



Stressor based water quality assessment using benthic macroinvertebrates as bioindicators in streams and rivers around Sebeta, Ethiopia

Master of Science Thesis

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Abstract

The increasing impact of human activities on the freshwater bodies of Ethiopia calls for efficient and cost effective method for water quality and ecological health assessment. Benthic macroinvertebrates are important group of aquatic invertebrates to show the level of degradation of aquatic ecosystems and in this study they were used to assess the impact of different stressors originating from industries (tannery, alcohol, brewery and textile factories) and agricultural activities on streams and rivers around Sebeta. A total of 27 benthic macroinvertebrates taxa (20 families, 1 genus and 6 species) were collected from nine sampling sites in four streams, representing different anthropogenic activities. From these, Family Planariidae, Caenidae, Baetidae, Hydropsychidae, Gyrinidae, Dystiscidae, Hydrophilidae, Naucoridae and Corixidae were distributed mostly from reference site to minimally impacted upstream sampling sites and considered as indicators of minimally impacted streams and rivers. Family Syrphidae and Thiaridae were dominant in streams with high turbidity and can be an indicator of turbid streams and rivers. Family Chironomidae, Lymnaeidae and Oligochaeta were dominant in highly polluted sites (brewery and textile effluent receiving sites) and can be indicator of highly polluted streams and rivers. From lower taxonomic level of Family Chironomidae, *Chironomus alluaudi* and *Chironomus imicola* were dominant in highly polluted sites (brewery and textile effluent receiving sites), and considered as an indicator of highly polluted streams and rivers. The distribution of *Polypedilum wittei*, *Polypedilum bipustulatum* and *Dicrotendipus septemmaculatus* were high in moderately impacted sites and considered as indicators of moderately polluted streams and rivers. The genus *Conchapelopia* and *Chironomus cliptres* were mostly distributed in reference and less impacted upstream sampling sites and can be indicators of good water quality. Metrics composed of sensitive group of taxa (No. of Ephemeroptera, No. CET and %ET) were able to differentiate reference sites, agricultural impacted sites and some instream activities (washing/bathing and cattle watering site). Metrics composed of tolerant taxa like number of Oligochaeta individual and %Diptera individual distinguish highly impacting industrial stressors (tannery, beer, textile and alcohol). Margalefs index may detect toxic effect of industrial wastes in addition to organic pollution. Total number of ind/m², number of Taxa (Family), ETHbios, and FBI were able to segregate stressors originated from different sources (agriculture, washing/bathing and industries). Freshwater bodies are highly deteriorated and research should focus on waste water treatment technologies and adequate waste treatment structures must be put in place at the industries and factories located along streams and rivers around Sebeta.

Key words: Bioindicator, Benthic macroinvertebrate, Chironomidae, Sebeta, Stressor

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ACRONYMS

APHA	American Public Health Agency
ASPT-BMWP	Average Score Per taxon of Biological Monitoring Working Party
ASPT-ETHbios	Average Score Per taxon of Ethiopian Biotic Score
ASPT-SASS	Average Score Per taxon of South Africa Scoring System
BMWP	Biological Monitoring Working Party
CTE	Coleoptera, Trichoptera and Ephemeroptera
DO	Dissolved Oxygen
ET	Ephemeroptera and Trichoptera
ETHbios	Ethiopian Biotic Score
HFBI	Hilsenhoffs Family Biotic Index
Indi.	Individuals
NTU	Nephelometric Turbidity Unit
RDA	Redundancy Analysis
SASS	South Africa Scoring System
SRP	Soluble Reactive Phosphorus
TP	Total Phosphorus

1. Introduction

1.1. Background of the study

Streams and rivers are the most important freshwater ecosystems being used for a variety of life sustaining purposes. In Ethiopia, streams and rivers supply water for: domestic consumption, agriculture production, industrial purposes, generating electricity, recreation, fish production and birds of great tourism attraction as well as several other species. In recent years, however, rapid development activities and human population growth in the country have affected the water quality and ecological health of these lotic systems. Two decades ago water pollution was not reported as a problem in Ethiopia (Harrison and Hynes, 1988). However, recent studies showed that degradation of streams and rivers in urban areas is increasing at alarming rate because of rapid human population increase and associated waste production (Zinabu Gebremariam and Elias Dadebo, 1989; Getachew Beneberu, 2013). Deforestation in the upstream of rivers, erosion, sedimentation, different agricultural activities, industrial and domestic waste, diversion and water abstraction are described as the threats for Ethiopian rivers and streams (Zinabu Gebremariam and Elias Dadebo, 1989; Solomon Akalu *et al.*, 2011; Aschalew, Lakew 2012; Aschalew Lakew, 2014). These activities cause a detrimental impact on the total ecosystem ranging from deteriorating water quality to partial or total destruction of river biota. These impacts also cause adverse effects on human health through increasing water treatment cost and decreasing aquatic food production like fish (Aschalew Lakew, 2014).

In Ethiopia, river water quality monitoring totally depends on conventional method using physicochemical analysis for streams and rivers. Increasing anthropogenic pressure on water bodies initiated researchers to develop holistic water quality assessment methods for the country (Seyoum Mengistou, 2006). The use of bioassessment method of decision making for river monitoring is nonexistent in contrary to the recommendation given by many researchers to apply it in developing countries like Ethiopia (Getachew Beneberu, 2013).

In developing countries, water quality assessment using physicochemical method has many drawbacks, particularly related to limited financial and technical resources available compared to the large number of streams and rivers. In addition, the overall ecological quality of streams and rivers cannot be fully reflected through physicochemical analysis. Thus, biological assessment methods are recommended because it integrates the overall biogeochemical components of the lotic aquatic ecosystem (Harrison and Hynes, 1989; Solomon Akalu *et al.*, 2011; Getachew Beneberu, 2013; Aschalew Lakew, 2014).

Benthic macroinvertebrate based biomonitoring studies conducted in many streams and rivers of Ethiopia shows their potential use for water quality and ecological health assessment (Tesfaye Berhe, 1988; Getachew Beneberu and Seyoum Mengistou, 2010; Solomon Akalu *et al.*, 2011; Aschalew Lakew, 2012; Getachew Beneberu, 2013; Aschalew Lakew and Moog, 2015). Unlike nektons benthic macroinvertebrates tend to stay in a specific location through most of their life cycles and therefore they enable scientists to show the intensity of localized pollution and respond with respect to their degree of tolerance to different anthropogenic impacts (Getachew Beneberu, 2013). In addition benthic macroinvertebrates are easy for identification, they require cheap equipment and easy to manipulate the sample. Therefore, in this study they are used for assessing the impacts of different human activities on the water quality and habitat integrity of streams and rivers around Sebeta.

For very polluted and disturbed aquatic environments the most dominant benthic macro invertebrates are chironomids, due to their tolerance to a variety of disturbances. In addition, chironomids have representative taxa from each water quality class due to their high diversity and ubiquity. Therefore, water quality classification using chironomids at family level is difficult and it is recommended to identify these taxa to lower taxonomic level, genus or species to fully describe the water quality and ecological health of streams and rivers exposed to different anthropogenic activities (Getachew Beneberu, 2013; Aschalew Lakew, 2014). In addition, the study of the taxonomy of chironomids, their distribution and potential use for biomonitoring is limited in Ethiopia except some studies (Harrison, 1989 and Getachew Beneberu *et al.*, 2014). So, studying chironomidae taxonomy and their potential use as biomonitoring tool has paramount importance for the development of science and the identification of the levels of

degradation of streams and rivers. In the present study the family chironomidae was identified to genus level (sub-family Tanypodinae) and species level (for all the others) to assess the impact of different human activities on streams and rivers found around Sebeta town, Ethiopia.

In Ethiopia, many development activities are designed to improve the socioeconomic conditions of the society and most of which are established near water bodies for water consumption during production process and for dumping the finished wastes. Moreover, the unwise agricultural activities through the catchment of rivers and streams can be mentioned as one of the major stressors to the aquatic ecosystems through sedimentation, increasing the nutrient level from fertilizers and pesticides. The health of these water bodies are increasingly deteriorating, since there is no continuous monitoring related to the high cost incurred to physicochemical parameter and absence of bioassessment based water quality assessment policy for mitigation and control measures. Above all, most rivers and streams in Ethiopia are not sufficiently studied and there is limited knowledge on ecological health for proper management to develop a systematic overall picture of the status of these lotic environments. Thus, assessing the impact of different stressor types on the water quality of streams and rivers by using various bioassessment methods has paramount importance for scientific community, river managers and policy makers to set proper river utilization strategy.

Thus, in this study, the impact of different stressors originating from industries (tanneries, alcoholic beverage industries, and textile factories) and agricultural activities on streams and rivers around Sebeta town were assessed using benthic macroinvertebrates community structure as bioindicators.

1.2. Research questions

- What is the structure of benthic macroinvertebrates in relation to stressors in streams and rivers around Sebeta town?
- What is the overall health of streams and rivers exposed to stressors based on biotic indices and scores?
- What are the physico-chemical characteristics of rivers and streams exposed to a stressor found around town?
- What is the relationship between benthic macroinvertebrate data and physicochemical parameters?
- Which metrics and indices best reflect the impact of stressors identified in the study site?

1.3. Objective

1.3.1. General objective

To assess relationship between benthic macroinvertebrate community structures and different stressor types found in streams and rivers around Sebeta town.

1.3.2. Specific objectives

- To describe macroinvertebrate distribution in relation to stressors in streams and rivers around Sebeta town.
- To calculate indices and biotic scores from the macroinvertebrate data for determining the overall health of streams and rivers in the study site.
- To determine physicochemical characteristics and occurrence of heavy metals in streams and rivers exposed to stressors.
- To relate benthic macroinvertebrate data with physicochemical parameters.
- To identify indices and metrics that best reflects the impact of different stressors

2 Literature review

2.1 Biomonitoring

Biomonitoring, or biological monitoring, is the systematic use of the responses of living organisms to stressors to determine the overall health of the environment (Rosenberg, 1998). It is a method of observing the impact of external factors (stressors) on ecosystems health and its development over a period of time. The impact is recognized through its effect on the survival and morphology of the biota living there. Bio-indicators provide information on the impact of different anthropogenic activities on the ecological health of aquatic ecosystems that often cannot be reflected by physiochemical variables, because they integrate the biogeochemical changes within the system (Barbour *et al.*, 1999). Organisms from any level of biological organization (sub organismal, organismal, population, community, and ecosystem) can serve as bioindicators, but the historical focus was on ecosystem and higher level of organization (Li *et al.*, 2010).

2.1.1 Advantages of biomonitoring

Biological communities reflect overall ecological quality of the aquatic ecosystem and integrate the effects of different stressors, providing a broad measure of persistent impact and an ecological measurement of fluctuating environmental conditions. In addition using bioindicators for community health assessment is reliable and less expensive than assessing toxicant pollutants (Barbour *et al.*, 1999). The major advantages of biological monitoring include:

- Biological monitoring techniques are cheap particularly when compared to the cost of chemical or toxicity tests.
- Biological monitoring indicates the history of water quality over a period of time unlike physicochemical method which only provides the water chemistry at the time of sampling.
- The technique is repetitive enabling continual assessment before and after the program or after remedial work has been completed.

- Results are comparable where ever the same biological monitoring protocol system is used. It may eventually be used to assess ecological impacts of planned and actual developments and will assist in establishing a 'desired' state objective.
- Biological communities reflect overall ecological integrity (i.e., chemical, physical, and biological integrity).
- The status of biological communities is of direct interest to the public as a measure of a pollution free environment.
- Where criteria for specific ambient impacts do not exist, biological communities may be the only practical means of evaluation (Barbour *et al.*, 1999).

2.1.2 Disadvantages of biomonitoring

Although the advantages of using biological communities as bioindicators are elaborated widely elsewhere, the limitations are also indicated for proper indicator selection. These include indicator organisms cannot exactly identify the source of pollution and the effect of specific pollutant rather they show cumulative impacts. Moreover the abundance and diversity of specific biotic community may be influenced by temporal and spatial variations. Some biomonitoring techniques are also time consuming, and thus it is always important to identify and then develop those techniques that provide the greatest amount of useful information at the lowest time and cost.

2.2 Biomonitoring based on macro invertebrates

Benthic macroinvertebrates are stream-inhabiting organisms, easily viewed with the naked eye and spend at least part of their lives, in stream bottom. Since the invertebrates inhabit the stream bottom, any modification of the stream bed by pollutants, deposited sediment and water shed degradation will most likely have a profound effect upon these communities. This makes them attractive water quality study subjects, with advantages over other community members (Rosenberg and Resh, 1993).

Benthic macroinvertebrates are key components of aquatic food webs that link organic matter and nutrient resources in streams and rivers (Wallace and Webster, 1996). These organisms have mostly sedentary habits and are therefore representative of site specific ecological conditions. With the sensitive life stage and relatively long life span they have the ability to integrate the effects of short-term and long term environmental changes (Hutchinson, 1993). In addition, benthic macroinvertebrate assemblages are made up of many species among which there is a wide range of trophic levels and pollution tolerances. So it is possible to know the potential impact of developmental activities on the receiving aquatic ecosystem using these organisms.

According Bode *et al.*, (1996) some of the advantages of benthic macroinvertebrates in biomonitoring and stream ecology studies are:

- They are large enough to be seen with the unaided eye, making them relatively easy to identify and inexpensive to collect.
- They are relatively abundant; there is little danger of depleting sparse populations through sampling.
- Small order streams often do not support fish but do support extensive macroinvertebrate communities.
- As a group, macroinvertebrate communities are sensitive and respond to both natural and man-induced changes in their environment.
- Their assemblage consists of a broad range of pollution tolerances, thus they provide strong information on cumulative effect of pollution and habitat degradation. Most of the species that make up the benthic community are more-or-less confined to a specific area and exhibit little movement out of the area, thus localized degradation and pollution levels are easily detectable
- Since benthic macroinvertebrates retain (bioaccumulate) toxic substances, chemical analysis of them will allow detection where levels are undetectable in the water resource.
- Sampling of macroinvertebrates is easy, requires few people and minimal equipment, low cost and does not adversely affect on other organisms.

On the contrary, Bode *et al.* (1996) explained the disadvantages of using macroinvertebrates as bioindicators as follows.

- Benthic macroinvertebrates do not respond to all disturbances.
- Seasonal variations may prevent comparisons of samples taken in different seasons.
- Drifting may bring benthic macroinvertebrates into waters in which they would not normally occur.
- Problems with taxonomic identification in some group of macroinvertebrates.

Benthic macroinvertebrates assemblages changes in response to environmental disturbances in predictable ways. The responses are reduction in diversity, retrogression to dominance by opportunistic species. Streams and rivers affected by anthropogenic activities like organic matter and heavy metal pollution shows reduction of species richness and diversity of macroinvertebrate community and increase the dominance of tolerant taxa. Benthic macroinvertebrates, especially aquatic insects, are good indicators of various environmental stress types, such as organic pollution, heavy metals, hydro-morphological degradation, nutrient enrichment, acidification and general stressors (Barbour *et al.*, 1999; Li *et al.*, 2010).

2.3 Biomonitoring approaches based on macroinvertebrates

Different biomonitoring techniques are employed to assess the water quality and ecological integrity of river ecosystems. But, selection of appropriate approach depends on the issues being addressed and the available resources (Li *et al.*, 2010). Biomonitoring techniques developed using benthic macroinvertebrates are sabrobic approach, biotic approach, multimetric approaches and multivariate approaches.

2.3.1 Saprobic approach

The saprobic systems indicate oxygen deficits caused by biologically decomposable organic pollution in running waters, on the basis of saprobic values of indicator species. The saprobic index (SI) gives values 1-4 to represent the saprobic classifications namely oligosaprobic, β and

α mesosaprobic, and polysaprobic. The relative abundance of species was taken into account as a weighting factor for deriving the saprobic index of the site. In the mid-1970s, these indices have been rejected by most European countries for its limits (Li *et al.*, 2010).

2.3.2 Diversity approach

Many diversity indices have been developed to describe responses of a community to environment variation, combining the three components of community structure, namely: Richness (number of species present), Evenness (uniformity in the distribution of individuals among the species) and Abundance (total number of individuals present) and these can be expressed in Shannon-Wiener Index, Simpson Index and Margalef Index.

Diversity indices assume that “undisturbed environments are characterized by high diversity or richness, an even distribution of individuals among the species, and moderate to high counts of individuals” (Li *et al.*, 2010). Use of diversity-related indices in river and stream monitoring is an indicator of changes in species composition when comparing impacted and reference assemblages (Stevenson, 1984). Using diversity indices separately in assessment of river systems is not efficient and it is preferable to use it in combination with other indices e.g. Multimetric approaches is highly recommended (Gayraud *et al.*, 2003 as cited in Li *et al.*, 2010).

2.3.3 Biotic approach

Biotic approach, combines the relative abundance on the basis of certain taxonomic groups with their sensitivities or tolerances into a single index or score (Tolkamp, 1985). Species-specific pollution indications can be used to know the status of the environment because the sensitivity and tolerance of indicator assemblages to a number of stressors, like organic pollution, heavy metals, pesticides, and eutrophication are known to vary from species to species. There are many biotic indices developed using benthic macroinvertebrates. These include:

- Trent Biotic Index (TBI) and Extended Biotic Index (EBI)
- Chandler’s Score System

- Biological Monitoring Working Party Score System (BMWP) and ASPT (Average Score per Taxon)
- Hilsenhoff's Biotic Index (HBI)
- South Africa Scoring System (SASS) and
- Ethiopian biotic score (ETHbios)

2.3.4 Multimetric approaches

Multimetric indices integrate a set of variables or metrics, which represent various structural and functional attributes of an ecosystem (such as taxa richness, relative abundance, dominance, functional feeding groups, pollution tolerance, life history strategies, disease, and density). Therefore it provides robust and sensitive insights into the responses of an assemblage to natural and anthropogenic stressors. Benthic macroinvertebrates based multimetric approaches have been widely used approach for river biomonitoring in USA and Europe and recently used in other parts of the world as well. Because of its popularity, all continents and regions, except Antarctica, have used this index for bioassessment purposes (Li *et al.*, 2010).

The multimetric approach is based on reference site approach and classifies reference sites based on geographic and physical attributes. Geographic regions, termed ecoregions, are predefined largely using geomorphologic characteristics such as climate, physiography, geology, soils and vegetation (Omerick 1987). This approach assumes that the test site characteristics match the chosen ecoregion reference sites (Reynoldson *et al.* 1997). Naturally occurring biotic assemblages as components of the ecosystem would be expected to differ among ecoregions but be relatively similar within a given ecoregion. The ecoregion concept thus provides a geographic framework for efficient management of aquatic ecosystems and their components and establishes homogeneous regions within which biomonitoring is conducted and for which ecological reference conditions are derived (Ollis *et al.*, 2006).

Establishment of reference conditions is the most critical issue during development of the index of biotic integrity (Davis, 1995). Reference sites act as benchmarks against which other sites are compared to determine the degree of their impairment (Stoddard *et al.*, 2006). Completely

undisturbed sites are virtually nonexistent and even remote waters are impacted by factors such as atmospheric pollution (Roux, 1997). Getting minimally impacted sites are also very difficult due to widespread human influence and non accessibility of the site. When reference condition does not exist and need to construct Barbour *et al.* (1999) recommend two approaches:

- Use of literature and expert opinion or local knowledge to reconstruct conditions in terms of habitat and water quality conditions expected in least-disturbed sites; however, this is difficult in developing countries like Ethiopia because of lack of historical data and expert.
- Best attainable ecological health: Data is usually collected on water quality and habitat characteristics across a gradient of human influence to detect biological responses to changes in environmental conditions; “the a posteriori approach”. The reference conditions are then selected based on the best values observed.

2.3.5 Multivariate approaches

The predictive multivariate approach to bioassessment is based on the association between bioindicator communities and the environmental attributes of sampling sites (Metcalf 1989). The basis for the multivariate approach is the similarity index, with classification, ordination and discriminate analysis being the most common multivariate techniques used. Multivariate approaches have been initially introduced to assess the biological status of rivers within the UK, with the development of RIVPACS (River Invertebrate Prediction and Classification System) Wright (2000).

