

**EFFECTS OF BEER FACTORY EFFLUENT DISCHARGE ON BENTHIC
MACROINVERTABRATE DIVERSITY AND WATER QUALITY OF
META RIVER IN SEBETA TOWN, ETHIOPIA**

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**Effects of Beer Factory Effluent Discharge on Benthic MacroInvertebrate
Diversity and Water Quality of Meta River in Sebeta Town, Ethiopia**

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MASTER OF SCIENCE DEGREE IN BIOLOGY**

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DEDICATION

I dedicate this thesis manuscript to my Brothers, for their love, affection, unrestricted encouragement during this research work and every success in my life. It is also dedicated to my friend Bineyam Hailu and all the researchers who have shown their ingenuity to change our world.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this Thesis is my own work. I have followed all ethical technical principles of scholarship in the preparation, data collection, data analysis and completion of this Thesis. Any scholarly matter that is included in the Thesis has been given recognition through citation.

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BIOGRAPHICAL SKETCH

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ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
APHA	American Public Health Association
BMI	Benthic Macro invertebrates
BOD	Biological Oxygen Demand
CLI	Community Loss Index
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
EC	Electrical Conductivity
EPA	Environmental Protection Authority
EPT	Ephemeroptera, Plecoptera and Trichoptera
FBI	Family Biotic Index
GFF	Glass Fiber Filters
H-FBI	Hilsenhoff Family Biotic Index
NTU	Nephelometers Turbidity Unit
SPSS	Statistical Package for Social Sciences
TDS	Total Dissolved Solids
TSS	Total Suspended Solids
UNEP	United Nations Environmental Program
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
WHO	World Health Organization

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Effects of Beer Factory Effluent Discharge on Benthic Macro Invertebrate Diversity and Water Quality of Meta River in Sebeta Town, Ethiopia

ABSTRACT

*The use of benthic macroinvertebrates together with physico-chemical parameters are considered as the reliable method for water quality assessment. The main objective of this study was to determine the water qualities of Meta River using biological and physico-chemical parameters. One reference site which is less affected by the beer factory effluent discharge and two affected sites in the down stream were used to collect the water samples. A total of three water samples were collected from the three sampling sites. Each water sample was subjected to various physico-chemical analyses. Nine physico-chemical parameters pH, temperature, E.C, TDS, salinity, turbidity, nitrate-nitrogen, nitrite-nitrogen, phosphate were used. There were significant differences in most of the physico-chemical parameters ($p < 0.05$) between reference and impacted sites. Higher value of EC, Salinity, TDS, nitrate-nitrogen, and Nitrite-nitrogen was recorded at affected sites (site II and site III) than reference site (site I). Most of the physico-chemical parameters were out of WHO and EPA water quality standard. Total of 392 benthic macro invertebrates belonging to 10 species were collected. In addition, identification and quantification of benthic macro invertebrates was carried out for each sampling sites. Most of the benthic macro invertebrates significantly correlated with almost all physico-chemical parameters. *Chironomus riparius* was correlated positively with Temperature ($r=0.851$), TDS($r=0.9270$), Nitrate ($r=0.858$) and Nitrite ($r=0.898$). The Hilsenhoff biotic index categorized the impacted sites under poor very substantial pollution water quality but site I was categorized under good water quality in contrast to results of physico-chemical parameters. Reference site I had large number of macro-invertebrates with moderate tolerance and pollution sensitive groups. Waste water discharged to the nearby Rivers has enormous effect on the degradation of the ecosystem. From this study we conclude that Beer factory Effluent affects the quality of Meta River. To sustain the ecological conditions of the nearby Rivers, wastewater treatment and environmental audit are suggested.*

Keywords – Biological parameters, Macro- invertebrates, Meta River, Sebeta, Turbidity.

1. INTRODUCTION

The earth contains approximately 1.39 billion cubic kilometers (331 million cubic miles) of water, with 96.5 percent stored in the oceans of the world. Approximately 1.7 percent is stored in glaciers, permanent snow, sea ice and polar ice caps, while another 1.7 percent exists as ground water, in rivers, lakes, wetlands and in the soil (Smol, 2002).

Fresh water is a finite resource exploited in all possible way (Wetzel, 1983). It can be used for agriculture, industry and even human existence. Without fresh water of adequate quantity and quality, sustainable development will not be possible. Water pollution and wasteful use of fresh water threaten development projects and make water treatment essential in order to produce safe drinking water (Bartram and Balance, 1996). Only 0.01 percent of the vast amount of fresh water on this planet is available as surface water contained in lakes and rivers (Smol, 2002).

Water is critical for sustainable livelihoods and it is impossible to live without water. Furthermore, there is a pressure to use these resources with maximum effort to feed the fast growing population and to improve the standard of living of citizens. However, It is argued that the physical and chemical condition of many streams and rivers in tropical countries is deteriorating as a result of human population explosions, changes in land use, intensified agricultural practices and increased industrialization, all of which cause changes to natural flow regimes of rivers directly or indirectly (Pringle *et al.*, 2000; Wishart *et al.*,2000).

In different parts of the world, streams and Rivers are the major sources of water to satisfy human needs. And are the main supply of water for domestic uses, agriculture, transport, industries, power production and recreation. Rivers and streams are the most important fresh water resources. They support and maintain macro and micro ecosystems. They carry water from mountains to the sea, fueling the water cycle, coupling land, ocean and the atmosphere (Karr, 1999).

Ethiopia is a country in the horn of Africa with an estimated area of around 1.1 million km². It is often referred to as the “Water Tower of Africa” (Tenalem, 2009). Around

70,000 km² of area is covered with natural inland water bodies including rivers, lakes and associated wetlands (Wood and Talling, 1988). The wetlands cover 1.14 % of the total landmass of the country (Hillman and Abebe, 1993). In Ethiopia, human activities such as land use and modification, urbanization, human settlement and other practices associated with rapid population growth are the major water quality degrading factors (Zinabu and Elias, 1989).

Rivers and streams are open dynamic ecosystems whose physical, chemical and biotic characteristics are greatly influenced by anthropogenic activities taking place within their drainage basins (Mokaye *et al.*, 2004). Urban centers put huge amount of organic and synthetic waste in to rivers with little or no treatment. In general, the effect of human activities in rivers and their ecosystem affect one or more of the five attributes of water sheds and streams: Water quality, habitat structure, stream flow patterns, sources of energy and nutrients, and biotic interaction (Karr, 1991). Altering these attributes in turn upset the whole river ecosystem integrity. A river in which its ecosystem cannot sustain itself not only troubles the aquatic biota, but also it cannot support human affairs (Karr and Chu, 1997).

Today, most of the water bodies including ponds are heavily polluted by industrial wastes. Many of the industries discharge huge quantities of chemically or thermally polluted water which disturbs the biodiversity of the fresh water habitats. Benthic macro-invertebrates which are main faunal component and play an important role in nutrient recycling and energy flow in aquatic ecosystem, are primarily affected by these polluted industrial effluents (Chakravorty *et al.*, 2014).

The use of biological tools for environmental impact assessment is not familiar in Ethiopia. However, Baye Sitotaw (2006) used various macro-invertebrate metrics and habitat scores in the assessment of environmental degradation in some rivers of Ethiopia. Solomon Akau (2006) also assessed the biological integrity of Great Akaki River using macro-invertebrates. Admasu Tasew (2007) also investigated the biological integrity using physico- chemical parameters and macro-invertebrate community index in Sebeta River. Water quality monitoring is required for pollution control and to assess long term trends and environmental impacts. To monitor river or inland water body, it is necessary to assess its actual condition.

Fresh water environment can be monitored by physical, chemical and biological parameters. Although physical and chemical variables are commonly used to determine water quality, these parameters by themselves can only express the condition of water at the moment of sampling. On the other hand, biological monitoring can give information about the water conditions for a longer period and better represent the responses of aquatic habitats making biotic monitoring indices excellent tools for the sustainable management of water resources. In this regard, benthic macro-invertebrates (BMI) are useful in evaluating water quality and the overall health of flowing water systems. They are affected by changes in a stream's chemical and/or physical structure (Karr and Kerans 1991).

Previously, most of the biological methods for river monitoring are based on their trophic level through analysis of nutrient concentrations and/or pelagic primary producers or through analyses of consumers' community (oligochaetes, dipteran, chironomidae and fishes) whose characteristics considered as a trophic level result (Seather, 1979; Wiederholm, 1980). Then this type of biological method of river monitoring is changed and replaced by benthic macro invertebrate as water quality indicators.

Benthic macro-invertebrates have been found as the most common faunal assemblages for bioassessment and provide more reliable assessment of long term ecological changes in the quality of an aquatic system compared to its rapidly changing physico-chemical characteristics. Benthic macro-invertebrates respond differentially to biotic and abiotic factors in their environment. Consequently, the structure of macro-invertebrate has long been used as bio-indicators to assess the water quality of a water body (Reynoldson *et al.*, 1989; Duran, 2006).

In Sebeta Town there are different factories. Most of them are located around the water bodies and releases their effluent discharge directly in to the river. Some of the factories release treated waste while others release untreated waste in to the river. Among the factories in Sebeta town that release effluent discharge in to river is Meta Beer Factory and it is located near Meta River. Meta River is found 3 KM far from the center of Sebeta town to the west and originated from Mogolle Mountain, drain in to rural areas of Sebeta from north to southwest direction.

Peoples use this river for irrigation, for drink their cattles, to wash their clothes and their body and some families also use this river water for drinking. Studies on macro-invertabretes communities in rivers in response to brewery effluent have not been given much attention in Ethiopia. Therefore the purpose of this study was to assess the water quality of Meta River by using benthic macro-invertebretes and related physico-chemical parameters. The finding of this study would give baseline information on the quality of Meta River in Sebeta Town, Oromia Regional State, Ethiopia.

General objective

To assess the effect of Beer Factory effluent discharge on water quality of Meta River using benthic macro-invertebrates and physico-chemical parameters at Sebeta town, Ethiopia.

Specific objectives

1. To determine physico-chemical parameters of the Meta River water samples
2. To identify the benthic macro-invertebrate species in Meta river
3. To determine the diversity and distribution of the benthic macro-invertebrate in the Meta River.
4. To correlate physico-chemical properties with that of biological parameters of Meta River.

2. LITERATURE REVIEW

2.1. Sources of Water Pollution

Aquatic ecosystems are threatened world-wide by pollution, as well non-sustainable land-use and water-management practices that are reaching critical levels (Mayes *et al.*, 2007; Devi *et al.*, 1995). Pollution of the aquatic environment is defined as introduction of substances (wastes) and/or energy (like thermal) in to 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 and Halmer, 1996).

