Wetlands as nature based solutions for in situ water purification: The case of urban wetlands, Jimma, Ethiopia

A thesis submitted to Addis Ababa Institute of Technology, for the partial fulfilment of Msc degree in Civil Engineering, Water Supply and Environmental Engineering stream

By: Adey Sileshi

Advisor: Dr. Agizew Nigussie

July, 2018

Addis Ababa, Ethiopia

Addis Ababa University, Addis Ababa Institute Of Technology
School Of Civil and Environmental Engineering, Water Supply and Environmental Engineering Stream

Wetlands as nature based solutions for in situ water purification: The case of urban wetlands, Jimma, Ethiopia

By: Adey Sileshi

Advisor: Dr. Agizew Nigussie

Approved by Board of Examiners:

Dr: - Agizew Nigussie____	_____	_____
Advisor	Signature	Date
_____	_____	_____
Internal Examiner	Signature	Date
_____	_____	_____
External Examiner	Signature	Date
_____	_____	_____
Chairman	Signature	Date

Abstract

Nature provides valuable solution to tackle different socio- environmental challenges. Wetlands have a potential to purify water which is mainly influenced by its hydro-system, including landform setting and the water regime, and different biogeochemical processes. In East Africa, it's estimated that approximately 80% of wastewater is discharged untreated or partially treated. Although there are some studies dealing with the water purifying effect of wetlands, its application for in-situ water purification is still in its infancy.

The main objective of this study is to determine the effect of natural riverine wetlands to reduce nutrient and organic pollutant concentrations. And to assess the difference among the study wetlands.

A cross sectional study on water samples collected from different locations before entering the wetlands, within the wetlands and after passing through the wetlands, was conducted between February 5 and February 25, 2017. Data collection check list and standard field protocol were used to collect data from three urban wetlands (urban downstream wetland and two urban upstream wetlands). After compiling, data analysis was done using R statistical software.

The results of this study showed a lower concentration of nutrients, and BOD in sites after joining the wetlands compared to those before the confluence of the wetland. This implied the presence of pollution attenuation by the wetlands. Among the three wetlands, the one located in the urban downstream revealed a higher level pollution. The PCA model showed nutrient and pollution gradient along its axis. Urban downstream wetland was positively correlated with nutrients as shown in the PCA biplot.

In conclusion, the study revealed the potential of urban wetlands to reduce/ attenuate pollution from the water which passes through them. However, discharging unlimited amount of nutrients and other toxicants to wetlands may affect the functioning of wetlands.

Acknowledgment

I am very grateful to my advisor Dr. Agizew Nigusie for allowing me to work with him. I appreciate his valuable insights, comments and suggestions during the preparation of this thesis. I would like to extend my gratitude to Addis Ababa Institute of Technology for providing me this chance to develop my analytical skill through working on a research project. I am also indebted to Jimma town administration office staffs for providing me the necessary background information for the study.

This thesis would not have been possible without Mohamud Ahmednur, Seyoum Derib, and Yifrashewa Mengesha, members of Jimma University, Environmental Health Science and technology laboratory, who helped me a lot during the fieldwork. Their unreserved contribution also continued during my laboratory analysis in Jimma University.

Finally, I would like to thank my families for providing me emotional support during the stressful moments.

Contents

List of figures

List of Tables

1. Introduction

1.1 Background

Nature provides valuable solution to tackle different socio- environmental challenges such as climate change, disaster prevention, sustainable cities and water resource management. Fresh water pollution is one of the worsening environmental issues in developing countries (Hanasaki et al., 2013; Babayemi et al., 2016). WHO (world health organisation) recommends the application of green infrastructures, such as wetlands, for water purification.

'Wetland' is a term used to express areas which are permanently or temporarily wet, shallow water and land-water margins. They can be found as naturally occurring or manmade. These areas are mostly characterised by the presence of high ground water or surface water inundation, hydrophytic plants and hydric soils (Keith, 2012). These Wetland ecosystems are estimated to cover 8-10 million km^2 globally (Lehner and Döll, 2004).

Wetlands are among the most productive environments in the world (Chapman et al., 2001; Rebelo et al., 2010; Moges et al., 2016). They have significant contribution for high quality water provision, flood mitigation, waste assimilation and detoxification; soil formation and maintenance; control of pests and disease and climate regulation. Water purification potential of wetlands is mainly influenced by its hydro-system, which include landform setting and the water regime, and different biogeochemical processes including microbial transformation, nutrient uptake by plants, microbial degradation of organic compounds and sedimentation (Keith, 2012). They retain sediments; can be used as a sink and transformer of nitrogenous compounds, and removal phosphorus through different chemical, biological and physical processes (Day et al., 2004).

Therefore this study will provide information on the application of wetlands for domestic waste water purification.

1.2 Statement of the problem

In East Africa, its estimated that approximately 80% of wastewater is discharged untreated or partially treated (Bateganya et al., 2015). This is due to inadequate or lack of access to appropriate on-site sanitation or centralized "wastewater treatment systems". Such conditions are jeopardising the valuable fresh water resources resulting in high nutrient and other organic and inorganic pollutant loadings. Fresh water nutrient loading is mainly aggravated by intensive agricultural practices and indiscriminate discharge of human wastes. These pollutants mostly reach the aquatic ecosystem in a diffused form (Strokal et al., 2016; Strokal et al., 2017). Such non-point source pollutants are more challenging for both containment and treatment (Verhoeven et al., 2006).

Naturally, eutrophication is known to occur due to natural factors such as lake age (thousands of years), climate change and geology (Dorgham, 2010; Chislock et al., 2013). However, the rate of eutrophication is accelerated by different anthropogenic activities. Runoff water from agricultural and urban areas typically contains large amounts of nitrate-nitrogen (NO_3–N) and phosphorus nutrients that stimulate algal growth in water bodies. If the runoff, containing nutrients, joins nearby rivers; it will make the water body prone to eutrophication. With eutrophication, the decay of algae lowers oxygen concentrations, sometimes causing fish kills and disrupting the aquatic ecosystem. Such conditions are unappealing and occasionally toxic to humans (Joy and Suzanne, 2005).