Multivariate approaches adopt statistical analyses to predict site-specific fauna patterns, which are expected in the absence of major environmental stress and the biological evaluations are then performed by comparing the observed fauna at the site with the expected fauna (Norris and Hawkins, 2000). RIVPACS (River Invertebrate Prediction and Classification System) uses a small number of site-specific environmental features to predict the macroinvertebrate fauna to be expected in the absence of major environmental stress. Predictions of the expected taxa can be undertaken at a species or family level, and the expected BMWP indices (BMWP Score, Number

of Taxa, ASPT) can also be predicted. Macroinvertebrate taxa collected at a site (or the biotic indices calculated), following the BMWP sampling protocol, are compared with those expected to determine the degree of impairment. RIVPACS also includes a site classification based on the macroinvertebrate fauna of the component reference sites.

In Australia, the development and use of a RIVPACS-type approach to the biomonitoring of river ecosystems has been advocated within their National River Health Programme, as part of the component based on aquatic macroinvertebrates known as the AUStralianRIVER Assessment Scheme (AusRivAS). Fundamental to AusRivAS are predictive models, based on the British RIVPACS models (Wright, 2000). In each state or territory, lead agencies have been given responsibility for developing models relevant to their region, which are used to predict the potential number of taxa and SIGNAL value at a site. The potential value of implementing a predictive multivariate system similar to RIVPACS or AusRivAS for the management of aquatic ecosystems in South Africa, with SASS as a possible tool to be used in the development of such a system, has been emphasized (Ollis *et al.*, 2010).

2.4 The Family Chironomidae as bioindicators

Numerous human activities have an impact on the quality of surface waters and consequently on the organisms living in these habitats. As any other benthic organisms chironomidae are affected by this activities and therefore, can serve as convenient biological indicators of the various environmental stresses on these ecosystems (De Pauw & Hawkes, 1993). The effects of pollution on the structure of chironomids are discussed by many authors and it has been found that chironomids have wide range of tolerance to specific sources of pollution (Fitter and Manuel, 1986). For example Williams & Feltmate (1992) reported that chironominae and some tanypodinae are very tolerant to low levels of dissolved oxygen, *Chironomus plumosus* larvae can survive in a pH value of 2.3 while *Cricotopus bicinctus* is known for its tolerance for electroplating wastes and crude oil. Other members of the family are very sensitive for poor water quality and only exist in relatively good water quality. In addition, chironomids are potential indicators for physical habitat disturbance and heavy metal contamination through mouth part deformities (Martinez *et al.*, 2002).

From the macroinvertebrates collected in fresh water ecosystems, the family chironomidae constitute almost 50% of the population and it is difficult to classify water quality of streams and rivers based on this taxa distribution (Armitage. *et al.*, 1983). This is also elaborated in Getachew Beneberu and Seyoum Mengistou (2010) work, that almost equal abundances of chironomid larvae have been found in the relatively unpolluted Chacha River and the moderately polluted Tikur Wuha River. Therefore, separate analysis of these taxa is mandatory to fully describe the water quality and ecological health of streams and rivers exposed to different anthropogenic activities.

Chironomids are a keystone group (Jones and Grey, 2004) that plays a key role in the cycling of nutrients in freshwater ecosystem and form a vital link between primary producers and secondary consumers (Porinchi and Macdonald, 2003). Chironomids (Insecta: Diptera; non-biting midges) are key macroinvertebrates in indicating the level of perturbations in fresh water bodies of the world. However their utilization in tropics is limited, because of incomplete inventory of their taxonomy, ecology of local species, and scarcity of detailed descriptions of the aquatic larvae (Verschuren and Eggermont, 2006, Getachew Beneberu, *et al.*, 2014).

There are about 15,000 species of chironomidae, which possess different degree of tolerance to organic pollution, acid mine drainage, heavy metal contamination. The macroinvertebrate survey in Ethiopian rivers and streams showed that, chironomids are prevalent in water bodies of different trophic status and differently respond to many antropogenic activities that could potentially affect the health of a water body (Getachew Beneberu *et al.*, 2014). Most diagnostic features of chironomidae larva are found on the ventral part of scleretized head capsule. In fact, the diagnostic structures differ from taxa to taxa. For example, in sub family chironominae, the number and shape of the inner, apical and dorsal teeth, the presence or absence of a seta interna, the morphology of the seta subdentalis, the pecten mandibularis, and antennae ratio are importance structures for identification. Where as members of the subfamily Tanypodinae differ from all other subfamilies in having retractile antennae and numerous other uniquely modified structures such as the ligula, paraligula and the M appendage (Epler, 2001).

2.5 Major stressors of streams and rivers

The impact of humans on water resources takes different forms. It includes physical alteration and pollution from industries and residential areas. Also, it includes changes in riparian vegetation and stream morphology, sedimentation, nutrient additions, organic enrichment and pesticide contamination from agricultural land uses (Chu and Karr, 2001 and Whiles *et al.*, 2000). In Ethiopia land degradation, urban sanitation, industrial and chemical pollution are the major environmental problems (Zinabu Gebremariam and Zerihun Desta, 2002) that cause adverse impact on aquatic resources of the country.

2.5.1 Industry

It is estimated that industry is responsible for dumping 300-400 million tons of heavy metals, solvents, toxic sludge, and other waste into waters each year worldwide (UNEP,1991). Industrial effluent can alter the physical, chemical and biological nature of the receiving water body leading to deterioration in water quality and quantity that causes adverse impact on the water chemistry and biological elements (Carr and Neary, 2008).

Even though, Ethiopia has few industries and few developed urban areas, water bodies near cities such as Addis Ababa, have shown severe pollution problem (Baye Sitotaw, 2006) and the same problem face in Sebeta town, which is recognized as one of the industrial zone of the country. The effects of industrial activities on aquatic environment are becoming evident through the pollution of water bodies and human habitat in the major cities of the country and its rivers and lakes (Seyoum Leta *et al.*, 2003).

For example, the tanning industry impacted the environment by the discharge of high volumes of wastewater in the process of converting a putrescible animal by-product in to a stabilized and marketable material (UNIDO, 1991). They dispose their wastewater in to aquatic environment which result in the accumulation of pollutants. The low pH of tannery effluents causes corrosion of the water-carrying system and can lead to metal dissolving in the water that adversely affect

aquatic life and impair recreational use of water. The high pH water can also cause sealing in the sewers. Large fluctuation in the pH value is detrimental to some aquatic species. In addition, tannery waste water causes depletion of oxygen which is fatal to aquatic life (Khan *et al.*, 1999). High amount of nitrogen in tannery effluent causes proliferation of water weeds and algae, which in turn, leads to various water purification and health problems and eutrophication which adversely affect the aquatic biota. Nitrogen in the ammonia form is toxic to certain aquatic organisms, the sulfide content of effluent causes the creation of hydrogen sulfide which creates unpleasant smells, and cause toxicity too for many forms of life. Suspended materials discharged in the wastewater of tannery forms a layer on the bottom of water course and covers natural fauna, which causes depletion of oxygen, reduces light penetration and thus photosynthesis in the water. High amounts of dissolved salts increase the salinity of the receiving water bodies which result in adverse ecological effects on aquatic biota (Lefebvre and Moletta, 2006).

The textile industries are one of the largest water users and polluters industries which adverse environmental problems. They have the potential to affect water transparency and gas solubility, (Banat *et al.*, 1996). Dyes contributed to overall toxicity at all process stages and they constitute a small fraction of total liquid effluent, but may contribute a high proportion of total contaminants (Yusuff and Sonibare, 2004). Textile industries also release heavy metals, which is carcinogenic to the resident biota and the metals of most immediate concern are chromium, zinc, iron, mercury and lead which tends to bioaccumulate in organisms and cause endocrine disruptions in aquatic fauna (Masud *et al.*, 2001).

Brewery and alcohol effluent causes oxygen depletion, increase in plant and animal biomass, reduction of the amount of light available for aquatic vegetation, decrease in species diversity and favors the dominance of tolerant biota. Microorganisms gradually break down the organic component of wastewater by consuming the available oxygen and make the environment anoxic and there is proliferation of disease causing microorganisms which will pollute rivers, lakes, streams and deep-water aquifers (Ekhaise and Anyansi, 2005).

2.5.2 Agriculture

Agriculture is one of the major human activities responsible for nonpoint-source of pollution in streams and rivers of Ethiopia (Aschalew Lakew, and Moog, 2015). Poor agricultural practices around rivers and streams can lead to soil erosion and subsequent runoff of fine sediments, nutrients and pesticides (Lowrance *et al.*, 1984). Studies showed that fine sediment accumulation affect macroinvertebrate assemblages by affecting substrate composition and by favoring only for the tolerant taxa. Suspended sediments accumulation have an impact on stream fauna by interference with filter feeding mechanisms or reducing visual feeding efficiency and by reducing light levels to the point of triggering drift behavior (Waters, 1995). In addition streams and rivers in Ethiopia serve for cattle watering site and their banks for grazing area due to all year availability of green grasses.

2.5.3 Domestic waste

Domestic sewage contains a wide variety of dissolved and suspended impurities such as organic materials and plant nutrients. The main materials of domestic waste are food and vegetable wastes, plant nutrients come from chemical soaps, washing powders, etc. Domestic sewage is also very likely to contain disease-causing microbes. Most detergents and washing powders that we use to clean our houses and other utensils contain phosphates and other toxic chemicals that affect the health of all forms of life in the water. Domestic waste contained water causes eutrophication, which is the increase in concentration of nutrients. The nitrates, phosphates, and organic matter found in human waste and other organic source serve as a food for algae and bacteria. This causes these organisms to overpopulate to the point where they use up most of the dissolved oxygen and makes the environment anoxic and difficult to survive. Some of the organisms that do overpopulate from this can also be disease-causing microorganisms (planetary Notions, 2002).

2.6 The trend of biomonitoring in Ethiopia

The history of biomonitoring started during Aristotle that he observed the reaction of fresh water fish to sea water and the first toxicity experiment was on survival of fresh water molluscs in different salt concentration (Mandaville, 2002). Use of fresh water community structure for assessing human impact started by Kolkwitz and Marsson (1902) by their publication of saprobity that lead to the development of indicator organism concept. A variety of indicators are used for bioassessment but, benthic macroinvertebrates are the most popular. Indices developed by benthic macroinvertebrates are from simple diversity measurements to multivariate mathematical models. The trend of biomonitoring shows towards more rapid bioassessment techniques, using semi-quantitative collecting methods through sub-sampling process (Mandaville, 2002). Today biomonitoring are conducted at a molecular level in which the level of pollution and change in community is observed through genetic diversity. It has been explained that finer taxonomic resolution gives better picture of the ecosystem health Li *et al.* (2010). All these bioassessment techniques from saprobic approach to molecular techniques have been developed and used in USA and European countries to see the adverse impact of different human activities on aquatic environment. Also many of these countries incorporate it as their legal and policy frame work for aquatic ecosystem monitoring.

In Ethiopia, studies have been conducted on faunal diversity of macroinvertebrates and their potential use for biomonitoring. For example, Harrison and Hynes (1988) studied the benthic fauna of highland streams of Ethiopia and tried to establish reference taxa composition found in least impacted streams and rivers. They also pinpoint that population increase cause soil erosion and this in turn leads to the elimination of benthic fauna. Human activities such as washing/bathing using synthetic detergents replacing traditional "cake of soap on the rocks" techniques of earlier times are also the cause for elimination of Gerridae and Veliidae. The extinction of crabs in the rift valley which were present in 1940's is related to the impact of intense agricultural activities. In that time, the level of industrial and domestic pollution was not as such apparent in Ethiopia except some tributaries of Akaki (particularly Abo-Kebena) in Addis Ababa (Harrison and Hynes, 1988), unlike today that all rivers that pass through towns are

extremely polluted. Therefore industrial and domestic waste was not mentioned as a problem for the destruction of rivers and streams of Ethiopia before two decades.

Baye sitotaw (2006) assess the ecological status of streams and rivers of Modjo, Kebena, Akaki, Chacha, Megecha, Wabe, Ghibe, Dabena and Sor, using structures of benthic macroinvertebrates and found that their community structure can able to show the intensity of human impact in streams and rivers of Ethiopia. The degradation level and the relationship between physicochemical parameters and biological assessment of the Kebena River are also stated by Tesfaye Berhe (1988) and Worku Legesse *et al.* (2004).

Other studies in different rivers, streams and wetlands of Ethiopia showed that benthic macroinvertebrates structures and composition are effective tools to assess the degradation of the countries water bodies (Hayal Desta and Seyoum Mengistou, 2009; Aschlew Lakew, 2012; Getachew Beneberu, 2013; Assefa Wosnie and Ayalew Wondie, 2014). In any case the effectiveness of biomonitoring in assessing the impact of different human activities has been assured by the above listed researchers even if all the method and protocols used are developed in temperate region and prone to error due to geographical difference.

Recently in Ethiopia, macroinvertebrate based biotic score system (ETHbios) was developed by Aschalew Lakew and Moog (2015) for assessing the ecological status of rivers in the Ethiopian highlands. It is developed on the principle of the BMWP approach (version of the South African Scoring System) but excludes taxa that don't occur in Ethiopia and includes some of Ethiopian fauna. It defines river quality classes as high, good, moderate, poor and bad. ETHbios which is rapid, inexpensive and scientifically sound monitoring method is believed to evaluate the ecological conditions of streams and rivers in the highland parts of Ethiopia.

2.6.1 Sampling tools and protocol

Most biomonitoring studies in Ethiopia use Rapid bioassessment protocol of Barbour *et al.* (1999) and use 80m-200m reach length which usually is assumed to be representative for stream or river under study. Most of these researchers consider pools and riffles in the reach and sample either the riffle or both. Others consider substrate type in addition to pool and riffle by giving percentage proportion for each. For example Aschalew lakew, (2014) considers habitat and hydrologic characteristics like substrate type (Megalithal, Macrolithal, mesolithal, microlithal, Akal, Psamal), width, depth and flow velocity of a river as important elements for structuring of benthic community. The most frequent sampling protocols used are quantitative, semi quantitative and qualitative. Different sampling tools (surber sampler, D-frame net, scoop net and square net) were used either in the same or different ecoregion for assessing the ecological health of water bodies. For example Solomon Akalu *et al.*, (2011) collect benthic macroinvertebrate samples from Greater Akaki from pools (using scoop net) and riffles (using surber sampler) and the collected biota from each biotype was pooled.

The use of different sampling tools and protocols may lead to produce low quality data that is difficult for comparison of the results, even within the same ecoregion. The application of these different sampling tools and protocols might produce errors and uncertainty to data collected in the same climatic ecoregions or biotopes (Clarke and Hering (2006) underscoring the question of accuracy in bio-monitoring programs. The use of standardized protocol together with available taxonomic knowledge is more recommended as they can easily govern the differentiation of site conditions or macro-invertebrate composition in the same biotope (Julius *et al.*, 2014).

2.6.2 Indices and taxonomic resolution

Almost all biomonitoring studies in Ethiopia use metrics and indices of benthic invertebrates developed in temperate region. In regard to metrics there may not be a problem on the results found as it is based on the proportion of collected taxa. But, use of tolerance/intolerance indices and index of biotic integrity ranges developed elsewhere in temperate region can have a problem as they are affected by geology and hydrology the study site.

Most of biomonitoring studies in Ethiopia use family level identification of the benthic macroinvertebrates (Baye Sitotaw, 2006; Solomon Akalu *et al.*, 2011; Assefa wosnie and Ayalew Wondie, 2014). Aschalew Lakew and Moog (2015) identified some of the macroinvertebrate taxa up to sub-family and genes level and most of these taxa get award of tolerance value based on Ethbios score. Getachew Beneberu *et al.* (2014) use one particular group of benthic macroinvertebrates (chironomids) to know the level of streams and river degradation in Ethiopia. He identified the taxa to gunes and species level and able to see the ecological preference of each taxa and their potential use for discriminating the degree of pollution between moderately polluted and heavily polluted sites.

The taxonomic resolution is based on cost benefit analysis that the cost saved in identifying macro-invertebrates to develop family level indices may not be justified if precision cannot be met by such indices at family level. Likewise, the cost expended to obtain species level indices may also not be warranted if cheaper family level indices can evaluate accurately the status of aquatic ecosystems. But, from large set of data and developed indices it has been described that lower taxonomic resolutions bet reflect the intensity of degradation and overall ecological health of aquatic ecosystem and it has been recommended for the sake of data accuracy and precision, index developed in tropical region shall be in lower taxonomic level resolution (Julius *et al.*, 2014).

Indices developed in the temperate region should be calibrated before use in Ethiopia. This is due to the fact that some macro-invertebrate species might occur abundantly in temperate region but not in tropical or Ethiopia climatic regions and vice versa. Even if the organisms in the temperate region occur in Ethiopia, due attention should be given as it may differ in diversity. This is emphasized by Julius *et al.* (2014), that for example Plecoptera is low in diversity in the tropics but high diversity in temperate and Mediterranean regions. In this regards, locally available freshwater organisms of high diversity should be considered in developing bio-monitoring index to represent that particular region.

2.6.3 Problems of biomonitoring in Ethiopia

The major problems for development of biomonitoring in Ethiopia share the problems of Africa. Problems are mainly related to logistics, financial and technical issues. The study of benthic fauna in Ethiopia started some 80 years ago by expatriates that come for colonization (Seyoum Mengistou, 2006). Even if the time started seems to long only some group of nematodes were studied and this is the main reason that most researchers in Ethiopia use benthic macroinvertebrate keys which are developed for temperate regions (Getachew Beneberu , 2013). Even if, Ethiopia is recognized as a classical example for its contrasting landscape and biodiversity, taxonomic inventory in its river biota are almost non-existent. These lack of knowledge hider the use of biota as indicator of water quality deterioration and habitat degradation (Baye Sitotaw, 2006). Therefore, poor taxonomic inventory of benthic macroinvertebrates were the main problem for their use as bioindicators.

In Ethiopia there is no biomonitoring supportive decision making process and there is no state support research. Therefore, there is problem of budget for the appraisal of bioassessment research. In addition, infrastructures for accessing the water body and equipments for sampling and processing can be mentioned as the bottlenecks for the development of biomonitoring method for water quality assessment in the country. The presence of technical, financial and logistical constraints have hindered the potential use of macro-invertebrate communities as indicators of water quality and thus, making bio-monitoring programs a remote possibility in tropical African region (Julius *et al.*, 2014). The other major challenge of biological monitoring in Ethiopia is absence of properly defined and selected reference sites. Baro basin which harbors high macroinvertebrate diversity was suggested as potential reference site for future biomonitoring studies (Baye Sitotaw, 2006).

2.6.4 Proposed solutions

- **Biomonitoring trainings:** As biomonitoring is a promising tool towards reflecting the intensity of anthropogenic activities on water bodies. In developing countries like Ethiopia training of professionals in biological water quality management may have paramount importance as it is cheap and easy.
- **Taxonomic inventory and development of identification key:** For a biomonitoring to be in use, full taxonomic inventory is mandatory. Taxonomic inventory will help to know the aquatic resource that we have and make biomonitoring easy.
- **State support:** Since biomonitoring is cheap, easy and robust method of water quality assessment, a state should incorporate this method as legal policy framework. For this to be in practice, intellectuals in the area should provide compiled material showing the cost benefit analysis of using biomonitoring method for water quality assessment.
- **Extrapolation of ETHbios:** As ETHbios is produced for monitoring highland streams and rivers of Ethiopia updating it to use for other parts of the country is important like South Africa Scoring System (SASS1, SASS2, SASS3, SASS4, and SASS5).
- **Adoption of indices with great care:** Before using indices developed elsewhere to Ethiopia calibration with great care is important as it is affected by regional geology and climate characteristics. Even if the same organisms are present, diversity will matter and adoption should take this into consideration. Such variation in both diversity and abundance of macroinvertebrates might affect the capability, functioning, and reliability of the existing non-tropical biomonitoring indices when applied in Ethiopian water bodies.
- **Development of ecoregion based indices:** as macroinvertebrate distribution is affected by ecoregion characteristics there is a need to develop and validate indices that will be more reliable in a specific ecoregion than adopting indices from other geographical areas which are inconsistent with regard to tools, research methods, taxonomic resolution and organisms involved.
- **Standardization of methods and reference conditions:** equipments starting from field sampling to laboratory processing should follow standard operating procedures and

reference conditions should be certified and accredited by a recognized governing body or ISO.

In the present study we try to identify the impact of specific stressors on the receiving streams and rivers using the community structure and assemblage of macroinvertebrates. Chironomids were identified to gene/species level that will alleviate some taxonomic problems and making biomonitoring more precise.