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 while diffuse sources consisting of many point sources of may also be controlled if all point sources can be identified. The major point source pollutions to fresh waters originate from the collection and discharge of domestic waste water and industrial wastes (Meyback and Helmer, 1996). Domestic wastes are those waste generated from commercial establishments and residential activities. They are primary source of organic waste released in to fresh water Tesfaye Berhe (1988).

Pollution of rivers and lakes with organic matter results in depletion of dissolved oxygen, and destruction of aquatic invertebrates and extensive fish kill. Industrial wastes polluting water bodies may contain inorganic nutrients, detergents, minerals compounds such as inorganic salts, heavy metals and natural organic compounds like carbohydrate, lipid and protein (UNEP, 1991). Inorganic nutrients like nitrates and phosphate have negative impact on aquatic ecosystem and human health. Nitrate is one of the most common contaminants in ground water. Since it is not strongly absorbed by sediments, it may move up to contaminate adjacent surface and ground waters. An excessive amount of nitrate and phosphate in rivers can induce eutrophication of surface waters leading to change in aquatic algal and macrophyte species composition and consequent decrease in dissolved oxygen (GSUQMP, 2002; Murphy, 2005; USGS, 2004).

River systems are the primary means for disposal of waste, especially the effluents, from industries that are near them. These effluent from industries have a great deal of influence on the pollution of the water body, these effluent can alter the physical, chemical and biological nature of the receiving water body. Increased industrial activities have led to pollution stress on surface waters both from industrial, agricultural and domestic source (Kanu *et al.*, 2011).

High Biological oxygen demand (BOD) levels leads to higher consumption of Dissolved oxygen (DO) by aerobic bacteria robbing the oxygen that other aquatic organisms need to get. Therefore, depletion of DO can cause major shifts in the composition and abundance of aquatic organisms. Families that cannot tolerate low levels of DO like mayfly, stone fly and caddis fly will be replaced by few kinds of pollution tolerant taxa such as worms and fly larvae (Barbour *et al.*, 1999).

Water consisting of high DO is usually considered healthy and capable of maintaining stable ecosystem with many taxa of organisms. However, a fall in DO level is an indicator of Organic pollution. Suspended solids and colloidal matter discharged with industrial wastes and sewage reduce water clarity and contribute to a decrease in photosynthesis in surface waters. In addition, they bind with toxic compounds and heavy metals, and rise water Temperature by absorbing sunlight. They may also clog the gills of fishes and benthic organisms, the benthic macro invertebrates are more adversely affected than fishes because of their small sizes (Murphy, 2005; USGS, 2003). The composition, abundance and distribution of benthic macro-invertebrates can be influenced by water quality (Hilman, 1993; APHA/WWA/WEF, 1998; Odiete, 1999).

2.2. Integrity of River Ecosystem

The integrity of river ecosystem refers to its biotic integrity. According to (Karr and Dudley, 1981) “biotic integrity is the ability of an aquatic ecosystem to support and maintain a balance, adaptive community of organisms having species composition, diversity and functional organization comparable to that of natural habitats within a region”. It can be fully characterized by the three major components: hydrology, physico-chemical and biology (De Berry and Perry 2005).

Excessive sediment load as the result of adverse human activities in the water shed, the riparian side and head water is a major factor for decline of benthic communities in rivers

and streams (USEPA, 1990). Sediments affects in stream biotic community by reducing habitat, altering water movement, food quality and interstitial spacing (Minshall, 1984). Fine sediments decreases the diversity of in stream biotic communities since the suspended solids absorb heat from sunlight, causing to increase and ultimately reduction in dissolved Oxygen (Murphy, 2000).

2.3. Nature and Types of Water Pollutant

Throughout history, the quality of drinking water has been a factor in determining human welfare. Fecal pollution of drinking water has frequently cause waterborne diseases that have decimated the populations. Currently, waterborne toxic chemicals pose the greatest threat to the safety of water supplies in industrialized nations. This is particularly true of groundwater in the USA which exceeds in volume the flow of all U.S. Rivers, lakes, and streams. In some areas, the quality of groundwater is subject to a number of chemical threats. There are many possible sources of chemical contamination. These include wastes from industrial chemical production, metal plating operations, and pesticide runoff from agricultural lands (Stanley, 1999).

Table 1. The major sources of pollution in aquatic system (Adopted from: Manahan, 1999).

Class of pollutant	Significance
Trace Elements	Health, aquatic biota, toxicity
Heavy metals	Health, aquatic biota, toxicity
Inorganic pollutants	Toxicity, aquatic biota
Algal nutrients	Eutrophication
Acidity, alkalinity, salinity (in excess)	Water quality, aquatic life
Trace organic pollutants	Toxicity
Polychlorinated biphenyls	Possible biological effects
Pesticides	Toxicity, aquatic biota, wildlife
Sewage, human and animal wastes	Water quality, oxygen levels
Biochemical oxygen demand	Water quality, oxygen levels
Pathogens	Health effects
Chemicals	Carcinogen, incidence of cancer
Sediments	Water quality, aquatic biota, wildlife

2.4. Source of Industrial Effluents

Industries are the major source of pollution in all environments. Based on the type of industry, various levels of pollutants can be discharged in to the environment directly or indirectly through public sewer lines. Waste waters are generated by many industries as a consequence of their operation and processing. Depending on the industry and their water use, the waste water contains suspended solids, both degradable and non biodegradable organic oils and greases; heavy metal ions; dissolved inorganics acids, bases and coloring compounds (Kanu *et al.*, 2011).

Industrial effluents are characterized by their abnormal turbidity, conductivity, chemical oxygen demand (COD); total suspended solids (TSS) and total hardness. The coastal residential environment in any industrial effluent site is always under considerable stress due to the prevailing harsh environmental conditions, especially high temperature and salinity, restricted benthic fauna diversity and over all development of a fragile intertidal ecosystem. The fauna inhabiting the intertidal zone is most likely dominated by a few species probably living at their limit of tolerance (Kanu *et al.*, 2011).

2.4.1. Industrial Effluents Discharged to River

The industrial discharge carries various types of contaminants to the River, lake and ground water. The quality of freshwater is very important as it is highly consumed by human for drinking, bathing, irrigation and etc. The presence of contaminants from industry within the water may reduce the yield of crops and the growth of plant and it will be harmful to the aquatic organism too (Jonathan *et al.*, 2008). Industrial effluents from beer factory are known to contain complex chemicals most of which are very toxic and capable of destroying the microbial habitats in a serious adverse way. Although studies on macro benthic invertebrates community in rivers in response to brewery have not been given much attention in Africa in general (Ogbeibu and Egborge, 1995).

The Rivers nearer industrial discharge point have adverse impact to the environment as well as to macro benthic communities. Toxic contaminants from surface runoff, sewage discharges and industrial discharge have caused negative impacts towards the fresh water macro benthic communities. The presence of substance chemical such as ammonia, chlorine, cyanide, metals, pesticides and phenols would caused a decline pattern on the

number of species and changes in the species composition. When industrial discharge and river regularly interact, benthic macro invertebrates will be highly exposed to the toxic contaminants. The living organism which will be deeply affected are shredders, which feed on coarse sedimentary detritus, and collector gatherers, which feed on fine sedimentary detritus, were the macro invertebrate functional feeding groups are most adversely affected (Camargo, 1992).

2.4.2. Composition of Brewery Effluent

Water with right quality runs through several different processes, but after absorbing materials or components from these process it becomes process waste water specific to the unit. Beer is a weak alcoholic drink obtained through fermentation, using selected Yeasts of the genera *Saccharomyces*, of wort prepared from malt cereals, mainly barley and other amylaceous or sugar-based raw materials, to which were added hop flowers, or their derivatives and adequate water (UNEP, 1995).

Resources consumed by the brewing industry include water, energy and grist materials (Barley, corn and rice), adjuncts and auxiliary materials such as Kieselguhr, caustic soda and detergents. Adjuncts are used to reduce the costs of production, to adjust the balance in the composition of the wort, and to produce (if desired) a “lighter” beer (UNEP, 1995).

Brewery wastes generated include Water treatment wastes, caustic boil-out solutions used for clean-ups in the brew house, and soak solutions and caustic rinses in the bottling area and organic loading, spent grain and hops, filter cakes in dry form and accidental losses from operational errors and leaking equipment (Ontario MOE, 1986). Solid waste mainly consists of organic material residuals from the process including spent grains and hops, trub, sludge, surplus yeast, label sludge, Kieselguhr, powdered carbon and broken glass (SEPA, 1991).

Other solid wastes from a brewery are glass cutlets from the packaging area, Kieselguhr from the filtration process, paper pulp from the bottle washer, paper, plastic and metal from received auxiliary material (especially packaging materials); and, waste oil and grease (Lenhardt, 1995). However, the quality and quantity of brewery effluent can fluctuate significantly as it depends on various different processes that take place within the brewery (raw material) handling, wort preparation, fermentation, filtration, clean in place

(CIP), packaging). The amount of wastewater produced is related to the specific water consumption (expressed as hectoliter water / hectoliter beer brewed). A part of the water is disposed with the brewery by-products and a part is lost by evaporation. As a result the wastewater to beer ratio is often 1.2-2 hectoliter/hectoliter less than the water to beer ratio. Organic components in brewery effluent (expressed as BOD) are generally easily biodegradable as these mainly consist of sugars, soluble starch, ethanol, volatile fatty acids and so on (SEPA, 1991).

The brewery solids (expressed as TSS) mainly consist of spent grains, kieselguhr, waste Yeast and ('hot') trub. Brewery effluent's pH levels are mostly determined by the amount and type of chemicals used at the clean in place (CIP) units (caustic soda, phosphoric acid, nitric acid and so on). Nitrogen and phosphorous levels are mainly dependent on the handling of raw material and the amount of spent yeast present in the effluent. Elevated phosphorous levels can also be the result of phosphorous containing chemicals used in the CIP unit (Lenhardt, 1995).

2.4.3. Environmental Impact of Brewery Effluent

The primary environmental impacts that can be attributed to the production of beer are the result of noise, emissions to air, wastewater discharges and inefficient waste handling system (USEPA, 1991). Wastewater treatment is a problem that has plagued man ever since he discovered that discharging wastes into surface waters can lead to many additional environmental problems. Intensified industrial and agricultural practices, as well as the exponential growth of the human population and explosive urbanization in the last few decades have led to an enormous increase in the discharge of nutrient (nitrogenous and phosphorus compounds) into the environment.

