AN ASSESSMENT OF THE EFFECTIVENESS OF THE USE OF BENTHIC INSECTS AS BIO-INDICATORS OF WATER POLLUTION IN SELECTED RIVERS OF LUSAKA DISTRICT

by

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A dissertation submitted to the University of Zambia in partial fulfillment of the requirements of the degree of Master of Science in Entomology.

Department of Biological Sciences
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DECLARATION

I, **Kochelani Saili**, hereby declare that this dissertation represents my own work and that it has not previously been submitted for a degree, diploma or other qualification at this or another University.

Signature

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Date

APPROVAL

This dissertation of **Kochelani Saili** is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Entomology by the University of Zambia.

Name	**Signature**	**Date**
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Internal Examiner		
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External Examiner		

DEDICATION

I dedicate this thesis to my Father and Mother, Mr. Kenny Basil Saili and Mrs. Bridget Salome Chilufya. I thank God everyday for giving me such loving and hard working parents. You are truely my inspiration and pillars of my strength; your support and continuous belief in me are what have kept me going.

ABSTRACT

Presently in Zambia, physico-chemical methods are employed to monitor water quality of the country's rivers and other freshwater bodies. These conventional methods have not only proved to be expensive, but also tell us very little about impacts of pollution on aquatic organisms. With this realization comes the need to develop cheaper monitoring methods for freshwater bodies. Biomonitoring of pollution in freshwater bodies using live organisms called bio-indicators is one such cheaper alternative method. This study investigated the effectiveness of using benthic insects as bio-indicators of water pollution in selected rivers of Lusaka district, as an alternative water quality monitoring method. The study hypothesized that there was; no significant differences in pollution levels due to human activities between rivers within and those surrounding Lusaka district; no significant differences in benthic insect macro-invertebrate diversity and richness among rivers outside Lusaka district boundaries and those within the district; no relationship between levels of water pollution and benthic insect macro-invertebrate diversity and; and no benthic insect macro-invertebrate groups that were sensitive to water pollution in Lusaka District rivers that could be used as bio-indicators of water quality. Benthic insect macro-invertebrates were collected from six different rivers in and around Lusaka using a dip-net. These were enumerated and identified. Water samples were collected at each sampling area and analysed for physico-chemical parameters. Benthic insect macro-invertebrate diversity and evenness was determined using the Shannon-Weiner Diversity Index (H). The relationship of benthic insect macro-invertebrate diversity and richness with levels of water pollution was determined by correlating the family richness and family diversity to physico-chemical water characteristics determined for the selected sampling areas on each river. A total of 40 different insect macro-invertebrate families were collected. There was no significant differences ($p \geq 0.05$) in the family richness of the benthic insect macro-invertebrates in the rivers of Lusaka. Further, there were no significant differences ($p \geq 0.05$) in pollution levels of the rivers within the city, and those outside the city. Nine benthic insect macro-invertebrate families had significant positive or negative correlations with physico-chemical parameters and hence were selected as bioindicators of water pollution in Lusaka District. Bioassay results showed the that less pollution tolerant macro-invertebrate species had high mortality levels in the river sites classified as 'Disturbed' whilst showing reduced mortality in river sites classified as 'Good' and 'Natural'. This proved that there are benthic insect macro-invertebrate groups that are sensitive to water pollution in Lusaka district freshwater bodies that could be used as bio-indicators of water quality.

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LIST OF ABBREVIATIONS AND ACRONYMS

DO:	Dissolved Oxygen.
LWSC**:**	Lusaka Water and Sewarage Company.
GPS:	Geographical Position System.
PCA:	Principle Component Analysis.
SASS:	South African Scoring System.
SAFRASS:	Southern African River Assessment Scheme .
SPSS:	Statistical Package for Social Scientists.
WHO:	World Health Organization.

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CHAPTER 1: INTRODUCTION

1.1 Background

The global freshwater resource is under continuing and conflicting pressures because human demand for potable water and freshwater for irrigation and other purposes, as well as for use by other freshwater and terrestrial biota, are on the increase (Clarke *et al.,* 2003). Zambia has one of the fastest human population growth rates in the world (United Nations, 2010) and pressure on catchments and associated river ecosystems in Zambia, is ever increasing. This in turn increases presssure on monitoring of potable water quality by municipal and city councils as well as other government agencies. Commonly, freshwater quality monitoring is done through chemical analyses which can provide very accurate measures of the concentrations of chemical substances in the water. However, a monitoring system of this kind has always suffered from some of the following major drawbacks;

1. Chemical analyses on their own, are expensive to undertake and in the long run, out of reach for many poor developing third world countries like Zambia (Dallas *et al.,* 2010; Day, 2000).

2. Chemical analyses tell us very little about impacts that pollution has on organisms in water bodies (Clarke *et al.*, 2003; Dallas *et al.*, 2010).

3. Results of chemical analyses reflect the conditions in the river only at the exact moment of sampling, and as such, are short-lived and catastrophic pollution events might easily be missed (Day, 2000).

4. It is virtually impossible to measure the thousands of different chemical substances present in water. This problem is further compounded by the fact that some of the most toxic substances occur in minute quantities, often below the detection limits of most chemical analytical techniques (Day, 2000).

With this realization comes the growing need to develop relatively simple, non-resource intensive monitoring methods for freshwater quality especially for a developing country like Zambia where rivers are often critically important to both rural and urban populations. Biomonitoring of pollution using live organisms in freshwater bodies is one such cheaper alternative method of ensuring proper water quality in natural water bodies (Dallas *et al.*, 2010; Day, 2000).

Biological monitoring or biomonitoring, in short, is defined as the systematic use of biological responses to evaluate changes in the environment with the intent to use this information in a quality-control programmes for the environment. It can also be defined as the use of a biological entity as a detector and its response as a measure to determine environmental conditions, usually done through biological surveys. In other words, biomonitoring is simply a toolbox of techniques that can be used to check the water quality of the aquatic ecosystems (Day, 2000; Dallas *et al*., 2010; Maseti, 2005).

Biological indicators form the basis of biomonitoring. Freshwater biological indicators or bio-indicators in short, are species of freshwater organisms that are particularly sensitive to change and so can be used to indicate problems in an area or water body which might lead to loss of biodiversity. They are communities, whether plant or animal, with a narrow range of ecological tolerance that may be monitored because their presence, relative abundance and/or behavior serve as barometer of ecological conditions (Allaby, 2005; Bonada *et al.,* 2006a). Diatoms, macro-invertebrates, fish and vegetation (i.e. macrophytes and/or riparian vegetation) have been used as bio-indicators of aquatic ecosystem conditions (Clarke *et al.*, 2003; Day, 2000; Dallas, *et al*., 2010).

Aquatic benthic macroinvertebrates are the most widely used organisms in freshwater biomonitoring. Macro-invertebrates are animals that are large enough to be seen with a naked eye and they lack a backbone. They inhabit all types of water bodies. Benthos or benthic organisms are those living at the bottom of a water mass (Allaby, 2005; Verma and Argawal, 2005) and are retained by net mesh sizes of less than 200 to 500µm (Rosenburg and Resh, 1993). They occupy tiny spaces between submerged stones, within organic debris, on logs and aquatic plants or within fine sediments such as silt and clay. The wide range of natural habitat preferences and pollution tolerances among benthic macroinvertebrates makes them excellent organisms for freshwater bioassessment. Benthic macroinvertebrates include insects (mostly in their immature stages), clams, shrimps, snails and worms (Braccia and Voshell, 2006; Carter *et al.,* 2009; Rosenburg and Resh, 1993). Aquatic macroinvertebrates are a major component of the biota of aquatic ecosystems and are associated with one or other aquatic habitat such as stony beds; marginal and instream vegetation; floating vegetation; gravel, sand and mud. They are mostly primary and secondary consumers near the base of the food chain and are therefore essential elements in the functioning of aquatic ecosystems. These macroinvertebrates are largely dependent on the aquatic environment in which they live, and may be sensitive to factors such as water quality, water quantity (environmental flows), habitat and food availability (Dallas *et al.,* 2010; Davies and Day, 1998).

There is a general consensus that macroinvertebrates are amongst the most sensitive components of aquatic ecosystems and hence their wide use in biomonitoring of aquatic ecosytems (Clarke *et al.,* 2003; Dallas *et al.,* 2010; Davies and Day, 1998; Rosenburg and Resh, 1993). There are several good reasons why freshwater macroinvertebrates are useful as indicators of the ecological quality of rivers:

1. They are ecologically diverse and widespread, occurring in almost all types of streams and rivers.

2. Qualitative or semi-quantitative sampling of macroinvertebrates are relatively inexpensive and macroinvertebrates are comparatively quick and easy to identify to the taxonomic level necessary to assess the ecological quality of a freshwater body.

3. Because most macroinvertebrate species do not move great distances, their presence or absence can be related to the environmental conditions in a given section of river/freshwater body.

4. The life cycles of most species are sufficiently long to integrate the environmental stresses that have occurred over an extended period of time (Clarke *et al.,* 2003; Dallas *et al.,* 2010).

5. Macroinvertebrates are also widely distributed in diverse communities, which constitute a broad range of trophic levels and pollution tolerances (Rosenburg and Resh, 1993; Dallas *et al.,* 2010).

According to Clarke *et al.* (2003) single samples of macroinvertebrates may be sufficient to indicate environmental problems over the previous few months, making aquatic macroinvertebrate sampling an ideal procedure for monitoring large numbers of sites. In particular, the community composition and the taxonomic richness observed in macroinvertebrate samples collected using standard protocols are considered to be sensitive indicators of alterations in aquatic ecosystems. The fact that the various invertebrate species comprising a sample have differing abilities to cope with different environmental stresses (e.g. organic pollution, heavy metal pollution, modified flow regime, loss of habitat richness) provides a route for diagnosing the form of the stress operating at a given site.

Being a developing third world country, Zambia has a problem in implementing the traditional monitoring approach for water quality. This approach which involves collecting regular stream/river/dam/lake water samples for analysis in the laboratory for physical and chemical pollutants has proved to be expensive and hence unsustainable. Further, due to the high cost of reagents and instruments required to capture the large number of potential pollutants, water chemistry testing may fail to capture changing concentrations of a particular pollutant. Compounding the problem is the fact that, although the traditional monitoring approach for water pollution can easily detect releases of point source pollutants such as heavy metals, sewage and other chemical wastes from industrial municipal and city origins, it cannot readily and reliably detect non-point source water pollution (pollution from sediment, fertilizer and pesticide runoff from farms, residential areas etc). This is because runoff does not come from a few easily identifiable sources but instead, stems from a number of locations scattered across a watershed. Non-point source pollution is therefore the most difficult type of water quality impairment that developing third world countries have to contend with at present, in their quest to protect human life and conserve the environment and biodiversity.

What Zambia needs in this quest is a simple, inexpensive and reliable water quality monitoring protocol, to supplement the traditional chemical water quality monitoring approach in use. The goal of this study was to assess the effectiveness of benthic insect macro-invertebrates as bio-indicators for water pollution in selected rivers of Lusaka district, Zambia, as an alternative water quality monitoring method for the country.

1.2 Statement of the Problem

Presently, Zambia has one of the fastest human population growth rates in the world, with a population projected to increase by almost 1000% by the end of the 21st Century (United Nations, 2010). This increase in population is undoubtedly increasing pressure on catchments and associated river ecosystems in the country. A medium-term aim in Zambia's national development plan is the development of integrated water resource management, and a decentralisation of water governance away from state level, towards management by local water user associations (WUAs) (Uhlendahl *et al.,* 2011). The development of relatively simple, non-resource intensive biomonitoring methodologies is therefore appropriate to support Zambia's water management aims. However, very little previous work has been undertaken on the riverine biota of Zambia i.e. fish, invertebrates and macrophytes, hence the need to build a relevant knowledge base (Kennedy *et al.,* 2012). The differential tolerance of aquatic insect invertebrates to pollution is a powerful tool for monitoring the quality of running waters and in future may form the underpinning of national legislation governing inputs of pollutants into streams.

Some developed countries and some developing Southern Africa countries such as South Africa, Swaziland, Namibia and Botswana, have or are in the process of producing and implementing biomonitoring strategies to assess river water quality and ecosytem integrity. This requires that a variety of taxonomic groups, including macroinvertebrates, be studied in assessing the water quality of rivers (Bonada *et al.,* 2006a; Clarke *et al.,* 2003; Dalas *et al.,* 2010). Zambia however, lags behind in this aspect despite the fact that biomonitoring is reported to offer a cheaper and easier alternative method for assessing the ecological or biological quality of rivers based on macroinvertebrate diversity.

1.3 Study Objectives

1.3.1 General Objective of the Study

The general objective of this study was to assess the effectiveness of the use of benthic insect macroinvertebrates as bio-indicators of water pollution in selected rivers of Lusaka district.

1.3.2 Specific Objectives of the Study

Specific objectives of the study were to:

i. Assess levels of pollution in selected rivers of Lusaka district using conventional physico-chemical methods of laboratory chemical analyses.

ii. Determine the diversity, abundance and distribution of benthic insect macroinvertebrate species of selected rivers in Lusaka district.

iii. Select benthic insect macroinvertebrate groups to test as bio-indicators of water pollution in Lusaka district.

1.4 Study Hypotheses

The study tested the hypotheses that:

i. There was no significant difference in pollution levels due to human activities between rivers within and those surrounding Lusaka district.

ii. There were no significant differences in benthic insect macroinvertebrate diversity and richness among rivers outside Lusaka district boundaries in pristine habitats and those found in disturbed and/or degraded habitats within the district.

iii. There existed no relationship between levels of water pollution and benthic insect macroinvertebrate diversity and richness in selected rivers of Lusaka district.

iv. There were no benthic insect macroinvertebrate groups that were tolerant to water pollution in Lusaka District rivers that could be used as bio-indicators of water quality.

1.5 Significance of the Study

The importance of the study is that it:

i. Provided the relevant knowledge base that will eventually lead to the development of a relatively simple, non-resource intensive biomonitoring methods based on macroinvertebrate diversity, for monitoring freshwater quality in Lusaka district in particular and Zambia in general, that will stand on its own or be used integrated with the traditional physico-chemical water quality analyses methods.

ii. Led to the development of an inventory of benthic freshwater insect macroinvertebrate species of selected freshwater bodies of Lusaka district.

iii. Led to the establishment of a reference collection at the University of Zambia, in the Zoological museum of the Department of Biological Sciences, of benthic freshwater insect macroinvertebrate species that can be used as bio-indicators of water quality in Lusaka district.

iv. Contributed to the body of knowledge by filling in part of the knowledge gap on the distribution and composition of aquatic benthic insect macroinvertebrates of Zambia.

CHAPTER 2: LITERATURE REVIEW

2.1 Water Pollution in Zambia

Pollution is defined as the contamination of Earth's environment with materials that interfere with human health, the quality of life, or the natural functioning of ecosystems (Engelking, 2009). It can also be defined as the defilement of the natural environment by a pollutant; a pollutant being a by-product of human activities which enters or becomes concentrated in the environment, where it causes injury to humans or desirable species (Allaby, 2005). Pollution is categorised as either Point-source or non-point source pollution. Point-source pollution comes from specific, localized, and identifiable sources, such as sewage pipelines or industrial smokestacks. Non point-source pollution comes from dispersed or uncontained sources, such as contaminated water runoff from urban areas, farms or automobile emissions (Allaby, 2005; Engelking, 2009).

It is worth noting that there is currently very little published information on actual pollution levels in the rivers and streams of Lusaka District in terms of pollutants such as Nitrates, Sulphates, Phosphates and Ammonium and/or the presence of heavy metals in water bodies. Nonetheless, the Zambia Environmental Outlook Report 3 (Environmental Council of Zambia, 2008) recognised agriculture as one of the major sources of water pollution in Lusaka district because of the effluent from large volumes of chemicals (fertilizers and pesticides) used. Bäumle *et al.* (2012) lists "the uncontrolled use of agrochemicals" among the largest threats to freshwater resources.

Another source of pollution is untreated sewage which is discharged into rivers and streams by Lusaka Water and Sewarage Company (LWSC). The Ngwerere and Chunga rivers are examples of rivers that receive such effluents (Bäumle *et al.*, 2012).

Yet another major source of water pollution is uncollected solid waste. It is estimated that Lusaka produces about 765 tonnes of solid waste daily. Of this, only 76.5 tonnes i.e only 10%, is actually collected and properly disposed. The remainder is disposed off elsewhere, rivers and streams inclusive (Bäumle *et al.*, 2012). Other sources of water pollution in Zambia include industries involved in cement production, food-processing, tannary, fertilizer production, and paint manufacturing (Environmental Council of Zambia, 2008).

2.2 Physico-Chemical Water Quality Parameters

2.2.1 Turbidity and Total Suspended Solids

Turbidity refers to the cloudiness or haziness of a fluid caused by individual particles (suspended solids) that are generally invisible to the naked eye (Allaby, 2005) and is used as a measure of water quality in water bodies. Total Suspended Solids (TSS) refer to solid material, including organic and inorganic that are suspeneded in the water. Rivers and lakes prone to high bank erosion rates as well as those associated with urbanized areas, contribute large amounts of turbidity, through storm water pollution from paved surfaces such as roads, bridges and parking lots. Industries such as quarrying and mining can generate very high levels of turbidity and TSS from colloidal rock particles. Another major contributing factor of high turbidity levels is sewage pollution which is about 99.9 per cent water and 0.02-0.04 per cent solids (Allaby, 2005; Singh, 2005).

2.2.2 Ammonium (as NH_4-N)

Ammonium is considered one of the most important pollutants in the aquatic environment mainly because of its highly toxic nature and ubiquity in surface water systems (Russo, 1985). Ammonium, mainly in the form Ammonium nitrate (NH_4NO_3) or Ammonium Sulphate [$(NH_3)_2$ SO_4] can enter the aquatic environment by direct means such as municipal effluent discharges, and indirect means such as nitrogen fixation and the excretion of nitrogenous wastes from animals (Krebs, 2009).

2.2.3 Total Phosphates

Phosphorous is an essential nutrient to all living organisms. It is a major constituent of household detergents in which the complex sodium tri-polyphosphate (STPP) is used as detergent builders. The use of detergents has been responsible for the increase in the phosphorous in sewage effluents which includes municipal wastewater. Phosphate pollution of rivers and lakes causes extensive growth of algae (sometimes called algae blooms), which depletes the dissolved oxygen content and disrupts the natural food chain. Another major source of phospahtes are fertilizers used in modern agriculture (Krebs, 2009).

2.2.4 Sulphates

Sulphates are naturally occurring substance that contains sulphur and oxygen. Sulphate forms salts with a variety of elements including calcium, magnesium, potassium and sodium. In running waters, sulphates may be leached from the soil and are commonly found in most water supplies. Other sources of sulphates in running water include decaying plant and animal matter. Sulphates and sulphuric acid products are used in the production of fertilizers, chemicals, dyes, glass, paper, soaps, textiles, fungicides, and insecticides. They are also used in the mining, wood pulp, metal and plating industries, sewage treatment and leather processing (Krebs, 2009; Verma and Argawal, 2005).

2.2.5 Iron

Iron is the second most abundant metal in the earth's crust, of which it accounts for about 5%. It is used as constructional material, *inter alia* for drinking-water pipes. Iron oxides are used as pigments in paints and plastics whilst various iron salts are used as coagulants in water treatment (World Health Organization, 2008).

2.3 Use of Benthic Freshwater Insects in Biomonitoring

Biomonitoring strategies are increasingly being used to assess river water quality and ecosytem integrity in both developed and developing countries. For example, the United States Clean Water Act, the Canadian Protection Act, and the European Water Framework Directive, all require that the development of river catchment management plans include both chemical and ecological quality monitoring objectives. This requires that a variety of taxonomic groups, including macroinvertebrates, be studied in assessing the water quality of rivers (Bonada *et al.,* 2006b; Clarke *et al.,* 2003; Dallas *et al.,* 2010).

In Southern Africa, South Africa is well advanced in this area through its River Health Programme (RHP). The main purpose of the RHP is to serve as a source of information regarding the overall ecological status of South Africa's river systems in order to promote their management. It uses various biological indicators including aquatic invertebrates (South African Scoring System index or SASS); fish assemblages (Fish Assemblage Integrity Index or FAII); and riparian vegetation (Riparian Vegetation Index or RVI). It also uses physical indicators like the habitat (Index of Habitat Integrity IHI) to assess the ecological health of a river (Bonada *et al.,* 2006a; Dallas *et al.,* 2010; Maseti, 2005; Ollis *et al.,* 2006). Zambia, lags behind in this aspect despite the fact that biomonitoring is reported to offer a cheaper and easier method for assessing the ecological or biological quality of rivers based on the macro-invertebrate communities.

Traditionally, physico-chemical monitoring forms the backbone of most water quality monitoring programmes in Africa and the world (Dallas *et al.,* 2010). Limitations identified with this type of monitoring include:

i. The assessment is limited to the period of sample collection, therefore pulsed releases of effluents in freshwater bodies may be missed.

ii. The assessment is limited to the physical and chemical analyses performed and, since the number of constituents that could be present is vast, potentially toxic compounds may be missed.

iii. The sensitivity of chemical analytical methods when measuring very low concentrations of pollutants may be inadequate, particularly for substances that are characteristically present in low concentrations but which are persistent and tend to accumulate in the environment.

iv. Cost of a full spectrum of chemical analyses is high.

v. Synergistic (magnifying) and antagonistic (reducing) effects are difficult to establish, e.g. pH significantly alters the toxicity of trace metals, and

vi. Chemical analysis cannot readily and reliably detect non-point source water pollution for example, fertilizer and pesticide runoff (Dallas *et al.,* 2010; Day, 2000).

Biota are dependent on the medium in which they live and in case of benthic macro-invetebrates, the water body. They are sensitive to all alterations to the water body by, for example, pollution or habitat alteration. Such alteration will be reflected in the native biotic assemblages. Aquatic biota therefore, act as indicators of the overall ecological condition of the aquatic system. They act as continuous monitors of the water they inhabit, thereby enabling man to undertake long-term analysis of both regular and intermittent discharges, variable concentrations of pollutants, single and multiple pollutants, and synergistic or antagonistic effects (Dallas *et al.,* 2010; Day, 2000).

However, whilst indicating that a water body is impacted, biota do not provide insight into the specific cause of the problem. Further, the use of macro-invertebrates as bio-indicators presents the following challenges:

i. They have a relatively patchy or irregular distribution hence creating a potential variation between sites and over time.

ii. They are sensitive to factors other than water quality. Water flow, habitat availability and food availability are factors that can all strongly influence the diversity of macro-invertebrates. This in turn can complicate the interpretation of a particular biotic or diversity index.

iii. Macro-invertebrates may not respond to all impacts e.g. certain herbicides (Dallas *et al.,* 2010; Ismael and Dorgham, 2003; Lowe , 2012, pers. comm).

For the above reasons, bioassessment, which produces biological data, and physico-chemical monitoring, which produces physical and chemical data, should really be viewed as complementary in water quality assessments (Dallas *et al.,* 2010).

A major setback to developing biomonitoring programes for water quality in most African countries is that data about most aquatic species is either inadequate or not existing at all (Dallas, 2010, pers. comm.). This is not different for Zambia, which has not only incomplete data on most aquatic biota species but also has poorly documented data (Environmental Council of Zambia, 2008; Mbata, 2011, pers. comm.). Commenting on collection efforts of freshwater invertebrates north of the Limpopo River (Zimbabwe), de Moor *et al.* (2003a) states that records of many taxa are patchy and cannot be regarded as a good reflection of actual distributions. Because of this, Zambia lags behind in its taxonomic studies of aquatic macro-invertebrates.

It was not until recently through the Southern African River Assessment Scheme (SAFRASS) project (2010-2012), that aquatic invertebrate inventories had been undertaken to study the distribution and composition of such in Zambia. The SAFRASS project was a pilot study aimed at the long term development of biomonitoring protocols for Zambian rivers. The scheme is adapting the SASS systems for Zambian conditions and species This recent survey work represents the first extensive assessment of the riverine aquatic macro-invertebrates and macrophyte flora of Zambia (Kennedy *et al.,* 2012). Surveys through this project on 86 Zambian rivers has provided new information on the occurrence of aquatic macro-invertebrates, diatoms, and macrophyte and riparian plant species. However, much still needs to be done. There still remains an urgent need to build a knowledge base for riverine biota (Dallas *et al.,* 2010; Kennedy *et al.,* 2012).