The sustainability issue is bringing a "paradigm shift" towards green infrastructures and the application of nature based solutions. "Green" infrastructures are natural and semi natural ecosystems that provide water utility service that compliment augment, or replace those provided by grey infrastructures" (IUCN, 2015). Recent studies are recommending the application of nature based solutions for water purification (Kumar *et al*., 2015; Liquete *et al*., 2016). Wetlands are among the most important nature based solutions. Wetlands, if managed properly, have a potential to reduce pollutant load and to retain sediments. Wetlands are one of the

multipurpose ecosystems, characterized by a unique hydrology, soil, and vegetation (Huang et al., 2015). The loss and degradation of wetlands however, has been affecting its pollutant reduction potential. The major factors for the degradation of the wetlands include agriculture, uncontrolled discharge of untreated wastewater, overgrazing and deforestation (Millennium Ecosystem Assessment, 2005). Extensive use of fertilizers to improve agricultural production leads to eutrophication of surface waters (Crumpton, 2001; Zhenlou et al., 2002). Moreover, in intense agricultural areas, riparian transport has been shown to contribute for the deposition of large amounts of sediment to the wetlands, which contributes to the degradation of water quality downstream (Heimann and Roell, 2000). These sediment loads result in sedimentation problems to reservoirs and dams as it reduces water storage capacity (Devi et al., 2008). Although wetlands have the capacity to assimilate different pollutants there normal functioning will be affected if there is uncontrolled release of nutrients and other pollutants (Nyenje et al., 2009; Ansari et al., 2010).

In most of developing countries wetlands are threatened with increased inflow of nutrients and extensive encroachment for various land use activities especially agriculture and high settlement densities. In Ethiopia, wetlands are estimated to cover about 1.4% of the country's land mass (Gebresllassie *et al*., 2014). Like in many other African countries (Beuel *et al*., 2016), wetlands in Ethiopia are under continuous pressure of degradation (Mereta *et al.*, 2012; Moges *et al.*, 2015).

In Ethiopia, the use of nature based solutions, such as wetlands, for in-situ water purification is still in its infancy. Although wetlands have a potential to reduce pollutant load, indiscriminate release of pollutants might affect its normal functioning. Environmental variables are important to ensure the functioning of wetlands and, thus to monitor and protect this multi-purpose ecosystem (Justus *et al*., 2016). These variables provide information about the status of the water during the sampling event (Resende *et al*., 2010).

1.3 Objectives

1.3.1 General objectives

To provide evidence based information on the effect of natural riverine wetlands to attenuate nutrient and organic pollutants so that to enhance the application of wetlands for on-site water purification.

1.3.2 Specific objectives

- To characterise the study wetlands based on physicochemical parameters
- To analyse the presence of significant reduction/retention of nutrients and organic pollutant by natural riverine wetlands of Jimma
- To find out the difference in percent reduction of pollutants among the study wetlands

1.4 Research questions

- Is there a significant difference in concentration of nutrients, biochemical oxygen demand, and electrical conductivity and pH?
- Is there a significant difference in the level of nutrients (nitrogen and phosphorus), BOD, and DO in sites before joining the wetlands, within the wetland, and after passing through the wetland?
- Is there a difference in percent reduction of nutrients, BOD, turbidity, and EC, and improvement in the level of DO?

1.5 Significance of the study

There are various natural and technological waste water purification techniques. The technological waste treatment option needs a relatively high operational and maintenance cost compared to the natural purification technique. This study is intended to provide evidence based information on the application of natural riverine wetlands to reduce/ retain nutrients and organic pollutants. So that to promote its application for in-situ water purification. It can be used as an input for water resource management which happens to integrate wetlands. This study also provide information on the major disturbance of the wetlands and can be used as a guide to design appropriate wetland management plan. The study will also serves as a baseline information for further research and as an input for policy makers.

2. Literature Review

"Wetland are areas of marsh, fen, peatland or water whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt including areas of marine water the depth of which at low tide does not exceed six meters" (Article 1.1 of the Ramsar convention on wetlands). Wetlands have a wide variety of functions which includes provision of products; micro climate stabilisation; flood control; ground water recharge; water quality improvement and maintenance of biodiversity (Fisher and Acerman, 2004).

Wetlands occupy approximately 6% of the land surface of the world (Mitchell, 2012), and 4% of Africa's land mass. About 65% of wetlands in Sub-Saharan Africa occur within four major river basins namely, Lake Chad, Congo River, Nile River and Niger River (Robelo et al., 2009).

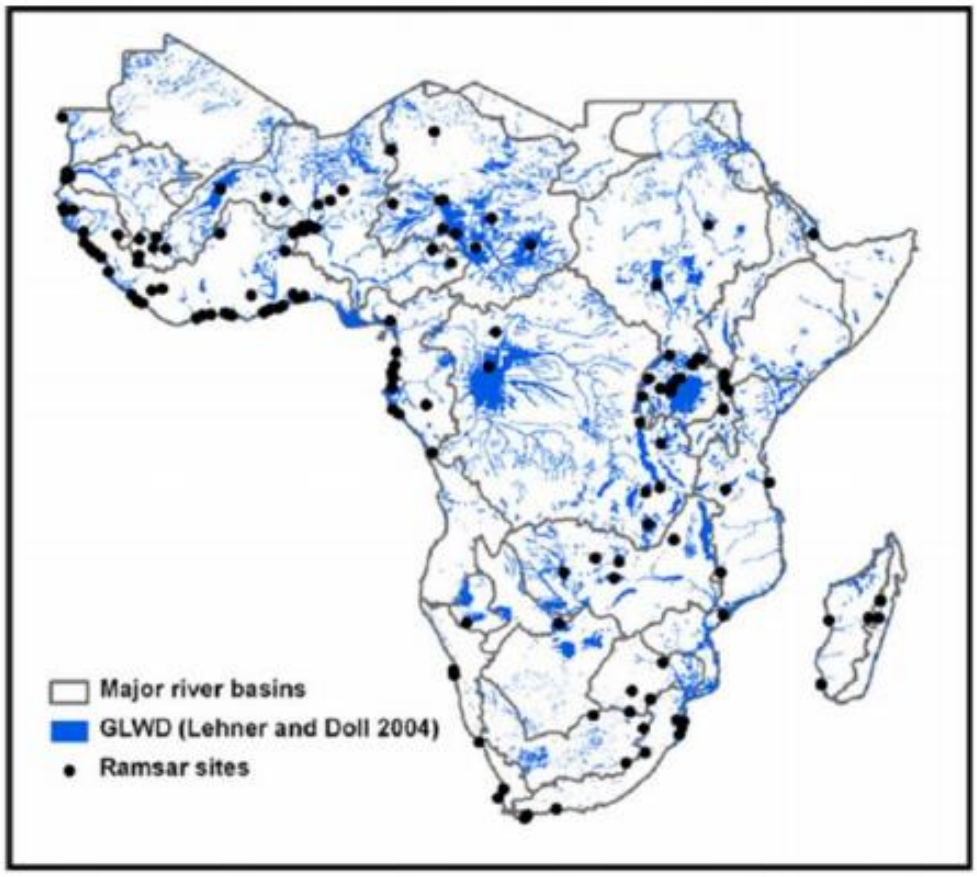


Figure 1. The distribution of wetlands and location of Ramsar wetland sites across major river basins in Sub-Saharan Africa (Adopted from Robelo et al., 2009)