Severe environmental problems which are of global concern arise from these excessive loadings due to their pollution effects on the receiving ecosystems. Owing to microbial processes in wastewater nitrogen is present in raw wastewaters mostly in reduced form as ammonia nitrogen ($\text{NH}_3\text{-N}$ or $\text{NH}_4\text{-N}$) and organic nitrogen (urea, amino acids, proteins, and nitrogen heterocyclic compounds). However, in well established treatment systems, the oxidized forms of nitrogen ($\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$) exist in wastewaters.

Phosphorus in wastewater exists both as inorganic and organic forms, with orthophosphate (PO_4^{3-}P) form that is easy to assimilate dominating raw waste water. The discharge of nutrient rich waste water of domestic and industrial origins can have deleterious consequences on the ecological balance and functioning of the receiving environment as well as the public health of downstream end-users of the polluted water sources. Such devastating consequences manifest as: toxicity to fish and other aquatic organisms; depletion of the dissolved oxygen in receiving water bodies as ammonia or ammonium ions are oxygen consuming; eutrophication when nitrogen and phosphorus are made available to aquatic plants as nutrients; and potential public health risk (methaemoglobinemia) in drinkingwater, especially when consumed by infants (Bitton, 1999; Horan, 1990; and NebGuide, 1998).

Due to bacterial reduction of consumed nitrate to nitrite in the digestive system nitrite oxidizes iron in the haemoglobin of red blood cells to form methaemoglobin, which lacks haemoglobin's oxygen-carrying ability. Thus, the methaemoglobin formed in this interaction cannot carry sufficient oxygen to the infant's cells and tissues leading to its blue appearance (blue baby syndrome). Not only does consuming drinking water contaminated with nitrate-nitrogen above the maximum containment level (MCL) of 10mg/L have the potential to result in methaemoglobinemia, but recent studies also have indicated a possible risk of cancer, as well as the potential to be a contributing factor in Spontaneous abortions. Nitrates can react with amines or amides in the body to form Nitrousamine which is known to cause cancer (NebGuide, 1998).

2.5. Physico-Chemical Parameters

Understanding Rivers physical, chemical and biological properties is essential to determining the River's condition and in making informed River management decisions. The most commonly used physico-chemical parameters for water quality measurement of Rivers were,

- Physical measurements like temperature and total dissolved solids (TDS), PH and conductivity.
- Chemical measurements such as nutrients (nitrates and phosphate).

These parameters are important to study water quality at the moment of study only because its result fluctuates with the diurnal and seasonal variation of the weather condition and based on level of input of pollutants at a certain time

A) Temperature

Most aquatic organisms have adapted to survive within a range of water temperature. Organisms like Stoneflies and Mayflies prefer cooler water, while others like Dragonflies need warmer condition. As the temperature of water increases, cool water species will be replaced by warm water organisms. Temperature also affects aquatic life sensitivity to toxic wastes and disease, either due to rising water temperature or the resulting decrease in dissolved oxygen. Water temperature influences aquatic weeds, algal blooms and surrounding air temperature (Gupta *et al.*, 1993).

B) pH

pH value of water is important to many organisms. Aquatic insects are extremely sensitive to pH value below five. The Gastropods, Mayflies, Stoneflies and Caddis flies are some of macro invertebrate groups that prefer pH level from 7-9.5. One of the most significant environmental impacts of pH is involvement on synergistic effects. For example, very acidic water can cause heavy metals, such as copper and aluminum to be released into the water. These heavy metals may accumulate on the gills of fish or cause deformities in young fish, reducing their chance of survival (Siva Kumar and Karuppasamy, 2008). High pH could alter the toxicity of other pollutants. For example, ammonia is much more toxic in alkaline water than acid because free ammonia (NH_3) at high values ($\text{pH} > 8.5$) is more toxic to aquatic biota than when it is in the oxidized form (NH_4^+) (Deshu Mamo, 2004).

C) Conductivity

Conductivity is a measure of the waters ability to conduct an electric current. It is also useful for estimating the concentration of total dissolved solids (TDS) in the water. Because the measurement is made using two electrodes placed one centimeter apart, conductivity is generally reported as microsiemen's per centimeter ($\mu\text{S}/\text{cm}$). The lakes with high alkalinity often have high conductivity (Bronmark and Hansson, 1998)

D) Phosphate (PO_4^{-3})

Phosphorus is a limiting nutrient for algal growth and, therefore controls primary productivity. It is rarely found in high concentrations in fresh waters as it is actively taken up by plants. Its seasonal fluctuations in surface waters are considerable. In most natural waters, phosphorus ranges from 0.005 to 0.02mg/L. Concentrations as low as 0.001mg/L may be found in some pristine waters and as high as 200mg/L in some enclosed saline waters (Chapman and Kimstach, 1996). Small amount of phosphate (to the level 0.01mg/L) can have measurable effect on aquatic communities. High phosphate concentration in Rivers can lead to eutrophication (USEPA, 2006).

E) Nitrates (NO_3^-)

Natural Nitrates (NO_3^-) concentrations seldom exceed 0.1mg/L. The amount may be enhanced by municipal and industrial wastes. The two important source of nitrate is fertilizers and the runoff from cattle feedlots and dairies. Nitrate concentration is indicates organic pollution in an area. Nitrogen concentrations in lakes and Rivers vary widely from about 100 $\mu\text{g/l}$ to over 6000 $\mu\text{g/l}$. In most lakes the concentration is usually from 4-1500 $\mu\text{g/l}$ but in polluted lakes, the level extends to more than 5000 $\mu\text{g/l}$ (Bronmark and Hansson, 1998).

F) Turbidity

Turbidity is a measure of the relative clarity of water. In its 1984 Guidelines, WHO recommended turbidity should be maintained at less than 5NTU, but if water was disinfected it would be better to aim for values of less than 1 NTU. Drinking water with turbidity less than 1NTU was considered safe if it was disinfected by chlorine with free residual of 0.5 mg/l at pH less than 8.0 (WHO, 1984).

2.6. Biological Parameters

Biological assessment (bio assessment) is defined as an evaluation of condition of a water body using biological surveys and other direct measurements of the resident biota in surface waters (Braccia and Voshell, 2006). The determination is by gathering multiple measures of biological data, converting the data in to single numerical index, and then comparing the index with an index developed for a reference condition. Reference

condition is established by characterizing the biology and water quality of reference sites with unimpacted water bodies (Barbour *et al.*, 1999). Taxa richness, composition, tolerance/ intolerance measures and feeding groups together with various diversity indices such as community loss index (disappearance of certain taxa in impacted conditions with respect to the reference one) and Shannon weaver diversity are the most frequently employed metrics in bio assessment (Barbour *et al.* 1999, Mandeville, 2002; peitz, 2003; Nwra 2005). Biological parameters are increasingly studied as more sensitive indicators of ecosystem integrity than physico-chemical parameters (Craft *et al.*, 2007; Flinders *et al.*, 2005; Smith *et al.*, 2007).

2.6.1. Biological indicators of water quality

Bioindicator measures the change of biological or non-biological factors in ecosystem focusing on a living thing in some circumstances. Bioindicator indicates a living thing or a group of living things. It is used as a representative to understand and estimate general status of ecosystem. But more specifically or generally, it means the impact of environmental change in habitats, community, or ecosystem as species or group of species representing the status of living things or inanimate objects in the environment. It could also indicate living things or group of living things that show the diversity of taxonomic group in an area or a subset in entire diversity (Gerhardt, 2002).

Macro invertebrates have long been used as indicators to assess water quality. A well-balanced and functioning biological community is one of the best indicators of a healthy stream, capable of providing vital ecosystem services. Benthic macro invertebrates (bottom-dwelling organisms including aquatic insects, crayfish, clams, snails, and worms) are often used in studies to determine the quality of water because of their high numbers, known pollution tolerances, limited mobility, wide range of feeding habits, varied life spans, and dependence on the land environment around the stream. When chemical grab samples are taken, they are really a snapshot of the water at that moment, that can change rapidly, but the macro invertebrates are living there all the time. Their composition will be affected by either periodic episodes of poor water quality or continuous poor water quality. Many watershed monitoring programs include biological indicators as well as chemical and physical tests. Water resources with high water quality generally have diverse and rich macro invertebrate fauna; certain pristine environments have low

diversity of macro invertebrate fauna because of the cold temperature and/or relatively low nutrient levels (Peckarsky *et al.*, 1990).

Macro benthic invertebrates are useful bio indicators providing a more accurate understanding of changing aquatic conditions than chemical and microbiological data, which at least give short term fluctuations (Raven, 1998, 2000, Ikomi *et al.*, 2005).

2.6.2. The Role of Benthic Macro-invertebrates for Bioassessment

Aquatic macro invertebrates are good indicators of stream quality because:

- They are affected by the physical, chemical, and biological conditions of the stream.
- They can't escape pollution and show the effects of short- and long term pollution events.
- They may show the cumulative impacts of pollution.
- They may show the impacts from habitat loss not detected by traditional water quality assessments.
- They are a critical part of the stream's food web.
- Some are very intolerant of pollution.
- They are relatively easy to sample and identify several biological communities including microphytobenthos, macrophytes and fishes have been considered in assessments of water quality.

However, the use of benthic invertebrate communities as indicators of environmental degradation or restoration has become widespread and reliable for bio-assessment since the benthos broadly reflect environmental conditions (Jackson, 1993; Rosenberg and Resh, 1993).

Freshwater benthic macro-invertebrates or "benthos" are animals that inhabit the bottom of substrates (for example, sediments, debris, macrophytes and filamentous algae) of their habitats for at least part of their life cycle and are larger than 500µm. They are retained by mesh sizes from 200-500 micrometers. Benthic macro-invertebrates include insect larvae, annelids (leeches), oligochaetes (worms), crustaceans (crayfish and shrimp), mollusks, (clams and mussels), and gastropods (snails). Insect larvae tend to be the most abundant

benthic macro-invertebrates in freshwater aquatic ecosystems (Rosenberg and Resh, 1993).

Aquatic invertebrates are morphologically, physiologically and ecologically diverse and therefore exhibit a wide range of responses to toxicants (Maciorowski and Clarke, 1977). As benthic macro-invertebrates tend to remain in their original habitat, they are affected by local changes in water quality. Some are capable of tolerating higher loads of pollution than others. If the pollution is severe, the whole community structure may simply in favor of tolerant species. Although the abundance of certain species may increase, the diversity and species richness decreases. By assessing indicator species, diversity, and functional groups of the benthic macro-invertebrate community, it is possible to determine water quality (Lange, 1994). Collections of macro invertebrates from more than one habitat type may introduce variation that can potentially mask water quality difference among sites (Jeffrey and Charles, 2003).

