LEAF LITTER DECOMPOSITION AND MACROINVERTEBRATE ASSEMBLAGE IN STRESSED TROPICAL URBAN STREAM: IMPLICATIONS FOR STREAM ECOLOGICAL INTEGRITY ASSESSMENT.IN CASE OF AWETU STREAM, JIMMA SOUTH WESTERN ETHIOPIA

By

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Leaf Litter Decomposition and Macroinvertebrate Assemblage in Stressed Tropical Urban Stream: Implications for Stream Ecological Integrity Assessment. In Case Of Awetu stream, Jimma, South Western Ethiopia.

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Dedication

Dedicated

To

My beloved Grandmother Werkitu Tekeste.

Bibliographical Sketch

The author, Armaye Biresaw Melese was born on July 12, 1990 in Addis Ababa, Ethiopia. She attended her elementary education at Misrak Ber Primary and Junior Secondary School. She completed her secondary school at Lem Secondary and her preparatory class at Ginchi secondary and preparatory school. Armaye joined Haramaya University and graduated with BSc. degree in Natural Resource Management on July 2011.

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Acrimony and Abbreviations

AFDM	Ash Free Dry Mass
ANCOVA	Analysis Of Covariance
ANOVA	Analysis Of Variance
APHA	American Public Health Association
BMWPs	Biological Monitoring Working Party Score
BOD	Bio Chemical Oxygen Demand
CPOM	Course Particulate Mater
DCA	Detrended Correspondence Analysis
DNRE	Department Of Natural Resources And Environment
DO	Dissolved Oxygen
FPOM	Fine Particulate Matter
IHF	Fluvial Habitat Index
PCA	Principal Component Analysis
QBR	Riparian Habitat Quality
RDA	Redundancy Analysis
SRP	Soluble Reactive Phosphorus
TSS	Total Suspended Solids

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Abstract

The impact of urbanization activities on streams has substantially increased in recent years. As result, most urban streams are losing their ecological integrities. Effective characterization of ecosystems integrity requires information from both structural and functional components an ecosystem. In this work we examined the ecological integrity of Awetu stream impacted by urbanization using changes in macroinvertebrate community as structural integrity measures and leaf litter decomposition as a measure of functional integrity. Macroinvertebrate derived metrics and biotic indices were used as measures of structural integrity. Leaf litter decomposition rates of exotic *Eucalyptus globulus*, native *Salix mucronata and Syzgium guineense*, and a standard cotton strip were used as ecosystem functional integrity indicator. A total of six study sites were selected along a gradient of increasing nutrient enrichment and habitat degradation in the study stream. Selected pollution indicator parameters like TSS, conductivity, BOD, SRP, ammonium and nitrate were highly increased from upper stream to downstream sites. Measures of structural integrity, biotic indices and invertebrate metrics clearly discriminated upstream sites from heavily impacted downstream sites. The invertebrate community was sensitive to nutrient enrichment and habitat quality degradations as it responded negatively to increases in nutrient concentration, changes in the riparian vegetation from native to absent or exotic, and to reduction in the habitat quality. Litter decomposition rates also were sensitive to these changes, and lowest values were observed at the upper stream sites, which were classified as having a less impacted ecosystem functioning. The functional and structural approaches used in this study gave the same results for the most impacted and unimpacted sites. The results suggests that data on both stream structure and function are important for assessing ecological integrity of impacted tropical urban streams. Therefore, the incorporation of litter decomposition as a functional measures in evaluations of tropical urban streams ecological integrity is important.

Keywords: Ecological integrity, Urban stream, Functional integrity, Litter decomposition, Structural integrity

Chapter One: Introduction

1.1 Background Information

Multiple anthropogenic pressures pose an increasing threat to aquatic ecosystems ever nowadays than the past. Freshwater ecosystems have been increasingly modified from their historic condition by human activities. These changes often have unintended and undesirable consequences such as reducing biodiversity, altered ecosystem functioning, and losses of ecosystem services (Dudgeon et al., 2006). Nowadays, streams throughout the world are under strong pressures linked with both point and nonpoint pollution (Vorosmarty et al., 2010). Above all major change in land covers associated with urbanization have been regarded as a key factor responsible for stream ecosystem degradation.

The impact of urbanization activities on streams ecosystem has substantially increased worldwide linked with population booming. Urban stream syndrome, a phenomena used to refer the ecological disintegrity of urban streams due to urbanization impacts, have been illustrated in many studies that examine urbanization impacts (Paul and Meyer, 2001;Chadwick et al., 2006).

Modern stream ecologists have long recognized that streams are well interconnected with and influenced by their surrounding activities in their watershed (Castela et al., 2008). They are profoundly affected by changes in the vegetation and land cover of their riparian zones. For instance, the effects of agriculture, clearing riparian vegetation and urbanization on aquatic systems have been well documented to affect aquatic ecosystem integrity (Moggridge et al., 2014). Besides this factors, it is well known that natural factors like geographical variation can also affects ecosystems components and ultimately stream functioning. Hence, to optimize management options and better understanding of the alterations of this factors ecosystem integrity evaluation is necessary.

In ecology, the importance of understanding and predicting the effects of pressures on stream ecosystem integrity is well recognized (Dudgeon et al., 2006). However, still it remains a central challenge to develop rapid, sensitive, low-cost and ecologically relevant tools that can assess the ecological integrity a given ecosystem, considering both functions and ecosystem structures at the same time (Castela et al., 2008). River monitoring programs, in most cases, focuses on ecosystem structures than focusing on ecosystem functions to assess ecosystem integrity (Gessner et al., 1999). Bio-assessment tools rely exclusively on structural aspects of the aquatic ecosystem to evaluate their ecological integrity (Hladyz et al., 2010). Benthic macroinvertebrates have been extensively used in this way through the application of metrics and biotic indices (Lecerf et al., 2006). However, nowadays there are attempts to change these problems in aquatic ecosystem integrity assessment programs. One way consists of assessing the effects of community attributes (e.g., species richness) on ecosystem-level processes, such as primary production, litter decomposition, or nitrogen mineralization and release, and metabolism assessment (Masese et al., 2014).

Thus, in any aquatic ecosystem monitoring program there is urgent need to find assessment method which helps to foreseen stressors impact on ecosystem-level process, rather than solely depending on structural integrity, which will be important from the point view of ecological integrity assessment as well as restoration activities. Adequate characterization of ecosystems integrity requires information on both the structural and functional components (Gessner and Chauvet, 2002), because stressors might cause changes to structure but not function, to function but not structure, or to both (Boyero et al., 2015). In this study, we assessed the ecological integrity of a tropical urban stream using benthic macroinvertebrate as a measure of structural integrity, and leaf litter decomposition rates as a measure of functional integrity so as to compare the use of functional and structural indicators to detect changes in water and habitat quality in tropical urban stream.

1.2 Statement of the problems

Human population growth and landscape developments have led to severe pressures being placed on freshwater systems globally (Vorosmarty et al., 2010). Change in land covers associated with urbanization have been regarded as a key factor behind the alteration of stream hydrology, geomorphology, physico-chemistry, and consequent deterioration of water and habitat quality (Yule et al., 2015). These changes typically affect the structural and functional aspects of stream ecosystem to various extents (Castela et al., 2008; Moggridge et al., 2014). Therefore, evaluations of their ecological integrity are a vital step for a better understanding of these alterations and optimize management options.

In streams, the traditional ecological integrity assessment method has relied on measurements of physical and chemical variables, and of community structures e.g MI community assemblage (Barbour et al., 1999; Bonada et al., 2006)) and neglects the assessment of ecosystem functions e.g., litter decomposition (Gessner and Chauvet, 2002). Ecological integrity of an ecosystem refers to both the structural and functional components (Castela et al., 2008; Masese et al., 2014) and it represent a holistic approach for ecosystem health assessment.

The functional component of ecosystem includes important processes occurring at ecosystem level such as decomposition rate, whereas structural component refers to the composition of communities and their resources (Gessner and Chauvet, 2002). More importantly, the structural and functional integrity indicators are not necessarily concordant (Nelson, 2000; Castela et al., 2008) emphasizing the need to consider both components in stream ecological integrity assessment programs. Focusing only on ecosystem structure preclude comprehensive ecosystem integrity assessment (Gessner and Chauvet, 2002; Bonada et al., 2006). This is the cardinal reason why several studies (Castela et al., 2008; S. D. Tiegs et al., 2013) recommend the incorporation functional indicators into ecological integrity assessment programs.

Litter decomposition in streams is an important ecosystem level process that links riparian vegetation, environmental conditions, microbial and invertebrate activities (Hladyz et al., 2010) and has been suggested and used as indicator of functional integrity of temperate urban streams (Castela et al., 2008; Young et al., 2008). However, their study in the tropics are limited (Silva-Junior et al., 2014; Yule et al., 2015). In tropical streams, litter decomposition rates often have been reported to be faster than temperate streams, because of increased microbial activity consequent to higher water temperatures. Thus, microbial breakdown of leaf litter may be more important in tropical streams than in their temperate counterparts (Boyero et al., 2015) and this difference may extend into urbanized streams. Therefore, studies of urban stream ecology are vital for a better understanding of the various urbanization processes that alter stream ecosystem function

Few studies have been done in tropical urban streams, and most of them focused on water quality and stream hydrology. The extent of urbanization influences on ecosystem functions of tropical urban streams remain limited (Masese et al., 2014) and no studies regarding the effects of urbanization on stream ecosystem functioning in Ethiopia have been published. Furthermore, no comparative studies have been carried using litter decomposition and macroinvertebrates in stream along a gradient of habitat degradation and nutrient enrichment towards developing tools that can be used by water quality managers in developing countries from a tropical regions.

In this study, the ecological integrity of a tropical urban stream were examined using benthic macroinvertebrate as a measure of structural integrity, and three species of leaf litter and cotton strip decomposition rates as a measure of functional integrity –— exotic *Eucalyptus globulus*, native *Salix mucronata* and *Syzgium guineense*, and a cotton strip. Further, it aimed to compare the use of functional and structural indicators to detect changes in water and habitat quality in tropical urban stream.

1.3 Significance of the Study

The ecological integrity of rivers and streams has become a subject of key importance for the maintenance or rehabilitation of these resources worldwide. Specifically linked with urbanization, protecting ecological quality of rivers in urbanized areas are growing problems whose solutions will require extensive effort and research. Many goals in the conservation and restoration of aquatic ecosystems relate to ecosystem processes. In addition to structural biological parameters (e.g. macroinvertebrate, fish and diatom community structure), it is desirable to determine which ecosystem processes respond to anthropogenic stresses, and which may be good indicators of functional ecosystem damage.

Assessment schemes targeted at ecosystem processes are few (Gessner and Chauvet 2002), especially for highly impacted urban streams . This gap is a fundamental limitation of current ecosystem assessment. Thus, studies of urban stream ecology are vital for a better understanding of the various urbanization processes that alter stream ecosystem function and to identify potential conservation, remediation, and recovery strategies. The study results is important in (1) Explaining the relative importance of structural (macroinvertebrate) and functional (leaf-break down rate) approaches of ecosystem assessment methods for highly impacted urban streams. (2) The role of leaf-breakdown rate as a measure of ecological integrity for urban streams . (3) The response of macroinvertebrate community for impacted urban stream system. (4) Strengthening the local water quality monitoring programs.

Chapter Two: Literature Review

The conversion of land use from rural and forested to urban can negatively affect stream ecosystems by altering trophic resources, hydrology, geomorphology, biodiversity, and water chemistry (Chadwick et al., 2006; Dudgeon et al., 2006). Along with urbanization, increases in impervious surfaces tend to heavily influence the ecology and health of urban streams. As Many studies have illustrated that an increase in imperious surfaces, which is a side effect of urbanization, decreases stream biotic health by reducing biodiversity, increasing runoff, simplifying channel morphology, increasing pollutants, and decreasing invertebrate diversity; this phenomena is known as the urban stream syndrome (Chadwick et al. 2006). Aspects of stream health can be derived from observation of stream functions, one key functions being the decomposition of leaves (Young et al. 2008).

Along with urbanization comes an increase in pollutants (chlorides) and nutrients (N and P) due to impervious surfaces and agricultural runoff (Pickett et al., 2011). This increase in pollutants and nutrients can affect stream ecosystem in many ways. More sensitive and essential invertebrate species can be eliminated while more tolerant species thrive due to an intermediate increase in nutrients and pollutants (Pascoal et al., 2003). However, too large of an increase can eliminate the majority of species which can negatively affect leaf decomposition rates (Masese et al., 2014). In either case, increasing nutrients and pollutants due to urbanization can affect the invertebrate diversity and thus the rate of leaf breakdown in urban streams.

