DECLARATION

STUDENT’S DECLARATION

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SUPERVISOR’S DECLARATION

This research thesis has been submitted with our approval as the university supervisor.

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DEDICATION

I dedicate this work to my father Francis O. Maranga and my mother Esther Moraa, for their immeasurable contribution to my academic life. To my sisters, Joyce Kwamboka and Florence Kerubo and brothers Tony Nyanchoka, Joshua Machuka and David Nyamwange. To you I owe everything!

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ABSTRACT

At the University of Eldoret, wastewater is treated using sewage treatment ponds. These ponds were constructed to cater for a few students when the university was a Teacher's Training College. Since then, the treatment ponds have never been expanded despite the ever rising population of the university and therefore creating the need for evaluation of the ponds' efficiency. In this study, macro-invertebrates pollution tolerance index and selected physicochemical parameters were used to evaluate the efficiency of the University of Eldoret sewage treatment ponds. Wastewater samples were collectedmonthly in triplicates and transported to the laboratory for a period of six months. Nitrite, nitrate, dissolved reactive phosphorus and BOD were determined in the laboratory using standard methods and procedures. Temperature, pH, DO and conductivity were measured *in situ* monthly for a period of six months. Macro-invertebrates were collected monthly in plastic containers and preserved in 70 % alcohol and taken to the laboratory for identification for a period of six months. Data on physicochemical parameters was subjected to, One-way ANOVA test to determine (pond variation) for sampling spots and principle component analysis to determine metabolic processes in the sewage treatment ponds. The results of one way ANOVA test demonstrated that temperature ($p \leq 0.001$), pH ($p \leq 0.002$), DO ($P \leq 0.001$), conductivity ($p \leq 0.003$), BOD ($P \leq 0.022$), nitrates ($p \leq 0.04$) and nitrites ($p=0.003$) varied significantly among ponds except dissolved reactive phosphorus ($p \geq 0.822$) and TSS ($p \geq 0.992$) did not vary significantly. Principle Component Analysis results indicated three major components were extracted from water quality data. Component 1 was controlled by DO, pH BOD and temperature with loading values; 0.894, 0.865, 0.824 and 0.778 respectively. Component 2 was controlled by conductivity, TSS and phosphates with loading values; 0.85, 0.811 and 0.739 respectively while component 3 was controlled by nitrates and nitrites with loading values; 0.718 and 0.715 respectively. Pollution Tolerance Index resultsrated wastewater of the ponds as poor with range of values between 7.7 and 11, suggesting inefficiency of the sewage treatment ponds in wastewater restoration. It is therefore recommended that the sewage treatment system to be expanded and accumulated sludge to be disposed after every two to three years hence to improve its performance in wastewater restoration.

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LIST OF ACRONYMS

ANOVA	Analysis of Variance
B-IBI	Benthic-Index of Biotic Integrity
BOD	Biological Oxygen Demand
DO	Dissolved Oxygen
DRP	Dissolved Reactive Phosphorous
IBI	Index of Biotic Integrity
JICA	Japan International Cooperation Agency
NEMA	National Environmental Management Authority
PTI	Pollution Tolerance Index
TSS	Total Suspended Solids
UNDESA	United Nation Department of Economic and Social Affairs
UNEP	United Nation Environmental Programme
UNESCO	United Nation Educational, Scientific and Cultural Organization
UNICEF	United Nation Children’s Fund
UoE	University of Eldoret
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
WSP	Wastewater Stabilization Ponds

CHAPTER ONE

INTRODUCTION

1.1Background information

The world is facing a global water quality crisis, a trend further aggravated by continuous anthropogenic population growth, industrialization, food production practices, increased living standards and poor strategies in water use (WHO/UNICEF, 2010). These crises have contributed largely to water scarcity and water pollution in today's world.

Over half of the world's hospital beds are occupied by people suffering from waterborne diseases and more people die as a result of water pollution than those from all other forms of violence (Corcoran, Nellemann, Baker, Osborn and Savelli, 2010).Worldwide,nearly 900 million people still do not have access to safe water (UNDESA 2009), and some 2.6 billion, which amounts to almost half thepopulation of the developing world do not have access to adequate sanitation (WHO/UNICEF, 2010).

At least 1.8 million children aged five years and below die annually due to water related ailments, comprising 17 percent of deaths in this age group (Corcoran *et al*., 2010). Cholera, typhoid and hepatitis A are the most common diarrheal disease caused by usage of contaminated water. In addition an estimated 2.2 million people die globally each year from diarrheal diseases due to poor hygiene and unsafe water (Corcoran *et al*., 2010).

Two-thirds of the human population in the developing world has no hygienic means of disposing wastesas well as adequate means of disposing wastewater(Jhansi, Campus and

Mishra, 2013). In the context of the present trends in urban development, wastewater treatment deserves greater emphasis. Currently, there is a growing concern of the impact of sewage contamination on rivers and lakes. As such, wastewater treatment is now receiving greater attention from the World Bank and other government regulatory bodies(Jhansi *et al*., 2013).

In sub-Saharan Africa, most of the wastewater treatment infrastructure in many of the fastest growing cities is inadequate or inefficient (Jacobsen, Webster and Vairavamoorthy, 2012). In many cities, the infrastructure is either outdated, poorly designed to meet local conditions, inadequatelymaintained and entirely unable to keep pace with rising urban populations. Careful and comprehensive integrated water and wastewater planning and management at national and municipal levels must be in place to tackle scarcity and related water problems (UNEP, 2009).

In Kenya, wastewater treatment ponds also known as wastewater stabilization ponds (WSP) are used for treatment and restoration as they are cost effective and therefore, the cheapest method that can be used in management of wastewater (Norton, Bjonberg, Kibirige and Raja, 2012). The ponds utilize natural processes to remove a wide range of contaminants including harmful pathogens as well as organic pollutants (WHO, 1987).