2.6.3. Macro Invertebrates and Water Quality Assessment

The major reason for using benthos in toxicity test is that information of the effects of toxicants on macro invertebrates is essential in the protection of aquatic ecosystems. Toxicity tests help in evaluating the nature and degree of harmful effects produced on aquatic organisms by toxicants since toxicants alter the distribution, density and behavior of aquatic invertebrates by direct lethal or sub lethal action on a particular species, or indirectly by affecting a species food, competitors, predators, or habitat (Maciorowski and Clarke, 1977).

According to Maciorowski and Clarke (1977); Metcalf's (1989) and Bode *et al.*, (1996) the criteria and/or advantages why benthos should be used in water quality assessment include: The Sampling procedures of benthoses are relatively well developed and it can be operated by someone working alone. There are identification keys for most groups of macro invertebrates and benthoses are reasonably sedentary with comparatively long lives, so that they can be used to assess water quality at a single site over a long period of time. The size of benthos is almost ideal for water quality testing, since many are macroscopic and can even be recognized with the naked eye.

The diversity of aquatic invertebrates provides several attributes that can be utilized as responses in laboratory toxicity test. Reproduction and life-cycle of benthos may be completed with 2 to 4 weeks with genera such as *Daphnia* and *Chironomus* whereas life cycle studies with rapidly reproducing fish may require 3 months to 2 years. The group is heterogeneous and so a single sampling technique may catch a considerable number of species from a range of phyla. Since taxa differ in their tolerance to pollutants, particular taxa make useful indicators of conditions. In addition benthic invertebrates respond sensitively not only to pollution, but also to a number of other human impacts (physical modification, recreational and others) (Roy *et al.*, 2003).

In Ethiopia, benthoses of streams and Rivers have been studied for various reasons. For instance, Baye Sitotaw (2006) studied some benthic macro invertebrate structures in relation to environmental degradation in some rivers; Birenesh Abay (2007) assessed pollution of downstream effluent along TikurWuha River using macro invertebrate indicators; Harrison and Hynes (1988) were pioneers to study benthos in Ethiopia, especially the benthic fauna of highland streams and Tesfaye Berehe (1988) studied degradation of Kebena river using macro invertebrate structures and composition. Hayal and Seyoum (2009) studied water quality parameters and macro invertebrate index of biotic integrity of the Jimma wetlands and Tujuba (2010), studied macro invertebrates abundance and community structure of Lake Kuriftu. Evaluating the status of water quality and climate change by examining fauna has been well established in both theory and practice for several decades (Davis, 1995). In this regard, benthic macro invertebrates are useful in evaluating water quality and the overall health of flowing water systems. They are affected by changes in a stream's chemical and/or physical structure (Karr and Kerans, 1991).

3. MATERIALS AND METHODS

3.1. Descriptions of the Study Area

Sebeta town is located in Oromia National Regional State, Oromia Special Zone surrounding Finfinne at a distance of 24 Km from Addis Ababa to Jimma main road. Its' location is 8o 55' north latitude and 38o37' East longitude with elevation of 2356 meters. Above the sea level. The town was founded in 1942. According to the Ethiopian major cities and town statistics carried out in 2013 the population of the town was 63,400. Out of this 31,301 were males and 32,099 were females'. Meta Beer Factory is located 1 Km far from the main road to the west. Similarly the study site is located 3 Km far from the center of Sebeta town. Meta River is originated from Mogole Mauntain and flows down from the north to south west region and mixed with Awash River (Figure 1).

3.2. Design of the Study

Laboratory based cross sectional study was conducted to assess the effect of Beer Factory effluent discharge on benthic macro invertebrate diversity and water quality of Meta River using physico-chemical parameters and benthic macro invertebrates in sebeta town, Oromia Regional State, Ethiopia. Water samples were collected from three sampling sites; Site I (reference site), Site II and III (affected Sites) purposively. Each water sample was subjected to laboratory analyses including determination of various physico- chemical parameters. These include pH, temperature, electrical conductivity, salinity turbidity, total dissolved solids, nitrate- nitrogen, nitrite nitrogen and phosphate.

The design based on laboratory investigation was carried out by water samples from three sites using purposive sampling in two times for physical measures like temperature, turbidity, pH, electrical conductivity, salinity and total dissolved solids on site. Chemical measurements such as nitrate, nitrite and phosphate were done in sebeta polytechnic college of water laboratory. In addition identification and quantification of benthic macro invertebrates (BMI) species as well as their diversity estimation was investigated for each sampling sites. Benthic macro invertebrates' collection was done by using D-net (net with 500 µm mesh size).

First round River water sample collection were done at the beginning of December 2016 and second round were done at the end of December 2016. Identification of benthic macro invertebrates' species was done using a binocular microscope at Sebeta Research Station using identification keys (Bucharads key) to their respective taxonomic levels (species level). The study was conducted in Oromia special zone surrounding Finfinne at Sebeta Town. From November 2016 up to February 2017.

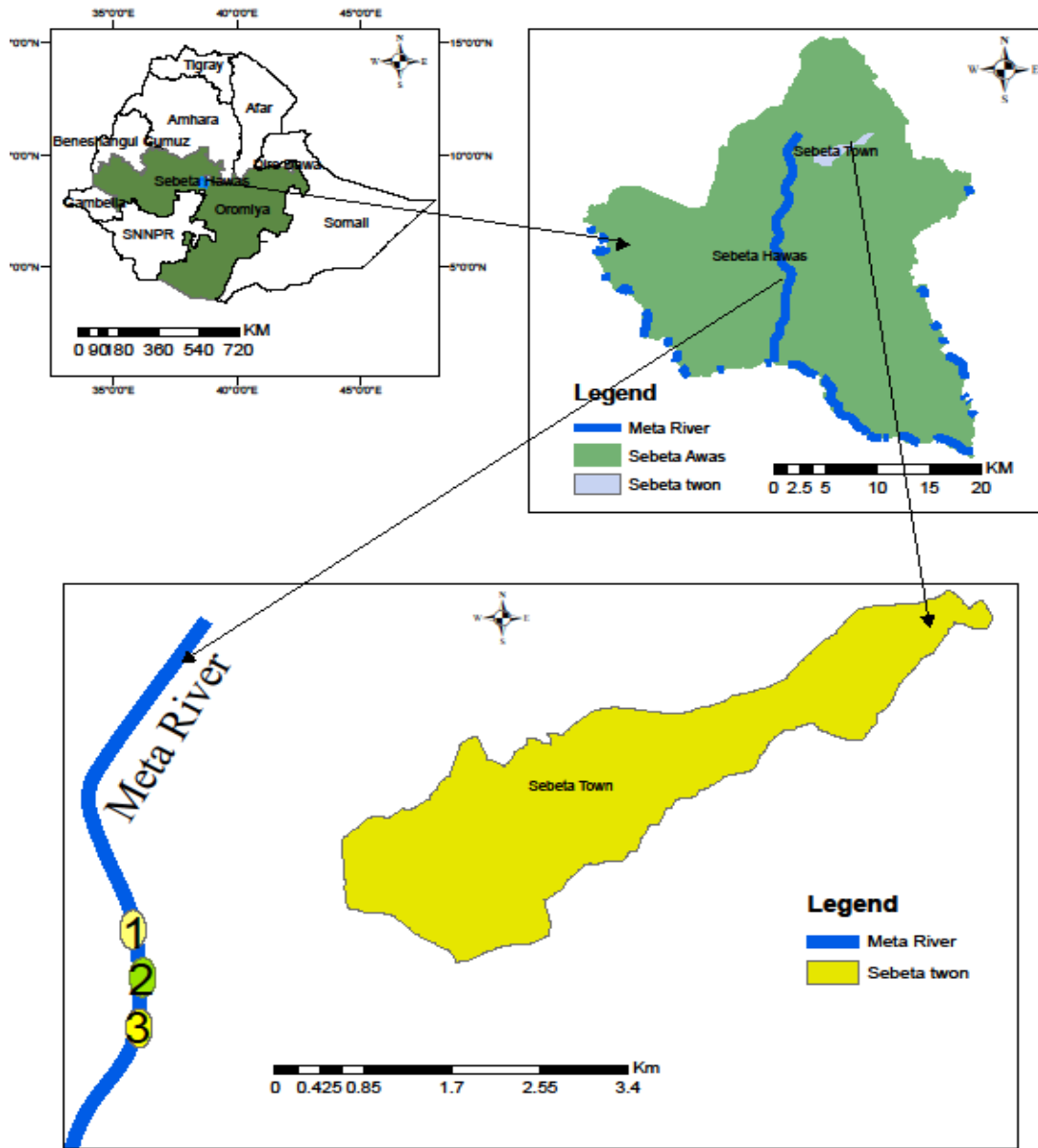


Figure 1. Map showing Sebete Town and the study area within Oromia Regional State Ethiopia.

3.3. Sampling Sites and Collection of River Water Samples

There were three sampling sites selected based on purposely sampling methods (Site-I, Site-II and Site-III). The reference condition collectively refers to the range of quantifiable ecological elements (i.e. chemistry and biology) that were found in minimally disturbed environments. Finding reference sites in streams and rivers are a difficult task, because no regions are entirely without areas of human disturbance. Therefore, the reference site has to be selected based on minimally or least disturbed attributes (Gregory *et al.*, 1991).

Thus, the Reference site was identified based on the following criteria as there was a problem getting a reference site that fulfills all the criteria indicated by Jennifer *et al.*, (2003):

- Same water body type, size, and chemical characteristics as treated site
- Within same watershed as treated site,
- Minimal application of aquatic pesticides within the last few years, and
- Limited anthropological inputs.

According to the above criteria, a reference site one (Site I) was selected to compare against the impacted sites two (Site II) and site three (Site III). Chemical and biological measures taken in impacted sites were compared to reference site. Site I was chosen because it is found in the upper stream of the river with good habitat quality and had minimal anthropogenic interventions. The watershed of Meta River is highly exposed to several anthropogenic interventions including- agricultural and industrial activities. Among this, waste water from industry together with agricultural activities cover all the watershed of the river and are more likely to affect the habitat qualities of the river. Agricultural activities (both farming and grazing) are known for their diffuse source pollution and a riparian vegetation removal. The riparian side was slightly covered by different vegetation type like Eucalyptus and other trees in the upper stream and mainly Eucalyptus, Shrubs and grasses in the downstream along the river bank.