2.1 Wetlands hydro-morphology

Wetland's hydrology is governed by water source (precipitation, runoff, ground water), water movement (seepage, overland flow), and geomorphic setting (landscape position, soils, geologic setting). Wetland conditions occur where topographic and hydrogeologic conditions are favourable and a sufficient, long-term source of water exists. Geologic conditions which may be favourable for wetland development include areas that have fine textured surficial soils with low hydraulic conductivity and sufficient thickness to store water. Water may enter the wetland directly as precipitation, surface flow, interflow (water flowing through the soil profile), groundwater (including deep and/or perched groundwater) or any combination of these (Williams, 1993). A wetland's interaction with surface water and ground water together with its landscape position accounts for its unique hydrologic functions (Bodoque et al., 2016).

The Storage of the water might occur in the channel, the basin and ground water table, which coexist with the hydric soils and create specific conditions suitable for growth and establishment of hydrophytic vegetation (Brander, 2004). The hydrophytic vegetation refers to plants adapted to wet conditions and areas that are covered by water for at least part of the growing season. A soil is considered hydric if it has been flooded of saturated with water long enough to become anaerobic. The hydric soils are volatile and are continually changing with decomposition of the vegetation and the erosion of sediment with river flow and flooding. Wetlands water purification function is a combined effect of different hydrological and biogeochemical processes (Bodoque et al., 2016).

Wetlands hydrology can be managed actively by measuring or estimating the following parameters;

$$dS = P + Q_i + G_i - E - Q_o - G_o$$

Where: dS = change in water storage; P = rainfall E = evapotranspiration losses; Qi/Qo = surface groundwater inflow/outflow; Gi/Go = subsurface groundwater inflow/outflow.

2.1.1 **Indicators of wetland hydrology**

a. Visual observation of inundation – Simply observing the areal extent of inundation is among the most obvious and revealing hydrologic indicator. When applying this indicator, seasonal conditions and recent weather conditions should be considered since they can contribute to surface water being present on a non-wetland site.

b. Visual observation of soil saturation - Examination of this indicator requires digging a soil pit to a depth of 16 inches and observing the level at which water stands in the hole after sufficient time has been allowed for water to drain into the hole. Depending on the soil texture, the required time varies. In some cases, the upper level at which water is flowing into the pit can be observed by examining the wall of the hole. This level represents the depth to the water table. Due to the capillary fringe the depth to saturated soils will always be nearer to the surface. For soil saturation to impact vegetation, it must occur within a major portion of the root zone (usually within 12 inches of the surface) of the prevalent vegetation. The major portion of the root zone is that portion of the soil profile in which more than one half of the plant roots exist.

c. Drainage patterns within wetlands - This indicator, which occurs primarily in wetlands adjacent to streams, consists of surface evidence of drainage flow into or through an area. In some wetlands, this evidence may exist as a drainage pattern eroded into the soil, vegetative matter (debris) piled against the thick vegetation or woody stems oriented perpendicular to the direction of water flow, or the absence of leaf litter. Scouring is often evident around roots of persistent vegetation. Debris may be deposited in or along the drainage pattern

d. Watermarks - occur as stains on bark or other fixed objects (e.g., bridge pillars, buildings, fences, etc.). They are most common on woody vegetation. When several watermarks are present, the highest reflects the maximum extent of recent inundation.

e. Drift lines - This indicator is most likely to be found adjacent to streams or other sources of water flow in wetlands. Evidence consists of deposition of debris in a line on the surface or as debris entangled in above ground vegetation or other fixed objects. Debris usually consists of remnants of vegetation (branches, stems, and leaves), sediment, litter, and other waterborne materials deposited parallel to the direction of water flow. Drift lines provide an indication of the minimum portion of the area inundated during a flooding event; the maximum level of inundation is generally at a higher elevation than that indicated by a drift line.

f. Sediment deposits - Plants and other vertical objects often have thin layers, coatings, or depositions of mineral or organic matter on them after inundation. This evidence may remain for a considerable period before it is removed by precipitation or subsequent inundation. Sediment deposition on vegetation and other objects provides an indication of the minimum inundation level. When sediments are primarily organic (e.g., fine organic material, algae), the **detritus** may become encrusted on or slightly above the soil surface after dewatering occurs.
(Source: Navitzki 1982 and 1998)

2.2 Classification of wetlands

Wetlands could be classified based on their hydrology, topography, nutrient status, substrate type and floristics (Stephenson et al, 1983; Thompson, 1987). Among these Hydrology is the most important determinant in wetland classification. The main wetland hydrosystems are:

Riverine – Flowing shallow water systems and their margins; **lacustrine** – More or less static shallow water systems and their margins. **Estuarine** - brackish systems and freshwater wetlands influenced by tidal effects; **Palustrine** - typically not, or rarely, flooded 'land'-based systems, such as swamps and marshes; **Seep/Spring/Artesian** – A sub-set of palustrine systems, but supplied from subsurface water sources. Seeps and springs typically tap unconfined subsurface aquifers; artesian sources (properly called artesian springs) derive from confined aquifers under pressure (i.e. with positive piezometric heads) at depth. These hydro-systems are usually the ultimate source of rivers but, where they break surface, they create unique wetland systems, such as dune slacks and spring mires.

2.3 Water quality improvement by wetlands

Wetlands water purification function is a combined effect of different hydrological and biogeochemical processes. Which includes flow velocity reduction, which is related to the relatively flat topography and presence of vegetation in the wetlands, microbial degradation and transformation, and nutrient uptake by wetland vegetation (Verhoeven et al., 2006).