3 Materials and Methods

3.1 Description of the study area

This study was conducted on rivers and streams around Sebeta town, 25 km southwest of Addis Ababa. The altitude in the study area ranges from 2124 to 2277 m asl. Streams and rivers of the area are used for a variety of activities. Agricultural activities (e.g. crop cultivation and cattle farming) are some of the major stressors which cause pollution of the river through release of agrochemicals, organic wastes and sediments. In addition, streams are used for intensive irrigation in the dry season mainly for growing a stimulant locally known as 'Chat' or 'Khat' (*Catha edulis*) and vegetable production. Streams in study area also serve as various domestic activities (washing/bathing, drinking, raw leather moistening, dumping of domestic wastes) and industrial purposes like water usage in the production process and dumping of the finished wastes.

Most area of these streams and rivers are devoid of riparian vegetation, due to the intense human activities taking place in the area. In the stream and river banks studied only a few remnant plants like *Acacia sp.*, *Eucalyptus*, *Syzygium guineense*, shrubs and some grasses are observed.

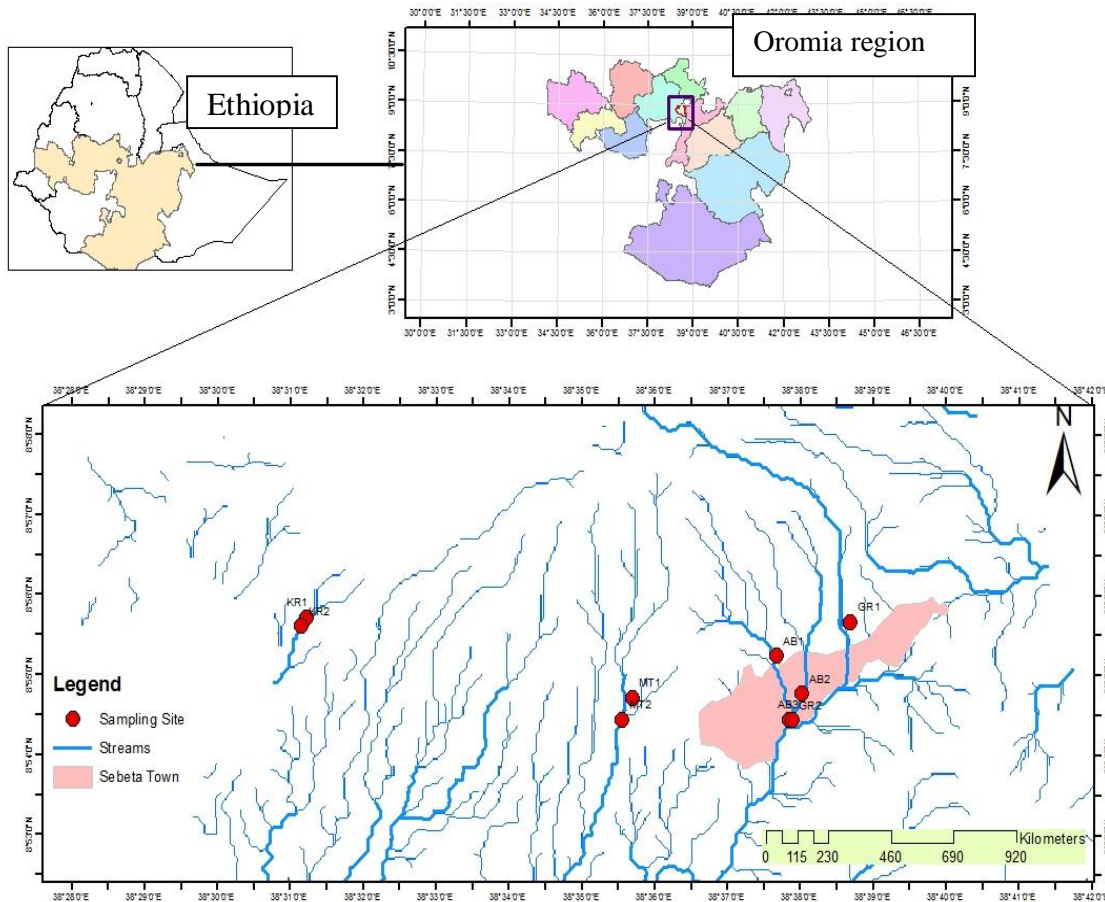


Figure 3. 1. Location map of the study area (obtained from satellite image)

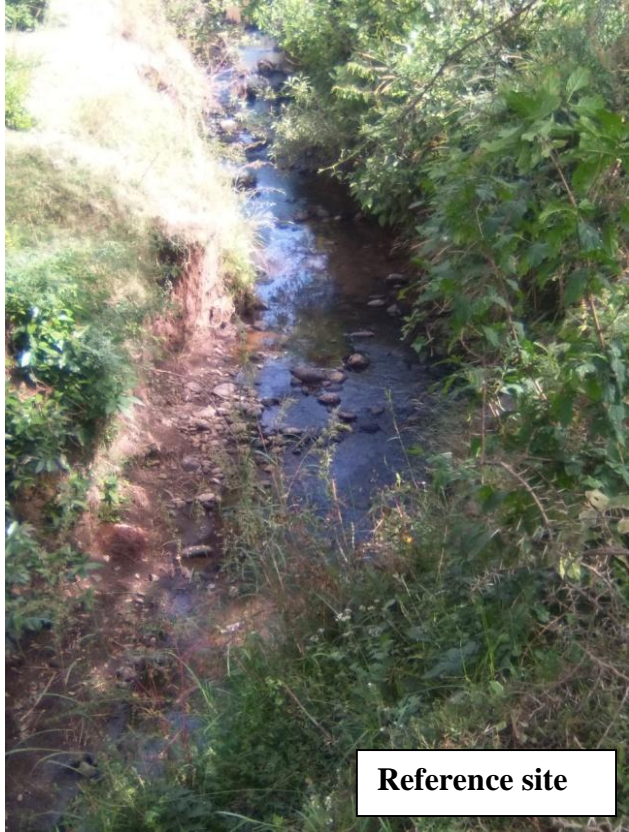
3.2 Sampling site description

In total nine sampling sites from four streams representing different anthropogenic activities (stressors) were selected. Site KR1 and KR2 are found in rural part of Sebeta, in Kersa stream, which originate from the Suba Forest. KR1 is relatively less disturbed, characterized by better riparian vegetation cover, utilized for drinking and considered as reference site. KR2 was utilized for cattle watering, washing and bathing purpose. Sites, MT1 and MT2 are found in Meta Stream which receives effluents from Meta Abo Brewery. MT1 is immediately above the effluent discharge of Meta Abo Beer Factory, and it is utilized for cattle watering and washing while MT2 is situated some 100m below the effluent discharge of Meta Brewery.

Sites AB1, AB2, and AB3 are found along the gradient of Abeyi Stream. AB1 is upstream of Abeyi stream with no impact from factory effluents while AB2 and AB3 are exposed to effluents of alcohol factories (National Alcohol, Balezaf Alcohol & Liquor Factories) and Tannery (Jafar Leather Industry), respectively. Sites GR1 and GR2 are found in Gerado Stream. GR1 is the upstream parts in high gorge and utilized for washing/bathing purpose while GR2 is located below the effluent discharge of Ayika Textile Factory.

Table 3. 1. Study sites with geographic location and major stressors description

River/stream	Site code	Altitude (m)	Latitude(E)	Longitude(N)	Major features and stressors
Kersa	KR1	2124	08.92867	038.52033	Well developed riparian vegetation, limited in stream activities and farming in the catchment. Used for household activities including drinking
	KR2	2124	08.92678	038.51897	Mainly affected by agricultural activities. in addition used for Cattle watering, washing and bathing activities and water abstraction. Remnant riparian vegetation cover.
Meta-Abo	MT1	2176	08.91195	038.59489	Well developed riparian vegetation cattle watering and washing/ bathing site
	MT2	2153	08.90740	038.59255	Meta beer waste effluent receiving site
Abeyi	AB1	2234	08.92068	038.62773	Limited riparian vegetation, cattle watering and domestic washing site
	AB2	2198	08.91286	038.63369	Limited riparian vegetation, receive effluent from Alcohol factories (National Alcohol factory and Balezaf Alcohol and Liquer Factory)
	AB3	2170	08.90747	038.63081	Below effluent discharge from Jafar Tannery effluent
Gerado	GR1	2277	08.92776	038.64478	Limited riparian vegetation but intensive domestic cloth washing and bathing
	GR2	2169	8.083455	38.616912	Below effluent from Ayika Textile factory confluence



Reference site



Cattle watering site



Textile effluent receiving site



Cloth washing site



Plate 1. Photographic image of some sampling sites

3.3 Data collection

3.3.1 Field Data collection and laboratory analysis

3.3.1.1 Sampling period

The frequency and time of benthic invertebrate sampling depends on the objective of the study and climate conditions. For instance variability of discharge volume of rivers caused by seasonal changes affect the distribution pattern of benthic macroinvertebrate and limit accessibility to sampling sites. If the purpose of the study is to assess the impact of anthropogenic activities, it is usually recommended to avoid sampling during high water levels and shortly after flooding. In this sense sampling was conducted in the dry season from November 2016 to January 2017 based on the recommendation for Ethiopian highland streams and rivers (Aschalew Lakew, 2014).

3.3.1.2 Environmental variables

Environmental variables (physical, chemical and hydro-morphological parameters) have relationship with the diversity and abundance of indicator organisms (BMI) and were measured during the sampling period. Latitude, longitude and altitude were determined with the help of Global Positioning System (GPS) instrument. Substrate composition of each site was visually estimated in accordance with particle size: psammal (<0.2 cm), akal (0.2–2 cm), microlithal (2–6 cm), mesolithal (6–20 cm), macrolithal (20–40 cm), megalithal (>40 cm). Water quality parameters including temperature, pH, dissolved oxygen, conductivity and turbidity were measured *in situ* using a portable WTW multi-parameter probe before sampling the macroinvertebrates.



Plate 2. Onsite measurement of physicochemical parameters

For nutrient analysis of NH_4^+ , NO_3^- , SRP, and TP, two liters of water were collected from each site and stored in ice box, and transported to the National Fishery and Aquatic Life Research

center (NFALRC, Sebeta) and Addis Ababa University, limnology laboratory for analysis. Chemical nutrients were analyzed using standard procedures as outlined in APHA (1995). For all nutrient variables except total phosphorus (TP), 300 ml water samples were first filtered through glass fiber filters (GF/F) and then analyzed for nitrate (NO_3^-), ammonium (NH_4^+) and soluble reactive phosphorus (SRP). For Total phosphorus (TP) a separate 50 ml unfiltered sample was used. For heavy metal analysis one liter water sample was taken from three sampling sites using polyethylene bottle. The sampling was performed to determine the specific effect of the tanning industry and textile factory on water quality of receiving streams. Water samples from those sampling sites were analyzed for the presence of heavy metals (Chromium, Lead, Zinc, Nickel, Cadmium and Copper). For this, a 100-ml water sample acidified with nitric acid was filtered through glass-fiber filters and the filtrate was analyzed using Analyticjena ZEE nit700P at Addis Ababa University, Department of Chemistry.

3.3.2 Benthic macroinvertebrate

3.3.2.1 Field sampling

Benthic macroinvertebrate samples were collected using square frame hand net with frame width of 25*25 cm and mesh size of 500 μm . During sampling multi habitat sampling scheme (MHS) was implemented to include major habitat types in proportional representation within 100 m sampling reach following Moog (2007). From each site 20 sampling units were sampled with at least 5% share of each major habitat types. Sampling unit is a sample collected within the surface area of a net by disturbing the substrate. Sampling of benthic macroinvertebrates starts at the downstream of the reach against water current to avoid disturbance of the upstream sampling units. Sampling unit collection depends on the substrate type: for macrolithal and mesolithal substrates complete disturbance and washing was performed while megalithal substrates were sampled by brushing the surfaces complimentary to the net size. Sediments were disturbed to an adequate depth that capable of capturing the macroinvertebrate sample.



Plate 3: Field sampling of benthic macroinvertebrates

Samples collected from each sampling units were pooled in to one container. For the sake of inventory rapid on site taxa identification was performed. The composite samples were preserved in 4% formaldehyde (final concentration) and all mandatory information (stream name, sampling site code, collectors name and date of collection) was labeled using water proof marker.

3.3.3 Laboratory analysis

The macroinvertebrate samples collected and preserved in the field was subjected to process in the laboratory for further analysis. Before processing, the information in the sample container was copied to data sheet. A composite macroinvertebrate sample was allowed to pass through a set of sieves (500, 300 and 150 μm mesh size) to separate size class of macroinvertebrates taxa and wash the preservative through tap water. By placing the sample in white tray, sorting was

performed through naked eye. Organisms trapped in the smaller fraction of the sieve were sorted with help of light microscope. In some sites where the density of some taxa was very high (e.g. Simuliidae, Chironomidae, Baetidea and Caenidae), sub sampling was applied according to Barbour *et al.* (1999). Identifications were performed using Aquatic Invertebrates of South African Rivers (2002) field guide.



Plate 4. Benthic macroinvertebrate sample processing and sorting

In the laboratory, like other macroinvertebrates chironomids were properly sorted, counted and preserved in 75% ethanol. For further analysis Chironomids were picked from the preservative and put and heated in 10% KOH solution for 5 minutes using hot plate. This is to make their body transparent and to clearly see the diagnostic structures. After heating, the samples were washed by distilled water, dehydrated and mounted by glycerin by inverting upside down. The cover slips were sealed with nail polish. This is to fix the cover slip to slide and to make it permanent for validation, future education and research. The most diagnostic feature used for identification was the head capsule. The muntum was used for identification of sub family Chironomus while Ligulia and cephalic setation was used for identification of sub family Tanypodinae. Identification was made under compound microscope with the help of identification keys (Epler, 2001); Eggermont and Verschuren, 2003a 2004a; Getachew

Beneberu, 2013) and chironomid expert (Dr. Getachew Beneberu). Images of representative specimens were taken with digital camera (YWcamera 1.4.3).



Plate 5. Lower taxonomic level Chironomidae identification

3.4 Statistical analyses

The distribution of benthic macroinvertebrate taxa in relation to the sampled environmental variables was analyzed using Detrended Correspondence Analysis (DCA) followed by Redundancy Analysis (RDA). Before multivariate analysis the environmental variables were log transformed and the macroinvertebrate data was square root transformed. Spearman's correlation was used to examine the relationship between macroinvertebrate metrics and measured environmental variables. Based on the Spearman correlation coefficients, the redundant physicochemical parameter were removed for further analysis in RDA. For example, dissolved oxygen concentration and oxygen saturation values were strongly correlated, soluble reactive phosphorus and total phosphorus also highly correlated thus only dissolved oxygen concentration and total phosphorous were retained for analysis in RDA. Microsoft excel were used to calculate the metrics. Mean and significance tests were analyzed using SPSS version 20. All statistical analyses were carried out in the statistical software packages Canoco version 4.5, SPSS version, 20 and PAST and Excel.

4. Results

4.1. Environmental parameters

Most of the physicochemical parameters measured in the field and laboratory showed significance difference between sampling sites (Table 4.1). There was no significant difference ($p>0.05$) in temperature between sampling sites. But, the temperature in reference and agricultural impacted sites was low compared to other sampling sites. The distribution of pH was nearly similar among sampling sites with a range of 7.30-8.26. There was a significant difference ($p<0.05$) in oxygen concentration (saturation) between sampling sites. The highest record was at agricultural impacted and reference sites (7.76mg/l, 7.62mg/l respectively) while lower record was at sites receiving effluents of industrial wastes particularly tannery (AB3) and alcohol (AB2) with a reading average of 1.54mg/l and 2.77mg/l respectively. There was a significant difference ($p<0.05$) in conductivity with highest recorded at Meta Brewery (MT2) and Ayika Textile (GR2) factory effluent receiving sites with a record of 1848.67 μ S/cm and 2518.00 μ S/cm respectively. Otherwise there were almost similar conductivity readings for the other sampling sites.

There was no significant difference ($p>0.05$) in turbidity and NO_3^- concentration between sampling sites. But, there was high record of turbidity at sites receiving alcohol (AB2) and textile (GR2) effluents with a reading of 107.6 NTU and 50.8 NTU respectively. The concentration of NH_4^+ were significantly different ($p<0.05$) between sampling sites with high record at AB3 (1.58mg/l) and GR2 (4.6mg/l). The concentration of SRP and TP were also significantly different ($p<0.05$) along sampling sites with a high record at industrial effluent receiving sites particularly at MT2 (29.13mg/l of SRP and 35.14mg/l of TP) and GR2 (27.68mg/l of SRP and 29.9 mg/l of TP).

Table 4. 1 Mean value of physicochemical parameters measured in the sampling sites

Physicochemical Parameters	Sampling sites								
	KR1	KR2	MT1	MT2	AB1	AB2	AB3	GR1	GR2
Temp (°c).	13.23±3.89	13.57±4.15	17.83±4.48	17.23±5.96	20.83±6.93	22.37±0.90	20.57±0.42	20.70±1.11	21.57±2.38
pH	7.67±1.15	7.30±1.03	7.56±1.06	8.00±1.44	7.72±1.29	8.26±1.66	7.59±1.11	7.68±1.17	7.78±1.31
DO (mg/l)	7.62±0.16	7.86±0.89	5.95±0.31	5.08±1.34	6.49±0.73	2.77±2.26	1.54±0.50	5.01±0.90	4.59±0.92
DO (%)	91.33±5.85	98.13±18.43	78.90±7.21	65.97±14.21	93.37±2.97	41.43±33.76	22.37±7.13	74.80±9.55	67.93±13.07
Conductivity (µS/cm)	155.17±132.42	207.07±201.59	152.47±136.96	1848.67±1750.98	142.93±130.41	570.17±427.38	548.57±447.47	117.23±94.11	2518.00±2043.42
Turbidity (NTU)	20.2±15.37	14.5±0.57	15.0±7.73	12.9±5.09	21.6±2.54	107.6±48.65	29.075 ±14.88	32.1±9.44	50.8±40.62
NH₄⁺(mg/l)	0.10±0.03	0.11±0.03	0.09±0.06	1.39±0.97	0.14±0.13	0.30±0.26	1.58±0.96	0.11±0.03	4.63±3.57
NO₃⁻(mg/l)	0.15±0.00	0.18±0.04	0.14±0.00	0.28±0.01	0.33±0.04	0.15±0.01	0.15±0.00	0.14±0.01	0.16±0.00
SRP (mg/l)	0.69±0.55	0.46±0.32	1.50±1.36	29.13±11.05	0.58±0.47	4.02±3.64	4.15±3.48	0.45±0.43	27.68±7.81
TP (mg/l)	3.69±3.58	5.13±3.93	5.77±5.15	35.14±8.06	4.10±1.86	11.16±6.25	12.22±2.48	4.34±2.20	29.29±9.58

Human activities degrade the aquatic ecosystems through various activities like water diversion, abstraction and dumping of household and industrial wastes which affects the community structure and assemblage of benthic macroinvertebrates. In this study there was a significant difference ($p < 0.01$) in bed visibility among sampling sites, with totally visible at agricultural impacted and reference sites (100%) and slightly or totally invisible at industrial impacted sites (0%). There was no significant difference ($p > 0.05$) in substrate composition between sampling sites. More than 50% of the substrate were composed of mesolithal but, the contribution of megalithal was high at AB2 (80%) and low in KR1 (5%). Other substrates have almost equal contribution for all sites.

Table 4. 2. Substrate characteristics of the sampling sites

Habitat parameters	Sampling sites									p-value
	KR1	KR2	MT1	MT2	AB1	AB2	AB3	GR1	GR2	
Bed visibility (%)	100	100	77	25	98	0	0	100	0	0.001
% Megalithal	5	10	0	0	0	80	0	0	0	0.433
% Macrolithal	25	10	40	20	20	0	20	40	20	0.433
% Mesolithal	50	50	60	50	50	20	60	40	60	0.433
% Microlithal	20	30	0	30	30	0	20	20	20	0.433

Industries release wastes that contain not only high organic matter but also other toxic metals that cause carcinogenic effect on the near biota. In this study attempts were made to see the concentration of heavy metals released in to the streams by Ayika Textile factory (GR2) and Jafar Leather Industry (AB3). Upstream site (AB1) was taken as a comparison for the parts of streams below those two industries. The immediate heavy metal concern released from Textile industries are copper, chromium, cadmium, zinc, iron, mercury and lead. Incidentally, it should be known that in Tannery effluents chromium and cadmium are the most frequently suspected heavy metals.

