JIMMA UNIVERSITY
COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES
DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCE & TECHNOLOGY

RELATIONSHIP OF MACROINVERTEBRATE COMMUNITIES AND FECAL COLIFORM ORGANISMS: AS AN ALTERNATIVE MEANS TO PREDICT FECAL CONTAMINATION, GILGEL GIBE WATERSHED, SOUTHWEST ETHIOPIA

BY
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THESIS SUBMITTED TO DEPARTMENT OF ENVIRONMENTAL SCIENCE & TECHNOLOGY, COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCE, JIMMA UNIVERSITY; IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTERS DEGREE IN ENVIRONMENTAL HEALTH SCIENCE

RELATIONSHIP OF MACROINVERTEBRATE COMMUNITIES AND FECAL COLIFORM ORGANISMS: AS AN ALTERNATIVE MEANS TO PREDICT FECAL CONTAMINATION

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Jimma, Ethiopia

Declaration

I, the undersigned, declare that this thesis was my original work, has not been presented for a degree in this or other university and that all sources of materials used for this have been acknowledged.

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This thesis has been submitted for examination with my approval as university advisors

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1. Dr. Argaw Ambelu _____
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Abstract

Water quality assessments in pollution profiles of rivers and streams have been undertaken in different parts of Ethiopia, using fecal coliform, physicochemical and macroinvertebrate as indicator organisms However, there are no studies on assessment of relationship between macroinvertebrate diversity and fecal Coliform counts. Therefore, this study was aiming to show the relationship of fecal coliform with macro invertebrates, so that they could be a potential indicator for fecal contamination of streams and rivers. For this purpose Field assessment and laboratory based cross-sectional study was conducted from June to September 2012.

Macroinvertebrate assemblage score together with the data of fecal Coliform and physicochemical parameters all together analyzed using SPSS statistical software. One-way ANOVA was computed to identify mean difference among sites .Also Bivariate Pearson correlation, regression and correspondence analysis were used to describe relationship of fecal coliform with macroinvertebrate indices, families and physicochemical parameters. Results of BMWP, FLBI and ASPT showed that all the study sites were in the range of poor water quality. But Gibe Seka had relatively better water quality while Mite Seka had more poor water quality. There was significant mean difference among sample sites in Coliform colonies (p=0.00) at significance level of 0.05.

The count of fecal Coliform was positively correlated with % CHIR (r= 0.77, p=0.003) and FLBI (r=0.6,p=0.016) while negatively correlated with BMWP(r=-0.591,P=0.021) Chironomidae,Gomphidae,sphaeridae,Coenagridae and Ashinidae were observed in sites where high fecal coliform colonies were counted while Cordullidae, Tripulidae Leptoceridae were in streams that had releatively low Fecal coliform colonies .

Fecal Coliform count was significantly correlated negatively with Turbidity(r=-0.899, p=0.000) and water T^{O}(r=-0.619, p=0.032). In contrast, positively correlated with EC(r=0.591, p=0.043) and Nitrate(r=0.794, p=0.002).Generally based on this finding and with further model development ,relationships will be used in areas where peoples who have been used surface water for drinking and not have well equipped laboratory for bacteriological analysis.

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Abbreviations

ASPT	Average score per taxon
BMI	Benthic Macro invertebrate Indices
BMWP	Biological monitoring working party
CHIR	Chironomide
DO	Dissolved Oxygen
EPA	Environmental Protection Agency
H-FBI	Hilsenhoff Family Level Biotic Index
ML	Mill liter
SDI	Shannon Diversity Index
SPSS	Statistical Package for Social Sciences
UNEP	United Nations Environmental Program
UNESCO	United Nations Educational, Scientific and Cultural Organization
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

CHAPTER ONE
INTRODUCTION

1.1. Background

Water is the basic component of life. About 70% of the earth's surface is water, and 3% of this is fresh water. Yet, out of this small portion of fresh water, high portion of fresh water (99%) is unavailable for utilization (Jarrett, 1995). Clean water is an essential resource for drinking, irrigation, industry, transportation, fishing, support of biodiversity, recreation and esthetic enjoyment (UNEP/DFID, 2003).

Industrialization and increase in human population both have resulted in greater demands of high quality water for range of activities. In addition, the scale and diversity of human activities such as agriculture, urbanization, and industry have increased rapidly. As human activities and industrialization increased, the water pollution problem becomes more critical, since these things result in habitat loss and the excessive addition of pollutants into the water bodies; and this affects the use and the natural balance of the aquatic ecosystem (Richards & Bacon, 1994).

Worldwide the scarcity of sanitary waste disposal facility and clean water for drinking, cooking, and washing is responsible for over 12 million deaths each year (USAID, 2008). The most common cases to human health related to water is due to pathogens such as viruses, bacteria, and protozoa. Most of the time these pathogens originate from water polluted with human excrement (Revenga & Mock, 2000). Human feces can contain a variety of intestinal pathogens that may cause diseases such as amoebic dysentery, bacillary dysentery, diarrheal diseases, cholera, hepatitis-A, paratyphoid and typhoid and polio (POPLINE, 2000).

Pathogens and contaminants related with the discharge of sewage ,agricultural runoff and domestic waste water when released in to water bodies such as rivers may present the above mentioned health risks to users (Chapman, 1996). Therefore, water sources such as streams and rivers need regular monitoring to improve their quality so that they will provide the intended service without affecting human health. There have been different methods of water quality monitoring using a number of indicators such as physicochemical parameter, macroinvertebrates and fecal coliforms.

1.2. Statement of the problem

Water quality is a critical factor affecting human health. Studies showed that approximately 3.1% of deaths (1.7 million) and 3.7% of disability-adjusted-life-years (54.2 million) worldwide are attributable to unsafe water, poor sanitation and hygiene (WHO, 2005).

Disease and mortality are not the only consequences of polluted and scarce water. Less attention is paid to the fact that women and children bear much of the cost of dirty water and water shortages. Children are more likely to become ill, and women have to look after them. Women and girls carry out most water collection, and many spend long hours that could have been used for other productive activities, such as food production or education. As a result, there is a high opportunity cost to the lack of clean water (UNICEF, 2000).

Water resources in general are not properly managed, especially in the developing world. For example, in many urban areas more than 50 percent of distributed water is wasted through leaking pipes. Water and sanitation technologies used in the developed world, such as extensive sewer systems and large wastewater treatment plants, are frequently expensive or impractical for developing countries (IRC, 2000). Due to this, many people in developing countries are forced to use water sources such as rivers and streams, which could have been easily contaminated by pathogenic microorganisms. Use of raw water before it receives treatment presents a sanitary risk and may be unsafe (USEPA, 1999).

In addition, the type and extent of treatment employed depends on the raw water quality. Therefore, the quality of raw surface water needs frequent monitoring so that it will be possible to minimize the burden on human health and to anticipate water treatment mechanisms to be used. Fecal coliforms and macroinvertebrates are advantageous water quality indicators over the use of costly methods such as physicochemical parameters.

Presence and load of fecal coliforms in bodies of water are common indicators of fecal contamination, which shows the presence of pathogenic organisms. However, this method need well equipped laboratory settings making it difficult to be applied in conditions with limited resources and require minor duration (<8 hours) to analyze after sampling (WHO, 1997; EPA, 2000).

Use of benthic macroinvertebrates and rapid assessment procedures can provide accurate information in surveys of pollution effects at a fraction of the cost and technical expertise than is required when using fecal Coliform assessment approaches. Likewise, macroinvertebrates can easily be identified and give more comprehensive results regarding surface water quality (Barbour et al., 1999).

To the best of our knowledge there is no study done to show the relationship between macroinvertebrate diversity and fecal Coliform count. Therefore, this study is aiming at describing this relationship so that macroinvertebrates can be used as alternative means of indicating fecal contamination.

CHAPTER TWO
LITERATURE REVIEW

2.1. Water Quality

The water in a stream is always moving and mixing, both from top to bottom and from one side of the stream to the other. Pollutants that enter the stream travel some distance before they are thoroughly mixed all over the flow (EPA, 1997).

Pollution is broadly categorized into Point source pollution in which pollutants comes from clearly known sources And Nonpoint source pollution comes from a wide area and thus can be difficult to identify (EPA, 1997).

Rapid population growth, urbanization ,industrialization, and uncontrolled waste disposal; systems as well as leachate from open solid waste dumps which are usually located on edges of rivers causes serious water quality deterioration (Koukal et al., 2004; Hamze et al., 2005).

Watersheds are regions that drain to a particular water course or body of water. Humans depend on range of services provided by rivers, tributaries and Surrounding lands. Land use changes described by, uncontrolled agriculture, excessive fertilizers and pesticide application, alters the physicochemical quality of rivers, dams, streams and their ecological integrity. In general, the effects of human activities on water shades and their ecosystem affect water quality, habitat structure, stream flow patterns, nutrients, and biotic interactions (Karr, 1991).

However, excessive inputs of phosphorus (P) and nitrogen (N) into surface waters from various human activities made water bodies unsuitable for intended uses such as drinking, irrigation, industry, recreation, or fishing. These pollutants may enter into water bodies as raw sewage, effluents from sewage treatment plants, urban and rural runoff, septic tanks, landfills, open damps, and agricultural practices (Hassan et al., 2005).

According to Lemly, (1998).Aquatic life use impacted by nutrient pollution due to several mechanisms, like

- High algal biomass can

  Physically alter the habitat by covering the stream bottom

  Have a net-negative effect on DO

  Increase pH, and high pH is toxic for invertebrates and fish

  With nutrients can stimulate bacteria, which respire and reduce DO

2.2. Methods of water quality monitoring

Water quality monitoring was generally considered the principal way of identifying water pollution problems, approaches that combine chemical, physical, and biological monitoring methods to achieve the best picture of water quality conditions (EPA, 1997; UNICEF, 2010).