2.3.1 Nitrogen removal

Two main biological processes are involved in the removal of NO_3 from groundwater:

(i) Uptake by plants and microorganisms, representing temporary retention;

(ii) Microbiological denitrification, representing permanent nitrogen loss from soils and subsurface waters (Clement et al., 2003).

Denitrification is generally the most important process for nitrate removal, whereby dead organic matter is decomposed by bacteria in the absence of oxygen, using nitrate as an electron acceptor. Nitrate is converted to nitrous oxide (N_2O) and, subsequently, to atmospheric nitrogen (N2), which is emitted by the wetland (Verhoeven et al., 2006).

Wetlands are known to reduce nutrient loading and thereby play a significant role in reducing eutrophication of downstream and adjacent water bodies. This has led to wetlands being managed as buffers for rainfall run off or for treatment of domestic / industrial waste (Fisher and Acerman, 2004). Wetlands are very effective at removing nitrogen from the system. This is because nitrate is readily converted to volatile gases under anaerobic conditions and in the presence of organic carbon, which is abundant in most wetlands. Most nitrogen is transported in its soluble forms.

Organic-N/NH_3/NH_4 ➔ N_2 ➔ NO ➔ NO_2 ➔ NO_3^-

Increasing oxidation state

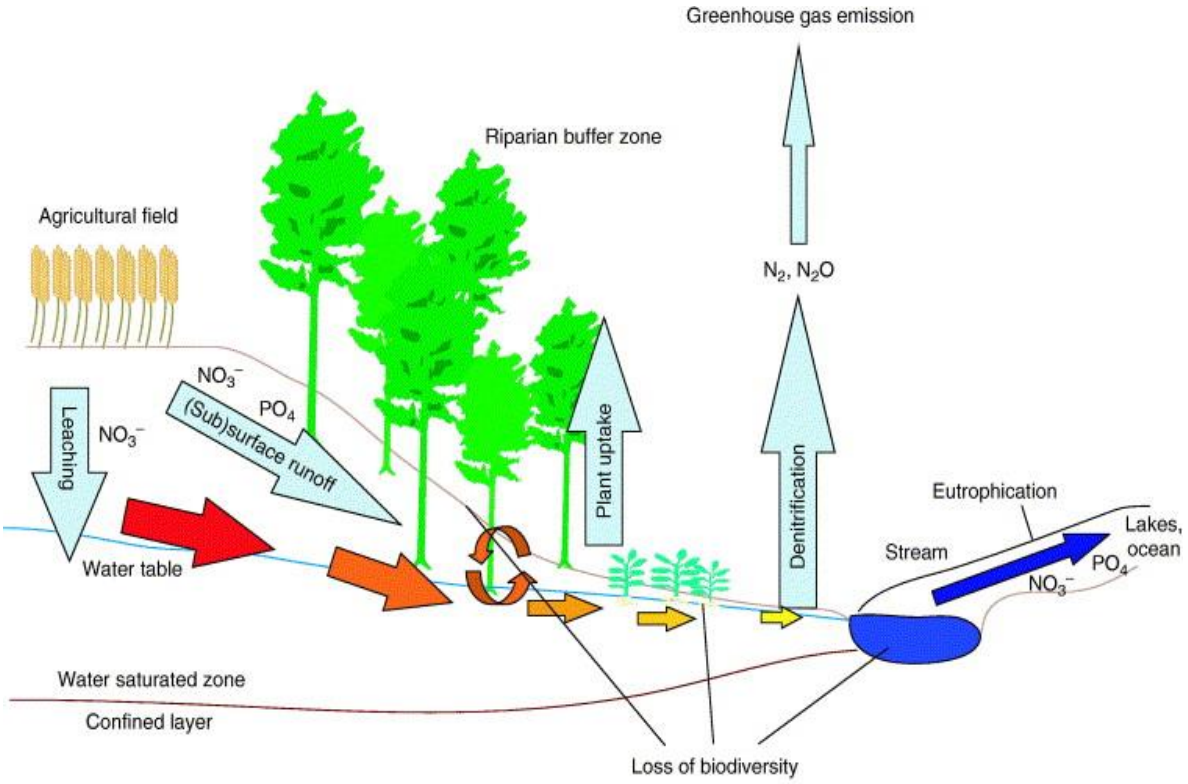


Figure 2. Wetlands water purification processes (Adopted from Verhoeven *et al.*, 2006)

2.3.2 Phosphorus retention/removal

Phosphorus, on the other hand, is not the same as the denitrification mechanism and phosphorus is largely transported by surface waters in its particulate form adsorbed to sediments. Phosphorous removal in riparian habitats has also been reported, with sedimentation, soil adsorption and plant uptake being the most important mechanisms. Its interaction in wetlands are complex, but in general, it is mostly immobile in its organic form and when bound to aluminum, iron or calcium. So the best strategy for removing phosphorus is to trap sediment-rich water and hold it long enough for soil particles to settle out (Joy, 2005).

A study done in Uganda on waste water buffering capacity of natural wetlands indicated the a critical role of the wetlands in reducing both pulse and intermittent pollution loads from urban environments of sub-Saharan Africa whose sanitation systems are defective and inadequate. The results implied, the reduction in suspended solids (73%), organic matter (BOD5, 88% and COD, 75%), and nutrients (total nitrogen, 74% and total phosphorus, 83%) (Bateganya et al., 2015).

Water quality assessment in streams and rivers of Jimma town shows that there was some evidence that indicates its purification potential. A decrease in nitrogen levels is observed along the wetland. This pattern is also verified by Teferi et al. (2010), who constructed a physicochemical profile for Boye pond. The consumption of these nutrients leads to proliferation of vegetation in the wetland and prohibits downstream areas from becoming overgrown by vegetation. Compared to the standards, nutrient levels are sufficiently low to protect the natural environment. In addition, the concentration of fecal and total coliforms is reduced by 68.8% and 91.9% after the water has passed Boye wetland, indicating its removal potential of pathogens.

2.3.3 Sediment retention

Wetlands are effective in trapping sediments; they intercept and retain more sediments than they export (Christopher and David, 2004). The retention of suspended solids in wetlands is controlled by particle size, hydrologic regime, flow velocity, wetland morphometry and residence time (Verstraeten et al., 2006). Hydraulic resistance from the vegetation and soil decreases the velocity of water entering a wetland and enhances the settling and deposition of suspended solids (Reinelt and Homer, 1995; D'Arcy et al., 2007). Wetlands function in reducing pollutants and sediment build up in culverts, streams and harbours.