Effective management of sewage treatment ponds allows maintenance of ecosystem integrity thus leading to the desired water quality before being released to other environments. The desired water quality is achieved if a wastewater treatment plant is efficient and meets the recommended microbiological and chemical guidelines (Munyenyembe,Mtethiwa, Jere and Nyari, 2006). Appropriate risk assessment must be performed before reuse of wastewater. Such risk assessment should involve extensive monitoring of the treated water before being released to other ecosystems (Jhansi *et al.*, 2013).

Chemical analysis method is expensive and hazardous to health (Norton *et al.*, 2012). Therefore alternative monitoring procedures that use bioindicators, which are less costly and environmentally friendly are recommended in water quality assessment (Aura, Raburu and Herrman, 2010).Bioindicators such as macro-invertebrates, lichens,birds and bacteria, are commonly used to monitor the health status of differentwatersheds (Holt and Miller, 2010). The development and application of bioindicators has been in use since 1960s(Hilsenhoff, 1988). Among the most commonly used bioindicators for assessing water quality status include macro-invertebrate communities. Unlike chemical analysis that gives snapshot status, macro-invertebrates provide cumulative effects of long term status of watersheds (Andem, Esonowo and Bassey, 2015).

Macro-invertebrates possess hallmark traits making them ideal biological monitoring tools for assessment of aquatic ecosystems' integrity (Carignan, 2002 and Holt 2010). The traits include several stages of life cycles, which undergo metamorphosis making them easy to study (Vertessy and Rissman 2000). Macro-invertebrates also comprise families with varying sensitivity/response to pollution. The concept of varying sensitivity/response among different macro-invertebrate taxa to varying levels of pollutants make macro-invertebrates ideal bioidicators in evaluating health status of aquatic environments (Harding, Young, Hayes, Shearer and Stark, 1999).

The University of Eldoret uses stabilization ponds for treating wastewater that originate from the University and the surrounding area before the treated water is discharged to Marula River. Marula River is the source of water to Sogomo residents and the riparian communities. During the dry season the shallow wells dry up forcing the community to depend on the river water to perform domestic chores such as washing utensils, bathing and for irrigation exposing them to contamination. Due to this reason, it was necessary to evaluate the efficiency of the University of Eldoret sewage treatment ponds through carrying out physicochemical analysis and an inventory of macro-invertebrate abundance and diversity.

1.2 Statement of the problem

The University of Eldoret sewage treatment plant has not been expanded despite the ever rising population of students and staff over the years. The rise of population has lead to increased discharge of effluent to the treatment ponds hence reducing retention time. Reduced retention time of wastewater in sewage treatment ponds reduce theefficiency of treatment systems in wastewater restoration. If poorly treated wastewater drain into another aquatic ecosystem, the wastewater could lead to species loss to some riparian communities on the receiving water body due to pollution. The polluted water may also cause outbreaks of waterborne disease to individuals depending on such water to perform domestic chores.As a result, assessment of the University of Eldoret sewage treatment ponds in wastewater restoration through the use of affordable and simpler way of assessment using macro-invertebrates as bioindicators was necessary.

1.3 Justification of the study

The University of Eldoret population has grown tremendously over the past 15 years due to high intake of students and increased staff recruitment. The growth of the institution has led to increased wastewater discharge to the treatment system, with subsequent reduction in retention time of wastewater in the sewage treatment ponds. Reduced retention time hinders maximum removal of pollutants from the wastewater before discharging to Marula River. The Sogomo community depends on the river water for irrigation and in performing of domestic chores. As a result, there isneed toassess the discharge from the University's sewage ponds to ascertain its quality status before draining to Marula River. However, due tofinancial constraints and health issues experienced when carrying outchemicalsanalysis, macro-invertebrates are used as a cost effective biological monitoring tool for regular assessment of aquatic ecosystems includingwastewater. Therefore, this research was carried out to investigate the efficiency of the University of Eldoret sewage treatment ponds in wastewater restoration.

1.4 Objectives of the study

1.4.1 Overall objective

The overall objective was;

To investigate the efficiency of University of Eldoret sewage treatment system in wastewater restoration using macro-invertebrates as bioindicators.

1.4.2 Specific objectives

The specific objectives were;

i) To investigate the effect of physicochemical parameters of the University of Eldoret sewage treatment ponds on the abundance and diversity of the ponds.

ii) To investigate the effect ofnutrients of the University of Eldoret sewage treatment ponds on the abundance and diversity of the ponds.

iii) To find out if there is correlation between physicochemical parameters and nutrients on macro-invertebrates abundance and diversity at the University of Eldoret sewage treatment ponds.

1.5 Hypothesis

H_1. Physical parameters of the University of Eldoret sewage treatment ponds have no effect on abundance and diversity of macro-invertebrates communities of the ponds.

H_2. Nutrient levelsof the University of Eldoret sewage treatment ponds have no effect on abundance and diversity of macro-invertebrates communities of the ponds.

H_3. There is no correlation between physicochemical parameters and nutrients on macro-invertebrate abundance and diversity

CHAPTER TWO

LITERATURE REVIEW

2.1 Wastewater and treatment

In the last two decades, the availability of suitable water for domestic use is reducing faster than its provision by nature (Kumar, Pinto and Somashekar, 2010). As a result most parts of the globe are heading towards water crisis, especiallyin urban areas of the third world countries. The situation is exacerbated by the ever increasing anthropogenic activities that impair the natural water bodies (Khambete and Christian, 2011).

Pollution levels are increasing due to increased wastewater discharge from households, which comprise human waste, oil, food scraps, chemicals and other domestic wastes(Kumar*et al.,*2010*).* Industrial activitiesalso release high quantities of pollutants that cause eutrophicationin natural water bodies (Norton*et al.,* 2012). Pollution has been a nuisance and health hazard to the people exposed to contaminated waters (Munyenyembe *et al.,* 2006).

To improve the availability of utilizable water, wastewater must be purified to supplement the natural sources. Though self purification has the ability to cope with certain amounts of contaminants, there is need to treat the billions of gallons of wastewater, emanatingfrom homes and industries before being released back to the natural water systems (Kumar*et al.,* 2010). Third world countries whose economies are struggling are mostly affected by problems associated with poor wastewater management.As such, these countries use wastewater treatment ponds in wastewater restoration as they are cheap to construct and economical to manage.