The magnitude of their effects can be influenced by properties such as pH and temperature. For example, temperature influences the quantity of dissolved oxygen that water is able to contain, in natural condition and pH affects the toxicity of ammonia (EPA, 1997; WHO, 2004).

Water quality monitoring, however, might be inadequate for determining whether aquatic life uses are being met in a stream. While some constituents (such as dissolved oxygen and temperature) are important to maintaining healthy fish and aquatic insect populations, other factors, such as the physical structure of the stream and the condition of the habitat, play an equal or greater role. Biological monitoring methods are generally better suited to determining whether aquatic life is supported (EPA, 1997; WHO, 2004).

2.2.1. Physico- chemical parameters

pH

Acids and base can affect the PH of a water body and may eliminate those aquatic organisms that are PH change intolerant. Besides, a decline in PH will increase the mobility of trace metals and makes them bio-available for organisms (Manahan, 2000).

Mean pH levels of all the water bodies within the Newmont Ghana Gold Limited concession area varied between 5.80 and 11.60, with Tano downstream recording the highest mean pH value of 7.61 and the lowest at Subika stream. These differences in pH for all the water bodies were not statistically ($P>0.05$) significant (Asamoah & Emmanuel, 2009).

Permissible range of pH in natural surface water is (6.0-8.5), and (6.0-9.0) not to have adverse effect to be used for recreational purposes and aquatic organisms (WHO, 1993; EPA, 2003).

Temperature

An increase in temperature changes the physical environment in terms of reduction in oxygen concentration of water bodies while increasing the metabolism of species such as fish that are very sensitive to changes in temperature (Harrison, 1990).

Conductivity

Conductivity can be affected highly by geology since it is mainly influenced by mineral salts However, an increase in conductivity possibly occurs when additional wastes containing ions enter the stream section. Thus, it is highly probable that increase in conductivity in the stream is due to additional waste from residence (Kalyoncu et al., 2009).

Nitrate

The nitrate ion (NO_3-) is the common form of combined nitrogen found in natural waters. It may be biochemically reduced to nitrite (NO_2-) by denitrification processes, usually under anaerobic conditions. Natural concentrations (0.1 mg l-1 NO_3-N) increases may be due to municipal and industrial waste-waters, including leachates from waste disposal sites, sanitary landfills and use of inorganic fertilizers in rural and suburban area can be a significant source (UNESCO/WHO/UNEP, 1996).

Also according to (Harrison, 1990) Natural waters have very low concentrations of nitrate (a soluble form of nitrogen) and phosphate, because they exist in forms not readily available to the biota.

Phosphate

Based on (UNESCO/WHO/UNEP, 1996) report, Phosphorus is an essential nutrient for living organisms and exists in water bodies as both dissolved and particulate species **.**Phosphorus is rarely found in high concentrations in freshwaters as it is actively taken up by plants. As a result there can be considerable seasonal fluctuations in concentrations in surface waters. In most natural surface waters, phosphorus ranges from 0.005 to 0.020 mg l-1 PO_4-P.

Concentrations as low as 0.001 mg l-1 PO_4-P may be found in some pristine waters and as high as 200 mg l-1 PO_4-P in some enclosed saline waters. Average groundwater levels are about 0.02 mg l-1 PO_4-P. As phosphorus is an essential component of the biological cycle in water bodies, it is often included in basic water quality surveys or background monitoring programmes. High concentrations of phosphates can indicate the presence of pollution and are largely responsible for eutrophic conditions.

2.2.2. Macroinvertebrate

Biological measurements, called metrics, represent elements of the structure and function of the bottom dwelling macro invertebrate assemblage. They include specific measures of diversity, composition, functional feeding group representation and ecological information on tolerance to pollution. If there is predictable way with increased human influence, Metrics change is occurred.

Using benthic macro invertebrates for biological assessment have the following advantages

1. Their assemblages are good indicators of localized conditions. Because many benthic macro invertebrates have limited migration pattern and they are particularly well suited for assessing site-specific impacts
2. Assemblages are made up of species that constitute a broad range of atrophic levels and pollution tolerances thus providing strong information for interpreting cumulative effects
3. Also they integrate the effects of short-term environmental variations. Most species have a complex life cycle of approximately one year or more. Sensitive life stages will respond quickly to stress; the overall community will respond more slowly (Barbour et al., 1999).

Using macro invertebrates in Bioassessment also has a number of potential disadvantages:

- They do not respond to all impacts due to natural stressors and disturbances such as drought (Feminella, 1996) and display seasonal variation, which can present constraints for timing of sampling and comparing samples, (Linke et al., 1999).

Study showed that chemical evaluations failed to detect 50% of the damage to surface waters when compared with application of more comprehensive, sensitive and objective biological criteria (Davis et al., 1996; USEPA, 2005). Karr and Chu, (2000) asserted that living communities reflect watershed conditions better than any chemical or physical measure because they respond to the entire range of biogeochemical factors in the environment

According to study done by Beyene and Legesse, (2005) in Borkena River around Dessie and Combolcha town, the decline in taxa richness of macroinvertebrates was reported with increasing load of pollution and again rise at recovery site. Also 19 and 5 taxa were collected from rural area above Dessie and below Dessie town respectively. In River Awetu, Upstream of Jimma town, family taxa as high as 25 have been reported (Hailu & Legesse, 1997).

Study on Environmental influences on macroinvertebrate assemblages in headwater streams of northeastern Ohio (USA), a total of 12,691 individuals comprising 12 orders and 45 families of macroinvertebrates were collected in the eight sample reaches. Diversity indices indicated downstream reaches have higher mean richness of macroinvertebrate families,

higher mean Shannon diversity index values, and assemblages that on average are more evenly distributed than the upstream reaches (Ohio EPA, 1987) .

As study done on Benthic Macro invertebrate Structures along Tikur Wuha River, a total of 18,651 macro invertebrate individuals belonging to 34 families were collected from 6 sites. Taxa richness at the sites ranged from 10 to 30 families (Birenesh, 2007).

At Hiwane second order stream in north Ethiopia, A total of 1139 individuals composed of 8 order of insects and 4 orders of non insects were collected during the study time. Among the insect orders Trichoptera and Diptera were the most dominant with 34 and 27% of the macroinvertebrate community. Based on this study result the highest diversity was observed in the sampling site with a good water quality (Tsegazeabe & Teferi, 2012).

According to study done around Addis Ababa, FLBI value of two sites was 5.1, both located in the upstream rural area above Sebeta Agro-industry ,so each was in the range of fair class of Hilsenhoff, 1988.while other Sites fall into the category very poor (Tasew, 2007).

Table 1: Standard Evaluation of water quality using the family-level biotic Index (Hilsenhoff, 1988**)**

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

2.2.3. Fecal Coliform (indicator organisms)

Bacterial monitoring of surface waters is done continuously to ensure public safety. The Environmental Protection Agency (EPA) recommends not more than 1.26 colony-forming units (CFU) per ml of water, and a sample maximum of 3.94 CFU/ ml for freshwaters that will be used for "primary contact," such as recreation (WHO, 1997; EPA, 2000).

When results show unacceptable levels, Action should be taken to ensure public health, restriction of recreational access to the water, and identifying source of contamination (Tiefenthaler et al., 2009).

In Ethiopia, water shade resources play a major role in the lives of adjacent communities by helping them to achieve ecosystem services. However, many water shades throughout the country are facing degradation as population growth rate increases the need for more fertile agricultural land. Analyzing the health and diversity of streams, dams, rivers and tributaries, based on the presence of macroinvertebrates, could therefore indicate the state of the ecosystem and the related services (Feld et al., 2010).

