



**ASSESSMENT OF BIOLOGICAL INTEGRITY USING PHYSICO-CHEMICAL
PARAMETERS AND MACROINVERTEBRATE COMMUNITY INDEX
ALONG SEBETA RIVER, ETHIOPIA**

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DEDICATION

This work is dedicated to those who fight for the conservation of natural resources and the welfare of the environment.

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Acronyms and Abbreviations

BOD	Biochemical Oxygen Demand
Chl. 'a'	Chlorophyll 'a'
CLI	Community Loss Index
cm ²	Square Centimeter
DO	Dissolved Oxygen
EC	Electrical Conductivity
EMA	Ethiopian Mapping Agency
EPA	Environmental Protection Authority of Ethiopia
FBI	Family Level Biotic Index
GPS	Global Positioning System
GSWQMP	Great Swamp Water Quality Monitoring Program
H-FBI	Hilsenhoff Family Level Biotic Index
km	Kilo meter
km ²	Square Kilo meter
MCISR	Macroinvertebrate Community Index of Sebeta River
m a.s.l.	Meter above sea level
m	Meter
m/s	Meter per second
mg/L	Milligram per liter
mm	Millimeter
NMSA	National Meteorological Service Agency
NWRA	Nevada Water Resources Association
pH	Measure of acidity or alkalinity
SPSS	Statistical Package for Social Sciences
SR ₁ , SR ₂ ...	Selected study sites on Sebeta River
TDS	Total Dissolved Solids
UNEP	United Nations Environmental Program
\$	The United States Dollar
USEPA	The United States Environmental Protection Agency
USGS	The United States Geological Survey
WHO	World Health Organization
WWDSE	Water Works Design and Supervision Enterprise
µg/L	Microgram per liter
µs/cm	Micro siemens per centimeter
°c	Degree Celsius

ABSTRACT

The level of water pollution and ecological disturbance of Sebeta River was evaluated using some important physico-chemical and biological parameters. The Physico-chemical parameters were: flow velocity (m/s), dissolved oxygen (DO) (mg/L), pH, temperature ($^{\circ}$ C), electrical conductivity (EC) (μ s/cm), nitrate-nitrogen (mg/L) and phosphate (mg/L). The mean flow velocity and nitrate-nitrogen measures showed no significant difference between the study sites of Sebeta River ($P > 0.05$). DO records of the river at the upstream rural sites above Sebeta Agro-industry (SR₁ and SR₂) were significantly higher than the DO records of the downstream sites ($P < 0.001$). The mean pH values of SR₃ (3.60 ± 0.81) (immediately downstream from Bale Zaf Alcohol and Liquor Factory and Soap Factory) and SR₄ (4.75 ± 0.88) (downstream of National Alcohol and Liquor Factory and Blue Nile Tannery) were significantly lower than the pH values of the rest of the sample sites ($P < 0.001$). The mean temperature and EC records of SR₃ (33.83 ± 1.17 $^{\circ}$ C, 16050.00 ± 2270.77 μ s/cm) and SR₄ (27.63 ± 0.68 $^{\circ}$ C, 15400.00 ± 316.60 μ s/cm) were significantly higher than the respective records at the rest of the study sites ($P < 0.001$). Phosphate concentration at SR₃ (8.59 ± 1.16 mg/L) was significantly higher than the phosphate concentrations recorded from the other sites ($P < 0.001$). Flow velocity and nitrate-nitrogen of Sebeta River were in the natural range, except for extreme values at sites SR₃ and SR₄. Habitat integrity assessment was done according to the US Environmental Protection Agency Rapid Bioassessment Protocol. Percent comparability of habitat scores classified the upstream reference sites to good, while it categorized the entire downstream sites to very poor. The mean chlorophyll 'a' measures of upstream reference sites (SR₁ and SR₂) showed significantly higher values than those of the impacted downstream sites ($P < 0.001$). Macroinvertebrate community index of Sebeta River (MCISR) was developed using selected metrics. The MCISR score classified both of the upstream sites (SR₁ and SR₂) to very good biological integrity, while it classified all the downstream sites to very poor biological integrity, but SR₈ (further down in Atebela peasants association) to poor.

1. INTRODUCTION

Rivers are the most important freshwater resources. They support and maintain macro- and micro-ecosystems. They carry water from the mountains to the sea, fueling the water cycle coupling land, ocean and the atmosphere (Karr, 1999). Social, economic and political development of mankind has, in the past, been largely related to the availability and distribution of freshwater contained in riverine systems. The Egyptian, Mesopotamian, Chinese and Indian civilizations were centered along the rivers of Nile, Euphrates and Tigris, Yellow and Yangtze Rivers and the Ganges River, respectively. The ancient myths and rituals were centered on rivers that people believed them as holy and have the power to wash away sin (Hooker, 1996). Urban centers and the world's most productive agricultural lands are tied to rivers. Rivers also generate electric power and commerce partly depends on rivers for transportation. They serve as sources of water for recreational activities such as bathing and/ or fishing. They are also the most suitable media to clean, disperse, transport and dispose of wastes (domestic and industrial wastes, mine drainage waters, irrigation returns, etc.) (Meybeck and Helmer, 1996).

Until recently, deterioration of water bodies by pollution was not a serious problem because human populations were small, lived in scattered communities and wastes dumped into rivers were subjected to dilution and natural self-purification (Mason, 1990). With ever increasing population and industrialization, however, human societies affect rivers and their ecosystem structure and function in an ever alarming way (Roy *et al.*, 2003). Urban centers put huge amounts of organic and synthetic waste into rivers with little or no treatment. Uncontrolled agriculture, excessive fertilizers and pesticide application alter rivers and their ecological integrity. Land use change such as canalization or damming and diversion also contribute to the deterioration of river ecosystems (Meybeck and Helmer, 1996). In general, the effects of human activities on rivers and their ecosystem affect one or more of the five attributes of watersheds and streams: water quality, habitat structure, stream flow patterns, sources of energy and nutrients, and biotic interactions (Karr, 1991). Altering these attributes in turn upsets the whole of 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). Therefore, it demands employment of biological assessment integrated with

physico-chemical data for comprehensive and successful monitoring of water quality and ecosystem integrity of a river.

Using biological criteria to assess environmental impact was developed in the USA since the classical studies of S.A Forbes on Illinois River. Forbes limnological investigations began in the 1870s and demonstrated the indicator value of benthic fauna (Cairns and Pratt, 1993). The use of indicator organisms to help classify trophic status of rivers and streams is also developing in Europe (Davis, 1995). The use of biological tools for environmental impact assessment is not familiar in Ethiopia. However, Tesfaye Berhe (1988) used some biological parameters in the evaluation of the degradation of Abo-Kebena River in Addis Ababa. Baye Sitotaw (2006) used various macroinvertebrate metrics and habitat scores in the assessment of environmental degradation in some rivers of Ethiopia. Solomon Akalu (2006) also assessed the biological integrity of Great Akaki River using macroinvertebrates.

Sebeta River is one of the freshwater bodies under high pollution impact in Ethiopia. It is subjected to municipal, domestic and industrial sources of pollution. Factories like National Alcohol and Liquors Factory, Balezaf Alcohol and Liquors Factory, Soap Factory, Agro-industry that processes dairy products and Blue Nile Tannery are established near the river for easy discharge of the effluent into the river. Among these firms, Blue Nile Tannery is the only one that has effluent treatment plant, the rest of them release untreated waste effluents into the river. Those factories are jointly posing series pollution problem on the river ecosystem and local communities.

Local people use the river water to irrigate their farmlands, drink their cattle and for other domestic purposes (plates 1 to 6). The main victims of the river pollution problems are rural communities found in Dalati, Geme and Atebela Peasants Association. The farmers have constructed traditional dams on the river (at SR₆ and SR₇) to divert river water to their farmlands. Diverted water, which carries industrial residue and pollutants, is directed to farmlands to irrigate vegetables. Urban dwellers in and around Sebeta including Addis Ababa may consume the vegetable crops grown. In addition to factories, domestic and municipal wastes from hotels and individual households together with toilet discharge join the river. Some town dwellers excrete on the riverbanks contributing additional organic load. Wastes

generated from these all sources degrade the river ecosystem together with the physical alterations. The disagreeable odor that someone may sense from a distance on his closure to the main bridge of Addis Ababa-Jimma highway is the easiest indication of the air pollution associated with the factories in and around Sebeta town. Despite the increased pollution and multi-purpose of the river, no monitoring actions were undertaken except pollution assessment by Deshu Mamo (2004). The present study, therefore, is an assessment of the water quality and biological integrity of the river using physico-chemical data, habitat integrity scores, periphyton biomass and macroinvertebrate community structures.

Objective of the Study

The general objective of the present study is to assess the degree of environmental impairment of Sebeta River and its effect on the biological integrity of the river's ecosystem.

The specific objectives of the study include

- Evaluating chemical and physical status of different sampling sites by measuring flow velocity, dissolved oxygen, pH, temperature, electrical conductivity, nitrate-nitrogen and phosphate.
- Evaluating the status of macroinvertebrate communities and periphyton biomass of different sampling sites.
- Developing benthic macroinvertebrate community index of Sebeta River (MCISR) using metrics such as taxa richness, composition, tolerance/intolerance measures including Hilsenhoff family level biotic index (H-FBI) and community loss index (CLI).
- Determining the habitat integrity of the river using principles of Rapid Bioassessment Protocol.
- Interrelating physico-chemical measures, the macroinvertebrate community index, periphyton biomass measures and habitat scores in assessing pollution impacts.

2. LITERATURE REVIEW

2.1. Major Sources of Water Pollution

Pollution of the aquatic environment is defined as *introduction of substances (wastes) and/or energy (like thermal) into the system which can result in such deleterious effects* as: harm to living resources, hazards to human health, hindrance to aquatic activities including fishing, impairment of water quality with respect to its use in agricultural, industrial and often economic activities and reduction of amenities (Meybeck and Helmer, 1996). According to the World Health Organization (WHO), wastes are usually referred to "*something*" which the owner no longer wants at a given time and space and which has no current or perceived market value (Deshu Mamo, 2004). Pollution may result from point sources or diffuse sources (non-point sources). An important difference between a point and a diffuse source is that a point source may be collected and treated or controlled (diffuse sources consisting of many point sources may also be controlled if all point sources can be identified). The major point source pollutions to freshwaters originate from the collection and discharge of domestic wastewaters and industrial wastes (Meybeck and Helmer, 1996). Some agricultural activities such as animal husbandry are also point source pollution of freshwaters. Most other agricultural activities like pesticide spraying or fertilizer application are considered as diffuse sources. The atmospheric fall-out of pollutants also leads to diffuse pollution of the aquatic environment. In general, pollutants can be released into the environment as gases, dissolved substances and/or in the particulate form. Ultimately, pollutants reach the aquatic environment through variety of pathways, including the atmosphere and the soil (Fig. 1).

Domestic wastes are those wastes generated from commercial establishments and residential activities. They are primary source of organic waste released in to freshwater (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, mineral compounds such as inorganic salts, heavy metals and natural organic compounds like carbohydrate and protein (UNEP, 1991).

Inorganic nutrients like nitrate 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 adsorbed by sediment, 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 (GSWQMP, 2002; Murphy, 2005; USGS, 2004).

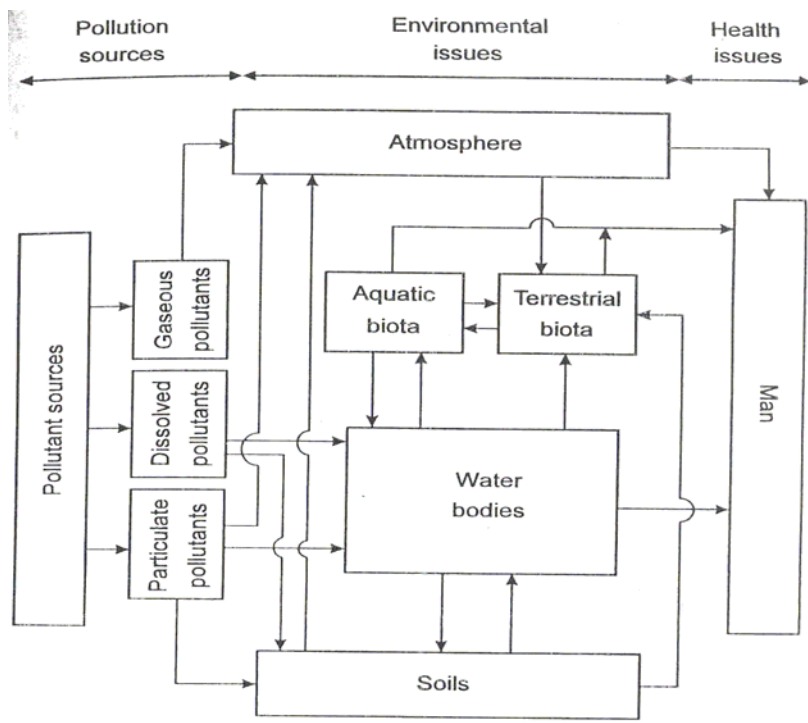


Figure 1. The pathways of pollutants to the environment (after Meybeck and Helmer, 1996)

The presence of high organic matter in wastewater has also an adverse impact on aquatic life. High BOD levels lead to higher consumption of 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, stonefly and caddisfly will be replaced by few kinds of pollution tolerant taxa such as worms and fly larvae (Barbour *et al.*, 1999; Delzer and McKenzie, 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 macroinvertebrate are more adversely affected than fishes because of their small sizes (Murphy, 2005; USGS, 2003).