2.4 The Use of Diversity Indices in Determining Ecological Effects of Pollution

Whilst biological indicators give some idea of the environmental conditions of a system and how it reacts to change and disturbance, an index summarizes the ecological condition into a single number (Todd and Roux, 2000). There are three basic types of indices: diversity indices, comparison (similarity or dissimilarity) indices and biotic indices. Of these, the biotic and diversity indices are the most widely used (Todd and Roux, 2000; Ollis *et al.*, 2006).

In biotic indices such as the South African Scoring System Index (SASS), each taxon from a particular group of organisms is assigned a sensitivity weighting based on the tolerance or sensitivity to particular pollutants. Biotic indices are generally specific to the type of pollution and/or the geographical area and are used to classify the degree of pollution by determining the tolerance of an indicator organism/s to a pollutant. Biotic indices assume that polluted sites or systems will contain fewer species than unimpacted sites or systems and the species that are present will reflect their particular sensitivity to a pollutant (Allaby, 2005; Dallas *et al.,* 2010; Ollis *et al.,* 2006).

A diversity index on the other hand, is defined as a mathematical expression of the species diversity of a given community or area, which includes relative abundance of the different species present (Allaby, 2005). These indices are based on vital features of a population such as number of existing species (Richness), distribution of individuals among species (Evenness) and the total number of existing individuals. As such, any changes in any of these three features will affect the whole population, hence, the diversity index (Türkmen and Kazancı, 2010; Southwood and Henderson, 2006). Diversity indices are used in biological monitoring based on the assumption that more species occur at unimpacted habitats and the total number of individuals is distributed more evenly among the species than in impacted habitats (Allaby, 2005; Cao *et al.,* 1996). Hence, diversity indices have proved vital in determining the ecological integrity of streams and rivers.

The three most commonly used diversity indices include the Shannon-Weiner Diversity Index, Simpson Diversity Index (also refered to as the Simpson-Yule) and the Margalef Diversity Index. The Pielou Evenness Index derived from the Shannon-Weiner Index is a common evenness index. Of the diversity indices, the most preferred and widely used in ecological studies is the Shannon-Weiner Index (Allaby, 2005; Krebs, 2009; Türkmen and Kazancı, 2010).

The Shannon-Weiner Index is widely used in ecological studies because it is an index that combines richness with evenness i.e. the number of species and the equitability of allotment of individuals repectively. It is relatively easy to calculate and fairly sensitive to actual site differences (Türkmen and Kazancı, 2010). However, despite this index being the most widely used and preferred, Türkmen and Kazancı (2010) showed that there are many instances where the value of the index is similar between sites despite differences among the sites. Similarly, Southwood and Henderson (2006) argued that the Shannon-Weiner index is the least sensitive to actual change in the community and hence, less useful as an indicator of environmental change. This is especially so with small sample sizes as it strongly influenced by the species number (Southwood and Henderson, 2006).

Earlier studies however, e.g. To'thme'resz (1995; cited from Southwood and Henderson, 2000) and Cao *et al.* (1996) and more recent studies e.g. Türkmen and Kazancı (2010), Krebs (2009) and Payne *et al.* (2005) show that the Shannon-Weiner Index and other non-parametric diversity indices can be particularly useful for diversity ordering i.e. comparing the diversity between communities. For example, according to Türkmen and Kazancı (2010), values of the Shannon- Weiner Index (H) for aquatic systems are generally between 1.5 and 3.5 but can exceed 4.5. Values above 3.0 indicate that the structure of habitat is stable and balanced; the values under 1.0 indicate that there are pollution and degradation of habitat structure.

As such, three classes of habitat structure can be devised as shown in Table 2.1.

Table 2.1: Classes of habitat structure devised from the Shannon-Weiner Index Range (Türkmen and Kazancı, 2010).

Shannon-Weiner Index Range	Habitat structure
Below 1	**Disturbed**: habitat degradation and high levels of pollution
Between 1.1- 2.9	**Fairly good:** Habitat moderately modified to natural, with moderate levels of pollution
Above 3	**Natural:** habitat stable and balanced; low pollution

Using the above categories then, the diversity between communities of different rivers sites can be made.

Parametric indices are based on measurable values or parameters in a population. They involve measurements, counts and scores and inferences made using them are based on specific distributions, their shapes and clusters (e.g. normal distribution). Non-parametric diversity indices, on the other hand, are 'distribution free'. They are sometimes referred to as 'Ranking Indices" to show that they may involve scores which are not exact in any numerical sense but simply ranks. They are useful where exact measurements or counts are difficult to set e.g. when animal signs or effects in a habitat are used to estimate densities. They are also useful as indicated above, for diversity ordering or ranking between communities.

2.5 Taxonomic Resolution in Macro-invertebrate Studies

The term taxonomic resolution refers to the identification of organisms in a community to a taxonomic level adequate to meet the objectives of a study. It is also refered to as taxonomic sufficiency (Bowman and Bailey, 1997).

A review of literature shows that there have been conflicting views on the level of taxonomic resolution necessary for community studies involving macro-invertebrates for both freshwater and marine benthic research. For example, Chessman *et al.*

(2007), Marshall *et al.* (2006) and Resh and Unzicker (1975) have advocated species-level identification in the context of freshwater monitoring of water quality. These scholars show that biotic or diversity indices calculated mainly at the species level had stronger correlations with potential stressors than a similar indices derived mainly at the family level for macro-invertebrates in freshwater ecosystems. It must be noted however, that identification to the species level is not always feasible because of the following limitations:

i. Lack of taxonomic expertise.

ii. Limitations in time and/or money and the necessary laboratory equipment.

iii. Incomplete or non-availabilty of comprehensive taxonomic keys. This is a particular problem for many freshwater aquatic macro-invertebrates (Chessman *et al.*, 2007; Dallas *et al.,* 2010; Davies and Day, 1998).

iv. A lack of the biological features to identify the organisms collected at a given place and time most likely due to poor handling or preservation of the organism (Bowman and Bailey, 1997).

As such, many biomonitoring protocols are based on biotic indices developed at family level identification e.g SASS. Gayraud *et al.* (2003), O'Leary *et al.* (2004) and Waite *et al.* (2004) all report that family-level assessments differs only slightly from species-level or genus-level assessments in their ability to distinguish different levels of human impact including pollution. This, apart from the reasons of time constraint, lack of taxonomic expertise and a general lack of taxonomic information pertaining to aquatic invertebrates, justifies the identification of aquatic macro-invertebrates to family level in this particular study.

2.6 Conducting Bioassays on Benthic Macro-invertebrates

One way of testing the effectiveness of selected benthic macro-invertebrates as indicators of pollution is to subject them to laboratory bioassays or toxicity tests. A bioassay or toxicity test is a biological test using a living organism, such as an animal or a plant. More specifically, bioassays are used to make quantitative and/or qualitative measurements of the amounts or activity of substances (Allaby, 2005). In studies such as this one, a bioassay tests the relative effectiveness of indicator organisms by subjecting them to the the different toxicities of the water samples as found naturally from different rivers.

One advantage of using bioassays is that they are integrative i.e., the test organism/s can react to multiple contaminants in a single sample. This is in contrast to chemical analyses which measure only one chemical at a time. However, bioassays lack specificity i.e. it is not possible to identify exactly which contaminant is causing the particular toxic effect. This is because bioassays do not usually indicate which toxic substances are present or their exact concentrations in a water sample (Markowitz, 1999).

Literature review shows that the most commonly used organism in aquatic bioassays is *Daphnia magna*, a micro-crustacean (Class: Branchiopoda, Order: Cladocera) commonly refered to as the Water Flea. Other organisms that have been used include *Chironomus tentans* (Order: Diptera; Family: Chironomidae), *Ceriodaphnia dubia* (Class: Branchiopoda, Order: Cladocera) and *Hexegenia limbata* (Order: Ephemeroptera; Family: Heptegeniidae) (Bennet and Cubbage, 1992; Harvey-Clarke, 2011; Markowitz, 1999). According to Markowitz (1999) and Bennet and Cubbage (1992), *D. magna* is particularly useful in bioassays because it is easier to rear. Other aquatic invertebrates are particularly difficult to rear and transportation from one place to another is not always easy. For example, Ephemeroptera and Plecoptera, although being among the most sensitive to pollution of the aquatic insects, are fragile, easily losing their gills or legs. This consequently affects their mortality rate if used in bioassays (Gerber and Gabriel, 2002; de Moor *et al.*, 2003a). It must be recognized and appreciated that there is very little published information providing guidelines on overcoming the challenges of transporting aquatic insects from one place to another particularly for the purpose of bioassays with many a literature focusing on terrestrial invertebrates.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study Area

This study was conducted in and around Lusaka, the capital city of Zambia (latitude, 15^0 – 16^0S; Longitude, 28^0-30^0E.). Lusaka city is an urban agglomeration which, due to a large population also forms Lusaka District (Figure 3.1). According to the last census conducted in 2010, Lusaka had a population of 2, 198, 996 people (Central Statistical Office, 2011). It is a fairly flat inland city at an altitude of about 1,300m above sea level, with no major rivers. There is significant agricultural activity within the urban area (Beddow, 2012).

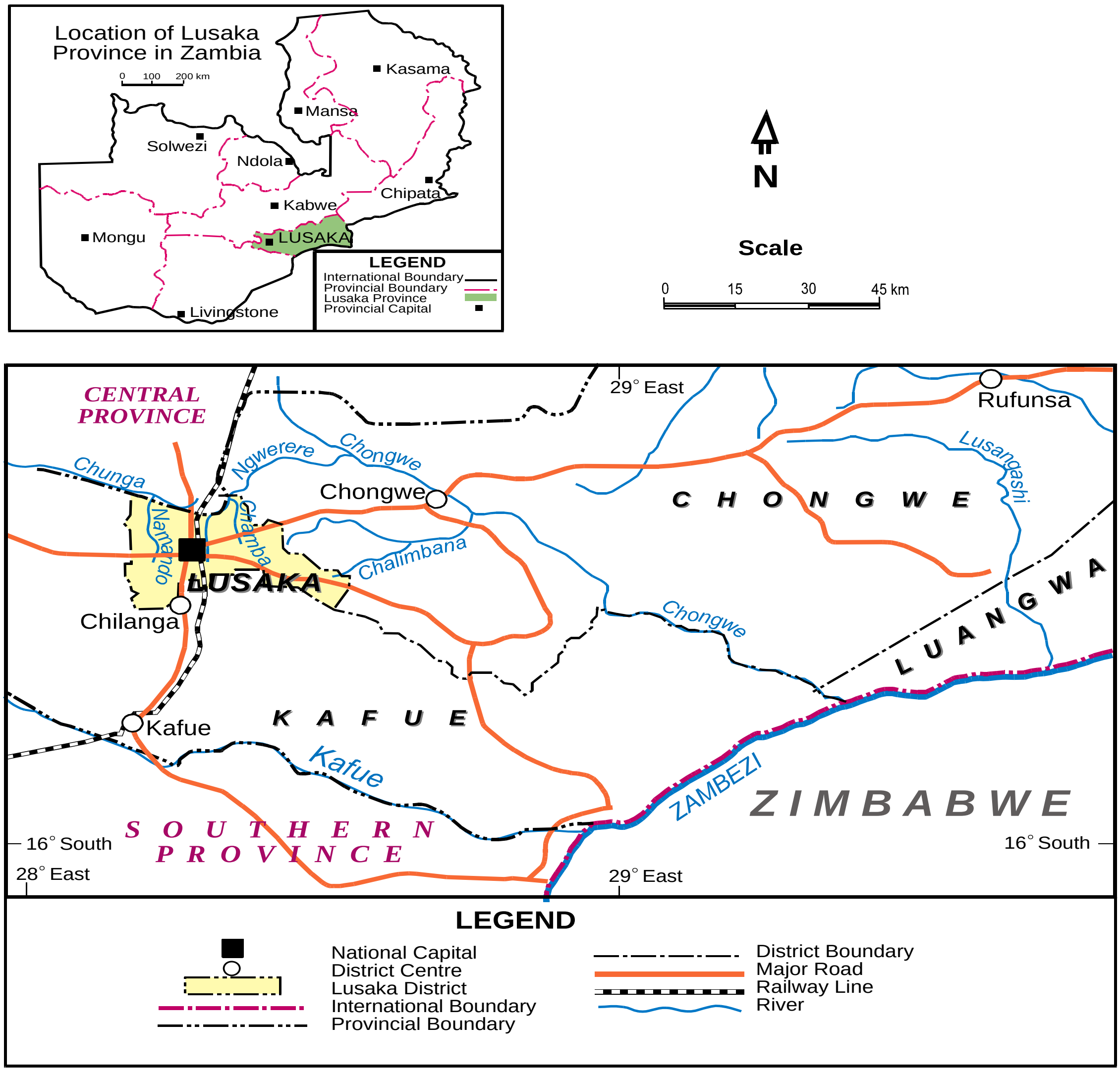

Figure 3.1. Location of the study area in Zambia.

3.1.1 Climate

Due to its high altitude, Lusaka city has a humid subtropical climate and is generally warm. The coldest month is July with a monthly average temperature of 16°C, whilst the city's warmest month is October with a monthly average temperature of about 25°C. Lusaka, like most parts of Zambia, has three different seasons; Cool and Dry, spanning from May to August, Hot and Dry from September through to October and the Warm and Wet from November to April. The average annual rainfall is 830mm falling mainly between November and March (Environmental Council of Zambia, 2008). Lusaka falls in Region IIa of Zambia's agro-economic zones, a region commonly classified as the medium rainfall region of Zambia. It forms a central band stretching from the Western border to the Eastern border of the country. The region is characterized by a mean annual rainfall of between 800 and 1000mm, and has a rain-fed cropping season of 90-150 days (Bunyolo *et al.*, 1995).

3.1.2 Geology and Drainage

The geology of Lusaka city consists of limestone in the western and southern parts of the city. In the northern and central parts, there are outcrops of schists and granites. The areas underlain by limestone are generally flat and have no surface streams or dambos (seasonally flooded areas) over them. The areas where schist and granites are found are elevated and streams emerge from these areas which flow towards the northeast (Nachiyunde *et al.*, 2013).

The following rivers that flow in and out of Lusaka city were selected for this study:

i. **Chamba River**

The Chamba river has its origins near the University of Zambia, Great East Road Campus in the city and includes three Anglin Lakes (now silted and dried up) and two Goma Lakes within the University of Zambia campus. It flows northwards, through Kalundu residential area, Ngombe Compound and part of Chudleigh residential area and it then drains off into the Ngwerere River in the north-east. Chamba river is subjected to non-point source pollution and surface water pollution from Ng'ombe Compound and the Chudleigh residential area.

ii. Chunga River and Tributaries

The Chunga river is located in the north of Lusaka city. Its source is near the Lusaka North Forest reserve Number 28 near the Great North Road. It is joined by streams emerging near the Independence Stadium and from Matero township. The river was particularly selected because it is subjected to point source pollution from sewage treatment works in Chunga and Matero township. It is also subjected to non-point source pollution and surface water pollution from the nearby townships. The Namando river is a tributary of the Chunga River near Barlastone Compound.

iii. Ngwerere River and Tributaries

The Ngwerere River has its origin near the marshy areas in the middle of Lusaka city (near the Main Post Office). It flows northward and is joined by a river and other streams (e.g. Chamba river and unnamed streams) and then turns north-east near Ngwerere township and drains into the Chongwe River. Ngwerere river is subject to point source pollution, particularly to chemical pollution from factories and untreated sewage from the Lusaka Water and Sewerage Company, Machinchi Station. It is also subjected to non-point source pollution from agricultural activities particularly near its confluence with the Chongwe River.

iv. Chongwe River and Tributaries

The Chongwe River has its source north of Lusaka but flows south-east through Chief Nkomeshya's area, part of the Lower Zambezi

National Park and and finally flows into the Kafue river. Most rivers draining Lusaka district, flow into the Chongwe. The Chongwe River also flows through many agricultural areas.

v. Musangashi River

The Musangashi River is a small river within the Lower Zambezi National Park. It occurs in the South-east of Lusaka district and flows into the Zambezi River.

3.1.3 Vegetation and wildlife

Like most of Zambia, vegetation in Lusaka district is mainly open Miombo woodlands and grasslands. The Miombo woodlands are dominated by *Julbernardia* and *Brachystegia* tree species (Fanshawe, 1969). Grasslands, cover less than 27% of land in Lusaka and mainly occur in poorly drained dambos or swampy areas. The dominant grasses are *Themeda triandra, Hyparrhenia* spp. and *Heteropogon contortus* (Aregheore, 2009).

3.1.4 Livelihoods

The economy of the City of Lusaka provides formal employment to only a small proportion of its labour force. In 2000, the number of people in formal employment in Lusaka was at 120,233 or 35 per cent of the labour force. Hence, the majority (65 per cent) of the city's labour force, earns its livelihood from informal economic activities, which predominantly consist of unregistered and unregulated small-scale non-agricultural economic activities ranging from petty trading and street vending to metal fabrication and wood processing. The bulk of the informal economic activities are, however, essentially in trading (Mulenga, 2003).

3.2 Assessment of levels of pollution in selected rivers of Lusaka district using physico-chemical methods

In order to assess the different levels of pollution due to human activities between rivers within and those surrounding Lusaka district, physico-chemical water quality analyses was conducted. Water samples were collected at each sampling area using standard procedures for laboratory water quality tests. Standard procedure for collection of water samples for laboratory tests involves first rinsing the collecting water bottle with the river water to avoid prior contamination. This is followed by the complete submersion of the bottle into the water and once completely filled, an air tight seal is placed over the bottle whilst still submerged. This procedure avoids the dissolving of any atmospheric gases into the water, hence giving false measurements of the dissolved oxygen and/or other gases. The water sample is then kept at freezingly low temperature to slow down microbial activities.

The collected water samples were sent to the Environmental Engineering Laboratory of the School of Enginerring at the University of Zambia for analysis of the following parameters;

i. Levels of Pollutants: Nitrates, Sulphates, Phosphates and Ammonium in the water

ii. Levels of the physical paramenters: total dissolved solids, and total suspended solids.

iii. Levels of heavy metals such i.e. Zinc, Lead and Copper.

iv. The state of turbidity, conductivity and dissolved oxygen (DO).

Also at each sampling site, direct water quality measurements namely; pH using a portable pH meter and temperature using a mercury thermometer were also made. Temperature and pH are important facets of water physico-chemical characteristics which affect the behaviour and distribution of aquatic organisms.

3.3 Benthic Insect Macro-invertebrate Sampling

On each of the rivers, in and around Lusaka city described above, three sampling sites were established and their locations geo-referenced using a Global Positioning System (GPS) for mapping of the whole study area. At each sampling site, 10 sampling quadrats (each measuring 1 x 1 m) were established at right angles to one bank of the river (Figure 3.2). Three of the ten quadrats were randomly selected for benthic insect macro-invertebrates sampling. In order to avoid collecting invertebrates clinging on to marginal vegetation (otherwise not benthic), sampling was conducted 0.5-1m away from the river bank.

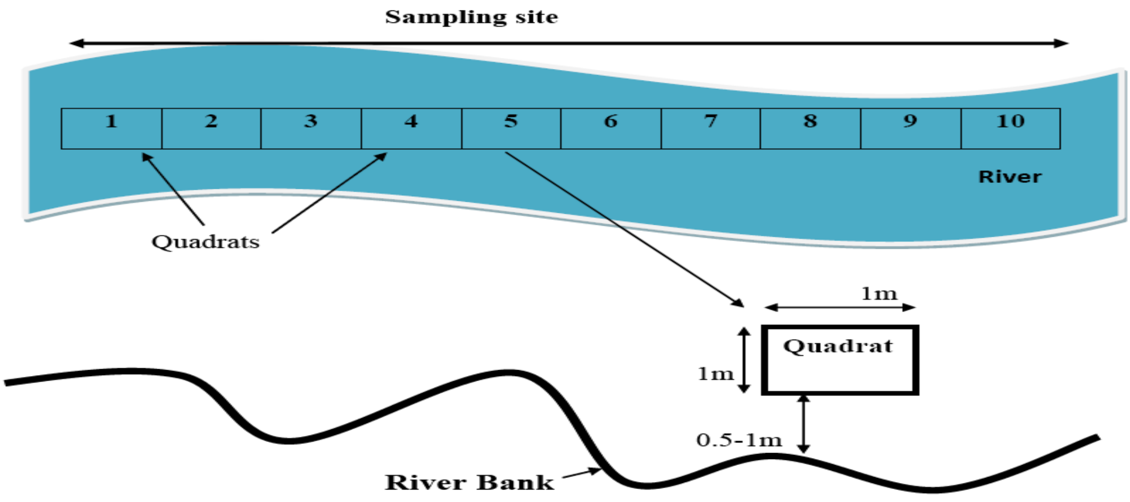

Figure 3.2. Sampling design: The location of sampling quadrats on a selected sampling site on a river

3.4 Sampling Site Selection

The following criteria were used when choosing sampling sites on selected study rivers:

1. Three sampling sites were established on each selected study river in the city. The first site was located immediately near the source to serve as a control site for the river. The second site was located immediately below the river source while the

third was located further downstream at different distances from the second sampling area depending on the condition of the study river.

2. Human-made modifications of the river such as a bridge, channel, dam or pipeline crossing were avoided by setting up the sampling site at least 50m upstream of such modifications.

3. Areas on the river that were strongly influenced by other factors, such as tributary confluences, backwater areas or eddies immediately upstream or downstream of large boulders or debris jams were avoided.

4. Ease of accessibility to the sampling site, perennial flow of water in the area and availability of suitable range of habitats in the area were other factors that influenced the establishment of sampling sites on the selected study rivers (Betty *et al.*, 2006).

3.5 Site descriptions

A total of 13 different sites, described in tables 3.1 to 3.15 and figures 3.3 to 3.16, were selected and sampled between the months of August and December, 2012.

3.5.1 Chamba River

3.5.1.1 Site I of the Chamba River

Table 3.1: Site description of Site I of the Chamba River

Location	Site Coordinates	General Site description
Behind Marshlands flats, opposite the University of Zambia, Great East Road Campus main entrance.	Latitude 15° 23' 10" S Longitude 028° 19' 53.2" E	Close by were dense mats of *Potamagedon, Cyperus, Typha, and Phragmites.* The active channel width was ~1.5m wide, with a depth of ~0.5m.

Figure 3.3. The diversity of habitat and macrophytes at Site 1 of the Chamba River.

3.5.1.2 Site II of the Chamba River

Table 3.2: Site description of Site II of the Chamba River

Location	**Site Coordinates**	**General Site description**
Chudleigh	Latitude 15° 22' 26.6" S Longitude 028° 19' 57.2" E	Active channel width was narrow, approximately 1m wide but deeper than at Site I (~0.75m). Diversity of macrophytes was generally low, with *Cyperus* dominating. Shallow parts of the river are invaded by dense mats of *Lycopersicon esculentum* (Tomato). There was some agriculture activity in the riparian area of the river, with some patches cleared up for vegetable farming. The river substratum is generally sandy.

Figure 3.4: The diversity of habitat and macrophytes present at Site II of the Chamba River.

3.5.1.3 Site III of the Chamba River

Table 3.3: Site description of Site III of the Chamba River

Location	Site Coordinates	General Site Description
Ng'ombe Compound; ~400m from confluence with the Ng'ombe stream	Latitude 15° 22' 06.9" S Longitude 028° 19' 58.9" E	The active channel width is narrow (~1m) and very shallow (approximately 0.25m deep). There were plenty of local disturbances both up and downstream of this site; washing of clothes, crossing points, water drawing points and garbage damping among other things. The river bed substratum was mainly stones which were well covered with *Spirogyra* (algae). No true macrophytes present.

Figure 3.5. Site III of the Chamba River.

3.5.2 Chunga River and tributaries

3.5.2.1 Site I of the Chunga River and its tributary

Table 3.4: Site description of Site I of the Chunga River

Location	Site Coordinates	General Site Description
Lusaka West, adjacent to the Chingwere/Chunga Cemetery	Latitude 15° 21' 02.7" S Longitude 028° 15' 28.0" E	Approximately 1km away from the Chunga Sewage Settlement ponds. The river formed a 7m high incised valley and was highly shaded. There were no true macrophytes except for a few patches of *Azolla*. The stretch within the site is highly subjected to water surface pollution. Active channel width was approximately 2m with boulder/stone substrate and lots of decaying plant material.

Figure 3.6a. Site I of the Chunga River

Figure 3.6b. Surface water pollution at Site I of the Chunga River.