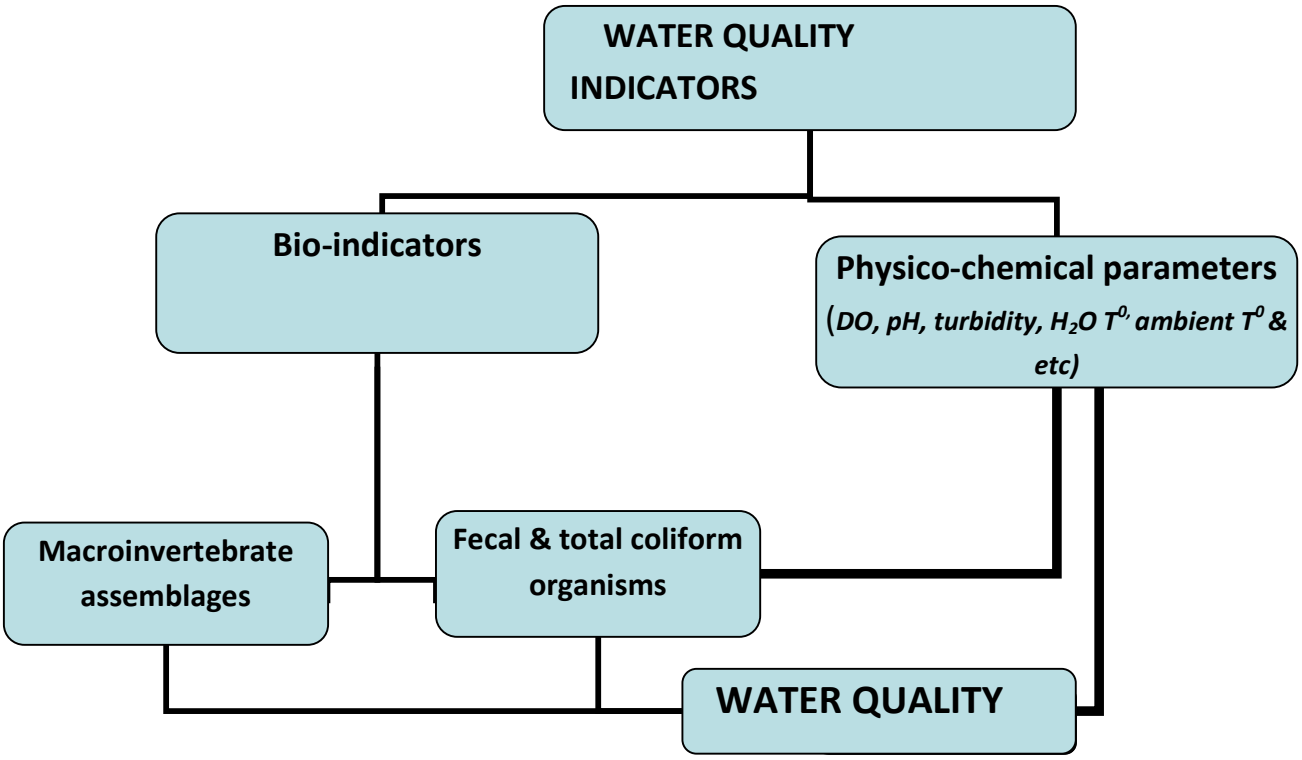


Fig. 1: Conceptual *frame work of water quality indicators*

Significance of the study

There is no study done to show the relationship between macroinvertebrate diversity and fecal Coliform counts.

Finding of this study can be used to show the potential application of macroinvertebrate diversity to indicate fecal contamination in surface water sources like streams and rivers. Especially in Developing countries that have limited laboratory, for water quality analysis such as Ethiopia will be the first to benefit from such findings.

Helps to identify which type of macroinvertebrate taxa directly correspond to fecal contamination as the result it will be helpful to identify the source of contamination

Therefore, this study finding could be also used as a baseline data for future study

CHAPTER THREE
OBJECTIVES AND HYPOTHESIS

3.1. General objective

The main objective of this study was to describe the relationship between benthic macroinvertebrate community assemblages, Coliform and physicochemical parameters as indicator of surface water quality within Gilgel Gibe watershed.

3.2. Specific objectives

- To determine turbidity, conductivity, DO, pH, Temperature, N and P
- To investigate total and fecal Coliform counts
- To determine diversity, evenness and richness of benthic macro invertebrate in stream water sources (Gibe Seka, Mite Seka, Arrer and Naada).
- To describe the relationship between macroinvertebrate communities, indices and Physico-chemical parameters with fecal Coliform

3.3 . Hypothesis

There are specific macroinvertebrate taxa and indices that have significant relationship with fecal Coliform organisms.

CHAPTER FOURE

METHODS

4.1. Study area and period

The study area is located in Jimma Zone of Oromia National Regional State, in southwestern part of Ethiopia. Jimma town is the capital and administrative center of the zone, located 335km away from Addis Ababa. The town is by far the largest urban center in the zone(ESA,2002)

Study was conducted from June to September 2012 in four streams located in Gilgel Gibe watershed, Jimma Zone, Southwest Ethiopia

4.2. Study design

Field assessment and laboratory based cross-sectional study was performed in the selected streams.

4.3. Study variables

- Macro invertebrate assemblages
- Fecal Coliform counts
- physic-chemical parameters

✓ DO pH

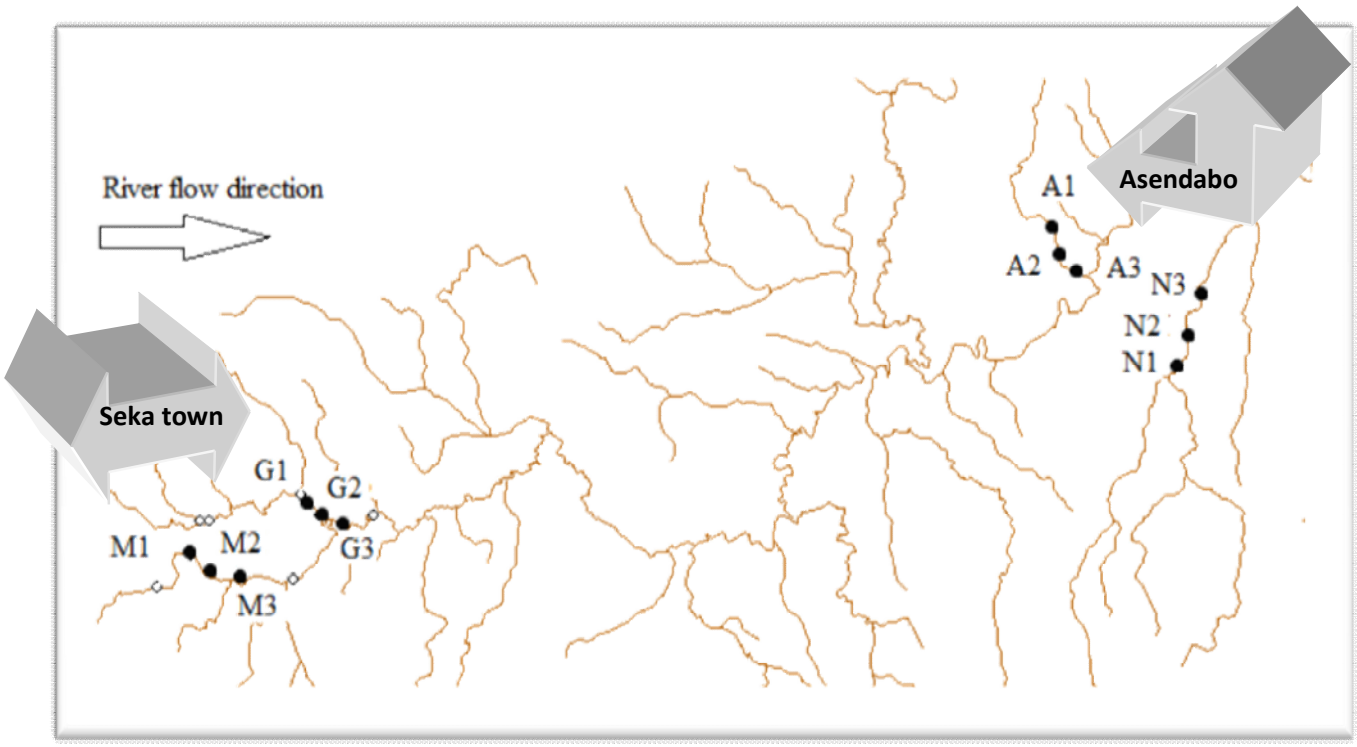
✓ Phosphate Nitrate

✓ Conductivity Temperature

✓ Turbidity

4.4. Sample Site Selection

Three sampling sites were chosen at each selected river as recommended by (Barbour et al., 1999) suggesting a minimum of three sampling points for river and stream.



NB: G: Gibe Seka, M: Mite Seka, A: Arrer, N: Naada

Fig. 2: Free sketch map of sampled sites

4.5. **Sample Collection and Laboratory analysis**

4.5.1. Macroinvertebrates

Triplicate samples were collected from each sites using a D frame kick net (mouth = 20cm to 30 cm and mesh size= 0.25 mm) by disturbing the substrate with kick net. Sampling was done against the water flow so that the dislodged invertebrates were carried into the net by the water current.

Sampling was conducted based on wadable river protocols for streams, i.e. for 5 minutes at a distance of 10 meters (King et al., 1996; Hilsenhoff, 1987). Individual stones was also be picked up and then scraped to dislodge attached invertebrates.

Then, Macroinvertebrate samples was cleaned to remove debris, transferred to plastic tray and a small amount of the sample randomly placed in a Petri dish with forceps to be identified. The kick samples were transferred in to a single bottle to preserve with 70% ethanol, and then transported to laboratory with a label identifying the location, date and time.

In the laboratory, all macro invertebrates were sorted, identified and enumerated using a binocular dissecting microscope and identification keys to family level (Bouchard, 2004). The identified benthic macro invertebrates were preserved in glass jars containing 70% ethyl alcohol for further use as specimen.

4.5.2. Physicochemical parameters

Physicochemical variables such as pH, temperature, turbidity, conductivity, DO, were measured in situ at each sampling site from which macroinvertebrate sample was taken by multi parameter probe (HACH HQd). Nitrate and Phosphate was analyzed using PG-T 80 UV/VIS spectrophotometer, from water sample by phenoldisulfonic and stannous chloride method respectively. Methods and procedures for chemical analysis indicated in Annex part.

4.5.3. Fecal Coliform

Water sample for fecal Coliform analysis was collected by using sterile glass bottles from the same site where macro invertebrate samples were taken. All the water samples were kept in an icebox to preserve the samples in cool condition, during transportation to Environmental biology laboratory of Jimma University. One ml of water sample, from each site was serially diluted up to 10^{-5}, two replicates of 0.5ml per dilution was inoculated in maconkey agar. Then, it was incubated for 24 h at 37 ^{o}C for total Coliform test and 48 h at 44.5^{o}C to identify fecal Coliform; the number of colony forming unites of fecal Coliform and total Coliform bacteria was calculated using standard plate count method.