The sampling site selection was conducted which based on 100m length with riffle zone. This site was selected according to the USEPA Rapid Bio assessment protocol criteria (Barbour *et al.*, 1999). Three sites were sampled along the course of the river.

Site-I - is 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 relatively unpolluted. It was a reference Site for bio assessment with less human activity which was around 300m upstream far from the factory.

Site-II - is situated in the downstream behind the beer factory and was covered by grasses and Eucalyptus trees. It was the contact point of the Meta brewery effluent with the river water before it flows down to site III.

Site-III - is around 300 m far from site-II and mainly covered with grasses shrubs, and Eucalyptus trees.

Sampling at 3-5 points is usually sufficient and fewer points were needed for narrow and shallow rivers and streams. Thus a composite sampling technique was employed to take water samples at 3 points across the width of rivers for chemical analysis (Bartram and Balance, 2001). The samples were transported to laboratory within 1-6 hours for analysis and chemical analysis following the standard methods (APHA, 1998). Composite one liter water was taken from each site using a one liter plastic bottle and stored in ice box prior to analysis.

3.4. *In situ*-Physico-Chemical Measurements

Physico-chemical variables measure the assessment of the extent of pollution of Meta River. These include; electrical conductivity (EC), pH, temperature ($^{\circ}\text{C}$) Nitrate- Nitrogen ($\text{NO}_3^- \text{N}$), Nitrite-nitrogen ($\text{NO}_2^- \text{N}$) and Phosphate (PO_4^{3-}). Electrical conductivity was determined with a field conductivity meter and temperature was measured by digital thermometer in which all were measured on site. pH of water was measured using portable digital pH meter and turbidity by digital turbido meter (USGS, 2003).

3.5. Water Chemistry

Before analysis samples were first filtered through glass fiber filters (GF/F). Then the filtered water sample was used for measurements of chemicals like-Nitrate-nitrogen (NO_3^-N), nitrite-nitrogen (NO_2^-N), Phosphate (PO_4-P). For total phosphorus determination (TP) unfiltered water sample was used. Nitrate, Nitrite and Phosphate were measured by using spectrophotometre (DR, Japan, 2008). To measure the concentration of Nitrate-nitrogen First 10ml sample water were added in to cuvet then add Nitriver 5 nitrate reagent powder pillow and wait for 5 minutes then measure. To measure the concentration of Nitrite-nitrogen add 10 ml of sample in to cuvet then add Nitriver 3 Nitrite reagent powder pillow and wait for 20 minutes for reaction then measure. The same procedure was applied to measure the concentration of phosphate except the reagent add phosver 3 phosphate reagent and the reaction time this process take 2-8 minutes for reaction and finally the result was measured.

3.6. Sampling of Benthic Macro Invertebrates

Collections of macro invertebrates from more than one habitat type may introduce variation that can potentially mask water quality difference among sites (Jeffrey and Charles, 2003). To minimize this variation, all samples were collected from the same habitat types of riffle zones of streams and rivers in areas where there was the best canopy coverage and side bank macro vegetation. Riffle communities of streams and rivers are also more diverse in invertebrate forms than pools (Gerth and Herlihy, 2006).

First round macro invertebrate collection was done at the beginning of December, 2016 and second round was done at the end of December, 2016. At each site, qualitative macro invertebrates sample were collected from a River section of about 100 meter length, using D-net (net with 500 μ m mesh size) and finally preserved in 70% ethanol. For numerical analyses, all samples from 100-m River section were pooled as one sample. Identification of benthic macro invertebrate was done using a binocular compound microscope and various available keys were assigned to their respective taxonomic levels (family level or species level) identification (Hailu and Legesse, 1997; Hering *et al.*; 2006; Flinders *et al.*; 2008). For each group diagnostic structures were photographed.

3.7. Identification and Quantification of Macro Invertebrates

The macro invertebrate metrics representing composition, richness, rate of organic pollution of the river, and taxa lost (intolerant) due to perturbation was used. Metrics (or indices) allow the investigator to use meaningful indicator attributes in assessing the status of assemblages and communities in response to perturbation. Metrics were review based on description in the EPA, and Hilsenhoff (1988) from the data. The criteria for metric selection were the ability to be associated with physical degradation, ability to provide unique information about the River and ability to discriminate reference site from impacted sites (Chihart, 2003). Thus, this study was restricted to indices focused on the determination of water quality. The indices to be selected were Shannon-Weaver Diversity index, Hilsenhoff-Family Biotic Index (H-FBI) and Community Loss Index. The Shannon-Weaver Diversity Index (H) was evaluating the abundance and evenness of benthic macro invertebrates among the study sites.

This was calculated as: $H = - \sum_{i=1}^S (P_i) (\log P_i)$

Where “Pi” is the proportion of individuals in the “ith” taxon of the community and “S” is the total number of taxa in the community.

To analyze the degree of organic load among the study sites, Hilsenhoff-Family Biotic Index was computed using Hilsenhoff (1988). The Family Level Index has been modified for the Rapid Bio Assessment Protocol II to include organisms other than just arthropods using the genus and species level tolerance values adopted by the State of New York (Bode *et al.*, 1996). Although the FBI may be applicable for toxic pollutants, it has only been evaluated for organic pollutants.

The formula for calculating the Family Biotic Index is:

$$FBI = \frac{\sum X_i T_i}{N}$$

Where: xi= number of individuals within a taxon

Ti= tolerance value of a taxa

N=total number of organisms in the sample

To measure the loss of benthic taxa and dissimilarity between sample sites, community loss index (CLI) was used. Community loss index measures the loss of benthic taxa in a study site with respect to a reference site. Values range from 0 to infinity and increase as the degree of dissimilarity between the sites increases (Plafkin *et al.*, 1989).

Community loss index was calculated as: $CLI = (D - A)/E$

Where, A- is the number of taxa common to both sites

D- Is the total number of taxa present in reference site

E- Total numbers of taxa present in the study sites.

3.8. Data Analysis

For sampling site comparisons, the physico-chemical variables and benthic macro invertebrate community structures were analyzed for all of the three sampling sites. All data collected were subject to statistical analysis appropriate for the multimetric approaches. For all statistical tests, a probability of $P < 0.05$ was considered significant.

Relationship between the environmental and biological (macro invertebrates) data were assessed. One way Analysis of variance (ANOVA) was used to test for significant difference in physico-chemical parameters and macro-invertebrates among the study sites and to locate site(s) of significant difference, respectively. SPSS and Microsoft Excel sheet on computer were used to analyze data obtained from physico-chemical and biological parameters. Bivariate Pearson correlation was used to inter-relate the physico-chemical parameters and macro-invertebrates.

4. RESULTS AND DISCUSSION

4.1. The Physico- chemical Parameteres of Meta River water samples

4.1.1. Temprature

Water quality profiles for each of the three sites are presented in Table 2. The Maximum and Minimum values of the temperature are presented, the maximum and minimum temperature measurement for site I was 17.4 °c and 17.2 °c. The maximum and minimum temperature for site II was 18.8, and 17.5 °c respectively. For site III the maximum, minimum and mean temprature recorded were 18.2, °c 18 °c and 18.1 °c respectively. The temperature values were approximately the same at the impacted sites except site I which showed slightly lower temperature than site II and III. The mean temperature of site I was 17.3 ± 0.10 . In this study, relatively the highest mean value for temperature was recorded in Site II ($18.15 \pm 0.65^{\circ}\text{c}$) appendix 2. There was a mean temperature difference between the uppestream reference site and down stream sampling sites. All the temperature value was within the EPA (2003) standared for effluent discharge to surface water. Despite slight change in temperature of the river water at the reference sites and the impacted sites was normal (within the range of ambient standared for surface water) with no significant difference in the three sites ($P > 0.05$). So temperature of the effluent has no effect on the Meta River Table 2.

4.1.2. pH

As the result shown in Table 2, the pH value of the study sites were within EPA (2003) standards for surface water (6.0-9.0) and WHO drinking water quality standards and ambient standard for surface water for pH (6.5-8.5). The maximum and minimum and pH value for the three sampling sites were presented in the table below. The maximum and minimum pH value for site I was 7.8 and 7.4 for site II it was 6.75 and 6.66. Similarly the pH value recorded in site III was 6.98 and 6.78 respectively. The mean pH value recorded at the three sites was 7.6, 6.7 and 6.88 these revealed that the pH value become decreases as we go from reference site to the impacted sites appendix 2. This indicates that the waste water discharges mainly from Beer Factory cause decreament of pH which directly affect water quality as well as the distribution of the benthos.

All of the pH values of the effluent during the sampling period were within the Brewery effluent discharge limits. The low pH value is due to acidic discharges (i.e. beer and by-products) resulting from beer production. Thus, the effluent is acidic and has the potential to acidify the River water. Apart from high organic content of brewery effluent, spent wash generated from the fermentation step also contains nutrients in the form of nitrogen. Spent wash is the dark brown distillery wastewater generated during the fermentation step of beer production (Satyawali, 2008).

The result in table 2 indicates that significant variation in pH was seen during first round and second round data collections between site I&II ($p=0.03$) and site I &III (0.061). These permit an accumulation of discharge of organic waste in to the river which brings change in pH. Naturally, occurring fresh waters have a pH range between 6 and 9; the concentration range suitable for the existence of most biological life is quite narrow and critical. Most fresh waters are relatively well buffered and more or less neutral.

The pH of the water is important because it affects the solubility and availability of nutrients and how they can be utilized by aquatic organisms. It also alters the ionic and osmotic balance of individual organism and determine the type of the chemicals (and thus the potential toxicity) of numerous elements and molecules (e.g. ammonia) found in water. Aquatic organisms are very sensitive to the pH of the aquatic environment because most of metabolic activities are pH dependent (Wang *et. al.*, 2002). Analysis of variance (ANOVA) showed that a statistical difference at $p<0.05$ in the three sites. Sites III and Site II were similar, while sites I was significantly different from sites II and Site III (Table 2).

4.1.3 Electrical Conductivity

Conductivity is the ability of the water to conduct an electric current, and is an indirect measure of concentration of ions. The more ions present, the more electricity can be conducted by the water. As the results shown in table 2 the average electrical conductivity value for the three sites were presented. The maximum and minimum mean E.C recorded in Site I was 265 and 260 $\mu\text{S}/\text{cm}$, site II 965 and 915 $\mu\text{S}/\text{cm}$ and E.C of site III was 786 and 782 $\mu\text{S}/\text{cm}$ respectively. The average electrical conductivity of the three sampling sites was within the EPA standard discharge to surface water. Mean value of site I was 262.5 $\mu\text{S}/\text{cm}$, site II, 940 $\mu\text{S}/\text{cm}$ and site III, 784 $\mu\text{S}/\text{cm}$ appendixes 2.