Six heavy metals (Cr, Cd, Zn, Ni, Pb, and Cu) were measured and from these only four heavy metals (Cr, Cd, Zn, and Ni) were detected. The concentration of cadmium was 98.37 μ g/l, 760.20 μ g/l and 827.73 μ g/l for AB1, AB3, and GR2 respectively. This means the Jafar Leather industry and Ayika Textile factory release seven and eight times more cadmium concentration respectively than found in the natural environment. The chromium concentration was not detected at the upstream site (AB1) and 3.61 μ g/l and 25.99 μ g/l were recorded for tannery (AB3) and textile (GR2) effluent receiving sites respectively. The measurement of the rest of the heavy metals was limited to the Textile Industry waste receiving site (GR2) and the upstream (AB1) because it is not expected from tannery effluent (AB3). Based on this, 349.61 μ g/l and 415.01 μ g/l for nickel and 13.19 μ g/l and 43.09 μ g/l for zinc were recorded at AB1 and GR2 sampling sites respectively. Pb and Cu were not detected in either of the sampling sites (Table 4.3).

Table 4. 3 Mean heavy metal concentration (μ g/l) of water samples from selected sampling sites (AB1, AB3 and GR2 refer to sites upstream of Abeyi stream, Abeyi stream receiving Jafaf Tannery effluent and Gerado stream receiving Ayika Textile Factory effluent respectively) ND refers to not detected and – indicates not measured. ND means either the water sample has no metal or it is below the detection limit of the measuring instrument.

Heavy metals	Sampling sites		
	AB1	AB3	GR2
Cadmium(Cd)	98.37 \pm 0.65	760.20 \pm 1.16	827.73 \pm 1.17
Chromium(Cr)	ND	3.61 \pm 0.03	25.99 \pm 0.05
Nickel(Ni)	349.61 \pm 1.58	-	415.01 \pm 0.65
Zinc (Zn)	13.19 \pm 0.03	-	43.09 \pm 0.12
Copper(Cu)	ND	-	ND
Lead (Pb)	ND	-	ND

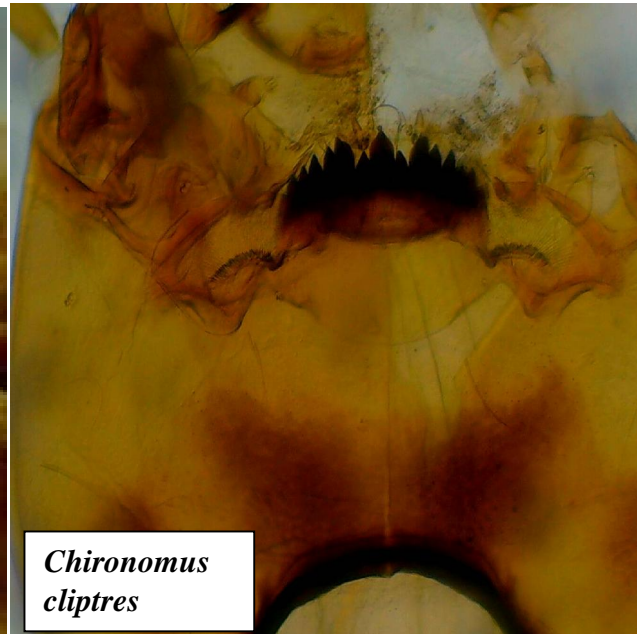
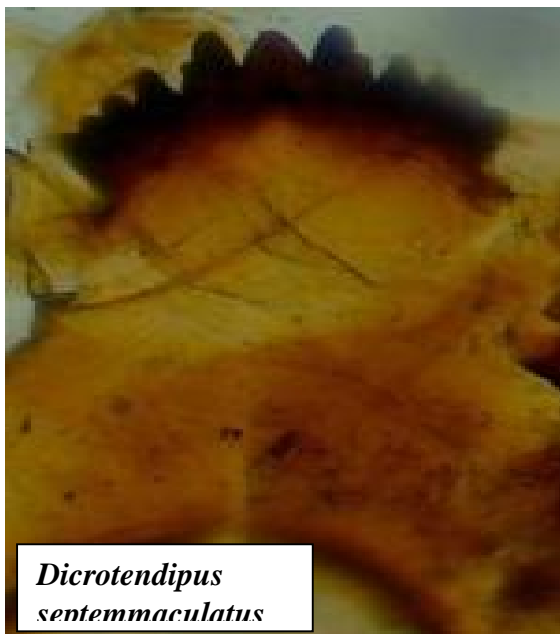
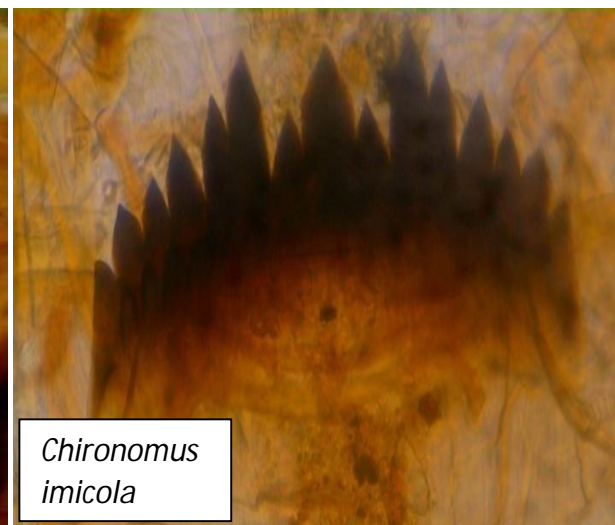
4.2. Benthic macroinvertebrate community structure

During the sampling period from November 2016 to January 2017, a total of 27 benthic macroinvertebrate taxa were collected. Among 27 taxa, 20 were identified to family level while the rest which are under family chironomidae were identified to genera (1) and species (6) level. A detail of benthic macroinvertebrate diversity and abundance are shown in Table (4.4). Higher numbers of taxa (18) were recorded at less impaired sites (considered as reference) upstream sites (KR1) while lower number of taxa was recorded at sites affected by industrial wastes (7 at AB3 and 9 at AB2).

Family Planariidae was found only at reference site (KR1). The Dipterans were more or less found from minimally impacted to highly impacted sites in which Family Tipulidae, Muscidae and Simuliidae distributed mostly on the minimally impacted sites and the Family Psychodidae, Syrphidae and Culicidae were dominant on highly impacted sampling site while Family chironomidae was distributed throughout all sampling sites. Gastropoda (Lymnaeidae and Thiariidae) were restricted to highly impacted site. From the pollution intolerant group, ET (Ephemeroptera and Trichoptera) which constitute Caenidae, Baetidae and Hydropsychidae were distributed mostly in the upstream sampling sites (KR1, KR2, AB2 and GR1) and agricultural impacted site (KR2). Family Gyridae, Dystiscidae and Hydrophilidae were distributed along minimally impacted sampling sites and the Hemiptera group (Naucoridae and Corixidae) were restricted to the reference site (KR1) but, Notonetidae have a wide range of distribution. Oligochaeta was found in 89% of the sampling sites.

From the Chironomidae taxa only two sub families were observed (sub family Chironominae and Tanypodinae) unlike what other researchers found in Ethiopia subfamily Ortocladinae in slightly polluted sites (Harrison, 1992; Aschalew Lakew, 2012; Getachew Beneberu, 2013). Sub-family chironominae were comprised of *Chironomus alluaudi*, *Chironomus imicola*, *Chironomus cliptres*, *Polypedilum wittei*, *Polypedilum bipustulatem* and *Dicrotendipu septemmaculatus* while subfamily Tanypodinae were represented by genus *Conchapelopia*. From genus *Chironomus*, *Chironomus alluaudi* and *Chironomus imicola* were distributed along all sampling sites except

absence of *Chironomus imicola* in agricultural impacted site (KR2). *Polypedilum wittei*, *Polypedilum bipustulatem* and *Dicrotendipus septemmaculatus* were highly abundant in moderately polluted sites and also found in fewer amounts at highly polluted sampling sites but, absent in dissolved oxygen < 2.5mg/l (AB2 and AB3) sites. The distribution of *Chironomus cliptres* and genus *Conchapelopia* were limited to reference and minimally impacted upstream sampling sites, but, genus *Conchapelopia* was also found in less proportion at textile effluent receiving site (GR2).



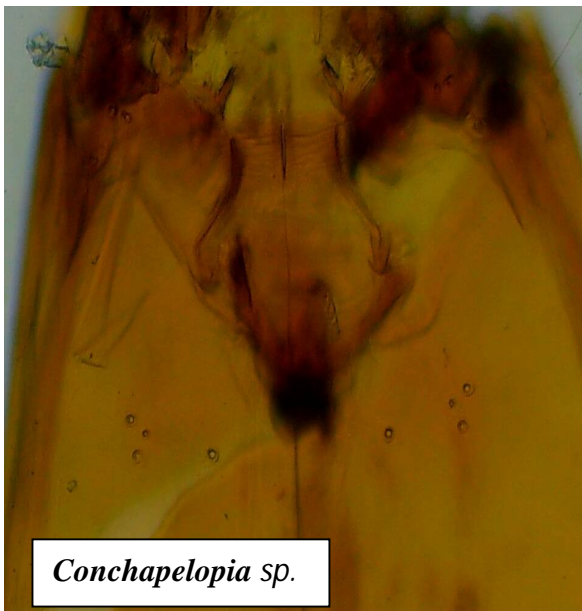
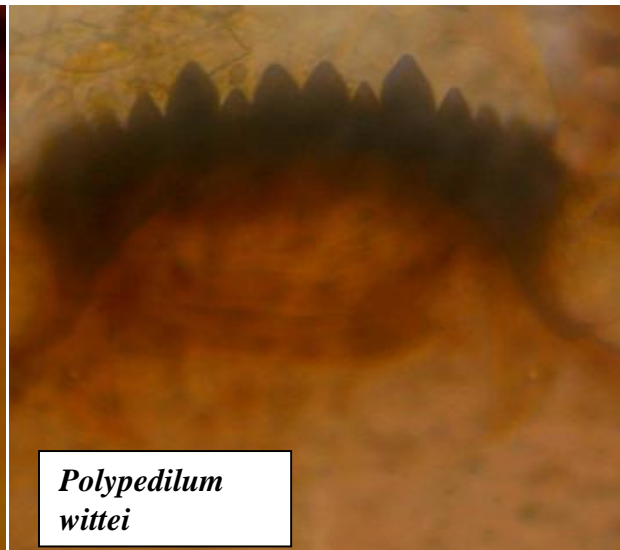
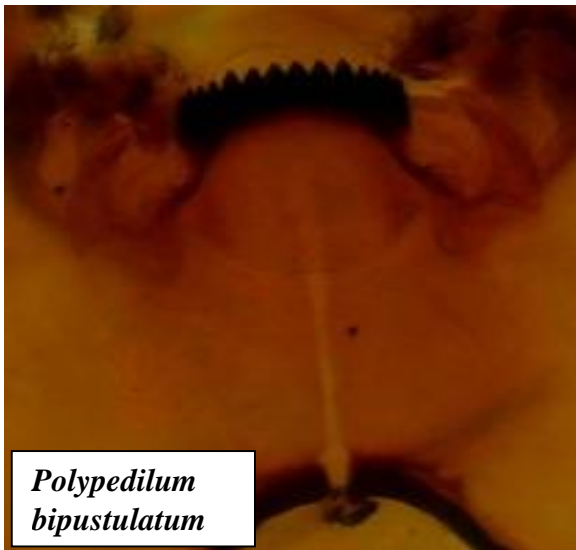


Plate 6. Species and genus of Chironomid taxa identified during the study period

The relationship between morphological deformities of chironomidae by heavy metal and pesticides contamination of aquatic ecosystem has been described by Hamilton and Sæther (1971) and mouth parts deformity of Chironomidae larvae has been described as successful methods for biological assessment of water quality. In this study mouth part deformity particularly on the muntum of sub family chironominae was recorded at upstream sites (AB1, GR1) and textile effluent receiving sites (GR2).

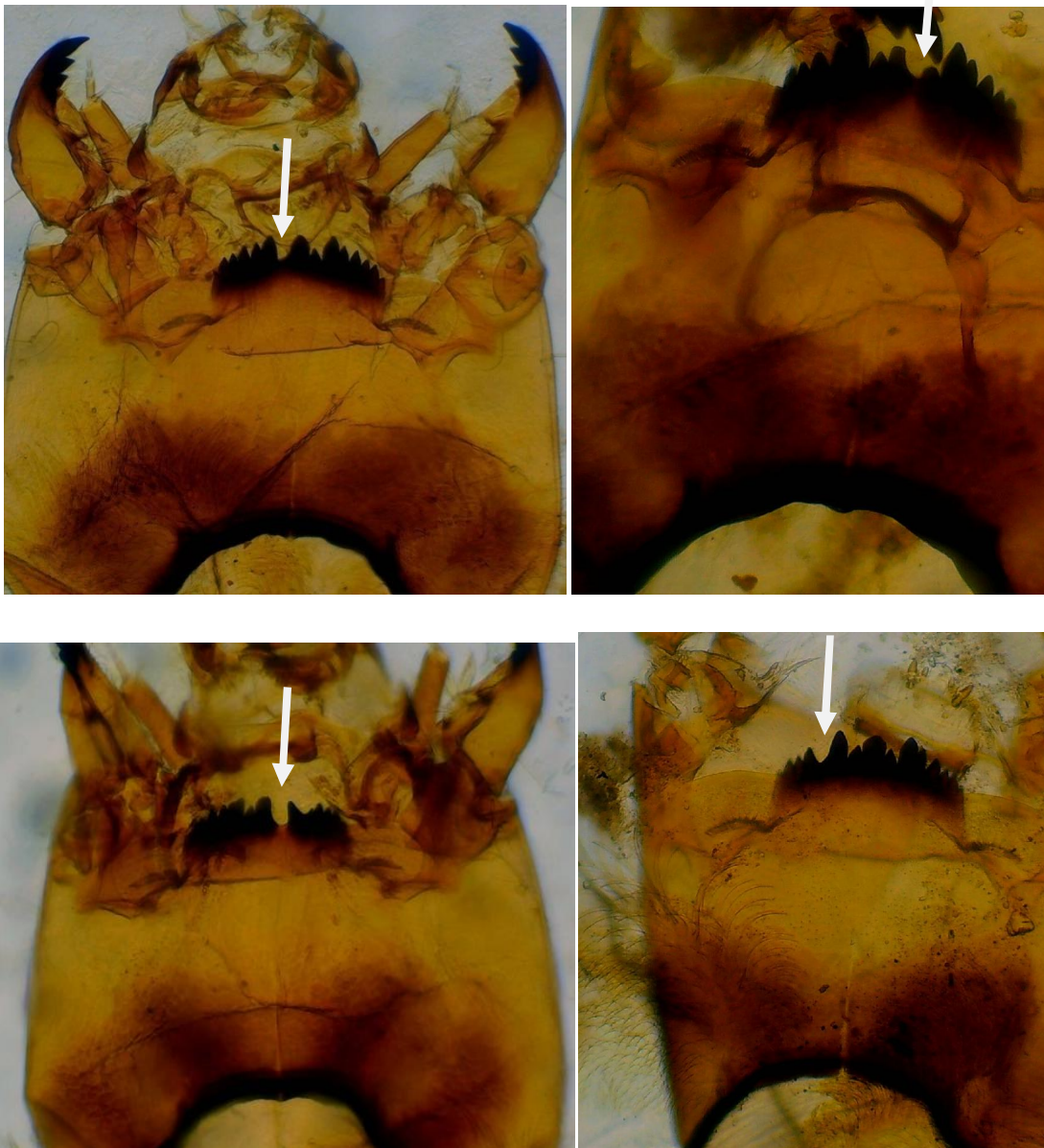


Plate 7. Muntum deformities of some chironominae taxa from some of sampling sites

Table 4. 4. Benthic macroinvertebrate data recorded during the sampling season. Each individual taxon is recorded in individual/ m²

Benthic macroinvertebrates		Sampling sites								
Order	Family	KR1	KR2	MT1	MT2	AB1	AB2	AB3	GR1	GR2
Planaria	Planariidae	5	0	0	0	0	0	0	0	0
Gastropoda	Lymnaeidae	0	0	0	0	0	0	0	0	116
	Thiaridae	0	0	0	0	0	5	0	0	0
Oligochaeta	Oligochaeta	7	4	4	453	9	565	109	0	668
Ephemeroptera	Baetidea	616	237	531	0	1194	0	0	365	34
	Caenidae	223	39	0	0	727	0	0	121	0
Trichoptera	Hydropsychidae	403	8	0	0	0	0	0	3	0
Coleoptera	Gyrinidae	3	2	0	0	2	0	0	0	0
	Dystiscidae	0	0	4	0	0	0	0	0	0
	Hydrophilidae	0	0	4	0	0	0	0	0	0
	Notonetidae	0	2	0	0	46	0	0	10	7
Hemiptera	Corixidae	9	0	0	0	0	0	0	0	0
	Naucoridae	5	0	0	0	0	0	0	0	0
	Chironomidae	328	49	1242	5457	1807	27	235	809	4385
	Chironominae									
	<i>Chironomus alluaudi</i>	11	11	366	2354	942	9	127	288	3459
	<i>Chironomus imicola</i>	0	0	107	2616	446	10	102	206	1187
	<i>Chironomus cliptres</i>	0	0	17	0	13	0	0	0	0
	<i>Polypedilum wittei</i>	40		581	98	149	0	0	91	174
	<i>Polypedilum Bipustulatem</i>	30	0	121	196	14	0	0	63	0
	<i>Dicrotendipus septemmaculatus</i>	6	00	0	0	25	0	0	0	118
	Tanypodinae									
	<i>Conchapelopia sp.</i>	267	86	48	0	281	0	0	163	30
	Tipulidae	69	7	13	0	5	0	0	3	0

Benthic macroinvertebrates		Sampling sites								
Order	Family	KR1	KR2	MT1	MT2	AB1	AB2	AB3	GR1	GR2
	Muscidae	12	10	0	0	0	0	0	23	0
	Psychodidae	0	0	4	11	0	0	0	3	0
	Culicidae	21	0	30	4	104	41	0	101	6
	Syrphidae	0	0	0	0	0	48	23	0	0
	Simuliidae	1336	225	26	128	2	0	0	0	19
Total No. of Indi/m ²		3037	583	1858	6053	3898	686	367	1438	5236
Total No. of Taxa (family)		14	12	11	7	11	7	5	11	8
Total No. of Taxa with chiro spp.		19	14	17	11	18	9	7	16	13

4.3. Benthic macroinvertebrate metrics selection and calculation

Metrics are biological attributes that reflect the impact of anthropogenic activities and respond in predictable way. The response may be either to a single impact factor or to the cumulative effects of multiple human impairments within a watershed. Selection of metrics that fit to the collected taxa, geography and socioeconomic aspects of the watershed is very important. In the present study we included metrics of taxa abundance, richness/diversity, and sensitivity/tolerance. Functional feeding groups were not included as most of taxa identification was takes place at family level and different feeding groups may be within the same family. About 44 metrics were selected and calculated using Microsoft Excel (Table 4.5). The tolerance values of benthic macroinvertebrate for family biotic index, BMWP, SASS and ETHbios were calculated according to the values awarded to each benthic invertebrate fauna.