2.4 Threats for the wetlands

Because hydrologic conditions define wetlands, any alteration of water volume (increases, decreases, or timing of high and low waters) threatens the area and integrity of wetlands. And because the quality of the water further defines the type of wetland, increases in nutrient loadings (eutrophication) often threaten wetland integrity. Eutrophication is a common problem for wetlands downstream from agricultural and urban lands, in part because nutrients allow aggressive plants to gain a competitive advantage and displace native species (Joy, 2005). Wetlands are sometimes drained and filled for development; others are polluted from dumping of wastes from various sources (e.g. industry, agriculture, household, etc.). Wetlands which are in close proximity to urban centers are threatened with increased inflow of nutrients and

extensive encroachment for various land use activities especially agriculture and high settlement densities. All these activities have altered the wetland ecosystems (Kanyiginya, 2004).

In a countries like Ethiopia, a wise use wetland management program would need a responsible agency to co-ordinate national action. Because wetlands fall within the scope of a crosscutting issue like environmental protection, both public and private institutions would need to contribute their expertise and work together. The development of a management plan for Ethiopia's wetlands will need basic studies, including awareness, surveys, and inventories, which should be part and parcel of a wetland development program (Ramsar, 1997; Ambelu et al., 2013).

2.5 Modelling relationships between Environmental variables and wetland sites

R statistical software

R is a language and programming environment for statistical analysis and graphics that is distributed under the GNU General Public License*a* and is largely modeled on the powerful proprietary S/Splus (from ATT Bell Laboratories). R is an object oriented language and everything in R is an object. For example, a single number is an object, a variable is an object, output is an object, a data set is an object that is itself a collection of objects, etc**.** R provides a flexible and powerful environment consisting of a core set of integrated tools for classical data manipulation, analysis and display. It includes different statistical packages and it has rigorous quality control (Logan, 2010; R development core team, 2015, version 3.2.2).

3. Methods and Materials

3.1 Study area description

The study was conducted in three permanent riverine urban wetlands namely Boye (urban downstream wetland), Kitto (urban upstream wetland 1) and Fisho (urban upstream wetland 2) wetlands. These wetlands are located in Gilgel Gibe watershed, between the geographic coordinates of 7°41'N and 36°50'E, Jimma town. The average annual rainfall is estimated to be 1500mm and 2300mm (Seleshi and Zanke, 2004). Jimma town covers an area of 4623ha (Yimer and Mengistou, 2010) with a total population of 207,573 (Jimma City administration, 2016). There is no liquid waste treatment plant in the town. Therefore, liquid wastes from the residential areas join the nearby streams and wetlands, and will be drained to the different water bodies.

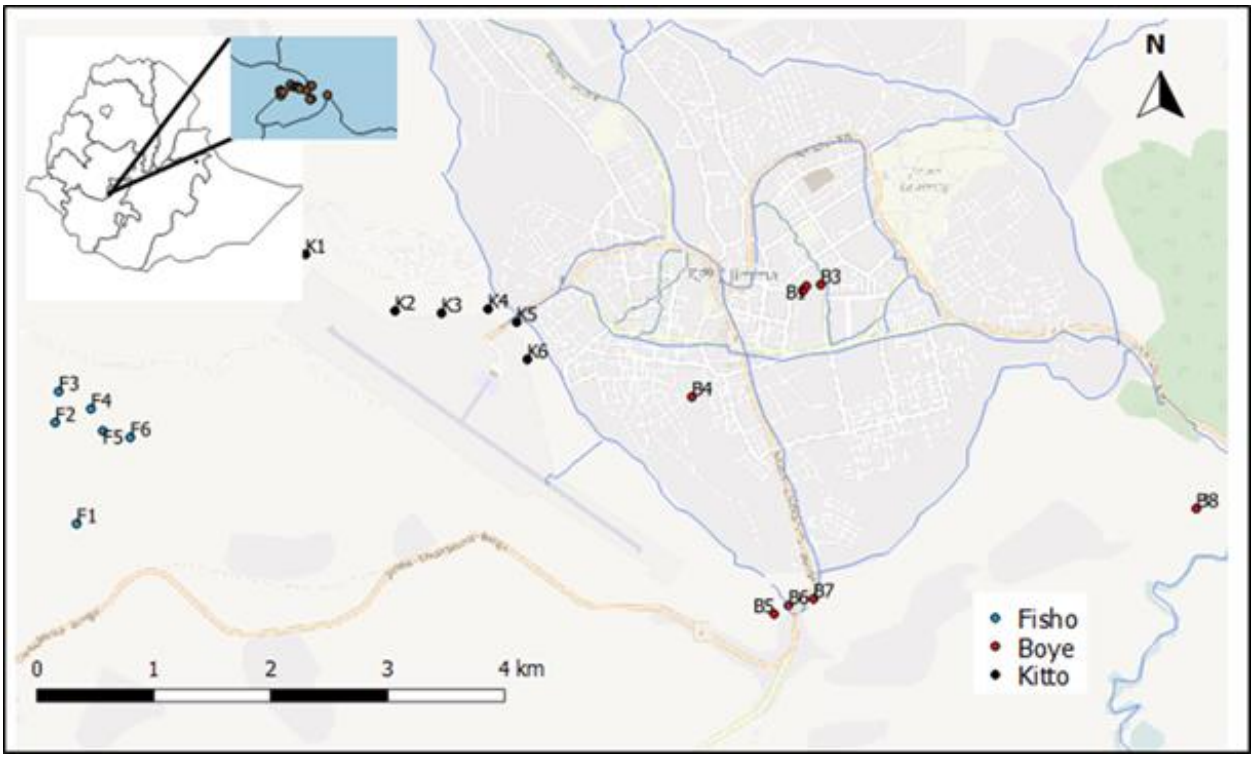


Figure 3. Map of study area with sampling locations, Jimma, Ethiopia

3.1.1 Boye Wetland

Boye wetland covers an estimated area of 60ha. This wetland is located downstream of residential and commercial areas. The wetland is classified as permanent riverine wetland, which is connected to streams/ rivers flowing into the Gilgel Gibe River, and finally it enters Gilgel Gibe hydro-power dam. There are four streams/ river joining this wetland namely, Kochie stream (b1), Dololo stream (b2), University stream (b3), and Awaitu River (b4). These streams pass through residential and commercial areas of the town, where they act as waste disposal sites, especially liquid waste. Awaitu River is the major carrier of Jimma town's liquid waste (Ambelu et al, 2012).