The result of this study was different from the study made by Seyoum Leta *et al* (2003) reported E.C.value of $14330 \pm 1182.3 \mu\text{S}/\text{cm}$ at tannery waste water receiving sites of Modjo River. The variation in the level of conductivity is associated to the total dissolved solids (TDS) in water (Bauder *et al.*, 2003). This fact is best seen in this study. The EC provides an ideal about the exchangeable elements present in the water. The source of EC might be ions from industry or fertilizers. Conductivity at 25°C is $0.055 \mu\text{S}/\text{cm}$ in absolute pure water. The optimum EC for stream water is $100\text{-}1000 \mu\text{S}/\text{cm}$ at 20°C (EPA, 2003).

In this study, electrical conductivity values varied between $262.5 \pm 2.5 \mu\text{S}/\text{cm}$ (Site I) and $940 \pm 25 \mu\text{S}/\text{cm}$ (Site II). The highest electrical conductivity value at Site II ($940 \pm 25 \mu\text{S}/\text{cm}$) and Site III (784 ± 2.00) indicate the effect of industrial wastes discharge into the River. Meta Brewery Factory release wastes to the Meta River thus it is responsible for the increasing values of the conductivity at Site II. There was significant difference between the reference site and impacted site at $p < 0.05$ table 2.

4.1.4. Nitrate- Nitrogen (NO_3^- -N)

As the result indicated below the levels of nitrate ranged from $1.35 \pm 0.045 \text{ mg}/\text{L}$ to $7.4 \pm 1.00 \text{ mg}/\text{L}$ appendixes II. The maximum, minimum and mean Nitrate concentration recorded in the impacted site was greater than the amount recorded in the reference site. The maximum and minimum Nitrate concentration for site I was 1.39 and 1.3 mg/l, respectively and site II, 8.4 and 6.4 respectively. Nitrate concentration in site III was 7.1 and 6.8 mg/l. The mean nitrate concentration for the three sampling site was 1.35, 7.4 and 6.95 mg/l respectively appendix 2. Analysis of variance (ANOVA) showed that there was statistical difference (at $p < 0.05$) in the mean values of nitrate for impacted site and reference site ($p = 0.011$) and ($p = 0.013$) but there was no significant difference between site II and site III ($p = 0.856$) (Table 2).

According to Ayers and Westcott (1976) the required maximum concentration of NO_3^- for live stock and irrigation were 100 and 30mg/l respectively and the concentration below 5mg/l will not affect flora and the soil. The level of NO_3^- among sites showed very high variation. Nitrate generally occurs in trace quantities in surface waters, must coming from organic and inorganic waste discharges. An excess nitrate in river water promotes high primary productivity and is taken as a warning for algal blooms (eutrophication). A

massive growth of aquatic plant life can change the chemistry of water significantly (Ranvidra, 2003).

In the present study, the decrease in mean concentration of nitrate from site II to site III might be due to dilution and self purification along the stream. Thus all of the sampling sites meet the WHO drinking water quality standards and ambient standard for surface water for nitrate (45mg/l). All the nitrate values were within the EPA standards for effluent discharges to surface water (Appendix 2).

4.1.5. Nitrite-Nitrogen

The Nitrite concentration of the Meta river water samples were summarized and presented in table 2. The maximum and minimum nitrite - nitrogen concentration for site I, was 0.02 and 0.02, site II, 0.17 and 0.12 and site II, 0.14 and 0.11 mg/l respectively (table 2).

There was a significant difference in nitrite-nitrogen ($P < 0.05$) between reference site and impacted sites II ($p = 0.027$) and site III ($p = 0.043$). According to the result of ANOVA, Nitrite-Nitrogen was not significantly differ ($p > 0.05$) at Site II and site III ($P = 0.708$). The average value increased from Site I (0.025 ± 0.007 mg/L) to Site II (0.145 ± 0.025 mg/L) but decreased in site III (0.125 ± 0.015 mg/L) (Appendix II). The standard nitrite-nitrogen concentration is 3mg/L (WHO, 2006). The concentration of nitrite in the three study sites were within WHO standard.

4.1.6. Phosphate (PO_4^{3-})

As the result shown in table 2, the maximum and minimum phosphate concentration at the three sites was presented. As indicated in the table below the maximum and minimum phosphate concentration at site I was 5.2 and 3.8, site II, 72.9 and 44.5 and site III, 27.4 and 23.36 mg/l (table 2). The levels of phosphate in the downstream of Meta River was high and of great concern. Relatively lower phosphate levels were recorded in the upstream of the River. The mean phosphate concentration at site I was 4.5 ± 0.70 mg/l Site II 58.7 ± 14.20 mg/l and site III 7.95 ± 2.02 mg/l respectively. The lower mean phosphate concentration was recorded in site I (4.5). The higher levels of phosphate concentration in site II (58.7 ± 14.2 mg/L) and site III (25.38 ± 2.86 mg/L).

The discharge of phosphate salts and detergents used for washing in the factory was a regular source of phosphate at the discharge point. Water quality standard levels must be below 0.05 mg/L of phosphate concentration to prevent downstream eutrophication (Australia Water Watch, 2002). Analysis of variance (ANOVA) showed that there was significant difference ($p < 0.05$) in the mean phosphate concentrations for the reference site and impacted sites. All the three sites found to be varied significantly P value between site I and site II (0.038), between site I and site III (0.315) and between site II and site III (0.127) table 2. In all samples, the phosphate concentrations were higher than 0.05mg /L, which is considered as the lower limit for River water to pose risk of eutrophication. Phosphate values in Site II to Site III and site I were significantly above the EPA standards for effluent discharges to surface water (Appendex 2).

4.1.7. Salinity

As the result showed that the average ion concentration of site I, site II and site III was 74.5 ± 5.6 , 259.2 ± 12.90 and 172 ± 8 mg/l respectively (appendix2). Table 2, showed that the maximum and minimum ion concentration at the three study sites. Ion concentration at Site I was 80.1 and 68.9 mg/l, the ion concentration at site II was 272.1 and 246.3 mg/l. Similarly at site III 180 and 164 mg/l ion concentration was recorded. Salinity is an important measurement in the analysis of certain industrial wastes in the stream and river. As the result indicated in (Table 2) significant difference in salinity ($P < 0.05$) between reference site I and site II ($P = 0.002$) and between site I and site III ($P = 0.011$) and between site II and site III ($P = 0.014$). According to the result from ANOVA, there was a difference in salinity among the three sites. Higher salinity value was recorded at impacted sites due to chemical effluents from the factory.

4.1.8. Total Dissolved Solids (TDS)

As the result indicated in appendix 2 the highest mean TDS values was 468.5 ± 26.30 mg/L in the site II where the river and the effluent mix together and followed by that of the down stream point III (390.7 ± 8.7 mg/L) and the lower TDS value (131.45 ± 2.55 mg/L) was recorded from Site I. Table below showed that the maximum and minimum. TDS value at the three study sites. The maximum and minimum TDS value of site I, site II and site III was, site I 134 mg/l and 128.9 mg/l, site II, 494.8 mg/l and 442.2, mg/l site III 399.4 mg/l and 382 mg/l respectively.

In this study, all sites have shown high amount of total dissolved solids, beyond WHO's maximum allowable concentration (80mg/l) (appendix 2). But according to FAO recommendation, the acceptable range for livestock drinking is 100-1,500mg/l. There was Significant difference in TDS ($P < 0.05$) between reference site and impacted sites as well as between the two impacted sites (site I and site II), ($P = 0.001$), ($P = 0.003$) and ($P = 0.082$) respectively (Table 2). The total dissolved solid concentration at the impacted sites and reference site were above the EPA standards for effluent discharges to surface water (Appendex 2). Like conductivity, the increase in TDS might be due to increased amount of the Meta Brewery effluent discharges, erosion and organic detritus load.

Table 2. The Physico- chemical Parameteres of Meta River water samples

Parameters	Site I		Site II		Site III		P- Value		
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	SiteI&II	SiteI&III	SiteII&III
Temperature	17.4	17.2	18.8	17.5	18.2	18	0.383	0.416	0.995
pH	7.8	7.4	6.75	6.66	6.98	6.78	0.03	0.061	0.657
E.C	265	260	965	915	786	782	0.00	0.00	0.01
Salinity	80.1	68.9	272.1	246.3	180	164	0.002	0.011	0.014
Turbidity	31	25	110	98	98	70	0.019	0.043	0.379
Nitrate	1.39	1.3	8.4	6.4	7.1	6.8	0.011	0.013	0.856
Nitrite	0.02	0.02	0.17	0.12	0.14	0.11	0.027	0.043	0.708
TDS	134	128.9	494.8	442.2	399.4	382	0.001	0.003	0.082
Phosphate	5.2	3.8	72.9	44.5	27.4	23.36	0.038	0.315	0.127

4.2. Identified Benthic Macro Invertebrate Species in Meta River

4.2.1. Identified Benthic Macro Invertebrates Species

During this study a total of three Meta river sampling sites were selected and examined for detection of any BMI species. The results are summarized and presented in (Table 3). From the result a total of 392 macro-invertebrate organisms that belongs to 10 different species were collected from the three sampling sites. Of the total taxa 178 Benthic Macro Invertebrates were collected from the references sites, while 110 and 104 were in the impacted sites (Site II, and Site III) respectively. A wide fluctuation was observed in the abundance of BMI from site to site. Four taxa viz- Mollusca, Diptera, Annelida and Odonata were recorded during this study. Ephemeroptera, Plecoptera and Trichoptera were not observed at site II site III (Table 3).

The presence of gastropods at site II can be related to their tolerance to some levels of pollution. The existence of positive correlation between the benthic macro-invertebrate and the Physico-chemical parameters confirmed this assertion. The occurrence of *Chironomus* species in all sites, site I, site II and site III was 50, 78 and 62 respectively (Table 3). This perceived to be normal as they are usually present in any water bodies (Emere and Narisu, 2007). However, their abundance at site II may be linked to their high tolerance to anoxic conditions. This may probably be because of their possession of haemoglobin; a pigment that transports dissolved oxygen (Tyokumbur *et al*, 2002).