Table 4. 5. Observed values of all metrics in streams and rivers around Sebeta exposed to different anthropogenic impacts

Metrics	Sampling sites								
	KR1	KR2	MT1	MT2	AB1	AB2	AB3	GR1	GR2
Total No. of indi/m2	3037	583	1858	6053	3898	686	367	1438	5236
No. of Taxa (family)	13	10	9	5	9	5	3	9	7
No. of Taxa with chiro spp.	18	12	15	9	16	7	5	14	12
No. of Ephemeroptera indi.	840	276	531	0	1922	0	0	486	34
No. Trichoptera indi.	403	8	0	0	0	0	0	3	0
No. of Coleoptera indi.	3	2	9	0	2	0	0	0	0
No. of ET taxa	3	3	1	0	2	0	0	3	1
No. of CTE taxa	4	4	3	0	3	0	0	3	1
No of ET indiv/m2	1243	285	531	0	1922	0	0	490	34
No.CTE ind/m2	1246	286	540	0	1924	0	0	490	34
Number of Oligochaeta indi.	7	4	4	453	9	565	109	0	668
No. of Chironomidae indi.	328	49	1242	5457	1807	27	235	809	4385
No. of Tolerant taxa indi.	2406	536	1858	6053	3171	686	367	1314	5236
No. of Intolerant Taxa indi	631	47	0	0	727	0	0	124	0
%Ephemeroptera indi.	28	47	29	0	49	0	0	34	1
%Trichoptera indi.	13	1	0	0	0	0	0	0	0
% ET taxa indi.	41	49	29	0	49	0	0	34	1
%Diptera indi.	58	50	71	93	49	17	70	65	84
%CTE taxa	41	49	29	0	49	0	0	34	1
% Tolerant indi.	79	92	100	100	81	100	100	91	100
% Intolerant indi.	21	8	0	0	19	0	0	9	0
%Dominant taxa indi.	44	41	67	90	46	82	64	56	84
% Chironomids and Oligochaeta indi.	11	9	67	98	47	86	94	56	97
ET/Chironomidae	4	6	0	0	1	0	0	1	0
Margalef's Index	2.12	1.73	1.86	1.03	1.81	1.07	1.01	1.79	1.40

Metrics	Sampling sites								
	KR1	KR2	MT1	MT2	AB1	AB2	AB3	GR1	GR2
Family Biotic Index	2.82	5.27	6.63	7.96	6.27	7.57	8.06	6.15	7.90
ETHbios	42.00	34.00	25.00	4.00	27.00	7.00	10.00	29.00	14.00
ASPT-ETHbios	4.20	3.78	3.13	1.00	3.59	1.40	2.00	3.63	2.30
SASS	44.00	32.00	29.00	14.00	32.00	11.00	11.00	27.00	23.00
ASPT-SASS	3.67	3.50	3.20	2.30	3.50	1.60	2.20	3.00	2.80
BMWP	49.00	38.00	31.00	17.00	34.00	9.00	15.00	32.00	24.00
ASPT-BMWP	4.45	4.75	3.88	3.40	4.25	2.25	3.00	4.57	3.40
No. of chironomidae indi/m2	354	97	1239	5265	1870	18	229	812	4968
No. chironominae taxa	4	1	5	4	6	2	2	4	4
No. of Chironominae indi.	87	11	1191	5265	1589	18	229	649	4938
No. of Tanypodinae indi.	267	86	48	0	281	0	0	163	30
No. of Chironomus indi.	11	11	490	4970	1402	18	229	494	4646
%Tanypodinae ind.	75	88	4	0	15	0	0	20	1
% of chironominae indi.	25	12	96	100	85	100	100	80	99
% Chironomus indi.	3	12	40	94	75	100	100	61	94
TotalChironomus/total benthic indi.	0	2	26	82	36	3	62	34	89
%Tanypodinae/chironomidae	21	91	0	0	1	0	0	2	0
Chironomus/chironominae	12	100	41	94	88	100	100	76	94

4.4. Multivariate analysis of sampling sites

The impact of major environmental parameters on the community structure of benthic macroinvertebrates was analyzed using the multivariate analysis RDA ordination. From physicochemical parameters pH, temperature, O₂ (%), NO₃⁻, conductivity and SRP were rejected because of high inflection factor and multi co-linearity with other variables. The other parameters TP, NH₄⁺, dissolved oxygen and turbidity were selected as main parameters that affect the community structure of benthic macroinvertebrates. The first axis of RDA expressed 51.0% the faunal variation where as the second axis explained 15.5% of the variation (Table 4.8). The species environmental correlation of axis 1 and 2 explained 0.899% and 0.885%

respectively which showed a high predictive power of selected physicochemical parameters in structuring benthic macroinvertebrate community.

Table 4. 6. Summary statistics of Redundancy Analysis (RDA) for species environment relationship

Axes	1	2	3	4
Eigenvalues	0.510	0.145	0.032	0.006
Species-environment correlations:	0.899	0.885	0.928	0.613
Cumulative percentage variance				
of species data	51.0	65.5	68.7	69.3
of species-environment relation:	73.6	94.5	99.1	100.0
Sum of all eigenvalues	1.000			
Sum of all canonical eigenvalues	0.693			

The RDA ordination plot also showed the distribution of benthic macroinvertebrates along with physiochemical parameters. Planariidae, Caenidae, Baetidea, Simuliidae, Notonetidae, Naucoridae, Corixidae, Hydropsychidae, Dystiscidae, Gyrinidae, Tipulidae, Muscidae, Culicidae, and Hydrophilidae go with streams and rivers with high concentration of dissolved oxygen. This depicted that these organisms can be indicator of good water quality. Syrphidae and Thiaridae go with sampling sites experiencing high suspended particulate matter and can be considered as an indicator of turbid streams and rivers. Lymnaeidae, Oligochaeta, psychodidae and chironomidae were dominant in streams and rivers suffering from high organic load (high concentration of NH_4^+ and TP). Therefore, the dominance of these taxa can predict the presence of high organic load in the respective water body.

Genus/species level of chironomidae taxa also showed varying distribution with respect to the response of environmental variables. Based on this *Chironomus alluaudi* and *Chironomus imicola* were more prevalent in high phosphorus and ammonia experiencing streams and rivers

while *Polypedilum bipustulatem*, *Polypedilum wittei* and *Dicrotendipu septemmaculatus* became dominant in moderate oxygen concentration and organic load (NH_4^+ and TP) level. *Chironomus calipters* and genus *Conchapelopia* were abundant in streams and rivers rich in dissolved oxygen concentration (Figure 4.2).

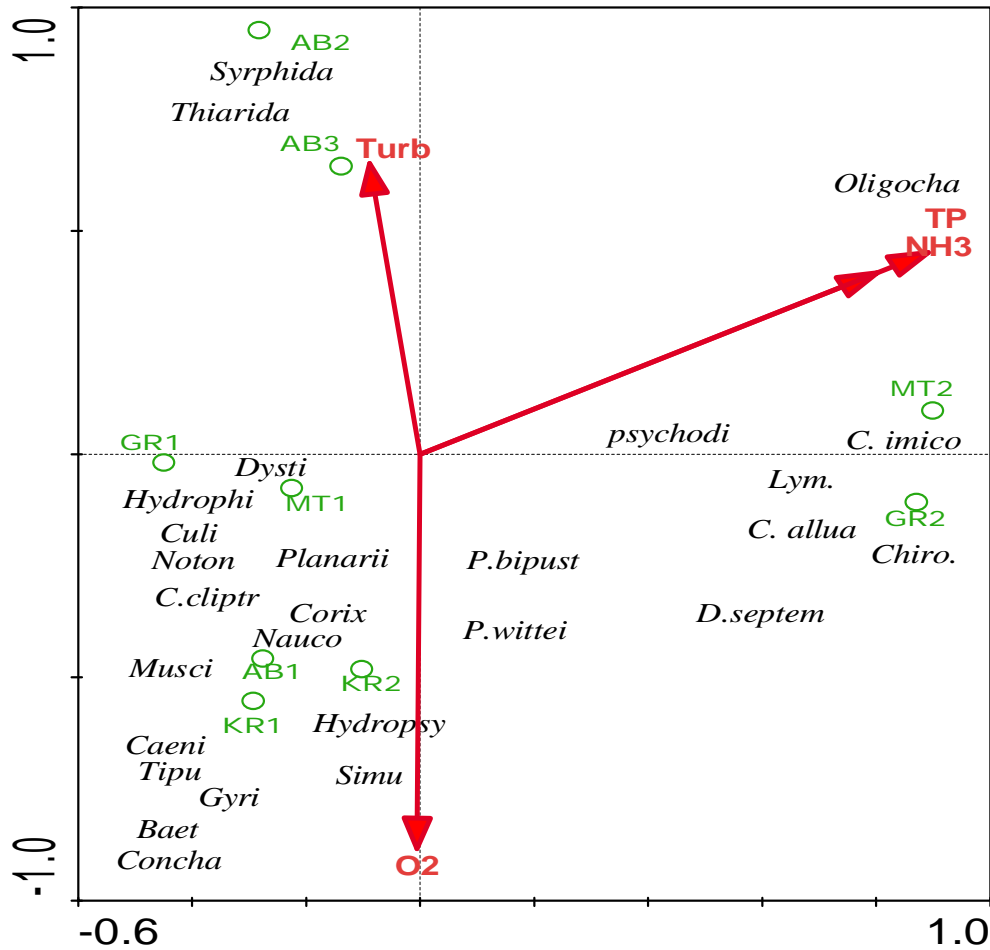


Figure 4. 1. Triplot of Redundancy Analysis (RDA), between species-environmental and sampling sites. Circles indicate sampling sites. The arrow shows the major physicochemical parameters that structure the benthic community: DO=dissolved oxygen, Turb=Turbidity, TP=total phosphorous, NH_4^+ =ammonia. Macroinvertebrates are abbreviated (Planarii=Planariidae, Caeni=Caenidae, Baet=Baetidea, Simu=Simuliidae, Noton=Notonetidae, Nauco=Naucoridae, Corix=Corixidae, Hydropsy=Hydropsychidae, Dysti=Dystiscidae, Gyri=Gyrinidae, Tipu=Tipulidae, Musci=Muscidae, Culi=Culicidae, Hydrophi=Hydrophilidae, Lym=Lymnaeidae, Syrphida=Syrphidae, Thiaida=Thiaridae, Oligocha=Oligochaeta, Psychodi=Psychodidae, Chiro=Cchironomidae, *C.allou*=*Chironomus alluaudi*, *C.imico*=*Chironomus imicola*, *P.bipusti*=*Polypedilum bipustulatem*, *P.wittei*,=*Polypedilum wittei*, *D.septem*=*Dicrotendipu septemmaculatus*, *C.caliptr*=*Chironomus calipters* and concha=Chonchapelopia).

5. Discussion

5.1. Environmental parameters

Physicochemical parameters give information about the status of a water body at the time of sampling. These parameters are affected in geomorphology, climate and hydrology of the catchment. Sites within the same ecoregion mostly experience similar physicochemical characteristics unless affected by human activities. Thus, physicochemical measurements can show the changes even if it is snapshot. In this study most of the physicochemical parameters measured in field and laboratory show significance difference among sampling sites.

The variation of temperature in surface waters is affected by latitude, altitude, season, time of day, air circulation, cloud cover and the flow and depth of the water body. In addition, industries which use surface waters as cooling and dumping of waste purpose can aggravate the temperature of receiving water bodies. The change in temperature affects the biogeochemical processes in the aquatic ecosystem. For example, increase in temperature decreases the solubility of gases in water, increase the metabolic rate of organisms and decomposition of organic matter which leads to the depletion of oxygen and destruction of the aquatic biota (Aschalew Lakew, 2012). The water temperature fluctuation during the study time was not significant between sampling sites ($p > 0.05$). Reference (KR1) and agriculture impacted sites (KR2) showed lower in temperature than other study sites. This can probably be due to their location upstream at Kersa, a higher altitude in an area that has riparian vegetation cover with cooling effect on the water. Aschalew Lakew, 2014) also reported that sites with high riparian cover show lower temperature than impacted sites.

pH is an important parameter in aquatic life. An increase or decrease in pH has a great effect on aquatic organisms like fish and benthic fauna. Low pH levels can encourage the solubility of heavy metals which are carcinogenic to aquatic life, while high pH levels can damage gills and skin of aquatic organisms and cause death (Aschalew Lakew, 2012). At $\text{pH} > 9$ the nitrogen compounds will be converted to ammonia (NH_3), which is fatal to aquatic life. In the present

study there is no significance difference ($p>0.05$) in pH among sampling sites. Therefore, temperature and pH was not important parameters to show the intensity of human activities on streams and rivers of the study site.

Dissolved oxygen which is expressed in mg/l or percent of saturation is essential for all forms of life. It is affected by temperature, turbulence, partial pressure of solutes, photosynthesis, respiration, the available organic matter and the decomposer biota living there (Aschalew Lakew, 2012). The concentration/saturation of dissolved oxygen highly varied between sampling sites. Reference and agricultural impacted sites have high amount of dissolved oxygen, 7.62mg/l, and 7.76mg/l, respectively. These sites were relatively less disturbed by anthropogenic activities and there was less organic load as the river bank was covered by riparian vegetations.

Streams receiving effluents of the Alcohol (AB2) and Tannery (AB3) Factories showed low oxygen levels which were recorded as 2.77mg/l and 1.54mg/l respectively (Table 4.1). The low dissolved oxygen concentration of these sites need some further explanation, because these sites have low nutrient load compared to other industrial effluent receiving sites (MT2 and GR2). The possible explanation is as these sites are highly turbid and with canopy cover, entrance of light to the bottom planktons may be prevented. Therefore, as there is no addition of oxygen through photosynthesis and turbulence and the available oxygen is consumed by decomposition, the dissolved oxygen level in these sites can be depleted. This was also confirmed from the RDA ordination result that the two sites were expressed as highly turbid (Figure 4.2). In addition, these two industries might cause direct toxicity as low diversity and abundance of benthic invertebrates also observed (Table 4.4). The dissolved oxygen concentration of MT2 (5.08 mg/l) and GR2 (4.59 mg/l) which receive wastes from Meta Brewery and Ayika Textile Factories respectively was not as such low. This is because the stream channel of these sites was mostly riffle and there may be addition of oxygen from the atmosphere through turbulence.

The conductivity of water is mainly affected by the geology of the catchment and human activities. Intense human activities may increase the entrance of ions to the water body. High conductivity was recorded at streams affected by industrial effluents particularly Ayika Textile (GR2) and Meta Brewery (MT2). These industries contribute high amount of ions to the

receiving water body. In Textile factories dies contribute the release of high amount of ions and heavy metals to the receiving water body. The high organic load of rives in the town and watershed disturbance increase the ionic concentration of receiving water body and subsequently conductivity (Dyer *et al.*, 1982).

The concentration of NO_3^- was not significant ($p>0.05$) in the sampling sites, while NH_4^+ show a significant difference with high concentration at GR2 (4.63 mg/l) and then MT2 (1.39mg/l) and AB3 (1.58mg/l) which receives wastes of textile, brewery and tannery respectively. This is because these industries release high amount of waste water with a considerable amount of organic matter. The high ammonia contribution of these industries is a clear indication that they cause direct intoxication on the resident biota in addition to the indirect deterioration of the water quality and habitat destruction. Solomon Akalu *et al.* (2011) also suggest that industries and urban domestic waste in Addis Ababa affect aquatic life in the greater Akaki through direct intoxication and indirect water quality deterioration. There was almost similar concentration of ammonia to all the other remaining upstream sampling sites which ranges from 0.09mg/l to 0.10mg/l. The possible reason may be headwater streams and rivers surrounded by riparian vegetation have a considerable efficiency to capture nitrogen and reduce it to N_2 gas though the process of denitrification (Vitousek *et al.*, 2002)

Turbidity is a measure of how cloudy is a water body. It is caused by particles suspended or dissolved in water. Particulate matter clay and silt, fine organic and inorganic matter, soluble colored organic compounds, algae, and other microscopic organisms can cause turbidity (Minnesota Pollution Control Agency, 2008). There was high record of turbidity at sites receiving alcohol (AB2) and textile (GR2) effluents. The impact was reflected by reduction in diversity and abundance of macroinvertebrates and low dissolved oxygen.

Phosphorus is one of the limiting nutrients for algal growth in aquatic ecosystem. Increasing in concentration of phosphorus leads to eutrophication of water bodies which is the cause for aquatic life destruction particularly mass fish kill. As like conductivity, the concentration of phosphorus was high in sites which receive industrial wastes. This is because the tannery, textile, alcohol and beer factories use phosphorus containing raw materials in the production process and

detergents for washing purpose. The trend of Total Phosphorus (TP) and Soluble Reactive Phosphorus (SRP) was the same along sampling sites (Table 4.1). This means the available form of phosphorus (PO_4^-) is in parallel to the total phosphorus released. The level of nitrogen and phosphorous in industrial effluent mainly depends on the handling of raw material, the amount of spent yeast present in the effluent and the amount of phosphorous containing chemicals used in the clean in place units (caustic soda and phosphoric acid) (Tesfalem Fikresilasie, 2011).

Even if agriculture contributes phosphorus through production inputs (like fertilizers), the concentration was nearly similar to reference and other upstream sampling sites. This may be all the upstream sites may experience similar phosphorus load like the agricultural sites. This is because of population growth, most rivers and streams are affected by human activities like washing/ bathing that may increase the phosphorus level as like agriculture contributes. The increases of urbanization and industrialization have pronounced impact in deteriorating water quality than agricultural activities (Jiao Ding *et al.*, 2015).

The distributions of benthic macroinvertebrates are also affected by the nature of the habitat. In this study, bed visibility was significantly different from reference site (100%) to industrial impacted sites (0%). Bed visibility is based on the amount of suspended solids in the water body and it is known that suspended solids affect the overall ecology of aquatic life through interfering feeding habit, preventing light penetration and food production. Therefore, presence/absence of organisms depends on their intensity of tolerance to the nature and amount of dissolved and suspended matter in the water body. The substrate grain sizes difference between sampling sites was not significantly different which supports the premise that study sites within the same ecoregion have similar habitat type. Anthropogenic activities are the major causes for the degradation of hydro morphological characteristics of streams and rivers and this disrupts the dynamic movement of water (Poff *et al.*, 1997). In Ethiopian highlands, the hydro morphological alterations are damming, diversion and water abstraction. These activities are highly intensified for the purpose of irrigation, livestock watering, domestic consumption and electric power generation (Aschalew Lakew, 2014).

Effluents containing heavy metals from industries are one of the principal sources of pollution for surface water, ground water and soil (Szefer, 1997). In developing countries like Ethiopia, untreated or partially-treated wastewaters of industries are directly discharged to the nearby wetland and /or water bodies (Solomon Sorsa *et al.*, 2015). In the present study the cadmium concentrations of AB3 (760.20 μ g/l) and GR2 (827.73 μ g/l) sampling sites were above the minimum permissible limit (100 μ g/l) for surface waters according to World Bank (1998). Cadmium is a non-essential heavy metal that is not used by biological systems. It tends to bioaccumulate in both aquatic and terrestrial ecosystems and builds up in invertebrates, algae and plants. The effects of cadmium on aquatic organisms can be directly or indirectly lethal and can impact populations and ecosystems as well as individuals and it causes also morphological deformities (Frances Solomon, 2008).

The other heavy metals were below the minimum permissible limit for surface waters (Table 4.3): chromium total (500 μ g/l), copper (500 μ g/l), lead (100 μ g/l), nickel (500 μ g/l), and zinc (2000 μ g/l) according to World Bank (1998). The concentration of metals released from industries varies from industry to industry and the raw materials used. Most factories in Ethiopia, including textile and leather industries, have no effluent treatment plants (Ethiopian Environmental Protection Authority, 2003). The major problem associated with textile processing effluents is presence of heavy metal ions, from dyeing process or usage of metal containing dyes (Correia, 1998).

5.2. Benthic macroinvertebrate community structure

The result of the present study showed that benthic invertebrates were distributed from least impacted to highly impacted streams and rivers implying that they have different ecological needs and different degree of tolerance to various anthropogenic impacts. Therefore, their use as pollution indicator tool will not come in to question.

From benthic macroinvertebrate taxa, Family Planariidae was found only at reference site (KR1). Caenidae, Baetidae and Hydropsychidae were distributed mostly on the upstream sampling sites

and totally absent at highly impacted industrial effluent receiving sites. This may be due to the high organic pollution of industries. The loss of most sensitive taxa (Ephemeroptera, Plecoptera, and Trichoptera) was also observed because of excessive organic loading, increased total dissolved solids and conductivity from households and industrial wastes which are harmful to these organisms (Baye Sitotaw, 2006). The same thing was also reported by Tesfaye Berhe (1988) and Worku Legesse *et al.* (2004) at Kebena River. Therefore, these organisms can be an indicator of good water quality. But, few number of Baetidae was also observed at highly polluted site (GR2) indicating that there may be some genes/ species of the family very tolerant to pollution. Baye Sitotaw, (2006) also recorded these group in highly polluted site. Therefore, lower taxonomic identification is mandatory to fully utilize the bioindicator value of family Baetidae.