In streams there are two main energy sources: in stream photosynthesis and imported organic matter from overhead forest cover or streamside vegetation (Lecerf et al., 2006). Forested streams rely heavily upon imported organic matter through the decomposition of CPOM to FPOM (leaves to small particulates) (Smith and Chadwick, 2014). Larger streams and rivers not surrounded by forest therefore rely on the transformation of CPOM to FPOM in forested streams as their main energy source. Without the ecosystem service of leaf decomposition provided by small streams, large streams can become unable to sustain their inhabitants (collectors) while being burdened with excess unusable CPOM (Boyero et al., 2015). Because imported organic matter is such a pivotal part of stream function, it is necessary to observe leaf litter decomposition in order to assess a stream’s function and health. The decomposition of leaf litter is heavily influenced by the presence of invertebrates, bacteria, temperature, nutrients, and the type of leaf present (Hladyz et al., 2010). Invertebrates are observed to play one of the main roles in the decomposition of leaves; they actively break down leaves into smaller pieces by exposing more surface area to microbial colonization, therefore increasing the decomposition rate (Li and Dudgeon, 2009).

A healthy stream, therefore, can be characterized as one with a rich diversity of invertebrates that actively break down leaf litter (Chadwick et al. 2006). Likewise, the more nutrients (organic pollutants) that are found in streams, there were found to be a more diverse presence of invertebrates (Silva-Junior et al. 2014).

2.1 Ecological integrity

Ecosystems are extremely complex. The three primary elements of an ecosystem are its structure, composition and function: Ecosystem structure refers to all of the living and non-living physical components that make up that ecosystem. The more components that make up an ecosystem, the more complex its structure becomes. Ecosystem composition refers to the variety of living things found within an ecosystem and Ecosystem function refers to all of the natural ecological processes that occur within an ecosystem.

There is more than one way to define ecological integrity. A few different definitions follow: A report by the Panel on the Ecological Integrity of Canada's National Parks in 2000 proposed that "an ecosystem has integrity when it is deemed characteristic for its natural region, including the composition and abundance of native species and biological communities, rates of change and supporting processes." In 1999, the BC Parks Legacy Panel determined that an ecosystem has ecological integrity when "the structure, composition and function of the ecosystem are unimpaired by stresses from human activity; natural ecological processes are intact and self-sustaining, the ecosystem evolves naturally and its capacity for self-renewal is maintained; and the ecosystem's biodiversity is ensured." According to Lecerf et al. (2006), ecological integrity (also known as bio- logical, biotic, or ecosystem integrity) refers to a given state of a stream along a gradient of impairment that ranges from strongly impacted to pristine. More recently, the term ecological integrity has been reserved for the pristine endpoint of the impairment gradient only, whereas all other states represent different states of ecosystem health (Castela et al., 2008). Gessner and Chauvet (2002) define Ecological integrity as, it is an ecosystem property that reaches the highest level when its structure is complete and when all processes inside it work perfectly.

2.2 Benthic macroinvertebrate assemblage

A wide range of organisms (bio indicators) are employed to assess aquatic ecosystems. Among the biological communities, macroinvertebrates have proven to be useful indicators to determine the status of rivers. , since differences in environmental requirements among taxa produce community characteristics that reflect ecological conditions (Bouchard, 2004). Macroinvertebrate communities can respond to nutrient enrichment, oxygen availability, food quantity and quality , and changes in habitat structure (Dudgeon et al., 2006).

Macroinvertebrates such as snails, crustaceans and the larvae of many insects that have an aquatic life stage respond to a broad range of environmental conditions, are relatively immobile and live in close contact with both bottom sediments and the water column, thereby having the potential for exposure to stressors via both sediment and aqueous pathways. Benthic macroinvertebrates have been used in several bio monitoring and bio assessment programs (Barbour et al., 1999). According to Barbour et al. (1999), macroinvertebrates are relatively easy to identify to family level, are good indicators of localized conditions and integrate the effects of short-term environmental variations, among others. In studies where invertebrates have been eliminated by insecticides, the decomposition rates of leaves significantly decreased, suggesting that invertebrates play the main role in decomposition in the majority of streams (Lewin et al., 2013).

2.3 Leaf Litter Decomposition in Streams

Leaf litter decomposition is a critical ecosystem level process in streams and other aquatic environment. Many forested headwater streams are heterotrophic ecosystems in which inputs of plant litter from the surrounding forest are a major source of energy (Masese et al., 2014). Detrital inputs generally exceed within-stream primary production because light is limited by riparian shading (Woodward et al., 2012). Leaf litter entering the stream is transformed by a combination of biotic and abiotic processes, including the leaching of soluble leaf constituents, physical fragmentation, decomposition by fungi and bacteria, and consumption primarily by leaf-shredding detritivorous invertebrates (Gessner et al., 1999). These invertebrates are a major link between terrestrial litter and the aquatic food web because they consume leaf litter and their feeding activity accelerates the production of fine particulate organic matter, which is the main food source of other detritivores, such as gatherer–collectors and filter-feeders (Hepp and Santos, 2009).

Among the qualities that an ecosystem process should have in order to qualify as a candidate for assessing the ecological condition of streams is sensitivity to human impacts. Litter decomposition a key ecosystem level process in streams meets this key criterion in many situations. Litter decomposition responds to highway runoff, eutrophication, mine runoff, invasion of exotic species and alterations of land use such as timber harvest among other anthropogenic activities and agriculture (Maltby and Booth, 1991; Hagen et al., 2006;Bahar et al., 2008).

Leaves entering streams generally break down via a multi-step process: chemical leaching of soluble compounds, aerobic degradation by microbial organisms (conditioning), physical abrasion, and physical fragmentation by leaf-shredding macroinvertebrates (shredders) (Gessner et, al. 1999 ;Hagen et al. 2006). Leaching is the rapid loss of soluble leaf constituents shortly after immersion (typically within 24 h), conditioning the modification of leaf matrix by microorganisms enhancing leaf palatability for detritivorous macro invertebrates called shredders, and fragmentation the physical break up and removal of pieces from the original coarse litter particle, whether mediated by shredder feeding or shear stress and abrasion (Gessner et,al.1999.).

Alterations to leaf litter decomposition rates can affect the timing and mass of benthic organic matter standing stocks and, thus, the seasonal feeding patterns of shredders. This effect can ultimately limit shredder abundance and diversity, which can have bottom-up effects on the food web. Alterations to organic matter stocks also affect nutrient export, dissolved organic C composition and availability, and habitat for microorganisms and larger biota (Paul and Meyer, 2001; Roy et al., 2003). In general, leaf-litter breakdown includes leaching of soluble compounds, microbial decomposition and conditioning, and feeding by aquatic invertebrates. Several studies demonstrate that microbial activity and leaf decomposition in streams are regulated by leaf litter quality and environmental factors such as temperature, concentration of dissolved nutrients and pH (Paul and Meyer, 2001; Dobson et al., 2002; Roy et al., 2003;Moggridge et al., 2014).

Larger streams and rivers not surrounded by forest therefore rely on the transformation of CPOM to FPOM in forested streams as their main energy source (Gonçalves et al., 2006). Without the ecosystem service of leaf decomposition provided by small streams, large streams can become unable to sustain their inhabitants (collectors) while being burdened with excess unusable CPOM. Because imported organic matter is such a pivotal part of stream function, it is necessary to observe leaf litter decomposition in order to assess a stream's function and health. The decomposition of leaf litter is heavily influenced by the presence of invertebrates, bacteria, temperature, nutrients, and the type of leaf present (Li and Dudgeon, 2009). Invertebrates are observed to play one of the main roles in the decomposition of leaves; they actively breakdown leaves into smaller pieces by exposing more surface area to microbial colonization, therefore increasing the decomposition rate (Masese et al., 2014).

2.3 Litter as functional ecological integrity indicator

Functional integrity is a complement to structural integrity and refers to the rates, patterns, and relative importance of different ecosystem-level processes under reference conditions. Decomposition of litter, a key ecosystem level process in small woodland streams, has been shown to have potential to be used as a functional tool to assess organic contamination (Lecerf et al., 2006). Several studies demonstrate that anthropogenic stress affects leaf breakdown rates (e.g. Gessner & Chauvet, 2002). Some authors find that nutrient enrichment stimulates leaf breakdown (Hladyz et al., 2010), while other working groups have demonstrated that this is not always the case. In a Hong Kong stream, the presence of organic pollution led to an increase in leaf breakdown rates in summer and a decrease in winter, and no effect of pollution was found in an Indian river (Li and Dudgeon, 2009). Leaf breakdown rates were lowered by mine effluent discharge and they were negatively correlated with the concentration of dissolved zinc in stream water. However, high values for leaf breakdown rates were found in a moderately heavy metal polluted stream, which was explained by the presence of an adapted fungal community and high N and P concentrations in the stream water (Smith and Chadwick, 2014).

2.4 Standardize Cotton Strip as functional ecological integrity indicator

The use of standardize cotton strip is clearly discussed by different researchers (S. D. Tiegs et al., 2013). Some of the shortcomings of the litter-bag approach especially those relating to a lack of standard organic matter substrate − have been overcome with a cotton-strip assay (Tiegs et al., 2013). One shortcoming of the litter-bag assay is its limited consistency. The cotton-strip assay was first developed by the textile industry as a test to evaluate the effectiveness of fungicide treatments and loss of tensile strength was measured rather than mass loss to indicate decay.

Eventually, a material produced by the Shirley Company (Manchester, UK), Shirley Burial Test Fabric, became used as a standard in soil studies as an index of decomposition. More recently, the Shirley material has been adopted for use in aquatic habitats, including streams (Tiegs et al., 2007). Along with the Shirley material, and published protocols for its standard use, came values of tensile-strength loss that were comparable across studies, and large-scale syntheses followed. The cotton-strip assay offers numerous advantages over the litter-bag assay. Being 95% cellulose, the cotton-strip assay allows a degree of standardization of the material that is not possible with plant litter. Further, cellulose is a highly ecologically relevant compound because it constitutes the bulk of plant and is the most abundant organic polymer on Earth. cotton-strip assays have been shown to be sensitive to diverse human activities including urbanization and agriculture contamination.

2.5 Effect of eucalyptus species litter

The decomposition rates of leaf litter vary considerably among the diverse vegetation species. This process is influenced by the physical and chemical characteristics of the litter and the environment as well as the micro and macro fauna of aquatic environments. Such relationship influences directly the breakdown of organic matter performed by fungi, bacteria and invertebrates (Gessner et al. 1999).

The aquatic ecosystems have suffered impacts due to the replacement of native riparian vegetation by exotic species. Such changes affect the amount and quality of the leaf litters (Paul and Meyer, 2001), causing alterations in the aquatic ecological processes, especially those related to leaf decomposition and nutrients cycling processes performed by the microbial communities and benthic detritivorous macro-invertebrates (Hladyz et al., 2010).

Some authors have reported the negative impact of Eucalyptus sp. leaves on the decomposition micro-organisms; it reduces the colonization rate of filamentous fungi and yeasts, also demonstrated that for the benthic macro-invertebrates the consumption of Eucalyptus sp. leaves causes the breakdown of the trophic chain and reduction of the organism diversity (Masese et al., 2014). Some of the problems associated with the decomposition and consumption of eucalyptus leaves by benthic macro-invertebrates involve physical characteristics such as hardness and the presence of cuticle, besides the chemical composition of the plant, which has low concentration of nitrogen and phosphorous, high contents of tannins and dense oil glands that may act as a toxic substance and cause a low decomposition rate (Hladyz et al., 2010). Therefore, based on the premise that Eucalyptus sp., when compared to other native species, has a greater amount of chemical compounds that inhibit the biological activity (Masese et al., 2014).

Chapter Three: Objectives

3.1 General Objective

- To evaluate litter decomposition rate and macroinvertebrate community as indicators of urbanization influences on ecological integrity of tropical streams. Implications for stream ecological integrity assessment tool.

3.2 Specific Objectives

- To determine the physicochemical water quality parameters of the study stream
- To evaluate the habitat quality of the study stream based on Quality of the Riparian Corridor Index (QBR) and the Fluvial Habitat Index (IHF).
- To determine abundance and distribution of macroinvertebrate community in impacted urban stream
- To determine leaf litter decomposition rates in impacted urban stream.
- To compare exotic and native leaf litter decomposition rate in impacted urban stream
- To determine the standard cotton strip decomposition rate in impacted urban stream
- To compare litter decomposition rate and MI community structure in impacted and unimpacted stream sites
- To identify natural and anthropogenic influencing factors on macroinvertebrate distribution and litter decomposition process in the study stream.