4.5. Data analysis

A number of biotic metrics and indices were generated that described the macro invertebrate community at each site. The data about physicochemical parameters, fecal coliforms and macroinvertebrate scores were coded and entered into Statistical software SPSS version 16. One-way ANOVA was used to differentiate mean differences in macro invertebrate indices between sample sites. Also Bivariate Correlation, regression and correspondence analysis were used to measure the strength of relationship between macroinvertebrate assemblage, Physico-chemical parameters and fecal Coliform count.

4.6. Data quality control

Aseptic technique was employed during sampling, transporting and processing of water samples for microbiological tests. Three replicates were used for each experiment. Sampling bottles were labeled to differentiate between sampling sites and time. Sterile water in sterilized containers were used as blank during sample transportation in pre determined sites with specific notification and analyzed as regular sample. Field blank was used to identify errors or contamination in sample collection and analysis. The labeling was consistent for macroinvertebrate, fecal Coliform and physicochemical parameters. Parameters such as pH, Temperature, turbidity etc were measured in situ to minimize the variations during transport. Macro invertebrates were immediately be preserved in 70% ethanol, after sampling and sorting

4.7. Ethical consideration

Ethical clearance was obtained from Ethical Committee of Jimma University, College of Public Health and Medical Sciences.

4.8. Dissemination plan

The final result of this study will be presented to Jimma University, College of Public Health and Medicinal sciences, Department of Environmental Health Science and Technology

CHAPTER FIVE

RESULT

5.1. Macroinvertebrates

Total of 707 macroinvertebrate individuals belonging to 18 families and 7 orders were present in the samples collected from study sites, namely Gibe Seka, Arrer, Naada and Mite Seka. Odonata was the most abundant order represented by 207 (29%) organisms (Table 2).

Table 2: Distribution of macroinvertebrate orders along the sampling streams

Order	Site name					
	Gibe Seka	Mite Seka	Arrer	Naada	Total	Percentage (%)
Odonata	105	83	12	7	207	29
Ephemeroptera	31	0	34	98	163	23
Trichoptera	24	8	4	14	50	7
Coloptera	8	0	3	0	11	2
Hemipetra	49	1	0	1	51	7
Mollusca	19	29	34	16	98	14
Diptera	32	54	15	26	127	18
Total	268	175	102	162	707	100

The dominant families were Coenagriidae (17.4%) and Baetidae (15.3%). The richest site was Gibe Seka containing 14 different families followed by Nadda (11), Arrer (10) and Mite (10). (Annex table 14). The result reveals that the Simpson reciprocal (1/D) of Gibe Seka (8.99) was greater than all other sites. Likewise the Shannon H' Log Base 10 index was 2.29 which is slightly greater than other sites. Evenness index (0.906) was also higher than other sites. This implies that Gibe Seka was more diversified than all sites while Naada was less diversified (Fig.3).

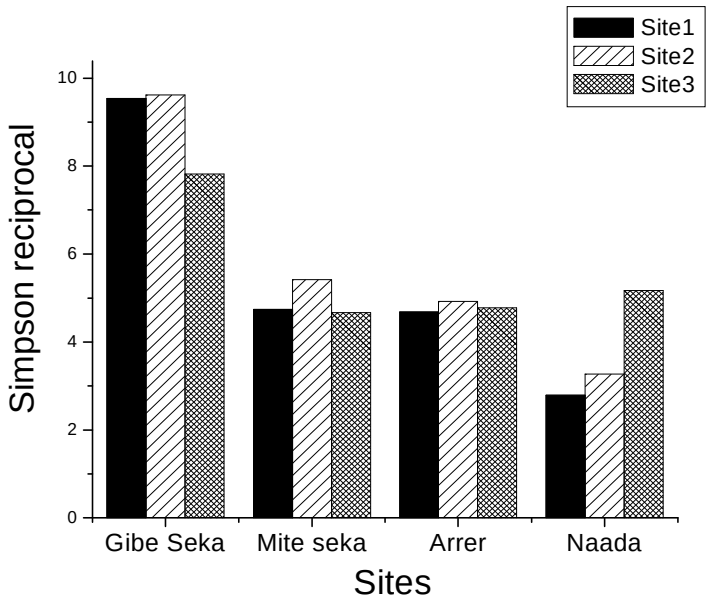


Fig. 3: Distribution of Simpson reciprocal scores among study streams

From all the study streams, value of BMWP was highest at Gibe Seka while lowest at Naada and %CHIR was highest at Mite Seka but non at Gibe Seka. FLBI value of Gibe Seka lie in the range of (3.76-4.25), Naada (00-3.75) and the rest two streams lie in the range of 4.26-5.00(Table3)

Table 3: BMWP, ASPT, FLBI and %CHIR scores among different sample sites

	Gibe Seka			Mite Seka			Arrer			Naada		
Indices	G1	G2	G3	M1	M2	M3	A1	A2	A3	N1	N2	N3
FLBI	3.9	4.2	4.3	4.9	4.5	4.6	4.3	4.5	4.5	3.6	3.5	4.4
%CHIR	0.0	0.0	0.0	32	28	25	18	12	15	9	14	32
BMWP	46	51	48	27	25	25	33	32	26	26	24	21
ASPT	3.5	3.6	4.4	3.9	3.9	3.6	4.1	4.6	4.3	3.7	2.7	3

5.2. Total and Fecal coliforms

The Highest number of total (31,136Cfu/ml) and fecal Coliform (6,161 Cfu/ml) colonies were recorded at Mite Seka followed by Arrer 19,325 total and 3,879 fecal Coliforms. The lowest values of colonies were recorded at Gibe Seka where an average of 4691 total and 1020 fecal coliforms found in 1 ml. Statistical tests indicated that there were significant difference (p=0.00) in mean of total and fecal coliform counts among the sites.

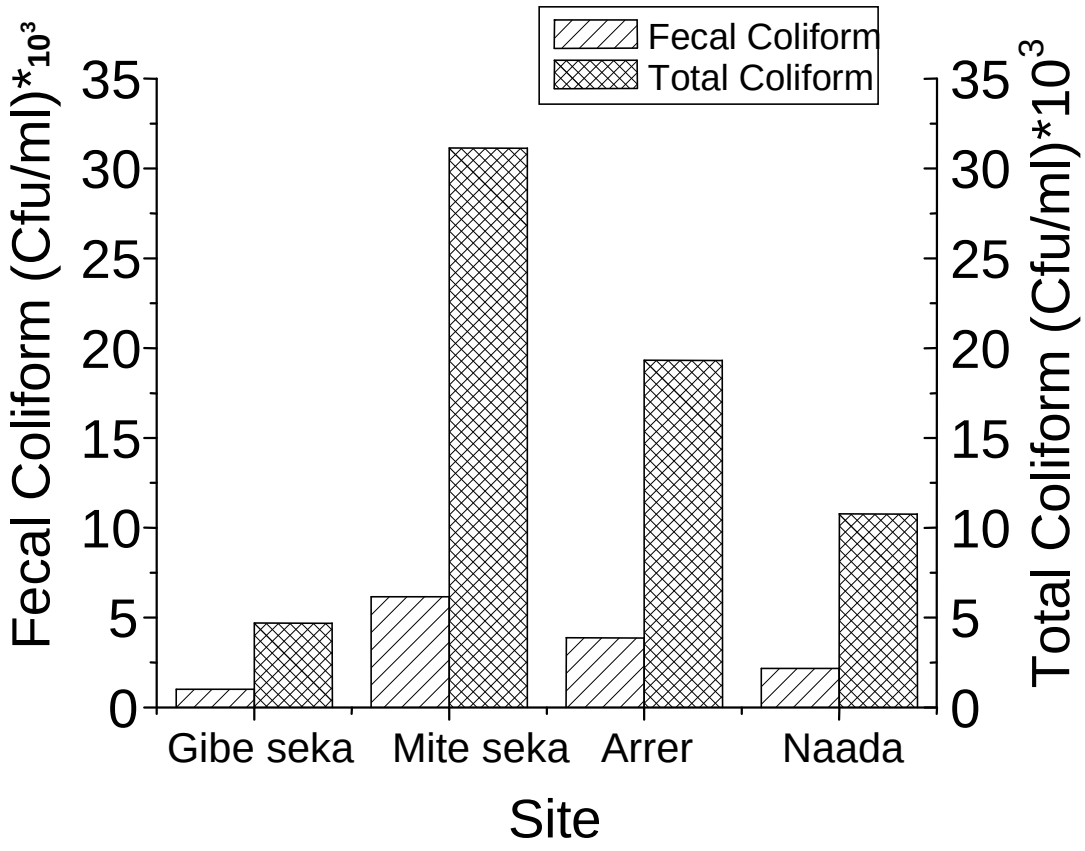


Fig. 4: Total and Fecal Coliform Cfu/ml of water samples among study streams

5.3. Physico-chemical parameters

The highest temperature (22.73^{O}C) and lowest temperature (19.23^{O}C) was measured in Arrer and Mite Seka sites respectively. pH among the course of the streams was in the range of 6.32 to 6.83. The highest DO was 7.56mg/l at Mite Seka site, followed by 7.2 mg/l at Gibe Seka. Lowest Do value was 6.42mg/l at Naada site. Highest conductivity was measured at Arrer site (101.43 µs/cm) while lowest at Naada site (38.87 µs/cm).