Anthropogenic activities which take place within and surrounding of this wetland include uncontrolled livestock grazing, plant harvesting (especially papyrus), solid and liquid waste disposal, car and other vehicle washing, and, water use and extraction.

3.1.2 Fisho wetland

Fisho wetland covers approximately 40ha area. The location of this wetland is upland of the residential and commercial areas. This is also a permanent riverine wetland connected to two streams (upstreams) and finally flows to Kitto River, then to Gilgel Gibe River and end up to Gilgel Gibe dam. The streams joining this wetland are namely, Degoye stream 1 (f1), and Degoye stream 2 (f2).

The major anthropogenic activities in and around this wetland include land conversion to cropland, brick mining, vegetation clearance, grazing, cultivation, and conversion of flow direction.

3.1.3 Kitto wetland

Kitto wetland covers an estimated area of 50ha. Like Fisho wetland, this wetland is also located upstream of the urban area. The geomorphic setting of this Kitto wetland is a permanent riverine wetland and the streams connected to this wetland are Kitto stream 1 (k1) and Kitto stream 2 (k2). The second stream, which is Kitto stream 2 is a collection of small streams which pass through residential areas. The outlet from this wetland flows as Kitto River and pass through Gilgel Gibe River to Gilgel Gibe dam.

Major threats to this wetland include, water use (cloth washing inside the wetland) and extraction, Papyrus harvesting, and grazing.

3.2 Selection of sampling points

The sampling locations were selected as those before joining the wetland, within the wetland, and after passing through the wetland. Those points considered as “before joining the wetland” are the incoming streams before they confluence the wetlands. As it is presented in the study area map, the streams associated with Boye wetland (b1, b2, b3, and b4) are somehow in a dispersed location. From the incoming streams, the nearest accessible points (i.e. from the points which can be accessed, the one which is close to the inlet of the wetland) were selected for sampling. Within the wetland points (b5, b6, b7) represent those points taken after the streams confluence the wetland and traveled a considerable distance within the wetland. The limitation here is that due to lack of accessibility, it was not possible to take samples from the exact middle point (across the width) for Boye wetland. “After passing through the wetlands” (b8) represent the outlet point of the wetlands. The other two wetlands (Kitto and Fisho) were relatively accessible. Therefore samples were taken from the streams around the inlet (before joining the wetland) (k1 &k2; f1 & f2); from the middle part of the wetland (k3 & k4; f3 & f4) and at the out let points (k5 & k6; f5 &f6). Finally a total of 20 sampling points were selected for this study.

3.3 Study design and period

A cross sectional study on water samples collected from different locations before entering the wetlands, within the wetlands and after passing through the wetlands, was conducted between February 5 and February 25, 2017. Laboratory investigations were done on water samples taken from different sampling points and the level of disturbance on the wetlands was assessed by using wetlands field protocol.

3.4 Study variables

Table 1. Physico-chemical parameters

Parameters measured on site	
	Temperature
	pH
	DO
	Conductivity
	Turbidity

Parameters analysed in the laboratory	
	Chloride
	BOD
Nitrogen	Total Nitrogen
	Nitrate
Nitrogen	Ammonium
Phosphorus	Total phosphorus
	Orthophosphate

3.5 Sampling and Data Collection Techniques

Representative samples were taken from the water before confluence of the wetlands, within the wetland, and after passing through the wetlands. Sampling for water quality parameters was done following American Public Health Associations (APHA, 2005) standard procedure for wetland assessment and WHO guideline for water sampling and chemical analysis. At each of the sampling locations, the first 30 to 40 minutes from the data collection period was given to do sanitary survey and record the major disturbances around of the study wetland following the

field protocol. Geographic positioning system (GPS) was used to record the coordinates for each sampling location and Q-GIS was used to build the map of the study sites.

3.6 On-site and laboratory measurements

At each sampling site, in-situ measurements of dissolved oxygen (DO), electrical conductivity (EC), potential of hydrogen(pH), and Water temperature (T°) was done with HACH Lange multi - parameter probe following APHA *et al.* (2005) procedure. These measurements were done by inserting the probe into a 3L capacity bucket filled with the water sample, followed by gentle stirring for about 30 seconds. Turbidity was also measured on-site using turbidity meter. For the physicochemical analysis which was done in the laboratory, 1.5 L of unfiltered water samples were collected from each site by inserting clean polyethylene plastic bottle, and facing it in opposite direction of the current flow. Water samples were taken at three points across the width in order to get a well-mixed and representative sample. The collected samples were kept in an ice-box and transported to Jimma University, Environmental Health Science, and Technology laboratory, within six hours after collection. In the laboratory, samples were placed in a deep freezer until further processing and analysis was done.

In order to minimise the effect of freezing on the bacterial activity, BOD_5 was analysed immediately after collection (before freezing). Samples were un-frozen at room temperature. Total nitrogen (TN), and total phosphorus (TP), were determined from unfiltered samples.

Water samples were filtered using Whatman glass microfibre filter having a pore size of 0.45µm and transferred to a 150 ml of polyethylene bottles for the analysis of nitrate (NO_3-N), ammonia (NH_4-N), orthophosphate (PO_4-P), and chloride (Cl^-). Spectrophotometer was used to read the concentration of each parameter. All the parameters were analyzed following APHA's standard protocol (APHA *et al*., 2005).

3.7 Quality control

Collected samples were transported to the laboratory, within 6hours, aseptically in order to reduce the risk of further contamination.

To assure the quality of the tests during analysis a blank test was run at the start of analysis and in between each sample run. Each reading was read at least three times to see precision between results.

3.8 Statistical analysis

The collected data was compiled and recorded in **excel.** After wards, the statistical analysis was done using **R** statistical software. Packages which were used in the analysis include stat (Shapiro-Wilk test), Vegan (multivariate analysis), Packfor (forward selection) and Hmisc (correlation).

Descriptive statistics was used to determine the overall status of the water chemistry along the sampling locations.

