the balance of the community since EPT is considered to be more sensitive and Chironomidae less sensitive to environmental stress (Plafkin, 1989). A community considered to be in good biotic condition will display an even distribution among these four groups, while communities with disproportionately high numbers of Chironomidae may indicate environmental stress (Plafkin, 1989). The EPT/C index is calculated by dividing the sum of the total number of individuals classified as Ephemeroptera, Plecoptera, and Trichoptera by the total number of individuals classified as Chironomidae.

The highest number of EPT individuals was found at the upstream sites of Study Rivers. The % EPT Index or the Ephemeroptera, Plecoptera, and Trichoptera (EPT) index shows the taxa richness within these groups which are considered to be sensitive to environmental stressors. Their number should increase with increasing water quality (Mandaville, 2002). Initially developed for species-level identifications; this index was valid for use at the family-level (Plafkin, 1989). The percentage composition of EPT constituted (35.25%) for Ketalla river and (32.56%) for Ajacho river. The high proportion of % EPT taxa found in Ajacho and Ketalla upstream sampling sites 1 and 2 (0.849) and (0.839) respectively. The family Baetidae, which constituted the mayfly taxa, collected in this study site is moderately tolerant. This family is known to increase with moderate pollution as reported by (Gallardo, 2006).

Therefore, the abundance of species at a given site is more a reflection of past environmental conditions (Richard, 1997), while measurements of physical and chemical factors may be more of an indication of present conditions (Karr & Chu, 2000).

Therefore pollution) and Baetidae species are removed from the taxa since mayfly nymphs (Plecoptera) and Caddis larvae (Trichoptera), which are believed to be intolerant to organic pollution (Cao, 1996) and will better indicate the pollution status of the rivers.
This result indicates that the water had good water quality conditions.

The Hilsenhoff Family Biotic Index, a weighted measure of individuals in a population, has also increased considerably at the downstream sites. Higher scores of percentage tolerant organisms at the downstream site assure the abundance of pollution tolerant organisms.

The lowest FBI score at the upstream sites of Ajacho River (3.64) is related to the absence of any pollution tolerant species. In general, the deterioration of river water quality at the downstream sites relative to upstream sites indicated the abundance of pollution tolerant organisms and the presence of organic pollution as a result of wet coffee processing effluent.

The highest percent composition of order Odonata was (0.37% and 0.204%) at the downstream sites of Ketalla river and the value almost completely decreased into zero (0) - (0.01) at majority of upstream sampling sites of both rivers. The percent composition of Odonata at the upstream was lower than the downstream points indicates upstream has high water quality. While percent composition of the Odonata species increased from upstream. The Odonata species has high tolerance value and can survive stress (Plafkin, 1989).

5.2 Correlation Analysis

5.2.1 Cluster Analysis

The clustering technique is found to follow closely the number of individuals that the sites contain in that sites similar in species richness are clustered together (K-DS_1 and K-DS_2) but sites that contain a higher number of species are failed to be clustered together. Thisclassification confirmed that different macroinvertebrate communities occurred at these sampling sites with different water quality. This suggests that water quality has a determinative effect on macroinvertebrate community structure in the study area. In this analysis the highest similarity was found between sites K-DS_1 and K-DS_2 of Ketalla River and while the lowest similarity was found between sites (K-UP_1 and K-UP_1). In general 3 types of clustering can be seen from the diagram. The first extends from site (K-UP_1) to (A-UP_1), the second from site (K-ENP) to (A-ENP) and the third one from (A-DS_1) to (A-UP_2). The cluster analysis was effective in clustering the impacted points of (K-$DS_{1,}$ K-$DS_{2,}$ A-$DS_{1,}$ and A-ENP). These sites were also relatively found a similar diversity index.