Table 4: Result of Physico-chemical parameters in four streams around GilGel Gibe

Site name		Physico-chemical parameters						
		EC (µs/cm)	DO (mg/l)	DO (saturation) (%)	water (T^{O})	Ambient (T^{O})	pH	Turbidity (NTU)
Gibe Seka	G 1	59.9	7.19	99.8	21.2	23.2	6.51	196
	G2	58.8	7.12	98.8	22.1	23	6.42	197
	G3	60.5	7.3	99.6	21.5	23.1	6.35	188.5
	Mean	59.73	7.20	99.40	21.60	23.10	6.43	193.83
Mite Seka	M1	81.1	7.66	103.3	19.3	23.6	6.58	88.1
	M2	80	7.63	102.1	19.4	23.3	6.54	90.1
	M3	80.5	7.4	100.9	19	22.8	6.56	89
	mean	80.53	7.56	102.10	19.23	23.23	6.56	89.07
Arrer	A1	101.35	6.54	93.4	23.2	24.5	6.99	89
	A2	102.4	6.58	94.3	22.1	24.3	6.52	109.5
	A3	100.53	6.48	94	22.9	23.6	6.97	110.9
	mean	101.43	6.53	93.90	22.73	24.13	6.83	103.13
Naada	N1	39.7	6.53	91.5	22.2	24.4	6.5	202.5
	N2	38.2	6.32	90.4	23.4	23.2	6.2	203
	N3	38.7	6.42	90.8	21.5	23.5	6.25	201.5
	mean	38.87	6.42	90.90	22.37	23.70	6.32	202.33

Nitrate and phosphates

The highest concentration of Nitrate was measured at Mite Seka (0.268) while lowest at Naada (0.161).high concentration of phosphate was measured at Gibe Seka (0.173) but lowest at Arrer (0.076).there is significant mean difference in concentration of both Nitrate and Phosphate in all study sites at significance level 0.05 (p=0.00)

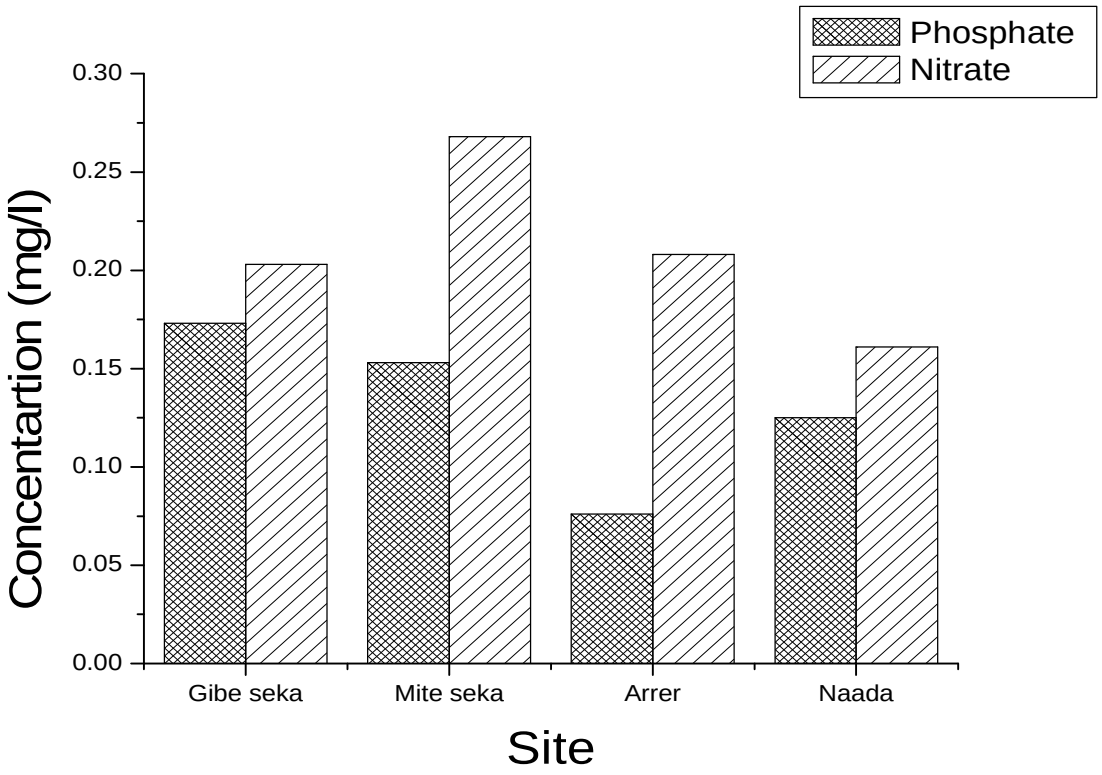


Fig. 5: Concentration of Nitrate and Phosphate among study streams

5.4. Relationship among macroinvertebrate, Coliform counts and Physico chemical parameters

5.4.1. Relationship between Macroinvertebrate and Coliform counts

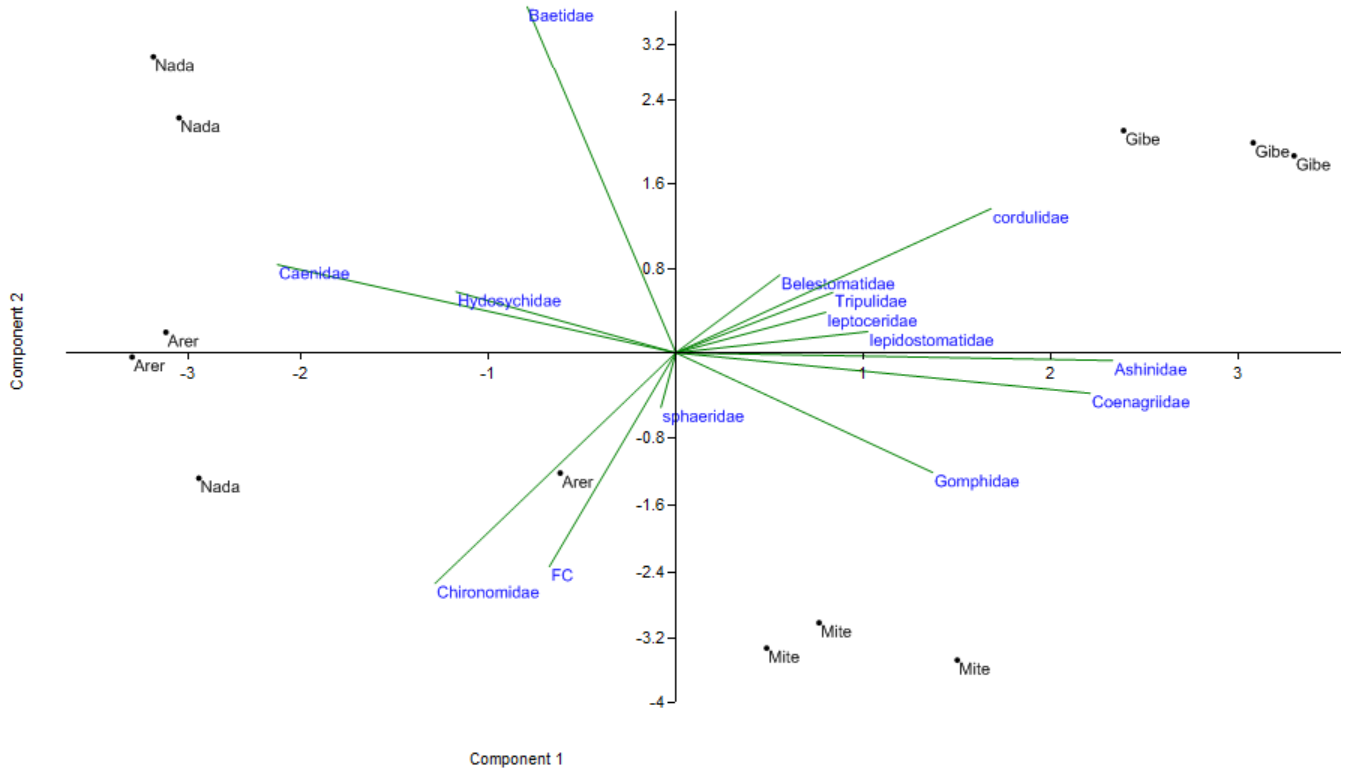
The count of fecal and total Coliform was positively correlated with % CHIR (r= 0.77, p=0.003) and FLBI (r=0.6, p=0.016). In contrast, there was significant negative correlation of BMWP with the number of total (r=-0.602, p=0.039) and Fecal (r= -0.591, p=0.043) coliforms. The correlation of coliform numbers with macroinvertebrate abundances, Shannon index and Simpson reciprocal was not significant (p>0.05). (Table 5)

Table 5: Relationship of Fecal and total Coliform counts with macroinvertebrate indices

Macroinvertebrate indices	Fecal Coliform		Total Coliform	
	R – Value	P –value	R – Value	P –value
BMWP	-0.591	0.043	-0.602	0.039
FLBI	0.673	0.015	0.680	0.016
Simpson (1/D)	-0.486	0.109	-0.497	0.1
Shannon	-0.544	0.068	-0.554	0.062
Macroinvertebrate abundances	-0.457	0.135	-0.453	0.139
% CHIR	0.771	0.003	0.775	0.003

The result of correspondence analysis indicated that the probability of finding Chironomidae is highest at sites with relatively higher number of fecal coliforms, such as Arrer and Mite. Other macroinvertebrate families which were relatively concentrated at sites with higher fecal and total coliforms were Spharidae, Gomphidae Coenagriidae and Ashinidae. Similarly regression analysis showed that Gomphidae (p=0.03) and Coenagriidae (p=0.005) were significant positive predictor families for fecal coliform (Table 6).

In contrast, Cordulidae, Belestomatidae, Tripulidae, Leptoceridae and Lepidostomatidae preferred Gibe Seka, a site with the lowest number of total and fecal coliforms. Other macroinvertebrate families such as Caenidae, Hydrosychidae and Baetidae were more concentrated at Nadda than other 3 sites (Figure 6). Corixidae was the most negative predictor (p=0.0003) of fecal coilform count. (Table 6)



Component 1 and 2 represents 78% of variance.