Being a third world economy, Kenyaexperiences water scarcity and sanitation issues due to poor wastewater management practices.Most municipal councils in Kenya are unable to sufficiently supply their population with safe waterfor domestic and agricultural use (Kaluli, Githuku, Wahome and Mwangi, 2011). As a result, there is water scarcity and health related complications caused by contaminated water (Kaluli *et al.*, 2011 and Kenya National Bureau of Statistic, 2014). Therefore, the councils should treatwastewater as per the set guidelines, to supplement the scarcely available potable water in the country to prevent water related disease outbreaks (NEMA, 2006). Kenyan towns such as Eldoret and others with a population of more than 100,000 people can produce enough restored wastewater for irrigation and industrial use (JICA, 1998 and Kaluli *et al.*, 2011) ifwastewater is treated as required.

2.2 Use of wastewater treatment ponds

Wastewater treatment ponds are designed to stabilize wastewater before releasing to other ecosystems or recycled for reuse (Norton *et al*., 2012). The ponds involve both biological and chemical processes where inorganic pollutants andorganic matter are broken down by bacteria. The organic matter serves as a source of food for the microbes (Norton *et al.*, 2012). Wastewater treatment ponds provide suitable conditions for removing disease causing organisms,suspended solids and dissolved solutes (Sperling, 2007). The degree of purification of wastewaterin the ponds depends upon the type and number of ponds used as well as the retention time of the wastewater in the ponds. WSP can be used as the sole type of wastewater treatment or can be used in conjunction with other forms of wastewater treatment technologies to meet the desired water quality criteria (Norton *et al.*, 2012).

2.3 Classification of wastewater treatment ponds

Wastewater treatment ponds are classified with respect to the type(s) of biological activity occurring in the ponds. The ponds are of three types namely; anaerobic, facultative and maturation ponds (Ramadan and Ponce, 2003). Anaerobic and facultative ponds are designed for BOD removal although some BOD removal occurs in maturation ponds while some pathogen removal occurs in anaerobic and facultative ponds. Usually anaerobic and facultative ponds are used mostly in wastewater treatment. Maturation ponds are used when wastewater containing BOD> 150mg/l is treated before being discharged for purposes such as irrigation and aquaculture (Ramadan and Ponce, 2003). Anaerobic ponds are deep enough to exclude oxygen and encourage the growth of anaerobic bacteria, which breakdown organic matter anaerobically with subsequent release of ammonia, methane, hydrogen sulphide and other reduced gases (Phuntsho, Shon, Vigneswaran and Kandasamy, 2001).

Facultative ponds are of two types: primary facultative ponds, which receive raw wastewater, and secondary facultative ponds which receive wastewater with reduced total suspended solids usually from effluent from anaerobic ponds (Phuntsho *et al*., 2001). Facultative ponds are designed for BOD removal on relatively low BOD loading at temperature between 20^{o}C and 25^{o}C. Such range of temperature permits the development of a healthy algal population that provides oxygen through the process of photosynthesis for BOD removal (Phuntsho *et al*., 2001). Organic matter entering the facultative ponds from the anaerobic ponds is converted into carbon dioxide, water and new bacterial and algae cells in presence of oxygen, aerobically.

The use of anaerobic and facultative ponds for wastewater treatment has been proven to be satisfactory for reuse in agriculture and aquaculture (Phuntsho *et al*., 2001). However, when the output water quality is not satisfactory, it will be necessary to choose alternative technologies to improve the quality of the treated wastewater. Maturation ponds are much shallower than anaerobic and facultative ponds and are primarily designed for removal of pathogens, nutrients and possibly algae through the outflow (Boney, 1998). Maturation ponds act as buffer for facultative ponds and are verysuitable for nutrient removal (Phuntsho *et al*., 2001). Additional technology that may replace maturation ponds to improve wastewater pond system performance is the use of constructed wetlands(Bailey, Busulwa and Williams, 1994). The purpose of constructed wetlands is the removal of nitrogen and phosphorous nutrients (Kalff, 2002). Nitrogen and phosphorousnutrients cause eutrophication when discharged to aquatic ecosystems (Masese, 2007).

2.4 Macro-invertebrates as biological indicators

Focus is now being directed towards aquatic organisms (macro-invertebrates), which are used as ecological indicators (bioindicators) of water quality (Wenn, 2008). Biomonitoring of ecosystems require the use of bioindicators that are biologically and methodologically user friendly, and can effectively be used to provide early warnings (Burger, 2006).Bioindicators are developed for ecosystem health assessment, for human effects and interventions, human health assessment, and for evaluating sustainability (Burger, 2006). Macro-invertebratepopulations have families with differential responses to pollution and thus their relative abundance is used to infer the nature, load and severity of pollution (Wenn, 2008).

2.5 Indices of biological integrity

Macro-invertebrate assemblages have been used to develop indices of biotic integrity (IBI) as a tool for assessment of aquatic ecosystems' health (Orwa, Raburu, Kipkemboi, Rangoei, Okeyo-Owour and Omari, 2013). Different versions of IBI have been developed for different regions and for varying ecosystems. The most commonly used assessment indices are those of benthic-index (B-IBI) and pollution tolerance index (PTI)(Mark, Mitchell and Stapp, 1997).

B-IBI is used in assessment of samples from deep regions of streams and rivers (Kerans and Karr, 1994). PTI is used in sampling from riffles and other shallow areas to detect moderate to severe stream quality degradation (Mark *et al*., 1997). PTI is useful in developing an information data base and the concept of developing tolerance ranges of organisms (Mark *et al.*, 1997).

The PTI groups macro-invertebrates into three categories on the basis of pollution; sensitive, moderately sensitive, and tolerant groups (Mark *et al*., 1997). These groups are assigned numerical values depending on their pollution tolerance values. For wastewater, a PTI value greater than 23 is considered as excellent condition, a PTI of 17-22 indicates that the water quality is good; a PTI between 11 and16 indicates fair water quality while water with PTI below 10 is considered as poor quality.