The presence of haemoglobin is advantageous to their proliferation and colonization of this site effectively out-competing other taxa. Victor and Onomivbori (1996) documented some species as opportunistic fauna with high reproductive rates, short life span, and high dispensability and reduced long-term competitive abilities occupying disturbed habitats. *Chironomus* species are among the tolerant groups of dipteran taxa with the red blood being more tolerant than the pale one (tolerance values of 8). Higher tolerance of the blood red chironomidae is due to its pigment that helps the organism to get oxygen from the atmosphere hence the name “blood red” (Barbour *et al.*, 1999; Bouchard, 2004).

Generally 8 species and total of 178 macro invertebrates were identified from site I. Relatively small number of chironomous species which is a total of 50 (28.1%) were

collected and identified from reference site I. These revealed that they were the dominant taxa in impacted site (Site II & III) than less polluted area (site I).

From table 3, Total of 6 species was identified from the impacted site III and 5 species were identified from site II. The second dominant taxa in the impacted site were *Microchaetus sp.* The result in Table 3, showed that the absence of intolerant species indicates that Meta River was so polluted from near by Beer factory effluent. From the biological parameters one can deduce the presence or absence of macro invertebrates in rivers or streams that indicate the quality of water. The occurrence of *Chironomus* species in all sites observed is perceived to be normal as they are usually present in water bodies (Samson, 1982; Emere and Narisu, 2007). However, their abundance at site II may be linked to their high tolerance to anoxic conditions. This property is advantageous to their proliferation and colonization of this site effectively out-competing other taxa. Victor and Onomivbori (1996) documented some species as opportunistic fauna with high reproductive rates, short life span, and high dispersability and reduced long-term competitive abilities occupying disturbed habitats. The physico-chemical parameters and habitat status of site II was some what favorable to allow them to reproduce and grow.

Table 3 . Number of BMI Species Identified in Meta River at the three sampling sites

Identified BMI Families	Identified Species	BMI	Site I		Site II		Site III	
			<u>No of Inds</u>	Abundance (%)	No of Inds	Abundance (%)	No of Inds	Abundance (%)
Dipterian	Chironomus sp		50	28.1	78	70.9	62	59.6
Chironomidae	Ablabesymia		20	11.2	10	9.09	11	10.58
Mollusca	Melanoid tuberculata		18	10.11	0	0	11	10.58
Annelida	Glycera dibrancaire		18	10.11	0	0	0	0
Gyrinidae	Dinewute sp		6	5.6	0	0	0	0
physidae	Physela sp		0	0	0	0	5	4.8
Sialidae	Sialis sp		4	2.25	0	0	0	0
Baetistidae	Baetis harrisoni sp		20	11.2	5	4.55	0	0
Odonata	Macromia magnifica		0	0	4	3.64	6	5.7
Acanthodrilid ae	Microchaetus sp		42	23.59	13	11.8	9	8.65
Total			178		110		104	

4.3. The Diversity and Distribution of BMI in Meta River

4.3.1. The Benthic Macro Invertebrate Metrics

The diversity and distribution of the benthic macro-invertebrate species were estimated by calculating the Benthic Macro Invertebrate index (Shannon weaver diversity index, Family biotic index and community loss index). The results are summarized in (Table 6).

According to Shannon –weaver index (Table 6) the result obtained in site I (1.852) was indicates that diversity among species with even distribution relative to other sites whereas there was low “H” value in site II (0.914) and site III (1.199) which describes less diversity in which a community dominated by one family relative to other sites. High values of “H” would be representing of more diversity community as well as even distribution. This provides valuable clues for water quality monitoring. The Shannon-Weaver community index revealed that more number of organisms (abundance) was found in the reference site than impacted sites. In the same way taxa richness and diversity ($H'=1.852$) characterize this site as well. The higher the index, the more diversity an organism has. But in the impacted site relative to reference site there was less diversity of organisms. A little bit dominancy shown by *Chironomus* species and *Acanthodrilidae* (*Microchaetus* sp) in both impacted sites.

Increment in the total abundance does not show better environment because disturbance may favors some tolerant, opportunistic and less competent taxa with reduction in sensitive taxa (community dominated by few taxa). When streams become excessively acidic or alkaline, the change can adversely affect the biota. As those fish and macro invertebrates unable to tolerate the altered conditions decline, a tolerant organism increases in number due to lack of competition for food and habitat. This results in un healthy biological community dominated by few tolerant taxa (Kimmel, 1983).

Tolerance values range from 0 to 10 for families and increase as water quality decreases. The index was developed by Hilsenhoff (Hilsenhoff, 1988) to summarize the various tolerances of the benthic arthropod community with a single value. The Modified Family Biotic Index (FBI) was developed to detect organic pollution and is based on the original species-level index (BI) of Hilsenhoff. In unpolluted streams the FBI was higher than the

BI, suggesting lower water quality, and in polluted streams it was lower, suggesting higher water quality (Hering *et al* 2006).

These results occurred because the more intolerant genera and species in each family predominate in clean streams, whereas the more tolerant genera and species predominate in polluted streams. Thus the FBI usually indicates greater pollution of clean streams by overestimating BI values and usually indicates less pollution in polluted streams by underestimating BI values. The FBI is intended only for use as a rapid field procedure. It should not be substituted for the BI; it is less accurate and can more frequently lead to erroneous conclusions about water quality (Hilsenhoff, 1988). The family-level index has been modified for the RBP II to include organisms other than just arthropods using the genus and species-level tolerance values adopted by the State of New York (Bode *et al.*, 1991, 1996, 2002). Although the FBI may be applicable for toxic pollutants, it has only been evaluated for organic pollutants.

From FBI (Table 6), the macro invertebrates collected revealed that the water quality of Meta River in site I (upper stream) falls into category of Good some organic pollution probable (4.89). This means that the upper stream site of the River was less impaired relative to the other sites. Due to the fact that, sewage from households and Agricultural waste were contributed for the presence of organic pollution that reduce the quality of water which can affect human, aquatic biota and the environment. This may be pollution from agriculture and or water treatment plant. The impacted site II and site III was (6.73 and 6.68) both fall in to category of 'Poor Very substantial pollution likely which arise from factory, residential and agricultural wastes.

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 *et al.*, 1983; Ohio Department of Natural Resources, unpublished; Rosenberg and Resh, 1993). Biotic index systems have been developed which give numerical scores to specific "indicator" organisms at a particular taxonomic level (Armitage *et al.*, 1983; Ohio Department of Natural Resource). Presence of numerous families of highly tolerant organisms usually indicates poor water quality (Hynes, 1998).

Table 4. Evaluation of water quality using the family-level biotic index**(Hilsenhoff, 1988)**

Family Biotic Index	Water Quality Degree of Organic Pollution of study sites
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 Site I
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 site II and site III
7.26-10.00	Very poor severe organic pollution

Table 5. Common BMI identified from Reference site and impacted sites

Macro-invertebrat species	Number of organisms		
	Reference site	site II	Site III
1. Chironomous species	50	78	62
2. Microchaetu species	42	13	9
3. Ablabesymia sp	20	10	11

In order to measure the degree of dissimilarity between reference site and impacted site as well as loss of benthic taxa, community loss index was computed. From result obtained (Table 6), the degree of dissimilarity increases from 0.819 (reference site I) to 1.818 and 1.0007 (site II&III) respectively. But the two impaired sites were more akin in terms of loss benthic taxa as well as degrees of similarity. Form the above result, benthic macro invertebrates that were sensitive to pollution are core to measure and monitor water quality.

Community loss index tends to decrease with increasing water quality to maintain healthy aquatic ecosystem (Mandaville, 2002). Therefore lower scores at the downstream sites are associated with high CLI which intern confirms higher ecosystem disturbance at these sites. Due to the difference in their tolerance to pollution, the presence or absence of certain macro invertebrates can provide valuable information on stream water quality. The result from the study indicates that benthic index designed for Rivers and streams assessment also works for discriminating impacted sites in River.

Table 6. Diversity indices of benthic macro invetebrate during the study

Diversity indices	Site I(SI)	Site II(SII)	Site III(SIII)
No. of individual species	178 (8)	110 (5)	104 (6)
Shannon –Waever index (S-W)	1.852	0.914	1.199
H-FBI	4.89	6.73	6.68
CLI	0.819	1.818	1.0007

Note: H-FBI (Hilsenhoff-Family Biotic index), S-W (Shannon – waever diversity index), CLI (community loss index)

4.4. Correlation between Physico-Chemical Properties and BMI Parametres

Pearson correlation coefficient ($r > 0.9$ or $r < -0.9$) was taken as a line to reject a metrics (Mandaville, 2002). For those metrics with $r > 0.9$ or $r < -0.9$, only the one believed to be more information was taken (Shearer, 2006).

Chironomus species showed significant correlation (Table 7) with all physico-chemical parameters. Negatively correlated with pH, E.C, Salinity, and Phosphate, and positively with temperature, Turbidity, TDS, Nitrate and nitrite. This may be due to chironomus species were not sensitive to physico-chemical parameters considered. This indicates they were the most tolerant taxa at the discharged point and down stream of the River.

Melanoid tuberculata species showed negative correlation with all physico-chemical parameters. And positively with pH ($r = 0.896$). Except pH, the rest parameters indicated (EC, salinity, TDS) are highest in the impacted sites (Appendix 1).

The blood worm, *Glycera dibranchaire*, was the only Annelid observed. It may have been transported by water currents to the site. This and the fact that the organism prefers sandy or silty substrata may probably be the reason for their presence in site I. And also *Glycera* species negatively correlated with all physico-chemical parametres except pH $r = 0.982$ (Table 7).

From the result in Table 6, *Microchaetus* species were negatively correlated with physico-chemical parameters such as turbidity, TDS and salinity and positively correlated with pH ($r = 0.955$) as well as phosphate ($r = 0.891$). Since majority of them could obtained from site I and good habit quality relative to others scored in this site, there is strong attachment between the two with respect to the abundance of oxygen. Habitat could be among the key factors that influencing the abundance, diversity and distribution of the plecoptera family.

Healthier habitat integrity may insure adequate amount of allochthonous food source (Ward, 1989) and better stream conditions with respect to total solids entering the River system. Most commonly they are tolerant organism (Merritt and Cummins, 1996). As the rate of organic pollution increases, there are few tolerant taxa that would survive to overcome the problem (example-*chironomus* sp).

Ablabesymia species correlated significantly with all physico-chemical parameters such as pH ($r = 0.59$), *Chironomus riparius* correlated inversely with pH, E.C, Salinity, and Phosphate positively correlated with Temp, Turbidity, TDS, Nitrate and nitrite.