Fig. 6: Correspondence analysis with square root transformed data of macroinvertebrate and fecal Coliform

Table 6: Summary of regression analysis for fecal Coliform prediction with Macroinvertebrate communities

N=12	R=0.984, R^2=0.967, Adjusted R^2=0.943 F(5,6)=37.402 P<.00019 Standard Error of estimate 483.01					
	Beta	Std Err of Beta	B	Std Err of B	t (6)	p-level
Intercept			228.72	258.30	8.63	0.0001
Coenagriidae	0.76	0.18	175.59	40.94	4.29	0.0051
Coroxidae	-1.47	0.20	-964.50	131.99	-7.30	0.0003
Belestomatidae	0.43	0.15	637.96	223.48	2.85	0.0290
Gomphidae	0.33	0.12	229.57	82.17	2.79	0.0314
Lepidostomatidae	-0.25	0.10	-197.15	80.81	-2.44	0.0505

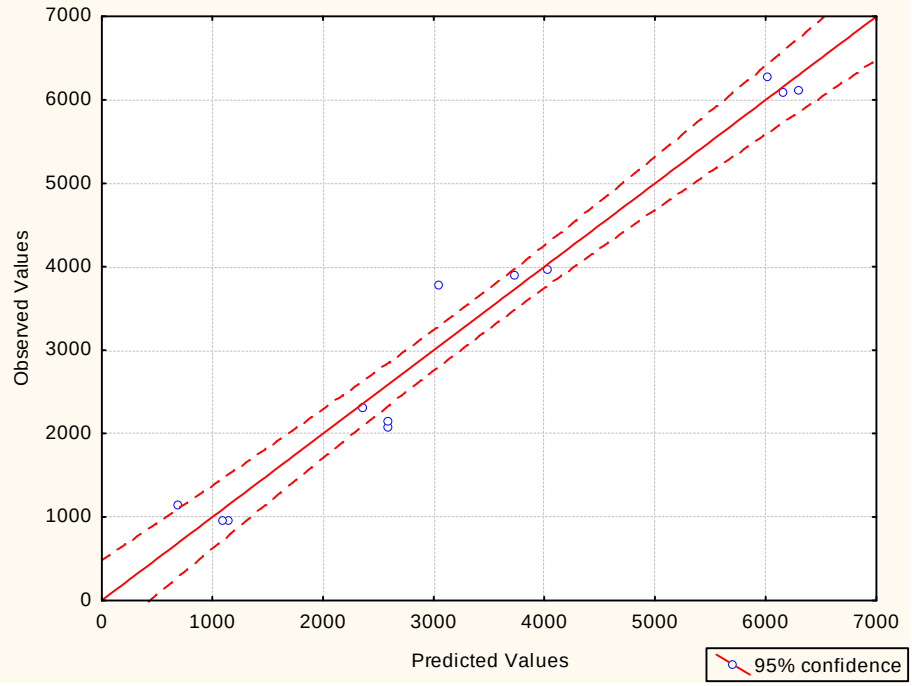


Fig. 7: Fecal coliform prediction with macroinvertebrate communities

Gibe Seka, a site with lowest total and fecal coliform numbers, has relatively higher BMWP (48) & ASPT (3.86) scores than other sites. Lowest mean FLBI was scored at Naada (3.83) followed by Gibe seka (4.07), Arrer (4.46) and Mite (4.67). Similarly, the number of coliforms was increasing from Gibe Seka to Arrer and Mite.

Table 7: Score of macroinvertebrate indices, water quality class and fecal coliform load among sample sites

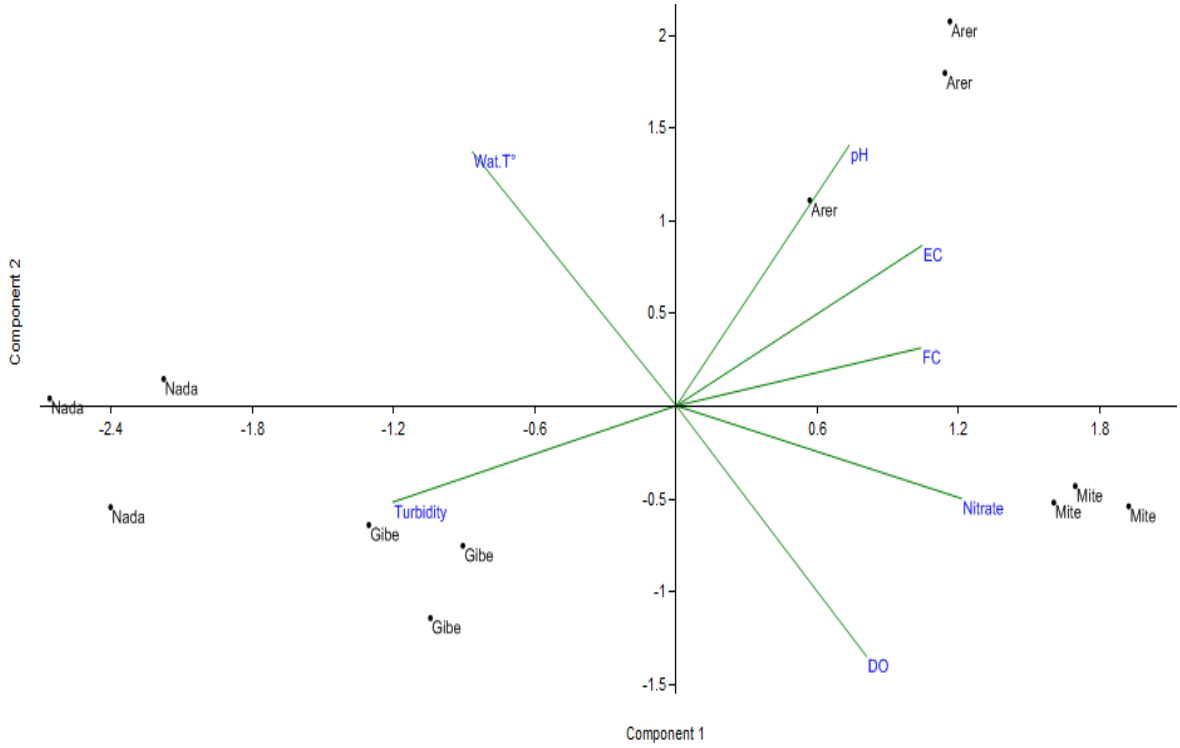
Site	**BMWP**	Water quality class	**ASPT**	Water quality class	**FLBI**	Water quality class	Degree of organic pollution	FC(Cfu/ml)	Water quality index
G1	46	Moderate	3.54	Poor	3.88	very good	Possible slight organic pollution	1137	Moderate
G2	51	moderate	3.68	Poor	4.02	very good	Possible slight organic pollution	965	Moderate
G3	48	moderate	4.36	Moderately poor	4.32	Good	Some organic pollution probable	959	Moderate
M1	27	poor	3.86	Poor	4.89	Good	Some organic pollution probable	6272	very poor
M2	25	poor	3.86	Poor	4.52	Good	Some organic pollution probable	6120	very poor
M3	27	poor	3.57	Poor	4.59	Good	Some organic pollution probable	6090	very poor
A1	33	poor	4.125	Moderately poor	4.3	Good	Some organic pollution probable	3784	Poor
A2	32	poor	4.57	Moderate	4.55	Good	Some organic pollution probable	3960	Poor
A3	26	poor	4.3	Moderately poor	4.54	Good	Some organic pollution probable	3892	Poor
N1	26	poor	3.71	Poor	3.57	Excellent	Organic pollution unlikely	2072	moderately poor
N2	24	poor	2.66	Poor	3.48	Excellent	Organic pollution unlikely	2312	Moderately poor
N3	21	poor	3	Poor	4.44	Good	Some organic pollution probable	2140	moderately poor

5.4.2. Relationship of physicochemical parameters with Coliform count

The number of fecal and total coliforms had strong negative correlation with physicochemical parameters such as turbidity (r=-0.89, p=0.000) and water temperature (r=-0.62, p=0.032), (Table 8). According to correspondence analysis higher turbidity and water temperature was recorded at Gibe Seka and Nadda, respectively (Figure 8). These sites had relatively lower number of fecal and total Coliform counts (Figure 4). In contrast, nitrate and electric conductivity had significant positive correlation with the number of coliforms (Table 8). Similarly, the result of correspondence analysis demonstrated that there is relatively higher nitrate concentration and electric conductivity at Arrer and Mite (Figure 8), sites with relatively higher number of fecal coliforms (Figure 4). Other parameters such as phosphate, ambient temperature, pH, DO, had no significant correlation with Coliform counts (Table 8). Regression analysis showed that EC (p=0.043), turbidity (p=0.000) and nitrate (p=0.002) are the major predictors of fecal coliforms. (Table8).