3.5.2.2 Site II of the Chunga River and its tributaries

Table 3.5: Site description of Site II of the Chunga River

Location	Site Coordinates	General Site description
Just after the Lusaka Water and Sewerage Company (LWSC) Chunga Waste Water Treatment Plant, adjacent to Barlastone area, Lusaka West	Latitude 15° 20' 38.4" S Longitude 028° 14’ 56.7’’ E	The river received large amounts of discharge from the sewage treatment plant and was very voluminous. There was clearly no true macrophyte. The water is black in colour with an extremely foul smell/stench. Parts within the site are dangerously steep whilst others are relatively very shallow. The site had considerable parts highly shaded and was mainly of stony substrate.

Figure 3.7. Site II of the Chunga River.

3.5.2.3 Site III of the Chunga River and its tributaries

Table 3.6: Site description of Site III of the Chunga River

Location	Site Coordinates	General Site description
Barlastone area, Lusaka West	Latitude 15° 20' 38.4" S	Dry site.
	Longitude 028° 14’ 56.7’’E	

No samples were taken at Site III of the Chunga River. This was because at the time of sampling, the river had dried up at this site and beyond. This could be attributed to damming, sand extraction and/or stream diversion upstream (Per obs.; Bäumle *et al.*, 2012).

3.5.2.4 Namando River Site

The Namando River is a small tributary of the Chunga River in Barlastone area, approximately 1km in length. Because of its small size, only one sampling point was selected on the river.

Table 3.7: Site description of the Namando River Site

Location	Site Coordinates	General Site description
Barlastone area, near the Chunga LWSC water treatment plant and close to the confluence with the Chunga River.	Latitude 15° 20' 49.0"S Longitude 028° 14' 49.4''E	This site was near the confluence of Namando/Chunga River. The active channel width was approximately 1m wide. The site was generally a limestone area with no true macrophyte and was highly shaded. The substratum was mostly sand and stone.

Figure 3.8. Namando River Site.

3.5.3 The Ngwerere River

3.5.3.1 Site I of the Ngwerere River

Table 3.8: Site description of Site I of the Ngwerere River

Location	Site coordinates	General site description
Near the source of the Ngwerere River, near the Zambia Electricity Supply Cooperation (ZESCO) National Headquarters	Latitude 15° 24' 25.8" S	High velocity flow, active channel width is ~2m wide with a depth of 0.5m. River substrate is mainly sand and stone. There is clear surface water pollution and sewer pollution. *Cyperus* is the most dominant macrophyte.
	Longitude 028° 17' 03.1" E	

Figure 3.9a. Site I of the Ngwerere River.

Figure 3.9b. Water surface and sewer pollution at Site I of the Ngwerere River

3.5.3.2 Site II of the Ngwerere River

Table 3.9: Site description of Site II of the Ngwerere River

Location	Site coordinates	General site description
In Garden Compound, roughly 1km away from LWSC Machinchi Waste water Treatment Centre and Sewage Settlement Ponds.	Latitude 15° 22' 21.1"S	The active channel width was~ 1.70m wide and is ~0.5m deep. The substratum was mostly Gravel, Sand and Mud with lots of decaying matter. The water is murky, dark green and has a pungent smell, characteristic of sewer polluted water. No true macrophytes present.
	Longitude 023° 17' 41.2" E	

Figure 3.10. Site II of the Ngwerere River.

3.5.3.3 Site III of the Ngwerere River

Table 3.10: Site description of Site III of the Ngwerere River

Location	Site coordinates	General site description
Just before Kalimba Reptile Farms, before confluence with the Chongwe River.	Latitude 15° 18' 25.7" S Longitude 028° 21' 47.5" E	Characterised by plenty of *Eichornia*, and *Phragmites*. The water is brownish-green in colour and highly silty. Active channel river width ~3m with a depth of 0.5m. Made up mainly of stone substrate.

Figure 3.11. Site III of the Ngwerere River.

3.5.4 Chongwe River

3.5.4.1 Site I of the Chongwe River

Table 3.11: Site description of Site I of the Chongwe River

Location	Site coordinates	General site description
Just before confluence with Ngwerere River, near Kasisi Girls Secondary School	Latitude 15°13' 59.3"S Longitude 028°37' 02.8"E	Dominated by dense thickets of *Phragmites. V*ery deep, slow moving waters, making it difficult to sample. Small patches of *Nyphaea divaricarta, Persecaria senegalensis* and *Persecaria hydropiper* are seen. Active channel width ~5 to 6m wide and more 3m deep.

Figure 3.12. Site I of the Chongwe River.

3.5.4.2 Site II of the Chongwe River

Table 3.12: Site description of Site II of the Chongwe River

Location	Site coordinates	General site description
Just after confluence with the Ngwerere River, near Kasisi Girls Secondary School	Latitude 15° 13’ 34.1”	Dominated by *Phragmites*. Active channel width ~4m and ~0.7m deep. Mainly stoney substratum.
	Longitude 028° 30’ 44.2”	

Figure 3.13. Site II of the Chongwe River

3.5.4.3 Site III of the Chongwe River

Table 3.13: Site description of Site III of the Chongwe River

Location	Site coordinates	General site description
Near the Chongwe River-Great East Road bridge (Chongwe town).	Latitude 15° 19' 23.1" S	Lots of stony habitat. Plenty of *Eichhornia crassipes* & *Phragmites.* Active channel width changes from over 15m to less than 5m.
	Longitude 028° 42' 09.6 "E	

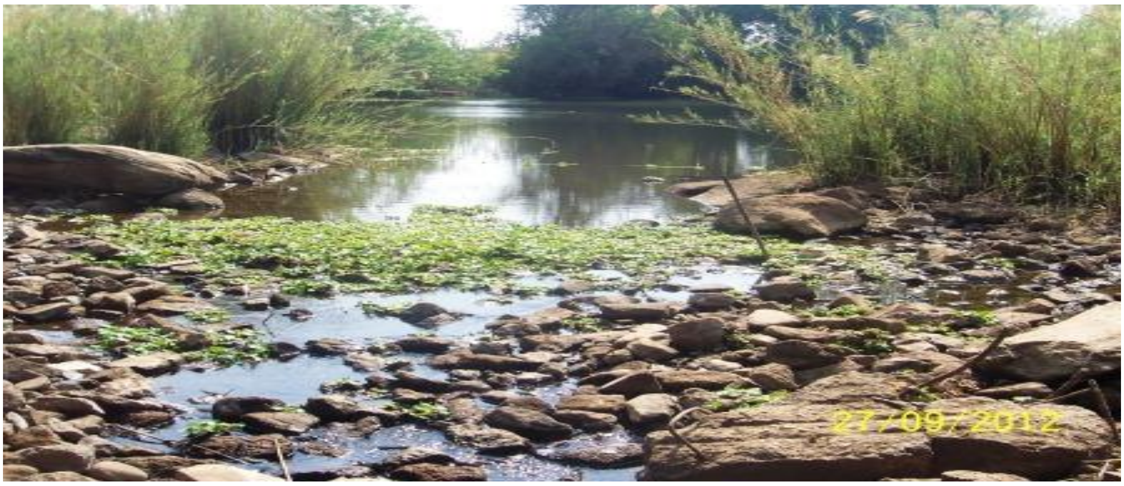

Figure 3.14. Site III of the Chongwe River.

3.5.5 Musangashi River

The number of sites on the Musangashi River was reduced to just one due to the unprecedented drying up of the river during the time of sampling.

3.5.5.1 Site I of the Musangashi River

Table 3.14: Site description of Site I of Musangashi River

Location	Site coordinates	General site description
Near Chilimba Community School within the Lower Zambezi National Park	Latitude 15° 35' 64.0" S Longitude 29° 21' 97.4" E	Dry at the time of sampling. *Phragmites* the dominant macrophyte.

Figure 3.15. Dry River bed at Site I of the Musangashi River.

3.5.5.2 Site II of the Musangashi River

Table 3.15: Site description of Site II of the Musangashi River

Location	Site coordinates	General site description
Near Musangashi Wildlife Camp within the Lower Zambezi National Park	Latitude 15° 24' 19.7" S Longitude 29° 23' 09.7" E	The river has been reduced to a single pool of water of ~5m in width and over and just over 40m in length. The pool is ~0.6m deep with a substratum of mainly sand/mud/gravel and bedrock filled with decaying plant matter. Water is murky with *Phragmites* as the most dominant macrophytes.

Figure 3.16. Site II of the Musangashi River; reduced to a single pool of (stagnant) water.

3.6 Benthic insect macro-invertebrates Collection

Benthic insect macro-invertebrates were collected using the following two methods:

3.6.1 Dip net sampling method

The first method, (which was the main method by which benthic insects macro-invertebrates were collected in the study) involved the use of a D-framed Dip net (Net dimensions: 30cm x 30cm with a mesh size of 1000 μm). In each randomly selected quadrant, a 4-5 minute timed sample was taken by pointing the net downstream and dragging it upstream i.e. against the water current whilst disturbing the substrate, and in the process allowing the benthic macro-invertebrates insects to collect in the net as shown in Figure 3.17. The net contents were then emptied into a tray half filled with water and followed by the removal of large organic debris and stones from the net contents (Figure 3.18). The tray was then visually searched and a record of the collected macro-invertebrate families made (Figure 3.19). Using a 500 μm sieve, the contents were then sieved out, and placed in well labelled plastic containers with 70% ethanol preservative and taken to the laboratory for enumeration and identification (Figure 3.20).

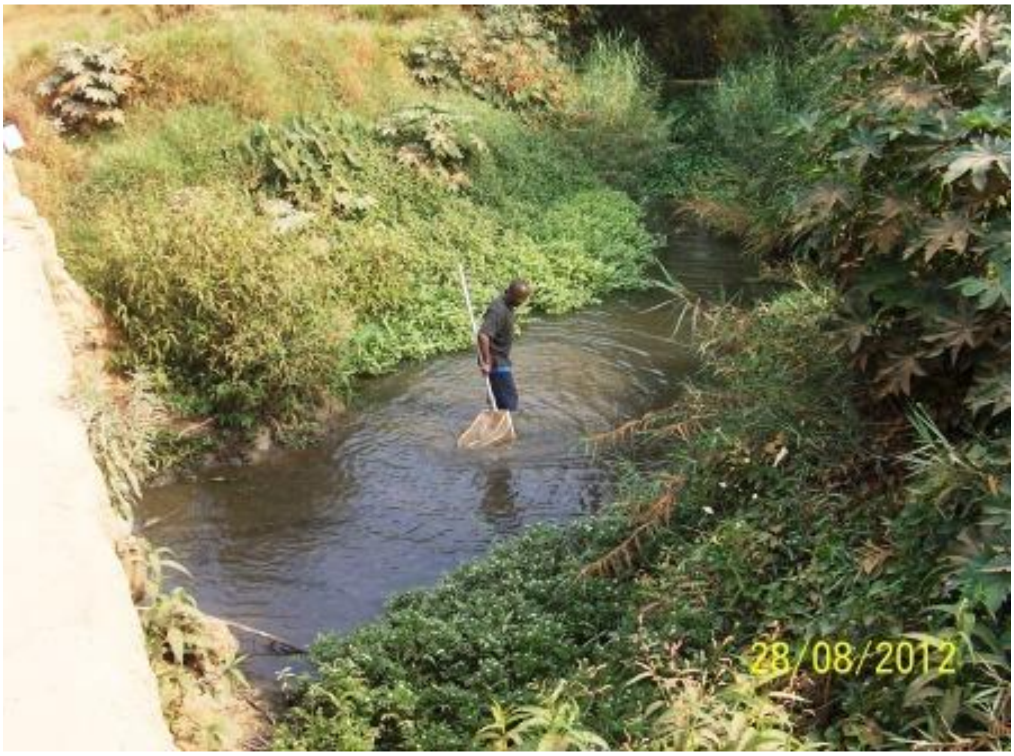

Figure 3.17. Collecting benthic insect macro-invertebrates, using a dip net along the Ngwerere River.

Figure 3.18. Emptying net contents obtained from the Chongwe River into a tray half filled with water.

Figure 3.19. Visual examination of dip net contents.

Figure 3.20. The dip net contents being sieved out and placed in well-labelled plastic containers with ethanol preservative.

3.6.2 Dredging sampling method

The second method involved a hand-pulled dredge to collect benthic insect macro-invertebrates in each randomly selected quadrat of a study site. The hand-pulled dredge was used in deeper waters of the study area. As such, this method was only used once in this study, in the Chongwe River Site II. All bottom soil of each selected quadrat were dredged for benthic insect macro-invertebrates. Dredged contents of each selected quadrat were emptied into a tray half filled with water followed by the removal of large organic debris and stones as before. The tray was visually searched and a preliminary record of collected macro-invertebrate insect families made. The dredged benthic insect macro-invertebrates were picked from the riverbed soil using hands and forceps and placed in well labelled preserving bottles containing 70% ethanol and taken to the laboratory for enumeration and identification.

3.6.3 Identification and preservation of benthic insect macro-invertebrates

In order to identify benthic insect macro-invertebrates from the selected rivers of Lusaka district, the collected river material was placed in labelled plastic containers and preserved in 70% ethanol and taken to the laboratory. In the laboratory, the benthic insect macro-invertebrates were hand sorted from this river material using the naked eye and with the aid of a magnifying glass and counted. The numbers of organisms in each taxon were recorded and the data were stored on Excel spreadsheets for later analyses (Microsoft Excel, version 2010). Good light and a white sorting tray were necessary for optimal recognition and identification of insects during the sorting. The benthic insect macro-invertebrates were identified to family level using both pictorial and dichotomous taxonomic keys. Gerber and Gabriel (2002) provides a useful field guide (a pictorial key with brief taxonomic information) to the identification of aquatic macro-invertebrates insects to family level whilst *Guides to the Freshwater Invertebrates of Southern Africa,* Volumes 5-9 (de Moor *et al.*, 2003a and b; Stals and de Moor, 2007; Day *et al.,* 2002) provide more taxonomic details at both family and genus level.

3.7 Determination of Benthic Insect Macroinvertebrate Family Richness

Species richness refers to the number of species present in a community, measured as the number of species per unit area of ground area. It is simply a count of species (Allaby, 2005; Southwood and Henderson, 2006). For this study, the total number of benthic insect macro-invertebrate families for each sampling site and for individual rivers were counted.

3.8 Creation of a reference collection of identified benthic insect macro-invertebrates from selected rivers in Lusaka district

A reference collection of benthic macroinvertebrates that was deposited at the University of Zambia, Department of Biological Sciences, Zoological Museum was created. Each macro-invertebrate family per river site was placed in a well labelled 50ml plastic container with 70% ethanol as the preservative. The following information was placed on each container for identification purposes:

i. **Collector:** Identified the person responsible for the collection, in this case the author of this dissertation.

ii. **Computer Number:** This is the unique code given to all University of Zambia students for the sake of identification. Because this was part of an academic study, the computer number of the author of this dissertation was included on every specimen label.

iii. **Date of Collection:** This gave the day, month and year of specimen collection.

iv. **Location:** Identifies the river name from which the specimen was collected.

v. Order and Family and whenever cardinal, other important taxonomic information such as Suborder and Subfamily.

Below is an example of the convention used**.**

Collector: Saili, Kochelani

Computer #: 531001173

Date of collection: 4th Sept, 2012

Location: Chamba River; near UNZA

Order: Diptera Suborder: **Nematocera** Family: ***Culicidae***

3.9 Data Analysis

3.9.1 Determination of Benthic Insect Macroinvertebrate Family Diversity

Using data collected from section 3.5.1 above, benthic insect macro-invertebrate diversity was determined using the Shannon-Weiner Diversity Index (represented by the symbol H) (Allaby, 2005). This is represented by the expression:

$$H = -\sum_{i}^{s} (pi).log\rho i$$

Where,

pi = proportion of species i represented by each taxon in the community,

S = total number of species in the community.

3.9.2 Determination of Benthic Species Evenness

Species evenness refers to how close in numbers each species in an environment are. It is represented by the letter J (and is restricted between 1 and 0) is given as (Allaby, 2005):

$$J = \frac{H}{Hmax}$$

Where,

H is the Shanon-Wiener index value,

H_{max} is the possible maximum possible diversity for a given number of species and individuals and is given as (Allaby, 2005):

$$H_{max} = \ln S$$

For this study, invertebrates were identified to family taxonomic level and as such diversity was calculated at this level.

3.9.3 Assesssing the Relationship of Benthic Insect Macroinvertebrate Diversity, Richness and Abundance to Levels of Pollution in Selected Rivers

To assess the relationship of benthic insect macroinvertebrate diversity, richness and abundance to levels of pollution, the following commonly used ecological characteristics were correlated with physico-chemical water characteristics for selected sampling areas on each river:

1. Number of families (family richness)

2. Total insect abundance in each sampling area

3. Diversity at family level

The physico-chemical water characteristics used in the assessments were reduced to four components using Principle Component Analysis (PCA) ordination method using the Varimax rotation with Kaiser normalization.

The above Physico-chemical water characteristics were hence used for correlation analyses using the statistical tools of Statistical Package for Social Scientists (SPSS), version 15.0 (SPSS Inc, 2006). Significant correlation was determined by a *p* value greater than 0.05 at 95% significant level i.e. the H_0 was rejected if $p \leq 0.05$.

3.9.4 Selection of Benthic Insects for use in Monitoring Water Quality in Selected Rivers of Lusaka District

Individual benthic insect macroinvertebrate families showing significant positive correlations with high levels of pollution as exhibited by the five variables mentioned above in the rivers were selected for use as bio-indicators of poor water quality in selected rivers of Lusaka district. Those showing significant negative correlations were selected as bio-indicators of good water quality. Significant correlation was determined by a *p* value greater than 0.05 at 95% significant level i.e. the H_0 was rejected if $p \leq 0.05$.

3.9.5 Testing the Effectiveness of Benthic Insects as Indicators of Pollution

To test the effectiveness of the benthic insect macro-invertebrates as indicators of pollution, laboratory bioassays or toxicity tests were conducted.

Before carrying out the test, heirarchical cluster analysis was performed on the physico-chemical water characteristics of each river site to produce a dendrogram or tree diagram using the SPSS. Cluster analysis forms a natural grouping of data based on similarity amongst separate samples (e.g. replicates and sites). A dendrogram is a graph ploted with the y-axis representing the full set of samples and the x-axis defining the level of similarity at which samples are considered to have fused (Southwood and Henderson, 2006). Basically, river sites within a group are more similar than rivers sites from a different group.

To carry out the bioassay, the following procedure was followed:

A total of 191 aquatic macro-invertebrates were collected from the Chongwe River Site III. This is because this site represented the highest diversity of aquatic macro-invertebrates, comprising of groups known to be highly tolerant to pollution, moderately tolerant to pollution and very low tolerance to pollution. The collected aquatic macro-invertebrates were then transported to the University of Zambia, Department of Biological Sciences laboratory in a large open container (10L) and upon arrival, selected macro-invertebrate families were distributed in six different open containers containing water collected from the above named river sites, each with different levels of pollution.

Aeration (sometimes refered to as oxygenation), is the process of putting a gas into a liquid. This is usually done by means of an electrically powered device called an aerator. This process is important to increases the amount of dissolved gases, especially oxygen, in a liquid, in this case river water. For this study, due to a limited number of aerators (only one was available), each container was aerated for only 10 minutes. Temperature was kept constant at room temperature to reduce any variation due to temperature.

The number of benthic insect macroinvertebrate families and actual numbers of individuals in each of these families was noted at the beginning of the experiment, after a period four hours and later after a 24 hr cycle. Levels of mortality of the benthic insect macro-invertebrates, expressed through immobility and in some cases floating, were noted and correlated to the levels of pollution of the six variables mentioned in section 3.8 on page 38.

CHAPTER 4: RESULTS

4.1 Physico-Chemical Water Quality Assessment

The four physico-chemical water characteristics that were highly correlated with the physico-chemical parameters of water, using Principle Component Analysis (PCA) ordination method in this study are presented in Table 4.1 and Figure 4.1 below.

Table 4.1: Rotated Component Matrix table showing four components and the most highly correlated variables of the physico-chemical proportion of water assessed.

Water Variable	**Component**			
	1	**2**	**3**	**4**
Temp	-0.222	0.674	-0.510	-0.065
Ph	-0.183	-0.842	-0.332	-0.103
Turbidity	**0.897**	-0.189	0.141	0.229
Conductivity	0.721	0.283	0.573	0.164
Total Dissolved Solids	0.719	0.282	0.576	0.172
Total Suspended Solids	**0.930**	-0.146	0.102	0.166
Dissolved Oxygen	-0.337	-0.585	-0.277	0.341
Ammonia	**0.970**	0.113	0.149	0.040
Sulphates	0.193	0.161	**0.847**	0.291
Nitrates	0.570	-0.391	0.464	-0.338
Total Phosphates	-0.181	**0.803**	0.010	-0.037
Zinc	0.263	0.044	0.061	0.701
Copper	-0.131	-0.238	0.162	**0.762**
Iron	0.482	0.124	0.145	**0.744**

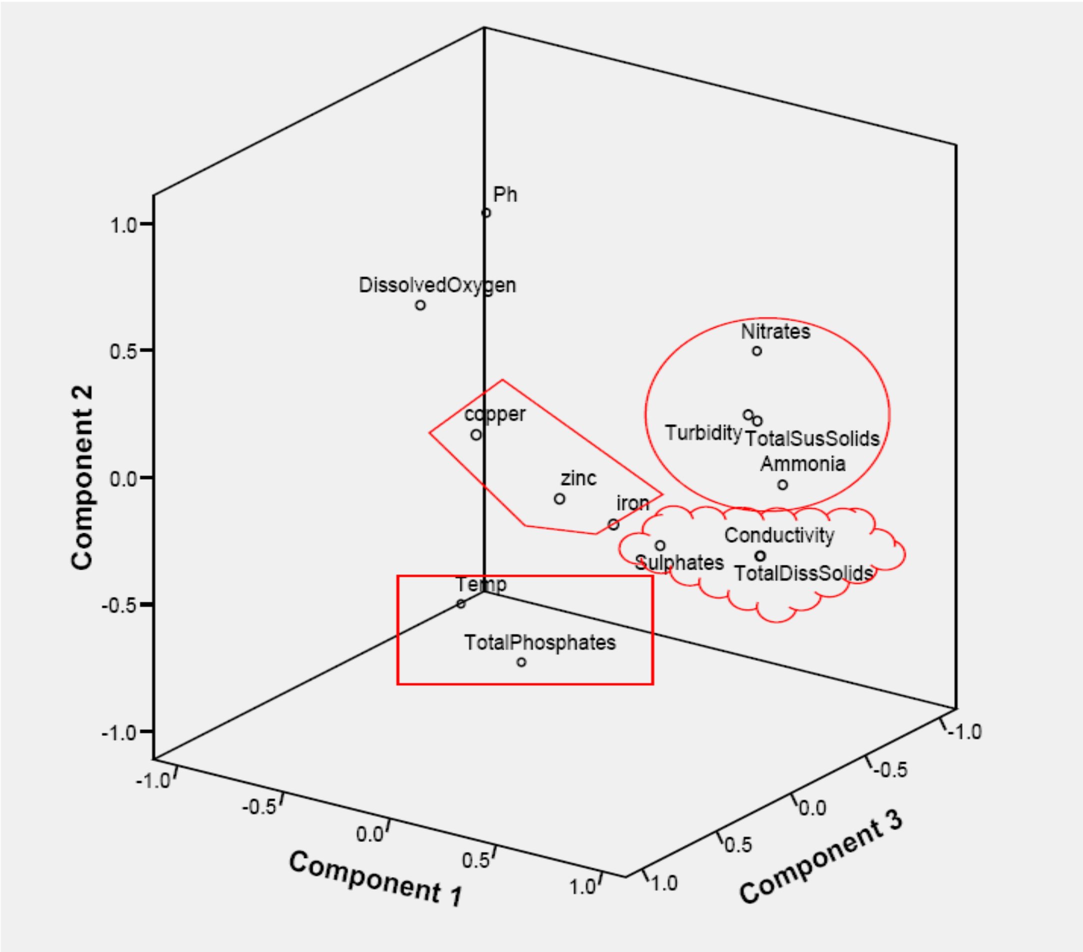

Figure 4.1: Factor loading plots of the physico-chemical characteristics of the rivers of Lusaka showing four components to which the physico-chemical parameters were most correlated.

These were:

Component 1: Total Suspended Solids, Ammonia, and Turbidity

Component 2: Total Phosphates.

Component 3: Sulphates.

Component 4: Iron

The seven physico-chemical water characteristics were hence used for further analysis. Table 4.2 summarises the physico-chemical characteristics of the rivers of Lusaka District sampled in the study. Specific site to site variations of physico-chemical water characteristics are presented in Appendix B.