Currently the United States Environmental Protection Agency (USEPA) uses PTI to determine quality of water using macro-invertebrates (Idroos and Manage 2012). Macro-invertebrates that are used to calculate the PTI include aquatic worms, backswimmers, water boatman, riffle bettles, scud, leech, blackfly, midge larvae and gilless snails (Mark *et al*., 1997). Large numbers of these types of organisms normally, in absence of sensitive and somewhat sensitive organisms to pollution indicates poor water quality that is organically polluted (Burger, 2006). Some of these organisms including aquatic midge and blood worms are adapted to polluted water as they have hemoglobin that enhances the efficiency of oxygen extraction from water and allows them exist in hypoxic environments (Welch 1992).

Macro-invertebrates that are sensitive to pollution include, Caddisflies, Stoneflies, Mayflies, Dobsonflies and Alderflies. In goodwater quality, macro-invertebrates, which are sensitive to pollution are found in abundance, evenly distributed, and in high diversity (Wenn, 2008). Somewhat sensitive macro-invertebrate include Dragonflies, Craneflies, Crayfish, Aquatic Sow bugs and Damselflies (Burger, 2006) and are found in good or fair quality water. The absence of macro-invertebrates that are sensitive to pollution and the presence of the moderately sensitive organisms is an indication of fair water quality. Presence of pollution-tolerant macro-invertebrates only is an indication of poor water quality (Wenn, 2008). Macro-invertebrates also possess certain advantages as indicators for water quality health compared to other bioindicators. The advantages include, group diversity that make it possible for some members to respond to pollution; long life span that allow the observation of temporal changes in communities due to pollution (Wenn, 2008). In addition, macro-invertebrates are cost effective monitoring tool that can be used for regular assessment of ecological integrity of aquatic ecosystems (Orwa, Raburu, Njiru and Okeyo-Owour, 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

3.1.1 Study site

The study was carried out at the University of Eldoret Sewage treatment plant. The University is located in the highlands of UasinGishu County, 9 kilometers north of Eldoret Town and at latitude $1^{o}30'$N and $0^{o}05'$S and longitude $34^{o}15'$W and $35^{o}45'$E. It lies at an altitude of approximately 2000m above sea level.

Effluent from the treatment plant drains into major wetland, the Marula Swamp before draining to Marula River which is a major source of domestic water to the surrounding communities. The vegetation around the ponds is common papyrus (*Cyperus papyrus*) and short emergent vegetation dominated by Cyperus spp. (*C. rotundus*, *C. triandra*, and *C. laevigatus*). The area experiences an average annual rainfall of 1000 mm and average temperatures of 24^{0}C during the day and 10^{0}C at night.

3.1.2 Macro-invertebrates and physicochemical analysis

Sampling of macro-invertebrates and physicochemical analysis were carried out at the University of Eldoret sewerage treatment ponds. A large quantity of wastewater draining to the ponds originates from the University of Eldoret. Two sampling points were selected on pond one i.e., the inlet and the outlet while only the outlets of the other three ponds were used as sampling points.

The inlet of pond one was used as a reference point to the outlets of all the four ponds to evaluate the condition of the wastewater leaving each of the four ponds. A comparison of wastewater at the inlet of pond one to the outlets of all the other four ponds was carried out to determine the efficiency of each pond in terms of wastewater restoration process.

The following physicochemical parameters were analyzed *in situ* in triplicate at each sampling point; temperature, pH, dissolved oxygen (DO), and conductivity using a multi parameter analyzer WTW 340i (Wetzel and Likens 2000).

3.2 Determination of physicochemical parameters

One litre of wastewater samples was collected in plastic bottles in triplicates and transported to the laboratory in a cool box to determine total suspended solids (TSS), BOD, nitrites, nitrates and dissolved reactive phosphorus. Analysis was done within six hours after sample collection except for BOD, which requires five days incubation period. Temperature, pH, DO and conductivity were measured *in situ* at the sampling points.

3.2.1 Total suspended solids

The initial weight of glass fiber filter (5.5 cm) was weighedusing an electronic weighing machine (TT-A837-3). Triplicate one litre of well-mixed wastewater sample was filtered through theglass fiber filter (5.5 cm) in the laboratory. The residue retained on the filter was dried in an oven at 105^{O}C and weighed again using an electronic weighing machine (TT-A837-3) to determine TSS. The increase in weight of the filter represented TSS.

3.2.2 Biological oxygen demand

One hundred milliliters of wastewater samples were placed in well labeled 250ml beakers in triplicates for designated sampling point and DO determined for day 1. The beakers were then covered with aluminum foil and incubated in complete darkness at 20^{o}C for 5 days. After 5 days, DO of the wastewater sample was determined again.The value for DO day 5 was subtracted from the value of DO day 1 and BOD was obtained. The three samples from each sampling point were averaged to get a representative water quality value for each site.

3.2.3 Dissolved reactive phosphorous

Dissolved reactive phosphorouswas determined through digestion method. Ten (10) milliliters of wastewater was poured into 250ml volumetric flasks and prepackaged powder reagent weighing 100mg consisting of sulfuric acid, potassium antimonyl tartrate, ammonia molybdate and ascorbic acid was added to the volumetric flasks and swirled vigorously to mix thoroughly.

The mixture was left to stand for 10 minutes and then poured into a clean sample cell test tube. The spectrophotometer (BioMate 3S UV-Vis) was zeroed using a blank standard solution (wastewater sample with no reagent in it) and the sample cell test tube placed into the sample cell and covered. Absorbance was read at a wavelength of 885 nm and recorded.

The absorbance value obtained represented the amount of dissolved reactive phosphorous in the wastewater. The sample cell was rinsed thoroughly with distilled water while avoiding touching its lower side and wiped with clean cotton wool. The above procedure was repeated for all the sampling sites.