Table 8: Correlation between Coliform counts and physicochemical parameters

Physicochemical parameters	Fecal Coliform		Total Coliform	
	r	p-value	r	p-value
Turbidity	-0.9	0.000	-0.9	0.000
Water (T^O)	-0.6	0.032	-0.6	0.032
Nitrate	0.8	0.002	0.8	0.002
EC	0.6	0.043	0.6	0.049
Phosphate	-0.2	0.53	-0.2	0.54
Ph	0.4	0.199	0.4	0.212
DO	0.4	0.184	0.4	0.186
Ambient temperature	0.1	0.847	0.1	0.852



Component 1 and 2 represent 86 % of all variability

Fig. 8: Correspondence analysis with log transformed data of physicochemical data and fecal coliforms

Table 9: Summary of regression analysis for fecal Coliform prediction with Physico-chemical parameters

	R=0.995 R^2=0.999,Adjusted R^2=0.984 F(4,7)=169.53 P<0.0001Standard Error of estimate =256.37					
N=12	Beta	Std Err of Beta	B	Std Err of B	t (7)	P-value
Intercept			9707.7	2077.05	4.67	0.002
EC	0.97	0.09	80.2	7.57	10.59	0.000
Turbidity	-0.94	0.18	-35.2	6.63	-5.31	0.001
Nitrate	0.77	0.18	38572.8	9041.35	4.27	0.004
Phosphate	-0.53	0.12	-28169.	6541.43	-4.31	0.003

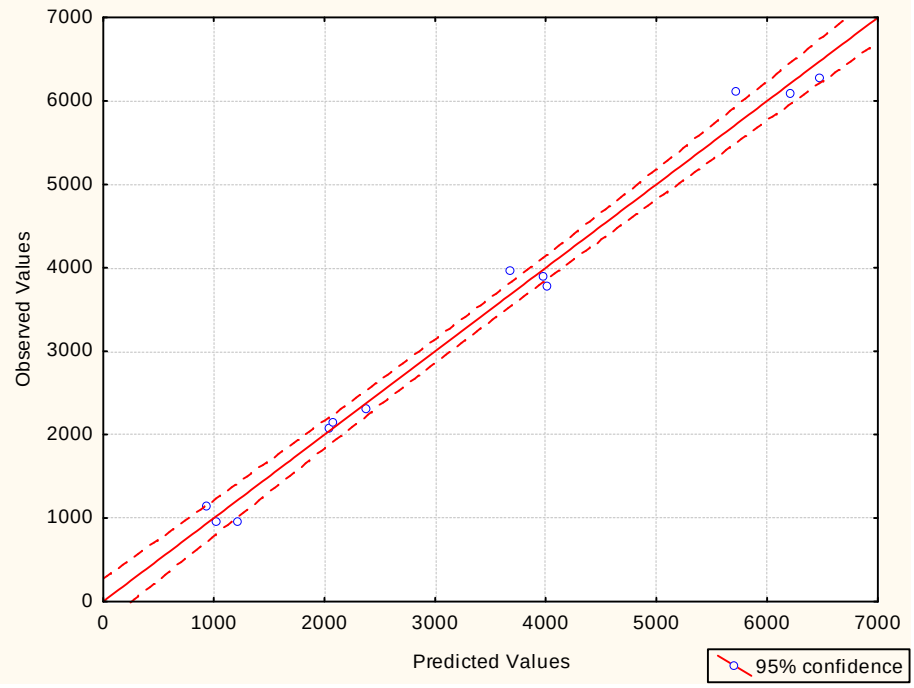


Fig.9: Fecal coliform prediction with Physico-chemical parameters

5.4.3. Relationship between macroinvertebrate indices and physico-chemical parameters

Turbidity and phosphate had strong negative correlation with FLBI (r=-0.76, p=0.004) and Shannon indices (r=-0.63, p=0.02), respectively. EC had strong positive correlation with FLBI (r=0.675, p=0.016) and ASPT (r=0.76, p=0.004). In addition, there was positive correlation between nitrate and FLBI (r=0.76, p=0.004); as well as pH and ASPT (r=0.601, p=0.039). DO is not significantly correlated with any of the macroinvertebrate indices calculated.

Table 10: Correlations between macroinvertebrate indices and Physico-chemical parameters

Physico-chemical parameters	Macroinvertebrate indices			
	FLBI	ASPT	Simpson reciprocal	Shannon
Turbidity	r= -0.76	r = -0.512	r= +0.305	r= +0.379
	p= 0.004	P=0.09	P=0.33	p=0.22
EC	r= 0.675	r= +0.76	r= -0.051	r= -0.131
	P=0.016	P=0.004	p=0.875	p=0.68
Phosphate	r= -0.1	r= -0.282	r=0.599	r= -0.63
	p=0.765	p=0.378	p=0.004	P=0.02
PH	r= +0.377	r= +0.601	r= -0.136	r= -0.230
	p=0.23	P=0.039	p=0.774	p=0.472
Nitrate	r= +0.76	r= +0.399	r=+0.085	r= +0.022
	p=0.004	p=0.198	P=0.794	p=0.946
DO	r= +0.46	r= +0.202	r=+0.423	r= +0.402
	P=0.13	p=0.53	P=0.17	p=0.195

CHAPTER SIX
DISSCUTION

Water quality monitoring was generally considered the principal way of identifying water pollution problems, approaches that combine chemical, physical, and biological monitoring methods to achieve the best picture of water quality conditions (UNICEF, 2010). This study focused on showing the relationship among macroinvertebrate families, fecal indicator microorganisms and Physico-chemical parameters.

The total number of macroinvertebrate families identified in this study was 18 belonging to 7 orders. Another study conducted in upstream of Awetu river at Jimma reported relatively higher (25) taxa richness, (Hailu & Legesse, 1997). Similar number of macroinvertebrate families was found in Borkena River; a rural site above Dessie town (Beyene & Legesse, 2005).

FLBI result of two study sites Naada (3.83) and Gibe Seka (4.1) were in the ranges of water quality class of very good (3.76-4.25) with possible slight organic pollution while other two sites Mite Seka (4.7) and Arrer (4.5) lie in a range for good water quality class (4.26-5.00) with probability of some organic pollution(Hilsenhoff, 1988) .This finding was better as compared with study done in the upstream rural area above Sebeta Agro-industry with FBI of 5 (Tasew , 2007).

These variations might be due to difference in study sites with respect to load of pollution and habitat quality differences. Based on calculated diversity index for each sampling site, the species diversity increases with water quality (i.e. the highest diversity was observed in the sampling site with a good water quality). This result is in agreement with a study done on second order stream called Hiwane located in northern part of Ethiopia (Tsegazeabe & Teferi, 2012).

Mean pH values of the two sites (Mite Seka and Arrer) were 6.56 and 6.83, so these value lie within the range of 6.50 - 8.50, stipulated for recreational purposes (WHO, 1993). But other two sites were a little bit lie out of this standard range. pH value of all sites were lie in permissible range for natural water (6.0-8.5), and standards for surface water (6.0-9.0) not have adverse effect to be used for recreational purposes and habitat for aquatic organisms (EPA, 2003).

Over all, the mean pH values were not significantly different in all study sites and in line with study done at Newmont Ghana (Asamoah & Emmanuel, 2009).

Electrical conductivity in freshwaters range between 10-1000 μs/cm and EPA set a standard to EC of 1000 μs/cm for surface waters (Chapman & Kimstach, 1996; EPA, 2003). Electrical Conductivity Levels for all the water bodies ranged from 38.9 to 101.4 μs/ cm. The highest mean conductivity values were recorded at Arrer and Mite Seka sites while lowest at Naada. It is argued that conductivity can be influenced highly by mineral salts and an increase in conductivity indicates pollution of streams containing wastes containing ions (Kalyoncu et al., 2009). Therefore, high conductivity in Mite Seka and Arrer might be due to the additional waste from residence.

According to UNESCO/WHO/ UNEP, (1996) report, natural surface water has concentrations of nitrate and phosphate 0.1 mg/l and 0.005 to 0.020 mg/l, respectively. Nitrate and phosphate concentrations at all of the studied sites were higher than these values. The use of nitrogen fertilizers on farmlands and pollution by human or animal waste can contribute to elevated nitrate concentrations in water bodies (Chapman & Kimstach, 1996). Similarly, a highest nitrate concentration in this study was recorded at Mite where relatively more human settlements and agricultural activities were observed.

Total and fecal coliform counts in all studied sites were higher than those recommended by American EPA, 1.26cfu/ml, (EPA, 2000) and 3.94 cfu/ml (WHO, 1997) guideline values for fresh water to be used for primary contact such as recreation. Highest number of coliforms was recorded at Mite and Arrer. This might be due to more anthropogenic activities as observed at these sites. For example, sand extraction and agricultural activities were observed around stream of Arrer, while Mite is nearest site to human localities, i.e., Seka town.

The relationship between macroinvertebrates and coliform organisms was studied here. The number of total and fecal coliforms showed positive and significant correlation with macroinvertebrate indices % CHIR and FLBI.

Chironomids are pollution tolerant organisms and their number tends to increase with a decrease in water quality or increase with perturbation (Barbour et al., 1999; Gallardo et al., 2006). Similarly, the results of this study indicated that there is higher probability of finding percentage chironomids in sites with high number of fecal coliforms, such as Arrer and Mite Seka.

Macroinvertebrate indices such as, BMWP, ASPT and FLBI, indicated that the quality of the studied water bodies ranges from very poor (Mite) to moderate (Gibe Seka). In relation to these macroinvertebrate indices score and respective water quality category fecal coliform index for water quality was developed. Based on this index, fecal coliform in the range of 959 to 1137Cfu/ml and 6090 to 6272Cfu/ml indicate moderate and very poor water quality classes, respectively (Table 7).

Table 11: Water quality of sites with respect to Macroinvertebrates indices and corresponding number of fecal coliforms

Sites	INDICES			Fecal Coliform	Water quality	Reference
	FLBI	ASPT	BMWP			
Gibe Seka	4.07	3.86	48.33	959-1137	Moderate with possible organic pollution	(Hilsenhoff, 1988)
Nadda	3.83	3.12	23.67	2072-2312	Moderately Poor with less likely of organic pollution	(Friedrich et al., 1996)
Arrer	4.46	4.33	30.33	3784-3960	Poor with probable organic pollution	
Mite	4.67	3.76	26.33	6090-6272	Very poor with some organic pollution	

The results indicated that Nitrate, EC, turbidity and water temperature were major predictors for fecal coliforms. In theory, higher EC and elevated concentrations of nitrate may result from discharges of households (Chapman & Kimstach, 1996).