5.2.2 Canonical correspondence analysis

Although a slight difference was observed for the results of environmental variables and biological indicators to depict the pollution gradient along the river separately, the combination of both were able to clearly define and explain the pollution gradients. CCA is correspondence analysis of a site/species matrix where each site has given values for one or more environmental variables. The ordination axes are linear combination of the environmental variables CCA is thus an example of direct gradient analysis, were the gradient in environmental variables is known *a* prior and the species abundance is considered to be a response to this gradient (Ryan, 1995). The interpretation of the ordination diagram is that sampling sites close to each other contained similar measurements in terms of water quality, while those separated apart have different water quality. The first and the second canonical axes explained 59.39% (eigenvalue of 0.18) and 37.14% (eigenvalue of 0.11) of the variation in the species data, respectively.

The first axis was positively correlated with TN (r = 0.83), NO_3^--N (r = 0.74), %Odonata (r = 0.73), TDS (r = 0.65), FBI (r = 0.63), Dominance (r=0.6), COD (r=0.39) TSS (r=0.14) and BOD (0.09). %EPT (r=-0.95), NH_4^+-N (-0.89), WT (r=-0.86), $PO4_3^-$-P (r=-0.73) and Evenness (r=-0.64) respectively were negatively correlated with CCA axis 1. CCA axis 2 was strongly positively correlated with %EPT (r=0.63), Shannon (r=0.54), Simpson (r=0.52) DO (r=0.48) and pH (0.44). Several studies indicate that Ephemeroptera, Plecoptera, and Trichoptera show a strong negative response to anthropogenic disturbances in aquatic ecosystems (Ode, 2005). Ephemeroptera is an order of aquatic insects commonly used in bio-assessment and bio-monitoring of freshwater ecosystems all over the world (Arimoro & Muller, 2010).

They are considered an ecologically important group that is of high importance for assessing biodiversity in aquatic ecosystems. Among the water quality variables, most of the concentration of the variable like (Water temperature), (pH) (EC), (TURB) and Dissolved oxygen were negatively related to all the core metrics. The decrease of macro-invertebrate species along a gradient has repeatedly been reported (Carpenter, 1998).

5.2.3 Principal Component Analysis (PCA)

The PCA routine finds the eigenvalues and eigenvectors of the variance-covariance matrix or the correlation matrix, with the SVD algorithm. Use variance-covariance if all variables are measured in the same units (e.g. centimeters). Use correlation (normalized var-covariables) if the variables are measured in different units; this implies normalizing all variables using division by their standard deviations. The eigenvalues give a measure of the variance accounted for by the corresponding eigenvectors (components). The percentages of variance accounted for by these components are also given. If most of the variance is accounted for by the first one or two components, you have scored a success, but if the variance is spread more or less evenly among the components, the PCA has in a sense not been very successful.

5.2.4 Correlation Analysis between physicochemical Parameters and benthic macro-invertebrates Indices

From this study, nutrient enrichmentand organic loading of physicochemical parameter were important in shaping community index in Ketalla and Ajacho Rivers.

The distribution of macroinvertebrates and the disappearance of some sensitive taxa were associated with depletion of dissolved oxygen and availability of food might have effect on it. The correlation coefficient (r) measures the degree of association that exists between two variables, one taken as the dependent variable. To interpret the strength of correlations, parameters were used, which consider perfect (high correlations) to be where r = 1; strong correlations where r is between 0.7 and <1, moderate where r is between 0.4 to <0.7, and week where r is less than 0.3 (Daney and Reidy, 2004). When two or more variables were highly correlated (Spearman r > 0.075, p < 0.05), only one of such variables were retained for further analysis (ODUME, 2014).

The overall Pearson correlation coefficients (r) calculated for the quantification between various pairs of the physicochemical parameters of surface water samples from Ketalla and Ajacho rivers were provided in the (Table 6). Results of correlation analysis (Table 7) showed that there was statistically significant positive correlation with a strong relationship between TDS with WT, TURB and %Odonata shows “r” in the ranges between 0.4 to 0.7 and No_3^--N with PO_4^{3-}-P, BOD, COD, WT, EC and & Odonata shows week correlation in the ranges of 0.4 to 0.7 similar to TDS. And also, NH_4^+-N with PO_4^{3-}-P, TN with PO_4^{3-}-P, BOD, COD, WT, EC, and TP with BOD and COD shows a similar trend of those mentioned above.