Chapter Four: Material and Methods

4.1 Study Sites Descriptions

The study was conducted on Awetu stream which is located in Southwestern, Ethiopia. This stream bisects the center of the Jimma city to make its outlet southward and plays an important role on the day-to-day life of the town and surrounding population. The stream is subjected to different types of anthropogenic pressures varying in extent from upstream to downstream, thereby creating spatial variability of water and habitat quality in the stream segment (Haddis et al., 2014). The upstream segment is regarded as slightly impacted compared to downstream sections which receive a lot of pollutants from abattoir, street runoff, and small scale industries in the town. The stream is predominantly surrounded by urban area in down- and middle stream sections, with only a small percentage of agricultural land use. The upstream section had good riparian corridor, covered with natural native vegetation. Using selected physicochemical and biological parameters the downstream section of the stream is classified as an impacted system (Dejene and Legesse, 1997; Haddis et al., 2014). The climate in the study area is tropical. The annual rainfall ranges from 1138 mm to 1690 mm (Alemu et al., 2011). Maximum precipitation occurs during the three months period, June to August, with minimum rainfall in December and January. The landscape is topographically heterogeneous, consisting of Afromonte forest cover. The riparian forest in the urban stream catchment has been severely affected by high levels of human activity at the riverbanks and the presence of exotic riparian species such as Eucalypts globules.

A total of six sites were selected along 7 km reach of Awetu stream stretch, distributed upstream (A1, S and A2), middle (A3), and downstream (A4 and A5) along a downstream increased nutrient and habitat degradation gradient (Figure 1). Sites A1 and S, had canopy cover and highly diverse riparian vegetation including eucalyptus species; sites A2 and A3 had light anthropogenic disturbance like grazing, swimming, light agriculture, and small weirs, and sites A4 and A5 had a distinct odor and color with strong bank channelizing and flow regulation characterized with input of raw municipal wastewater effluent, carwash effluent, and municipal solid waste.

Riparian vegetation gradually decreased downstream leaving the stream increasingly exposed, and most of the riparian vegetation was replaced with exotic species in downstream sections.

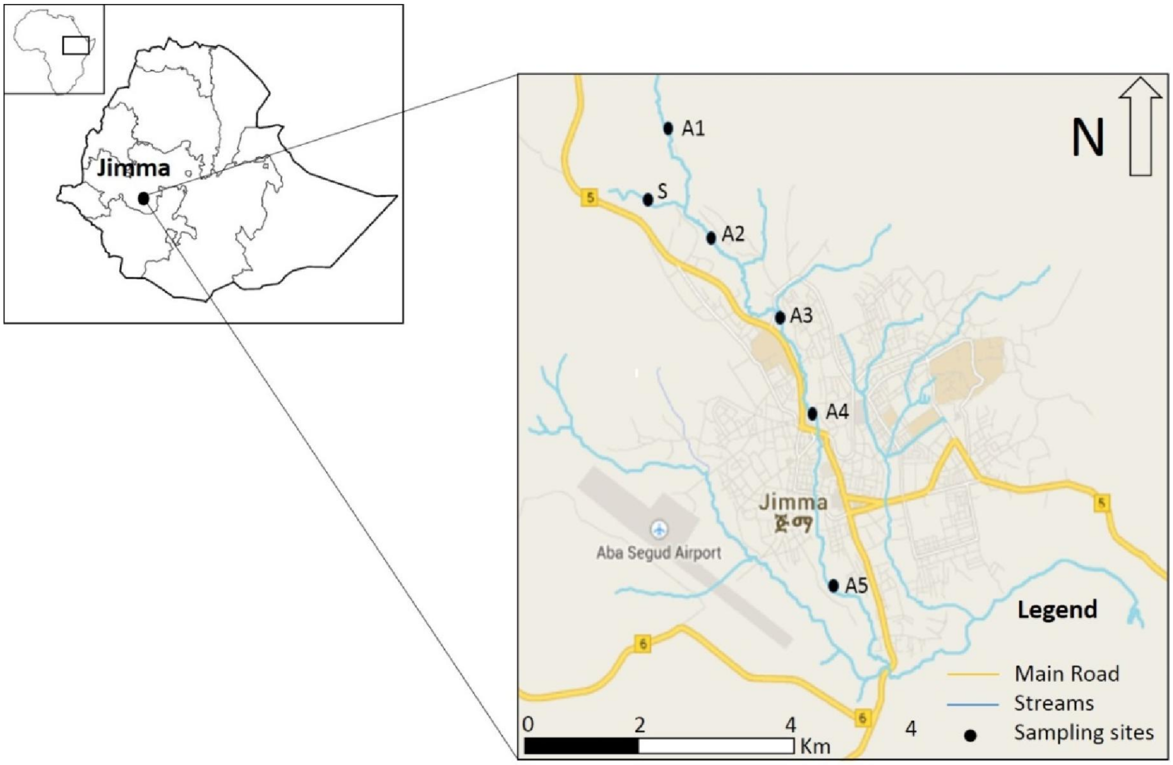


Figure 1. Study area and locations of sampling sites on the Awetu stream.

4.2 Experimental Design and Period

In this work, a rapid stream assessment was performed to evaluate stream ecological integrity using benthic macroinvertebrate derived metrics and biotic indices as measures of structural integrity, and *Syzgium guineense, Eucalyptus globules* and *Salix mucronata* leaves litter decomposition rates as a measure of functional integrity. In addition, a standard cotton strip was deployed to assess the functional integrity of the studied sites. Sampling sites was selected on purpose to present gradient of habitat and water quality degradation (See section 4.1). The study was conducted from June 07 to July 08, 2017 following a standard leaf litter decomposition test at each sampling station. To conform to stream rapid assessment characteristics, benthic samples were taken only once, and the decomposition of leaf litter was assessed three times along a 33-day period, at each site. Both structural and functional approaches used here are in the line with the criteria defined by Bonada et al., (2006) and should therefore give an accurate picture of the ecosystem health status. The habitat and water quality of the sites were assessed and then response of litter decomposition and benthic invertebrate to the habitat and water quality was evaluated.

4.3 Water Quality Assessment

Water samples were collected on June 07, 2017. In each sampling site, three 200 mL water samples were filtered (Whatman GF/F) and stored in clean bottles. At the same time unfiltered 1-L samples were collected using clean bottles. The filtered and unfiltered samples were stored in an ice box (below 4 °C) and were transported to Environmental Health Laboratory unit at Jimma University, Ethiopia, within 1 to 6 h for analysis. Chemical analysis followed standard methods by APHA (1998). Filtered water samples were analyzed for chloride, soluble reactive phosphorus (SRP), and ammonium and nitrate concentration according to the standard methods as prescribed by APHA (1998). Correspondingly, the unfiltered 1-L samples will analyzed for total suspended solids (TSS), and five day biochemical oxygen demand (BOD) according to the standard methods as prescribed by APHA (1998). Dissolved oxygen (DO), electric conductivity, pH and water temperature were measured in-situ using a multi-probe meter (HQ30d Single-Input Multi-Parameter Digital Meter, Hach). The physical features measured included stream width, depth, and adjacent land use pattern. Current velocity was measured with Vale port's Flow Meter Model 001. Discharge was calculated as the wetted width of each site multiplied by its average depth and velocity.

4.4 Riparian Habitat Quality Assessment

The habitat quality of the sample sites were assessed by using the Fluvial Habitat Index (IHF) and the Quality of the Riparian Corridor Index (QBR). Theses indices are the best indices currently available for the purpose of such study, and they have also been commonly used by water agencies and consultancies in tropical region and elsewhere (Barquın et al., 2011). The IHF evaluates in-stream habitat heterogeneity and considers seven items related to substrate, current velocity and depth, shadow, presence of elements of heterogeneity and aquatic vegetation. The final IHF score is the sum of the scores obtained for each item. The higher the habitat heterogeneity of a stream, the better the final IHF score indicating little impacts (Barquın et al., 2011). In our study, the final IHF score decreased in a downstream direction indicating degradation of habitat quality.

The QBR index is an easy-to-use field method for assessing the habitat quality of riparian forests. It was developed to be used in Mediterranean streams of Spain and applied in several regions of the world with satisfactory results. Some changes have been introduced by several authors in order to adapt it to other geographical areas although the basic structure and the assessment procedure have not significantly changed. Here, the original version of the QBR index was used. The index is based upon four main aspects of the riparian area being studied, and unlike indices currently in use which assess the water quality itself or the habitat directly adjacent to the stream; the QBR index assesses a site's entire floodplain. It generates a score that can then be used to contrast sites, to compare sites to ideal conditions, or to assess the success of impacts of human activities over time.

The QBR classifies the riparian corridor quality into five classes. Class-I (QBR $\geq$ 95), riparian habitat in natural condition, excellent quality; class II (90 > QBR > 75), some disturbance, good quality; Class III (70 > QBR > 55), disturbance important, fair quality; Class IV (50 > QBR > 30), strong alteration, poor quality; class V (QBR $\leq$ 25), extreme degradation, bad quality. Natural riparian corridors without alteration increases habitat complexity, improve the quality and quantity of leaf litter inputs, and maintain water temperature which results in increased habitat quality and is translated to higher QBR score (Castela et al., 2008). In contrast, building of dams, channelization of streams, agricultural conversion, and urban development destroy natural vegetation and floodplains, and alter flooding cycles for the riparian area, which will result low QBR scores. The IHF and QBR value of site A1 was higher when compared with the rest sites which indicate that site A1 has good habitat quality and heterogeneity. Likewise, this was confirmed with the water chemistry analysis where site A1 has better water quality when compared with the rest of the sites. Therefore, based on the results of habitat and water quality assessments, sites A1 was considered as a reference site.

4.5 Benthic Macroinvertebrates Sampling

Macroinvertebrates were collected at each sampling site using a rectangular frame net (20 × 30 cm) with a mesh size of 300 µm in June 07, 2017. Each collection needed a 10-minute kick sampling over a distance of 10 m. Time is allotted proportionally to the cover of different habitats of the sites such as bare edge, open water, and emergent and submerged vegetation. Macroinvertebrates were sorted in the field, stored into vials containing 80% ethanol and labeled. To increase sorting efficiency in the field each sample was wash through a series of sieve (0.5-1.0-2.0 mm). Afterward, in the laboratory, macroinvertebrates were identified to family level using a stereomicroscope (10× 186 magnification) and the identification was based on the key by Bouchard (2004).

4.6 Litter Decomposition Test

Leaves of *Syzygium guineense*, *Salix mucronata*, and *Eucalyptus globules* were collected from a single plant of each species on one day to minimize spatial and temporal variation in leaf nutrient status. Green leaves were collected from live branches of each species because this tree species is evergreen and does not have a seasonal peak in litter fall, abscised leaves could not be collected. Only similarly sized, mature leaves without blemishes, breached cuticles, or galls were selected. *Syzygium guineense* in its local name "Dokima" and *Salix mucronata* (willow) "Aleltu or wonz adimik "chosen because they were a common riparian tree species throughout Southwestern, Ethiopia, on the other hand *Eucalyptus globules* is chosen because it is the best known prevalent exotic species in the country.

The collected leaves were air dried in the laboratory for two weeks and weighed into 5g- packs and placed in coarse mesh (10 mm mesh) bags (16 x 20 cm) to measure the combined microbial and invertebrate driven decomposition rates in each sites for each leaves species (Figure 2). A triplicate litter bag per species of leaves were prepared in the laboratory. Thus, a total of 162 litter bags were sealed and distributed at each sampling sites. The bags were secured to the bank with hard rope and tied to boulders on the stream bed. Three litter bags were collected from each sampling site per species of leaf at the day of 11, 22 and 33 incubation periods. During the retrieval date, the recovered litter bags were collected and transported to the laboratory and the leaves were removed from the bags, rinsed with tap water individually to remove the sediments and adhering invertebrates. Then the clean leaf material were dried at 105 °C to constant mass (24 h) and weighed to the nearest 0.01g to determine the mass loss, burned at 550^{o}C for 4 h, and reweigh to determine ash content and ash free dry mass (AFDM) remaining.

Figure 2 Coarse mesh size 10 mm bags (16 x 20 cm) used for litter deployment

4.7 Standard Cotton Strip Decomposition Test

Cotton strips (4cm×6cm) were wrapped in aluminum foil and dried in oven for 2hours at 105 ºC. Cotton strips were secured to the bank with hard rope and tied to boulders on the stream bed in each sampling sites. During the retrieval day of 11, 22 and 33 the incubated cotton strip, were collected and transported to the laboratory and the cotton were rinsed with tap water by using brush to remove the sediments and adhering invertebrates. A total of 18 cotton-strips were be deployed at each site. The cotton strip used in this study were brought from Oakland University and was prepared from bolts of Fredrix-brand unprimed 12-oz. See Tiegs et al. (2013) for the detail of thee fabrication the cotton strip procedures.