Both nitrate concentration and EC in this study were positively correlated with the number of fecal coliforms. Similarly, higher nitrate concentration and EC were recorded at sites where there is higher ASPT and FLBI, indices which indicate higher number of pollution tolerant macroinvertebrate organisms. High phosphate concentration in rivers can lead to Eutrophication and can alter aquatic fauna (USEPA, 2000). Similarly, Shannon index, which shows the diversity of organisms, was negatively correlated with phosphate concentrations.

CHAPTER SEVEN
CONCLUSION AND RECOMMENDATION

7.1. CONCLUSION

Macroinvertebrate indices, physicochemical parameters and number of Coliform organisms revealed that the quality of the studied streams ranges from very poor to moderate. Non point sources, like fertilizers from agriculture, cattle grazing, sand extraction activities and surface runoff might be main factors. According to the findings, Gibe Seka was under moderately polluted water quality class While Mite Seka was under highly impacted water quality class.

The findings indicate that there is relationship between macroinvertebrate and the level of fecal contamination. At the individual family level, higher number of Chironomidae and Spharidae were observed at sites with higher number of fecal coliforms. Thus, these macroinvertebrate families can be considered as indicators of higher degree of fecal contamination. In contrast, Cordulidae which showed higher probability of being found at sites with lower fecal coliforms can indicate lower degree of fecal contamination.

Moreover, macroinvertebrate indices such as % CHIR, FLBI and BMWP had significant correlation with the number of fecal coliforms. Therefore, these indices can be used to predict the level of fecal contamination in surface waters.

In general, this study gives an insight for the application of macroinvertebrate families and indices to indirectly measure the quality of water bodies with regard to fecal matter pollution.

7.2. RECOMMENDATIONS

- ❖ To Minimize consequences of sand extraction and small scale agricultural activities;
- Jimma zone health office should motivate HEWs in order to focus implementation of sanitation packages in the community who have been adjacent to streams and assigned in sand extraction
- Jimma zone agriculture office in collaboration with woreda offices should try to motivate farmers in order to use compost to reduce nutrient pollution
- Further studies should be done at large scale to come up with best model

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ANNEX

PROCEDURES FOR DETERMINATION OF PHOSPHATE AND NITRATES

NITRATE NITROGEN

Phenoldisulfonic method

1. first the chloride content of the water sample was determined by treating 100ml of water sample with equivalent amount of silver sulfate solution(1ml for 1mgcl) in order to precipitate the chlorides
2. precipitated chlorides removed by filtration
3. 100ml of sample from clarified filtrate was added in to evaporating Dish.
4. Evaporated to dryness over hot water bath
5. 2 ml phenoldisulfonic acid reagent was added in residue ,then rubbed thoroughly to insure dissolution of all solids
6. After diluted with 20 ml distilled water,7ml of NH4OH solution was added until maximum yellow color was developed
7. In order to remove any flocculent hydroxide EDTA was added drop by drop WITH stirring until turbidity re dissolves.
8. Filtrate of clear solution was transferred in to 50ml volumetric flask
9. Diluted to 50ml mark with distilled water and mixed thoroughly
10. The absorbance was measured at wave length of 410nm against a blank prepared from the same volumes of reagents as used for the sample.
11. A calibration curve was constructed in the range 0-2mg/LNO3-N by adding 0,0 .2,0.5, 1.0,3.0,5.0,and 10ml of standard nitrate solution to separate evaporating dishes and treated same as sample.
12. Finally concentration of NO3-N IN the sample by reference to calibration curve.

PHOSPHATE

Stannous chloride method

- Determination of orthophosphate

1. The following series of phosphate standards were prepared by measuring indicated volume of standard phosphate solution in to separate 100ml volumetric flasks.

Table 12: Shows standard phosphate solution in ml and concentration in) µg /100ml

Standard phosphate solution. Ml	Phosphate(PO_4) µg /100ml
0	0
1	5
2	10
3	15
4	20
5	25
6	30

2. 0.05ml 1 drop of phenolphathalein indicator solution was added in the sample
3. 4ml of acid- molybdate solution was added in to each of the standards and sample by measuring pipette.
4. Mixed thoroughly by inverting each flasks six times
5. 0.5ml(10 drops)of stannous chloride solution was added in to each standards and sample by medicine dropper
6. Again each flasks mixed by inverting for six times
7. After 10 minutes, phosphate absorbance was measured by spectro-photo meter at wave length 690nm; distilled water was used as blank.
8. Finally calibration curve was constructed by using standards and the amount of phosphate present in the sample was determined.

Procedure for preparing samples for Macroinvertebrates

1. Pour the entire contents of the bucket into a sieve bucket with 500 μm mesh size**.**

1. Using a wash bottle filled with river water, rinse all the organisms from the bucket into the sieve. This is the composite sample for the reach
2. Estimate the total volume of the sample in the sieve and determine how large a jar will be needed for the sample
3. Fill in a sample label with the Sample ID and date of collection. Attach the completed label to the jar and cover it with a strip of clear tape. Record the sample ID number for the composite sample on the Sample Collection Form. For each composite sample, make sure the number on the form matches the number on the label.
4. Wash the contents of the sieve to one side by gently agitating the sieve in the water
5. Place a waterproof label inside each jar with the following information written with a number 2 lead pencil:
    - Site ID
    - Type of sampler and mesh size used
    - Name of site
    - Date of collection
    - Collectors initials
    - Number of stations s
6. Organisms will be properly preserved with 70% ethanol
7. Store labeled composite samples in a container with absorbent material that is suitable for use with 70% ethanol until transport or shipment to the laboratory

Table 13: One-way ANOVA in different macroinvertebrate indices around sample sites

		Sum of Squares	Df	Mean Square	F	Sig.
BMWP	Between Groups	1113.000	3	371.000	52.376	.000
	Within Groups	56.667	8	7.083		
	Total	1169.667	11			
ASPT	Between Groups	2.219	3	.740	5.231	.027
	Within Groups	1.131	8	.141		
	Total	3.349	11			
FBI	Between Groups	1.279	3	.426	4.371	.042
	Within Groups	.781	8	.098		
	Total	2.060	11			
Simpson reciprocal	Between Groups	48.127	3	16.042	22.882	.000
	Within Groups	5.609	8	.701		
	Total	53.736	11			
Simpson index of diversity	Between Groups	.047	3	.016	7.574	.010
	Within Groups	.017	8	.002		
	Total	.063	11			
Shannon index	Between Groups	1.041	3	.347	33.478	.000
	Within Groups	.083	8	.010		
	Total	1.124	11			
Macro invertebrate abundance	Between Groups	4711.583	3	1570.528	9.395	.005
	Within Groups	1337.333	8	167.167		
	Total	6048.917	11			
Percent CHI	Between Groups	1251.674	3	417.225	9.790	.005
	Within Groups	340.935	8	42.617		
	Total	1592.609	11			

Correlation is significant at 0.01 and 0.05

Table 14: Correlation between Coliform organisms and macroinvertebrate abundance in study site

Correlations				
		Fecal Coliform	Total Coliform	Macroinvertebrate abundance
Fecal Coliform	Pearson Correlation	1	.999**	-.457
	Sig. (1-tailed)		.000	.067
	N	12	12	12
Total Coliform	Pearson Correlation	.999**	1	-.453
	Sig. (1-tailed)	.000		.069
	N	12	12	12
Macroinvertebrate abundance	Pearson Correlation	-.457	-.453	1
	Sig. (1-tailed)	.067	.069	
	N	12	12	12

**. Correlation is significant at 0.01 levels (1-tailed).

Table 15: Spatial variation of macroinvertebrate taxa and abundance of the study sites

Taxa	Gibe Seka			Mite Seka			Arrer			Naada		
	G1	G2	G3	M1	M2	M3	A1	A2	A3	N1	N2	N3
Coenagriidae	18	24	19	15	17	17	1	3	2	2	3	2
Coroxidae	5	7	8	0	0	0	0	0	0	0	0	0
Baetidae	5	9	10	0	0	0	3	5	4	39	33	0
Belestomatidae	1	3	4	0	0	0	1	2	0	0	0	0
Gomphidae	2	4	3	5	9	5	0	0	5	0	0	0
Epherimedae	3	4	0	0	0	0	0	0	0	0	0	0
Hydrometridae	0	1	2	0	0	0	0	0	0	0	0	0
Chironomidae	0	0	0	19	18	13	6	5	4	6	9	10
Caenidae	0	0	0	0	0	0	12	10	0	7	7	8
Hydosychidae	0	0	0	0	0	0	1	2	0	3	4	4
Heptageniidae	0	0	0	0	0	0	0	0	0	1	2	1
Ashinidae	12	9	14	4	8	3	0	0	1	0	0	0
Simulidae	5	13	4	3	0	0	0	0	0	0	0	0
Cordulidae	13	7	9	1	0	0	0	0	0	1	0	0
Tripulidae	7	3	0	0	1	0	0	0	0	0	1	0
Sphaeridae	6	9	4	12	7	10	9	15	10	9	2	5
Lepidostomatidae	5	7	0	0	5	1	0	0	0	0	2	0
Leptoceridae	4	3	2	0	0	2	1	0	0	0	0	1
Total	86	103	79	59	65	51	34	42	26	68	63	31