TSS with TURB, DO with Simpson and Shannon with EPT were also fitted in the range of 0.4 to 0.7. But in this linear correlation COD and BOD show strong relationship r = 0.97. Similarly, Joshi et al., (2009) had reported that turbidity showed strong positive significant correlation with TSS (r=0.9976) and an increase in the suspended particles in the water body increases the turbidity of water, which interferes with the penetration of light. In general, an increase in TSS concentrations affects an increase in turbidity levels and suspended solids have the ability to obstruct the transmittance of light in a water sample when TSS concentration increases; light scattering intensifies (Lawrence K & Phyllis., 2007). In general, the current result shows that an increase in TSS concentrations affecting an increase in turbidity levels.

The sensitive EPT dominated due to sufficient DO and less pollution.
This was evidenced by their positive correlation with (pH and DO), r = (0.608 and 0.528) respectively above in the (table. 7) while the negative correlation with (water temperature, EC and BOD), r = (-0.571, -0.651 and -0.737) respectively in the table above.

A community considered to be in good biotic condition will display uniform distribution among these four groups, while communities with disproportionately high numbers of Chironomidae may indicate environmental pollution (Plafkin, 1989). In this study, the ratio of pollution sensitive taxa (EPT) to pollution tolerant (Chironomidae) shows a good or even relation among both groups ($r = 0.568$) positive relationship between them.

5.2.5 Physical habitat assessment

The physical habitat conditions assessed using the integrated habitat assessment system (IHAS) revealed that the physical habitat did not vary much between the sampling sites (Table 7). On the basis of the QHEI score, the habitat score was in the range (55% and 66%) at upstream samplings site and (47% and 69%) at downstream sampling sites. The physical habitat assessment result recorded in this study displays the physical environment of Study Rivers was in good condition.

6. CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Wet coffee processing industries have beneficial contribution on the economic growth of the country. However, the wastewater generated from wet coffee processing plants that are often found along the banks of rivers and streams, when discharged back into natural water bodies. Most of the measured physicochemical parameters were higher in industry waste receiving sites. Depleted dissolved oxygen concentration (4.47mg/l and 5.12mg/L) were recorded at sites receiving wet coffee processing effluent (A-DS_2 and K-DS_1), which resulted in low diversity and abundance of benthic macroinvertebrates. Consequently, the wastewater has an opposing effect on the natural environment near the wet coffee processing plants and downstream settlers. While the results of this study revealed; some of physicochemical parameter was within the permissible limit of (WHO, 2010) standards of surface "water which was slightly polluted". Also some degradation or impairment of same entry/outlet and downstream sites due to the activity of wet coffee processing plant stations.

The higher abundance of sensitive species and lower abundance of tolerant species like Chironomidae in both study rivers indicated heterogeneous habitat structure and good water conditions. However, in the wet coffee processing season, macroinvertebrate diversity was different between impacted and un-impacted (reference) sites. The ratio of pollution sensitive taxa was relatively decreased as wet coffee processing season. In this study from the two selected methods conducted to assess river water quality level the benthic macroinvertebrates indices more reflect the pollution status of the sites that of physicochemical parameter analysis. Therefore, environmental agencies, researchers have a good option of using macroinvertebrate communities with the aim of assessment and monitoring programs of rivers. However, the construction of lagoon for the control of wet coffee pulp and coffee wastewater around study rivers, location of processing station at considerable distance from the study rivers and good qualitative habitat condition of riverine bank made the study rivers relatively un-impacted.

6.2 Recommendations

Based on the results of this study, the following recommendations are forwarded to prevent further deterioration of water quality of both rivers.