Table 4.2: Summary of Physico-chemical water parameters of Lusaka rivers. The figures indicate the mean ± SE

	Physico-chemical water parameters	**Chamba River**	**Chunga and Namando Rivers**	**Ngwerere River**	**Chongwe and Musangashi Rivers**
1.	Turbidity (NTU)	6.63 ± 1.12	32.65 ± 3.40	10.86 ± 2.12	8.20 ± 1.92
2.	Total Suspended Solids (mg/l)	2.4 ±0.67	17.93 ± 2.5	4.66 ± 1.31	5.52 ± 1.46
3.	Ammonium (as NH_4-N mg/l)	0.01 ± 0	8.26 ± 1.84	1.09 ± 0.5	1.02 ± 0.09
4.	Total Phosphates (mg/l)	0.01 ± 0	29.03 ± 3.5	2.92 ± 1.07	1.21 ± 0.54
5.	Sulphates (mg/l)	39.01 ± 3.38	72.56 ± 1.69	57.67 ± 1.90	2.72 ± 0.63
6.	Iron (mg/l)	0.73 ± 0.31	1.43 ± 0.41	1.26± 0.38	1.21 ± 0.24

i. Turbidity and Total Suspended Solids

Chunga and Namando River Sites had the highest turbidity followed by the Chongwe and Musangashi River sites. The lowest turbidity values were obtained at the Ngwerere River, particularly Site III.

The highest turbidity and TSS values were at Site II of the Chunga River followed by Site 1 of the Ngwerere River (please see Appendix B). This may be attributed to the large amounts of sewage discharge of the Chunga River received from the Chunga wastewater treatment plant and from storm water runoff from the Lusaka city business area running into the Ngwerere River (Bäumle *et al*., 2012).

The lowest turbidity values were at Site III of the Ngwerere River and the Chongwe River Sites (please see Appendix B). At these points, both rivers were subjected to more non-point source pollution due to the large proportion of cultivated and irrigated land through which these rivers passed (Bäumle *et al*., 2012).

ii. Ammonium (as NH_4-N)

Chamba River Sites had the lowest levels of ammonium (<0.01 mg/l) followed by the Chongwe and Musangashi Rivers. Chunga and Namando River had the highest ammonium values, particularly Site II. This is followed by the Ngwerere River, high values particularly coming from Site III.

iii. Total Phosphates

The Chamba River Sites had the least values (< 0.01mg/l) of total phosphate. Other low values were found at the Chongwe and Musangashi River Sites. Ngwerere River had the highest value of phosphates with site II standing out.

iv. Sulphates

The highest value of sulphates was found at the Chunga and Namando River sites followed by the Ngwerere River. The Chongwe and Musangashi River Sites had the lowest sulphate values.

v. **Iron**

The highest values of the iron were obtained at the Chunga and Namando River Sites followed closely by the Ngwerere River particularly from site I. Chamba River recorded the lowest values of iron content.

4.1.2 Variation in Water Characteristics

Analysis of variance (ANOVA) of the data (Table 4.3) shows that there were **no significant differences in pollution levels** due to human activities among the rivers in pristine habitats outside Lusaka district boundaries and those found in disturbed and/or degraded habitats within the district i.e. the six different rivers showed similar levels of pollution.

Table 4.3: Analysis of variance for physico-chemical data from different rivers of Lusaka district.

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	7383.7	12	615.3	1.4	0.2**NS**	1.9
Within Groups	29261.6	65	450.2			
Total	36645.3	77				

NS= Not significant at 5% level.

4.2 Macroinvertebrate Families of Rivers in the Study Area

A total of 3836 individuals from 40 different macroinvertebrate families were collected. Chongwe River Site II had the highest number of families, followed by

Musangashi River II. The lowest number of families was found at Ngwerere River Site I and Chamba River Site III.

The macro-invertebrate families were distributed as follows in terms of numbers:

1. Order Diptera (true flies): **9**
2. Ephemeroptera (Mayflies): **8**
3. Trichoptera (Caddisflies): **4**
4. Hemiptera (Bugs): **5**
5. Coleoptera (Beetles): **4**
6. Lepidoptera (Butterflies): **1**
7. Gastropoda (Snails): **5**
8. Other Non-insect families: **4**

4.2.1 Chamba River

4.2.1.1 Site I of the Chamba River

A total of 13 macroinvertebrates families and five non-insect families were recorded from this site shown in Table 4.4. The Coenagrionidae (Order: Odonata, Suborder: Zygoptera) and Chironomidae (Order: Diptera; Suborder: Nematocera) dominated the site with relative abundances of 34.5% and 25.4% respectively.

Table 4.4: Macroinvertebrate orders and families, and their relative abundances present at Site I of the Chamba River

Order/Class	**Family**	**Number of individuals**	**Relative abundance (%)**
Ephemeroptera	Caenidae	1	0.24%
Odonata	Gomphidae	5	1.22%
	Coenagrionidae	141	**34.47%**
Diptera	Chironomidae	104	**25.43%**
	Simulidae	44	10.76%
	Culicidae	7	1.71%
Hemiptera	Gerridae	2	0.49%
Lepidoptera	Pyralidae	2	0.49%
Class Gastropoda	Ancylidae	6	1.47%
	Thiaridae	55	13.45%
	Bulininae	36	8.80%
	Lymnaedae	5	1.22%
Class Hirudinae	Unknown	1	0.24%

4.2.1.2 Site II of the Chamba River

A total of five insect families and four non-insect families were found at this site, as shown in Table 4.5. The Coenagrionidae (Order: Odonata, Suborder: Zygoptera) and Chironomidae (Order: Diptera; Suborder: Nematocera) dominated this Chamba river site with relative abundances of 54.3% and 24.7% respectively.

Table 4.5: Macroinvertebrate orders and families and their relative abundances at Site II the Chamba River.

Order/Class	**Family**	**Number of individuals**	**Relative abundance (%)**
Odonata	Coenagrionidae	57	**54.29%**
Diptera	Culicidae	1	0.95%
	Chironomidae	26	**24.76%**
Class Oligochaeta	Unknown	8	7.62%
Class Hirunidae	Unknown	2	1.90%
Hemiptera	Veliidae	1	0.95%
	Nepidae	2	1.90%
Decapoda	Potamonautidae	4	3.81%
Class Gastropoda	Ancylidae	1	0.95%
	Thiaridae	3	2.86%
		Total= 105	

4.2.1.3 Site III of the Chamba River

This site recorded among one of the lowest numbers of macroinvertebrate families (Table 4.6). The Chironomidae (Diptera; Nematocera) dominated this site with 96% relative abundance.

Table 4.6: Macroinvertebrate orders and families, and their relative abundances present at Site III of the Chamba River.

Order	Family	Number of individuals	Relative abundance (%)
Diptera	Chironomidae	120	96
Class Oligochaeta (Phylum Annelida)	Tubificidae	3	2.4
	Hirudinea (leaches)	5	0.04
	Total	**125**	

4.2.2 Chunga River and its tributaries

4.2.2.1 Site I of the Chunga River

A total of 10 macroinvertebrate families and one non-insect family (Physidae) were recorded from this site (Table 4.7). The most dominant family were the Chironomidae (Diptera) with a relative abundance of approximately 90%. However, the site also recorded an insect family with a low tolerance to pollution, the Blepharoceidae (Diptera) (Gerber and Gabriel, 2002).

Table 4.7: Macroinvertebrate orders and families, and their relative abundances at Site I of the Chunga River.

Order	**Family**	**Number of individuals**	**Relative abundance(%)**
	"unknown"	5	1.16
Hemiptera	Nepidae	2	0.46
Odonata	Libellulidae	1	0.23
Class Gastropoda	Physidae	15	3.47
Coleoptera	Dytiscidae	3	0.69
Diptera	Muscidae	3	0.69
	Blepharoceridae	1	0.23
	Chironomidae	*388*	**89.81**
	Simulidae	13	3.01
Ephemeroptera	Baetidae	1	0.23
	Total	**432**	

4.2.2.2 Site II of the Chunga River

Table 4.8 shows that a total of eight macroinvertebrate families were recorded from this site. Dipterans dominated the site, with Culicids (mosquitoes) and Psychodidae (sewage flies/moths) the most dominant, together accounting for 90% of individuals found. The site also recorded a rare and moderately tolerant family to pollution taxa, the Hydrosychidae (Order: Trichoptera) (Gerber and Gabriel, 2002).

Table 4.8: Macroinvertebrate orders and families, and their relative abundances at Site II of the Chunga River.

Order	**Family**	**Number of individuals**	**Relative abundance (%)**
Diptera	Syrphidae	7	4.05
	Psychodidae	6	3.47
	Simulidae	1	0.58
	Psychodidae pupa	12	6.94
	Culicidae	141	**81.50**
	Chironomidae	1	0.58
Trichoptera	Hydrosychidae	1	0.58
Class Gastropoda	Planorbidae	1	0.58
	Physidae	3	1.73
	Total	**173**	

4.2.2.3 Namando River

A total of 17 macroinvertebrate families and one non-insect family were recorded at this site (Table 4.9). Chironomids dominated the site, accounting for 65.33% of the total number of individuals.

Table 4.9: Macroinvertebrate orders and families, and their relative abundances present at the sampling site on Namando River.

Order	Family	Number of individuals	Relative abundance (%)
Diptera	Simulidae	12	3.02%
	Muscidae	10	2.51%
	Psychodidae	2	0.50%
	Chironomidae	260	**65.33%**
	Culicidae	13	3.27%
Odonata	Libellulidae	11	2.76%
	Gomphidae	25	6.28%
	Coenagrionidae	5	1.26%
	Chlorocyphidae	5	1.26%
Ephemeroptera	Caenidae	2	0.50%
	Telogonadidae	1	0.25%
	Lestidae	3	0.75%
Trichoptera	Hydrosychidae	30	7.54%
Hemiptera	Gerridae	11	2.76%
	Veliidae	2	0.50%
Coleoptera	Dytiscidae	1	0.25%
Decapoda	Potamonautidae	2	1.26%
	Total	**398**	

4.2.3 Ngwerere River

4.2.3.1 Site I of the Ngwerere River

No insect families were recorded from this site. Oligocheates (Annelida) and the Gastopod family Thiaridae occured in the waters but in very small numbers; two and three individuals respectively. As such no collections were made at this site.

4.2.3.2 Site II of the Ngwerere River

A total of five macroinvertebrate families were recorded at this site (Table 4.10). Dipterans dominated the site, with Chironomids accounting for 98% of the total number of individuals collected.

Table 4.10: Macroinvertebrate orders and families and their relative abundances at Site II of the Ngwerere River.

Order/Phylum	Family/Class	Number of individuals	Relative abundance (%)
Diptera	Chironomidae	1269	**98.37%**
	Syrphidae	1	0.08%
	Culicidae	5	0.39%
Coleoptera	Dytiscidae	3	0.23%
Phylum Annelida	Class Hirudinae	12	0.93%
	Total	**1290**	

4.2.3.3 Site III of the Ngwerere River

Table 4.11 shows that a total of 12 macroinvertebrate families were recorded from this site. Baetidaes (Ephemeroptera) dominated the site perhaps due to the high presence of the macrophytes *Eichornia* and *Phragmites*. Chironomids accounted for 33% of the total number of individuals whilst another dominating class is the Hirudinae, which accounted for 12.6%.

Table 4.11: Macroinvertebrate orders and families and their relative abundances present at the Site III of the Ngwerere River.

Order/Phylum/ Class	Family/Class	Number of individuals	Relative abundance (%)
Ephemeroptera	Baetidae	160	**45.85%**
Hemiptera	Corixidae	3	0.86%
	Belostomatidae	3	0.86%
	Veliidae	3	0.86%
Diptera	Culicidae	10	2.87%
	Chironomidae	118	**33.81%**
Phylum Annelida	Class Hirudinae	44	12.61%
Odonata	Aeshnidae	3	0.86%
Coleoptera	Gyrinidae	1	0.29%
	Limnichidae	2	0.57%
	Dytiscidae	1	0.29%
Class Gastropoda	Hydrobiidae	1	0.29%
	Total	**349**	

4.2.4 Chongwe River

4.2.4.1 Site I of the Chongwe River

A total of 13 macroinvertebrate families and two non-insect families were found at this site (Table 4.12). Although Chironomids dominated the site, it worth noting the presence of Atyidae (Order Decapoda; Common name: Shrimps), Calamoceratidae (Trichoptera) and Leptophlebidae (Ephemeroptera) which are good indicators of well oxgenated waters and are particularly sensitive to tempertature flactuations and pollution (Gerber and Gabriel, 2002).

Table 4.12: Macroinvertebrate orders and families and their relative abundances present at Site I on Chongwe River.

Order	**Family**	**Number of individuals**	**Relative abundance (%)**
Odonata	Libellulidae	2	4.55%
Decapoda	Atyidae	3	6.82%
Class Gastropoda	Thiaridae	4	9.09%
	Lymnaedae	1	2.27%
Trichoptera	Calamoceratidae	1	2.27%
Ephemeroptera	Caenidae	2	4.55%
	Leptophlebidae	1	2.27%
	Baetidae	1	2.27%
Diptera	Chironomidae	23	**52.27%**
	Blepharoceridae	1	2.27%
	Ceratopogonidae	1	2.27%
Coleoptera	Dytiscidae	3	6.82%
Hemiptera	Veliidae	1	2.27%
	Total	**44**	

4.2.4.2 Site II of the Chongwe River

A total of 12 macroinvertebrate families and one non-insect family were recorded from this site (Table 4.13). The Order Diptera was the most abundant, typically dominated by the Chironomids (~38%), followed by Anthericidae (at 18% proportion). In addition to the Anthericidae, it was worth noting the presence of Elmidae (Order Coleoptera) and the Hydrosychidae (Trichoptera) which are considered to have a very low tolerance to pollution (Gerber and Gabriel, 2002; Stals and de Moor, 2007).

Table 4.13: Macroinvertebrate orders and families and their relative abundances at Site II of the Chongwe River.

Order/Phylum	**Family/Class**	**Number of individuals**	**Relative abundance (%)**
Coleoptera	Elmidae	14	13.73%
Trichoptera	Hydrosychidae	8	7.84%
Ephemeroptera	Caenidae	13	12.75%
	Baetidae	1	0.98%
Odonata	Coenagriniodae	2	1.96%
	Gomphidae	1	0.98%
Hemiptera	Veliidae	1	0.98%
Phylum Annelida	Class Hirudinea	1	0.98%
Diptera	Anthericidae	*19*	**18.63%**
	Ceratopogonidae	2	1.96%
	Culicidae	1	0.98%
	Chironomidae	39	**38.24%**
	Total	**102**	

4.2.4.3 Site III of the Chongwe

A total of 22 macroinvertebrate families and two non-insect families, were recorded at this site, the highest recorded number (Table 4.14). Noteworthy at this site was the high proportion of the Elmidae (Coleoptera), Baetidae (Ephemeroptera), Anthericidae and Simulidae (Diptera), taxa documented to have a very low tolerance to pollution (Gerber and Gabriel, 2002; Stals and de Moor, 2007).

Table 4.14: Macroinvertebrate orders and families and their relative abundances at Site III on Chongwe River

Order	Family	Number of individuals	Relative abundance (%)
Odonata	Aeshnidae	2	1.94
	Gomphidae	1	0.97
	Libellulidae	11	10.68
Coleoptera	Dytiscidae	9	8.74
	Gyrinidae	12	11.65
	Elmidae	40	**38.83**
Ephemeroptera	Leptophlebidae	3	2.91
	Oligonueridae	4	3.88
	Trichorithidae	4	3.88
	Caenidae	8	7.77
	Baetidae	22	**21.36**
Class Gastopoda	Thiaridae	2	1.94
Hemiptera	Belostomatidae	4	3.88
	Veliidae	5	4.85
Phylum Mollusca, Class Bivalvia	Spheridae	14	13.59
Trichoptera	Philapotamidae	2	1.94
	Hydrosychidae	12	11.65
	Leptoceridae	1	0.97
Diptera	Culicidae	7	6.80
	Simulidae	19	18.45
	Chironomidae	1	0.97
	Anthericidae	16	15.53
	Total	**199**	

4.2.5 Musangashi River

A total of 19 macroinvertebrate families and three non-insect families were recorded from the sampling site on this river (Table 4.15). Noteworthy at this site was the presence of Polymitarcidae (Ephemeroptera), Pyralidae (Lepidoptera) and Lestidae (Ephemeroptera), taxa considered to have a very low tolerance to pollution (Gerber and Gabriel, 2002; Stals and de Moor, 2007).

Table 4.15: Macroinvertebrate orders and families and their relative abundances at the Musangashi River sampling site.

Order/Class	Family	Number of individuals	Relative abundance (%)
Odonata	Lestidae	3	1.38%
	Coenagrionidae	2	0.92%
	Libellulidae	4	1.84%
Hemiptera	Belostomatidae	2	0.92%
	Nepidae		0.46%
	Veliidae	1	0.46%
Coleoptera	Elmidae	4	1.84%
	Dytiscidae	1	0.46%
Trichoptera	Leptoceridae	3	1.38%
Lepidoptera	Pyralidae	2	0.92%
Ephemeroptera	Polymitacidae	2	0.92%
	Caenidae	18	8.29%
	Heptagenidae	5	2.30%
	Baetidae	11	5.07%
Diptera	Chironomidae	110	*50.69%*
	Barborochithonidae	1	0.46%
Glass Gastropoda	Bulininae	21	9.68%
	Planobidae	22	10.14%
	Lymnaedae	9	4.15%
	Total	**210**	

4.3 DATA ANALYSIS

4.3.1 Benthic Insect Macroinvertebrate Family Richness

Table 4.16 summaries the family richness of macroinvertebrates found at each sampling site in each river of the study area.

Table 4.16: Summary of the family richness found at each sampling river per site.

River	Site	Family Richness	Dominant families
Chamba River	I	13	Coenagrioniodae and Chironomidae
	II	9	Chironomidae
	III	3	Chironomidae
Chunga River	I	10	Chironomidae
	II	8	Culicidae
Namando River	I	17	Chironomidae
Ngwerere River	I	2	none
	II	5	Chironomidae
	III	12	Chironomidae
Chongwe River	I	13	Chironomidae
	II	12	Anthericidae
	III	22	Elmidae
Musangashi river	I	19	Chironomidae

There was **no significant difference** ($p \geq 0.05$) in the family richness of the benthic insect/macroinvertebrates in the rivers of Lusaka i.e the six different rivers showed similar numbers of benthic insect macroinvetebrate families as shown in Table 4.17.

Table 4.17: Analysis of variance for macro-invertebrate families found in the different rivers of Lusaka district.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	250.308	5	50.062	2.218	0.164 **NS**	3.972
Within Groups	158	7	22.571			
Total	408.308	12				

NS= Not significant at 5% level

4.3.2 Benthic Insect Macroinvertebrate Diversity and Evenness

4.3.2.1 Benthic Insect Macroinvertebrate Diversity

Table 4.18 shows a summary of the family diversity and variations at each river site in this study.

Table 4.18: Summary of the Shannon-Weiner Index (H) of diversity and evenness for each river per site**.**

River	Site	Shannon-Weiner Index (H)
Chamba River	I	1.76
	II	1.383
	III	0.257
Chunga River	I	0.506
	II	0.788
Namando River	I	2.53
Ngwerere River	I	0.673
	II	0.101
	III	1.331
Chongwe River	I	1.806
	III	1.796
	III	3.896
Musangashi river	I	1.917

There was **no significant difference ($p \geq 0.05$)** in the family diversity of the benthic insect/macroinvertebrates found in the sampled rivers (Table 4.19) i.e the six different rivers showed similar family diversity of benthic insect and other macro-invetebrates.

Table 4.19: Analysis of variance (single factor ANOVA) for family diversity of the different rivers of Lusaka district.

Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	8.510	5	1.702	2.389	0.144**NS**	3.972
Within Groups	4.987	7	0.712			
Total	13.496	12				

NS= Not significant at 5% level.

Table 4.20: Classes of habitat structure of the different river sites as devised from the Shannon-Weiner Index.

River	Site	Shannon-Weiner Index	Habitat structure
Chamba River	I	1.76	Fair
	II	1.383	Fair
	III	0.257	Disturbed
Chunga River	I	0.506	Disturbed
	II	0.788	Disturbed
Namando River	I	2.53	Good
Ngwerere River	II	0.101	Disturbed
	III	1.331	Fair
Chongwe River	I	1.806	Good
	III	1.796	Good
	III	3.896	Natural
Musangashi river	I	1.917	Good

Using the Shannon-Weiner Index, four natural groupings of river sites, based on macro-invertebrate family diversity were observed in the study (Table 4.20). For example, the sites classified as 'Disturbed' were all highly polluted by organic matter, most notably sewage effluents. The sites classified 'Fair' were moderately modified and received a fair share of pollution. The Namando River site, Musangashi river and Chongwe River Sites were noted for the presence of benthic insect macroinvetebrates particularly intolerant to pollution.

4.3.2.2 Benthic Insect Macroinvertebrate Evenness

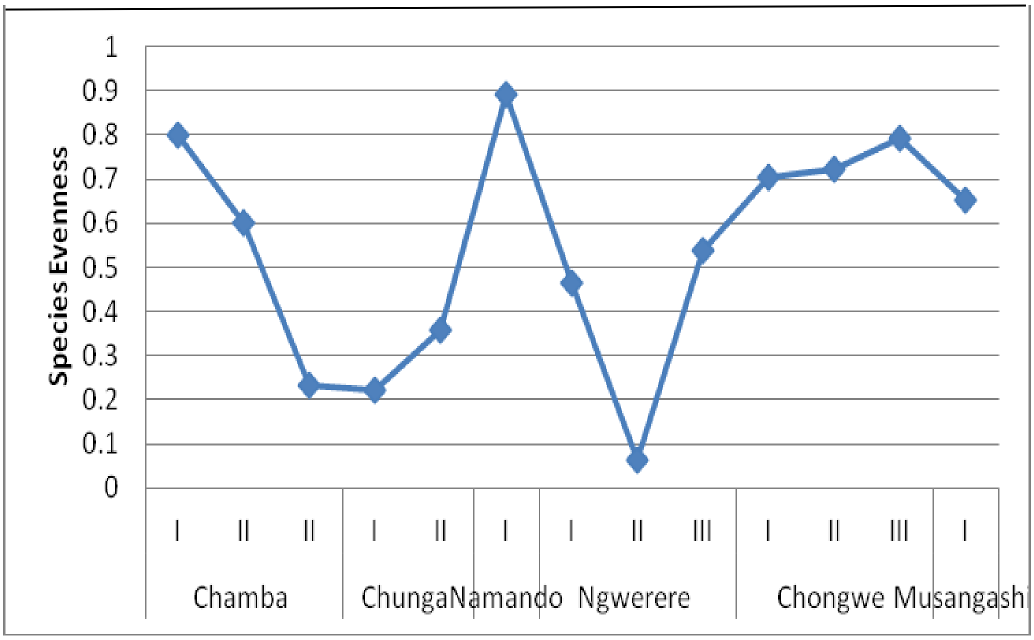

Figure 4.2: Species evenness variations between river sites.

A summary of species evenness variation is presented in Figure 4.2. There was no significant difference ($p \geq 0.05$) in the species evenness of the benthic insect/macroinvertebrates found in the sampled rivers (Table 4.21) i.e the six different rivers showed similar species evenness of benthic insect and other macroinvetebrates.

Table 4.21: Analysis of variance for species evenness of the different rivers of Lusaka district.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.486	5	0.097	2.197	0.167**NS**	3.972
Within Groups	0.310	7	0.044			
Total	0.796	12				

NS= Not significant at 5% level.

4.3.3 Assessment of the Relationship of Benthic Insect Macroinvertebrate Diversity, Richness and Abundance to Levels of Pollution in Selected Water Bodies

Table 4.22 below shows correlation coefficients of the physico-chemical parameters examined in this study with the number of families (family richness), total insect abundance and diversity at family level of the benthic insect macro-invertebrates of the study area.

Table 4.22: Spearman Rank (non-parametric) correlation coefficients of physico-chemical parameters with the total insect abundance, evenness and family richness and diversity.