3.2.4 Nitrite

Nitrite was determined through diazotization method. Nitriver 3 nitrate reagent powder pillow weighing 100mg was added to a sample cell with 10ml wastewater and the content swirled until pink color appeared. The mixture was left for 20 minutes for the reaction to be completed. The spectrophotometer (BioMate 3S UV-Vis) was zeroed using a blank standard solution (wastewater sample with no reagent in it). The sample cell test tube was placed in the sample cell and covered and the absorbance read at a wavelength of 543nm and recorded.

The absorbance value obtained represented the amount of nitrite in the wastewater. The sample cell was rinsed thoroughly with distilled water while avoiding touching its lower side and wiped with clean cotton wool. The above procedure was repeated for samples from all the sites.

3.2.5 Nitrate

Nitrate was determined through cadmium reduction method. Powdered pillow reagent weighing 100mg was added to a sample cell with 10ml wastewater and the mixture was swirled vigorously for three minutes and left for 10 minutes for the reaction to complete. The spectrophotometer (BioMate 3S UV-Vis) was zeroed using a standard solution (wastewater sample with no reagent in it) and the sample cell test tube was placed in the spectrophotometer and absorbance read at a wavelength of 420nm and recorded.

The absorbance value obtained represented the amount of nitrate in the wastewater. The sample cell was rinsed thoroughly with distilled water while avoiding touching its lower side and wiped with clean cotton wool. The above procedure was repeated for all the samples from the designated sampling sites.

3.3 Macro-invertebrates sampling

Samples of macro-invertebrates were taken alongside triplicate two litre wastewater samples from the four outlets of the sewage treatment ponds. This was done by the use of a scoop net measuring 0.5mm mesh size that was used to collect macro-invertebrates. Two litre sampling containers were used to carry macro-invertebrates to the laboratory for identification.The macro-invertebrates were fixed in 10% formalin solution in a sample collection container, hand sorted in a white plastic tray, placed into vials and preserved in 70% alcohol. They were later transported to the laboratory for further sorting, counting and identification.

Hand held magnifying lens was used to enlarge small specimen for easy identification. A pair of forceps was used for handling and sorting of the specimens that were kept in labeled sampling containers. Macro-invertebrates were counted and identified to order and family taxonomic unit to determine abundance and diversity. The counting and identification was done every time samples were collected from the field using identification key by IFM, 2006, and Aquatic invertebrates Identification Guide Walker, 2006.

3.4 Determination of pollution tolerance index

Pollution tolerance index (PTI) was determined to assess the overall wastewater quality status of the sewage treatment ponds to ascertain if the ponds were efficient in wastewater restoration. The index was computed by utilizing methods used by Olomukoro and Dirisu (2013). The PTI for the ponds was determined by assigning the organisms' abundance codes. The codes were assigned depending on the number of organisms sampled for each family for each pond. The codes assigned were R (rare) = 1 – 9 organisms; C (common) = 10 – 99 organisms and D (dominant) = 100 or more organisms.

The code numbers for each pond were added together and multiplied by standard multiplication factor for each code. The multiplication factors the codes are; 1.2 for R (rare), 1.1 for C (common) and 1.0 for D (dominant) (Andem *et al.,* 2015). The PTI value for each pond was arrived at by adding the products of each letter code and its respective multiplication factor. Unpolluted water would have values between 23 and above as excellent, 17 – 22 as good while the polluted water would have 11 – 16 as fair and below 10 as poor quality (Andem *et al.,* 2015).

3.5 Data analysis

Data storage and management was done using Microsoft Excel spreadsheet for windows 2007 while analysis was done using Minitab^{TM} Version 14.0 for windows. One-way analysis of variance (ANOVA) was used to test for significant difference between sampling sites for water quality parameters. Macro-invertebrates were identified at family taxonomic unit to determine family diversity for the wastewater treatment ponds. Macro-invertebrates were counted to determine order and family abundance for the treatment ponds. Spearman's rank correlation analysis was performed to correlate the relationship between macro-invertebrates taxa abundance with physicochemical parameters and nutrient levels. Significant differences for all inference tests were tested at 95% confidence level.

CHAPTER FOUR

RESULTS

4.1 Physical parameters

Table 4.1 shows results of the physical and chemical parameters measured in five sampling spots in the treatment ponds. Temperature ($p \leq 0.001$), pH ($p \leq 0.002$), conductivity ($p \leq 0.003$), DO ($p \leq 0.001$) and BOD ($p \leq 0.022$) varied significantly among ponds. TSS did not vary significantly among ponds ($P \geq 0.992$). Temperature, pH, DO and BOD increased from the inlet to pond 4 while conductivity decreased as illustrated on table 4.1.

Table 4.1: Summary of Means ± SD of water quality parameter for the sewage treatment ponds

Parameters	Inlet	Pond 1	Pond 2	Pond 3	Pond 4	Av means	F value	P value
Temp	21.0± 0.63^{a}	21.87± 0.5^{a}	22.43±1.15^{b}	22.56±1.36^{b}	22.87±2.06^{c}	22.15±1.41	5.38	0.001**
pH	7.0±0.51^{a}	7.86±0.53^{b}	8.03±0.43^{c}	8.26±0.44^{d}	8.31±0.59^{d}	7.78±0.67	16.569	0.002**
DO	2.15±0.41^{a}	5.49±3.81^{b}	5.55±1.07^{b}	8.43±6.96^{d}	6.85±2.3^{c}	5.69±4.19	6.164	0.001**
TSS	0.21±0.05^{b}	0.23±0.33^{b}	0.18±0.32^{a}	0.2±0.32^{b}	0.19±0.33^{a}	0.2±0.28	0.067	0.992
Conductivity	576.56±48.8^{a}	569.4±57.6^{a}	541.31±73.8^{b}	523±86.4^{c}	489.93±74.7^{d}	540.64±75.18	4.319	0.003**
BOD	1.21±0.88^{a}	3.2±4.5^{c}	2.86±2.19^{b}	5.68±6.06^{d}	4.51±3.52^{c}	3.49±4.08	3.04	0.022*
Nitrates	4.11±1.9^{d}	3.76±3.2^{c}	3.27±3.75^{c}	2.53±3.58^{b}	1.66±1.64^{a}	3.07±3	1.788	0.040*
Nitrites	0.17±0.1^{d}	0.12±0.01^{c}	0.08±0.35^{b}	0.04±0.09^{a}	0.01±0.14^{a}	0.084±0.1	7.263	0.003**
DRP	17.75±3.68^{b}	16.35±2.63^{a}	17.18±2.47^{b}	17.44±3.58^{b}	17.36±4.36^{b}	17.22±3.36	0.38	0.822