- Organizations which involved in the coffee growing and processing regions have to abide by the rules, regulations, and policies of environmental protection of the region or local administration to keep the water safeguarded.
- The government (stockholders) not only put rules and regulations for wet coffee processing plant owners and crews. However, prior to the punishment they should create awareness and support how to design & construct modern wastewater stabilization ponds/how to use modern wastewater treatment technologies.
- Environmental and water protection policies should have to use these research findings as an input for policy making and implementation practices and processes to improve the mild impact it has on the aquatic life and utilization of the people.
- The upcoming undergraduate and postgraduate Environment Health Sciences and Technology Institute students and the scientific community have to use as the primary reference for their new studies concerning this research topic.
- The environmental impact assessment should be strictly conducted before issuing a permit to newly establish processing stations.
- There should be strict rule and regulation to enforce the construction of at least more than two lagoons by each station for the containment of coffee pulp and coffee wastewater.
- Based on the findings of this study, the researcher highly suggested further extensive and comprehensive studies to be conducted in different study areas to determine and understand the variation or to comprehend the extraneous variables which contribute to these variations.

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Annex and Appendix

Annex 1 Description of sampling sites

	Assigned codes and their geographical location of Ketalla and Ajacho rivers			
Site Name	Abbreviations	Altitude (m)	North	East
Ketalla upstream one	K-UPS1	1776	70 12.241’	37045.910’
Ketalla upstream two	K-UPS2	1782	70 12.332’	37045.898’
Ketalla outlet/entry point	K-ENP	1772	70 12.217’	37045.890’
Ketalla downstream one	K-DS1	1769	70 12.190’	37045.874’
Ketalla downstream two	K-DS2	1768	70 12.189’	37045.870’
Ketalla effluent	K-EFU	1782	70 12.342’	37045.980’
Ketalla influent	K-INF	1780	70 12.322’	37045.960’
Ajacho upstream one	A-DS1	1935	70 11.927’	37048.890’
Ajacho upstream two	A-DS1	1940	70 12.956’	37048.364’
Ajacho outlet/entry point	A-DS1	1937	70 12.936’	37048.334’
Ajacho downstream one	A-DS1	1938	70 11.946	37048.375’
Ajacho downstream two	A-DS1	1937	70 12.966’	37048.324’
Ajacho effluent	A-DS1	1935	70 12.906’	37048.314’
Ajacho influent	A-DS1	1931	70 12.756’	37048.164’

Annex 2 National wet and dry coffee processing industries (June 2017)

Regions		Oromia	SNNP	Gambela	Total
Wet coffee processing	Privately owned	367	520	7	894
	Association	165	175	-	340
	State farm	15	-	-	15
	Sub-total	547	695	7	1249
Dry coffee processing	Privately owned	604	181	14	799
	Association	58	44	-	102
	State farm	6	-	-	6
	Sub-total	668	225	14	907
Grand	Total	1215	920	21	2156

Source: (EFEPA, 2003) and (US-EPA, 1989), Modified from, Dadi .D (2018)

Annex 3 Macroinvertebrate taxa abundance and composition along Study Rivers

Taxa	Selected sampling sites for benthic macroinvertebrates								
	K-UP$_2$	K-DS$_1$	K-DS$_2$	K-ENP	A-UP$_1$	A-UP$_2$	A-DS$_1$	A-DS$_2$	A-ENP
Perlidae	7	0	0	0	0	0	0	0	0
Perlodidae	9	0	0	0	0	0	0	0	0
Chloroperlidae	0	0	0	0	0	0	0	0	3
Baetidae	14	0	0	0	12	0	0	0	0
Caenidae	12	0	0	0	0	0	0	0	0
Oligoneuriidae	0	0	0	0	0	8	0	0	0
Potomanthidae	0	0	0	0	0	0	0	0	0
Heptageniidae	0	0	0	3	4	0	0	0	0
Ephemerllidae	0	0	0	0	0	0	0	0	0
Aeshnidae	0	6	3	4	0	0	0	0	0
Coengronidae	1	2	0	0	0	1	4	2	0
Gomphidae	0	8	6	1	0	0	1	0	5
Macromiidae	0	0	0	1	0	0	1	0	0
Lestidae	0	0	1	4	0	0	1	0	0
Philopotamidae	0	0	0	0	9	0	0	0	0
Hydropsychidae	0	0	0	0	2	0	0	0	0
Hydroptilidae	5	0	0	0	7	0	0	0	0
Leptoceridae	0	0	0	0	5	0	0	0	0
Brachycentridae	0	0	0	0	6	0	0	0	0
Elmidae	0	0	0	2	4	0	0	0	0
Chironomidae	8	17	19	31	4	6	20	6	16
Psychodidae	0	0	0	0	0	0	4	0	0
Ceratopogonidae	0	0	0	5	0	0	0	0	12
Tabanidae	0	4	1	0	0	5	0	8	0
Tipulidae	0	3	1	0	0	0	0	7	0
Simuliidae	0	1	0	0	0	0	0	0	0
Culicidae	0	0	4	0	0	0	0	0	0
Syriphidae	0	0	7	0	0	0	4	0	0
Chaoboridae	0	0	7	0	0	0	0	12	0
Muscidae	0	2	0	0	0	0	0	0	0
Relative total	56	43	49	52	53	20	35	35	36
Total				271					179