Figure 3. The cotton strip used for decomposition test in this study

4.8 Statistical Analyses

Differences in water chemistry were compared among sites using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison tests. Principal component analysis (PCA) was used to summarize variation in physico-chemical parameters and benthic macroinvertebrate communities among sites. Detrended correspondence analysis (DCA) was used to determine the appropriate response model (linear or unimodal) for benthic macroinvertebrate data. The performed DCA gives a gradient length less than three standard deviations, implying that taxa abundance exhibit linear response to environmental gradients. Macroinvertebrate abundance data were log transformed log(x + 1) prior to analysis to obtain homogeneity of variance. Furthermore, CCA analysis was performed to evaluate the relationship between measured environmental variable and species data. The statistical significance of eigenvalues and species–environment correlations generated by the CCA were tested using Monte Carlo permutations. All the multivariate analysis was performed using CANOCO ver 4.5 software (ter Braak and Smilauer).

The macroinvertebrate community structural and functional composition were described per site as total number of individuals, family richness, total number of EPT taxa (Ephemeroptera + Plecoptera + Tricoptera), total number of Trichoptera taxa, % Trichoptera individuals, % Ephemeroptera individuals, % Diptera individuals, % Chironomidae individuals, % Oligochaeta individuals, % intolerant individuals, % tolerant individuals, and the FFGs (collectors, predators, scrapers and shredders). The Marglef's index (M), Simpson's diversity index (1/d), Pielou's index (J), and Shannon's diversity (H') index was also calculated for each sites. BMWPS also used to classify the water quality of the sampling sites. All statistical analyses were performed with Statistica (Version 7, 2004, StatSoft, Tulsa, Oklahoma), unless otherwise indicated. Prior to analysis, data were checked for normality and homogeneity of variances to meet assumptions of the ANOVA. Level of significance was set at $P<0.05$.

Rates of leaf decomposition (k) were estimated using an exponential decay model Mt = Mi e-kt (Mt = remaining AFDM at time t (33 days); Mi = initial AFDM; −k = decay rate). Each litter species of mass remaining were compared among sites using analysis of variance (ANCOVA) followed by Tukey's post hoc multiple comparison tests. Decomposition rate was as a response variable, sites as treatments, and time as covariates for the ANCOVA. To determine the functional integrity of the sites as suggested by Gessner and Chauvet (2002), the ratio of leaf breakdown rate in impacted and reference sites were determined. As defined by Gessner and Chauvet (2002) kimpact: kreference score classifies the functional integrity of rivers into three classes: 0 score, severely compromised river functioning; 1 score, compromised river functioning and 2 score, no clear evidence of impact, uncompromised functioning.

4.9 Data Quality Control

Quality control was conducted on field procedures to ensure a high level of consistency and accuracy in all operations i.e. *in situ* field measurements; sample collection and field processing, human disturbance and habitat assessment. A standard procedure method and protocol was followed. For water sampling, measurement and analysis sampling bottles were labeled to differentiate between sampling sites. The labeling was consistent for macroinvertebrate, physicochemical parameters and the habitat quality assessment checklist were clearly recognized the sampling sites and identified the pollution gradient. Composite sampling techniques were employed to get representative data for each sampling sites. Appropriate procedures and guidance were used during analysis of water samples and during sampling, identification and counting of macroinvertebrates. Standardized checklist were adopted and used for the assessment of river habitat quality which has been used by researcher in Jimma university for a long period of time. Parameters such as pH, temperature, turbidity, DO and conductivity were measured in situ to minimize the variations during sample transport. For water, a triplicate samples were taken and the results were averaged for accuracy.

4.10 Ethical Consideration

Clearance of Ethical were taken from Ethical and Research Committee of Jimma University, College of Public Health and Medical Sciences to publicly make assured that the research was relevant and approved by the college as well as by the Department of Environmental Health Science and Technology.

4.11 Dissemination Plan

The final result of this study was presented to Jimma University, Faculty of Public Health, Department of Environmental Health Science and Technology. Actions will be made to publish the paper in international as well as local journals so as to provided important information for Biomonitoring of River Water Quality Programs for the local communities in particular and international level in general.

Chapter Five: Results

5.1 Water Quality

The average values for the measured water chemistry data and environmental variables for each site are presented in Table 1. Water characteristics differed significantly among sites (one-way ANOVA: $F = 5.44$, $P = 0.02$). Stream nutrient concentrations generally increased along the stream gradient from upper stream to down streams. The mean concentrations of ammonium increased along the gradient, ranging from 0.37mg/l in the upper stream (S) to 0.55 mg/l in downstream (A5) except a lower concentration on site A2 (0.03 mg/l). The mean of nitrate also increased along the gradient except sites of A1 and A2 (0.28 and 0.17 mg/l, respectively), ranging from 0.43 mg/l in upper stream (S) to 2.39 mg/l in downstream (A5).

Correspondingly, Chloride, BOD_5 , SRS, conductivity and turbidity were more elevated at all sites than at the upstream sites A1 and S. pH did not show significant change throughout the study sites. Similarly, water T° did not show significant change ($P > 0.05$). Discharge and current velocity also did not differ statistically among sites ($P > 0.05$). The biochemical oxygen demand (a measure of organic pollution) at the most impacted site reached 171 mg/L, i.e., far exceed than the recommended level (30mg/L.

Soluble Reactive Phosphorus (SRP) and Nitrate concentrations were always higher at sites A3, A4 and A5 when compared with upstream sites. Conductivity and TSS were higher in downstream sites where pollution load and habitat degradation were high comparatively. DO was significantly higher in upper streams than down streams ($p= <0.0001$; Table 1).

Table 1. Location and mean (±SE) values for measured physicochemical characteristics of the six study sites in Awetu stream

	S	A1	A2	A3	A4	A5	*F*-value	*p*-value
Latitude (N)	7°42'08.7"	7°42'14.7"	7°42'02.5"	7°41'52.3"	7°40'2.5"	7°39'23.6"		
Longitude (E)	36°49'18.1"	36°49'14.1"	36°49'09.5"	36°49'21.4"	36°50'10,1"	36°50'30.6"		
Width (m)[b]	1.8 ± 0.3	3.0 ± 0.01	4.3 ± 0.16	2.8 ± 0.35	3.8 ± 0.21	4.1 ± 0.02	34.8	0.0002*
Depth (m)[b]	0.16 ± 0.20	0.48 ± 0.04	1.26 ± 0.35	0.70 ± 0.14	0.48 ± 0.01	0.40 ± 0.02	9.388	0.008*
Current (m/s)[b]	0.04 ± 0.02	0.76 ± 0.62	0.04 ± 0.01	0.06 ± 0.02	0.73 ± 0.07	0.17 ± 0.01	3.7	0.072
Discharge (m^3/s)[b]	0.02 ± 0.01	1.12 ± 0.97	0.63 ± 0.03	0.64 ± 0.64	1.17 ± 0.01	0.68 ± 0.04	1.5	0.303
Temperature (^{o} C)[a]	19.9 ± 0.28	19.6 ± 0.99	20.2 ± 0.11	19.9 ± 0.28	20 ± 0.71	20.6 ± 0.07	0.8	0.607
Conductivity (µS/cm)[a]	96.0 ± 0.4	91.0 ± 10.0	97.1 ± 1.3	115.8 ± 3.6	131.0 ± 2.8	185.1 ± 5.4	93.0	< 0.001*
pH [a]	7.63 ± 0.49	7.97 ± 0.47	7.65 ± 0.16	7.29 ± 0.29	7.58 ± 0.39	7.28 ± 0.11	1.1	0.454
DO (%)[a]	84.8 ± 3.25	95.1 ± 4.03	92.7 ± 0.42	81.8 ± 1.70	27.6 ± 1.70	9.0 ± 1.41	268.4	<0.0001*
Turbidity (NTU)[a]	18.4 ± 4.3	27.2 ± 6.2	34.5 ± 1.0	24.6 ± 4.9	162.5 ± 3.5	182.5 ± 3.5	650.9	<0.0001*
SRP (mg/L)[a]	0.02 ± 0.0	0.02 ± 0.01	0.1 ± 0.52	0.13 ± 0.01	0.14 ± 0.02	1.35 ± 0.06	11.8	0.0046*
DO (mg/L)[a]	6.66 ± 0.20	7.35 ± 0.13	6.67 ± 0.32	5.62 ± 0.59	2.05 ± 0.06	0.73 ± 0.11	177.1	<0.0001*
BOD_5 (mg/L)[a]	4.5 ± 2.5	4.1 ± 2.5	3.7 ± 0.1	27.7 ± 14.6	52.0 ± 1.4	171.0 ± 1.4	221.5	<0.0001*
Ammonium(mg/L)[a]	0.37 ± 0.04	0.45 ± 0.22	0.03 ± 0.00	0.48 ± 0.06	0.58 ± 0.06	0.55 ± 0.01	8.2	0.012*
Nitrate (mg/L)[a]	0.43 ± 0.06	0.28 ± 0.08	0.17 ± 0.02	0.55 ± 0.14	0.79 ± 0.04	2.39 ± 0.24	87.2	<0.0001*
Chloride (mg/L)[a]	8.0 ± 1.41	13 ±1.41	7.5 ± 0.71	9.5 ± 0.71	9.0 ± 1.41	70.0 ± 1.41	821.1	<0.0001*
TSS (mg/L)[a]	56.0 ±11.3	57.3 ± 23.6	22.0 ± 1.4	27.7 ± 14.6	193.0 ± 1.4	250.0 ± 7.1	116.6	<0.0001*

[a] n = 3.

[b] n = 10.

The ordination analysis by PCA, discriminated sites S1, A1, A2 and A3 from sites A4 and A5 based on water quality parameters along Axis1, which explained 92.7% of the variation. BOD_5, SRP, NO3, chloride and conductivity were positively associated with sites A4 and A5, while DO negatively correlated to site A4 (Fig. 4). During the sampling periods, always the DO concentration at site A1 is as high as 7.33 mg/l and gradually decreases to the lowest value 0.73 mg/l at site A5, after crossing the town. The increased pollution load resulted in 37, 4 and 67 fold increase in BOD_5, TSS and SRP concentration, respectively at site A5 when compared to site A1 which is slightly impacted. Similarly, pollutant indicator parameters were increased along a stream indicating gradient of water and habitat quality degradation (Fig. 5).

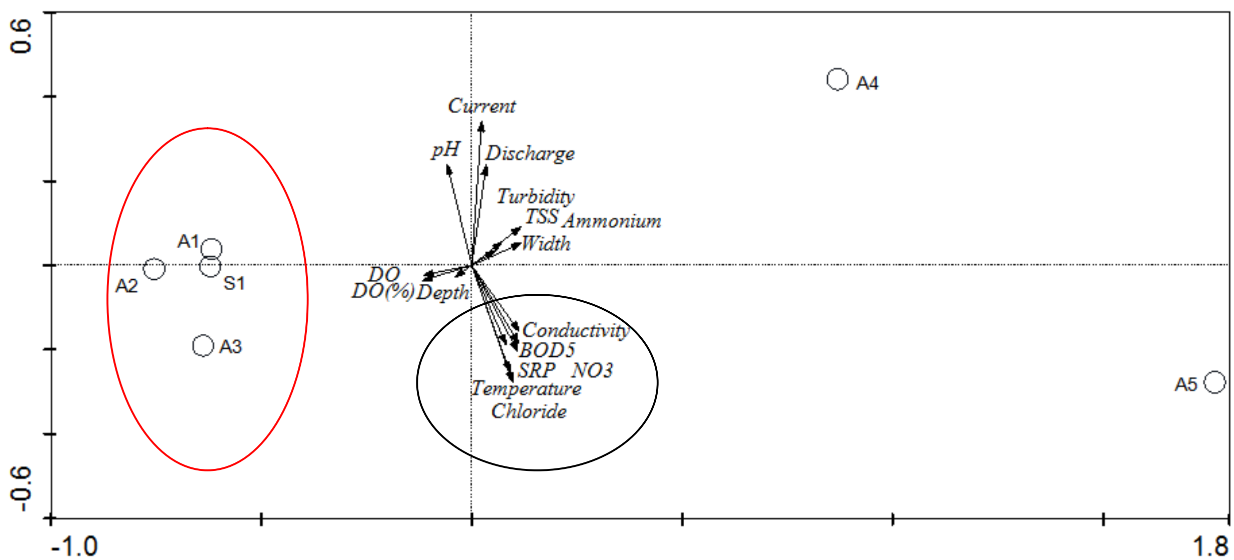


Figure 4 Principal component analysis on physicochemical parameters measured at six sites along a habitat degradation gradient in Awetu stream. PCA axis 1 explained 92.7% of variability among sites.