2.2. The Integrity of River Ecosystem

Rivers are characterized by unidirectional current with a relatively high average flow velocity ranging from 0.1 to 1m/s (Meybeck and Helmer, 1996). The integrity of river ecosystem refers to its biotic integrity (also called biological integrity). Biotic integrity according to Karr and Dudley (1981) is “*the ability of an aquatic ecosystem to support and maintain a balanced, adaptive community of organisms having species composition, diversity and functional organization comparable to that of natural habitats within a region.*” It can be fully characterized by the three major components: hydrology, physico-chemistry and biology. DeBerry and Perry (2005) gave summary of five attributes of river ecosystem (Fig. 2).

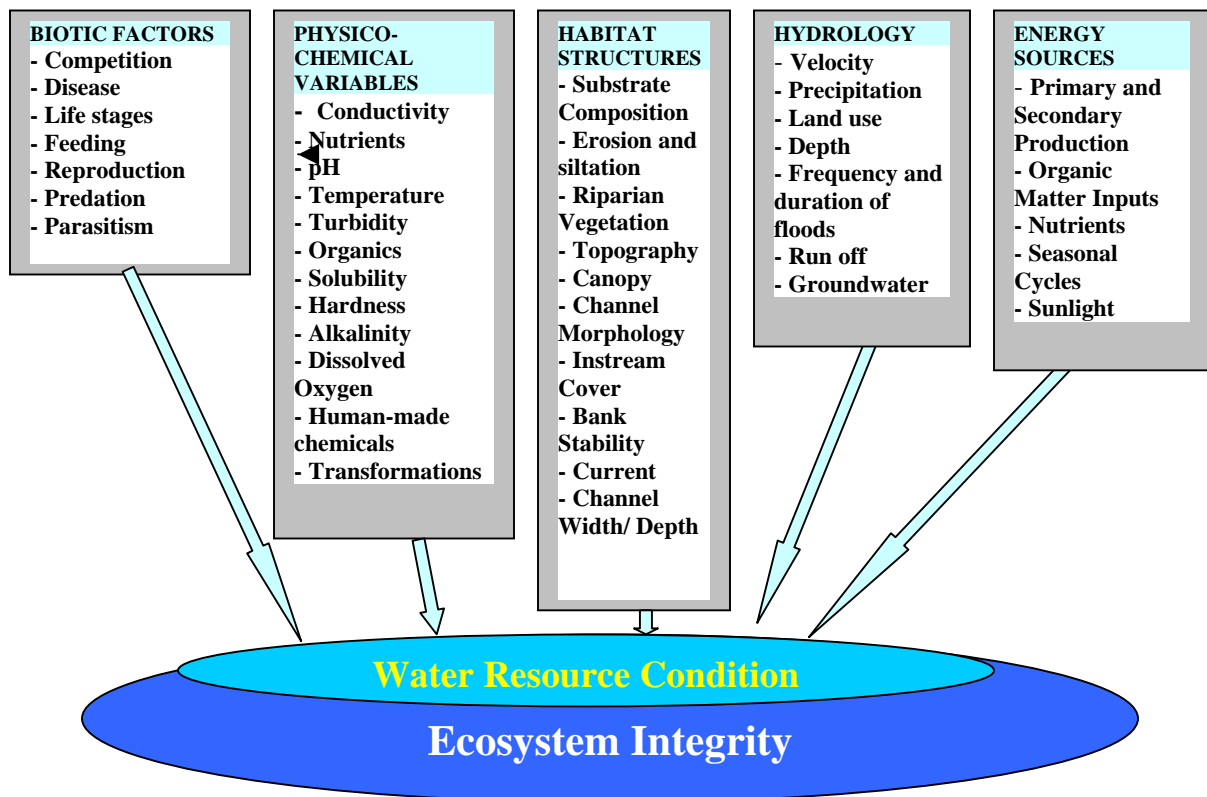


Figure 2. The schematic illustration of components contributing to the integrity of water resources and aquatic ecosystem (modified from DeBerry and Perry, 2005).

2.2.1. Hydrology

The hydrology of a river takes the hydrodynamics of the system such as stream flow (discharge), flow velocity and surface runoff. Discharge is a hydrological characteristic determined by size, form and geological feature of the basin and climatic conditions. It is a product of velocity (flow) and the cross-sectional area of the channel (Meybeck *et al.*, 1996).

Flow velocity is a fundamental property of rivers affecting everything from temperature and concentration of various substances to distribution of organisms. Rapid flow favors lower temperature through aeration, whereas low flow elevates temperature. Flow velocity directly affects the amount of dissolved oxygen in river water. Fast flowing waters, especially the “white waters” favor diffusion of atmospheric oxygen to the river water, thereby raises the amount of dissolved oxygen. Low flow conditions are much less conducive for oxygenation, especially when water temperature is high, DO (dissolved oxygen) lowers critically. The amount of sediment and debris a stream can carry depends up on flow since higher velocity increases stream channel scouring (<http://en.wikipedia.org/wiki/Streamflow>). Stream flows acting together with the slope (gradient) and the geology of the channel determine habitat types, channel shape and composition of the stream bottom (Cushing and Allan 2001). Stream flow is responsible for assimilation and transport of pollutants. It also determines light penetration by affecting turbulences. Therefore, flow indirectly influences the amount, type and distribution of photosynthetic organisms that can colonize various habitats (Chapman and Kimstach, 1996; Meybeck *et al.*, 1996). Nutrient cycling depends on stream flow since spiraling length is a function of flow and retention (Newbold *et al.*, 1981). Precipitation can cause high flows that may wash higher amounts of surface runoff from the watershed into the instream system (USGS, 2005).

Hydrology of rivers can be influenced by anthropogenic alterations like damming, channelization and bridge building (Dudgeon, 2000). Land disturbances directly influence the magnitude of storm water runoff and ultimately increase the amount of stream flow from surface runoff rather than from base flow and/or groundwater (Booth and Jackson, 1997). The results are higher and more frequent flow events in the wet seasons and low flow or even no flow during dry seasons. Higher flow rates result in an increase in sediment losses from the

disturbed areas, bank erosion and channel scouring. Consequently, the hydrological changes alter the habitat and geometry of the streams and increase the amount of sediment pollution (Knighton, 1984).

Excessive sediment load as the result of adverse human activities in the watershed, the riparian side and headwaters is a major factor for decline of benthic communities in rivers and streams (USEPA, 1990). Sediment affects the instream biotic community by reducing habitat, altering water movement, food quality and interstitial spacing (Minshall, 1984). Fine sediment decreases the diversity of instream biotic communities since the suspended solids absorb heat from sunlight, causing temperature increase and ultimately reduction in dissolved oxygen (Murphy, 2000).

2.2.2. Physico-chemical Properties

Composition and concentration of chemicals and nutrients, gases, temperature, pH, conductivity, erosion-deposition processes, the substrate and turbidity are among physico-chemical characteristics making up the integrity of river ecosystem (USEPA, 2002a). Presence and concentration of nutrients such as nitrate and phosphate are essential to the integrity of river ecosystem (GSWQMP, 2002). Natural $\text{NO}_3\text{-N}$ concentrations seldom exceed 0.1mg/L. The amount may be enhanced by municipal and industrial wastes. The use of nitrogen fertilizers on farmlands on the watershed can also contribute to elevated $\text{NO}_3\text{-N}$. Concentrations in excess of 5 mg/L $\text{NO}_3\text{-N}$ usually indicate pollution by human or animal waste or fertilizer runoff (Chapman and Kimstach, 1996). The Environmental Protection Authority of Ethiopia set a standard of 10 mg/L nitrate-nitrogen for surface waters.

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 (USEPA, 2006). High phosphate concentration in rivers can lead to eutrophication. The consequent depletion of DO can alter aquatic fauna.

Dissolved oxygen determines the abundance, diversity and distribution of zoobenthos and other biotic communities of the river ecosystem (Chapman and Kimstach, 1996). DO in natural waters varies with temperature, salinity, turbulence, the photosynthetic activities of algae and plants and the atmospheric pressure. It declines with increment in temperature and salinity. DO in unpolluted waters are usually close to, but less than 10mg/L. High organic and nutrient load reduces DO concentrations as a result of increased decomposer activities. DO concentrations below 5mg/L may adversely affect the functioning and survival of biological communities (Chapman and Kimstach, 1996). Temperature is another important physical factor determining the integrity of river ecosystem. It is directly related to rate of physiological processes. The microbial respiration responsible for self-purification that occurs in water bodies, for instance, depends on temperature. It also determines the dissolution of gases in water bodies. Temperature in turn can be influenced by the amount of shading, climate and elevation in lotic systems (Allan, 1995). In running waters, temperature usually increases gradually from source to mouth with few irregularities in the presence of effluents (Meybeck *et al.*, 1996).

Both high and low pH pose adverse effect on stream biota. Low pH in rivers affects aquatic life by increasing the solubility of many elements such as Al, Cd, Cu, Fe, etc. (Deshu Mamo, 2004). 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^+).

The EPA standard for EC in surface waters is 1000 $\mu\text{s}/\text{cm}$ (EPA, 2003). EC in fresh waters range between 10 and 1000 $\mu\text{s}/\text{cm}$, but it may exceed the maximum value of the range in polluted waters (Chapman and Kimstach, 1996). According to Stream Watch (2003) cited in Marshman and Abbott (2003), adverse impacts are likely in fresh waters when the EC reaches 1500 $\mu\text{s}/\text{cm}$. Higher values of EC generally results due to watershed disturbances like erosion and discharges from industries and urban households. The degree of dissociation, amount of electrical charge on each ion, ion mobility and temperature of the solution matter entering into a river all influence EC. EC is highly sensitive to and is related to Total Dissolved Solids (TDS) and major ions. In natural waters TDS can be estimated as the product of EC and a

factor between 0.55 and 0.75. The factor is 0.67 for waters in which the dominating ions are Na^+ and Cl^- , but it is higher in waters containing high concentration of SO_4^{2-} (Chapman and Kimstach, 1996).

The abundance and type of substrate in the streambed is a physical factor that determines the ability of the instream biota to colonize specific location especially for primary producers. Formation and deformation of the streambed and the channel line and the availability of different habitat structures are associated with the erosion and deposition processes. The effects are significant especially in fast flowing tropical rivers (Meybeck *et al.*, 1996).

The physico-chemical properties of water bodies are influenced by climate, geomorphological and geochemical conditions of the drainage basin and the underlying aquifer and the environmental influences posed on the basin. Natural phenomena bringing hydrothermal waters, acid volcanic lakes, peat bogs etc. together with several anthropogenic influences affect the ecological status of water bodies and their usability to man (Meybeck *et al.*, 1996).

2.2.3. Biological Characteristics

Biological characteristics comprise the flora and fauna living in the river system and their interactions (Meybeck and Helmer, 1996). Flora in rivers include macrophytes, phytoplankton and periphyton, whereas fauna mainly refers to fish and invertebrates. The development of these biological characteristics is governed by a variety of environmental conditions which determine selection of species as well as the physiological performance of individual organisms. The “environmental conditions” refer to both natural and anthropogenic factors affecting those hydrological and physico-chemical properties of river ecosystem. Therefore, river ecosystem integrity, in its generality, is a function of its components interconnected through the environmental conditions affecting the hydrological and physico-chemical properties and manifesting the effect on the biological characteristics.