NB: Means with same superscript across the rows are not significant different at $P \leq 0.05$ while * shows significant variation and **shows highly significant variation, between ponds ($P \leq 0.05$)

4.2 Variation of nutrients concentration

Nitrite and nitrate varied significantly among ponds with P values of 0.003 and 0.04 respectively as shown on Table 4.1. Dissolved reactive phosphorus did not vary significantly among ponds and had P value of 0.822. Nitrite and nitrate decreased from pond 1 through 4 as illustrated on Table 4.1.

4.3 Principle component analysis

Table 4.2 below shows three components that were extracted from the water quality data. The three components accounted for 70.156% of the total variance. Component 1 accounted for 32.847% of the total variance, component 2 accounted for 22.898% of total variance while component 3 accounted for 14.411% variance.

Table 4.2: Principle Component Analysis Results For Variance of Major Components

	Initial Eigen Values			Sums of square loadings		
Component	Total	% Var	Cum%	Total	% Var	Cum %
1	2.956	32.847	32.847	2.956	32.847	32.847
2	2.061	22.898	55.745	2.061	22.898	55.745
3	1.297	14.411	70.156	1.297	14.411	70.156

Table 4.3 shows DO, pH, BOD and Temperature had the highest loading values of 0.894, 0.865, 0.824 and 0.778, respectively in component 1. Conductivity, TSS and phosphates had highest loading values of 0.850, 0.811 and 0.739 respectively in component 2. Nitrates and Nitrites had loading values of 0.718 and 0.715 and controlled component 3.

Table 4.3: Principle Component Analysis Results showing Loadings of Major Components

	Component		
	1	2	3
DO	0.894	-0.097	0.204
Ph	0.865	-0.149	-0.149
BOD	0.824	-0.155	0.252
Temperature	0.778	0.325	-0.251
Conductivity	-0.16	0.85	0.146
TSS	0.134	0.811	-0.236
DRP	-0.102	0.739	0.103
Nitrates	0.156	0.073	0.718
Nitrites	-0.083	0	0.715

4.4 Macro-invertebrates abundance and diversity of the sewage treatment ponds

A total of 6516 macro-invertebrates were collected from the sewage treatment ponds. Five orders and thirteen Families were identified as shown on (Table 4.4). Diptera was the most abundant and diverse taxon. The order had 3845 macro-invertebrates belonging to five families. Diptera accounted for 59% of macro-invertebrates collected from the ponds. Hemiptera was ranked the second abundant and diverse taxon and had 2486 macro-invertebrates belonging to four families. Hemiptera accounted for 38% of macro-invertebrates collected from the ponds. Coleoptera had 95 macro-invertebrates belonging to two families. The order accounted for 1.5% of the total macro-invertebrates collected from the ponds. Ephemeroptera had 53 macro-invertebrates of the family Caenidae that accounted for 0.799% of the macro-invertebrates sampled from the ponds. Isopoda was the least abundant taxon, with 32 macro-invertebrates of Jarinidae, which accounted for 0.5% of the total macro-invertebrates.

Two-way cluster analysis diagram (Fig 4.1) depicts a decrease of Diptera from pond 1 through 4 on the contrary the other four orders increased in abundance from pond 1 to pond 4. Macro-invertebrate abundance did not vary significantly among ponds.

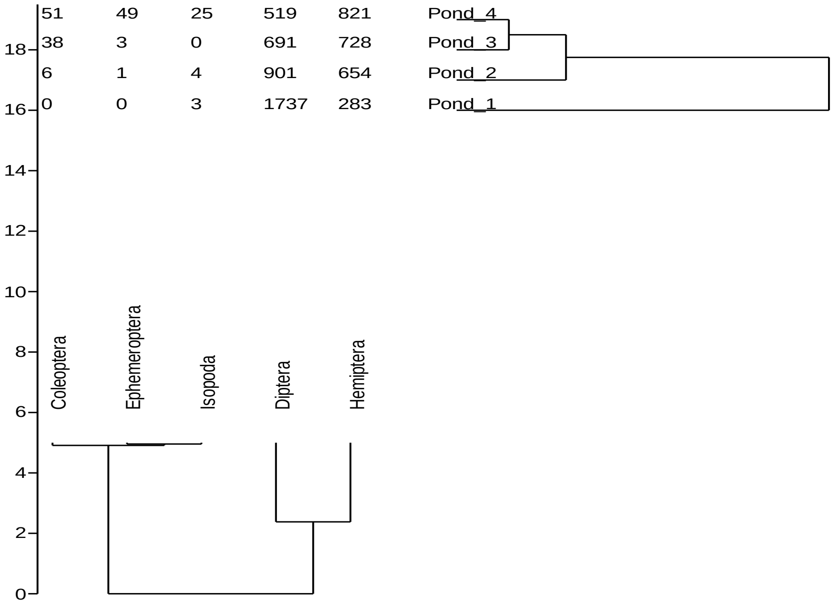

Figure 4.1: Two way cluster analysis diagram for macro-invertebrates order abundance per sewage treatment pond

Table 4.4 below depicts the family abundance and diversity of macro-invertebrates in the four sewage treatment ponds. Pond 1 had the highest number of macro-invertebrates with 2024. Ponds 2, 3 and 4 had 1567, 1460 and 1465 respectively. Pond 1 was least diversified with seven families while pond 4 was the most diverse pond with 10 families. Pond 2 had eight families while pond 3 had nine families. Chironomidae dominated ponds 1, 2 and 3 while Corixidae dominated pond 4. The abundance of Chironomidae decreased while that of Corixidae increased from pond 1 to pond 4.