3.8.1 Data standardisation

Since the measurement of those various environmental variables is different, there is a need to bring the data into a common (similar) format. There different data standardisation techniques including square root (sqrt), Hellinger (hell), and logarithmic (log) or log (x+1) transformations. All environmental parameters, except pH, were log transformed (log (x+1)) prior to analysis. pH is normally in a logarithmic scale so, there is no need for additional transformation.

3.8.2 Shapiro-Wilk normality test

Distribution of environmental parameters in each group was tested using Shapiro–Wilk normality test, which is the most powerful normality test (Razali *et al*., 2011). The null hypothesis of this test is that the data is normally distributed. The obtained p-value was less than the chosen alpha level (i.e $p < 0.05$). Therefore the null hypothesis was rejected and the data distribution was confirmed to be non-normal. Thus, the proceeding analysis was done using non-parametric tests.

3.8.3 Mann-whitney Wilcoxon test

Mann-Whitney Wilcoxon test is used to test for differences in the means of a response variable in two groups. This test assumes that one distribution is a horizontal shift of the other distribution. It is the non-parametric version of a t-test.

The first step of Wilcoxon test is to calculate the differences of the repeated measurements and to calculate the absolute difference. The next is ordering the cases by increasing absolute differences. Then each rank will be signed. If the original difference is less than zero, the rank will be multiplied by -1 otherwise it stay positive. The **W-statistics** indicate the sum of ranks (Logan, 2010).

In R statistical software, Mann-Whitney test uses the **Wilcox.test** function which is preinstalled in R. In this test the p-value is calculated using F distribution. P-value < 0.05 indicates the presence of significant difference among the comparison groups (R development core team, 2015, version 3.2.2).

3.8.4 Kruskal-wallis

It is the Extension of the MW Wilcoxon test which is used when you want to compare more than two groups. This test is the non-parametric equivalent of ANOVA. The test statistics of Kruskal-Wallis is the ANOVA “F” test statistics.

In R statistical software Kruskal-wallis test uses **kruskal.test** function. Unlike Mann-whitney, the P-value is calculated using a chi square distribution. Significance of a KW test (i.e p-value <0.05) indicates that there is at least one significant difference between the tested groups. To identify the groups having significant difference, Kruskal-wallis was followed by Mann-whitney Wilcoxon test (Logan, 2010; R development core team, 2015, version 3.2.2).

3.8.5 Spearman correlation

Correlation analysis tests the presence of association among variables. Unlike regression, correlation doesn’t tell the presence of causality between variables. Spearman correlation measures for monotonic association (not necessarily linear).

Spearman-correlation= Pearson correlation coefficient between the ranks of the values of each variable

$$\rho = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_i (x_i - \bar{x})^2 \sum_i (y_i - \bar{y})^2}}.$$

In R, spearman-correlation analysis is performed by installing Himisc package. Running the correlation was performed by using “cor” function and mentioning the method as “spearman”.

3.8.6 Bonferroni correction /correcting for multiple testing

When performing ten or more separate tests there is a need to consider correcting for multiple testing in order to reduce type I error. This is because, each time we perform a test there is a 5 % chance that we will be rejecting a null hypothesis incorrectly. If we do 10 tests there is a total probability of 50 % that at least one of the tests has incorrectly rejecting the null hypothesis becomes (10x5 %). Bonferroni correction is the simplest way to adjust P-values. It is simply done by multiplying the P-values by 10, then corrected P-values will be interpreted based on the 5% significance level (Logan, 2010).

*when we deal with 100 samples, the obtained P-values will be multiplied by 100 to reduce type I error.

3.8.6 Ordination analysis

Ordination uses distances and dissimilarity indices to visualize and assess variation between observations in relation to predictor variables. There are different ordination techniques. For this study Principal component analysis (PCA) was selected in order to see the correlation between environmental variables and sites (Logan, 2010).

3.8.6.1 Principal Component Analysis (PCA)

PCA is a mathematical procedure that identifies patterns in data, and highlights similarities and differences. PCA creates new artificial variables (principal components) that are linear combinations of the variables in a dataset. What is more these principal components are all constructed to be uncorrelated (orthogonal) and they reflect variable amounts of variation in the dataset (given by the eigenvalue of the principal component). They are ordered with PC1 explaining most variation and so on. PCA regression - regress the response variable against the principal components resulting from a correlation matrix for all the predictor variables. Each of

these principal components by definition are completely independent (Logan, 2010; R development core team, 2015, version 3.2.2).

PCA uses Euclidian distance to analyse similarity and differences between variables. In two dimensions Euclidian distance calculation is similar to Pythagorean theorem.

$$\mathrm{d}(\mathbf{p},\mathbf{q}) = \sqrt{(p_1 - q_1)^2 + (p_2 - q_2)^2}.$$

To analyse PCA, "vegan" package was installed and environmental variables were fed to the "rda" function. The PCA model was built by forward selection procedure (permutation, n = 999). In the PCA plot the relative length of the vectors shows the amount of variation in the variables that are shown in two dimensions. In the output, PCA eigenvalues (ƛ) also called variance of components (from a correlation matrix for all the predictor variables) close to zero indicate collinearity and component loadings may be useful in determining which predictor variables cause collinearity (Logan 2010; Field et al, 2012).

3.9 Percent reduction calculation

The potential of wetlands to reduce or remove nutrients and organic pollutants was calculated, for TP, PO4-P, NO_3-N, NH_4-N, DIN (NO_3-N + NH_4-N), EC, Cl^-, BOD, and DO, using the following formula:

$$\% \text{ Reduction } = \frac{C_{in} - C_{out}}{C_{in}}$$

Where C_{in} is the concentration in the incoming water and C_{out} is concentration in the outgoing water

3.10 Ethical consideration

A letter from Addis Ababa Institute of Technology was submitted to Jimma University Environmental health science and technology department to ask for their cooperation in providing the necessary sampling equipment's and to get permission for working in the laboratory. Copy of support letter was submitted to Jimma town Administration office in order to get permission to take water samples from the wetlands and to make professional observation on the surrounding environment.

3.11 Data dissemination plan

The findings of the study will be presented to Addis Ababa institute of technology. A copy of the study will be given to Jimma University meanwhile trying to present it on research conferences and looking forward for ways to publish the paper on scientific journals.