2.3. Biological Indicators for Water Quality Assessment

Many different methods have been employed to measure the impacts of human activities on the integrity of water resources including chemical, physical and toxicological measures. However, the traditional chemical evaluation of water quality have been largely inadequate because pollution from non-point sources may be transient and unpredictable, and interpreting the impact on biota may be confounded by the co-occurrence of physical habitat disturbances (Barbour *et al.*, 1996). Studies revealed that biological assessment tools have comparatively unique ability to integrate and thus reflect the aggregate condition of water bodies. For instance, Karr (1997) reported that chemical evaluations failed to detect 50% of the damage to surface waters when compared with the application of more comprehensive, sensitive and objective biological criteria. Biological communities are more informative about watershed conditions than physico-chemical measures because they respond to the entire range of biogeochemical factors in the environment (Karr and Chu, 2000). Another advantage of biological assessment is that it is cost effective. Costs per evaluation for ambient monitoring, for example, were found to be low; macroinvertebrates, (\$ 4,120), fish (\$ 3,700) compared to physico-chemical water quality (\$ 8,616-12,924) and bioassay (\$ 3,573-18,318) (Karr, 1991). Therefore, biological monitoring and assessment of aquatic systems (using periphyton, fish, zoobenthos and aquatic macrophytes) are widely accepted as complementary to the more traditional methods of evaluating human impact based on chemical and physical variables.

Biological assessment (also called bioassessment) 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 into single numeric index, then comparing the index with an index developed for a *reference condition*. Reference conditions are established by characterizing the biology and water quality of reference sites with unimpacted water bodies (Barbour *et al.*, 1999). *Metrics* (attributes of biological communities changing in predictable way in response to disturbances) are used to compare the biological condition of reference sites with impacted sites. 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 ones) and

Shannon-Wiener diversity are among the most frequently employed metrics in bioassessment (Barbour *et al.*, 1999, Mandaville, 2002; Peitz, 2003; NWRA, 2005).

Some of the general advantages of using biological indicators for environmental monitoring are also documented in Barbour *et al.* (1999). Accordingly, they reflect overall ecological integrity (i.e., chemical, physical, and biological integrity). They integrate the effects of different stressors and thus provide a broad measure of their aggregate impact. They also integrate the stresses over time and provide an ecological measure of fluctuating environmental conditions. Routine monitoring of biological communities can be relatively inexpensive, particularly when compared to chemical and toxicological methods. Where criteria for specific ambient impacts do not exist (e.g., nonpoint-source impacts that degrade habitat), biological communities may be the only practical means of evaluation.

Benthic macroinvertebrate assemblages and periphyton are among the most frequently used biological tools for environmental impact assessment having their own specific advantages. Benthic macroinvertebrates are animals without backbone inhabiting in or on the bottom substrate of an aquatic environment and are large enough to be seen with unaided eye (Beauchene, 2005). Periphyton (also called phytobenthos) is described as community of primary producers attached to substrate mainly composed of benthic algal assemblages (Stevenson and Bahls, 1999). Advantages of using benthic macroinvertebrates in water quality assessment according to Barbour *et al.* (1999) include: Macroinvertebrate assemblages are good indicators of localized conditions. Because many benthic macroinvertebrates have limited migration patterns or a sessile mode of life, they are particularly well suited for assessing site-specific impacts (upstream-downstream studies). They also integrate the effects of short-term environmental variations because most species have a complex life cycle of approximately one year or more. Sensitive life stages will respond quickly to stress; the overall community will respond more slowly. The identification of macroinvertebrates to family level requires less expertise; many "intolerant" taxa can be identified to lower taxonomic levels with ease. Benthic macroinvertebrates are abundant in most streams. Many small streams (1st and 2nd order), which only support a limited or no fish fauna, naturally support a diverse macroinvertebrate fauna. Sampling is also relatively easy, requires few people and inexpensive gear, and has minimal detrimental effect on the resident biota. Benthic macroinvertebrate

assemblages are made up of species that constitute a broad range of trophic levels and pollution tolerances, thus providing strong information for interpreting cumulative effects.

The advantages of using periphyton include: algae generally have rapid reproduction rates and very short life cycles, thereby can be valuable indicators of short-term impacts. As primary producers, they are most directly affected by physical and chemical factors. Algal assemblages are sensitive to some pollutants that may not visibly affect other aquatic assemblages, or may only affect other organisms at higher concentrations (i.e., herbicides). Relatively standard methods exist for evaluation of functional and non-taxonomic structural (biomass, chlorophyll measurements) characteristics of algal communities.

Despite the considerable advantages, the use of biological assemblages for environmental impact assessment has few limitations such as difficulty to apply in low gradient streams, problems with accuracy, time taken to process the samples etc (Beauchene, 2005). It may also omit taxon-specific ecological information, especially for rare taxa (Braccia and Voshell, 2006).

3. METHODS

3.1. Description of the Study Area

The study was conducted on Sebeta River, 25 km southwest of Addis Ababa. The river originates from the highlands of Wechecha Mountain, which is located north of Sebeta town, crossing through the town and its surrounding and finally joins Awash River. Sebeta town is located in Alem Gena Wereda of Southwest Shoa Zone in the Oromia Regional State. It is situated at 38⁰38' East and 8⁰54' North at an elevation of 2240 m a.s.l. and mean annual rainfall of about 1043.4 mm (NMSA, 2006). The main rainy seasons of the area fall in the months June to August, but small rains are often seen from February to April. During the dry period, the upper course of the river dries, whereas the lower part of the river course receives water from the spring just above the town. The spring water has been developed to supply the town population with tap water and partly joins the river at the main bridge and makes downstream flows perennial. The stream that comes from around Alem Gena town is also another source of water for the perennial flow during the dry season.

Highland mountains of Wechecha with hills and slopes and low-lying flat plains characterize the topography of the area. Basaltic trachyte rocks dominate the geology of Wechecha with shallow brown soil types on the steep slopes and deep red soil in the depressions and gentle sloping sites (Zewdu Eshetu, 2000).

The watershed of Sebeta River is highly exposed to several anthropogenic interventions including; agricultural, industrial, residential and recreational activities (Table 1). Among these agricultural activities together with human settlement 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 greater riparian vegetation removal. The riparian side is devoid of vegetation cover except remnants of *Acacia*, *Eucalyptus* and other tree species like *Arundo donax* and shrubs and grass along the riverbank. The riparian vegetation removal is more intensive in the downstream.

Table 1. Physical Characterization of the study sites

Physical parameters		Sites							
		SR ₁	SR ₂	SR ₃	SR ₄	SR ₅	SR ₆	SR ₇	SR ₈
Watershed features	<i>Land use</i>	Agricultural/residential	Agricultural/residential	Industrial/residential	Industrial/residential	Agricultural/Industrial/residential	Agricultural	Agricultural	Agricultural/residential
	<i>Watershed erosion</i>	None	Slight	Moderate	None	None	Slight	None	Moderate
Riparian vegetation	<i>Vegetation structure</i>	Shrubs & grass,	Tree, Shrubs & grass,	Tree, shrubs & grass,	Tree, shrubs & grass,	Tree, shrubs & grass,	Tree, shrubs & grass,	shrubs & grass,	Tree, shrubs & grass,
		<i>shrubs dominant</i>	<i>trees dominant</i>	<i>trees dominant</i>	<i>grass dominant</i>	<i>Trees dominant</i>	<i>grass dominant</i>	<i>grass dominant</i>	<i>Trees dominant</i>
Instream features	<i>Canopy</i>	None	High cover	None	None	None	partly	none	partly
	<i>Physical alteration</i>	None	None	None	None	None	Dam & diversion	Dam & diversion	None
	<i>Stream width(m)</i>	2	2.5	3.5	2.3	2.2	7	7	4
	<i>Riffle/Run/pool proportions</i>	50%,5%,45%	30%,0%,70%	55%,41%,4%	70%,20%,10%	25%,25%,50%	pool	pool	30%,40%,30%
Water quality	<i>Color</i>	Slight Sediment turbid	Slight Sediment turbid	Reddish brown	Reddish brown	Ashy turbid	Dark brown	Greenish brown	Dark green
	<i>odor</i>	none	none	Alcohol	Alcohol	Sewage	Faintly alcohol	Faintly alcohol	other♦

Note: ♦ is to indicate that the odor is different

The major crops grown in the study area in the main production season include cereals- Teff (*Eragrostis tef*), Barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), etc.; vegetables-lettuce (*Lactuca sativa L.*), “Zikune”, cabbage (*Brassica oleracea L.*), green pepper (*Brassica carinata*), etc.; pulses- field peas (*Pisum sativum*), chick pea (*Cicer arietinum*), cow pea (*Vigna unguiculata*), lentil (*Lens culinaris L.*) etc., and root crops - Red beet (*Beta vulgaris L.*), Potato (*Solanum tuberosum L.*), Onion (*Allium cepa L.*), etc. In addition to crop production, farmers grow cash crops like chat, coffee, fruit trees, etc. The crop products were collected and the ground was left bare in the study period (dry months).

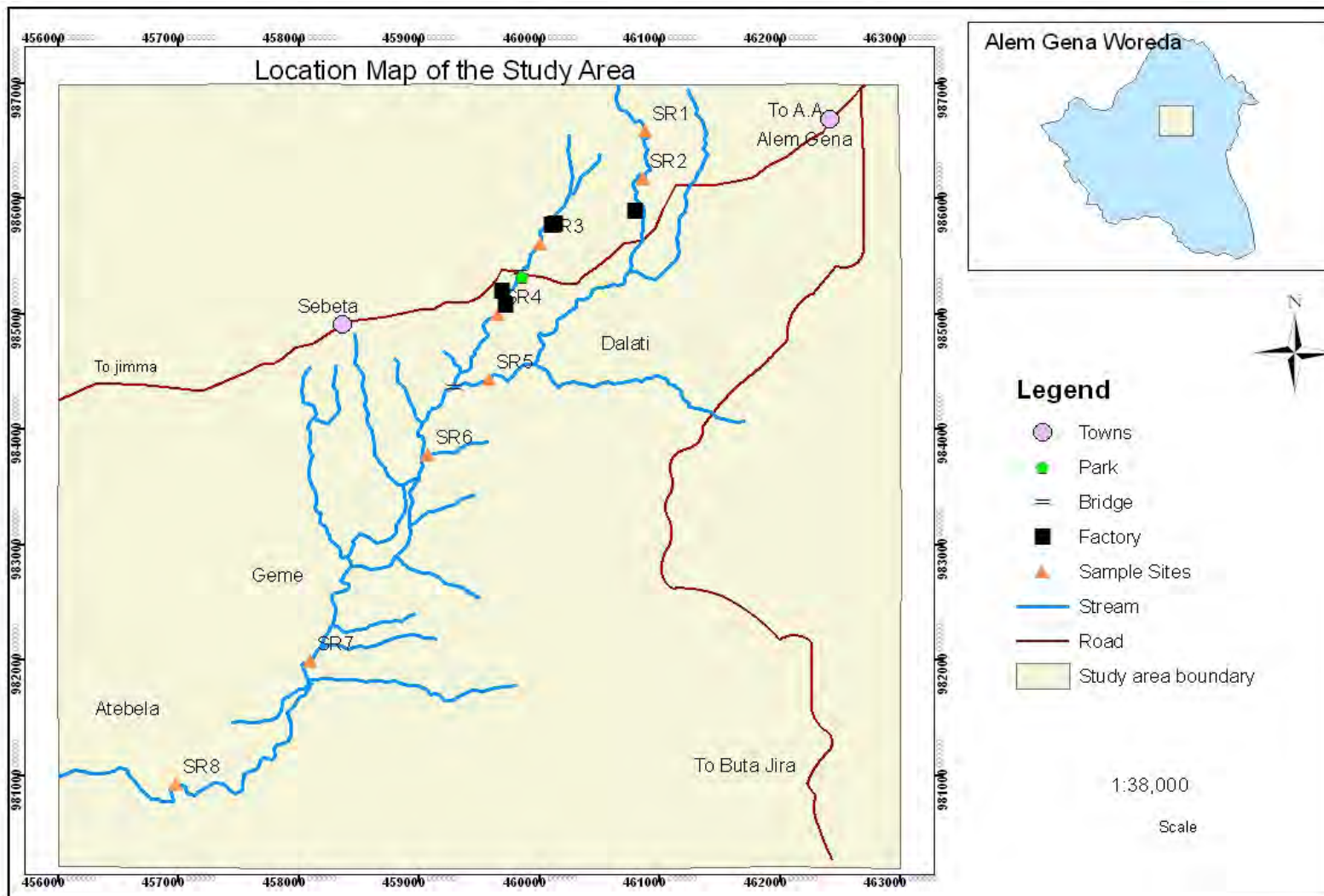


Figure 3. The location map of the study area with sampling sites indicated (developed from GPS readings collected and EMA, 2005 topographic map)

3.2. Sample Site Selection

Preliminary survey was conducted in August to gather general information on the physical characteristics of the study area such as stream characterization, watershed features, riparian vegetation, and instream features. Sample sites were selected based on their exposure to industrial and domestic wastes, instream features, stream geomorphology and other physical characteristics (Table 1). Coordinates of the study sites were taken using Global Positioning System Unit (Garmin etrex® GPS, Taiwan). The map of the study area was developed using ArcGIS 9.1 software.