Family Gyrinidae, Dystiscidae and Hydrophilidae were distributed along minimally impacted sampling sites and the Hemiptera group (Naucoridae and Corixidae) were restricted to the reference site (KR1) but Notonetidae have a wide range of distribution. This indicates that most of these groups possess intermediate pollution tolerance and proliferate in mild pollution and can be an indicator of moderately polluted streams and rivers. Syrphidae and Thiaridae were distributed in sampling sites experiencing high suspended particulate matter and can be considered as an indicator of turbid streams and rivers. Family Lymnaeidae, Chironomidae, Psychodidae and Oligochaeta were dominant in highly polluted sites may be due to low competition and at the same time the use of high organic matter as source of food. The ordination plot also gives similar information on the distribution of benthic macroinvertebrates along sampling sites and in relation of selected environmental parameters (Figure 4.2).

From benthic macroinvertebrates Family Chironomidae were found in all sampling sites and their qualitative use at family level to classify water quality is not important. But, identification to lower taxonomic level (genus and species) helped us to separate sampling sites possessing different water quality class. Many researchers also found that Family Chironomidae and the species thereof possess a wide range of tolerance to various perturbations like enrichment of organic nutrients (Helson *et al.*, 2006), heavy metals (Diggins, 2000), acid mine drainage (Heino *et al.*, 2003) and toxic organic compounds (Wright *et al.*, 1996). Williams and Feltmate, (1992) reported that Chironominae and some Tanypodinae are very tolerant to low levels of dissolved

oxygen, *Chironomus plumosus* larvae can survive in a pH value of 2.3 while *Cricotopus bicinctus* is known for its tolerance for electroplating wastes and crude oil. Other members of the family are very sensitive for poor water quality and only exist in relatively good water quality.

Sub Family Orthocladinae was not observed in our study unlike other investigations conducted in Ethiopia, which they found some group of Orthocladinae like *Cricotopus* sp (Harrison, 1992 and Getachew Beneberu, 2013). The same authors stated that the presence of this group was related to the available oxygen and its absence in slightly polluted water will not use as reliable indicator of water quality.

Results of the present study showed that *Chironomus alluaudi* and *Chironomus imicola* were distributed from less polluted to highly impacted sites with high proportion in most polluted sites, which indicates their wide range of tolerance to variety of disturbances. *Chironomus alluaudi* was the most dominant in extremely polluted sites as shown in Table (4.4). Getachew Beneberu (2013) also found that *Chironomus alluaudi* was the most dominant taxon constituting more than 95% of the chironomids taxa observed in highly polluted Modjo River. The dominance of *Chironomus spp.* in areas enriched with high detritus matter and subsequently low dissolved oxygen was also reported by Callisto *et al.*, (2001). The physiological and morphological adaptation of the *Chironomus* through its ability to slow their metabolic rates was supposed to allow it to survive in harsh and hostile environments (Hamburger *et al.*, 1994). A larger body size which helps in ventilation of larval tubes also allows chironomids to tolerate low dissolved oxygen levels (Int Panis *et al.*, 1996). Therefore, the dominance of *Chironomus* can be an indicator of high organic pollution.

Although the abundance of *Chironomus alluaudi* and *Chironomus imicola* were very high in polluted water bodies their abundance in streams (AB2 and AB3) with DO of 2.77 mg/l and 1.54 mg/l respectively was low. This showed that the species may reached its tolerance limit and these sampling sites were instead dominated by Oligochaeta and Syrphidae which are considerably tolerant to oxygen deficit by developing respiratory organ that able to use the atmospheric oxygen. Getachew Beneberu (2013) also found that the absolute abundance of *Chironomus* was

low in much polluted Sebeta River, even absent in some sampling sites in which DO level is <2mg/l.

Chironomus cliptres was found in less polluted upstream sampling sites (MT1 and AB1) and this species can be an indicator of good water quality. The distribution of *Polypedilum wittei*, *Polypedilum bipustulatem* and *Dicrotendipus septemmaculatus* were high in minimally impacted sites and in small proportion they also found highly impacted sites. But, none of these species were recorded in sites with low dissolved oxygen (<2.5 mg/l). Getachew Beneberu (2013) depicted that there seems to be an overlap in some groups of chironomids especially between slightly impacted and impacted sites by mentioning *Polypedilum wittei* that appeared in the slightly impacted sites and impacted Modjo River. There is no *Polypedilum wittei* in agricultural impacted sites in contrary to Getachew Beneberu (2013) hypothesis that this group may be an indicator of agricultural impacted sites.

The genus *Conchapelopia* were distributed mostly in less impacted sites and totally absent in oxygen deficit sampling sites for example alcohol (AB2) and tannery (AB3) waste receiving sites as shown in Table (4.3). Therefore these taxa can be indicator of a good water quality. Getachew Beneberu (2013) also reported that the presence of genes *Conchapelopia* can be an indicator of good water quality.

The level of impact of anthropogenic activities in streams and rivers was not restricted to reduction in abundance and diversity of benthic macroinvertebrates, but the intensity of the impact was also reflected through morphological deformities. Mouth part deformities particularly on the muntum of Sub family Chironominae was recorded at upstream sites (AB1, GR1) and Textile effluent receiving sites (GR2). In fact, the cause for deformity of this taxonomic group is a little bit ambiguous because deformities were observed in both slightly impacted (AB1 and GR1) and highly impacted sampling sites (GR2). Some researchers' considered deformities as indicators of heavy metals, pesticides, and organic pollution (Nazarova *et al.*, 2004) others still debate on the percentage frequency of deformities related to pollution (Burt *et al.*, 2003) because deformity will come due to feeding instead of some stress. Getachew Beneberu (2013) also found that deformed chironomids in upstream and downstream sampling

sites. Therefore, importance of deformity as indicator of stress was not included and its use as bioindicator can be conducted by considering the percentage of deformity, geographical area, feeding habit characteristics and possibly with the explanations from the physiological processes of the metabolism of the polluting chemicals in these deformed organisms.

5.3. Metrics used to differentiate stressors

Metrics are important tools as it allow the investigator to use pertinent attributes of the indicators to assess the ecological perturbation of streams and rivers. For a metric to be useful, it must be relevant to the community under investigation and to the specified program objectives and, sensitive: that able to discriminate stressors effect from natural variation (Mandaville, 2002). All the calculated metrics may not have equal importance and hence selections of the most potential metrics that enable to differentiate between stressors are mandatory.

Some metrics respond significantly to one type of stressor and show no or reverse response to the other stressor (Ofenboeck *et al.*, 2004). In our study also stressors are originated from different sources of industries (textile, tannery, alcohol, and beer) that use different raw materials for production and release wastes containing varying concentration of nutrient load, heavy metal and some habitat disruption materials. In addition agricultural activities (sedimentation, nutrient release) and some in stream activities like washing/ bathing, cattle watering and grazing site cause varying degree of perturbation and biological metrics are assumed to be respond differently. Therefore, the following metrics and indices were found to be very important to separate the intensity of pollution and degradation due to anthropogenic activities in streams and rivers around Sebeta town.

A. Total number of individuals/m²

Counting the total number of individuals/m² was able to differentiate the level of impact among the parameters of washing/bathing, agriculture, Brewery effluent and Alcohol effluent impacted sites. High amount of benthic macroinvertebrate individuals were found in Brewery effluent impacted sites. This is because of the Family chironomidae. Chironomids are highly populated in

beer effluent receiving streams as they use the organic load as a source of food and since decreasing competition favor for increasing their population. Alcohol effluent receiving sites support lower number of individuals/m², as these sites have lower dissolved oxygen level (2.77 mg/l) which may below the tolerance level of chironomidae. Streams and rivers affected by anthropogenic activities like organic matter and heavy metal pollution shows reduction of species richness and diversity of macroinvertebrate community and increase the dominance of tolerant taxa (Gray, 1989). Therefore, this metrics can differentiate minimally impacted, highly impacted and extremely impacted sampling sites.

B. Number of taxa (Family)

Taxa Richness indicates the health of the community through its' diversity, and increases with increasing habitat diversity, suitability, and water quality (Plafkin *et al.*, 1989). Number of taxa at family level differentiates streams affected by agriculture, textile and tannery effluents. The lower level of dissolved oxygen in tannery waste receiving site (AB3) provides lower number of taxa while the minimal impact of agriculture able to support higher number of taxa. Solomon Akalu *et al.*, (2011) also found a decrease in the number of taxa at sites experiencing depleted dissolved oxygen, nutrient enrichment and sedimentation. Therefore this metrics can segregate minimally impacted (agriculture and in stream activities) and highly impacted sites (like tannery and textile waste receiving sites).

C. No. of Ephemeroptera individuals

The number of Ephemeroptera was used to differentiate between references, agriculture impacted and textile effluent impacted (highly impacted) sites. This indicates that abundance of this group was dependent on the level of organic load unlike Baye Sitotaw, (2006) found that mild pollution has no effect on the distribution of Ephemeroptera and found similar percentage of Ephemeroptera between reference and minimally impacted sites. In this study the impact of agricultural activities on benthic macroinvertebrate structure was detected through the reduction in abundance of Ephemeroptera than reference site. Reduction in the abundance of Ephemeroptera is indicating perturbation as it is regarded as sensitive to pollution (Ofenboeck *et*

al., 2004). Therefore, these metrics can be used for assessing the impacts of agriculture related activities on the water quality of streams and rivers.

D. No. of Coleoptera, Trichoptera and Ephemeroptera (CTE) individual

The number of CTE (Coleoptera, Trichoptera and Ephemeroptera) was used to distinguish between reference sites, minimally impacted sites (agriculture and washing/ bathing sites) and highly impacted (Textile effluent receiving) sites. As the groups of CTE are considered to be pollution sensitive textile effluent affected site support lower number of CTE. Baye Sitotaw (2006) reported that may fly reduction may be related to general habitat degradation through channelization and riparian habitat loss in addition to organic pollution. Therefore this metrics can be used to identify the level of degradation of streams and rivers and mainly used to show the impact of in stream activities.

E. Number of Oligochaeta individuals

Oligochaeta are found almost in all sampling sites but, their abundance was very important to differentiate the impact of major stressors in streams and rivers around Sebeta town (agriculture and industries). These groups of macroinvertebrates are assumed to be very tolerant to pollution and the textile waste which contain high nutrient load and Cadmium metal support high abundance of this group. Brewery effluent and tannery waste affected streams also support high abundance of Oligochaeta. But agriculture impacted sites support low number of Oligochaeta. This is because the high nutrient load supports only few tolerant taxa and proliferate the number of Oligochaeta. Solomon Akalu *et al.* (2011) also found that increased abundance of Oligochaeta at downstream sites suffering from high nutrient load. Therefore, this metrics can used to know the level impact of industries which release high organic material (textile, beer, and tannery) on the receiving streams and rivers because the abundance difference observed in this study was complimentary to nutrient load of sites.

F. %ET(Ephemeroptera and Trichoptera)

The composition of these taxa differentiates reference and agricultural impacted sites. This metrics only able to separate between minimally impacted and moderately impacted sites because highly impacted sites with high nutrient load and low dissolved oxygen didn't support these taxa. Welch, (1992) stated that although there is a difference in the level of tolerance most species of Ephemeroptera and Trichoptera taxa are intolerant to high nutrient load. Solomon Akalu *et al.*, (2011) also observed total disappearance of these taxa in highly polluted sites of Great Akaki. Therefore, this metrics can be used to know the impact of agricultural and instream activities which cause moderate perturbation on the water quality of streams and rivers.

G. % Diptera individuals

Composition measures provide information on the relative contribution of a certain group to the total number of taxa and healthy habitats will support relatively similar proportion of the taxa (Barbour *et al.*, 1996). In the present study %Diptera vary between sampling sites, which can help to differentiate the intensity of stressors. As most of the Diptera are assumed to be tolerant to pollution and habitat degradation their percentage contribution is important to identify the level of impact that each stressor causes. Their percentage contribution was high at beer effluent receiving stream and then tannery and alcohol effluent waste receiving streams. The result is complimentary to the nutrient load in each sampling site as like number of Oligochaeta (Table 4.1). Therefore, this metrics can be a good indicator of organic load intensity of streams and rivers.

H. Margalef's index

This index was used to differentiate the impact of two major stressors (Tannery and alcohol industries) which were grouped together in both the ordination axis. This shows the potential of the index in analyzing the intensity of human impact on the community structure of benthic macroinvertebrates. Therefore, this index can be a good tool to segregate the impact of major polluting industries (Tannery and Alcohol) which may have the same nutrient load and dissolved oxygen concentration but, may vary by other waste component. In this study, the alcohol waste receiving site support better macroinvertebrate diversity than tannery waste receiving site. This

may be due to toxic effect of the Tannery waste as it contain high amount of NH_4^+ (1.58 mg/l) than alcohol effluent receiving site (0.30 mg/l). Therefore this index can be a good tool to see toxicity effect of industries on the receiving streams and rivers in addition to organic pollution.

I. Hilsenhoff Family Biotic Index

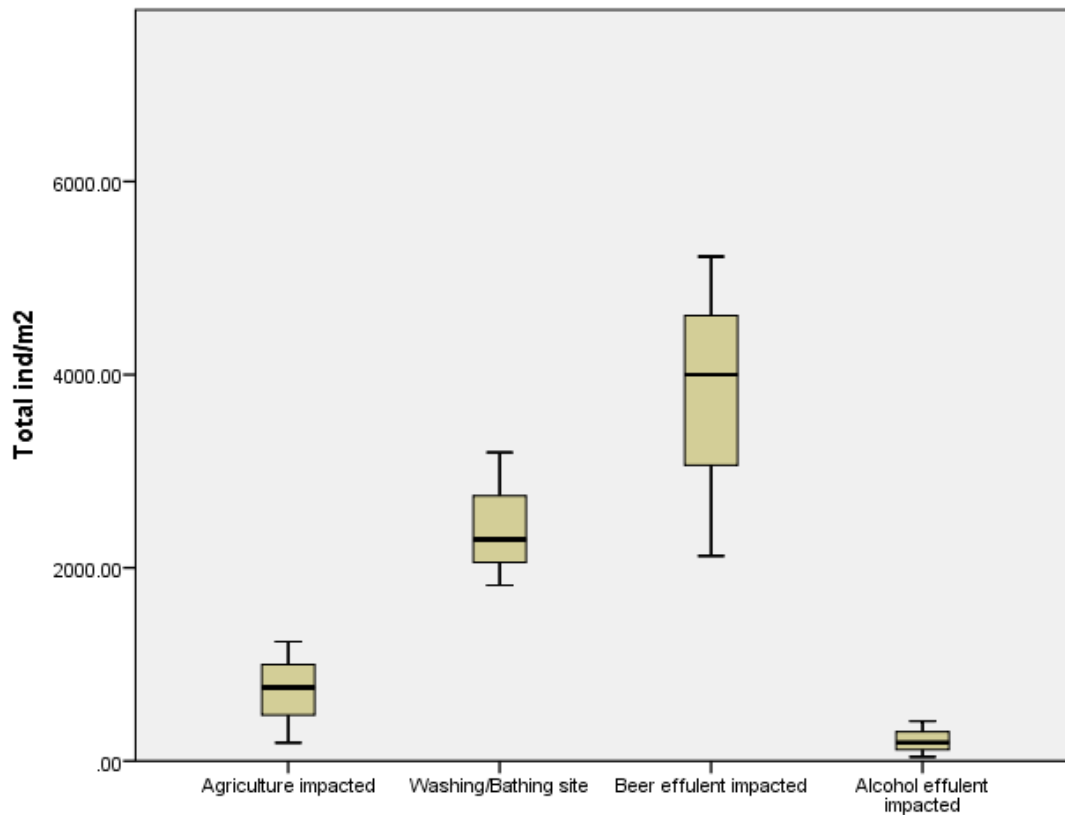
Hilsenhoff Family Biotic Index summarizes the overall pollution tolerances of the taxa in the sampling site. In this study, this index was able to separate the impact of different stressors originating from different sources (agriculture and industry) in streams and rivers around Sebeta. According to FBI value, the alcohol waste receiving site was highly dominated by very tolerant taxa and highly deteriorated while agriculture impacted site possess lower FBI value and dominated by pollution sensitive taxa and the other stressors are in between these two. This index is believed to detect nutrient enrichment, high sediment loads, low dissolved oxygen, and thermal impacts. Therefore, it is a very important tool to identify the impact of stressors originating from different source on the water quality and habitat integrity of streams and rivers.

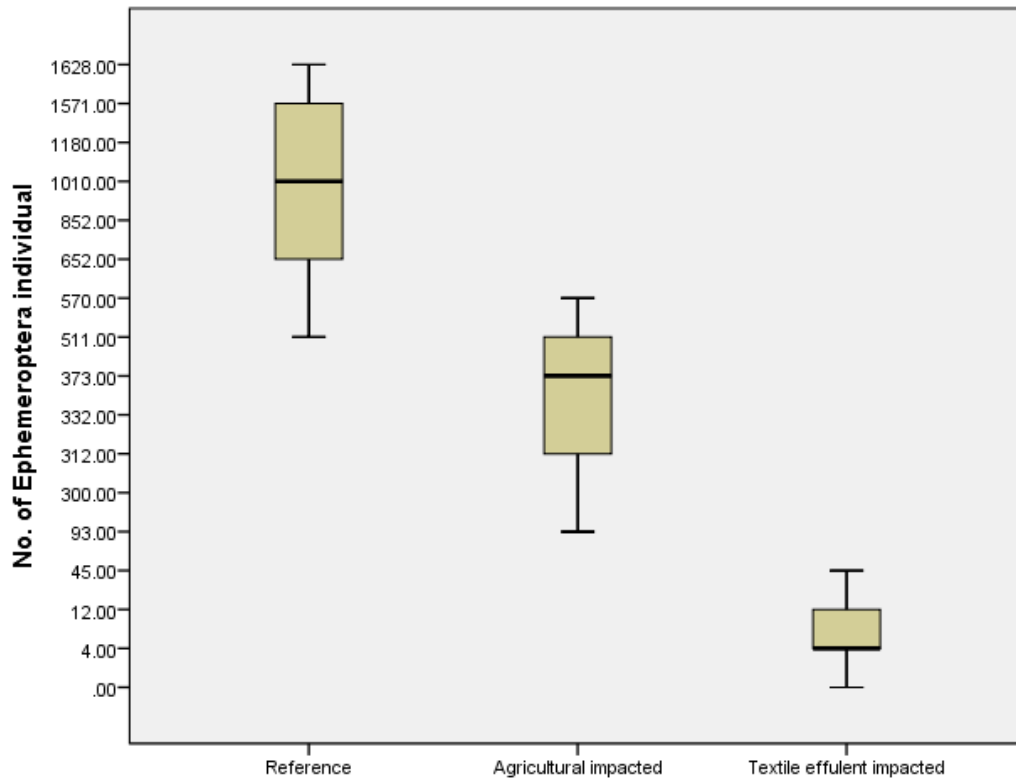
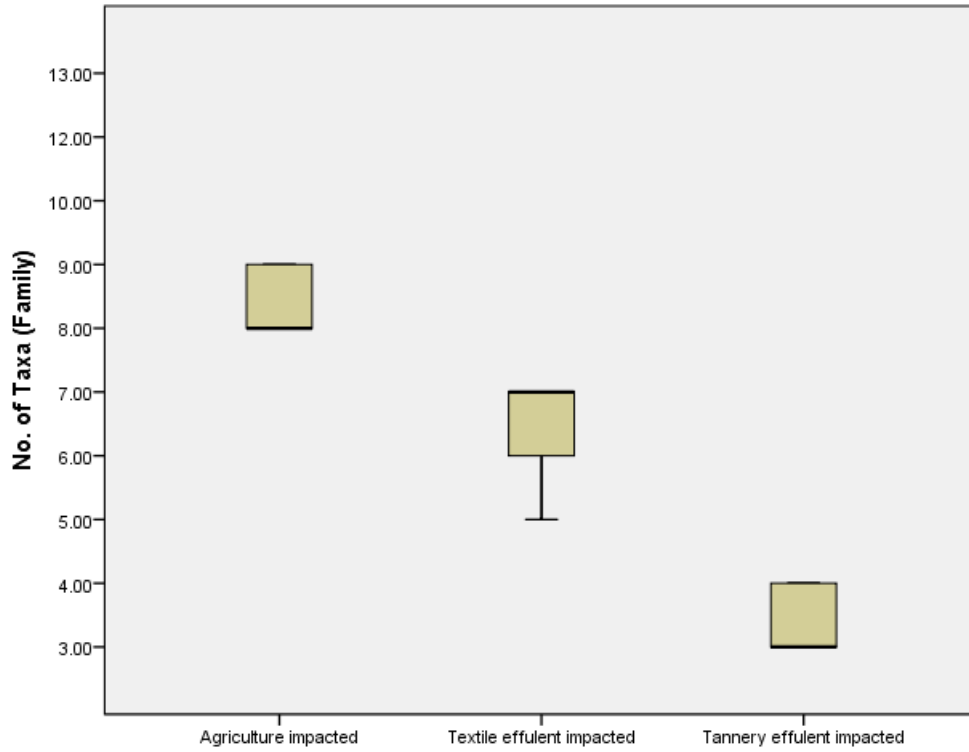
J. ETHbios