	Turbidity	**Total Suspended Solids**	**Ammonia**	**Total Phosphates**	**Sulphates**	**Iron**
Family Diversity	-0.17**NS**	0.02 **NS**	-0.01 **NS**	0.1 **NS**	**-0.53 NS**	-0.42 **NS**
Family Richness	-0.25 **NS**	-0.07 **NS**	-0.03 **NS**	0.2 **NS**	-0.45 **NS**	-0.5 **NS**
Evenness	-0.1 **NS**	0.07 **NS**	-0.34 **NS**	-0.18 **NS**	-0.33 **NS**	-0.41 **NS**
Total Macro-invertebrate Abundance	0.04 **NS**	-0.08 **NS**	0.02 **NS**	0.45 **NS**	0.48 **NS**	**-0.54** **NS**

NS= not significant at 5% level

There were **no significant correlations** between benthic insect macroinvertebrate diversity, richness and abundance to any of the physico-chemical parameters used to assess levels of pollution in selected water bodies. That is to say no significant relationship existed between benthic insect macro-invertebrat diversity, richness and distribution with the levels of pollution in the selected rivers of Lusaka.

Worth noting, however, was the fairly strong negative correlation between total macro-invertebrate abundance and iron. This negative correlation signifies a fairly strong negative relationship between the amount of iron in a water body and the total macro-invertebrate abundance i.e the more iron, the less the numbers of total macro-invertebrates expected.

4.4.4 Selection of Benthic Insects for use in Monitoring Water Quality in Selected Freshwater Bodies of Lusaka District

Results of correlation analyses of the six physico-chemical parameters used in the study Total Suspended Solids, Ammonia, Turbidity, Total Phosphates, Sulphates and Iron were used to select bio-indicators of pollution in freshwater bodies of Lusaka district.

4.4.4.1 Order Odonata

Only the family Coenagrionoidae showed significant negative correlation to the pollutant ammonium and the heavy metal Iron (Table 4.23). This means that a strong negative relationship exists between the amount of Iron in a water body and the family Coenagrionoidae. This means that high levels of iron and/or ammonium in a water body will negatively affect the abundance of the family Coenagrioniodae resulting in a reduction in population density.

Table 4.23: Spearman Rank correlations coefficient for physico-chemical parameters with Odonata famly densities.

Family	Turbidity	Total Suspended Solids	Ammonia	Total Phospates	Sulphates	Iron
Libellulidae	-0.072 **NS**	0.017 **NS**	0.003 **NS**	0.251 **NS**	-0.430 **NS**	-0.376 **NS**
Lestidae	0.399 **NS**	0.496 **NS**	-0.378 **NS**	-0.116 **NS**	-0.171 **NS**	-0.428 **NS**
Aeshnidae	-0.402 **NS**	-0.370 **NS**	0.356 **NS**	0.390 **NS**	-0.262 **NS**	-0.249 **NS**
Gomphidae	-0.040 **NS**	0.037 **NS**	-0.312 **NS**	0.085 **NS**	0.040 **NS**	-0.401 **NS**
Chlorocyphidae	0.231 **NS**	0.252 **NS**	-0.354 **NS**	0.000 **NS**	0.077 **NS**	-0.464 **NS**
Coenagrionidae	0.198 **NS**	0.164 **NS**	**-0.648***	-0.426 **NS**	0.047 **NS**	**-0.553***

*Correlation is significant at the 0.05 level.

NS= Not significant at p=0.05.

Figure 4.3: Coenagrionidae (Photo credit: Steven Lowe, 2012)

Based on the correlation results, the Odonata family **Coenagrionidae** (Figure 4.3) can be used as a bio-indicator of water pollution in Lusaka district i.e the absence or a low abundance of this family can be used as an indicator of pollution whilst their presence in high abundance shows good water quality.

4.4.4.2 Order Ephemeroptera

Significant negative correlations existed between the family Baetidae and Turbidity, Caenids and Sulphates including Leptophlebidae and Sulphates, as shown in Table 4.24. The negative correlation implies that as the Turbidity and amount of sulphates in a water body increases, the densities of the Ephemeropteran families Baetidae, Caenidae and Leptophlebidae decrease.

A significant positive correlation was observed between the Baetidae and Total Phosphates. Also, worth noting was the fairly strong negative correlations shown between the Baetidae and Total Suspended Solids. This means that an increase in Total Phosphates and/or Total Suspended Solids will result in an increase in the Baetidae population densities

Table 4.24: Spearman Rank correlation coefficients for physico-chemical parameters with Ephemeropteran family density.

Family	Turbidity	Total Suspended Solids	Ammonia	Total Phosphates	Sulphates	Iron
Baetidae	**-0.609***	**-0.535 NS**	0.466 **NS**	**0.729***	-0.370 **NS**	0.048 **NS**
Leptophlebidae	-0.498 **NS**	-0.370 **NS**	0.232 **NS**	0.257 **NS**	**-0.620***	0.026 **NS**
Oligonueridae	-0.231 **NS**	-0.252 **NS**	0.157 **NS**	0.391 **NS**	-0.386 **NS**	-0.232 **NS**
Caenidae	-0.200 **NS**	0.052 **NS**	-0.121 **NS**	0.106 **NS**	**-0.589***	-0.081 **NS**
Trichorithidae	-0.231 **NS**	-0.252 **NS**	0.157 **NS**	0.391 **NS**	-0.386 **NS**	-0.232 **NS**
Telegonadidae	0.231 **NS**	0.252 **NS**	-0.354 **NS**	0.000 **NS**	0.077 **NS**	-0.464 **NS**
Heptagenidae	0.309 **NS**	0.419 **NS**	-0.157 **NS**	-0.156 **NS**	-0.309 **NS**	-0.116 **NS**
Polymitarcidae	0.309 **NS**	0.419 **NS**	-0.157 **NS**	-0.156 **NS**	-0.309 **NS**	-0.116 **NS**

*Correlation is significant at 0.05 level.

NS= Not significant at 0.05 level.

Based on the correlation results, the Ephemeropteran families Baetidae (Figure 4.4), Caenidae (Figure 4.5) and Leptophlebidae (Figure 4.6) can hence be used as bio-indicators of pollution in Lusaka district.

1. **Baetidae**

Figure 4.4: Baetidae in thier two most common morphs (Photo credit: Steven Lowe, 2012).

2. **Caenidae**

Figure 4.5: Caenidae emphasising the characteristic square gill covers (Photo credit: Steven Lowe, 2012).

3. **Leptophlebidae**

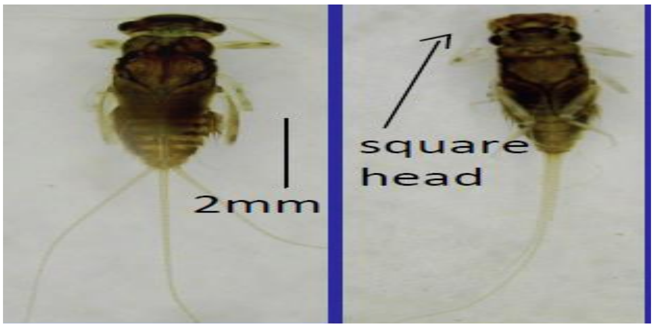

Figure 4.6: Leptophlebidae, showing the characteristic square head (Photo credit: Steven Lowe, 2012).

4.4.4.3 Order Trichoptera

Table 4.25: Spearman Rank correlation coefficients for physico-chemical parameters with Trichopteran family densities.

Family	Turbidity	Total Suspended Solids	Ammonia	Total Phosphates	Sulphate	Iron
Hydrosychidae	-0.18**NS**	-0.11 **NS**	-0.07 **NS**	0.37 **NS**	-0.31 **NS**	-0.23 **NS**
Philapotamidae	-0.23 **NS**	-0.25 **NS**	0.16 **NS**	0.39 **NS**	-0.39 **NS**	-0.23 **NS**
Leptoceridae	0.09 **NS**	0.16 **NS**	-0.02 **NS**	0.14 **NS**	-0.51 **NS**	-0.25 **NS**
Calamoceratidae	-0.46 **NS**	-0.25 **NS**	0.16 **NS**	-0.08 **NS**	-0.46 **NS**	0.31 **NS**

NS= Not significant at p=0.05.

No significant correlation existed between any Trichoptera family and a physico-chemical parameter (Table 4.25). As such, no trichopteran family was selected as a bio-indicator of water pollution in Lusaka district.

4.4.4.4 Order Diptera

Table 4.26: Spearman Rank correlation coefficients for physico-chemical parameters with Dipteran family densities.

Family	Turbidity	Total Suspended Solids	Ammonia	Total Phosphates	Sulphates	Iron
Syrphidae	0.26 **NS**	0.23 **NS**	0.37 **NS**	0.38 **NS**	0.35 **NS**	0.24 **NS**
Psychodidae	0.52 **NS**	**0.57***	0.13 **NS**	0.06 **NS**	0.30 **NS**	-0.13 **NS**
Anthericidae	-0.46 **NS**	-0.37 **NS**	0.23 **NS**	0.45 **NS**	-0.45 **NS**	0.21 **NS**
Simulidae	0.20 **NS**	0.13 **NS**	0.07 **NS**	0.19 **NS**	0.44 **NS**	-0.48 **NS**
Chironomidae	-0.15 **NS**	-0.23 **NS**	-0.17 **NS**	0.30 **NS**	0.25 **NS**	-0.30 **NS**
Blepharoceridae	-0.40 **NS**	-0.37 **NS**	0.41 **NS**	0.17 **NS**	-0.06 **NS**	0.23 **NS**
Muscidae	0.13 **NS**	0.03 **NS**	-0.01 **NS**	0.21 **NS**	0.32 **NS**	-0.37 **NS**
Ceratopogonidae	**-0.62***	-0.37 **NS**	0.23 **NS**	0.13 **NS**	-0.50 **NS**	**0.58***
Culicidae	0.12 **NS**	0.13 **NS**	0.15 **NS**	0.31 **NS**	0.27 **NS**	**-0.41 NS**

*Correlation is significant at the 0.05 level.

NS= Not significant at p=0.05.

Significant positive correlation existed between the family **Pyschodidae** and Total Suspended Solids (Table 4.26). The positive correlation implies that a strong positive relationship exists between Total Suspended Solids in a water body and the family Psychodidae. An increase Total Suspended Solids will result in an increase in the population densities of the Dipteran family whilst a decrease will result in the opposite.

A significant negative correlation existed between family **Ceratopogonidae** with Turbidity and Iron. This means that a strong negative relationship exists between the amount of Iron and Turbidity in a water body and the family Ceratopogonidae. An increase in these physico-chemical parameters will resut in a decrease in the population densities of the Dipteran families.

Based on the negative correlation results above, the Dipteran family **Ceratopogonidae** (Figure 4.7) can be used as a bio-indicators of water pollution in Lusaka district.

Figure 4.7: Ceratopogonidae (Photo credit: Steven Lowe, 2012)

Further, based on the positive correlation results above, the presence of the Dipteran family **Pyschodidae** (Figure 4.8) can be used as an indicator of pollution i.e the heavy abundance of this family can be used as an indicator of polluted waters.

Figure 4.8: Pyschodidae (Source: Gerber and Gabriel, 2002)

4.4.4.5 Order Hemiptera

Table 4.27: Spearman Rank correlation coefficients for physico-chemical parameters with Hemipteran family densities.

Family	Turbidity	Total Suspended Solids	Ammonia	Total Phosphates	Sulphates	Iron
Veliidae	-0.440**NS**	-0.329**NS**	0.040 **NS**	0.250 **NS**	**-0.675***	-0.404 **NS**
Belostomatidae	-0.197 **NS**	-0.117 **NS**	0.227 **NS**	0.298 **NS**	-0.442 **NS**	-0.301 **NS**
Corixidae	-0.342 **NS**	-0.372 **NS**	0.232 **NS**	0.462 **NS**	0.114 **NS**	-0.029 **NS**
Nepidae	0.192 **NS**	0.161 **NS**	0.143 **NS**	0.089 **NS**	0.017 **NS**	-0.092 **NS**
Gerridae	0.288 **NS**	0.313 **NS**	-0.521 **NS**	-0.239 **NS**	0.376 **NS**	**-0.577***

*Correlation is significant at the 0.05 level.

NS= Not significant at p=0.05.

Significant negative correlations were observed between the Family Veliidae, with Sulphates and between the family Gerridae and the heavy metal Iron (Table 4.27). This means that a strong negative relationship existed between the amounts of Sulphates and Iron in a water body with the families Veliidae and Gerridae respectively. An increase in these physico-chemical parameters will resut in a decrease in the densities of the Hemipteran families.

Based on the correlation results above, the Hemipteran families Veliidae (Figure 4.9) and Gerridae (Figure 4.10) can hence be used as bio-indicators of water pollution in Lusaka district.

1. **Veliidae**

Figure 4.9: Veliidae in their two most common morphs (Photo credit: Steven Lowe, 2012).

2. **Gerridae**

Figure 4.10: Gerridae in their two common morphs (Photo credit: Steven Lowe, 2012).

4.4.4.6 Family Coleoptera

Table 4.28: Spearman Rank correlation coefficients for physico-chemical parameters with Coleopteran family densities.

Family	Turbidity	Total Suspeded Solids	Ammonia	Total Phosphates	Sulphates
Dytiscidae	**-0.603***	-0.510**NS**	0.330 **NS**	**-0.718***	-0.446 **NS**
Unknown	-0.077 **NS**	-0.252 **NS**	0.393 **NS**	0.313 **NS**	0.386 **NS**
Gyrinidae	-0.231 **NS**	-0.252 **NS**	0.157 **NS**	0.391 **NS**	-0.386 **NS**
Hydrobiidae	-0.306 **NS**	-0.243 **NS**	0.310 **NS**	0.133 **NS**	0.044 **NS**
Elmidae	0.026 **NS**	0.085 **NS**	0.018 **NS**	0.204 **NS**	-0.515 **NS**

*Correlation is significant at the 0.05 level.

NS= Not significant at p=0.05

Only one family showed significant negative correlations with physico-chemical parameters (Table 4.28). This is Dysticidae, showing significant negative correlation with Turbidity and Total Phosphates. This means that a strong negative relationship exists between the amounts of Turbidity and Total Phosphates in a water body and the family Dytiscidae. An increase in this physico-chemical parameter will resut in a decrease in the densities of the Dytiscidae.

Based on the negative correlation anayses above, the Coleopteran family **Dytiscidae** (Figure 4.11) can hence be used as a bio-indicator of water pollution in Lusaka district.

Figure 4.11: Dystiscidae adult and larvae. Sizes vary greatly between species (Photo credit: Steven Lowe, 2012).

4.4.5 Testing the Effectiveness of Benthic Insects as Indicators of Pollution

Figure 4.12 below is a dendrogram constructed following hierarchical cluster analysis of physico-chemical characteristics of each river site in the study area.

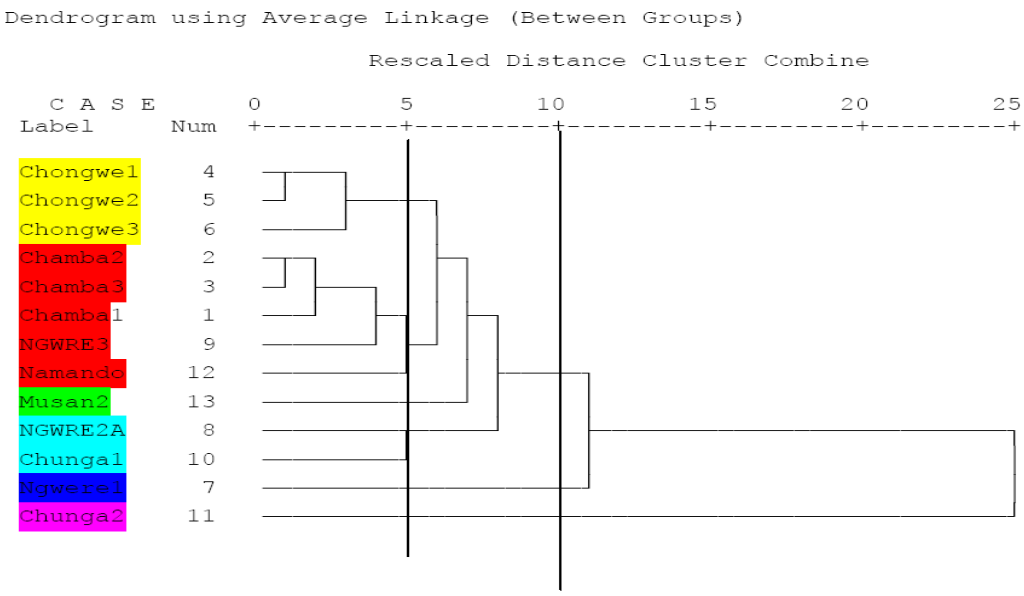

Figure 4.12: Dendogram for hierarchical cluster analysis of the physico-chemical characteristics of the rivers sites.

At 95 per cent similarity, six different clusters or groups, representing different levels of pollution, were identified. The clusters comprised the following river sites;

Cluster 1: Chongwe River Sites I, II, III

Cluster 2: Chamba River Sites I, II, III; Namando River Site, and Ngwerere River Site III

Cluster 3: Musangashi River Site

Cluster 4: Ngwerere River II, Chunga River I

Cluster 5: Ngwerere River I

Cluster 6: Chunga River II

From the above clusters, waters from the following six representative river sites were selected to be used as sample tests for the bioassay;

Cluster 1: Chongwe River Site III

Cluster 2: Chamba River I, Ngwerere River Site III

Cluster 3: Musangashi River Site

Cluster 4: Ngwerere River II

Cluster 5: Ngwerere River I

However, due to the pungent and potentially toxic fumes associated with the high volumes of chemicals used at the Lusaka Water Sewerage Company (LWSC), Chunga treatment plant, a bioassay was not conducted on Cluster 6 i.e Chunga River Site II.

i. **Cluster 1: Site III of the Chongwe River**

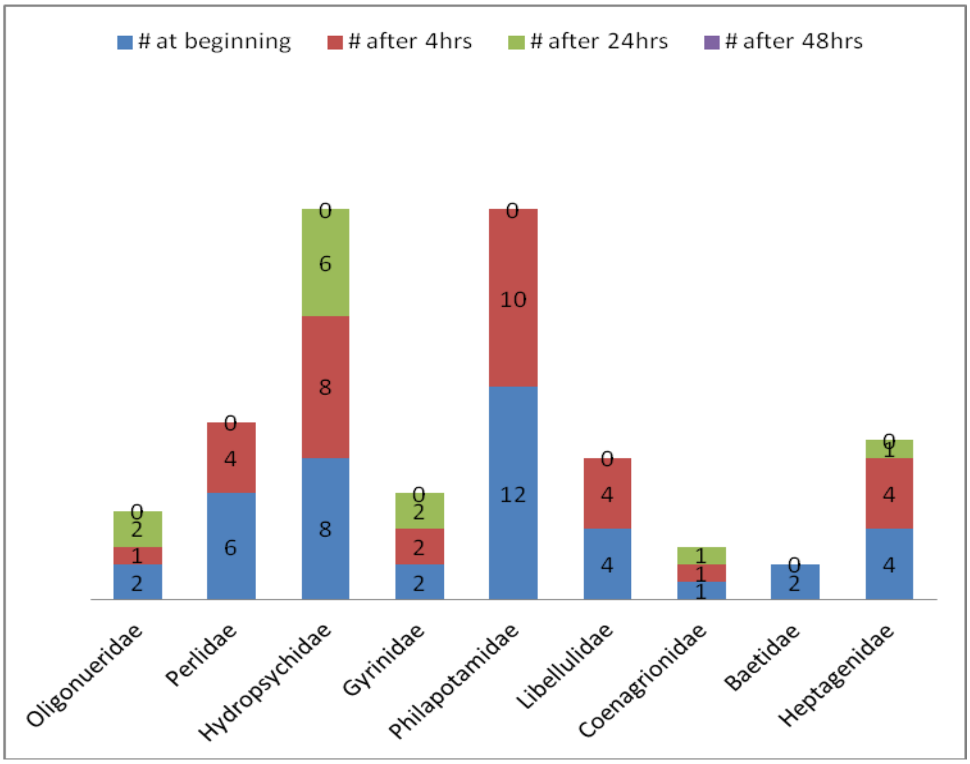

Figure 4.13: Results of Bioassay on Cluster 1, using Site III of the Chongwe River.

Figure 4.13 shows the numbers of representative live members of families in Site III of the Chongwe in four stages of the bioassay; at the beginning of the experiment, after 4hrs, 24hrs and 48hrs. Mortality was noted to determine tolerance of each insect macro-invertebrate family.

The Ephemeropteran families (Oligonueridae, Baetidae, Heptagenidae), Tricopteran families (Hydropsychidae and Philapotamidae) and Coleopteran families (Gyrinidae) generally showed moderate tolerance to the low pollution levels exhibited by these waters. For example, Heptagenidae, Oligonueridae, Hydropsychidae and Gyrinidae only died after a 24 hour period. However, the more pollution sensitive Baetidae, Philapotamidae, Libellulidae (Order Odonata) and Perlidae (Order Plecoptera) did not survive more than four hours.

ii. Cluster 2: Site III of the Ngwerere river and Site I of the Chamba River

Table 4.29: Results of bioassay on Cluster 2, using Site III of the Ngwerere River showing number of organisms at the beginning of the experiment, after a four, 24, 48 and 72 hour period.

Families present	# at beginnin g	# after 4hrs	# after 24hrs	# after 48hrs	# after 72hrs
Trichorithidae	2	2	1	0	
Oligonueridae	2	1	0		
Hydropsychidae	10	6	3	0	
Perlidae	2	2	0		
Tabanidae	1	1	1	1	0
Baetidae	4	4	0	0	0
Philapotamidae	4	4	1	0	
Pyralidae	1	0			
Leptophlebidae	4	4	4	3	0

Eight representative families were present in the Ngwerere river waters (Table 4.29). Ephemeropteran families (Trichorithidae, Oligonueridae and Baetidae) exhibited a low tolerance to pollution levels found in the Ngwerere River Site III. For example, Baetidae only lasted not more than fours hours. The Leptophlebidae and Tabanidae (Diptera), however, which survived for more than 24hours, showed a high tolerance.

Other families showing low tolerance were the Pyralidae (Order Lepidoptera) and the Perlidae which could not survive up to the four hour and 24 hour mark respectively.

Table 4.30: Results of Bioassay on Cluster 2, using Site I of the Chamba River showing number of organisms at the beginning of the experiment, after a four, 24hour period.

River and Site	Families present	# at beginning	# after 4hrs	# after 24hrs
Chamba River Site 1	Perlidae	4	2	0
	Hydropsychidae	7	3	0
	Oligonueridae	2	0	
	Libellulidae	1	1	0
	Caenidae	2	2	2
	Baetidae	2	2	0

The results of the bioassay on Site III of the Ngwerere River are very similar with those obtained from the Chamba River Site I (Table 4.30). For example, the Baetidae and Perlidae which lasted more than four hours but not more than 24 hours as was in the case of the Ngwerere River Site III.

iii. Cluster 3: Musangashi River Site

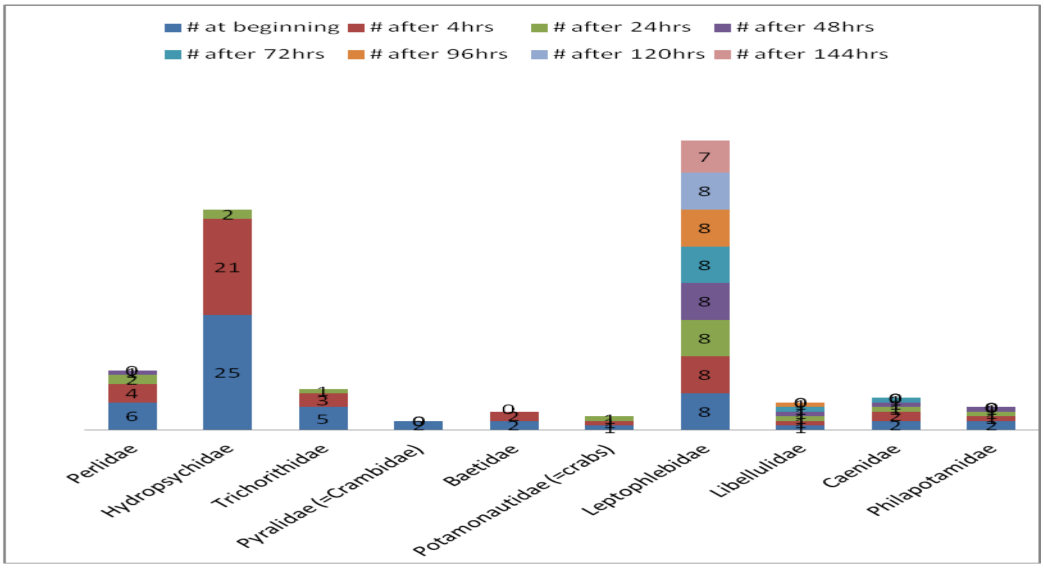

Figure 4.14: Results of bioassay on Cluster 3, using the Musangashi River site.