Baetis harrisoni sp correlate significantly with all physico-chemical parameters, positively with pH ($r=0.994$) and negatively with all other parameters. *Physelas sp* significantly correlated with all physico-chemical parameters, positively with pH ($r=0.982$) and TDS ($r=0.976$), Negatively with Temperature, E.C, Salinity, turbidity, Nitrate, Nitrite and Phosphate.

As the result shown in the Table 6, *Sialis sp* correlate negatively significantly with all physico-chemical properties except pH ($r=0.982$). *Dinewut sp* belongs to family Glyceridae and only found in less polluted water. This species also Correlate significantly with all physico-chemical parameters except with TDS ($r=-0.298$). This result indicates that in polluted water, all physico-chemical parameters were increased in level except pH. As the level of pollution increases pH value became decreases as a result the diversity and distribution of benthic macro-invertabrets become decreases. From this result it is possible to say Macro-invertabrets are inversely proportional to all physico-chemical parameters except the pH value. In the impacted sites (siteII and siteIII) the diversity and distribution of benthic macro-invertabretes were lower than referance site (siteI)

Table 7. Pearson's correlation co-efficient (r) values between the benthic macro-invertebrates and physico-chemical parameters

parametres	Chono mus sp	Dinewut sp	Michrochaitus sp	Beatis harrisoni sp	Glycera sp	Ablabesymia sp	Macromia sp	Physasp	Melanoid sp	Sialis sp
PH	-0.915	-0.982	0.955	0.994	0.982	0.59	-0.866	0.982	0.896	0.982
temp	0.851	0.999	-0.987	-0.970	-0.999	-0.999	0.926	-0.999	-0.826	-0.99
EC	-0.927	0.976	-0.945	-0.997	-0.976	-0.991	0.850	-0.976	-0.909	-0.976
salinity	-0.921	0.882	-0.824	-0.981	-0.882	-0.921	0.679	-0.882	-0.987	-0.882
turbidity	0.940	-0.967	-0.933	-0.999	-0.967	-0.986	0.967	-0.967	-0.923	-0.967
TDS	0.927	-0.298	-0.945	-0.997	-0.976	-0.991	0.850	0.976	-0.994	-0.976
nitrate	0.858	-0.428	-0.984	-0.974	-0.998	-0.907	-0.123	-0.998	-0.998	-0.998
nitrite	0.898	0.989	-0.966	-0.989	-0.989	-0.998	-0.989	-0.989	- 0.877	-0.989
phosphate	-0.989	0.473	0.891	-0.473	-0.473	-0.551	-0.710	-0.473	-0.910	-0.473

NB: **Bold font**: indicates significant correlation

5. SUMMARY AND CONCLUSIONS

5.1. Summary

This study focuses on the effects of beer factory effluent discharge in to Meta River and water quality. Meta River is a recipient of Brewery Effluent of poor quality that does not meet the stipulated minimum requirement for discharge in to surface water. The reference site is found at the upstream location, the second site was located at the discharged point and the third site was found downstream location. Benthic macro invertebrate species were collected by using D-net; they were identified by using compound microscope and categorized at species level. A total of 392 individual organisms were identified which belong to ten different species. 178 benthic macro invertebrates were collected from reference site I, 110 and 104 benthic macro invertebrates were collected from impacted sites, site II and site III respectively.

Abundance and distribution of those species were measured from the three study sites. This was done by using Indices like, Shannon Weaver diversity index (H), Family biotic index (FBI) and community loss index (CLI). The abundance and distribution of benthic macro invertebrate species were vary among reference site and impacted sites. The number of species were higher in reference site than impacted sites. Eight different types of species were identified from reference site and five and six species from impacted site II and site III.

Physico-chemical properties used to measure the qualities of Meta River were pH, Temperature, Electrical conductivity, Total dissolved solids, Turbidity, nitrate, nitrite and phosphate. The physical properties like, pH, Temperature, EC, TDS and turbidity of the River were measured at the study site whereas the Chemical properties of the River were measured in the Laboratory. Most of the physico-chemical properties were higher in the impacted site than reference site and are significantly correlated with Biological parameters.

5.2. Conclusions

Even if Beer Factories have positive impacts on economic development of the country and the surrounding community, they have negative impact on the aquatic environment: macro invertebrates' depletion or degradation of biodiversity (species abundance and diversity), disappearance of sensitive taxa in the downstream stretches, food chain of aquatic organisms' and consumption of water in the surrounding communities. This is due to the disposal of wastewater to the nearby Rivers.

Wastewater discharged to the nearby Rivers has enormous effect on the degradation of the ecosystem. To sustain the ecological conditions of the nearby Rivers, wastewater treatment and environmental audit are suggested. Environmental assessment and environmental auditing enables the floriculturist to keep humans and the environment safe. Taking care of workers, soil, water and the environment has to be seen with great care and caution because it is difficult to maintain a healthy community and carry out development in a degraded environment. Physico-chemical parameters such as Temperature, pH, EC, TDS, Salinity, $\text{NO}_3^- \text{N}$, and $\text{NO}_2^- \text{N}$ and Macro invertebrate metrics, total number of taxa, species richness, and loss of benthic macro invertebrate species were discussed.

The study showed the effectiveness of the benthic macro invertebrate protocol in assessing River water pollution condition. Therefore, environmental agencies and researchers have a good option of using macro invertebrate with the objective in assessment and monitoring programs of Rivers. The results indicated that pH and temperature were within the range of permissible limit for surface water. The water quality of Meta river shows spatial variation by which the water quality at site I is good that is interference that deteriorate the water quality is minimal and as the river crosses the brewery factory quality of certain samples reveals that it is getting deteriorated at the down stream where site II is the highly polluted site followed by site III. The levels of most parameters responsible for evaluation of water quality downstream were significantly higher than the corresponding levels upstream.

5.3. Recommendations

The River provides a lot of benefits in the agriculture and also it is habitat for large number of wildlife. Therefore, protection of River needs urgent intervention. The water condition can be maintained by using Best Management Practices or wastewater treatment methods. On the basis of this general assumption, it is recommended that:

Vegetation Buffer preparation: Vegetation buffer has to be prepared in place before the wastewater is discharged to the River to improve the discharged water quality.

Wastewater recycling: waste water from beer factory has to be recycled rather disposed to the environment with varieties of chemicals that pollutes water and soil of the surrounding environment.

Residue analysis: to know the disastrous effect of chemicals waste from factory residue analysis has to be done, there is information gap in residue released to the River.

Improve the performance of existing treatment systems through modifications and technological upgrades.

Environmental standards and effluent regulations for Brewery industries need to cover all parameters with adverse effects on the environment and should be implemented and monitored regularly.

It should be an urgent prerequisite to require Brewery factory to continuously monitor brewery effluents and take necessary actions to change wastewater to environmentally friendly form before discharging it into Meta River.

Despite the river is being contaminated with chemicals and toxic substances, it was observed that some people use the river water for different purposes like irrigation. The local communities using such polluted water for different economic activities.

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7. APPENDICES

Appendix Table 1. First and second round physico- chemical data collected from the three sampling sites.

Parametres	Site I		Site II		Site III	
	1 st round	2 nd round	1 st round	2 nd round	1 st round	2 nd round
Temprature	17.2	17.4	17.5	18.8	18	18.2
pH	7.8	7.4	6.75	6.66	6.98	6.78
EC	260	265	965	915	786	782
Salinity	68.9	80.1	272.1	246.3	164	180
Turbidity	31	25	98	110	70	98
Nitrate	1.3	1.39	8.4	6.4	7.1	6.8
Nitrite	0.02	0.02	0.17	0.12	0.14	0.11
TDS	128.9	134	494.8	442.2	382	399.4
Phosphate	3.8	5.2	44.5	72.9	27.4	23.36

Appendix 2. Mean values of physico-chemical parameters (n=2). Except EC ($\mu\text{S}/\text{cm}$) and temperature ($^{\circ}\text{C}$) all others measured in mg/L .

Parametres	Site I	Site II	Site III	WHO mac (2008)	EPA standared (2003)
PH	7.6 \pm 0.20	6.7 \pm 0.045	6.88 \pm 0.10	6.5-8.5	6-9
Temprature	17.3 \pm 0.10	18.15 \pm 0.65	18.1 \pm 0.10	12-25 $^{\circ}$ c	11-25 $^{\circ}$ c
Electrical conductivity	262. 5 \pm 2.5	940 \pm 25	784 \pm 2.00	1000 $\mu\text{S}/\text{cm}$	
Salinity	74.5 \pm 5.6	259.2 \pm 12.90	172 \pm 8		
Turbidity	28 \pm 3.00	104 \pm 6.00	84 \pm 14	>5NTU	
Nitrate	1.35 \pm 0.045	7.4 \pm 1.00	6.95 \pm 0.15	10 mg/l	10 mg/l
Nitrite	0.025. \pm 0.007	0.145 \pm 0.025	0.125 \pm 0.015	3 mg/l	
Phosphate	4.5 \pm 0.70	58.7 \pm 14.20	25.38 \pm 2.02		<0.05 mg/l
Total dissolved solids	131.45 \pm 2.55	468.5 \pm 26.30	390.7 \pm 8.7	80 mg/l	<1000 mg/l

Appendix 3. Species composition, distribution and abundance of benthic macro invertebrates at three study sites (during First and second round)

No	Species	First round			Second round		
		SI	SII	SIII	SI	SII	SIII
1	<i>Chironomus riparius</i>	27	44	24	23	34	38
2	<i>Baetis horrisoni sp</i>	14	5	-	6	-	-
3	<i>Melanoid tuberculata</i>	7	-	4	11	-	7
4	<i>Microchaetus sp</i>	23	6	5	19	7	4
5	<i>Glycera dibrachiare</i>	6	-	-	12	-	-
6	<i>Physela sp</i>		-	1		-	4
7	<i>Sialis sp</i>	3	-	-	1	-	-
8	<i>Ablabesymia sp</i>	9	4	6	11	6	5
9	<i>Dinewute sp</i>	2			4		
10	<i>Macromia sp</i>	-	2	3	-	2	3
	<i>Total</i>	91	61	43	87	49	61



SiteI



SiteIII



siteII

Appendix figure 1 . Image showing BMI identified in the three study sites.

Site III



Site II



Site I

Appendix figure 2. Image showing the three study sites and their vegetation cover



Appendix figure 3. Sampling BMI by using D- net from (site-1)



Appendix figure 4. Figure showing field and laboratory work