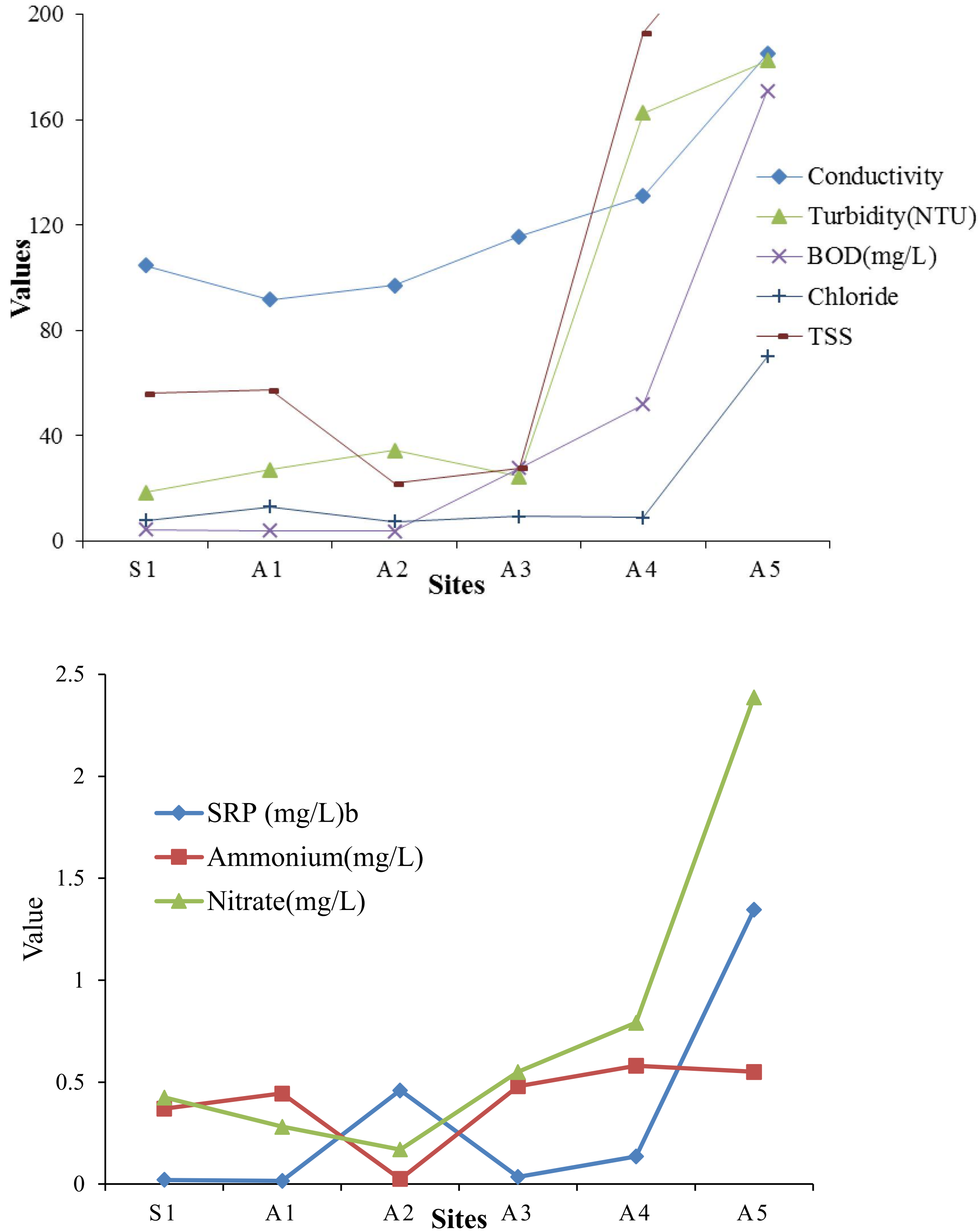


Figure 5 Chemistry data for selected pollutant indicator parameters measured at six sites along a habitat degradation gradient in Awetu stream.

5.2. Riparian Habitat Quality

The habitat quality of the six sites which were evaluated by QBR and IHF indices agreed with the physico-chemical characteristics of the sites. The sites are differed in habitat quality showing gradient of habitat degradation from upstream to downstream. Sites A1 and S had a native riparian corridor and high substratum heterogeneity with a minimal human alteration, site A2 has natural channel and good aquatic vegetation cover as well as good substratum heterogeneity with medium human alteration (grazing, swimming, and bathing), site A3 crossed light agricultural area with strong human alteration (Damming, bathing, washing clothing's, native forest removal and channelizing) and sites A4 and A5 were severely altered by human activities (channelization, removal of native riparian vegetation, municipal wastewater effluent input, municipal solid waste dumping, and carwash effluent input).

The QBR index clearly classified the riparian corridor quality of the studied stream sites into four classes: sample site A1 scored a total of 80 QBR score, that indicates the site had riparian habitat in some disturbance condition with good quality; sample site S scored 55 total QBR score means that the site had important disturbance with fair habitat quality ; site A2 had strong alteration indicates poor habitat quality and the last three sites(A3,A4 and A5) recorded < 25 total QBR score (15,10 and 5) respectively, had extreme degradation with bad habitat quality (Table 2).

Table 2 Habitat quality of the six study sites evaluated by Fluvial Habitat Index (IHF) and Quality of the Riparian Corridor Index (QBR) in Awetu stream.

	Sites					
	S	A1	A2	A3	A4	A5
Fluvial Habitat Index (IHF)						
Embeddedness in riffles/sedimentation in pools	15	20	20	15	5	5
Frequency of riffles	4	10	8	8	6	4
Composition of the substrate	14	17	17	14	14	14
Velocity/depth combinations	4	6	8	6	6	4
Percentage of shadow in the stream	3	5	5	3	3	3
Elements of heterogeneity	6	8	6	4	4	4
Aquatic vegetation cover and diversity	15	20	15	15	15	15

Final IHF Score	61	86	79	65	53	49
Quality of the Riparian Corridor Index (QBR)						
Degree of cover of the riparian corridor	10	10	10	0	0	0
Structure of the vegetation	10	25	5	5	0	0
Quality of vegetation cover	10	20	10	0	0	0
Degree of naturalism of the channel	25	25	20	10	5	5
Final QBR score [a]	55	80	45	15	10	5

[a] QBR score is the sum of the scores of the four items that compose it. QBR < 25, extreme degradation; 70 > QBR > 55, beginning of important alteration; QBR > 95, riparian vegetation without alterations as defined by (Colwell, 2007).

The IHF decreased in a downstream direction indicating degradation of habitat quality from site A1 to site A5 (Table 2). Like IHF index, the final QBR score decreased from upstream to downstream sites showing degradation of riparian corridor quality. Accordingly, downstream sites A3, A4 and A5 had a severely degraded riparian corridor, A2 had a low quality riparian corridor with important alteration, site S had a moderate quality riparian corridor with beginning of important alteration and site A1 was considered to have good a riparian corridor quality with no major degradation (Table 2). As expected, the sites from upstream to downstream exhibited gradient of water and habitat quality degradation which resulted spatial water quality variability in Awetu stream stretch. The IHF and QBR value of site A1 was higher when compared with the rest sites which indicate that site A1 has good habitat quality and heterogeneity. Likewise, this was confirmed with the water chemistry analysis where site A1 has better water quality when compared with the rest of the sites. Therefore, based on the results of habitat and water quality assessments, sites A1 was considered as a reference site.

5.3 Benthic Macroinvertebrate

A total of 1201 macroinvertebrate individuals belonging to 71 taxa and 30 families were collected during the sampling period along the stretch of Awetu stream. Of these, the largest number of individuals were recorded from the upstream and intermediate of Awetu sampling sites (S1, A1 and A3), and the lowest number of individuals were sampled at A4 and A5 which are the downstream of Awetu stream. A total of 21 taxa were recorded at sites A3, A4 and A5, which was the lowest number of taxa and the highest (50) taxa was recorded from the upper and intermediate sites (Table 3). Shredder taxa, number of EPT taxa, % of Ephemeroptera individuals, % of Trichoptera individuals and % of Intolerant individuals were totally absent at sites A4 and A5, while % of Chironomidae individuals, % of Oligochaeta individuals, % of Diptera individuals and % of tolerant organisms were higher when compared with upstream sites (Table 3). On the other hand, sites S and A1 had higher number of taxa, number of EPT taxa, % of Trichoptera individuals and % of intolerant individuals when compared with moderately disturbed sites A2 and A3.

BMWPS classified the water quality of the sampling sites into very good, good, moderate and poor classes. The highest score were recorded at site A1 (121 score) which is categorized under a very good (Unpolluted or unimpacted) class. Site S had good water quality (clean but slightly impacted), site A2 had moderate water quality (moderately impacted), sites A3, A4 and A5 had poor water quality (major degradation). Comparatively speaking, Species evenness, species richness and diversity of benthic macro invertebrate were also higher at the upstream site A1 than at sites S, A2, A3, A4 and A5, which has low diversity and species richness (Table 3). Shredder species abundance was very low to none along the sampling sites. Impacted sites A4 and A5 had no shredder species at all (Table 3). The calculated Shannon diversity index showed that at the upstream sites S, A1 and A2 (1.16, 2.5 and 2.07, respectively) have higher species diversity than at the downstream site A3, A4 and A5 (0.98, 0.97 and 0.9, respectively) (Table 4). Similarly, Simpson diversity index showed that site A1 had the highest score (8.25).

Table 3. Selected attributes of benthicinvertebrate collected from the six sites along habitat degradation gradient in Awetu stream.

	Sites					
	S1	A1	A2	A3	A4	A5
Total no. of individuals	399	208	171	208	64	160
Family richness	18	20	12	8	7	6
Total no. of EPT taxa [a]	6	6	4	3	0	0
Total no. of Trichoptera taxa	2	3	2	2	0	0
% Trichoptera individuals	5.3	10.1	4.7	4.8	0	0
% Ephemeroptera individuals	76.9	27.9	8.8	72.1	0	0
% Diptera individuals	7.8	13.0	9.4	7.3	70.3	31.3
% Chironomidae individuals	6.5	5.8	9.4	7.2	70.3	31.3
% Oligochaeta individuals	0.3	0	0.6	0	4.7	62.5
% Intolerant individuals [b]	7.5	24.1	5.2	4.3	0	0

% Tolerant individuals [c]	8.8	14.4	14.0	7.21	76.6	96.9
Total no. of shredder taxa	1	2	2	1	0	0
% Shredder individuals	1.503	6.25	4.67	4.32	0	0
No. of BMWP families	16	19	11	7	6	6
BMWP [d]	91	121	54	36	18	17
ASPT	5	6.1	4.5	4.5	2.6	2.8

[a] EPT = Ephemeroptera + Plecoptera + Tricoptera.
[b] Intolerant individuals belong to families with scores of 7, 8, 9 and 10 in the BMWPs
[c] Tolerant individuals belong to families with scores of 1, 2 and 3 in the BMWPs.
[d] BMWPs value is the sum of the tolerance scores of families (score = 1 - 10, the higher it is the more sensitive the family is to organic pollution). BMWPs >100, indicates high water quality (Unpolluted or unimpacted); BMWPs = 71 – 100, good water quality(Clean but slightly impacted); BMWPs 41–70, moderate water quality; BMWPs 11 – 40, poor water quality and BMWPs <10, bad water quality as Adapted from Walley and Hawkes, 1997).

Table 3. Benthic invertebrate diversity and shredder from six sampling sites along a habitat degradation gradient in Awetu stream

	Sites					
	S1	A1	A2	A3	A4	A5
Marglef's index, M	2.84	3.58	2.14	1.31	1.44	0.99
Simpson's diversity index, 1/d	1.75	8.25	2.07	1.84	1.88	2.04
Pielou's index J	0.40	0.85	0.51	0.47	0.50	0.50
Shannon's diversity, H'	1.16	2.54	2.07	0.98	0.97	0.90

The PCA ordination of all macro invertebrate communities (Fig. 6), clearly discriminated sites in to three groups ,site S, A2 and A3 in one group and site A1separetly from sites A4 and A5; sites were distributed along axis 1, which explained 50.1% of variability. Axis 2 explained 22.1 % of the variability observed among sites. Pollution tolerant species like Chironomidae, Oligochaeta, Glossiphonidae, Dytiscidae, Lymnaeidae, and Belostomatidae were positively associated with sites A4 and A5. Likewise, while pollution sensitive species like Heptagenidae, Gomphidae, Baetidae, Aeshnidae, Ephemerellidae, and Tipulidae were positively correlated with sites A1 and A2.