Eight sample sites were selected on about 10 km downstream length of Sebeta River as shown on the map of the study area and [Appendix 1](#). S₁ and SR₂ are reference sites (for the biotic integrity assessment) located in the upstream rural area with less human activities. These sites were selected according to the USEPA Rapid Bioassessment Protocol criteria (Barbour *et al.*, 1999) and are located on the tributary above Sebeta Agro-industry on which SR₅ was also located. The sites SR₃ to SR₈ are impacted sites. The air distance between sample sites was estimated using ArcGIS 9.1 software. Accordingly, the distance between SR₁ and SR₂ is 423 m. SR₃ is located above the main bridge of the Addis Ababa-Jimma highway. Above this site are Bale Zaf Alcohol and Liquor Factory and Soap Factory.

The main upper course dries in the dry season. An over-flow from spring water developed for tap water supply to the town also joins the river making the downstream flow perennial. The distance between SR₂ and SR₃ is 1026 m. SR₄ was located 709 m downstream from SR₃. Effluents from National Alcohol and Liquor Factory and Blue Nile Tannery together with toilet out-flow from Fikir Park join the river before this sampling site. SR₅ was located on Qera river (the tributary from Alem Gena on which the reference sites are located). The tributary joins the main course just at the wooden bridge at an estimated downstream distance of 300 m from SR₅. The distance between SR₄ and SR₅ was 574 m. SR₆ is located 842 m downstream from SR₅. Farmers built traditional dam to divert the polluted river water to their farmland. SR₇ is 2056 m downstream from SR₆ where another traditional dam was built to divert the river water for irrigation. The last site (SR₈) is 1550 m downstream from SR₇ in Atebela. The sampling reaches in all cases were estimated to be length of 100 m.

3.3. Sample Collection and Laboratory Processing

Monthly samples were collected beginning from January 2007 to April 2007 for both physico-chemical and biological parameters. Water samples taken after thorough mixing were brought to the laboratory with one-liter polyethylene bottle for immediate analysis of nitrate and phosphate. Nitrate and Phosphate were measured using Cadmium reduction and Ascorbic acid methods, respectively according to HACH, DR 4000 procedures in WWDSE laboratory. NitraVer 6 and NitriVer 3 reagents (differing in their reaction time) were used for the nitrate test. Phosphate was measured using PhosVer 3 reagent. Absorbances at 500 nm for nitrate and at 890 nm for phosphorus tests were determined using spectrophotometer (Jenway 6305). DO was measured with probe using Jenway 9150 DO meter. Temperature and flow velocity were measured using HANNA instruments, model 145 thermometer and standard mechanical flow meter with propeller, model 2030R, Eijkelkamp Agrisearch equipment, respectively. pH and conductivity were measured using the Oyster pH/conductivity + temperature meter, Extech instruments. DO, temperature, flow velocity, pH and conductivity were measured on-site.

Habitat features were scored using USEPA Rapid Bioassessment Protocol for high gradient streams (Barbour *et al.*, 1999). It qualitatively evaluates 10 important habitat components: epifaunal substrate/available cover, embeddedness (pool substrate characterization-for low gradient), velocity/depth regime (pool variability-for low gradient), sediment deposition, channel flow status, channel alteration, frequency of riffles (or bends) (channel sinuosity-for low gradient), bank stability, vegetation protection and riparian vegetation zone width. Four other components are also added: nutrient enrichment, water appearance, bank grass cover and human waste/manure load. Each habitat quality parameter was scored in 20-point scale to sum up to a score of 280. An overall percent comparability with this maximum possible score was calculated for each site (Gallardo *et al.*, 2006). For each parameter, high score indicates better habitat situation, whereas lower values represent habitat degradation.

Periphyton samples from hard substrates such as cobbles (with an estimated surface area of 16cm²) were taken by scrapping the attached algae with toothbrush into sample containers (Chiriac and Warren, 2005). Chlorophyll analysis was done in the laboratory to evaluate the algal biomass (Chlorophyll a). Absorbances at 665nm and 750nm were measured using PYE

UNICAM SP 6-350 visible spectrometer. The $\mu\text{g/L}$ chlorophyll 'a' concentration was calculated using the approximate equation of Talling and Driver (1963).

The macroinvertebrate samples were collected using Surber sampler (500 μm mesh size, sampling area = 0.09 m^2). The Surber sampler was held vertically facing upstream. The water was then vigorously disturbed with kicks so that the dislodged invertebrates are carried into the net by the current. Five kicks per reach for 5 minutes each were done. Individual stones were also picked up and then scraped to dislodge attached invertebrates. Number of traps were taken as standard for pool habitats. Accordingly, ten traps per pool habitat were taken with scoop net as an equivalent to the Surber sampling in riffles and runs. The habitat proportions were considered for the composited samples of each site. Habitats with proportion less than 5% were not considered (Barbour *et al.*, 1999). The samples were transferred to glass jars to be preserved with 70% alcohol and transported to laboratory. The samples were then transferred to a white tray to remove debris and obscuring detritus. The individual macroinvertebrates were picked out with forceps and placed in separate vials containing alcohol. Identification of macroinvertebrates was done using dissecting stereoscopic microscope to family level. Non-insect taxa were identified to higher taxonomic levels or to family level whenever possible. Taxonomic keys such as Bouchard, (2004); Jessup *et al.* (1999), and Thompson, (2004) were used for identification. The identified benthic macroinvertebrates were preserved in glass jars with 10% formaldehyde for further use as specimen.

3.4. Macroinvertebrates Metrics and Index Development

Nine metrics representing richness, composition and tolerance/intolerance measures (including Hilsenhoff family level biotic index, H-FBI) and community loss index (CLI) were used for the macroinvertebrate community index of Sebata River (MCISR) (Table 2) (Barbour *et al.*, 1999; Mandaville, 2002; NWRA, 2005; Peitz, 2003). The criteria for the metrics selection were ability to be associated with physical degradation, ability to provide unique information and ability to discriminate reference sites from those of the impacted sites (Chihart, 2003). Metrics with extreme low values (1 or less in number, generally < 5 in percent) in the reference (upstream) sites were not considered (USEPA, 2003). Repetitive metrics were avoided by

computing their correlation coefficient (Pearson correlation) (Barbour *et al.*, 1999; Chihart, 2003).

Table 2. Definition of candidate metrics and their expected responses to increasing perturbation

Category	Metrics Selected	Definition	Predicted response to increasing perturbation
Richness measures	Total number of taxa	Measures the overall variety of the macroinvertebrate assemblage	Decreases
	Number of coleopteran taxa	Number of taxa in order coleoptera	Decreases
	Number of diptera taxa	Number of taxa in order diptera	Decreases
Composition measures	% Ephemeroptera	Percent of mayfly nymphs	Decreases
	% Diptera	Percent of diptera larva	Increases
	% Chironomidae	Percent of midges larva	Increases
Tolerance/intolerance measure	% Tolerant organisms	Percent of macroinvertebrates tolerant to perturbation	Increases
Additional	Hilsenhoff family level biotic index (H-FBI)	Uses tolerance values to weight abundance in estimate of overall pollution (originally developed to evaluate organic pollution)	Increases
	Community loss index (CLI)	Measures loss of benthic taxa with respect to a reference site	Increases

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 informative was taken (Shearer, 2006). The amount of overlap of the interquartile ranges between the values of the upstream reference sites (SR₁ and SR₂) and the downstream impacted sites (SR₃ to SR₈) on box plots was then examined to judge the discriminatory power of each metrics (Barbour *et al.*, 1999, 1996; Shearer, 2006). Metrics with no overlap of the interquartile ranges were considered for the final macroinvertebrate community index. Those metrics with extensive interquartile range overlap (both medians within the overlap) between the upstream reference sites and downstream impacted sites were rejected (Pond *et al.*, 2003; Solomon Akalu, 2006).

Scoring for all metrics, but H-FBI was based on the continuous scoring method of Blocksom (2003). The metric scores on 0 to 10 continuous scales were then converted to 0 to 100 point scale in order to group the narratives very good to very poor (Pond *et al.*, 2003). The 0 to 10 score was calculated using upper and lower threshold of their distribution in the reference and impacted sites as follows (Blocksom, 2003).

$$\text{Metric score} = \frac{(\text{observed} - \text{lower threshold}^a) \times 10}{\text{Upper}^b - \text{lower threshold}^a}$$

where, ^aLower threshold for metrics that decrease (increase) with perturbation is 25th (75th) percentile of the impacted sites. ^bUpper threshold for metrics that decrease (increase) with perturbation is 75th (25th) percentile of the reference sites.

Metrics with values above the upper threshold for those decreasing with increased perturbation received a score of 10, whereas those below the respective lower threshold received a score of 0. For those metrics increasing with increased perturbation, values above the lower threshold (75th percentile of the impacted sites) received a score of 0, whereas those with metrics value below the upper threshold (25th percentile of the reference sites) received a score of 10. The other values were linearly scaled along the range between 0 and 10 before they were converted to 0 to 100 point scale for the narrative rating.

Study sites SR₃ (immediately downstream from Bale Zaf Alcohol and Liquor Factory and Soap Factory) and SR₄ (downstream next to National Alcohol and Liquor Factory and Blue Nile Tannery) were excluded from the index development because of the low macroinvertebrate collections from the sites (< 60 individuals), they were simply classified as of very poor biological integrity (Gallardo *et al.*, 2006).

The line between good and fair was taken from the quadrasection of the 25th percentile of the reference sites (SR₂) ($3/4 \times 87.74 = 65.81$). The three lower intervals of the quadrasection were categorized as fair, poor and very poor. MCISR scores ranging between 65.81 and 87.74 were classified as good. Scores that are at and above 87.74 were categorized as very good. Scores between 43.87 and 63.81 were classified as fair. Poor scores were those below 43.87, but scored at least 21.94. MCISR Scores below 21.94 were classified as very poor.

Hilsenhoff family level biotic index (H-FBI) was calculated separately using the following formulae. The scores ranging from 0 to 10 were grouped into 7 narratives (excellent to very poor) based on Hilsenhoff (1988). Tolerance values were taken from Bouchard (2004).

$$\text{H-FBI} = \sum_{i=1}^n \frac{X_i T_i}{N}, \text{ Where } X_i \text{ is the number of individuals of each taxon, } T_i \text{ is the tolerance value of the respective taxon and } N \text{ is the total of macroinvertebrates in a study site.}$$

The community loss index (CLI) was calculated using the formulae;

$$\text{CLI} = \frac{d - a}{e}, \text{ Where } d \text{ is the total number of taxa in the reference site, } e \text{ is the total number of taxa in the impacted site and } a \text{ is the number of taxa common to both sites.}$$

3.5. Data analysis

SPSS version 13.0 was used for data analysis. Bivariate Pearson correlation was used to interrelate the parameters considered. One-way ANOVA was computed to see significant difference between the sample sites for the physico-chemical parameters and algal biomass.

4. RESULTS

The physico-chemical variables, habitat assessment scores, periphytic algal biomass and benthic macroinvertebrate community structures were analysed for all of the 8 sample sites.

4.1. Physico-chemical Parameters

Physico-chemical variables measured for the assessment of extent of pollution of Sebeta River include; flow velocity (m/s), dissolved oxygen (DO) (mg/L), pH, temperature ($^{\circ}$ C), electrical conductivity (EC) (μ s/cm), nitrate-nitrogen (NO_3^- N) (mg/L) and phosphate (PO_4^{3-}) (mg/L). The average results of each with the standard error are given in table 3.

Table 3. Average physico-chemical analysis results (Mean \pm SE, n = 3).