Eight representative families were present in the Musangashi river waters (Figure 4.14). Most organisms survived for more than 24 hours, with the exception of the Pyralidae (Lepidoptera) and the Baetidae. The family Leptophlebidae showed the highest rate of survival, surviving up to 144 hours (12 days) with little mortality. Also noteworthy is the high rate of survival (compared to the previous clusters) of the Perlidae which survived up to 48 hours (2 days). The Caenids also showed a high survival rate, surviving up to 72 hours (3 days). Others, such as the Trichopteran families, Trichorithidae and Philapotamidae could only last up to 24 hours.

iv. Cluster 4: Site II of the Ngwerere River

Table 4.31: Results of Bioassay on Cluster 4, using Site III of the Ngwerere River showing number of organisms at the beginning of the experiment, after a four, 24, 48 and 72 hour period.

Families present	# at beginning	# after 4hrs	# after 24hrs	# after 48hrs	# after 72hrs	# after 96hrs
Hydropsychidae	12	9	5	0	0	
Oligonueridae	1	0	0	0		
Perlidae	4	0				
Baetidae	4	0				
Elmidae	2	0				
Libellulidae	1	1	1	1	1	0
Philapotamidae	4	2	0	0	0	0
Leptophlebidae	4	4	4	3	0	0
Caenidae	1	1	1	1	0	
Trichorithidae	1	1	1	1	0	

Ten representative families were present in the Ngwerere River Site III. The Ephemeropteran families (Oligonueridae and Baetidae), Perlidae and Elmidae (Coloeptera) showed little tolerance to these waters, dying off within just four hours (Table 4.31). However, the Trichorithidae, Caenidae, Leptophlebidae, Libellulidae, and Hydropsychidae generally showed moderate to high levels of tolerance, many surviving until a 48 hour period.

v. Cluster 5: Site I of the Ngwerere River

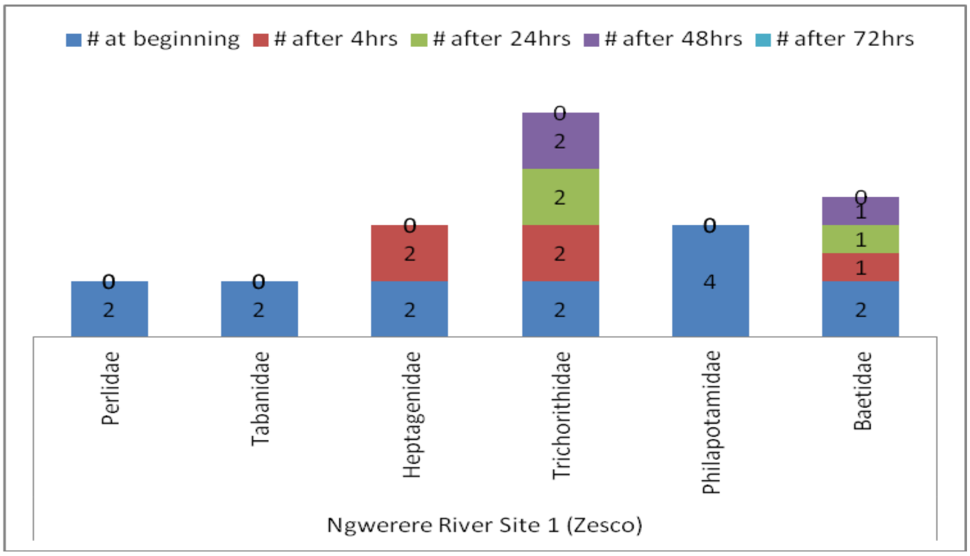

Figure 4.15: Results of bioassay on Cluster 5, using the Ngwerere River Site I.

Six representative families were present in the waters of the Ngwerere River Site I. The Perlidae, Tabanidae, Heptagenidae and Philapotamidae showed a low tolerance to the pollution levels found in these waters, not surviving up to the four hour mark (Figure 4.15). The Trichorithidae and Baetidae however, exhibited a moderate tolerance to pollution, surviving up to the 48 hours.

CHAPTER 5: DISCUSSION

5.1 Physico-Chemical Water Quality Assessments

5.1.1 Turbidity and Total Suspended Solids

Turbidity and Total Suspended Solids values were highest at Site II of the Chunga River followed by Site I of the Ngwerere River. This may be attributed to the large amounts of sewage discharge the Chunga River received from the Lusaka Water and Sewerage Company (LWSC) Chunga wastewater treatment plant. These results were in agreement with those of Krebs (2009) and Hynes (1960; cited from Singh, 2005) who showed that pollution with organic matter such as raw or untreated sewage, is strongly associated with an increase in the both Turbidity and Total Suspended Solids (TSS).

In addition to the raw sewage effluent that was received, the Ngwerere River was also subject to receiving storm water run-off from the Lusaka city business area (Bäumle *et al*., 2012). In a study of the urban storm water runoff, Shaver *et al.* (2007) consistently showed that storm water runoff can contain significant concentrations of harmful pollutants, mainly TSS, that can contribute to adverse water quality impacts in receiving streams and rivers.

The lowest turbidity values were at Site III of the Ngwerere River and the Chongwe River sites (Table 4.1 and Appendix B). At these points, both rivers were subjected to more non-point source pollution (as opposed point source pollution with organic matter) due to the large proportion of cultivated and irrigated land through which these rivers pass (Bäumle *et al*., 2012).

5.1.2 Ammonium (as NH_4-N)

The findings of this study showed that Site II of the Chunga River and Site III of the Ngwerere river had the highest levels of ammonium. Site II of the Chunga River was located just after the Chunga Sewage Treatment plant whilst Ngwerere River Site III was largely in a prime agricultural area. The sewage effluent discharges from the treatment plant and the probable high influx fertilizer and pesticide effluent at these sites respectively, are the likely reasons for the high levels of ammonium found at these sights.

The findings of this study are supported by the findings of those of the Zambia Environmental Outlook Report (Environmental Council of Zambia, 2008), which reported that the agricultural sector, through effluent from the large volumes of chemicals (fertilizers and pesticides) and animal manure used, contributes about 75% of ammonium (mainly as nitrates and sulphates) found in water bodies. Other major sources of ammonium (nitrates and sulphates) in surface waters are sewage, domestic and industrial waste effluent (Environmental Council of Zambia, 2008; Singh, 2005). The Ngwerere and Chunga rivers are examples of rivers receiving such effluents (Bäumle *et al.*, 2012).

The Chamba River had the lowest levels of ammonium despite sites I and III being subject to both domestic wastes and raw sewer effluent streaming in from the nearby Ng'ombe compound. This can be attributed to the dense presence of the macrophytes *Phragmites*, *Potamagedon*, *Cyperus* and *Typha* and the algae *Spirogyra*. According to Kennedy and Murphy (2012), the named macrophytes are associated with high to intermeditae nutrient levels i.e. eutrophic to mesotrophic waters. *Spirogyra* on the other hand is naturally associated with hyper-eutrophication (Kennedy and Murphy, 2012; Singh, 2005). The high uptake of the ammonium nitrate and sulphates by these plants may hence explain the low levels at these sites.

5.1.3 Total Phosphates

Ngwerere River Site III had the highest levels of total phosphates. This could be attributed to the untreated sewer effluent received by this river both from the LWSC Sewer ponds located in Garden Compound and from households located along the river which had septic pipes draining directly into the river.

The above explanation could also be used for the high total phosphates levels found at Chunga River Site I which equally had untreated sewer effluent streaming in from the Chunga sewage ponds.

The Chongwe River Site II recorded the second highest levels of total phosphates. This could be attributed to the possible large amounts of fertilizers used upstream of this point in the prime agriculture area of Chongwe town.

The Chamba river, specifically at site III was expected to show high levels of total phospahtes. This was because upstream to this site were many points used as domestic washing points. However, all three Chamba River sites had low levels of total phosphates. The possible explanation to this could be the same as that of ammonium content being low at this site; a high intake particulary by the algae *Spirogyra* which is associated with hyper-eutrophication.

5.1.4 Sulphates

Sulphates content showed great variation both between sites. It was expected that the Chunga River Site II, which was close to the sewage treatment plant, would exhibit the highest levels of sulphate content. Ngwerere River Site I, which collected waste waters emanating from the industries of Lusaka and hence was prone to chemical pollution, was equally expected to show high levels of sulphate pollution. However, it was Site I of the Chamba River followed by Site I of the Chunga River that had the two highest levels of sulphates. The Chunga River Site I was located near the LWSC Chunga sewage ponds, from which it receives a large amount of effluent, highlighting a possible source.

The Namando River Site equally had high levels of sulphate. This could possibly be due to the high levels of fertilizers (and pesticides) being used in the relatively active subsistence agricultural area (per. obs.)

5.1.5 Iron

The high values of iron content in the waters of Site II of the Chunga River could have been as a result of its extensive use in water treatment whilst the high values at Ngwerere River Site could be attributed to the waste water run-off collected from both ZESCO headquarters and the industrial area of Lusaka City.

It was hypothesized in this study that there would be no significant differences in pollution levels of the rivers within the city, as a result of human activities and those rivers outside the city, in more pristine habitats. Analysis of variance of the data (Table 4.3) proved this hypothesis true. According to Southwood and Henderson (2006) this could be attributed to the small sample size ($n<20$) used in the study. One limitation of this study was the small sample size used due to a reduction in the number of study sites. This was as a consequence of the unprecidented drying up of river sites. The Musangashi River for instance was reduced to only a single pool of water by the time this study was conducted whilst the Chongwe River was reduced to a number of small pools after Site III on the Great East Road. Another river that dried up by the time of study was the Chunga river, which beyond site II was a mere dry river bed. This was despite its being recorded as a perennial river with the large amounts of waste water received from the treatment plant contributing to this. However, daming and other forms of water extractions led to its drying up during the dry season (Bäumle *et al.*, 2012).

5.2 Benthic Insect Macroinvertebrate Richness, Abundance, Diversity and Evenness

5.2.1 Family Richness

Species richness refers to the number of species present in a community, measured as the number of species per unit area of ground area (Allaby, 2005). Site II of the Chongwe River had the highest family richness, with a total of 13 macroinvertebrate families. This could be attributed not only to good water quality but also to the extra habitat provided by the stones and vegetation in the water body. Stone and vegetation habitats generally support a higher diversity of benthic macro-invertebrates compared to sandy habitats (Dallas, 2007).

The lowest family richness was recorded at Chamba River Site III and the Ngwerere River Site I with only three and two families respectively. Both these sites had among the worst water quality as determined by physico-chemical parameters. The Ngwerere River for example, at this site received large volumes of industrial effluent and storm water pollution. There was also substantial amount of untreated sewage streaming in from sewer pipes draining directly from the nearby houses.

Equally, Chamba River Site III was highly subjected to surface water pollution and habitat degradation due to human activities such as washing clothes, river crossing points, water abstracting points and garbage damping sites among other things. The substratum at this point was mostly sandy and water flow was low. This could further explain the low family richness of insect macroinvertebrates (Dallas, 2007).

Various scholars have used family richness in diversity ordering i.e. comparing the diversity between communities. These have reported significant decreases in the species richness particularly in response to toxic exposure and organic pollution (Cao *et al.,* 1996; Payne *et al.*, 2005). This study reports similar findings. For example, Site III of the Chamba River, Site I and II of the Chunga River and Ngwerere River Site II were particularly subjected to gross organic pollution. This clearly was reflected in their low family richness. Sites with relatively less pollution such as Site III of the Chongwe and Musangashi River Site had high family richness values of 22 and 19 respectively.

However, as also demonstrated in this study, richness may not be a reliable source of diversity ordering. According to Krebs (1999) and Payne *et al.,* (2005) this is because it is sensitive to small sample sizes. This could perhaps explain the lack of significant difference in family richness among the river sites in this study.

5.2.2 Benthic Insect Macroinvertebrate Diversity

The highest diversity was at Site III of the Chongwe River followed by the Namando River Site. The Chongwe Sites I and II, Musangashi River Site and Chamba River Site I equally showed a relatively high Shanon-Weiner diversity index. Can this be attributed to relatively low levels of pollution? Possibly. The individual sites mentioned had comparably lesser pollution levels than the other sites. However, as mentioned earlier, although a major factor, pollution levels alone cannot be said to influence the benthic macroinvertebrate diversity. Water quantity, habitat and food availability affect the diversity of benthic insect macroinvertebrates (Dallas *et al.,* 2010; Davies and Day, 1998).

Notable among the mentioned sites were the Chongwe River Sites which had both plenty of stone habitat and good water quality. The Chongwe River Sites collectively had high Shanon-Weiner index values (Table 4.18).

Another site having a high Shanon-Weiner Index was the Namando River Site. The river was located in a former forest reserve and not until recently, was maintained in pristine conditions. Close to its confluence with the Chunga River, the Namando River however was subjected to non-point pollution due to agricultural activities taking place. The Namando River site had low pollution levels with exception of sulphate content. However, low water quantity and a closed canopy (Figure 3.8 on page 34) that reduced light penetration and hence reduced food availability may have resulted in reduced macroinvertebrate diversity (Davies and Day, 1998).

The Chamba River Sites particularly Site II, had a fairly high Shannon-Weiner index value (Table 4.18). This was despite the substratum being mostly sandy, which generally supports a lower diversity of macroinvertebrates (Davies and Day, 1998). However, this site was characterized by the heavy presence of the macrophyte *Cyperus.* This combined with good habitat integrity and water quality may have supported a higher diversity of macro-invertebrates than may have been expected.

As with the case of Family Richness, sites receiving sewage effluent had low Shannon-Weiner index values. These included, Site III of the Chamba River, Site I and II of the Chunga River, and Ngwerere River Site II. This demonstrated the effect that pollution had on macro-invertebrate diversity.

Similar to family richness, there was no significant difference in the family diversity of the benthic insect macro-invertebrates found in the sampled rivers i.e. the six different rivers showed similar family diversity of benthic insect and other macro-invertebrates. In this study, it was hypothesized that there was no significant differences in benthic insect macroinvertebrate diversity and richness among freshwater bodies outside Lusaka district boundaries in pristine habitats and those found in disturbed and/or degraded habitats within the district. The findings proved the hypothesis correct.

These findings are similar to those of Türkmen and Kazancı (2010) who showed that there were instances where the Shannon-Weiner diversity index values were similar between sites despite differences among the sites. Coa *et al.* (1996) equally use the index in community ordering despite its lack of consistency with increasing levels of pollution in the freshwater bodies of study. Southwood and Henderson (2006) argued that the Shannon-Weiner index is the least sensitive to actual changes in the community and hence is less useful as an indication of environmental change. This is especially so with small sample sizes as it is strongly influenced by the species number (Southwood and Henderson, 2000). This perhaps explains the lack of significant differences among the species diversity of the rivers of Lusaka district in this study.

There are three implications of the above observations, namely;

1. The Shannon-Weiner Diversity index should only be used when larger sample sizes (n>20) are involved (Southwood and Henderson, 2006).

2. Taxonomic resolution for the Shannon-Weiner Diversity Index should be increased to genus and/or species level in the context of freshwater monitoring of water quality (Chessman *et al.,* 2007; Marshall *et al.,* 2006; Resh and Unzicker, 1975).

3. The Shannon-Weiner diversity index and other diversity indices should be sparingly used in the context of freshwater monitoring of water quality and instead replaced with a biotic index (Dallas, 2013 per. com.). In biotic indices such as the South African Scoring System Index (SASS) and the recently developed Zambia Invertebrates Scoring System (ZISS) Index, each taxon from a particular group of organisms is assigned a sensitivity weighting based on the tolerance or sensitivity to particular pollutants. Biotic indices are generally specific to the type of pollution and/or the geographical area and are used to classify the degree of pollution by determining the tolerance of an indicator organism/s to a pollutant (Dallas *et al.,* 2010; Ollis *et al.,* 2006).

5.2.3 Site Classification based on the Shannon-Weiner Diversity Index

Various scholars have characterized sites based on the Shannon-Weiner Diversity Index (H). For example, according to Khan *et al.* (2007) and Türkmen and Kazancı (2010), values of H above 3.0 indicate that the structure of habitat is stable and balanced; between 1.1- 2.9 indicate that the habitat is between moderately modified to natural, with moderate levels of pollution and values under 1.0 indicate that there are severe pollution and degradation of habitat structure.

In this study, sites classified as ‘Disturbed’ were all highly polluted by organic matter, most notably in the form of sewage effluents. These included Site III of the Chamba River, Site I and II of the Chunga River, and Ngwerere River Site I and II. The sites classified “Good” were noted for the presence of benthic insect macroinvetebrates particularly those intolerant to pollution. These were habitats largely natural but with moderate pollution.

For example, the Namando River site, Musangashi River and Chongwe River Sites were noted for high Shanon-Weiner diversity index and the presence of benthic insect macroinvertebrates particularly intolerant to pollution. The Chamba River Site I equally had a high diversity index value and was classified under "fair". Only the Chongwe River could be said to be "natural" based on the Shannon-Weiner Diversity Index.

5.2.4 Benthic Insect Macroinvertebrate Evenness

In this study, evenness values ranged from 0.1-0.9. Chongwe river sites, Namando river site, Chamba river Site I had values of evenness closer to one. This implied that equitability of the macroinvertebrate community was high. This is in agreement with the species diversity. For remaining sites, equitability was understood to be low, agreeing well with the species diversity (Roozbahani *et al.,* 2010).

5.2.5 The Relationship of Benthic Insect Macroinvertebrate Diversity, Richness and Abundance to Levels of Pollution in Selected Water Bodies

This study hypothesied that there were no relationship between levels of water pollution and benthic insect macroinvertebrate diversity, richness and abundance in the selected freshwater bodies of Lusaka district. Analysis of variance showed that there were no significant correlations between benthic insect macroinvertebrate diversity, richness and abundance with any of the physico-chemical parameters used to assess levels of pollution in the selected water bodies. That is to say no significant relationship existed between benthic insect macro-invertebrat diversity, richness and distribution with the levels of pollution in the selected rivers of Lusaka. This proved the hypothesis correct, contrary to expectation.

It is, however, worth mentioning that many of the three ecological characteristics were negatively correlated to the physico-chemical parameters that were used to assess the pollution levels in the rivers of study. These findings were slightly similar to those by Chessman *et al.* (2007) who found the majority of the physico-chemical parameters used in water pollution assessment negatively correlated to the family and species richness in the rivers of study. These findings suggest that with a sufficient sample size (n>20), the physico-chemical parameters used in this study could still be used to access the relationship of benthic insect macroinvertebrates richness, diversity and abundance with water pollution (Southwood and Henderson, 2006).

5.3 Selected Benthic Insects for use in Monitoring Water Quality in Selected Freshwater Bodies

The following insect families were selected to be used as bio-indicators of water pollution in Lusaka District.

5.3.1 Order Odonata

The Order Odonata is divided into two subclasses, namely the Anisoptera which are the true dragonflies and the Zygoptera, the Damselflies. (Gerber and Gabriel, 2002).

Only the family Coenagrionidae (Suborder Zygoptera) showed significant negative correlation particularly to the pollutant ammonium and the heavy metal Iron. This suggested that high levels of iron and/or ammonium in a water body negatively affected the abundance of the family Coenagrionidae resulting in a reduction in density. It was on this basis that the Coenagrionidae were selected as the only odonatan bioindicators of water pollution in Lusaka District.

Coenagrionidae have a characteristically slender bodies, three leaf like gills with pointed tips at the end of the abdomen. Some species have jointed gills *(*de Moor et al., 2003a; Gerber and Gabriel, 2002), a characteristic of most found in Zambia genera (pers. obs., 2014). They are usually amid vegetation and hence green or brown in colour (Gerber and Gabriel, 2002).

Coenagrionidae are commonly used as bioindicators of water pollution. Gerber and Gabriel (2002) and a recent study by Lowe and Dallas (2012, unpublished) on macroinvertebrates in Zambia, show that Coenagrionidae are highly tolerant to pollution. Smith and Lamp (2008) describe them as indicators of headwaters in polluted urban streams. De Moor *et al.* (2003a) describes many species as being surprisingly tolerant of high silt loads. This suggests that Coenagrionidae can be used as bioindicators of moderately to heavily polluted waters.

In this study, the Coenagrionidae dominated site I and II of the Chamba River. The Chamba river sites are classified as fairly polluted sites whilst the Namando river and Musangashi river had classification of good implying the habitat was largely natural but with moderate pollution. This underscores the findings that Coenagrionidae are tolerant to pollution.

5.3.2 Order Ephemeroptera

Mayflies nymphs, as members of the order Ephemeroptera are commonly called, have a characteristically elongated body, large head and well developed mouthparts and stout legs. Paired gills on the abdomen are the most characteristic feature to distinguish Mayfly nymphs from other insects. The gills vary greatly; leaflike, oval, fringed. Three tails are always present except in the Baetidae (de Moor *et al.,* 2003a).

Significant negative correlations existed between the families Baetidae, Caenidae and Leptophlebidae. This suggested that an increase in turbidity and sulphates resulted in the densities of the Ephemeropteran families Baetidae, Caenidae and Leptophlebidae decreasing. As such these families were selected as bioindicators of water pollution.

The findings of this study supported that of many others. Mayflies play an important role in almost all freshwater ecosystems and are widely accepted as bioindicators of water quality and ecological integrity (Betty *et al.*, 2006; Dobson and Frid, 1998; de Moor *et al.,* 2003a). The high sensitivity of Mayfly taxa to various contaminants including metals, ammonia and other chemicals has been demonstrated in both observational and experimental studies (Beketov, 2004; Clements *et al.*, 2002).

i. Family Baetidae

Baetidae are perharps the most common Mayflies. They are widespread in the world except in New Zealand and other remote oceanic islands. 40 genera are recognised (de Moor *et al.*, 2003a).

Baetidae are generally light sand to dark brown and dart actively from stone to stone or cling on to vegetation (Gerber and Gabriel, 2002). Their nymphs are generally found in flowing waters with a few genera being found in still waters and temporary waterbodies (de Moor *et al.,* 2003a).

Observed were significant positive correlations between the Baetidae and Total Phosphates and Total Suspeneded Solids. This implied an increase in Total Phosphates and/or Total Suspended Solids resulted in an increase of Baetidae densities indicating that Baetidae may not be indicators of good water quality. This observation is supported by Gerber and Gabriel (2002) who described baetids as being moderately tolerant to pollution. The findings of this study are similar to those of Smith and Lamp (2008) who found Baetids in sites described as moderately polluted. In this study, Baetids domintaed Site III of the Ngwerere River which is described as fairly polluted using classifications of the Shannon-Weiner Index.

ii. Family Leptophlebidae

The family Leptophlebidae have a characteristic square heads with very long spreading tails. They have feathery leaf like gills on both sides of abdomen and swim in a dolphin-style for short distances. They inhabit stones or submerged pieces of wood, stones of gentle streams (de Moor *et al.,* 2003a; Gerber and Gabriel, 2002). They, however, are easily mistaken for Baetidae.

The Leptophlebidae are highly sensitive to pollution (Gerber and Gabriel, 2002) and score highly on many biotic indices like SASS (Dickens and Graham, 2002) and the recently developed Zambia Inverterbrate Scoring System (ZISS) (Lowe *et al.,* 2013). The selection of the Leptophlebidae as bioindicators of good water quality in this study is therefore well supported. In this study, the Leptophlebidae were found at Site I and III of Chongwe River. These sites are classified as being in 'Good' and 'Natural' states. This justifies the selection of the Leptophlebidae as indicators of good water quality.

iii. Family Caenidae

The Caenidae are generally small Mayflies with humped backs. The most prominent feature of the Cainflies (common name) are the two prominent square gills which give the naiads an appearance of wearing a skirt or a kilt. They are abundant, especially found in very slow streams and slow bodies of water. They can also be found among silty substrates of backwaters or among aquatic vegetation, where they feed on fine particulate detritus and periphyton (Gerber and Gabriel, 2002).

According to Gerber and Gabriel (2002), the Cainflies are moderately sensitive to water pollution and therefore are used only as indicators of moderately polluted waters, an observation supported by Maseti (2005) and Gerber and Gabriel (2002). In this study, the Caenidae were found at Chamba river Site I, Namando River, Site of the Chongwe River, Site II and Site III of the Chongwe River and Musangashi River site. Of these, only the Chongwe river is classified as Natural or in good water condition. The others are moderately polluted thereby supporting the findings of this study.