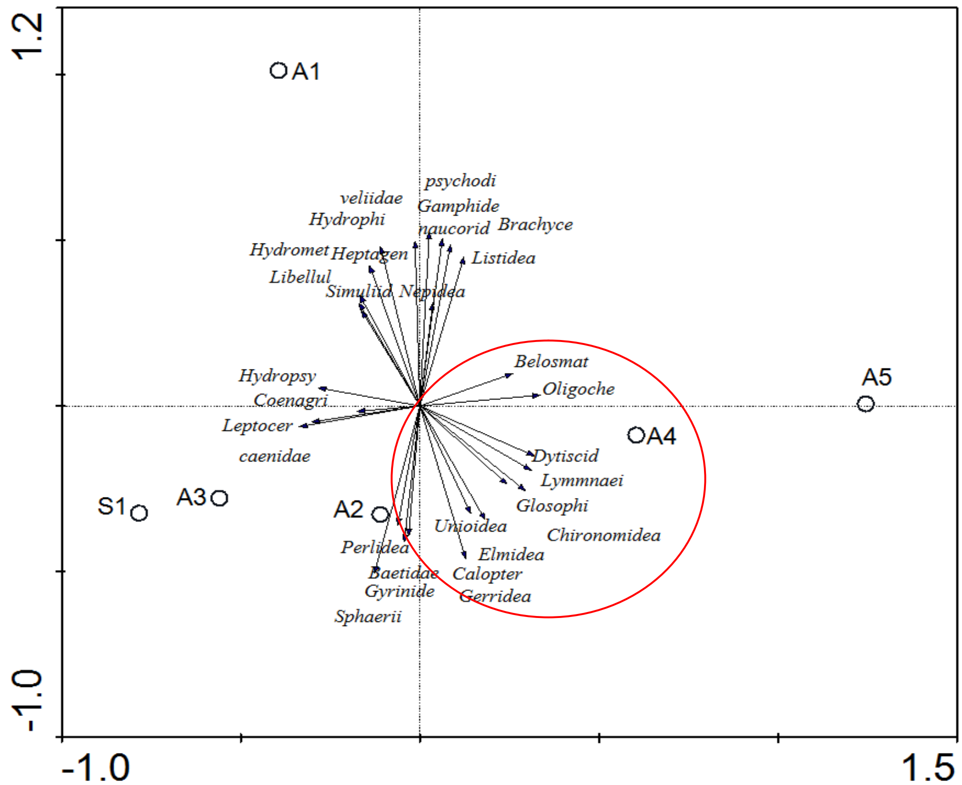


Figure 6. Principal component analysis (PCA) for benthic macroinvertebrates at six sites along a habitat and water quality degradation gradient in Awetu stream. PCA axis 1 explained 50.1% and PCA axis 2 explained 22.1% of variability among sites.

The RDA analysis showed that along Awetu stream drain land use type, clearly separated impacted and unimpacted sites in terms of water quality and land use type see Figure 7. The Pollution tolerant species like Chironomidae, Oligochaeta, Glossiphonidae, Dytiscidae and Belostomatidae were positively associated with heavy urbanization sites A4 and A5 and that of pollutant indicator phisco chemical parameters like SRP, chloride, TSS and nitrates. Likewise, while pollution sensitive species like Heptagenidae, Gomphidae, Baetidae, Aeshnidae, Ephemerellidae, and Tipulidae were positively correlated with forest and light agriculture sites (A1 and S, respectively) and good water quality indicators like DO,). However, the Monte Carlo test showed statistically insignificant relationship b/n the measured environmental variables and species variation along sites, land use gradients had direct relationship b/n habitat, water chemistry and MI community. DO (mg/L) is the only variable significantly influenced MI community in Awetu stream (P= 0.02) F = 6.64. The remaining habitant and water chemistry data didn't result a significant impact on MI community in Awetu stream.

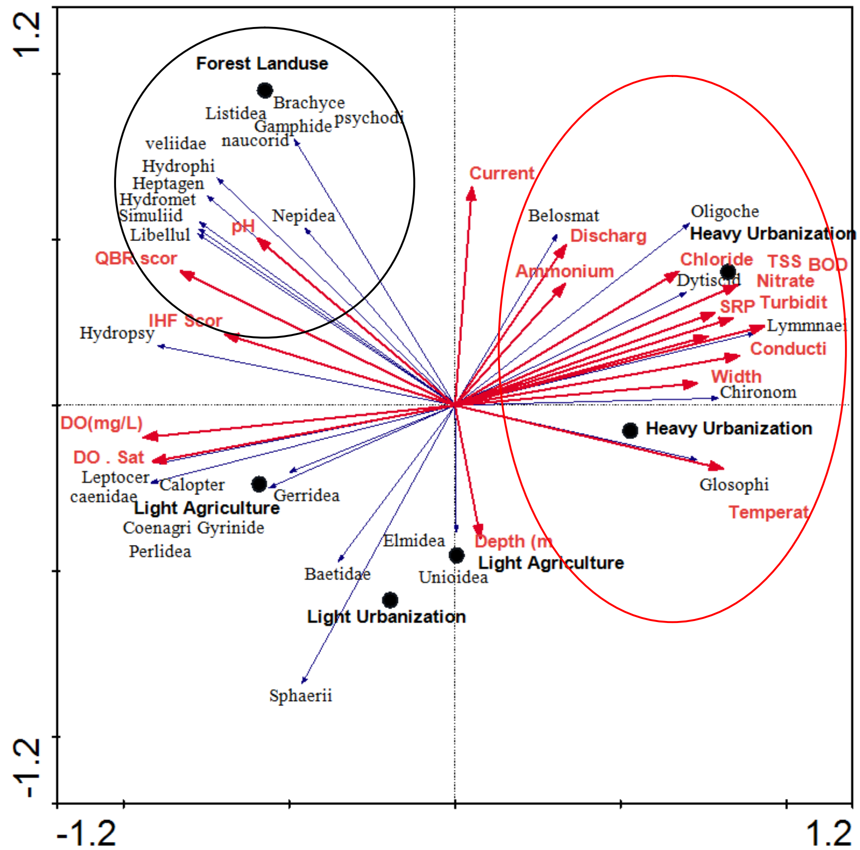


Figure 7. The RDA analysis for benthic macroinvertebrates at six sampling sites along a habitat and water quality degradation gradient in Awetu stream

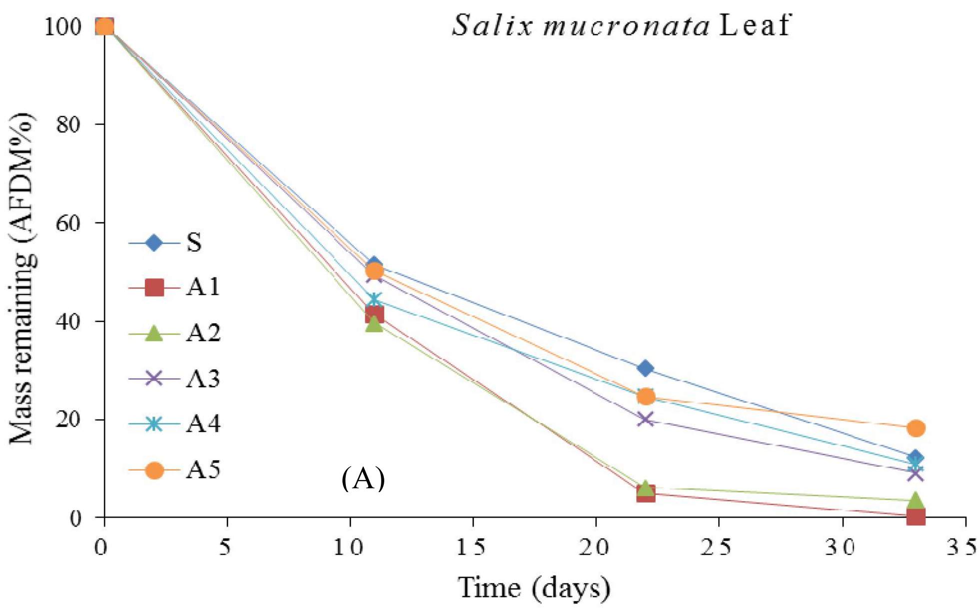
5.4 Litter Decomposition

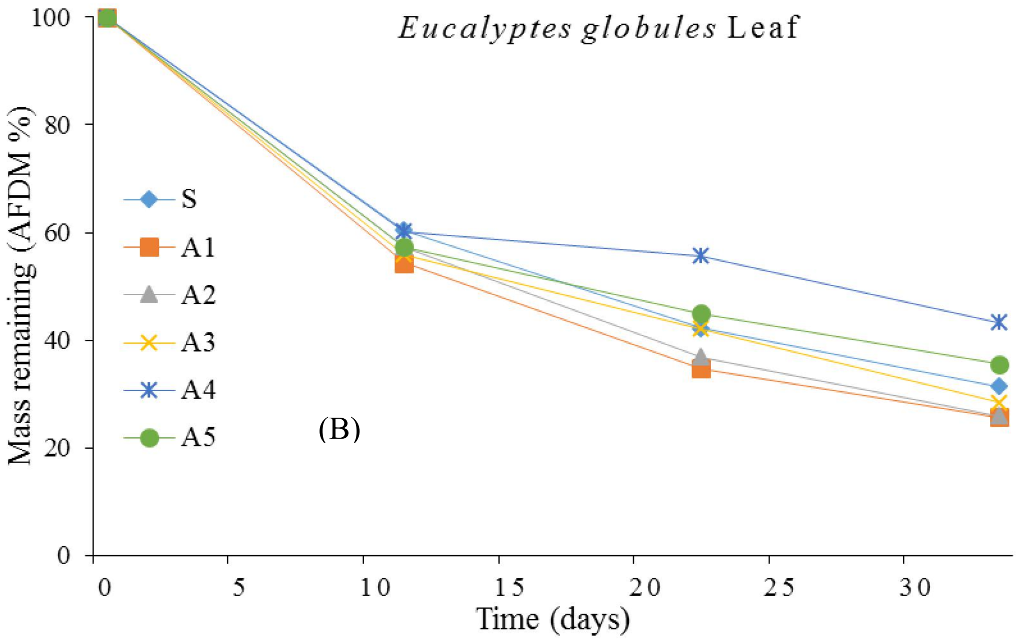
The results reported in Fig. 8 show that, regardless of the exposition time, The 3 plant species differed in the amount of ash free dry mass (AFDM) loss. On the day of 11, the leaves of *Salix mucronata* lost mass very rapidly *(*ranged from 48- 60%) in comparison to either *S.guineense* (ranged between 18 – 21%) or *E. globulus* (ranged from 39-42%). After 33 days of exposition the *Salix mucronata* leaves increased to 99 % and 93 % at the upper stream sites A1 and A2, respectively. In contrast, *S. guineense* increased to 82 % and 86% of AFDM loss at the most impacted sites A4 and A5, respectively, whereas at the reference site A1 reached only 21%. On the other hand, *E. globulus* showed the rapid AFDM loss at site A2 **(**93 %**),** while the reference site A1 (74.9%) exhibited the fast AFDM lost next to site A2. Correspondingly, at the most impacted sites A4 and A5 *E. globulus* showed the slowest AFDM loss.

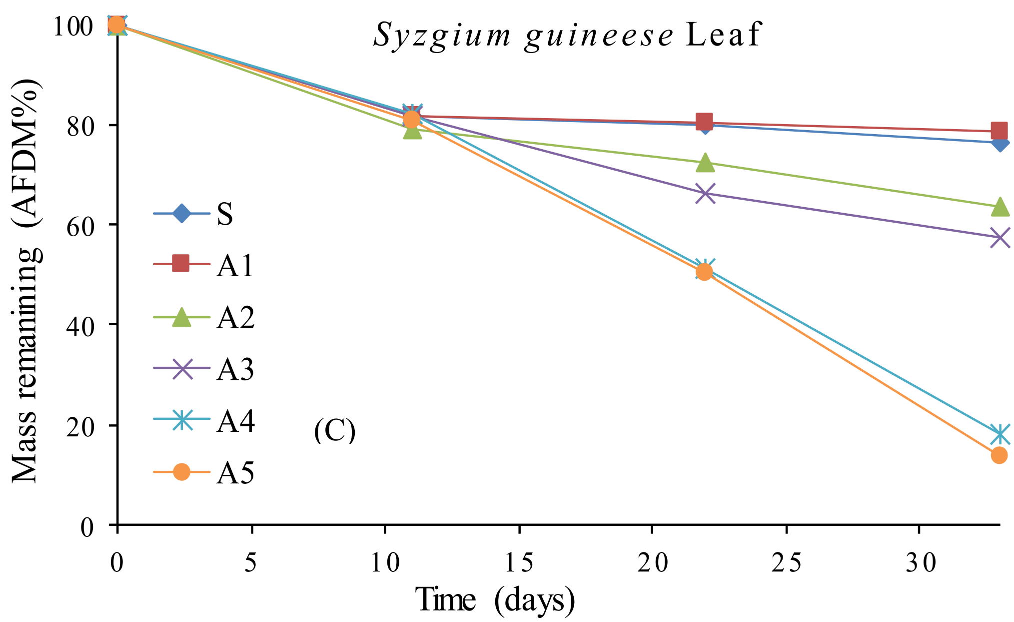
5.5 Standard Cotton Strip Decomposition

The mass loss of cotton strip was ranged from 2.4% – 21%. On day 11, the fastest mass loss was recorded at site A4 and A1 (21.9% and 18.5% respectively). While, the lowest mass loss was exhibited at site A5 and A3 (2.4% and 2.7%, respectively). After 33 days of exposition the mass lost increased to 69% and 70% at the most impacted sites A4 and A5, respectively, whereas at the upper sites S and A1 reached 67% and 68 % (Fig. 8D).