Sites	Variables						
	EC (μ s/cm)	Flow (m/s)	pH	T ($^{\circ}$ c)	DO (mg/l)	NO_3^- N(mg/l)	PO_4^{3-} (mg/l)
SR ₁	160.00 \pm 30.55	0.55 \pm 0.15	8.35 \pm 0.83	19.77 \pm 0.79	5.48 \pm 0.15	0.40 \pm 0.06	0.03 \pm 0.00
SR ₂	146.67 \pm 29.06	0.63 \pm 0.36	8.17 \pm 0.61	19.67 \pm 0.52	5.51 \pm 0.36	0.47 \pm 0.19	0.06 \pm 0.00
SR ₃	16050.00 \pm 2270.77	0.04 \pm 0.01	3.60 \pm 0.81	33.83 \pm 1.17	0.20 \pm 0.14	4.99 \pm 2.88	8.59 \pm 1.16
SR ₄	15400.00 \pm 316.60	0.17 \pm 0.04	4.75 \pm 0.88	27.63 \pm 0.68	0.21 \pm 0.11	4.14 \pm 2.39	2.16 \pm 1.17
SR ₅	4276.67 \pm 1006.58	0.18 \pm 0.04	7.24 \pm 0.17	21.90 \pm 0.95	0.33 \pm 0.20	1.15 \pm 0.08	1.04 \pm 0.18
SR ₆	5476.67 \pm 1118.47	0.09 \pm 0.02	5.72 \pm 0.39	19.63 \pm 0.38	0.45 \pm 0.28	1.92 \pm 1.11	2.76 \pm 1.18
SR ₇	3906.67 \pm 808.73	0.14 \pm 0.07	6.37 \pm 0.32	20.13 \pm 0.99	0.34 \pm 0.13	1.46 \pm 0.84	1.88 \pm 0.15
SR ₈	3813.33 \pm 1523.34	0.62 \pm 0.19	5.60 \pm 0.20	19.93 \pm 0.94	0.52 \pm 0.27	1.27 \pm 0.73	0.88 \pm 0.16

The lowest flow velocity was recorded at SR₃ (0.04 \pm 0.01m/s) which is immediately downstream from Bale Zaf Alcohol and Liquor Factory and Soap Factory. The highest average run was recorded at SR₂ (0.63 \pm 0.36 m/s) (in the upstream rural area in Alem Gena above the Sebeta Agro-industry) followed by SR₈ (0.62 \pm 0.19 m/s) (in the downstream in Atebela peasants association). There was no significant difference between the average flow velocity of the study sites (F = 2.612, df = 23, P > 0.05).

The maximum average concentration of DO in the river water (5.51 ± 0.36 mg/L) was recorded at SR₂ (in the upstream rural area above Sebeta Agro-industry). The minimum DO measure (0.20 ± 0.14 mg/L) was from SR₃ that is located immediately downstream from Bale Zaf Alcohol and Liquor Factory and Soap Factory. The mean DO concentrations of upstream sites (SR₁ = 5.48 ± 0.15 mg/L and SR₂ = 5.51 ± 0.36 mg/L) above Sebeta Agro-industry showed significantly higher values than the rest of the sample sites ($F = 170.192$, $df = 23$, $P < 0.001$).

The highest mean pH measurement was recorded at SR₁ (8.35 ± 0.83) (upstream site above SR₂), whereas the lowest measurement was from SR₃ (3.60 ± 0.81) (immediately downstream from Bale Zaf Alcohol and Liquor Factory and Soap Factory). High neutrality in pH was seen at SR₅ (7.24 ± 0.17) (downstream site on tributary from Alem Gena). The mean pH values of SR₃ (3.60 ± 0.81) and SR₄ (4.75 ± 0.88) (downstream of National Alcohol and Liquor Factory and Blue Nile Tannery) were significantly lower than the pH values of the rest of the sample sites ($F = 7.734$, $df = 23$, $P < 0.001$).

The average temperature measures of Sebeta River ranged from its highest (33.83 ± 1.17 °C) at SR₃ (immediately downstream from Bale Zaf Alcohol and Liquor Factory and Soap Factory) to its lowest (19.63 ± 0.38 °C) at SR₆ (downstream on the way to Dalati peasants association). At SR₆ farmers constructed traditional dam to divert river water to their farmlands. The mean temperature measures of SR₃ (33.83 ± 1.17 °C) and SR₄ (27.63 ± 0.68 °C) (downstream of National Alcohol and Liquor Factory and Blue Nile Tannery) were significantly higher than the mean temperature values of the rest of the sample sites ($F = 38.504$, $df = 23$, $P < 0.001$).

The maximum average EC was recorded at SR₃ (16050.00 ± 2270.77 µs/cm) (immediately downstream from Bale Zaf Alcohol and Liquor Factory and Soap Factory) followed by SR₄ (15400.00 ± 316.60 µs/cm) (downstream next to National Alcohol and Liquor Factory and Blue Nile Tannery). The lowest EC was recorded at SR₂ (146.67 ± 29.06 µs/cm) (in the upstream above Sebeta Agro-industry). The mean EC measures of SR₃ and SR₄ were significantly higher than the EC measures of the rest of the sample sites ($F = 29.390$, $df = 23$, $P < 0.001$).

The maximum average nitrate-nitrogen concentration (4.99 ± 2.88 mg/L) was recorded at SR₃ (immediately downstream from Bale Zaf Alcohol and Liquor Factory and Soap Factory) followed by SR₄ (4.14 ± 2.39 mg/L) (downstream next to National Alcohol and Liquor Factory and Blue Nile Tannery). The minimum concentration (0.40 ± 0.06 mg/L) was recorded at SR₁ (upstream site above SR₂). There was no significant difference between the mean nitrate-nitrogen concentrations of the study sites ($F = 1.388$, $df = 23$, $P > 0.05$). The pattern of NO₃-N in the downstream sites showed a general decline from SR₃ to SR₈.

The maximum phosphate concentration was recorded at SR₃ (8.59 ± 1.16 mg/L) (immediately downstream from Bale Zaf Alcohol and Liquor Factory and Soap Factory), whereas the minimum concentration was from SR₁ (0.03 ± 0.00 mg/L) (located upstream site above SR₂). The records of this study showed phosphate concentration at SR₃ (8.59 ± 1.16 mg/L) was significantly higher than the phosphate concentrations recorded from other study sites of Sebeta River sites ($F = 14.611$, $df = 23$, $P < 0.001$).

4.2. Habitat Integrity Assessment

The values obtained for the 14 parameters on 20-scale points were summed up according to the USEPA rapid bioassessment protocols and incorporated in Table 4. The percent comparability narrative of the habitat scores was expected to take six categories: those with percent comparability ≥ 95 % as excellent, ≥ 85 % as very good, ≥ 75 % as good, ≥ 60 % as fair, ≥ 50 % as poor, and ≤ 49 as very poor. Accordingly, both of the upstream sites above Sebeta Agro-industry (SR₁ and SR₂) fall into the category good, whereas the rest of the sites fall into one category (very poor).

Table 4. The mean habitat assessment results (with the total and percentage comparability).

Habitat Parameters	Sites							
	SR ₁	SR ₂	SR ₃ [♦]	SR ₄	SR ₅	SR ₆ [♦]	SR ₇ [♦]	SR ₈
ES	18	18	5	8	7	5	0	15
Em*	15	14	2	3	8	10	3	15
VDR*	10	10	2	1	7	16	15	10
SD	18	18	12	14	12	11	7	10
CFS	10	10	6	10	10	16	15	6
CA	20	20	20	18	18	8	6	17
FR*	12	15	6	4	6	6	6	6
BS	20	20	8	16	14	16	18	9
VP	18	16	13	10	9	17	12	8
RVZW	13	12	10	12	7	8	10	6
NEn	18	18	0	0	1	4	4	10
BGC	18	20	0	0	1	0	0	2
WA	10	10	5	2	2	1	1	2
FM	18	18	13	0	1	2	6	8
Total	218	219	102	98	103	120	103	124
% Comparability	77.9	78.2	36.4	35.0	36.8	42.9	36.8	44.3
category	Good		Very poor					

Note: ES = Epifaunal Substrate, Em = Embeddedness (Pool Substrate characterization (PS)-for low gradient), VDR = Velocity/Depth Regime (Pool Variability (PV)-for low gradient), SD = Sediment Deposition, CFS = Channel Flow Status, CA = Channel Alteration, FR = Frequency of Riffles/Bends (Channel Sinuosity (CS)-for low gradient), BS = Bank Stability, VP = Vegetative Protection, RVZW = Riparian Vegetative Zone Width, NEn = Nutrient Enrichment, BGC = Bank Grass Cover, WA = Water Appearance and FM = Faeces/Manure presence, ♦ = low gradient, * parameters replaced by PS, PV and CS in low gradient sites).

4.3. Algal Biomass

Higher mean chlorophyll 'a' concentrations ($78.8 \pm 6.1 \mu\text{g/L}$, $81.1 \pm 10.1 \mu\text{g/L}$) were recorded at SR₁ and SR₂ (the upstream sites above Sebeta Agro-industry), respectively. The records of this study showed significantly higher chlorophyll 'a' values at these two sites ($F = 49.031$, $df = 23$, $P < 0.001$).

Table 5. Average chlorophyll analysis result (Mean \pm SE, n = 3).

Sites	SR ₁	SR ₂	SR ₃	SR ₄	SR ₅	SR ₆	SR ₇	SR ₈
Chl. 'a' ($\mu\text{g/L}$)	78.8 ± 6.1	81.1 ± 10.1	7.0 ± 0.0	7.0 ± 4.0	11.6 ± 2.3	13.9 ± 0.0	11.6 ± 2.3	9.3 ± 2.3

4.4. Macroinvertebrates

A total of 2256 macroinvertebrate individuals representing 20 different taxa were collected from all representative habitat types of the study sites (Appendix 2). All were identified to family level with exception of class Hirudinea (leech). Three taxa (2 gastropod families and Hirudinea) comprised the non-insect group. Of the 20 taxa 16 belong to the reference sites, while the downstream sites were represented by only 9 insect families.

4.4.1. Macroinvertebrate Community Index of Sebeta River (MCISR)

Eight candidate metrics including the community loss index initially selected to develop the macroinvertebrate community index of Sebeta River (MCISR) are given in the table below.

Table 6. The candidate metrics with their respective values for each site (* not considered for MCISR)

Metrics	Sites							
	SR ₁	SR ₂	SR ₃	SR ₄	SR ₅	SR ₆	SR ₇	SR ₈
Total number of taxa	13	11	2	4	4	6	5	6
Number of coleopteran taxa	6	4	0	0	0	1	1	2
Number of diptera taxa	3	3	2	4	3	4	3	3
% Ephemeroptera	33.9	38.6	0.0	0.0	9.7	18.6	9.8	3.1
% Diptera	60.0	59.0	100.0	100.0	90.3	79.6	89.2	95.9
% Chironomidae	7.1	4.7	83.3	9.8	76.7	37.2	11.8	9.0
% Tolerant organisms	3.0	1.3	100.0	58.8	68.0	56.6	77.5	59.0
Hilsenhoff family level biotic index (H-FBI)*	5.1	5.1	8.3	8.2	7.4	7.0	8.4	8.1
Community loss index (CLI)	0.00	0.45	6.00	4.75	4.75	1.50	2.00	1.30

Total number of taxa was found to correlate within the rejection range with coleopteran taxa ($r = 0.974$), with percent ephemeroptera ($r = 0.915$), with percent diptera ($r = -0.935$) and with percent tolerant organisms ($r = -0.958$). Similarly, percent diptera showed high correlation with

percent ephemeroptera ($r = -0.997$) and percent tolerant organisms ($r = 0.901$). Therefore, these two metrics initially supposed to contribute for the MCISR were rejected.

Table 7. Bivariate Pearson correlation matrix of selected metrics

	Total no. taxa	No. Coleop. taxa	No.Dipter taxa	% Ephem.	% Diptera	% Chiron.	% Tolerant taxa	CLI
Total no. taxa								
No. Coleop. taxa	0.974							
No.Dipter. taxa	0.097	-0.076						
% Ephem.	0.915	0.844	0.065					
% Diptera	-0.935	-0.874	-0.062	-0.997				
% Chiron.	-0.627	-0.584	-0.454	-0.411	0.430			
% Tolerant organisms	-0.958	-0.886	-0.244	-0.894	0.901	0.637		
CLI	-0.858	-0.825	-0.232	-0.763	0.781	0.746	0.773	

From the box plots (Fig. 4) the median of both upstream reference sites and the downstream impacted sites were found within the interquartile overlap for number of dipteran taxa. Therefore, it was excluded from the candidate metrics for the MCISR. The rest five metrics; number of coleopteran taxa, percent ephemeroptera, percent chironomidae, percent tolerant organisms and community loss index (CLI) were taken as the core metrics to develop macroinvertebrate community index of Sebata River(MCISR).