Annex 4 Guidelines for Ambient Environmental Standards for Ethiopia and US-EPA Standards for Discharge of Environmental Pollutants

Parameter	Ambient Environment Standards for Ethiopia: Water Quality Standards (Surface Waters)	US-EPA Standards for Discharge of Environmental Pollutants to Inland Surface Waters
BOD_5	< 5 mg/L	30 mg/L, max (3 days at 27o$^{C)}$
COD, mg/L		Max 250
Conductivity	1000 µS/cm at 200c	
DO	Min 4-6	
NO_3^-N	50 mg/L	10 mg/L
Ammonical nitrogen		Max. 50 mg/L, (as N)
pH	6-9 (but no change of more than 0.2 units from natural level)	5.5-9.0
Dissolved phosphates (as P)		Max. 5.0 mg/L
Temperature	Discharge must not result in a variation of more than 1.5 °C - 3 °C temp downstream of thermal discharge.	Shall not exceed 5 o^{C} above the receiving water temperature
TSS	< 25 mg/L (annual mean) and 50 mg/L (max value)	100 mg/L

Source: (EFEPA, 2003) and (US-EPA, 1989), Modified from, Dadi .D (2018)

Annex 5 Tolerance values of macroinvertebrates in the Family Biotic Index (FBI)

Order	Taxa	Score	Order	Taxa	Score
Plecoptra	Perlidae	1	Tricoptera	Philopotamidea	3
	Perlodidae	2		Hydropsychidae	4
	Chloroperlidae	0		Hydroptilidae	4
Ephemeroptera	Baetidae	4		Leptoceridae	4
	Canidae	7		Brachycentridae	1
	Oligoneuriidae	2	Coleoptra	Elmidae	4
	Potomanthidae	4	Diptera	Chironomidae	6
	Heptageniidae	4		Psychodidae	10
	Ephemerllidae	1		Ceratopogonidae	6
Odonata	Aeshnidae	3		Tabanidae	6
	Coengronidae	9		Tipulidae	3
	Gomphidae	1		Simuliidae	6
	Macromidea	2		Culicidae	8
	Lestidae	9		Syriphida e	10
				Chaoboridae	8
				Muscidae	6
FBI=∑Xi*ti/n	x = number of individuals within a taxon t = tolerance value of a taxon’ n = the total number of organisms in the sample.				

Annex 6 Qualitative Habitat Evaluation Indexes

OWQ Biological QHEI (Qualitative Habitat Evaluation Index)

IDEM

Sample #	bioSample #	Stream Name	Location

Surveyor	Sample Date	County	Macro Sample Type	☐ Habitat Complete	QHEI Score:

1] *SUBSTRATE* Check ONLY Two predominant substrate TYPE BOXES; estimate % and check every type present

Check ONE (Or 2 & average)