Table 4.4. Order and family abundance and diversity for the ponds

Order	Family	Pond 1	Pond 2	Pond 3	Pond 4
Coleoptera	Dytiscadae	0	6	35	50
	Grynidae	0	0	3	1
Diptera	Ceratopogonidae	1	0	0	0
	Chironomidae	1534	846	686	489
	Cullicidae	0	8	4	0
	Ephyridridae	187	47	1	30
	Stratiomydae	15	0	0	0
Ephemeroptera	Caenidae	0	0	3	49
Hemiptera	Corixidae	280	338	353	719
	Hydrometridae	0	0	0	7
	Notonectidae	3	282	84	19
	Pleidae	0	36	292	76
Isopoda	Jarinidae	3	4	0	25
Total		**2024**	**1567**	**1460**	**1465**

4.5 Relationship between physico-chemical parameters and macro-invertebrates community

Table 4.5 shows the Spearmans' ranks correlation analysis, which indicated a significant relationship between some macro-invertebrate Orders and water quality parameters ($P < 0.05$). Temperature showed significant positive correlation with Dipteraand Hemiptera. TSS showed significant negative correlation with Coleoptera. Conductivity showed significant negative correlation with all the Orders except Diptera of which a significant positive correlation was obtained. DO, BOD and pH showed significant positive correlation with all the Orders except with Diptera which they showed significant negative correlation.

Table 4.5: Table showing Spearman's rank correlation coefficient (r) and significant values (p) observed between physicochemical parameters and macro-invertebrates order abundance of the wastewater treatment ponds

	Coleoptera	Ephemeroptera	Isopoda	Diptera	Hemiptera	
Temperature	0.175957	0.125706	0.074744	0.48421	0.52	r
	(0.35233)	(0.50804)	(0.69466)	(0.007)	(0.003)	p
TSS	-0.44753	-0.30133	-0.27882	0.215151	-0.168	r
	(0.01315)	(0.10562)	(0.13569)	(0.25354)	(0.376)	p
pH	0.569836	0.448845	0.404856	-0.64136	0.632	r
	(0.00101)	(0.01285)	(0.02647)	(0.00013)	(0.0000)	p
Conductivity	-0.6922	-0.49196	-0.41153	0.491218	-0.441	r
	(2.25^{-5})	(0.0058)	(0.0238)	(0.00584)	(0.015)	p
DO	0.492607	0.47778	0.530954	-0.7107	0.711	r
	(0.00568)	(0.00758)	(0.0025)	(1.1E-05)	(0.0000)	p
BOD	0.608672	0.43652	0.417086	-0.50586	0.469	r
	(0.000358)	(0.015878)	(0.02185)	(0.00435)	(0.009)	p
Nitrates	-0.0971	-0.16498	-0.15958	0.315908	-0.272	r
	(0.60974)	(0.38364)	(0.39961)	(0.08901)	(0.146)	p
Nitrites	-0.57662	-0.44092	-0.43422	0.888348	-0.843	r
	(0.000852)	(0.01473)	(0.01650)	(5.7E-11)	(0.0000)	p
DRP	-0.59326	-0.41193	-0.35781	0.232722	-0.199	r
	(0.00055)	(0.023709)	(0.05222)	(0.21587)	(0.291)	p

Table 4.6 shows pollution tolerance index (PTI) values of the four sewage treatment ponds. The pollution tolerance index (PTI) values of the ponds ranged between 7.7 and 11. Pond 1, 2 and 3 had similar value in terms of PTI rating. The (PTI) values for ponds 1, 2 and 3 indicated poor water quality status while that of pond 4 indicated fair water quality status.

Table 4.6: Pollution tolerance index (PTI) Rating for Wastewater Status of the Ponds

Pond	PTI	Status	Rating
1	7.7	Poor	< 11
2	8.8	Poor	< 11
3	10	Poor	< 11
4	11	Fair	≥11

CHAPTER FIVE

DISCUSSION

Eutrophication of rivers and lakes due to discharge of wastewater containing organic and inorganic substances is currently on the rise as a result of ineffective wastewater treatment and poor wastewater management. Third world countries mostly use sewage treatment ponds to process domestic water from sewage lines (Norton *et al.*, 2012).

Two major substances are contained in wastewater, organic matter from faeces and phosphorus in form of polyphosphates from detergents (Bodin, 2013). Organic matter is degraded into carbon dioxide, water and inorganic substances through the process of decomposition. Decomposition can be either aerobic, fermentation or anaerobic (Peavey, Donald and George, 1985). The results of the Principle Component Analysis in the present study indicated three principal components in which the major component was dominated by DO, BOD, pH and temperature with parameter loadings between 0.7 and 0.9. In this component BOD is a measure of organic matter in the wastewater. The high pH loading in component 1 resulted from detergents and soaps draining into the treatment ponds. Dissolved oxygen, temparature, BOD and pH were positively correlated to the principal component. The component indicated the activity of aerobic bacteria, which require DO, temperature and organic matter to function efficiently.

Most studies use BOD to measure the efficiency of sewage treatment ponds (Grady, 1980). High BOD in the receiving water bodies deplete oxygen, which has deleterious impacts on aquatic biota (Peavey *et al.*, 1985). The combination of the four parameters indicated the process of aerobic decomposition was the most dominant process of reducing organic matter in treatment ponds.

Although Component 3 was the least in terms of variance composition, it was closely related to component 1. They both involved inorganic electron acceptors in the oxidation of organic matter. Component 3 was controlled by nitrate and nitrite suggesting anaerobic respiration but to a lesser extent compared to aerobic respiration since it had the least variance compared to the latter. The high loading of nitrites and nitrates resulted from the consumption of nitrite and nitrate as hydrogen /electron acceptors in the process of nitrate respiration. Both nitrite and nitrate are denitrified to nitrogen. The data indicated that two decomposition processes of aerobic and anaerobic took place in the component.