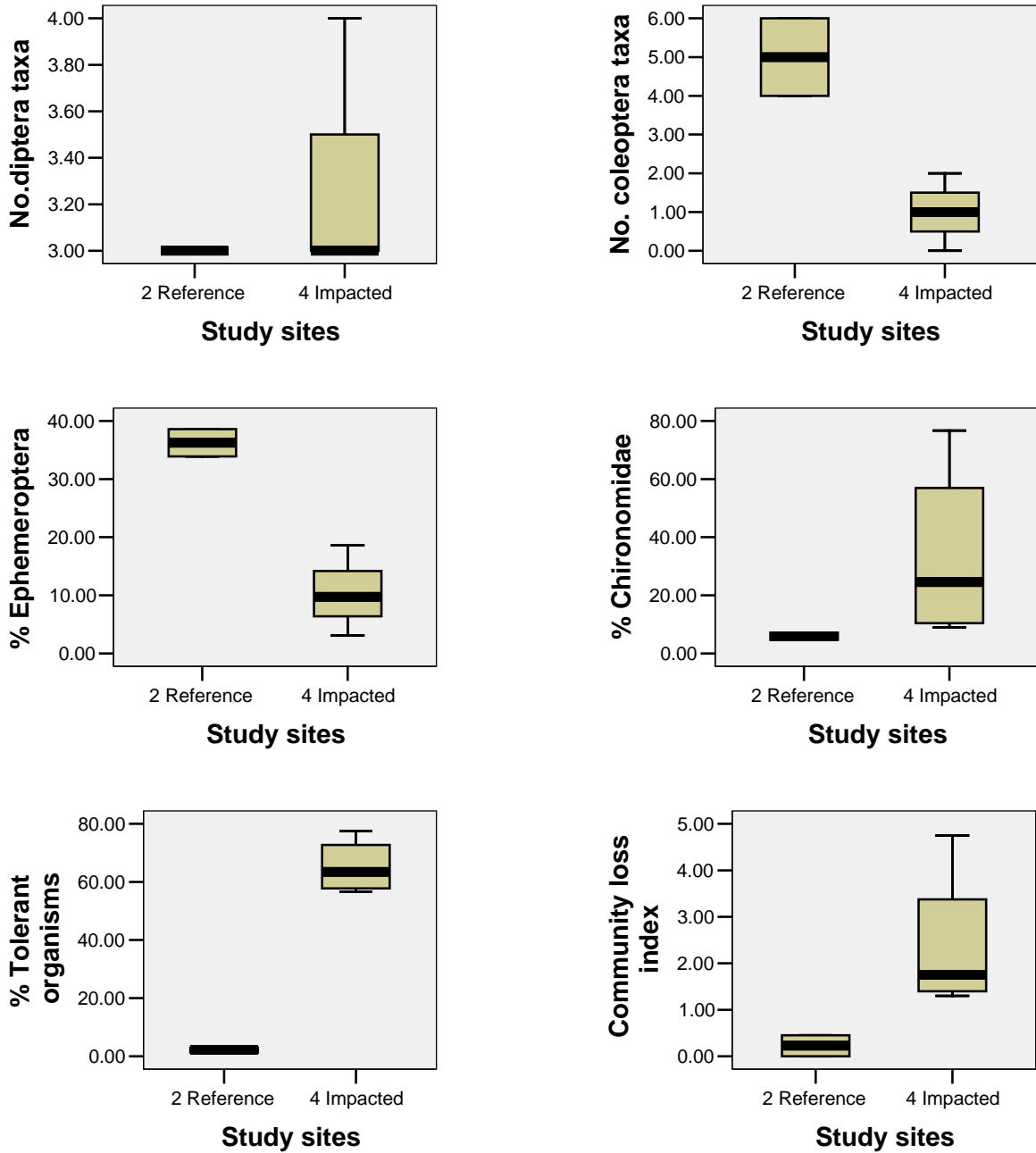


Figure 4. Discriminatory box plots of the candidate metrics considered for the MCISR

4.4.2. The MCISR Score

The MCISR scores for the six study sites of Sebeta River (with exception of SR₃ and SR₄) were developed from the five core metrics and the five class narratives made based on the 100-scale point rating were given in Table 8. Accordingly, SR₁ and SR₂ (upstream reference sites located above Sebeta Agro-industry) with MCISR scores of 94.8 and 87.4, respectively were classified as very good and SR₈ (the last downstream site located in Atebela peasants association) was categorized to poor, but the rest of the study sites (including SR₃ and SR₄) fall to the category very poor.

Table 8. Scores of the core metrics and the MCISR score for the study sites of Sebeta River.

Metrics	Sites					
	SR ₁	SR ₂	SR ₅	SR ₆	SR ₇	SR ₈
No. coleopteran taxa	10.00	6.67	0.00	0.00	0.00	1.67
% Ephemeroptera	8.40	10.00	0.00	3.10	0.03	0.00
% Chironomidae	9.30	10.00	0.00	0.00	7.8	8.7
% Tolerant organisms	9.70	10.00	0.00	1.70	0.00	1.30
Community loss index (CLI)	10.00	7.20	0.00	1.90	0.00	2.50
Total (out of 50)	47.40	43.87	0.00	6.70	7.83	14.17
MCISR score (out of 100)	94.8	87.74	0.00	13.40	15.66	28.34
	Very good		Very poor			Poor

4.4.3. Hilsenhoff Family Level Biotic Index (H-FBI)

SR₁ and SR₂ (both located in the upstream rural area above Sebeta Agro-industry) with equal FBI of 5.1 each were in the range of 5.10-5.75 (fair class of Hilsenhoff, 1988). The rest of the sites with exception of SR₆ fall into the category very poor (in the range of 7.26-10.00 H-FBI value). SR₆ (located downstream on the way to Dalati) with FBI of 7.0 was categorized as poor (within the H-FBI range of 6.51-7.25).

5. DISCUSSION

5.1. Physico-chemical parameters

Flow velocity records of most of the study sites of Sebeta River were within the natural range. Meybeck and Helmer (1996) reported that river flow velocity measures between 0.1 m/s and 1 m/s. Two sample sites, SR₃ (immediately downstream from Bale Zaf Alcohol and Liquor Factory and Soap Factory) and SR₆ (dam site downstream on the way to Dalati peasants association) measured flow velocity of 0.04 ± 0.01 m/s and 0.09 ± 0.02 m/s which were below this range. However, flow velocity of 0.01 m/s to 0.74 m/s was recorded in other studies (Braccia and Voshell, 2006). The flow velocity measures of different downstream sites were irregularly distributed. The low flow velocity at SR₃ could be due to large amount of total solid entering the river at the site. Deshu Mamo (2004) recorded total solid of 78000 ± 2309 mg/L and 26259 ± 3240 mg/L at around SR₃ and SR₄, respectively.

Dissolved oxygen concentrations of the upstream sites above Sebeta Agro-industry (SR₁ and SR₂) were higher than the minimum amount needed for survival and functioning of biological communities, 5 mg/L (Chapman and Kimstach, 1996). However, these might indicate mild pollution since the records were about half an amount of typical dissolved oxygen concentration in unpolluted natural waters (≈ 10 mg/L). The dissolved oxygen in the downstream sites (SR₃ to SR₈) were extremely low having the lowest at SR₃ (0.20 ± 0.14) (immediately downstream from Bale Zaf Alcohol and Liquor Factory and a soap factory) followed by SR₄ (0.21 ± 0.11) (downstream next to National Alcohol and Liquor Factory and Blue Nile Tannery). The decline in the DO at these sites could be attributed to the reduced flow velocity and increased temperature. The temperature records at these sites were out of the WHO (1984) guideline values (12-25 °C). The temperature record at SR₃ (33.83 ± 1.17 °C) was even out of the 0-30 °C reported by Meybeck *et al.* (1996) as typical characteristic of natural waters.

High organic load could be another factor contributed to the DO decline at downstream sites. Dense effluent discharge after processing of molasses for beverage production by Bale Zaf Alcohol and Liquor Factory is directly dumped to the river at SR₃ without any treatment. Effluents with high carbon content are among high BOD wastes (Sisay Bekele, 2000). Animal

fat thrown into the river from the soap factory at the site was also observed. Deshu Mamo (2004) recorded BOD of 7267 ± 6335 mg/L and 4414 ± 3223 mg/L at SR₃ and SR₄, respectively.

The DO records of this study are lower compared to the records of sites on other rivers under high anthropogenic impact (Modjo River, min. 6.1 ± 4.01 mg/L, Great Akaki River, min. 3.1 ± 2.4 mg/L, (Baye Sitotaw, 2006)). However, studies on Little Akaki River came up with DO values as low as 0.3 mg/L in its stretch within Addis Ababa (Deshu Mamo, 2004).

pH values of most of the study sites were in the permissible range for natural water (6.0-8.5). They also meet the EPA (2003) standards for surface water (6.0-9.0). Significantly low values (out of both the natural limit and the EPA standard) at SR₃ (3.60 ± 0.81) and at SR₄ (4.75 ± 0.88) may be attributed to high BOD at the sites (Deshu Mamo, 2004, Chapman and Kimstach, 1996).

Higher values of EC in the downstream sites may be associated with increased physical disturbances in the watershed such as agriculture-induced sedimentation and dumping of wastes from industries and households.

The EC records of some of the sampling sites of Sebeta River were higher than the hitherto tested rivers in Ethiopia. The maximum EC record of Modjo River was 910.2 ± 186.6 μ s/cm followed by Great Akaki River (873 ± 48.32 μ s/cm (Baye Sitotaw, 2006). Seyoum Leta *et al.* (2003) recorded EC of 14330 ± 1182.3 μ s/cm at tannery wastewater receiving sites of Modjo River. However, EC values recorded for industrial effluent receiving Alaro River in Nigeria (18000-22000 μ s/cm) (Fakayode, 2005) was much higher than the maximum EC recorded from Sebeta River.

Given that EC in freshwaters range between 10-1000 μ s/cm (Chapman and Kimstach, 1996) and the Environmental Protection Authority of Ethiopia (EPA, 2003) set a standard to EC of 1000 μ s/cm for surface waters, the downstream sites of Sebeta River seem to be highly polluted.

The nitrate-nitrogen of sites of Sebeta River were below the limit (5 mg/ L) above which nitrate pollution reported to cause adverse effects on aquatic system (Chapman and Kimstach,

1996). The records of all the sites were also in the permissible limit of EPA (2003) standard of 10 mg/L. Higher concentrations at SR₃ (4.99 ± 2.88 mg/L) and SR₄ (4.14 ± 2.39 mg/L) might be due to discharge of nitrogen containing wastes from the industries in the vicinity of the river bank. Further downstream decline in nitrate-nitrogen could be attributed to dilution from tributaries and denitrification process of the microbial communities.

The phosphate records of the study sites of Sebeta River were higher than the concentration in most natural waters. The upstream rural sites above Sebeta Agro-industry (SR₁ and SR₂) recorded values closer to the natural range, 0.03 ± 0.00 mg/L and 0.06 ± 0.00 mg/L, respectively. The slight increase from the natural condition at these sites could be due to diffuse source pollution from the farmlands in the watershed.

Significantly higher record at SR₃ (8.59 ± 1.16 mg/L) could be attributed to the use of phosphorus based chemicals in the factories such as detergents or the inclusion of shower and washing room wastes to the effluent joining the river water (Waterwatch Australia, 2002).

5.2. Habitat Integrity

Degraded habitat features and the low habitat integrity scores at the downstream sites (SR₃ to SR₈) were associated with watershed agriculture and the consequent shift and removal of riparian vegetation. Agricultural activities in the watershed of Sebeta River shifted the riparian vegetation from heterogeneous natural condition to few annual domestic crops and grass. Trees and shrubs which indirectly contribute to the instream habitat integrity by fencing floods and trapping sediments (Allan, 2004; Belsky *et al.*, 1999; Sweeney *et al.*, 2004; USEPA, 2000) are only limited to a narrower distance from the river bank. Better habitat integrity scores were recorded at the upstream rural sites with less human impact (SR₁ and SR₂). Increased degradation and removal of riparian vegetation in the downstream sites (SR₃ to SR₈) with increased human influence were the causes for the very poor habitat integrity. Habitat qualities such as bank stability, sediment deposition, pool substrate characterization and flow regime were adversely affected at those sites. The effects of industrial and household waste discharge, manure of grazing domestic animals near the river bank and human faeces altering habitat qualities such as water appearance all contributed to the lower habitat integrity at the downstream sites (Braccia and Voshell, 2006).

5.3. Algal Biomass

The lower periphytic algal biomass ($\mu\text{g/L}$ chlorophyll 'a') in the downstream sites (SR₃ to SR₈) may be because of the water chemistry and temperature (Chiriac and Warren, 2005). Water turbidity due to industrial and domestic pollution and sedimentation may be the most important factor that affected periphyton growth in the downstream sites by blocking penetration of light (Murphy, 2005; USGS, 2003).



Figure 5 Pictures of some of the study sites for visual comparison of turbidity in relation to primary productivity (SR₁ = upstream site in the rural area above Sebeta Agro-industry, SR₃, SR₄ and SR₆ = downstream sites immediately after Bale Zaf Alcohol and Liquor Factory and a soap factory, downstream from National Alcohol and Liquor Factory and Blue Nile Tannery and dam site on the way to Dalati, respectively).