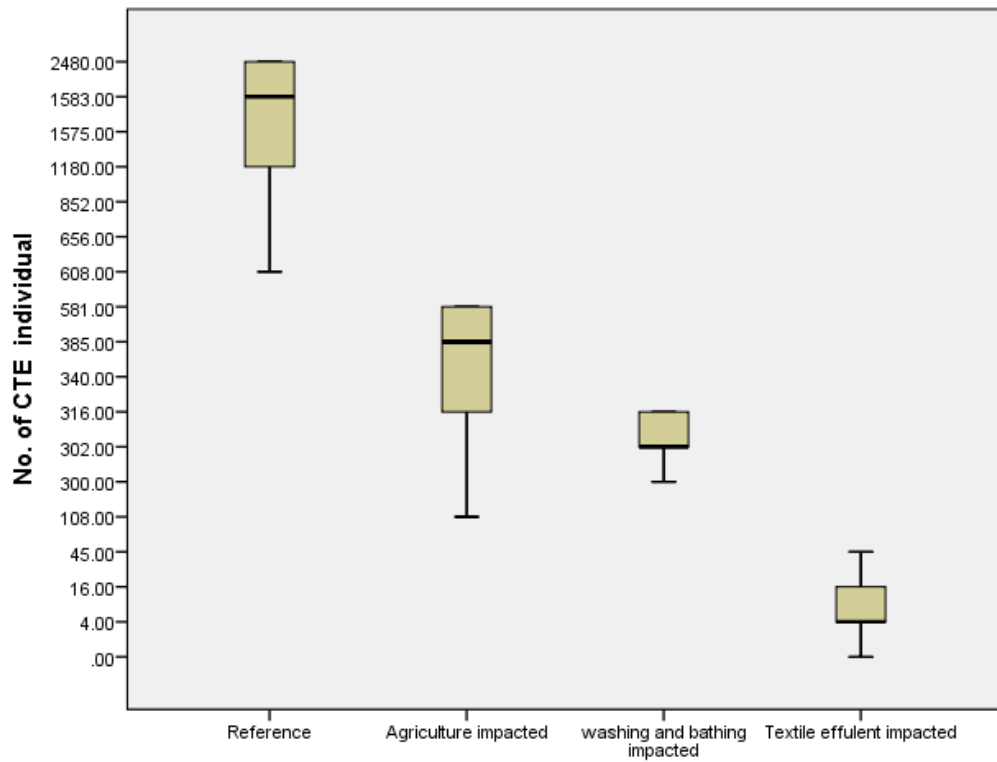
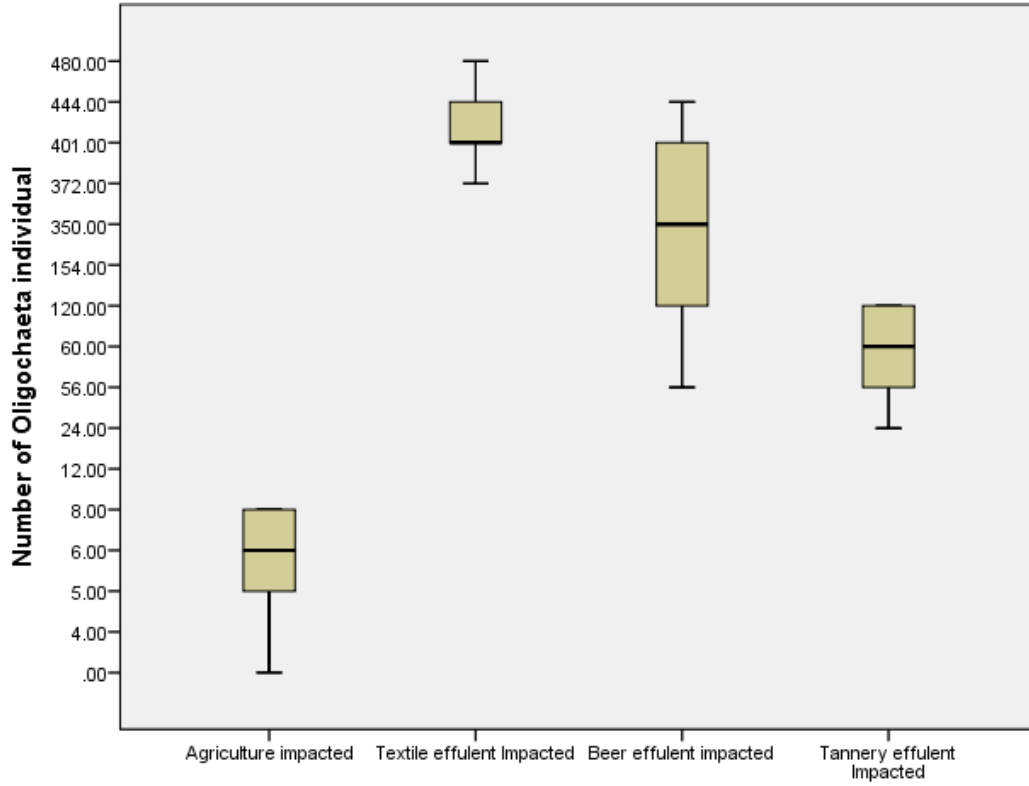
This scoring system is developed for assessing anthropogenic impacts on streams and rivers in central highlands of Ethiopia. The scoring system gives a score value of 1-10 in which tolerant benthic macroinvertebrates have lowest scoring value and the sensitive taxa possessing highest scoring value. The aggregate of each taxa score gives the water quality class of a site. It takes in to account organic pollution and different instream activities that affect the water quality and ecological health of streams and rivers.

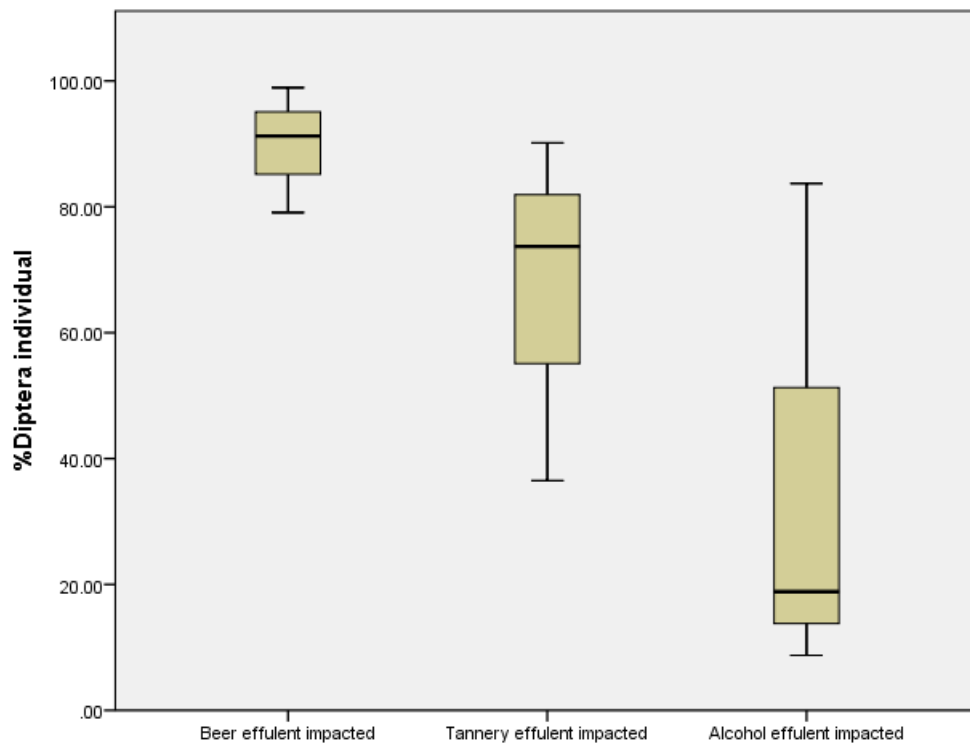
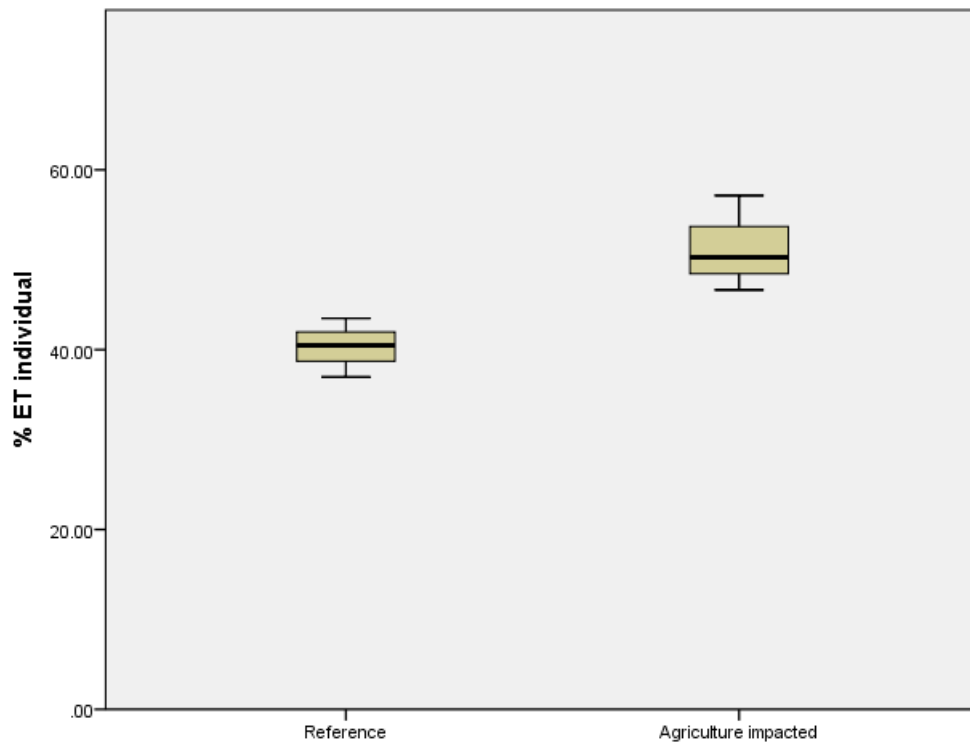
In this study it explicitly separates each potential stressor from the reference site and to other respective stressors. Each stressor is 100% separated from the other one. ETHbios able to show the intensity of each stressor on the receiving streams in the order of reference < agricultural impacted < washing/bathing < textile impacted < tannery impacted. Therefore, this index can able to detect the level of stream or river degradation or effectiveness of some management action as it responds to any kind of disturbance.

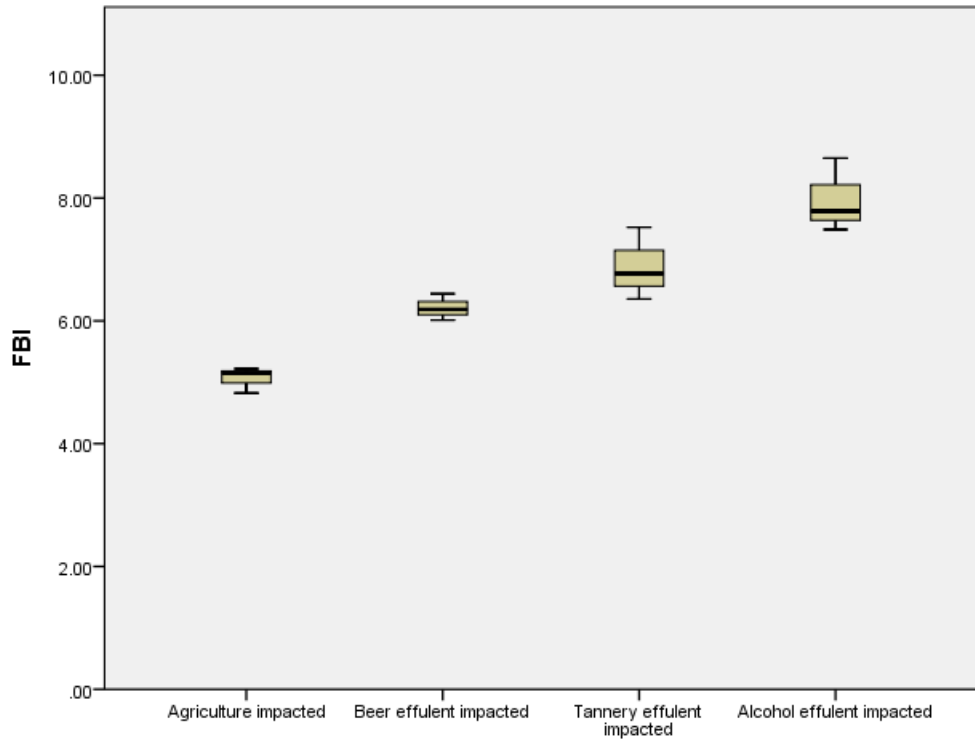
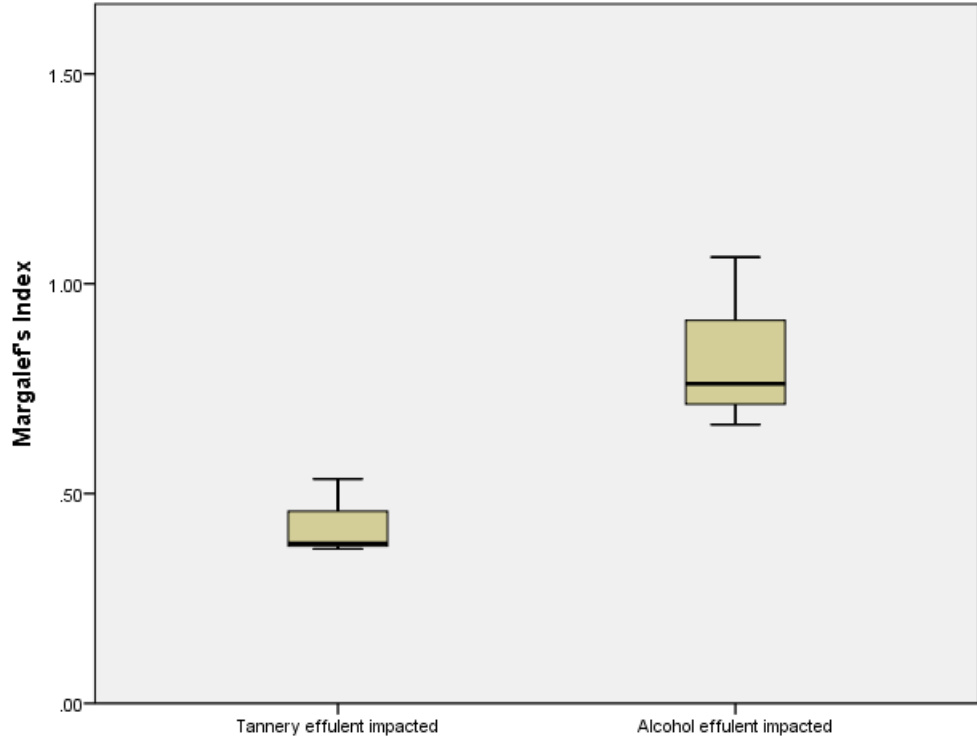
The box plots clearly show that metrics and indices can explicitly separate stressors based on their source and intensity of pollution or degradation to streams and rivers (Figure 5.6). Metrics composed of sensitive taxa able to separate reference and minimally impacted sites (agriculture, washing/bathing, cattle watering site) while metrics composed of tolerant taxa was able to differentiate the intensity of pollution between highly impacting industrial wastes. Sensitivity score metrics were able to separate the intensity of degradation from minimally impacted to extremely impacted sites.











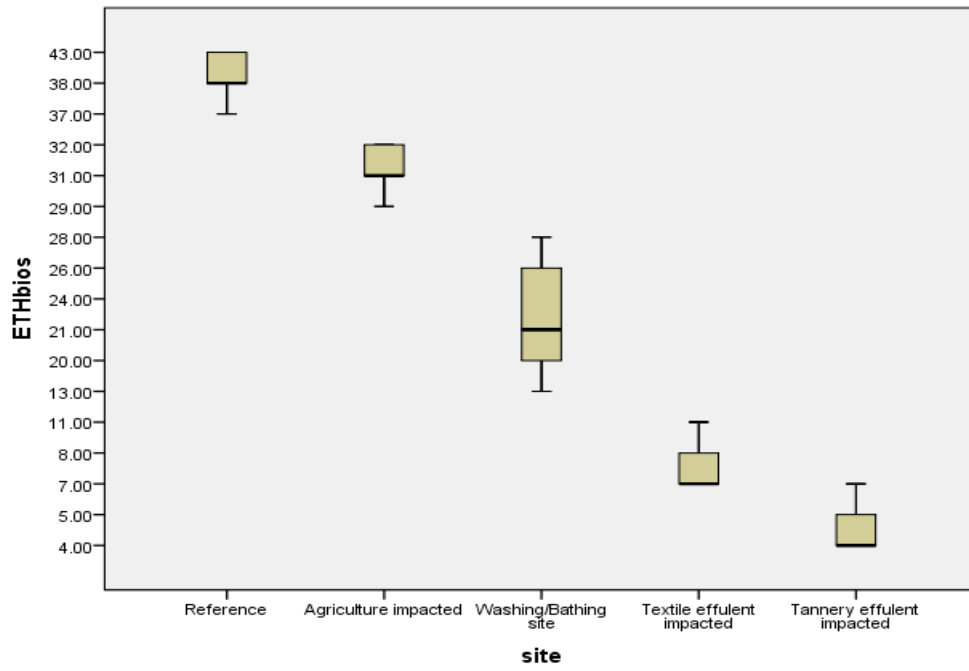


Figure 5. 1. Box plot illustration of some benthic macroinvertebrate metrics along different stressors

6 Conclusions and Recommendations

6.1 Conclusions

Most of the measured physicochemical parameters were higher in industry waste receiving sites. Very depleted dissolved oxygen concentration (<2mg/l) were recorded at sites receiving alcohol and tannery wastes (AB2 and AB3), that resulted in low diversity and abundance of benthic macroinvertebrates. Cadmium metal concentration was higher at textile and tannery effluent receiving sites with eight and seven times higher than the natural system concentration. Kersa stream (KR1) which was considered as reference site was used for household activities including drinking. However, sampling sites receiving industrial effluents are highly deteriorated and it was even difficult to walk around due to their pungent and irritating smell, which clearly indicated the severe impact of urban and industrial wastes on the water quality and ecological health of receiving streams and rivers. These highly impacted streams and rivers are tributaries of River Awash, which may support many downstream population and development activities. Therefore, due attention should be given on the establishment of industries by conducting environmental impact assessment. In addition, efficient waste treatment plant should be a mandatory for each industry before establishment.

The wide distribution and abundance of macroinvertebrates clearly indicates the variation of their tolerance to anthropogenic activities and their potential use as stressor indicator. Family Planariidae, Caenidae, Baetidae, Simuliidae, Notonetidae, Naucoridae, Corixidae, Hydropsychidae, Dytiscidae, Gyrinidae, Tipulidae, Musidae, Culicidae and Hydrophilidae were dominant in minimally impacted streams and rivers and can be an indicator of good water quality. Syrphidae and Thiaridae were dominant in highly turbid sampling sites and can be an indicator of turbid streams and rivers. Oligochaeta, Lymnaeidae, Psychodidae and chironomidae were dominant in high organic load streams and rivers (high concentration of NH_4^+ and TP) and can be an indicator highly polluted streams and rivers.