Although the following Ephemeropteran families were not selected as bioindicators, worth noting was their presence at the mentioned river sites.

iv. Family Polymitarcidae at the Musangashi River site

The Polymitarcidae, commonly called the pale burrowers are a rare Mayfly family with only three genera in Africa. They are found in pristine habitats, usually in slow moving waters where they burrow in the sand or in hard substrates such as wood (de Moor *et al.,* 2003a).

Although not showing any significant negative correlations with any physico-chemical parameter, this Ephemeropteran family has been considered to be a bio-indicator of water pollution i.e. its presence is an indicator of good water quality. This supposition is supported by recent study of 87 rivers throughout the country which revealed the Polymitarcidae only in three locations namely the Lunga River in West Lunga National Park, the Kafue River at its confluence with the Lunga River within West Lunga National Park and the Musangashi River in Lower Zambezi National Park, all considered pristine habitats (Lowe and Dallas, unpublished). Further, according to Gerber and Gabriel (2002), Polymictarcidae rank among the most intolerant to water pollution.

v. Family Heptagenidae, Musangashi River

The Heptageniidae or the Flat-headed Mayflies have an unusaully broad rounded head, long spread-out tails with large black eyes and a dorso-ventrally flattened body. They run swiftly over short distances and live in stones or submerged pieces of wood. Colour varies from grey, dark brown to black with speckles (Gerber and Gabriel, 2002; de Moor *et al*., 2003a).

Although not showing any significant negative correlations with any physico-chemical parameter, this Ephemeropteran family has been selected as a bio-indicator of water pollution i.e. its presence is a good indicator of good water quality. This is because the Family Heptagenidae is amongst the most sensitive aquatic macroinvertebrates (Gerber and Gabriel, 2002; de Moor *et al.*, 2003a), particularly to heavy metals (Stitt *et al.*, 2006). Its presence at only one site considered pristine, qualifies its selection as a bio-indicator of water pollution.

vi. Family Oligonueriidae, Chongwe River

Oligonueriidae or the Brushlegged Mayflies are large (up to 30.0mm) mayflies with pointed heads and long tufts of hair on the forelegs. They have large leaf-shaped gills on the sides. They are common in fast flowing, pollution free and high elavation rivers. They are brown in colour, getting darker as they get towards the last instar (de Moor *et al*., 2003a; Gerber and Gabriel, 2002).

Although not showing any significant negative correlations with any physico-chemical parameter, this Ephemeropteran family has equally been selected as a bio-indicator of water pollution i.e. its presence is a good indicator of good water quality. This is because the Family Oligonueridae is amongst the most sensitive aquatic macroinvertebrates (Gerber and Gabriel, 2002; de Moor *et al.,* 2003a) scoring very highly on various biotic indices such as SASS and ZISS. In this study, the Oligonueridae were only found at Chongwe River Site III, the only site characterized as being in its natural state. This justifies the selection of the Oligonueriids as bioindicators of water pollution.

5.3.3 Order Trichoptera

The order Trichoptera is commonly referred to as Caddisflies (caddis=shelter). This is evidently due to the fact that Trichopteran larvae use plant and sand material to build for themselves shelters where they hide from predators etc. The scientific name (*Trichos*=hair, *ptera*= wing) refers to the hair covering the wings in adults (de Moor *et al.*, 2003b).

No significant correlation existed between any Trichoptera family and a physico-chemical parameter. As such, no trichopteran family was selected as a bio-indicator of water pollution in Lusaka district. The findings of this study suggested that we do not have many trichopteran species within the study area that can be teased out and used as bioindicators.

5.3.4 Order Diptera

The order Diptera (True flies) is one of the largest orders of the Class Insecta. The majority of Diptera larvae have elongated, worm like bodies, with eyes and legs absent. The bodies are soft, naked or covered with bristles or scales (Day *et al.*, 2002; Gerber and Gabriel, 2002). Such a form is called Vermiform or Maggot-like form.

The Dipterans are further traditionally divided into two suborders, mostly on adult characteristics; Suborder Nematocera, the larvae head and body segments are clearly distinguished. In the Suborder Brachycera, larvae are maggot-like (Day *et al.*, 2002).

Based on the significant negative correlation that existed between family Ceratopogonidae with turbidity and iron and the positive correlation of the family Pyschodidae and total suspended solids, these two families in this order were selected as bio-indicators of water pollution in Lusaka District.

i. Family Ceratopogonidae

Family Ceratopogonidae (Suborder Nematocera) are sometimes classified as Family Heleidae and commonly called **Biting Midges** or punkies or "no-see-ums" They are a large family distributed worldwide with 89 genera, and a staggering 4732 species (Day *et al.*, 2002; Gerber and Gabriel, 2002).

The Ceratopogonidae are very thin, hair like and very difficult to see when dead. All body segments are equal in diameter. They possess neither prolegs nor respiratory tubes on the abdomen. The head is a sclerotized head capsule, which may not always be obvious. Ceratopogonidae swim with a curved body, like a snake and inhabit sand, mud, edges of streams. They are pale or brown, but usually so small that their colour becomes insignificant (Gerber and Gabriel, 2002).

According to Gerber and Gabriel (2002), Ceratopogonidae are moderately tolerant to pollution and hence used only as indicators of moderately polluted waters. Braccia and Voshell (2006) demonstrated that the Ceratopogonidae are moderately sensitive to water pollution.

In this study, this family was only found at the Chongwe River Site II, a site classified as "good" i.e. only slightly polluted and modified. These findings suggest that the family Ceratopogonidae should be used only as an indicator of moderately polluted waters. However, the occurrence of this family at only one site, despite being statistically supported, undermines its selection as a bio-indicator.

ii. Family Psychodidae

The Psychodidae are commonly called the Sewage flies or Moth flies. This is because they are particularly found where there is gross organic pollution such as near sewage purification works. The larvae are characterized by one or more dorsal plates on most segments with a short posterior siphon (Day *et al.*, 2002; Gerber and Gabriel, 2002).

In this study, the Sewage flies, true to their name, were particularly dominant at Site II of the Chunga River i.e. the site immediately after the Chunga Sewage Treatment plant. The findings are similar to those of Braccia and Voshell (2006) who found the Psychodidae most abundant in sewage drainpipes. This implies that Psychodidae are to be used as bioindicators of waters which are grossly polluted.

Although not statistically teased out, the Chironomidae were the most abundant family in this study, dominating a number of sites. As such, they have been selected as bio-indicators.

iii. Family Chironomidae

Family Chironomidae (Suborder Nematocera) is a large family which constitutes 50% or more of the total number of species of macro-invertebrates present in inland waters. They stand at a staggering 10, 000 to 15, 000 species worldwide with 300 to 400 species in Southern Africa (Day *et al.*, 2002).

The members of this family are typically worm like; slender, elongated and cylindrical. They possess small heads with prolegs and gill appendages on the tip of the abdomen. To move, they flicker the entire body. Colour varies from yellow to red, to green to brown (Gerber and Gabriel, 2002).

Among the collected macro-invertebrate families, the Chironomidae (Diptera) was the dominating family. These findings are similar to those of Wahizatul *et al.* (2011), Sharma *et al*. (2010) and Maseti (2005) who all reported that chironomids were the most abundant family in their studies of river systems in Malaysia, India and South Africa respectively. This is because they show no habitat restriction and they exhibit a great variety of feeding types. Further, chironomids are among the most pollution-tolerant of benthic aquatic invertebrates. As a result, they are highly distributed in both fresh and brackish waters (Day *et al.*, 2003; Hynes, 1960; Yule, 2004).

Concerning Chironomid relative abundance however, the following sites are noteworthy: Site III of the Chamba River, Site I of the Chunga River, and Site II of the Ngwerere River. All three sites had more than 90% chironomid abundance. This can be attributed to the treated and untreated sewer effluent received by these rivers from nearby townships and the LWSC sewage ponds. Similar results have been documented by several scholars (Maseti 2005; Sharma *et al.,* 2010; Hynes, 1970; Wahizatul *et al.*, 2011). This underscores the importance of Chironomids as bio-indicators of severe, particularly organic water pollution. According to Lencioni *et al*. (2012), chironomids can be the most useful indicators of the quality of surface water because the larvae are affected by both organic content and trace metal load in the sediments.

Closely associated with the Chironomids are the Tubificidae (Class Oligochaeta) commonly called the sewage or sludge worms. Together, these two families are sometimes collectively referred to as the 'pollution fauna' (Hynes, 1960; Singh, 2005; Braccia and Voshell, 2006). The Tubificidae were found at Site II and III of the Chamba River but were missing at Site I of the Chunga River and Site I of the Ngwerere River.

5.3.5 Order Hemiptera

Order Hemiptera are a large order with insects ranging from minute to large. They are usually oval or elongate and dorsal-ventrally flattened, terrestrial or aquatic. Eyes are large with or without ocelli. They undergo simple, gradual or incomplete metamorphosis (de Moor *et al.,* 2003b). Two families were selected from this order to serve as bioindicators.

i. Family Veliidae

The family Veliidae are commonly called the Broad-shouldered water striders or Water Crickets. They have small plump bodies with legs adapted for running (Cursorial adaptation). As such, Veliidae are commonly seen running (easily interpreted as floating) on the water. Veliidae prefer riffles in small streams whilst others prefer pools and largely open areas (Gerber and Gabriel, 2002; de Moor *et al.,* 2003b). Veliidae are not strictly benthic insects; they spend most of their time running on the surface of the water.

In this study, the Veliidae were found at Site II of the Chamba River, the Namando River site, Site III of the Ngwerere River, Sites II and III of the Chongwe and the Musangashi River Site. These are sites characterised as being fairly or moderately polluted. This may imply that Veliidae can be used as indicators of moderately polluted waters. This reasoning is supported by Gerber and Gabriel (2002) who described Veliidae as generalists and hence can be used only as bio-indicators of moderately polluted waters.

ii. Family Gerridae

The Gerridae are commonly called the Water Striders. The most salient features of the Gerridaes are that they have very long middle and hind legs which they use to skate or leap on the surface film of ponds and streams whilst their front legs are adapted for seizing prey (Raptorial adaptation). They are often found in shaded areas (Gerber and Gabriel, 2002; de Moor *et al.,* 2003b). Like their cousins the Veliidae, Gerridae are not strictly benthic insects; they spend most of their time skating on the surface of the water.

According to Gerber and Gabriel (2002), the Gerridae are moderately sensitive to pollution. This means they can be used as bio-indicators of moderately polluted waters. In this study, the Gerridae were found only at Site I of the Chamba River and the Namando River Site. Both these sites are classified as moderately polluted.

It must be noted, however, that the Gerridae are easily disturbed even by the mere casting of one’s shadow on them (de Moor *et al*., 2003b). This means that there could have been more sites with Gerridae than actually observed in this study.

5.3.6 Order Coleoptera

The Order Coleoptera, weevils and beetles, is the largest order of the Class Insecta with around 370, 000 species already described. Realistic estimation of beetles and weevils comes to a total of 1-5 million; one of every four kinds of animals is a beetle (Krebs, 2009; Stals and de Moor, 2007). Protected by their tough exoskeletons, beetles have entered virtually all none marine habitats of the world. The majority of these are terrestrial with the exception of a few families that are aquatic in both adult and larval stages. Others are pests with a few parasitic forms (Stals and de Moor, 2007).

Only the **Family Dytiscidae** was selected as a bio-indicator from among the Coleoptera. Commonly called Predacious Diving Beetles, they have oval shaped bodies, rounded backs and usually black or brown in their adult forms. The larvae have streamlined, spindle shaped bodies with large heads. They have well developed mouthparts and fringed legs for swimming (Natatorial adaptation). They are pale, light brown or dark brown and will be found amongst vegetation on the edges of ponds and pools (Bouchard, 2004; Gerber and Gabriel, 2002).

The selection of the Dytiscidae as bio-indicators of water pollution in this study is supported by Stals and de Moor (2007) and Bouchard (2004) in which Dytiscidae are described as being highly tolerant to pollution. In this study, the Dytiscidae were found at Chunga River Site I, Namando river site, Site II and III of the Ngwerere River, and Sites I and III of the Chongwe. With the exception of the Chongwe III, other sites are classified as moderately polluted or disturbed. Hence, the Dytiscidae can be used as indicators of moderately or highly polluted waters.

This study hypothesized that there were no benthic insect macroinvertebrate groups that were sensitive to water pollution in Lusaka district freshwater bodies that could be used as bio-indicators of water quality. The selection of the above insect macroinvertebrates groups proved the hypothesis false. These findings are similar to many other scholars highlighted in Chapter one who have proved that various benthic insect macro-invertebrates can be used as bioindicators of water pollution.

5.4 The Effectiveness of Benthic Insects as Indicators of Pollution

One way of testing the effectiveness of selected benthic macro-invertebrates as bio-indicators of pollution is to subject them to laboratory bioassays or toxicity tests (Bennet and Cubbage, 1992; Day, 2000; Harvey-Clarke, 2011; Markowitz, 1999). Rresults of the bioassay, show similar patterns of tolerance among the aquatic macroinvertebrates as expanded on below.

5.4.1 Cluster 1: Site III of the Chongwe River

Site III of the Chongwe River, represented a natural habitat. This means that the structure of the habitat was stable with negligible pollution. From this site, the Ephemeropteran family Oligonueridae and the Tricopteran families Hydropsychidae and Philapotamidae generally showed moderate tolerance to the low pollution levels exhibited by these waters, showing little mortality in the first four hours.

Showing more sensitivity were the Baetidae, Heptagenidae; Philapotamidae; Libellulidae (Order Odonata) and Perlidae (Order Plecoptera) many of which did not survive more than four hours. This underscores the selection of particularly the Baetidae and Heptagenidae as bioindicators of good water quality.

5.4.2 Cluster 2: Site III of the Ngwerere River

Eight representative families were present in the Ngwerere river waters bioassay. The Ngwerere River Site III represented sites classified 'Fair', i.e. moderately modified with a reasonable pollution mostly non-point.

The Ephemeropteran families Trichorythidae, Oligoneuriidae and Baetidae all exhibited a low tolerance to pollution levels found in the Ngwerere River Site III. For example, Baetidae had 100% mortality levels in the first 4 hrs whilst the Oligoneuriidae had 50% mortality in that short period. The more tolerant Tabanidae (Diptera) however, survived for more than 24 hours. This reinforces the general observation that Ephemeroptera are highly sensitive to water pollution and hence make good bioindicators of water pollution.

5.4.3 Cluster 3: Musangashi River Site

The Musangashi River site represented sites classified as good i.e. these sites had good water condition with minimal water pollution. Other river sites in this classification are the Chongwe River Sites I and II and the Namando River Site.

Eight representative families were present in the Musangashi river waters. Most organisms including Ephemeropteran families such as Caenidae, Trichorythidae and Leptophlebiidae survived for more than 24 hours suggesting that the water quality was good. The family Leptophlebiidae for example, survived up to 144 hours (12 days) with little mortality recorded. Also noteworthy is the high rate of survival (compared to the previous clusters discussed) of the Perlidae which survived up to 48 hours. The caenids also showed a high survival rate, surviving up to 72 hours.

The above observations emphasize the important role that aquatic macro invertebrates play as bioindicators of water pollution. The trend this far, was that of organisms not lasting an extended period e.g. more than four hours, especially so with the Ephemeropteran families. However, a close observation of the Musangashi River, a site classified as 'Good' shows the same types of invertebrates showing little or no mortality.

5.4.4 Cluster 4: Site II of the Ngwerere River

The Ngwerere River Site II represented 'Disturbed' sites. These are sites that were all highly polluted by high organic matter, most notably sewage effluents. Other sites in this category are Chunga River Sites I and II and Chamba River Site III.

Ten representative families were present in the Ngwerere River Site III. The Ephemeropteran families, Oligoneuriidae and Baetidae; Perlidae and Elmidae (Coleopteran) showed little tolerance to these waters, all showing 100% mortality levels within the first four hours. This observation underscores the low tolerance that these organisms have to water pollution thereby making them good bioindicators of water pollution.

The Trichorythidae, Caenidae, Leptophlebiidae, Libellulidae, and Hydropsychidae generally showed moderate to high levels of tolerance. This implies that they are more tolerant to water pollution especially with the fact that the waters in this bioassay represented the most grossly polluted. These findings cement the selection of particularly the Caenidae as bio-indicators of moderately polluted waters. Observations concerning the Leptophlebiidae and Trichorythidae are contrary to many findings (e.g. Gerber and Gabriel, 2002) which classify them as highly sensitive to water pollution.

5.4.5 Cluster 5: Site I of the Ngwerere River

The Ngwerere River Site I represented another 'Disturbed' site. This site was polluted most notably by chemical effluent from the nearby Zambia Electricity Supply Cooperation. It also received sewage effluent from sewer pipes from nearby houses that drained directly into the river.

Six representative families were present in the waters of the Ngwerere River Site I bioassay. The more sensitive Perlidae (Plecoptera), Heptageniidae (Ephemeroptera) and Philopotamidae (Trichoptera) showed a low tolerance to the pollution levels found in these waters, showing 100% mortality levels before the four hour mark. The more tolerant Trichorythidae and Baetidae (Ephemeroptera) however, exhibited a moderate tolerance to pollution, surviving up to the 48 hours.

The above observations emphasize the potential that freshwater macroinvertebrates have as indicators of the ecological quality of rivers. The trend of the above bioassays, was that of organisms showing high mortality levels in the river sites classified as 'Disturbed' whilst showing reduced mortality in river sites classified as 'Good' and 'Natural'. These observations further prove false the hypotheses that there were no benthic insect macroinvertebrate groups that were sensitive to water pollution in Lusaka district that could be used as bio-indicators of water quality.

However, it must be noted that testing the effectiveness of benthic insect macroinvertebrates as indicators of pollution using a bioassay in this study was severely hampered by the lack of laboratory equipment. Despite best efforts, an aquatic cage could not be obtained and this hampered the transportation of the aquatic macro-invertebrates. This poor transportation from their source (Chongwe River, Site III) may have caused both temperature and other physical stresses, perhaps leading to higher mortality levels than usual.

Many of the invertebrates collected were stream living and adapted to a high velocity flow of water. It is therefore recommended that water velocity be controlled (e.g. Hargeby, 1986). In this bioassay however, water velocity could not be controlled due the poor nature of the equipment used i.e. the absence of aquatic cages and velocity moderators. The lack of a high velocity current during the bioassay is an extenuating factor that should not be easily ignored.

Yet another challenge was the equal distribution of the aquatic macroinvertebrates among the selected representative sites. This is a recommended practice for bioassays (Bennet and Cubbage, 1992; Harvey-Clarke, 2011; Markowitz, 1999). In this study, this was made difficult due the nature of sampling and collection of the aquatic invertebrates which collected both debris and inverterbrates in the same net.

A lack of proper aeration (sometimes refered to as oxygenation) was yet another setback. Aeration is the process of putting a gas into a liquid. This is ussually done by means of electrically powered devices called aerators. This process was important to increase the amount of dissolved gases, particularly oxygen, in the liquid, in this case river water. However, with only one available aerator, this process could not be done to the best possible standards. This no doubt increased the stress levels of the invertebrates under investigation, possibly further distorting the true representation of the effectiveness of the selected aquatic insect macroinvertebrates as bioindicators of water pollution.

The above challenges, may hence suggest that results of the experiment to determine the effectiveness of invertebrates as bioindicators of water pollution could not be conclusive. It also points to the fact that the University of Zambia needs to invest more in basic laboratory equipment such as aerators and aquatic cages.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Findings of this study showed that; there are no differences in pollution levels of the rivers within the city, as a result human activities and those rivers outside the city, in more pristine habitats. There are also no differences in benthic insect macroinvertebrate diversity and richness among freshwater bodies outside Lusaka district boundaries in pristine habitats and those found in disturbed and/or degraded habitats within the district.

This study has however proved that there are benthic insect macroinvertebrate groups that were sensitive to water pollution in Lusaka district freshwater bodies that could be used as bio-indicators of water quality. The following families were selected as bioindicators of water pollution in Lusaka District: Coenagrionidae (Order Odonata, Suborder Zygoptera); Baetidae, Caenidae and Leptophlebidae (Order Ephemeroptera); Ceratopogonidae and Pyschodidae (Order: Diptera; Suborder: Nematocera); Veliidae and Gerridae (Order: Hemiptera) and Dytiscidae (Order: Coleoptera). The study has also demonstrated that Lusaka district as a whole does not have many trichopteran species that can be teased out and used as bioindicators. Nonetheless, the study has clearly demonstrated the potential that freshwater macroinvertebrates have as indicators of the ecological quality of rivers in Zambia.

6.2 Recommendations

This study recommends that;

i. Similar studies that test the effectiveness of benthic insect macroinvertebrates as bioindicators of water pollution should be embarked upon in other parts of Zambia to expand on the much needed knowledge base that will eventually lead to the development of a relatively simple, non-resource intensive biomonitoring methods based on macroinvertebrate diversity and distribution, for monitoring freshwater in Zambia.

ii. Future studies aiming to assess the effectiveness of benthic insect macroinvertebrates as bioindicators of water pollution using diversity indices such as the Shannon-Weiner Diversity index, should consider increasing the sample sizes to more than 20 (i.e. n>20). This will improve correlations between the diversity index and the physico-chemical parameters under assessment.

iii. To improve correlations with potential stressors in the context of freshwater monitoring of water quality, taxonomic resolution in studies similar to this should be increased to genus and/or species level.

iv. Future studies should consider assessing the effectiveness of the use of biotic indices such as SASS and the recently developed ZISS in the monitoring of water quality.

v. Future similar studies aiming, in part, to assess levels of pollution in selected rivers using conventional physico-chemical methods of laboratory chemical analyses should consider reducing the number of physico-chemical parameters being considered.

vi. Safety and security in the field: This study recommends that during the collection of benthic insect macroinvertebrates from different rivers sites, personnel should work in pairs as a minimum requirement. Protective foot wear such as steel-toes or gumboots, gloves and full waders should be worn at all times whilst carrying out the kick sampling. It is also important that one is vaccinated against diseases such as hepatitis and particularly when working in rivers that are highly impacted such as the Chunga and Ngwerere Rivers whilst maintaining a high levels of personal hygiene.

vii. This study was in part limited by the lack of aquatic laboratory equipment namely aquatic cages, velocity moderators and aerators. Future studies wishing to test the effectiveness of benthic insect macroinvertebrates as indicators of pollution using bioassays should consider investing in the aforementioned laboratory equipment. Further, it is recommended that the University of Zambia invests more in basic laboratory equipment including the aforementioned.

REFERENCES

Allaby, M. 2005. *Dictionary of Ecology*. Oxford University Press, Oxford.

Aregheore, E.M. 2009. *Country Pasture/Forage Resource Profiles- Zambia.* Food and Agriculture Organisation, Rome.

Bäumle, R., Nick, A., Shamboko-Mbale, B., Siwale, C., and S. Kang'gomba. 2012. *Groundwater Resources of the Mwembeshi and Chongwe Catchments including Lusaka Region.* Department of Water Affairs, Lusaka, Zambia and The Federal Institute for Geosciences and Natural Resources, Hannover.

Beddow, V. 2012. *Lusaka: Sanitation Status.* Water Wiki blog; http://www.waterwikilink. Accessed 3rd April, 2012.

Beketov, M. 2004. Different Sensitivity of Mayflies (Insecta, Ephemeroptera) to Ammonia, Nitrite and Nitrate: Linkage between Experimental and Observational Data. *Hydrobiologia,* **528**: 209–216.

Bennet J. and J. Cubbage. 1992. *Effects of Polycyclic Aromatic Hydrocarbons (PAHs) in Sediment from Lake Washington on Freshwater Bioassay Organisms and Benthic Macroinvertebrates.* Washington State Department of Ecology, Washington.

Betty, J.M., McDonald, L.E., Westcott, F.M. and C. J. Perrin. 2006. *Guidelines For Sampling Benthic Invertebrates in British Columbia Streams*. Ministry of Environment Kootenay Region, British Columbia, Canada.

Bonada, N., Dallas, H.F., Rieradevall, M., Prat, N. and J. Day. 2006a. A Comparison of Rapid Bioassessment Protocols in two 2 Regions with Mediterranean Climates, The Iberian Peninsula and South Africa. *The North American Benthological Society* ***25***: 487-500.

Bonada, N., Prat, N., Resh, V.H. and B. Statzner. 2006b. Developments In Aquatic Insect Biomonitoring: A Comparative Analysis of Recent Approaches. *Annual Review of Entomology* **51,** 495–523

Bouchard, R.W., Jr. 2004. *Guide to Aquatic Macro-invertebrates of Upper Midwest*. Water Resources Centre, University of Minnesota, St. Paul, MN.