5.4. The Macroinvertebrate Community Index and the Metrics

The core metrics considered for the MCISR well discriminated the upstream sites from the impacted downstream ones. Number of coleopteran taxa scored higher in the upstream sites ($SR_1 = 10$, $SR_2 = 6.67$). Except for SR_8 (with score of 1.67), the rest of impacted downstream sites received score of zero. Most of coleopteran taxa such as those collected from the study sites of Sebeta River are moderately tolerant (Bouchard, 2004). Therefore, higher number of coleopteran taxa can be an indicator of less impact, whereas low number of coleopteran taxa shows severe impact. Decrease in DO, flow velocity and habitat quality could be among the causes for the low scores of coleopteran taxa in the downstream. High temperature and EC measures could have also contributed to the decline in the coleopteran taxa in those sites.

Percent ephemeroptera also well discriminated the upstream sites from the impacted downstream sites. Percent ephemeroptera generally tends to decrease with decreasing water quality for healthy ecosystem since mayflies are among sensitive to moderately tolerant taxa (Barbour *et al.*, 1999; Bouchard, 2004). SR_2 was the richest in terms of percent ephemeroptera receiving score of 10 followed by SR_1 with score of 8.4 (both sites located in the upstream rural area above Sebeta Agro-industry). The sites SR_3 , SR_4 , SR_5 and SR_8 scored zero. The family Baetidae, which constituted the mayfly taxa, collected in this study is moderately tolerant. This family is known to increase with moderate pollution as reported by Gallardo *et al.*, (2006). Therefore, the low scores at the downstream sites (SR_3 to SR_8) could indicate highly impaired ecological condition. Higher percent mayflies at the upstream sites, on the other hand, may indicate mild pollution for they are moderately tolerant.

The poorest scores of percentage mayflies in the downstream sites were associated with decrease in DO, flow velocity and very poor habitat quality. Higher temperature and EC in most of the downstream sites could have also put its effect. In addition to this, degraded riparian community might have contributed its part through reduction in allochthonous energy inputs (Ward, 1989).

Higher scores for percent chironomidae at SR₁ (9.3) and SR₂ (10) indicate lower percent chironomidae at the upstream reference sites since it is negatively related to ecosystem integrity. Similarly, further downstream sites SR₇ (dam site in Geme peasants association) and SR₈ (the last site in Atebela peasants association) received relatively higher scores (7.8 and 8.7, respectively) indicating they have less percent chironomidae among the impacted downstream sites. The rest of the downstream sites scored zero implying higher ecological impairment due to closeness to factories discharging huge amount of waste to the river.

Chironomidae is among the tolerant families of dipteran taxa with the red blood being more tolerant than the pale one (tolerance values of 8 and 6 respectively). 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).

Higher scores of percentage tolerant organisms at the upstream reference sites (SR₁ and SR₂) testify the presence of few tolerant organisms. The impacted downstream site scores, on the other hand, show higher proportion of tolerant organisms, which in turn testify higher ecological impairment since percent tolerant organisms tend to increase with perturbation (Barbour *et al.*, 1999; Gallardo *et al.*, 2006).

Community loss index at SR₁ (10) followed by SR₂ (7.2) which shows overall taxa diversity differences even among the upstream reference sites. This metric tends to decrease with increasing water quality to maintain healthy aquatic ecosystem (Mandaville, 2002). Therefore, lower score at the downstream sites are associated with high community loss, which in turn confirms higher ecosystem disturbances at the sites.

The H-FBI narratives categorized sites with less pollution to a bit higher pollution and the sites with higher pollution; SR₆ for instance, into better category compared to MCISR. Therefore, it was not good enough in adequately discriminating between differently impacted sites of Sebeta River.

SR₃ and SR₄ for which macroinvertebrate community index was not developed received zero scores for all metrics for their extremely low water quality and severely disturbed ecosystem. Collection of only few numbers of macroinvertebrates was managed at both of the sites. SR₃

receives high organic pollution load from Bale Zaf Alcohol and Liquor Factory and a soap factory. Both of the factories put untreated solid and liquid wastes directly into the river water. SR₄, on the other hand receives pollutants from Fikir Park, National Alcohol and Liquor Factory and Blue Nile Tannery together with faecal contamination from the riparian community. The alcohol factory effluent together with hot water joins the river water. The tannery has treatment plant, but an intermittent liquid effluent is released to the river. Toilet discharge from Fikir Park is also directed into the river.

SR₅ scored MCISR of zero (equal with those excluded from the index development). The site receives polluted water with tannery waste from around Alem Gena. Sebeta Agro-industry also directs wastes and manure to the river (Fig. 3). Sites SR₆ to SR₈ do not receive industrial wastes directly hence they are in the rural area; instead, they face pollution from manure and faeces in the riparian side. They may also receive diffuse source pollution from the farmlands in the watershed. SR₈ scored better MCISR among the impacted downstream sites and was classified to relatively better category (poor). This may imply tendency of the river's biotic integrity to recover.

5.5. Interrelation between Physico-Chemical Data, Habitat Integrity Score and Biological Measures

Bivariate Pearson correlation was used to examine the interrelation between the parameters considered for the assessment of biological integrity of Sebeta River (Table 9). The core metrics selected for the development of the macroinvertebrate community index of the river, H-FBI and the MCISR were included as candidates of the macroinvertebrate community structure. The responses of the biological components (the macroinvertebrates and the algae) were strong with respect to the physico-chemical status and the habitat condition of the study sites.

Number of coleopteran taxa strongly correlated ($\alpha = 0.05$) to dissolved oxygen, flow velocity, pH, algal biomass, habitat integrity score, percent ephemeroptera, percent tolerant organisms, community loss index (CLI), H-FBI and the MCISR. Among those parameters, electrical conductivity, percent tolerant organisms, CLI and H-FBI showed negative relation.

Dissolved oxygen is one of the major determining factors in influencing the abundance diversity and distribution of macroinvertebrates (Chapman and Kimstach, 1996). The effect is high on more sensitive taxa to pollution. The coleopteran taxa collected from the study sites of Sebeta River were moderately tolerant with tolerance values 4 to 5 (Bouchard, 2004), which can be highly affected by the level of dissolved oxygen in the river water.

The strong relation of number of coleopteran taxa and flow velocity ($r = 0.794$) may be due to the effect of flow velocity on availability of dissolved oxygen. Fast flow favors oxygenation, whereas slow flows are less conducive to oxygenation, especially when temperature is high (Meybeck *et al.*, 1996; <http://en.wikipedia.org/wiki/Streamflow>).

Number of coleopteran taxa also showed strong positive correlation with pH ($r = 0.749$). This could imply that higher pH may be more suitable to the coleopteran taxa such as the ones collected in present study. It supports the fact that the higher pH measures of the study were within the permissible limit, while the lower measures such as at SR₃ (3.60 ± 0.81) and SR₄ (4.75 ± 0.88) were out the EPA (2003) standard of pH of 6-9 for surface waters.

Table 9 Bivariate Pearson correlation matrix of physico-chemical parameters, habitat score, periphytic algal biomass, core metrics of macroinvertebrate community index and the macroinvertebrate community index of Sebeta River.

	EC	FV	pH	T°C	DO	NO ₃ -N	PO ₄ ³⁻	HS	Chl. 'a'	Col.	Eph.	Chiro.	Toler.	CLI	H-FBI	MCISR
EC																
FV	-0.666															
pH	-0.899♦	0.623														
°C	0.915♦	-0.546	-0.772*													
DO	-0.625	0.727*	0.774*	-0.401												
NO₃-N	0.991♦	-0.669	-0.914♦	0.933♦	-0.592											
PO₄³⁻	0.784*	-0.668	-0.819*	0.866♦	-0.497	0.851♦										
HS	-0.668	0.773*	0.766*	-0.458	0.991♦	-0.630	-0.514									
Chl. 'a'	-0.641	0.701	0.793*	-0.419	0.998♦	-0.607	-0.503	0.987♦								
Col.	-0.711*	0.794*	0.749*	-0.532	0.928♦	-0.625	-0.558	0.949♦	0.917♦							
Eph.	-0.761*	0.580	0.850♦	-0.600	0.918♦	-0.722*	-0.556	0.925♦	0.937♦	0.844♦						
Chiro.	0.443	-0.632	-0.409	-0.575	-0.471	0.458	0.680	-0.494	-0.457	-0.584	-0.411					
Toler.	0.688	-0.778*	-0.819*	0.599	-0.922♦	0.692	0.732*	-0.933♦	-0.922♦	-0.886♦	-0.894♦	0.637				
CLI	0.850♦	-0.725*	-0.706	0.845♦	-0.679	0.817*	0.704	-0.744*	-0.682	-0.825*	-0.763*	0.746*	0.773*			
H-FBI	0.671	-0.625	-0.814*	0.487	-0.944♦	0.645	0.528	-0.950♦	-0.954♦	-0.860♦	-0.963♦	0.339	0.926♦	0.672		
MCISR	-0.709*	0.810*	0.774*	-0.511	0.975♦	-0.670	-0.557	0.988♦	0.969♦	0.977♦	0.898♦	-0.579	-0.921♦	-0.802*	-0.903♦	

EC is Electrical Conductivity, FV is Flow Velocity, pH is measure of alkalinity or acidity, DO is Dissolved Oxygen, NO₃-N is Nitrate-Nitrogen, PO₄³⁻ is Phosphate, HS is Habitat Score, Chl. 'a' is Periphytic algal biomass as chlorophyll 'a', Col. is Number of coleopteran taxa, Eph. is Percent ephemeroptera, Chiro. is Percent chironomidae, Toler. is Percent tolerant organisms, CLI is Community loss index, H-FBI is Hilsehoff Family Level Biotic Index, MCISR is Macroinvertebrate Community Index of Sebeta River, ♦ for significant correlation at the 0.01 level, * for significant correlation at the 0.05 level (2-tailed).

Number of coleopteran taxa showed the strongest correlation with habitat integrity score ($r = 0.949$). This indicates that habitat integrity could be among the key factors influencing the abundance, diversity and the distribution of the coleopteran families in the study sites of Sebeta River. Healthier habitat integrity may insure adequate amount of allochthonous food sources (Ward, 1989) and better instream conditions with respect to total solids entering the river system. Total solids entering rivers may affect water clarity, temperature, interstitial space and entrapment of toxic pollutants such as heavy metals (Murphy, 2005; USGS, 2003).

Number of coleopteran taxa showed strong correlation with algal biomass ($r = 0.917$). This may be due to effect of algae (primary productivity) on the concentration of dissolved oxygen (Chapman and Kimstach, 1996). The highest dissolved oxygen concentration (5.51 ± 0.36 mg/L) was measured at SR₂ with the highest algal biomass measure (81.1 ± 10.1 µg/L).

Strong negative relation was observed between number of coleopteran taxa and electrical conductivity ($r = -0.711$). High amount of total solid entering the river may be the main reason for the elevation in conductivity. Deshu Mamo (2004) recorded a total solid of 78000 ± 2309 mg/L and 26259 ± 3240 mg/L at around SR₃ (near Bale Zaf Alcohol and Liquor Factory and soap factory) and SR₄ (next to National Alcohol and Liquor Factory and Blue Nile Tannery), respectively. This can also be associated with depletion of dissolved oxygen, low pH, poor habitat qualities and elevated temperature. These adverse modifications of the physico-chemical and environmental factors not only affect the number of coleopteran taxa, but also they could alter the entire biotic community of the river.

Among the macroinvertebrate metrics, percent tolerant organisms, community loss index and H-FBI showed strong negative correlation with the number of coleopteran taxa due to the fact that they are directly related to perturbation (Barbour *et al.*, 1999; Mandaville, 2002).

Percent ephemeroptera showed strong response to DO, pH, electrical conductivity, nitrate-nitrogen, habitat integrity score, periphytic algal biomass, the macroinvertebrate metrics (except percent chironomidae) and the MCISR at $\alpha = 0.05$. The ephemeropteran family, Baetidae, collected from the study sites is among moderately tolerant mayflies (tolerance value of 4) (Bouchard, 2004). Its responses to dissolved oxygen, habitat integrity and algal biomass

were similar to the responses of the coleopteran taxa for similar adverse effects and identical biological reasons.

The percentage ephemeroptera (mayfly) showed stronger correlation to electrical conductivity and pH than the coleopteran taxa do. This may be due to the difference in their tolerance values. The significant correlation ($r = -0.722$) of percent mayfly to nitrate-nitrogen may also reveal that Baetidae was more sensitive to nitrate pollution in the study area than coleopteran taxa do. Percent ephemeroptera also showed strong relation to the macroinvertebrate community measures except chironomidae.