BEST TYPES (PREDOMINANT P/G R/R)	PRESENT P/G R/R	TOTAL %	OTHER TYPES (PREDOMINANT P/G R/R)	PRESENT P/G R/R	TOTAL %	ORIGIN	QUALITY
						☐ LIMESTONE [1]	SILT ☐ HEAVY [-2]
☐☐ BLDR/SLABS [10]	☐☐	____	☐☐ HARDPAN [4]	☐☐	____	☐ TILLS [1]	☐ MODERATE [-1]
☐☐ BOULDER [9]	☐☐	____	☐☐ DETRITUS [3]	☐☐	____	☐ WETLANDS [0]	☐ NORMAL [0]
☐☐ COBBLE [8]	☐☐	____	☐☐ MUCK [2]	☐☐	____	☐ HARDPAN [0]	☐ FREE [1]
☐☐ GRAVEL [7]	☐☐	____	☐☐ SILT [2]	☐☐	____	☐ SANDSTONE [0]	
☐☐ SAND [6]	☐☐	____	☐☐ ARTIFICIAL [0]	☐☐	____	☐ RIP/RAP [0]	EMBEDDEDNESS ☐ EXTENSIVE [-2]
☐☐ BEDROCK [5]	☐☐	____	(Score natural substrates; ignore			☐ LACUSTRINE [0]	☐ MODERATE [-1]
NUMBER OF BEST TYPES: ☐ 4 or more [2]			sludge from point-sources)			☐ SHALE [-1]	☐ NORMAL [0]
☐ 3 or less [0]						☐ COAL FINES [-2]	☐ NONE [1]

Substrate ☐ Maximum 20

Comments

2] *INSTREAM COVER* Indicate presence 0 to 3 and estimate percent: 0–Absent; 1–Very small amounts or if more common of marginal quality; 2-Moderate amounts, but not of highest quality or in small amounts of highest quality; 3-Highest quality in moderate or greater amounts (e.g., very large boulders in deep or fast water, large diameter log that is stable, well developed root wad in deep/fast water, or deep, well-defined, functional pools.)

% Amount		% Amount		% Amount		AMOUNT Check ONE (Or 2 & average)
___ __	UNDERCUT BANKS [1]	___ __	POOLS > 70cm [2]	___ __	OXBOWS, BACKWATERS [1]	☐ EXTENSIVE > 75% [11]
___ __	OVERHANGING VEGETATION [1]	___ __	ROOTWADS [1]	___ __	AQUATIC MACROPHYTES [1]	☐ MODERATE 25 - 75% [7]
___ __	SHALLOWS (IN SLOW WATER) [1]	___ __	BOULDERS [1]	___ __	LOGS OR WOODY DEBRIS [1]	☐ SPARSE 5 - < 25% [3]
___ __	ROOTMATS [1]					☐ NEARLY ABSENT < 5% [1]

Cover ☐ Maximum 20

Comments

3] *CHANNEL MORPHOLOGY* Check ONE in each category (Or 2 & average)

SINUOSITY	DEVELOPMENT	CHANNELIZATION	STABILITY
☐ HIGH [4]	☐ EXCELLENT [7]	☐ NONE [6]	☐ HIGH [3]
☐ MODERATE [3]	☐ GOOD [5]	☐ RECOVERED [4]	☐ MODERATE [2]
☐ LOW [2]	☐ FAIR [3]	☐ RECOVERING [3]	☐ LOW [1]
☐ NONE [1]	☐ POOR [1]	☐ RECENT OR NO RECOVERY [1]	

Channel ☐ Maximum 20

Comments

4] *BANK EROSION AND RIPARIAN ZONE* Check ONE in each category for EACH BANK (Or 2 per bank & average)

River right looking downstream

L R EROSION	L R RIPARIAN WIDTH	L R FLOOD PLAIN QUALITY	L R
	☐☐ WIDE > 50m [4]	☐☐ FOREST, SWAMP [3]	☐☐ CONSERVATION TILLAGE [1]
☐☐ NONE/LITTLE [3]	☐☐ MODERATE 10-50m [3]	☐☐ SHRUB OR OLD FIELD [2]	☐☐ URBAN OR INDUSTRIAL [0]
☐☐ MODERATE [2]	☐☐ NARROW 5-10m [2]	☐☐ RESIDENTIAL, PARK, NEW FIELD [1]	☐☐ MINING /CONSTRUCTION [0]
☐☐ HEAVY/SEVERE [1]	☐☐ VERY NARROW [1]	☐☐ FENCED PASTURE [1]	Indicate predominant land use(s) past 100m riparian.
	☐☐ NONE [0]	☐☐ OPEN PASTURE, ROWCROP [0]	