Component 2, which was second in terms of magnitude of variance, was dominated by TSS, conductivity and orthophosphates. TSS represented inorganic and organic particles. The organic particles included algae and bacteria (Peavey *et al*, 1985) while the rest of the suspended particles represented inorganic material which may have acted as substrates for bacteria. Some of the pollutants entering the treatment ponds were soaps and detergents. Soaps are salts of fatty acids of glycerol and bases of either sodium or potassium. Detergents contain compounds of polyphosphates.

Algae and bacteria were able to cleave the phosphorous from detergent to form orthophosphates by secreting an enzyme called alkaline phosphatase (Grady, 1980). The orthophosphates were responsible for eutrophication of other aquatic ecosystems. High loadings of orthophosphates may have resulted from cleavage of polyphosphate in detergents into orthophosphates with concomitant increase in conductivity.

Polyphosphates are neutral in charge while orthophosphates have high ionic charges which are responsible for high conductivity loadings in component 2.

Bioindicators have been used over time alongside physicochemical parameters to determine water quality status of aquatic ecosystems (Hilsenhoff, 1988) including wastewater treatment systems. Unlike chemical analysis that gives short term fluctuations, bioindicators reflect cumulative effects of the present and past conditions (Andem *et al*., 2015) of water bodies.

Among the most commonly used bioindicators for assessing water quality status include macro-invertebrate communities. Macro-invertebrates are useful in understanding the ecological health of aquatic ecosystem (Olomukoro and Dirisu 2013) and provide a continuous record of environmental degradation (Vertessy and Rissman 2000). Macro-invertebrates are widely used as indicators because they are ideal biological monitoring tools in that; they are relatively easily sampled and they usually occur in great diversity and numbers (Davey, 1980). Macro-invertebrates have short life cycles, and therefore many life stages (i.e. egg, larvae, pupae and adult) that may be studied in a short period of time (Vertessy and Rissman 2000). Macro-invertebrates also have different sensitivity/tolerance to different pollutants (Harding *et al*., 1999). In addition macro-invertebrates are relatively immobile and therefore unable to escape the effect of in stream pollution stresses (Davey, 1980).

In this study, macro-invertebrates abundance and diversity attributes were used to evaluate the performance of the sewage treatment ponds. The results of this study indicated that Diptera and Hemiptera dominated the sewage treatment ponds in abundance and taxa diversity. The high abundance of Diptera in polluted environment was attributed to the fact that most species belonging to the order were highly tolerant to pollution (Harding *et al*., 1999). The high density of Dipterans especially Chironomidae in the first three ponds was an indication of relatively highly polluted water compared to pond 4. Chironomidae being the most tolerant family to pollution was found in large numbers at highly degraded and polluted sites (Buss, Silveira, Nessimian and Dorville, 2002). High nutrients and reduced DO levels in the three ponds favored Chironomidae compared to other families. Most of the Chironomidae had enhanced red pigmentation suggesting that the wastewater had reduced oxygen levels (Welch 1992). Chironomidae appeared red because they synthesize hemoglobin to enhance oxygen absorption at low tensions (Buss *et al*., 2002). As the concentration of nutrients decreased, the abundance of Chironomidae decreased in the ponds.

Hemiptera was the second most abundant and diverse taxon after Diptera. The observed increase of Hemipterans may have been attributed to the decreasing nutrient concentration in the sewage treatment ponds. For the five families that were collected belonging to Hemiptera, Corixidae was the most dominant family. These aquatic bugs showed increasing trend in the treatment ponds. The increasing trend was attributed to the fact that most aquatic bugs have adaptation that enable them to survive in polluted aquatic ecosystems (Harding *et al*., 1999). In polluted environment, aquatic bugs do not depend on dissolved oxygen in water but obtain their oxygen directly from air (Chadde, 2009).

Coleoptera, Ephemeroptera and Isopoda were poorly represented in the sewage treatment ponds. The orders accounted for less than 5% of all macro-invertebrates that were collected from the sewage treatment ponds. The low percentage of Coleoptera, Ephemeroptera and Isopoda may have been attributed to similar water quality status of the first three treatment ponds which were rated poor. Though the PCA results indicated that decomposition process took place in the sewage treatment ponds, the ponds were not efficient enough in their performance. This explains the low representation of the three orders in ponds 1, 2 and 3.

Pollution tolerance index (PTI) is used to assess the overall health of aquatic environment through the use of macro-invertebrate abundance and diversity (Andem *et al.,* 2015). In this study, (PTI) was used to assess the status of wastewater in the sewage treatment ponds. The (PTI) results obtained from the study indicated that the wastewater of the treatment ponds was poor. The poor rating obtained was an indication that the treatment ponds were not effective in the restoration process. The poor rating may have resulted from large volumes of wastewater getting into the treatment ponds than what they were designed to hold. The large volume of wastewater reduced the retention time of wastewater in the ponds hence affecting negatively the ponds performance. The poor rating of the treatment ponds also resulted due to lack of proper management practices. If sludge is left to accumulate over a long period of time, the performance of treatment ponds is reduced. According to Quiroga (2004), performance of sewage treatment ponds is enhanced by disposing accumulated sludge every two to three years. Better pollution tolerance index would have been obtained for the sewage treatment ponds if the ponds were efficient and effective in carrying out their function.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The analyzed water quality parameters of the University of Eldoret sewage treatment ponds had an influence on the abundance and diversity of the ponds an indication of wastewater restoration process took place.

Some water parameters correlated positively while others had negative correlation with macro-invertebrates orders an indication that the University of Eldoret sewage treatment ponds were functional in wastewater restoration.

Though the treatment ponds were functional in the process of wastewater restoration, the PTI results showed that the University of Eldoret sewage treatment ponds were not efficient enough in the restoration process.

6.2 Recommendations

Since the PTI results showed that the sewage treatment ponds were not efficient in the restoration process, the university needs to:-

Expand the sewerage system to increase its capacity that will increase wastewater retention time that is required to enhance the efficiency in restoration of wastewater by the ponds.

Ensure sludge that accumulates in the sewage treatment ponds is disposed every two to three years to enhance the systems' efficiency in wastewater restoration.

Construct a wetland that will enhance the performance of the sewage treatment system.

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