From Family Chironomidae taxa *Chironomus alluaudi* and *Chironomus imicola* were more prevalent in high phosphorus and ammonium containing streams and rivers and can be an indicator of high organic pollution. *Polypedilum bipustulatum*, *Polypedilum wittei* and *Dicrotendipus septemmaculatus* became dominant in intermediate oxygen concentration and organic load (high NH_4^+ and TP) level and can be an indicator of intermediate organic pollution. *Chironomus calipterus* and genus Conchapelopia were abundant in streams and rivers rich in dissolved oxygen concentration and can be an indicator of good water quality.

Metrics and indices can separate the intensity of anthropogenic activities on the ecological health of receiving aquatic ecosystem. Metrics composed of sensitive group of taxa (No. of Ephemeroptera, No. CET and %ET) can enable investigators to differentiate reference sites and agricultural impacted sites and some in stream activities. Tolerant taxa metrics like number of Oligochaeta and %Diptera distinguish the intensity of highly impacting industrial stressors (tannery, beer, textile and alcohol) on the water quality of receiving streams and rivers. Margalefs index may detect toxic effect of industrial wastes in addition to organic pollution. Total number of ind/m², number of Taxa (Family), ETHbios, FBI can be able to segregate stressors originated from different sources (agriculture, washing/bathing and industries).

6.2 Recommendations

- As Ethiopia is a rapidly developing country, with plans of agricultural led industrialization economy, there will be immense development activities that may aggravate the deterioration of fresh water bodies. As a result of such development pace today streams and rivers around Sebeta town are highly deteriorated due to industrial and urban wastes. Therefore, it is recommended that adequate waste treatment structures be put in place at the industries and factories located along these rivers and streams waste.
- As human impacts are diverse and wastes released differ in quantity and quality, stressor specific studies are mandatory. This is to know the impact of a particular human activity on the overall health of aquatic ecosystem and to take prior management action. Nevertheless, in this study tannery, brewery, textile and alcohol industries were major stressors with different degree of impact and metrics that able to distinguish each stressor from the other were identified.
- It would be important to analyze most physiochemical parameters and toxicity tests to better establish relationship between the community structures of benthic macroinvertebrates.
- The impacts of water quality degradation due to stressors on the lifestyle of beneficiaries like farmers, fishermen, and domestic water consumers, have not been assessed. Therefore, studies should be conducted on this issue to see the cost benefit analysis of development activities.
- Lower taxonomic identification of chironomids able to separate sites with varying environmental characteristics and it has been suggested to identify some other cosmopolitan macroinvertebrate taxa (Baetidea, Oligochaeta and Notonetidae) to genus/species level to better use their bioindicator value because family level qualitative data of this taxa didn't give important information on the status of a water body. As it has also suggested that finer taxonomic resolution gives better picture of the ecosystem health Li *et al.* (2010).
- In this study mouth part deformity was observed in Sub family Chironominae. But, the deformity was both at minimally impacted upstream sites (AB1 and GR1) and heavily

impacted textile effluent receiving sites (GR2) and wasn't included as water quality assessment tool. Future research should focus on the bioindicator value deformity. This is by focusing on percentage of deformity, the deformed teeth part, the habitat and feeding habit of the organism and other situations that may cause deformity.

7 Reference

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8 Annexes

8.1 Spearman's Correlation between physicochemical parameters and benthic macroinvertebrate metrics

Metrics	physicochemical parameters									
	Temp (0c)	pH	O2(mg/l)	O2 (%)	Conductivity (µS/cm)	Turbidity(NTU)	NH ₄ ⁺ (mg/l)	NO ₃ ⁻ (mg/l)	SRP (mg/l)	TP(mg/l)
Total No. of indi/m2	0.016667	0.51667	0.2	0.11667	0.21667	-0.21667	0.15063	0.43414	0.38333	0.16667
Total No. of Taxa (family)	-0.52778	0.50224	0.87679	0.91084	-0.57034	-0.3405	-0.77789	-0.05217	-0.72357	-0.80869
Total No. of Taxa_ with chiro sp.	-0.31799	0.26778	0.71967	0.77825	-0.65273	-0.23431	-0.70168	-0.05129	-0.55231	-0.78662
No. of Ephemeroptera_	-0.23732	0.37293	0.69502	0.81368	-0.76282	-0.18647	-0.68942	-0.02597	-0.66111	-0.84758
No. Trichoptera_	-0.63369	0.46537	0.61389	0.57429	-0.38616	-0.23764	-0.56675	-0.177	-0.6535	-0.6634
No. of Coleoptera_	-0.54131	-0.6147	0.77067	0.74315	-0.50461	-0.50461	-0.81537	-0.09372	-0.41286	-0.62388
No. of ET taxa	-0.39857	0.49388	0.71916	0.8318	-0.64118	-0.16463	-0.62647	-0.09294	-0.84046	-0.80581
No. of CTE taxa	-0.54587	0.63251	0.85779	0.91845	-0.59786	-0.34658	-0.75263	-0.06196	-0.76248	-0.80581
No. of ET indiv/m2	-0.23732	0.37293	0.69502	0.81368	-0.76282	-0.18647	-0.68942	-0.02597	-0.66111	-0.84758
No.CTE ind/m2	-0.23732	0.37293	0.69502	0.81368	-0.76282	-0.18647	-0.68942	-0.02597	-0.66111	-0.84758
Number of Oligochaeta	0.5021	0.7113	-0.54394	-0.59415	0.85356	0.37657	0.81933	0.42314	0.82846	0.66109
No. of Chironomidae_	0.016667	0.3	0.066667	0.033333	0.11667	-0.28333	0.22594	0.34902	0.38333	0.3

No. of Tolerant taxa	0.016667	0.51667	0.2	0.11667	0.21667	-0.21667	0.15063	0.43414	0.38333	0.16667
No. of Intolerant Taxa	-0.23735	0.20083	0.63901	0.7303	-0.71204	-0.10954	-0.46752	0.20515	-0.76681	-0.89461
%Ephemeroptera	-0.15256	0.47464	0.71197	0.89843	-0.74587	-0.22037	-0.57026	0.12987	-0.83063	-0.72892
%Trichoptera	-0.7303	0.47926	0.70747	0.59337	-0.11411	-0.38797	-0.48127	0.069938	-0.38797	-0.54772
%_ET_taxa	-0.33199	0.50224	0.83423	0.96192	-0.65547	-0.28091	-0.5941	0.2	-0.80869	-0.81721
%Diptera	-0.21667	0	-0.21667	-0.33333	0.38333	-0.31667	0.28452	-0.0681	0.6	0.63333
%CTE	-0.33199	0.50224	0.83423	0.96192	-0.65547	-0.28091	-0.5941	0.2	-0.80869	-0.81721
%_Tolerant taxa	0.34689	0.23735	-0.65727	-0.71204	0.67552	0.1278	0.52252	-0.1119	0.74855	0.91287
%_Intolerant taxa	-0.34689	0.23735	0.65727	0.71204	-0.67552	-0.1278	-0.52252	0.1119	-0.74855	-0.91287
%Dominant taxa	0.45	0.66667	-0.65	-0.73333	0.65	0.2	0.57741	0	0.83333	0.85
%_Chironomidae and Oligochaeta	0.4	0.58333	-0.75	-0.83333	0.68333	0.2	0.72804	0.042563	0.88333	0.9
Ratio of _ET/Chironomidae	-0.50461	0.46791	0.77985	0.81655	-0.54131	-0.29359	-0.53897	0.16401	-0.80737	-0.7982
Margalef's_Index	-0.31667	0.36667	0.7	0.76667	-0.68333	-0.18333	-0.82846	-0.23835	-0.61667	-0.81667
Shannon Wiener index	-0.47699	0.62762	0.61088	0.67783	-0.67783	-0.15063	-0.61765	-0.1154	-0.82009	-0.89541
Family biotic index	0.41667	0.4	-0.78333	-0.83333	0.63333	0.18333	0.77825	0.11918	0.83333	0.88333
ETHbios	-0.46667	0.61667	0.71667	0.81667	-0.65	-0.16667	-0.67783	-0.15323	-0.81667	-0.86667
ASPT-ETHbios	-0.46667	0.61667	0.71667	0.81667	-0.65	-0.16667	-0.67783	-0.15323	-0.81667	-0.86667
SASS	-0.54624	0.49582	0.916	0.94121	-0.57145	-0.43699	-0.70465	0.12877	-0.63868	-0.79835

ASPT-SASS	-0.57741	0.54394	-	0.90377	0.92888	-0.57741	-0.46026	-0.68487	0.12822	-0.62762	-0.78662
BMWP	-0.58333	0.53333	-	0.88333	0.91667	-0.58333	-0.41667	-0.65273	0.11066	-0.7	-0.8
ASPT-BMWP	-0.54394	0.57741	-	0.80335	0.86193	-0.64436	-0.42678	-0.64706	0.008548	-0.81172	-0.69457
No. of chironomidae/m2	0.016667	0.3	0.066667	0.033333	0.11667	-0.28333	0.22594	0.34902	0.38333	0.3	0.3
No. of chironominae taxa	0.10492	0.1399	0.21859	0.2798	-0.42844	-0.11367	-0.2678	0.040193	-0.04372	-0.26231	-0.26231
No. of Chironominae indiv.	0.2	0.41667	-0.16667	-0.18333	0.18333	-0.13333	0.3682	0.29794	0.5	0.43333	0.43333
No. of Tanypodinae indiv.	-0.23732	0.33903	-	0.71197	0.84758	-0.76282	-0.13561	-0.6043	0.069265	-0.77977	-0.89843
No. of Chironomus	0.37657	0.5272	-0.29289	-0.26778	0.20921	0	0.4916	0.32483	0.45189	0.51046	0.51046
%Tanypodinae	-0.4916	0.59331	0.81368	0.89843	-0.61026	-0.28818	-0.68091	-0.03463	-0.83063	-0.79672	-0.79672
%_of chironominae	0.4916	0.59331	-0.81368	-0.89843	0.61026	0.28818	0.68091	0.034632	0.83063	0.79672	0.79672
%_Chironomus	0.6975	0.61347	-0.88238	-0.85717	0.55464	0.52103	0.82701	0.12877	0.63868	0.70591	0.70591
No. of Chironomus/total individual	0.43333	0.41667	-0.55	-0.5	0.43333	0.15	0.77825	0.31496	0.61667	0.7	0.7
%Tanypodinae/chironomidae	-0.51121	0.47469	-	0.74855	0.78507	-0.54772	-0.27386	-0.55002	0.09325	-0.82158	-0.78507
Chironomus/chironominae	0.31496	0.14471	-0.42563	-0.41712	0.57034	0.19579	0.64112	0.33913	0.29794	0.55332	0.55332

8.2 Biotic score values of benthic macroinvertebrates

A. South Africa Scoring System (SASS)

Taxon	Score	Taxon	Score	Taxon	Score
PORIFERA	5	HEMIPTERA		DIPTERA	
COELENTERATA	1	Belostomatidae*	3	Athericidae	10
TURBELLARIA	3	Corixidae*	3	Blepharoceridae	15
ANNELIDA		Gerridae*	5	Ceratopogonidae	5
Oligochaeta	1	Hydrometridae*	6	Chironomidae	2
Leeches	3	Naucoridae*	7	Culicidae*	1
CRUSTACEA		Nepidae*	3	Dixidae*	10
Amphipoda	13	Notonectidae*	3	Empididae	6
Potamonautidae*	3	Pleidae*	4	Ephydriidae	3
Atyidae	8	Veliidae/M...veliidae*	5	Muscidae	1
Palaemonidae	10	MEGALOPTERA		Psychodidae	1
HYDRACARINA	8	Corydalidae	8	Simuliidae	5
PLECOPTERA		Sialidae	6	Syrphidae*	1
Notonemouridae	14	TRICHOPTERA		Tabanidae	5
Perlidae	12	Dipseudopsidae	10	Tipulidae	5

EPHEMEROPTERA		Ecnomidae	8	GASTROPODA	
Baetidae 1sp	4	Hydropsychidae 1 sp	4	Ancylidae	6
Baetidae 2 sp	6	Hydropsychidae 2 sp	6	Bulininae*	3
Baetidae > 2 sp	12	Hydropsychidae > 2 sp	12	Hydrobiidae*	3
Caenidae	6	Philopotamidae	10	Lymnaeidae*	3
Ephemeridae	15	Polycentropodidae	12	Physidae*	3
Heptageniidae	13	Psychomyiidae/Xiphocen	t8	Planorbinae*	3
Leptophlebiidae	9	Cased caddis:		Thiaridae*	3
Oligoneuridae	15	Barbarochthonidae SWC	13	Viviparidae* ST	5
Polymitarcyidae	10	Calamoceratidae ST	11	PELECYPODA	
Prosopistomatidae	15	Glossosomatidae SWC	11	Corbiculidae	5
Teloganodidae SWC	12	Hydroptilidae	6	Sphaeriidae	3
Tricorythidae	9	Hydrosalpingidae SWC	15	Unionidae	6
ODONATA		Lepidostomatidae	10	SASS Score= sum of taxa score	
Calopterygidae ST,T	10	Leptoceridae	6	ASPT= SASS score/No. of Taxa	
Chlorocyphidae	10	Petrothrincidae SWC	11		
Chlorolestidae	8	Pisuliidae	10		
Coenagrionidae	4	Sericostomatidae SWC	13		
Lestidae	8	COLEOPTERA			
Platycnemidae	10	Dytiscidae*	5		

Protoneuridae	8	Elmidae/Dryopidae*	8		
Aeshnidae	8	Gyrinidae*	5		
Corduliidae	8	Haliplidae*	5		
Gomphidae	6	Helodidae	12		
Libellulidae	4	Hydraenidae*	8		
LEPIDOPTERA		Hydrophilidae*	5		
Pyralidae	12	Limnichidae	10		
		Psephenidae	10		

B. ETHiobios Tolerance value of benthic macroinvertebrates

Taxa	Score
Perlidae (Neoperla sp.), Philopotamidae, Lepidostomatidae, Scirtidae,	10
Heptageniidae (Afronurus sp.), Leptophlebiidae, Acanthiops sp., Hydropschidae(>2sp.) , Baetidae (>2sp.),	9
Hydracarina, Dryopidae, Tricorythidae, Leptoceridae, Psephenidae, Ecnomidae, Hydraenidae, Stenelmis sp., Microdinodes sp., Lestidae	8
Pisidium sp., Potamidae, Aeshnidae, Elmidae, Tipulidae,	7
Limpets (Ancylus, Burnupia), Tabanidae, Gomphidae, Caenidae, Baetidae (2 sp.), Naucoridae, Hydropschidae(2 sp.)	6
Gyrinidae, Haliplidae, Hydropschidae(1 sp.), Mesoveliidae, Veliidae, Gerridae, Dytiscidae, Hydrophilidae, Libellulidae, Ceratopogonidae excl. Bezzia-Gr.,	5

Corixidae, Coenagrionidae, Baetidae (1sp.), Pleidae	4
Bulimus sp., Bezzia-Group, Salifidae, Leeches, Belostomatidae, Notonectidae, Nepidae,	3
Musidae, Physidae, Chironomidae with predominantly Tanytarsini.and.Chironomini	2
Psychodidae soft/white, Ephydriidae, Oligochaeta (many or masses), Culicidae, Chironomidae (red), Syrphidae	1
ETHbios= sum of tolerance value of taxa	
ASPT-ETHbios=ETHbios/total No.of Taxa	

C. Biological Monitoring working party (BMWP) tolerance score of benthic macroinvertebrates

Group	Families	Score
Mayflies, Stoneflies, Riverbug, Caddisflies or Sedgeflies	Siphonuridae, Heptageniidae, Leptophlebiidae, Ephemerellidae, Potamanthidae, Ephemeridae, Taeniopterygidae, Leuctridae, Capniidae, Perlodidae, Perlidae, Chloroperlidae, Aphelocheridae, Phryganeidae, Molannidae, Beraeidae, Odontoceridae, Leptoceridae, Goeridae, Lepidostomatidae, Brachycentridae, Sericostomatidae	10
Crayfish, Dragonflies	Astacidae, Lestidae, Agriidae, Gomphidae, Cordulegasteridae, Aeshnidae, Corduliidae, Libellulidae	8
Mayflies, Stoneflies, Caddisflies or Sedgeflies	Caenidae, Nemouridae, Rhyacophilidae, Polycentropodidae, Limnephilidae	7

Snails, Caddisflies or Sedge flies, Mussels, Gammarids, Dragonflies	Neritidae, Viviparidae, Ancyliidae, Hydroptilidae, Unionidae, Corophiidae, Gammaridae, Platycnemididae, Coenagrionidae	6
Bugs, Beetles, Caddisflies or Sedgeflies, Craneflies/Black flies, Flatworms	Mesoveliidae, Hydrometridae, Gerridae, Nepidae, Naucoridae, Notonectidae, Pleidae, Corixidae, Haliplidae, Hygrobiidae, Dytiscidae, Gyrinidae, Hydrophilidae, Clambidae, Helodidae, Dryopidae, Elmidae, Chrysomelidae, Curculionidae, Hydropsychidae, Tipulidae, Simuliidae, Planariidae, Dendrocoelida	5
Mayflies, Alderflies, Leeches	Baetidae, Sialidae, Piscicolidae	4
Snails, Cockles, Leeches, Hog louse	Valvatidae, Hydrobiidae, Lymnaeidae, Physidae, Planorbidae, Sphaeriidae, Glossiphoniidae, Hirudidae, Erpobdellidae, Asellidae	3
Midges	Chironomidae	2
Worms	Oligochaeta (whole class)	1
BMWP= the sum of tolerance value of taxa		
ASPT- BMWP= BMWP/Total number of Taxa		

D. Tolerance values of macroinvertebrates in the Family Biotic Index(FBI)

Plecoptera	Score	Trichoptera	score	Amphipoda	Score
Capniidae	1	Brachycentridae	1	Gammaridae	4
Chloroperlidae	1	Calamoceratidae	3	Hyalellidae	8
Leuctridae	0	Glossosomatidae	0	Talitridae	8
Nemouridae	2	Helicopsychidae	3		
Perlidae	1	Hydropsychidae	4	Isopoda	
Perlodidae	2	Hydroptilidae	4	Asellidae	8
Pteronarcyidae	0	Lepidostomatidae	1		
Taeniopterygidae	2	Leptoceridae	4	Decapoda	6
		Limnephilidae	4		
Ephemeroptera		Molannidae	6	Acariformes	4
Baetidae	4	Odontoceridae	0		
Baetiscidae	3	Philpotamidae	3	Mollusca	
Caenidae	7	Phryganeidae	4	Lymnaeidae	6
Ephemerellidae	1	Polycentropodidae	6	Physidae	8
Ephemeridae	4	Psychomyiidae	2	Sphaeridae	8
Heptageniidae	4	Rhyacophilidae	0		
Leptophlebiidae	2	Sericostomatidae	3		
Metretopodidae	2	Uenoidae	3		
Oligoneuriidae	2				
Polymitarcyidae	2				
Potomanthidae	4				
Siphonuridae	7				
Tricorythidae	4				
		Diptera			
		Athericidae	2		
		Blephariceridae	0		

		Ceratopogonidae	6		
		Blood-red Chironomidae	8		
		(Chironomini)			
Odonata		Other Chironomidae	6		
		(including pink)			
Aeshnidae	3	Dolichopodidae	4		
Calopterygidae	5	Empididae	6		
Coenagrionidae	9	Ephydriidae	6		
Cordulegastridae	3	Muscidae	6		
Corduliidae	5	Psychodidae	10	Oligochaeta	8
Gomphidae	1	Simuliidae	6		
Lestidae	9	Syrphidae	10	Hirudinea	
Libellulidae	9	Tabanidae	6	Bdellidae	10
Macromiidae	3	Tipulidae	3	<i>Helobdella</i>	10
Megaloptera		Coleoptera		Polychaeta	
Corydalidae	0	Dryopidae	5	Sabellidae	6
Sialidae	4	Elmidae	4		
		Psephenidae	4		
Lepidoptera				Turbellaria	4
Pyralidae	5	Collembola		Platyhelminthidae	4
		<i>Isotomurus</i> sp.	5		
Neuroptera				Coelenterata	
Sisyridae				Hydriidae	
<i>Climacia</i> sp.	5			<i>Hydra</i> sp.	5
$FBI = \sum X_i * t_i / n$ <p>x = number of individuals within a taxon t; t = tolerance value of a taxon, n = total number of organisms in the sample.</p>					