Percent chironomidae showed no significant correlation with any of the parameters except CLI ($r = 0.746$). This may be due to chironomidae was not sensitive to the physico-chemical parameters considered and the habitat condition of the study sites of Sebeta River. Its response agrees with the fact that chironomidae is the most tolerant taxa among the core metrics of the MCISR. On the other hand, this family was not the most abundant even in the impacted sites. This could be as a result of biotic interactions, especially competition with Simuliidae. It may also need to measure wide range of physico-chemical parameters to identify the factor which is limiting to family chironomidae.

Percent tolerant organisms showed negative relation with environmentally positive parameters such as flow velocity, pH, dissolved oxygen, habitat integrity, algal biomass, positive metrics of the macroinvertebrate community and the MCISR at $\alpha = 0.05$. It showed strong direct relation with phosphate, CLI and H-FBI.

Community loss index (CLI) was significantly correlated with most of the parameters. Accordingly, high electrical conductivity, low flow velocity and poor habitat integrity could be the main factors for the loss of the taxa in the impacted sites which were present in the upstream reference sites. Nitrate nitrogen was also strongly correlated with CLI, but the records were within the EPA (2003) standard of 10 mg/L. Regardless of its significant correlation, temperature was also in the recommended limit of 12-25 °C except extreme values at SR₃ and SR₄.

As it was expected H-FBI showed strong negative response to DO, pH, habitat integrity, algal biomass, positive metrics and the MCISR since it is a tolerance measure. It showed strong direct relation to percent tolerant organisms.

The MCISR responses to physico-chemical parameters; DO, flow velocity, pH and electrical conductivity were strong. The relation was stronger with the former two, $r = 0.975$ and $r = 0.810$, respectively. Electrical conductivity was negatively related to the MCISR since it increases with perturbation (negative parameter). The strong response of MCISR to algal biomass could be due to the fact that primary productivity plays important role on the DO of river water. The MCISR and habitat integrity showed significant direct relation for both of them imply healthier ecosystem condition. The positive metrics (number of coleopteran taxa and percent ephemeroptera) showed significant direct relation to MCISR. Among negative metrics, only percent chironomidae failed to respond significantly to the MCISR.

Dissolved oxygen, pH and electrical conductivity were found to be the most important physico-chemical variables in this study. They were significantly correlated with most of the parameters measured for the assessment of the biological integrity of Sebeta River. Although flow velocity plays key roles in river ecosystem integrity by influencing almost everything from dissolved oxygen and temperature to concentration of chemical components, it significantly correlated to only about half of the parameters measured. Still its importance did not went out of concern for was it strongly correlated to the MCISR ($r = 0.810$).

The correlation matrix of all parameters revealed that habitat integrity and instream primary productivity (algal biomass) could be among the most important components of the biological integrity of the river. The relation between these two parameters was also strong ($r = 0.987$). Habitat integrity may be far more important than any other parameter measured in terms of its effect on macroinvertebrate community structure ($r = 0.988$) followed by DO ($r = 0.975$). Habitat conditions also affect the most important physico-chemical parameters such as DO, pH electrical conductivity and flow velocity.

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The results of this study revealed that the water qualities of Sebeta River were adversely affected and its biological systems were impaired due to various human impacts. Measures of most of the physico-chemical parameters, habitat scores, algal biomass and benthic macroinvertebrate community index all indicated severe water pollution and associated ecological impairment in the impacted sites. Some of the physico-chemical parameters such as flow velocity and nitrate-nitrogen measured within their natural background levels (0.1 m/s-1 m/s and < 5 mg/L, respectively). Even these parameters, however, measured extreme values at sites, which receive immediate industrial effluent discharges (SR₃ and SR₄). Temperature of the river showed dramatically high value at SR₃ and SR₄ (33.83 ± 1.17 °c and 27.63 ± 0.68 °c, respectively). Apart from these sites, temperature of the river was also in the natural range (12-25 °c).

Habitat scores, algal biomass and benthic macroinvertebrate community index best classified different study sites of the river. All of the impacted downstream sites received very poor for both the habitat score and benthic macroinvertebrate community index (except SR₈) indicating severe water pollution and ecological impairment of the river. Sites in the same category differ in their magnitude of the scores in accordance with their exposure and closure to discharge of pollutants. H-FBI was found to be less reliable for the assessment of biological integrity of Sebeta River. The correlation matrix of all parameters considered (Table 9) showed only DO, pH, electrical conductivity and flow velocity together with habitat integrity assessment, periphyton and macroinvertebrate community structures could be examined for monitoring in cases of resource shortage.

6.2. Recommendations

Sebeta River is found to be under high impact and is impaired. On the other hand, the river water is used for a variety of purposes such as irrigation, cattle drinking and domestic purposes without prior treatment. For sustainable management of this water resource, environmental protection agencies at different levels and other concerned administrative and/or non-governmental bodies should take strict as well as technical measures. Enforcement of law and propagating environmental education to the community with special target to those contributors of the present degradation could be one solution. Providing different advantages such as taxation, cooperativeness and market value for those industrial firms with treatment plant and good environmental management could be another option. It also necessitates avoiding establishment of additional industries near the river. Continuous monitoring using parameters such as those used in this study should be employed to assess timely status of the system.

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Plates



Plates 1 and 2: Domestic animals drinking diverted polluted water



Plate 3: cattle grazing on the riparian side



Plates 4 and 5: Polluted river water diverted for irrigation



Plate 6: Effect of polluted water on vegetable



Plate 7 (Surber sampler) and Plate 8 (Scoop net) facing against current



Plate 9: Riparian side at SR1



Plate 10: Riparian cover at SR2

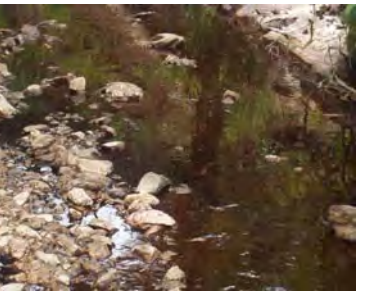


Plate 11: Effluents from **BZALF & SF**



Plate 12: Water appearance at SR3



Plate 13: SR3 in the wet months



Plate 14: Effluent from **BNT** above SR4



Plate 15: Water appearance at SR4



Plate 16: water appearance at SR5



Plate 17: Riparian vegetation at SR5



Plate 18: Dam at SR6 (dry months)



Plate 19: Dam site at SR6 (wet months)



Plate 20: Dam at SR7 (dry months)



Plate 21: Dam site at SR7 (wet months)



Plate 22: SR8 (dry months)



Plate 23: Water appearance at SR8



Plate 24: SR8 (wet months)

Photo by Admasu Tassew (Plates 7, 8, 13, 19, 21 and 24 were taken in October, 2006, the rest were taken in April, 2007),
BZALF = Bale Zaf Alcohol and Liquor Factory, SF = Soap Factory, BNT = Blue Nile Tannery

APPENDICES

Appendix 1. The altitude, coordinates and benchmark descriptions of the study sites

Site	Altitude(m)	37 P	UTM	North-East	Identifications
<i>SR1</i>	2265	0461009	0986616	38°38'43.22"E 8°55'34.12"N	Reference above Sebeta Agro- industry (Mama's)
<i>SR2</i>	2247	0460986	0986457	38°38'42.47"E 8°55'28.94"N	
<i>SR3</i>	2208	0460018	0985609	38°38'10.80"E 8°55'01.55"N	Bale Zaf Alcohol and Liquor + Soap Factory
<i>SR4</i>	2187	0459666	098500	38°37'59.30"E 8°54'41.46"N	National Alcohol + Blue Nile Tannery
<i>SR5</i>	2168	0459398	0984505	38°37'50.54"E 8°54'25.33"N	Above the wooden bridge
<i>SR6</i>	2149	0459086	0983786	38°37'40.35"E 8°54'01.90"N	Traditional Dam
<i>SR7</i>	2107	0458105	0981991	38°37'08.29"E 8°53'03.42"N	Traditional Dam
<i>SR8</i>	2086	0456986	0980923	38°36'31.68"E 8°52'28.60"N	Atebela Peasants Association

Appendix 2. Macroinvertebrates collected from each site (♦ = non-insect taxa)

Site	Taxa/Family	Common Name	Abundance	Tolerance
SR₁	Coleoptera			
	Dytiscidae (larva)	Predaceous diving beetles	8	5
	Elmidae (larva)	Riffle beetles	2	5
	Gyrinidae (adult)	Whirligig beetles	13	4
	Haliplidae	Crawling water beetles	4	7
	Hydrophilidae(larva)	Water scavenger beetles	2	5
	Psephenidae(adult)	Water pennies	1	4
	Diptera			
	Chironomidae (blood red)	Non-biting midges	9	8
	Chironomidae (pale)	Non-biting midges	51	6
	Simuliidae	Black flies	443	6
	Tipulidae	Crane flies	3	3
	Ephemeroptera			
	Baetidae	Small minnow mayflies	286	4
	Gastropoda			
	Planorbidae♦		15	7
	Physidae♦		1	7
	Hirudinea♦		5	10
	Total		843	xx
SR₂	Coleoptera			
	Dryopidae	Long-toed water beetles	1	5
	Dytiscidae	Predaceous diving beetles	5	5
	Gyrinidae (adult)	Whirligig beetles	3	7
	Haliplidae	Crawling water beetles	1	4
	Diptera			
	Chironomidae (pale)	Non-biting midges	37	6
	Simuliidae	Black flies	418	6
	Tipulidae	Crane flies	5	3
	Ephemeroptera			
	Baetidae	Small minnow mayflies	301	4
	Hirudinea♦		7	10
	Odonata			
	Aeshnidae	Darner dragonflies	1	3
Tricoptera				
Hydropsychidae	Common net-spinner caddisflies	1	4	
Total		780	xx	
SR₃	Diptera			
	Chironomidae (blood red)	Non-biting midges	35	8
	Syrphidae	Rat-tailed maggots	7	10
Total		42	xx	

SR₄	Diptera			
	Chironomidae (blood red)	Non-biting midges	5	8
	Phychodidae	Moth flies	4	10
	Simuliidae	Black flies	21	6
	Syrphidae	Rat-tailed maggots	21	10
	Total		51	xx
SR₅	Diptera			
	Chironomidae (blood red)	Non-biting midges	56	8
	Chironomidae (pale)	Non-biting midges	23	6
	Culicidae	Mosquitoes	2	8
	Syrphidae	Rat-tailed maggots	12	10
	Ephemeroptera			
	Baetidae	Small minnow mayflies	10	4
	Total		103	xx
SR₆	Coeloptera			
	Dytiscidae	Predaceous diving beetles	2	5
	Diptera			
	Chironomidae (blood red)	Non-biting midges	42	8
	Culicidae	Mosquitoes	5	8
	Simuliidae	Black flies	26	6
	Syrphidae	Rat-tailed maggots	17	10
	Ephemeroptera			
	Baetidae	Small minnow mayflies	21	4
	Total		113	xx
SR₇	Coleoptera			
	Dryopidae	Long-toed water beetles	1	5
	Diptera			
	Chironomidae (blood red)	Non-biting midges	12	8
	Simuliidae	Black flies	19	6
	Syrphidae	Rat-tailed maggots	60	10
	Ephemeroptera			
	Baetidae	Small minnow mayflies	10	4
	Total		102	xx
SR₈	Coleoptera			
	Dytiscidae	Predaceous diving beetles	1	5
	Hydrophilidae	Water scavenger beetles	1	5
	Diptera			
	Chironomidae (blood red)	Non-biting midges	17	8
	Chironomidae	Non-biting midges	2	6
	Simuliidae	Black flies	80	6
	Syrphidae	Rat-tailed maggots	114	10
	Ephemeroptera			
	Baetidae	Small minnow mayflies	7	4
	Total		222	xx
	Grand total		2256	xx

Appendix 3. Summary of H-FBI for evaluation of water quality and degree of organic pollution

Family Biotic Index	Water Quality	Degree of Organic Pollution
0.00-3.75	Excellent	Organic pollution unlikely
3.76-4.25	Very good	Possible slight organic pollution
4.26-5.00	Good	Some organic pollution probable
5.01-5.75	Fair	Fairly substantial pollution likely
5.76-6.50	Fairly poor	Substantial pollution likely
6.51-7.25	Poor	Very substantial pollution likely
7.26-10.00	Very poor	Severe organic pollution likely

Source: Hilsenhoff (1988)

Declaration

I, the undersigned, declare that this thesis is my original work and that all sources of materials used for the thesis have been correctly acknowledged.

Admasu Tassew

Signature

July, 2007