Bowman, M.F. and R.C. Bailey. 1997. Does Taxonomic Resolution Affect the Multivariate Description of the Structure of Freshwater Benthic Macroinvertebrate Communities? *Canadian Journal of Fish and Aquatic Sciences*, **54**: 1802–1807

Braccia, A. and J.R Voshell Jr. 2006. Benthic Macroinvertebrate Fauna in Small Streams Used by Cattle in the Blue Ridge Mountains, Virginia. *Northeastern Naturalist*, **13:** 269-286.

Bunyolo, A., Chirwa, B. and M. Muchinda. 1995. Agro-Ecological and Climatic Conditions. In: Muliokela, S.W. (Ed.), *Zambia Seed Technology Handbook*. Ministry of Agriculture, Food and Fisheries, Berlings Arlöv, Sweden.

Cao, Y., Bark, A.W. and W. P. Williams. 1996. Measuring The Responses of Macroinvertebrate Communities to Water Pollution: A Comparison of Multivariate Approaches, Biotic And Diversity Indices. *Hydrobiologia,* **341:** 1-19.

Carter, J.L., Purcell, A.H., Fend, S.V. and V.H. Resh, 2009. Development of a local-scale urban stream assessment method using benthic macroinvertebrates: an example from the Santa Clara Basin, California. *Journal of the North American Benthological Society,* **28:**1007-1021.

Central Statistical Office, 2011. *2010 Census of Population and Housing: Preliminary Population Figures.* Central Statistical Office, Ministry of Finance and National Planning, Lusaka.

Chessman, B., Williams, S. and C. Besley. 2007. Bioassessment of Streams with Macroinvertebrates: Effect of Sampled Habitat and Taxonomic Resolution. *Journal of the North American Benthological Society,* **26**: 546-565.

Clarke, R.T., Wright, J.F., and M.T. Furse. 2003. RIVPACS models for predicting the expected macroinvertebrates fauna and assessing the ecological quality of rivers. *Ecological Modeling,* **160:** 219-233.

Clements, W. H., Carlisle, D. M., Courtney, L. A. and E. A. Harrahy. 2002. Integrating observational and experimental approaches to demonstrate causation in stream biomonitoring studies. *Environmental Toxicology and Chemistry,* **21**: 1138–1146.

Dallas, H.F. 2007. The Influence of Biotope Availability on Macroinvertebrate Assemblages in South African Rivers: Implications for Aquatic Bioassessment. *Freshwater Biology,* **52**: 370–380.

Dallas, H.F., Kennedy, M.P., Taylor, J., Lowe, S. and K. Murphy. 2010. *Review of existing biomonitoring methodologies and appropriateness for adaptations to river quality assessment protocols for use in southern tropical Africa.* SAFRASS: Southern African River Assessment Scheme Project Report.

Davies, B.R. and J.A. Day. 1998. *Vanishing Waters*. University of Cape Town Press. Cape Town, South Africa

Day, J. 2000. *Biomonitoring: An Appropriate Technology for the 21st century*. 1st WARFSA/WaterNet Symposium, Maputo.

Day, J.A., Harrison, A.D. and I.J. de Moor (eds), 2002. *Guides to the Freshwater Invertebrates of Southern Africa* **9**: Diptera, Water Research Commission, Gezina

de Moor, I.J., Day, J.A. and F.C. de Moor (eds), 2003a. *Guides to the Freshwater Invertebrates of Southern Africa* **7:** Ephemeroptera, Odonata, & Plecoptera, Water Research Commission, Gezina.

de Moor, I.J., Day, J.A. and F.C. de Moor (eds), 2003b. *Guides to the Freshwater Invertebrates of Southern Africa* **8:** Hemiptera, Megaloptera, Nueroptera, Trichoptera & Lepidoptera. Water Research Commission, Gezina.

Dickens, C.W.S and P.M Graham. 2002. *The South African Scoring System (SASS) Version 5 rapid bioassessment method for rivers. African Journal of Aquatic Science,* **27**: 1–10.

Dobson, M. and C. Frid. 1998. *Ecology of Aquatic Sytems.* Prentice Hall, Essex.

Engelking, P. 2009. *Pollution*. Microsoft® Student 2009, Redmond, WA: Microsoft Corporation, 2008.

Environmental Council of Zambia. 2008. *Zambia Environmental Outlook Report 3*. Ministry of Tourism, Environment and Natural Resources, Lusaka.

Fanshawe D.B. 1969. *The Vegetation of Zambia.* Forest Research Bulletin No. 7. Government Printer.

Gayraud, S., Statzner, B., Bady, P., Haybach, A., Schöll, F., Usseglio-Polatera, P. and M. Bacchi. 2003. Invertebrate Traits for the Biomonitoring of Large European Rivers: An Initial Assessment of Alternative Metrics. *Freshwater Biology,* **48**:2045–2064

Gerber, A. and M.J.M. Gabriel. 2002. *Aquatic Invertebrates of South African Rivers Field Guide*. Resource Quality Services, Department of Water Affairs and Forestry, Pretoria.

Hargeby, A. 1986. A simple trick chamber for rearing aquatic invertebrates. *Hydrobiologia,* **133:** 271-274

Harvey-Clarke, C. 2011. IACUC Challenges in Invertebrate Research. *Institute of Laboratory Animal Rearing Journal,* **52:** 213-220

Hynes, H.B.N. 1960. *The Ecology of Running Waters,* Liverpool University Press, Liverpool.

Ismael, A.A and M.M. Dorgham. 2003. Ecological Indices as a Tool for Assessing Pollution in El-Dekhaila Harbour (Alexandria, Egypt). *Oceanologia*, **45:** 121–131.

Kennedy, M.P. and K.J. Murphy. 2012. *A Picture Guide to Aquatic Plants of Zambian Rivers- with preliminary allocation of trophic indicator scores.* Deliverable Report to the African, Caribbean and Pacific Group of States (ACP Group) Science and Technology Programme, Contract No. AFS/2009/219013. University of Glasgow, Glasgow, Scotland

Kennedy, M.P., Murphy, K.J., Soulsby, C., Lowe, S. and C. Gibbins. 2012. Multiple-scale Controls on Physical and Chemical Habitat Conditions and their influence on Biotic Assemblages in Zambian Rivers. *British Hydrological Society,* Eleventh National Symposium: Hydrology for a Changing World, Dundee 2012.

Khan, A. N., Kamal, D., Mahmud, M.M., Rahman, M.A. and M.A. Hossain. 2007. Diversity, Distribution and Abundance of Benthos in Mouri River, Khulna, Bangladesh. *International Journal of Sustainable Crop Production,* **2**: 19-23

Krebs, C.J. 2009. *Ecology.* 6th edition, Benjamin Cummings, San Francisco

Lencioni, V., Marziali, L. and B. Rossaro. 2012. Chironomids as bioindicators of environmental quality in mountain springs. *Freshwater Science,* **31**: 525-541

Lowe, S. 2012. *Photographic guide to the Aquatic Macroinvertebrates of Zambia.* SAFRASS Deliverable Report to the African, Caribbean and Pacific Group of States (ACP Group) Science and Technology Programme, Contract No. AFS/2009/219013. University of Glasgow, Glasgow, Scotland.

Lowe S., Dallas, H., Kennedy, M.P., Taylor, J.C, Gibbins, C., Lang, P., Sichingabula, H.M., Saili, K., Ntobolo, C., Kabangu, K., Day, J., Willems, F., Briggs, J.A. and K.J. Murphy. 2013. *The SAFRASS biomonitoring scheme: general aspects, macrophytes (ZMTR) and benthic macroinvertebrates (ZISS) protocols.* SAFRASS Deliverable Report to the African, Caribbean and Pacific Group of States (ACP Group) Science and Technology Programme, Contract No. AFS/2009/219013. University of Glasgow, Glasgow, Scotland. 20 pp.

Markowitz, D. 1999. *What's in our Water?* Community Outreach & Education Programs, University of Rochester, New York

Marshall, J.C., Steward, A.L., and B.D. Harch. 2006. Taxonomic resolution and quantification of freshwater macroinvertebrate samples from an Australian dryland river: the benefits and costs of using species abundance data. *Hydrobiologia*, **572**:171–194.

Maseti, P.P. 2005. *Biomonitoring in two contrasting catchments*. M.Sc. Thesis, Rhodes University, South Africa.

Mulenga, C.L. 2003. Urban Slums Report: The Case of Lusaka, Zambia. *Understanding Slums: Case Studies for the Global Report on Human Settlements 2003.* http://www.ucl.ac.uk/dpu-projects/Global_Report/pdfs/Lusaka.pdf.

Nachiyunde, K., Ikeda, H., Tanaka, K. and D. Kozaki. 2013. Evaluation of Portable Water in Five Provinces of Zambia Using a Water Pollution Index. *African Journal of Environmental Science and Technology*, **7***:* 14-29.

O'Leary, M., Vawter, A.T., Wagenet, L. P. and M. Pfeffer. 2004. Assessing water quality using two taxonomic levels of benthic macroinvertebrate analysis: implications for volunteer monitors. *Journal of Freshwater Ecology*, **19**:581–586

Ollis, D.J., Boucher, C., Dallas, H.F. and K.J. Esler. 2006. Preliminary testing of the Integrated Habitat Assessment System (IHAS) for aquatic macroinvertebrates. *African Journal of Aquatic Sciences*, **31:** 1-14

Payne, L.X., Schindler, D. E., Parrish, J.K. and S.A. Temple. 2005. Quantifying Spatial Pattern with Evenness Indices. *Ecological Applications*, **15**: 507–520

Resh, V. H. and J. D. Unzicker. 1975. Water quality monitoring and aquatic organisms: the importance of species identification. *Journal of the Water Pollution Control Federation*, **47**: 9–19

Roozbahani, M.M, Nabavi, S.M.B., Farshchi, P. and A. Rasekh, 2010. Studies on the benthic macroinvertebrates diversity species as bio-indicators of environmental health in Bahrekan Bay (Northwest of Persian Gulf). *African Journal of Biotechnology*, **9**; 510–523.

Rosenberg, D.M. and V.H Resh. 1993. Introduction to freshwater biomonitoring and benthic macroinvertebrates. In: Rosenberg D.M. and Resh V.H. (eds*) Freshwater Biomonitoring and Benthic Macroinvertebrates.* Chapman & Hall, New York, pp 1–9.

Russo, R.C. 1985. Ammonia, nitrite, and nitrate. In: Rand, G.M. and S.R. Petrocelli (eds). *Fundamentals of aquatic toxicology and chemistry*. Hemisphere Publishing Corp., Washington, D.C. pp. 455-471.

Sharma, S, Joshi, V., Kurde, S. and M.S. Singhvi. 2010. Biodiversity And Abundance Of Benthic Macroinvertebrates Community of Kishanpura Lake, Indore (M.P.) India. *Researcher,* **2**:57-67

Shaver, E., Horner, R., Skupien, J., May, C. and G. Ridley. 2007. *Fundamentals of Urban Runoff Management: Technical and Institutional Issues.* 2nd edition, North American Lake Management Society, Wisconsin.

Singh, H.R. 2005. *Environmental Biology*. S. Chand and Company ltd, New Dehli

Smith, R.F and W.O Lamp. 2008. Comparison of Insect Communities Between Adjucent Headwater and Main-Stem Streams in Urban And Rural Watersheds. *Journal of North American Benthological Society*, **27**: 161-175

Southwood, T.R.E and P.A. Henderson. 2006. *Ecological Methods*. 3rd edition, Blackwell Publishing, Oxford.

SPSS Inc. 2006. *SPSS 15.0 Command Syntax Reference*. Chicago, Illinois.

Stals, R. and I.J. de Moor. 2007. *Guides to the Freshwater Invertebrates of Southern Africa* **10**: Coloeptera, Water Research Commission, Gezina.

Stitt, R.P., Rockwell, R.W., Legg, D.E. and J.A. Lockwood. 2006. Evaluation of Cinygmula (Ephemeroptera: Heptageniidae) Drift Behavior as an Indicator of Aqueous Copper Contamination. *Journal of the Kentucky Academy of Science*, **67**: 102-108.

Todd, C. and D. Roux. 2000. *Design of an Aquatic Biomonitoring Programme, Using the South African River Health Programme as a Case Study*. 1st WARFSA/WaterNet Symposium, Maputo.

Türkmen, G. and N. Kazancı. 2010. *Applications of Various Diversity Indices to Benthic Macroinvertebrate Assemblages in Streams of a Natural Park in Turkey*. Balkan Institute for Water and Environment.

Uhlendahl, T., Salin, P., Casaroto, C. and J. Doetsch. 2011. Good Water Governance and IWRM in Zambia: Challenges and Chances. *Water Policy*, **13**: 845–862.

United Nations, Department of Economic and Social Affairs, Population Division. 2010. *World Population Prospects: The 2010 Revision*, New York. http://esa.un.org/unpd/wpp/country-profiles/country-profiles_1.htm

Verma, P.S and V.K. Argarwal. 2005. *Environmental Biology: Principles of Ecology*. S. Chand and Company, New Delhi.

Wahizatul, A. A., Long, S.H. and A. Ahmad. 2011. Composition and Distribution of Aquatic Insect Communities in Relation to Water Quality in two Freshwater Streams of Hulu Terengganu, Terengganu. *Journal of Sustainability Science and Management*, **6:** 148-155

Waite, I. R., Herlihy, A.T., Larsen, D.P., Urquhart, N. S. and D. J. Klemm. 2004. The Effects of Macroinvertebrate Taxonomic Resolution in Large Landscape Bioassessments: An Example from the Mid-Atlantic Highlands, U.S.A. *Freshwater Biology*, **49**:474–489

World Health Organization. 2008. *Iron in Drinking-water: Background document for development of WHO Guidelines for Drinking-water Quality*. World Health Organization, Geneva, 1996.

Yule, C. M. 2004. Insecta: Diptera. In Yule, C.M. and Yong, H.S. (eds.) *Freshwater Invertebrates of the Malaysian Region*. Malaysia: Academy of Sciences Malaysia, pp 610 – 612.

APPENDICES

Appendix A: Study Area Locations

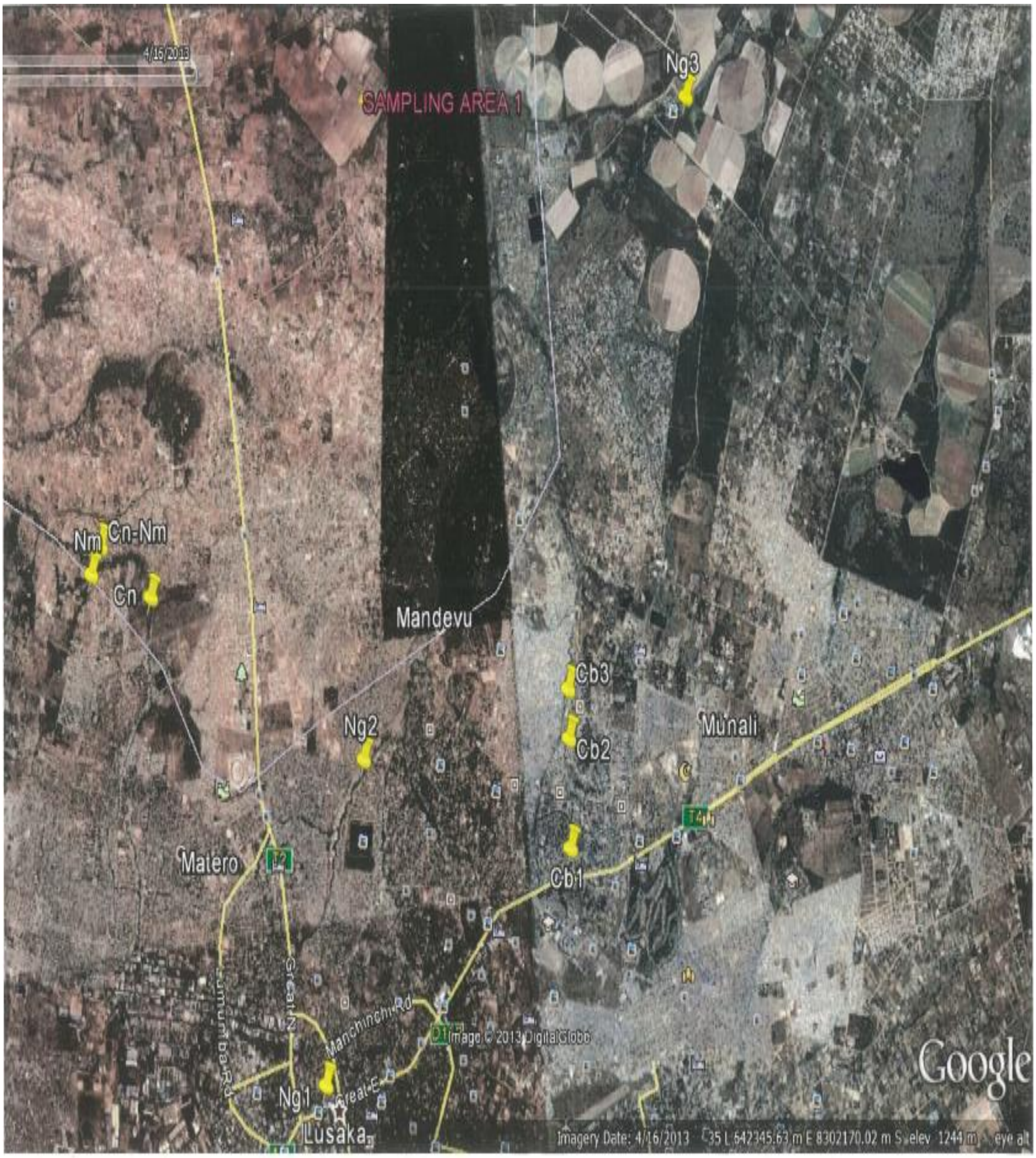

Figure A1: Google generated map (Google Maps Inc.) showing the specific sampling sites for Chamba, Namando, Chunga and Ngwerere Rivers (**Cb1**= Chamba River Site I; **Cb2**= Chamba River Site II; **Cb3**= Chamba River Site III; **Ng1**= Ngwerere River Site I; **Ng2**= Ngwerere River Site II; **Ng3**= Ngwerere River Site III; **Cn**= Chunga River Site I; **Cn-Nm**= Chunga River andNamando River Confuluence; **Nm**=Namando River Site).

Figure A2: Google generated map (Google Maps Inc.) showing the specific sampling sites for Chingwe, Ngwerere, and Musangashi Rivers (**Cg1**= Chongwe River Site I; **Cg2**= Chongwe River Site II; **Cg3**= Chongwe River Site III; **Ng-Cg=** Ngwerere River and Chongwe River Confluence; **Ng1**= Ngwerere River Site I; **Ng2**= Ngwerere River Site II; **Ng3**= Ngwerere River Site III; **Mu1=**Musangashi River, Mu2= Musangashi River site 2 [the intended site]).

Appendix B: Physico-Chemical Water Analysis Results

B. Chamba River Sites

Table B1: Physico-Chemical Water Analysis Results for the Chamba River.

Broad category of parameter	Parameter	Chamba Site I	Chamba Site II	Chamba Site III	WHO Guideline (Maximum Permissible value for drinking water)
Physical and Chemical Characteristics	Temperature (°C)	21.3	21.0	21.5	-
	pH	8.33	8.24	8.06	6.5- 8.5
	Turbidity (NTU)	11.80	3.02	5.08	5.0
	Conductivity (mMhos/cm)	506	494	602	1500
	Total Dissolved Solids (mg/l)	253	247	300	1000
	Total Suspended Solids (mg/l)	4.2	<1.0	2.0	-
	Dissolved Oxygen (as mg O_2/l)	4.6	4.8	5.0	500
Pollutants	Ammonia (as NH_4-Nmg/l)	<0.01	<0.01	<0.01	1.50
	Sulphates (mg/l)	85.94	4.55	26.55	250
	Nitrates (as NO_3 -Nmg/l)	4.86	5.66	5.09	10.0
	Total phosphates (mg/l)	<0.01	<0.01	<0.01	5.0
Heavy Metals	Zinc (mg/l)	0.009	0.015	0.018	3.0
	Copper(ml/l)	<0.003	<0.003	<0.003	0.2
	Iron (mg/l)	0.56	0.49	1.13	0.30

Table B2: Physico-Chemical Water Analysis Results for the Ngwerere River.

Broad category of parameter	Parameter	Ngwerere River Site I	Ngwerere River Site II	Ngwerere River Site III	WHO Guideline (Maximum Permissible value for drinking water)
Physical and Chemical Characteristics parameters	Temperature (°C)	21	21.7	21.3	-
	pH	7.70	6.80	7.51	6.5- 8.5
	Turbidity (NTU)	29.9	1.19	0.49	5.0
	Conductivity (mMhos/cm)	656	844	852	1500
	Total Dissolved Solids (mg/l)	338	424	426	1000
	Total Suspended Solids (mg/l)	12.0	<1.0	<1.0	-
	Dissolved Oxygen (as mg O_2/l)	5.2	4.1	4.0	-
Pollutants	Ammonia (as NH_4-Nmg/l)	0.41	0.73	2.15	1.50
	Sulphates (mg/l)	68.0	62.20	42.80	250
	Nitrates (as NO_3 -Nmg/l)	2.33	1.29	6.79	10.0
	Total phosphates (mg/l)	<0.01	7.77	0.99	5.0
Heavy Metals	Zinc (mg/l)	0.018	0.013	0.003	3.0
	Copper(ml/l)	<0.003	<0.003	<0.003	0.20
	Iron (mg/l)	1.84	1.09	0.84	0.30

Table B3: Physico-Chemical Water Results Analysis for the Chongwe River

Broad category of parameter	Parameter	Chongwe River Site I: Before confluence with the Ngwerere River	Chongwe River Site I; After confluence with Ngwerere River	Chongwe River Site III: Great East Bridge	WHO Guideline (Maximum Permissible value for drinking water)
Physical and Chemical Characteristics parameters	Temperature (°C)	24	22	24	-
	pH	7.86	7.73	7.61	6.5- 8.5
	Turbidity (NTU)	0.26	0.45	0.98	5.0
	Conductivity (mMhos/cm)	169	294	257	1500
	Total Dissolved Solids (mg/l)	85	147	128	1000
	Total Suspended Solids (mg/l)	<1.0	<1.0	<1.0	-
	Dissolved Oxygen (as mg O_2/l)	4.8	4.0	4.2	-
Pollutants	Ammonia (as NH_4-Nmg/l)	<0.01	<0.01	<0.01	1.50
	Sulphates (mg/l)	1.10	3.85	1.40	250
	Nitrates (as NO_3 -Nmg/l)	0.44	0.36	0.46	10.0
	Total phosphates (mg/l)	0.26	1.23	4.75	5.0
Heavy Metals	Zinc (mg/l)	0.010	0.012	0.011	3.0
	Copper(ml/l)	<0.003	<0.003	<0.003	0.20
	Iron (mg/l)	1.02	1.44	0.33	0.30

Table B4: Physico-Chemical Water Analysis Results for the Rivers Chunga, Namando and Musangashi.

Broad category of parameter	Parameter	Chunga River site I; Before Treatment Plant	Chunga River Site II: After confluence with Namando River	Namando River, Barastone Area	Musangashi River; Lower Zambezi National Park	WHO Guideline (Maximum Permissible value for drinking water)
Physical parameters	Temperature (°C)	22.0	21.0	21.5	20	-
	pH	7.64	7.28	7.56	**9**	6.5- 8.5
	Turbidity (NTU)	1.25	80.90	15.80	22.9	5.0
	Conductivity (mMhos/cm)	1195	1881	697	113.7	1500
	Total Dissolved Solids (mg/l)	598	942	351	56.8	1000
	Total Suspended Solids (mg/l)	<1.0	44.0	8.8	13.6	-
	Dissolved Oxygen (as mg O_2/l)	4.2	3.6	2.8	5.10	-
Pollutants	Ammonia (as NH_4-Nmg/l)	2.68	22.50	<0.01	0.05	1.50
	Sulphates (mg/l)	83.00	72.60	62.10	1.80	250
	Nitrates (as NO_3-Nmg/l)	0.47	8.44	1.79	1.82	10.0
	Total phosphates (mg/l)	2.23	0.74	0.43	0.23	5.0
Heavy Metals	Zinc (mg/l)	0.024	0.018	0.016	0.014	3.0
	Copper(ml/l)	<0.003	<0.003	<0.003	<0.003	0.20
	Iron (mg/l)	1.66	1.92	0.72	0.84	0.30