The analysis of covariance revealed that mass loss were significantly different among sampling sites (ANCOVA, P < 0.001), being higher at impacted sites A4 and A5 compared with upstream sites A1, A2, A3 and S (Tukey's test: P < 0.05) for all tested leaf litters. Since no replicate for the cotton strip, statistical test were not performed.




Eucalyptes globules Leaf
Mass remaining (AFDM %)
Time (days)
S
A1
A2
A3
A4
A5
(B)
100
80
60
40
20
0
0
5
10
15
20
25
30


Syzgium guineese Leaf
Mass remanining (AFDM%)
Time (days)
S
A1
A2
A3
A4
A5
(C)
100
80
60
40
20
0
0
5
10
15
20
25
30

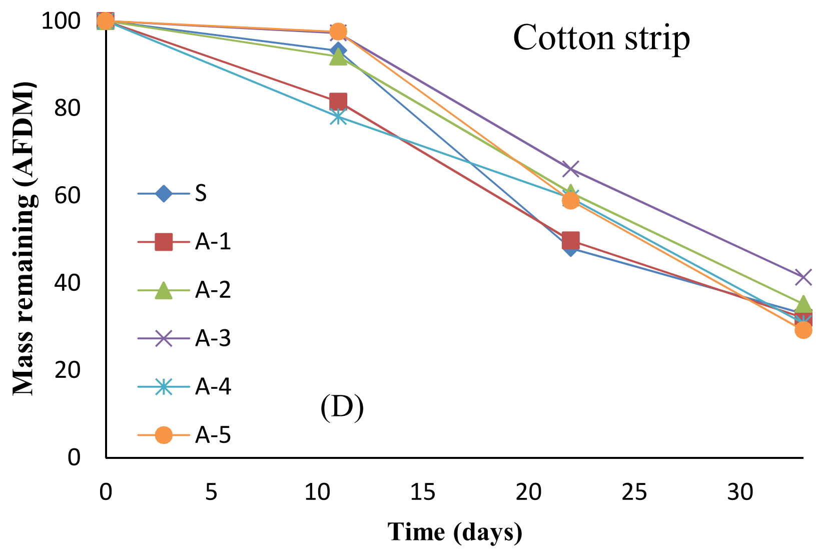


Figure 8 Remaining mass (mean ±SE) of litter decomposition test at the six sampling sites along a habitat degradation gradient in Awetu streams; (A) remaining mass of *S. mucronata* leaves; (B) remaining mass of *E. globules* (C) remaining mass of *S. guineense* leaves ; (D) remaining mass of cotton strip

Exponential decomposition rates of the three leaves and cotton strip is presented in Table 6 and Figure 9. The decomposition rate of *S. mucronata* were ranged from 0.051 to 0.162/d; *S.guineense* ranged from 0.007 to 0.061/d; *E. globules* ranged from 0.025 to 0.080 and cotton strip ranged from 0.014 to 0.037. Overall, *S. mucronata* decomposed faster in Awetu streams when compared with *E. globules, S.guineense* and Cotton strip (Figure 9).

There was statistical significant difference in decomposition rates (k value) across sites for the three species of leaf litter tested (One-way ANOVA: $P<0.05$). The multiple comparison test showed that *S. guineense* decomposition rate was faster at site A3, A4 and A5 significantly when compared with the rest of the sampling sites (Tukey test: $P<0.05$). However, for the *E. globules* leaf, the multiple comparison tests showed faster decomposition rate was measured only at A2 when compared with the rest of sampling sites. Inversely, faster decomposition rate for *S. mucronata* was observed at upstream sites A1 and A2, whereas slower decomposition rate measured at A5 (Tukey Test: $P<0.005$). For the cotton strip, statistical comparison was not performed as there were no replicates done.

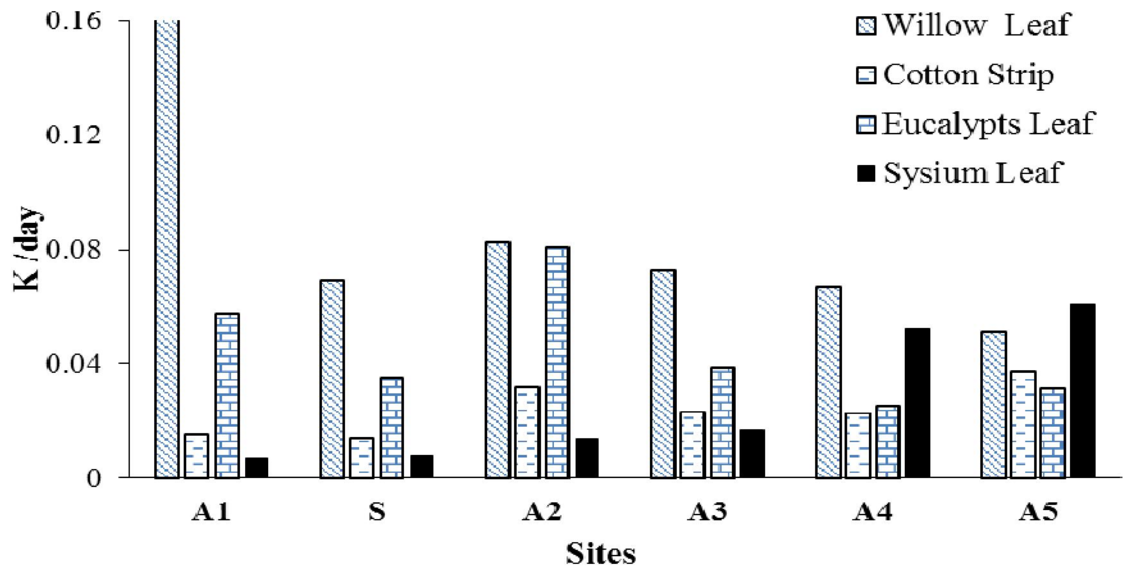


Figure 9. Exponential decomposition rates of the three leaves and cotton strip in the six sampling sites along Awetu stream

The calculated ratio between all litter decomposition rates at sites S, A2, A3, A4 and A5 to the decomposition rate at site A1 ($k_{impacted}$: $k_{reference}$), as proposed by (Gessner and Chauvet, 2002), site S was considered as having uncompromised stream functioning, sites A2 and A3 were considered as having a compromised stream functioning and sites A3, A4 and A5 were considered as having a severely compromised stream functioning (Table 6).

Table 4. Leaves decomposition rates (/d) in six sampling sites along habitat degradation gradient in Awetu stream, and breakdown rate ratio between impacted sites (S, A2 A3 A4 and A5) to a reference site (A1) (kimp: kref). R^2 refers to the fit of the exponential decay model.

Sites	Breakdown rate (/d)				R^2				$k_{imp}:k_{ref}$				Score [a]			
	WL	EL	CS	SL	WL	EL	CS	SL	WL	EL	CS	SL	WL	EL	CS	SL
A1	0.162	0.057	0.015	0.007	0.82	0.78	0.92	0.72	-	-	-	-	-	-	-	-
S	0.069	0.035	0.014	0.008	0.53	0.90	0.77	0.80	0.42	0.60	0.90	1.11	0	1	2	2
A2	0.082	0.080	0.031	0.014	0.76	0.90	0.86	0.96	0.51	1.40	2.03	1.85	1	1	0	1
A3	0.072	0.038	0.023	0.017	0.89	0.79	0.92	0.99	0.44	0.66	1.48	1.97	0	1	1	0
A4	0.067	0.025	0.022	0.052	0.789	0.85	0.79	0.89	0.41	0.44	1.46	7.23	0	0	1	0
A5	0.051	0.031	0.037	0.061	0.56	0.76	0.96	0.86	0.31	0.54	2.40	8.42	0	0	0	0

WL: *Willow Salix* leaves ,EL: *eucalyptus globulus* leaves ,CS: cotton strip , SL: *Syzygium Guineense*

[a] score proposed by Gessner and Chauvet (2002) as a measure of stream functional integrity

Chapter Six: DISCUSSION

Awetu stream exhibits all of the major ecological characteristics of urban stream syndrome that arise from channelization, decreased riparian vegetation, altered hydrology, increasingly impervious catchment, and elevated levels of nutrients and contaminants. Urbanization clearly had a significant impact on the ecological integrity of Awetu stream with respect to structural (species richness, elimination of sensitive taxa and dominance of tolerant taxa,) and functional measures (leaf litter decomposition). Spatial variability of urbanization effects along the stretch of Awetu stream has caused sites to differ with regard to physico-chemical characteristics and created habitat degradation gradient.

The higher concentration of SRP, BOD, conductivity, N03 and TSS in downstream (A3, A4, A5) showed spatial variability of water quality and impact of urbanization on the stream water quality. The result is also in accordance with studies by (Dejene and Legesse, 1997) and (Haddis et al., 2014). Streams that drain catchments of similar geology, variability in conductivity, turbidity, TSS and NO_3 are indicative of anthropogenic activities and land use (Minaya et al., 2013;Kilonzo et al., 2014), suggesting variability among sites in this study is linked with human activities.

The higher values observed for nutrients and lower level of DO in downstream sites A3 and A4 were most likely a consequence of the input of organic materials as result of rampant domestic waste discharge (both solid and wastewater), small-scale industrial discharge and street runoff, that have been reported to alter suites of environmental variables in other Ethiopian urban rivers and streams (Beyene et al., 2009).

The anthropogenic impacts were reflected in the IHF and QBR indices scores as well, which decreased from site A1 to A5, showing a gradient of impacts from upstream to downstream. These findings were consistent with (Haddis et al., 2014) observation which identified increased degree of anthropogenic impacts in the riparian corridor of Awetu stream. Overall, upstream sites (A1, A2 and S) had good water and habitat quality when compared with downstream sites which are characterized by increased in nutrient concentration and habitat degradation. Measures of the structural integrity were sensitive to water quality and have detected the differences in water and habitat quality among sites. Degradation of water and habitat quality causes predictable changes on macroinvertebrate community structure. For instance, reduction of macroinvertebrate diversity is found in streams affected by urbanization (Beyene et al., 2009). Indeed, sites located in upstream areas with native riparian vegetation, higher diversity of habitats and good water quality, showed greater taxonomic richness (reflected by the biotic index and derived metrics). In contrast, a decreased taxonomic richness and a correspondingly lower Shannon Diversity and Simpson's diversity index were observed in downstream sites. Overall, the measures of structural integrity differentiated sites into three groups; site S and A1 has good diversity, site A2 had moderate, and sites A3, A4 and A5 had poor structural diversity. The macroinvertebrate community responded to water and habitat quality degradation with a decrease in taxa richness and an increase in abundance of more tolerant individuals. This result is consistent with the findings of (Beyene et al., 2009; Castela et al., 2008) in impacted urban stream.

Pollution sensitive taxa (e.g. Ephemeroptera, Plecoptera and Trichoptera) were more abundant at sites S and A1, moderate at sites A2 and A3, and absent at sites A4 and A5 which resulted in higher ASPT scores in upstream section, while pollution tolerant taxa (e.g. Diptera and Oligochaeta) were higher at sites A4 and A5 indicating degradation of water quality. The sensitivity of this type of benthic derived metrics to stream impairment has been widely reported (Maxted et al., 2000). Moreover, (Niyogi et al., 2003) mentioned that the diversity of macroinvertebrate significantly decreases in a sites with poor water quality in urban streams. In our study, the differential distribution of taxa across sites was translated into differences in the BMWP scores that classified sites A1 and S as having high and good water quality respectively, site A2 as having moderate water quality, sites A3 as having poor water quality and sites A4 and A5 as having bad water quality.

The BMWP score differences between the five groups of sites can be explained by differences in water quality among sites. Streams under urban influence receive considerable amounts of nutrient and organic residues (Roy et al., 2003), which causes alterations in the physico-chemical characteristics of the water quality (Bahar et al., 2008) and these is reflected in change in BMWP score and macroinvertebrate diversity. The low water quality and sandy substrata with high organic matter of anthropogenic origin might led to the lowest scores at downstream sites than upstream sites. Considering the fact that macroinvertebrates in aquatic environment are exposed to mixture of multiple stressors which might have direct and/or indirect effects, therefore, a direct mechanism cannot explain the effect of water quality parameters on macroinvertebrates structure without further study.