Riparian ☐ Maximum 10

Comments

5] *POOL/GLIDE AND RIFFLE/RUN QUALITY*

MAXIMUM DEPTH Check ONE (ONLY!)	CHANNEL WIDTH Check ONE (Or 2 & average)	CURRENT VELOCITY Check ALL that apply		Recreation Potential (Circle one and comment on back)
☐ > 1m [6]	☐ POOL WIDTH > RIFFLE WIDTH [2]	☐ TORRENTIAL [-1]	☐ SLOW [1]	☐ Primary Contact
☐ 0.7 - < 1m [4]	☐ POOL WIDTH = RIFFLE WIDTH [1]	☐ VERY FAST [1]	☐ INTERSTITIAL [-1]	☐ Secondary Contact
☐ 0.4 - < 0.7m [2]	☐ POOL WIDTH < RIFFLE WIDTH [0]	☐ FAST [1]	☐ INTERMITTENT [-2]	
☐ 0.2 - < 0.4m [1]		☐ MODERATE [1]	☐ EDDIES [1]	
☐ < 0.2m [0] [metric = 0]		Indicate for reach – pools and riffles.		

Pool/ Current ☐ Maximum 12

Comments

Indicate for functional riffles; Best areas must be large enough to support a population of riffle-obligate species:

Check ONE (Or 2 & average) ☐ NO RIFFLE [metric = 0]

RIFFLE DEPTH	RUN DEPTH	RIFFLE/RUN SUBSTRATE	RIFFLE/RUN EMBEDDEDNESS
☐ BEST AREAS > 10cm [2]	☐ MAXIMUM > 50cm [2]	☐ STABLE (e.g., Cobble, Boulder) [2]	☐ NONE [2]
☐ BEST AREAS 5 - 10cm [1]	☐ MAXIMUM < 50cm [1]	☐ MOD. STABLE (e.g., Large Gravel) [1]	☐ LOW [1]
☐ BEST AREAS < 5cm [metric = 0]		☐ UNSTABLE (e.g., Fine Gravel, Sand) [0]	☐ MODERATE [0]
			☐ EXTENSIVE [-1]

Riffle/ Run ☐ Maximum 8

Comments

6] *GRADIENT* (ft/mi) ☐ VERY LOW - LOW [2 – 4] %POOL: ☐ %GLIDE: ☐

☐ MODERATE [6 - 10]

DRAINAGE AREA (mi^2) ☐ HIGH - VERY HIGH [10 - 6] %RUN: ☐ %RIFFLE: ☐

Gradient ☐ Maximum 10

IDEM 11/15/12

Annex 7 Summary of average, physicochemical parameters values for upstream and downstream sampling sites and their statistical values of wastewater samples at Study Rivers.

	Upstream				Downstream			
	Average	Max.	Min.	Stan. Dev.	Average	Max.	Min.	Stan. Dev.
TSS	67.5	87	55	13.67	70.3	87	48	26.1
TDS	55.7	60.4	50.5	5.17	112.25	130	104	12
No_3^--N	0.62	0.88	0.43	0.19	1.1075	1.19	0.99	0.086
TN	1.2225	1.74	1.74	0.39	2.485	2.69	2.25	0.22
$NH4^+$-N	0.037	0.052	0.027	0.011	0.03425	0.041	0.032	0.0045
PO_4^{3-}-P	0.1575	0.14	0.14	0.012	0.1925	0.26	0.1	0.072
TP	0.21	0.23	0.19	0.018	0.2	0.27	0.17	0.052
BOD5	12.9	25	7.6	8.6	399.5	454	344	53.67
COD	19.75	26	11	7.08	506.75	567	430	61.79
W T	21.85	22.1	21.1	0.5	22.4	22.9	21.9	0.41
EC	101.75	112	92	10.2	171.25	199	159	18.6
pH	6.9	7.1	6.7	0.18	6.65	6.9	6.1	0.36
TURB	29.73	46.7	9.12	17.3	29.525	40.1	20.5	8.05
DO	6.375	6.99	5.42	0.7	5.205	5.8	4.47	0.56