The QBR index showed that sites A1 and S had good habitat quality with abundant native riparian vegetation which plays crucial role in improving water quality and habitat heterogeneity for intolerant macroinvertebrates communities such as Plecoptera and Trichoptera. Although shredders taxa are sensitive to the presence of riparian vegetation (Wooster and DeBano, 2006), in our observation they were poorly represented in the upstream sites of Awetu stream. Studies (Dobson et al., 2002; Gonçalves et al., 2006; Li and Dudgeon, 2009; Wantzen and Wagner, 2006) showed that the abundance of shredder taxa are low in tropical streams, which might be the reason for the paucity of shredder taxa in the stretch of Awetu stream. However, comparatively, sites S, A1, and A2 had higher number of shredder individuals than site A3, and severally impacted sites A4 and A5 had no shredder taxa. Shredders are sensitive to water quality (Wooster and DeBano, 2006), which can partially explain the higher number of shredder individuals at the upstream sites when compared with downstream sites. As expected, good habitat quality at the upstream sites was translated into high species richness in our observations.

The PCA analysis also showed difference in macroinvertebrate community structure along the stretch of Awetu stream, sites distributed along axis 1 were positively correlated with DO concentration in water and negatively to turbidity and TSS concentration. These results agree with that of Hepp and Santos (2009) who reported a negative relationship between selected benthic metrics, and turbidity and TSS. A positive relation between DO concentration and macroinvertebrate community in upstream sites were observed. A result consistent with the findings of Connolly et al. (2004) who identified positive relation of macroinvertebrate community and DO concertation. Moreover, studies (Jacobsen et al., 2003; Kaller and Kelso, 2006) have highlighted DO availability is a widely recognized factor influencing the composition of macroinvertebrate and many freshwater communities because it critically affects the distribution of many species.

The decomposition rates of the three leaves were differed among sites and did show inconsistent a pattern with the gradient of impacts that was reflected in water and habitat quality in the stretch of Awetu stream. The mass loss of S. *mucronata* was slower at the most impacted site (A5, below the town) when compared with unimpacted site (A1, the most upstream). Inversely, the decomposition of Cotton strip and *S.guineense* leaf were faster at the most impacted sites when compared with the rest of the sampling sites. This may be due to variability of in leaf chemistry, and pervious study reported that soft leaf are more subject to higher mass loss due to physical abrasion than decomposition. Relatively S. *mucronata* leaf is characterized as soft and in upstream sites where abrasion might has caused huge mass loss of it. For the rest of litter tested, differences in decomposition among sites could be attributed to difference in water and habitat quality. However, more detailed information on microbial and detritivore assemblage composition at each site would facilitate further exploration of the potential sources of variation in decomposition among sites. Eucalyptus leaf decomposition rate was higher when compared to native *S.guineense* leaf litter and the standard cotton strip. However, statistically speaking eucalyptus leaf decomposition rates across sampling sites were not significantly different except at A2 unlike *S.guineense* leaf species. This might be due to high leaching properties of the leaf before conditioning phases which also was reported by Castele et al (2007), rather than responding to water and habitat quality degradation.

Based on Gessner and Chauvet (2002) classification of functional integrity, sites A2 and A3 had a compromised ecosystem functioning, site S had no clear evidence of impact with uncompromised ecosystem functioning, and sites A4 and A5 had severely compromised ecosystem function. In our study, the Gessner and Chauvet (2002) classification method accurately depicted impacted sites as having poor stream functional integrity when compared with undisturbed sites. The ratio of $K_{impacted}$: $k_{reference}$ calculated in this study were within the threshold value presented by Gessner and Chauvet (2002). Therefore, we recommends that Gessner and Chauvet (2002) method for stream functional integrity assessment can be used to assess functional integrity of tropical streams. Several studies (Castela et al., 2008; Young et al., 2008; Masese et al., 2014) have proposed leaf decomposition rates could be used as a tool to evaluate the impact of anthropogenic disturbances in lotic systems.

In our study, the disappearance of shredders at polluted sites did not result in a decrease in leaf exponential breakdown rates. On the contrary, significantly higher breakdown rates were found when compared with the upstream sites. The high decomposition rates at polluted sites may have been due to increased concentration of nutrients, which could have stimulated microbial decomposing activity, suggesting that microbial activity was a more important factor than invertebrate feeding in controlling leaf breakdown in organically polluted Tropical River. These results are in accordance with increased leaf breakdown rates in nutrient rich streams found by other authors (Meyer and Johnson, 1983; Suberkropp and Chauvet, 1995).

Chapter Seven: Conclusion and Recommendations

7.1 Conclusion

In conclusion, both the measure of structural and functional integrity responded to water and habitat quality degradation gradients. Urbanization has affected all aspects of the ecology of Awetu stream. The six sites along the stretch of Awetu stream differed in physico-chemical characteristics associated with urbanization effects; resulted habitat and water quality degradation gradient described by nutrient enrichment, decreased riparian vegetation quality and decreased fluvial habitat quality.

The measures of structural integrity, benthic macroinvertebrate metrics and indices, were sensitive to these changes as they responded negatively to increases in TSS concentration and turbidity, which are strongly linked with catchment land use disturbance. The decomposition of *S. guineense* leaf and cotton strip leaves identified the most downstream sites as having a severely compromised ecosystem functioning and were sensitive to changes in water and habitat quality too. However, *S. mucronata leaf* and exotic *E. globules* leaf did not detect change in water and habitat quality in the studied stream. Replacement of indigenous riparian vegetation with exotic *Eucalyptus* species has the potential to reduce nutrient cycling, as slow *Eucalyptus* leaf litter decomposition measured in the study stream.

The evaluation of the ecological integrity of the six sites, considering the structural (MI) and functional indictors (*S. guineense* leaf and cotton strip decompositions), showed that; sites A4 and A5 had were severely impaired in both structure and function; site A2 was as compromised in function and moderately impacted in structure, site A3 severely impaired in structure but compromised in function, and sites S good in structure and not compromised in function. Although the functional and structural indicator used in this study showed the same result of severely impacted sites (A4 and A5), they showed a contradicting result regarding moderately impacted site (A3). Measure of the functional and structural indicator used in this study showed the same result for severely impacted sites (A4 and A5) and slightly impacted site (S). However, they gave different result for moderately impacted site (A3). In general, the study showed that for accurate assessment of the functional integrity of impacted tropical urban streams, litter decomposition rates were a useful tool.

7.2 Recommendations

Based on the study results, the following points has to be considered:

1. In evaluation of the ecological integrity of impacted tropical urban river ecosystem using of both functional and structural indictors is important as both measures complement each other.

2. Further, assessment should be done in both dry and rainy seasons to assess seasonal influence on litter decomposition in tropical stressed river system.

3. The cotton strip decomposition assessment should be made in tensile strength loss rather than mass loss as sediment accumulation interferes with the weighing process.

4. A robust data to gain the study should have to be conducted on several streams representing different degree of impacts and land use.

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Annex

Annex 1. Stream assessment form

1. DD/MM/YYY---

2. Site code-------------------------Name of stream--

3. Stream description--

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4. Altitude (m) ------------------------coordinates--

6. Ambient temperature (0C) ------------------------water temperature (0C) -----------------------

7. DO (mg/l) -------------%-----------------EC (µS/cm) ---------------pH-----------------------------

8. Velocity (m/s) -------------water depth (m) --------------discharge (m^3/s) ------------------------

9. Turbidity (NTU) -------------color----------------------smell---------------------------------

Habitat assessment

10 River bank width (m) ------------------------------Bank height (m) ----------------------------

11. Riverbed (%)

a. Bed rock---------- e. Gravel------------ i. sticks------------------

b. Boulder------------ f. sand -------------- j. branches-------------

c. Cobble--------------- g. Silt---------------- k. loges-----------------

d. Pebble-------------h. Detritus-----------

11. Riparian vegetation

a. Trees>10m----------------------------------- d. grass--

b. Trees<10m------------------------------------ e. bare land------------------------------------

c. Shrubs--

12. Width riparian vegetation Right------------------------------ Left-----------------------------

13. Canopy cover--

14. Protection riparian vegetation Right-------------------------------- Left------------------------

15. %pool--

16. % riffle --

a. Water appearances

17. Sinuosity ---

18. Slope--

19. List the available anthropogenic disturbance---

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20. Upstream land use--

21. Adjacent land use Right------------------------------- Left--

22. Farming distance from the river bank--

23. Take picture (picture number) --

24. Anthropogenic activities	River	upland
a. Cultivation	---------------------------------------	----------------------------------
b. Tree removal	---------------------------------------	---------------------------------
c. Shrub removal	--	---------------------------------
d. Tree plantation	---	---------------------------------
e. Grazing	---	---------------------------------
f. Grass cutting	---	---------------------------------
h. Car washing	--	--------------------------------
j. Waste dumping	-------------------------------------	-------------------------------
l. Swimming	-------------------------------------	------------------------------

Annex 2: QBR Index form

Riparian habitat quality assessment form

Score of each part cannot be negative or exceed 25

1.	Site name	
2. 3.	Observer Longitude	
4.	Latitude	
5.	Altitude	

Total riparian cover **Part 1 score**

score		
25	> 80 % of riparian cover (excluding annual plants)	
10	50-80 % of riparian cover	
5	10-50 % of riparian cover	
0	< 10 % of riparian cover	
+10	if connectivity between the riparian forest and the woodland is total	
+ 5	if the connectivity is higher than 50%	
- 5	connectivity between 25 and 50%	
-10	connectivity lower than 25%	

Cover structure **Part 2 score**

score		
25	> 75 % of tree cover	
10	50-75 % of tree cover or 25-50 % tree cover but 25 % covered by shrubs	
5	tree cover lower than 50 % but shrub cover at least between 10 and 25 %	
0	less than 10% of either tree or shrub cover	
+10	at least 50 % of the channel has helophytes or shrubs	
+ 5	if 25-50 % of the channel has helophytes or shrubs	
+5	if trees and shrubs are in the same patches	
-5	if trees are regularly distributed but shrubland is > 50 %	
-5	if trees and shrubs are distributed in separate patches, without continuity	

-10	trees distributed regularly, and shrubland < 50 %

Cover quality **Part 3 score**

score		Type 1	Type 2	Type 3	
25	> 75 % of tree cover				
10	50-75 % of tree cover or 25-50 % tree cover but 25 % covered by shrubs				
5	tree cover lower than 50 % but shrub cover at least between 10 and 25 %				
0	less than 10% of either tree or shrub cover				
+10	at least 50 % of the channel has helophytes or shrubs				
+ 5	if 25-50 % of the channel has helophytes or shrubs				
+5	if trees and shrubs are in the same patches				
-5	if trees are regularly distributed but shrubland is > 50 %				
-5	if trees and shrubs are distributed in separate patches, without continuity				
-10	trees distributed regularly, and shrubland < 50 %				
Final score is sum of all level scores					

Annex 3 Fluvial Habitat Index

Sampling site	
Data	
Operator	

Parts	Score

1. Embeddedness in riffles and runs – sedimentation in pools

Riffles	Stones, pebbles and gravel embebed in fine sediment in 0 - 30%.	10	
	Stones, pebbles and gravel embebed in fine sediment in 30 - 60%.	5	
	Stones, pebbles and gravel embebed in fine sediment in > 60%.	0	
Pools	Sedimentation 0 - 30%	10	
	Sedimentation 30 - 60%	5	
	Sedimentation > 60%	0	
		TOTAL (only one score from pools or from riffles)	

2. Riffle frequency

High frequency of riffles. Ratio: distance between riffles / stream witdh < 7	10	
Medium. Ratio: distance between riffles / stream witdh 7 - 15	8	
Ocassional. Ratio: distance between riffles / stream witdh 15 - 25	6	
Scarce or null, laminar flow. Ratio: distance between riffles / stream witdh >25	4	
Only pools	2	
	TOTAL (only one score)	

3. Substrate composition

% Boulders and stones	1 - 10%	2	
	> 10%	5	
% Pebbles and gravels	1 - 10%	2	
	> 10%	5	
% Sand	1 - 10%	2	
	> 10%	5	
% Silt and clay	1 - 10%	2	
	> 10%	5	
		TOTAL (sum of scores of each class of substrate)	

4. Velocity/depth regime

4 classes present. Slow-depth, slow-shallow, fast-depth and fast-shallow.	10	
Only 3 of 4 regimes	8	
Only 2 of 4 regimes	6	
Only 1 regime	4	
TOTAL (only one socre)		

5. Shading of river bed

Shaded with some open areas	10	
Completely shaded	7	
Large open areas	5	
Not shaded	3	
TOTAL (only one score)		

6. Heterogeneity components

Leaf litter	> 10% or < 75%	4	
	< 10% or > 75%	2	
Presence of branches and wood in the stream		2	
Tree roots in the banks		2	
Natural dams		2	
TOTAL (sum of scores of each class)			

7. Aquatic vegetation cover

% Plocon + mosses	10 - 50%	10	
	< 10% or > 50%	5	
% Pecton	10 - 50%	10	
	< 10%or > 50%	5	
% Phanerogams + Charales	10 - 50%	10	
	< 10% or > 50%	5	
TOTAL (sum of scores of each class)			

FINAL SCORE (Addition of all previous scores)