4. Result and discussion

4.1 Association among environmental variables

Environmental variables indicate the status of the water during the sampling event. The result obtained from Kruskal-wallis test ($P<0.05$) showed the non-normal distribution of variables among groups. Therefore spearman correlation was employed to see the association among environmental variables. Correlation is a technique which is used to assess the degree to which variables vary together (Logan, 2010).

Table 2. Spearman correlation among environmental variables (based on the overall data; n=20).

	EC	TP	PO4	TN	BOD	DO	PH	T°	NO3	NH4	DIN	Cl
EC	1.00											
TP	**0.92**	1.00										
PO4	**0.91**	**0.99**	1.00									
TN	**0.98**	**0.98**	**0.97**	1.00								
BOD	0.28	0.07	0.06	0.13	1.00							
DO	-0.24	-0.15	-0.15	-0.21	-0.32	1.00						
PH	-0.06	0.50	0.45	0.48	0.19	-0.06	1.00					
T°	0.06	0.04	0.01	0.06	-0.14	0.36	-0.01	1.00				
NO3	0.21	0.08	0.06	0.15	0.50	0.19	0.22	-0.03	1.00			
NH4	**0.79**	0.48	0.43	**0.55**	0.53	-0.27	**0.58**	0.06	0.48	1.00		
DIN	**0.59**	0.40	0.36	0.48	0.59	-0.44	**0.53**	0.04	**0.73**	**0.95**	1.00	
CL	-0.31	-0.43	**-0.63**	-0.39	0.04	-0.27	-0.02	**-0.56**	-0.09	-0.49	-0.36	1.00

Key: TP = total phosphorus; PO_4-P = orthophosphate phosphorus; TN = total nitrogen; NO_3-N = nitrate nitrogen; NH4-N = ammonia nitrogen; DIN = dissolved inorganic nitrogen; BOD = biochemical oxygen demand; DO = dissolved oxygen; EC= electrical conductivity; Tur = turbidity; pH = potential of hydrogen, and T° = Temperature

Based on the overall spearman correlation analysis (n=20) of environmental variables, both total phosphorus and orthophosphate were highly correlated with total nitrogen (TP, r = 0.98; PO_4, r = 0.97) and electrical conductivity (TP, r = 0.92; PO_4, r = 0.91). Total nitrogen was highly correlated with electrical conductivity (r = 0.98). Dissolved inorganic nitrogen was correlated with nitrate (r = 0.73) and ammonia (r = 0.95). Chloride showed a negative correlation with orthophosphate (r = -0.63) and temperature (r = -0.56). The presence of positive correlation between variables tell us the increase in one variable might result in an increase in the other variable but it doesn't

differentiate between the cause and the response variable. The negative correlation implies that the increase in one variable causes a decrease in the other variable.

Table 3. Spearman correlation among environmental variables (based on data of Boye wetland data; n=8)

	EC	TP	PO_4	TN	BOD	DO	PH	T°	NO_3	NH_4	DIN	Cl^-
EC	1.00											
TP	**0.93**	1.00										
PO4	**0.93**	**0.99**	1.00									
TN	**0.97**	**0.99**	**0.99**	1.00								
BOD	0.35	0.09	0.08	0.22	1.00							
DO	-0.25	-0.21	-0.20	-0.24	-0.15	1.00						
PH	**0.88**	**0.70**	**0.70**	**0.78**	0.49	-0.15	1.00					
T°	-0.24	-0.21	-0.21	-0.23	-0.09	**0.77**	-0.03	1.00				
NO3	0.29	0.06	0.04	0.18	**0.96**	0.10	0.46	0.14	1.00			
NH4	0.55	0.29	0.27	0.41	**0.88**	-0.45	**0.63**	-0.45	**0.77**	1.00		
DIN	0.50	0.23	0.21	0.36	**0.95**	-0.29	**0.61**	-0.28	**0.88**	**0.98**	1.00	
Cl	-0.29	-0.09	-0.14	-0.19	-0.45	0.49	-0.36	**0.51**	-0.30	**-0.52**	-0.48	1.00

Here the correlation matrix was built by extracting the data of Boye wetland only. Similar to the overall correlation matrix, electrical conductivity showed a strong positive correlation with total phosphorus (r = 0.93), orthophosphate (r = 0.93), and total nitrogen (r = 0.97). Both TP (r = 0.99) and PO_4 (0.99) were correlated with TN. Although the strength of correlation is similar, the value of correlation coefficient (r) differs between the above two correlation matrixes. In this correlation matrix NO_3 (r = 0.96), NH_4 (r = 0.88), and DIN (r = 0.95), presented a strong correlation with biochemical oxygen demand. This is different from the value observed in the overall correlation (n=20)

Table 4. Spearman correlation among environmental variables (based on data of Kitto wetland data; n=6)

	EC	TP	PO4	TN	BOD	DO	PH	T°	NO3	NH4	DIN	Cl
EC	1.00											
TP	**-0.34**	1.00										
PO4	**0.64**	-0.34	1.00									
TN	-0.10	-0.47	-0.28	1.00								
BOD	0.33	-0.44	0.49	-0.56	1.00							
DO	-0.42	**0.96**	-0.43	-0.37	-0.46	1.00						

PH	-0.54	**0.89**	-0.44	-0.25	-0.49	**0.98**	1.00					
T°	0.13	**-0.82**	0.19	**0.61**	0.07	**-0.89**	**-0.83**	1.00				
NO3	-0.36	**0.95**	-0.44	-0.40	-0.41	**0.99**	**0.96**	**-0.92**	1.00			
NH4	-0.02	**-0.78**	0.05	0.15	0.59	**-0.81**	**-0.79**	**0.74**	**-0.80**	1.00		
DIN	-0.39	**0.95**	-0.46	-0.42	-0.39	**0.99**	**0.95**	**-0.91**	**0.99**	-0.33	1.00	
Cl	-0.31	0.51	-0.42	0.03	-0.36	**0.73**	**0.80**	**-0.74**	**0.74**	**-0.68**	**0.73**	1.00

Unlike the above two correlation matrixes (the overall (n=20) and based on Boye (n=8), here electrical conductivity was negatively correlated with TP (-0.34) and TN (-0.10) though the correlation is weak. TP showed a strong positive correlation with DO (0.96), pH (0.89), NO_3 (0.95), and DIN (0.95); and a negative correlation with T° (-0.82) and NH_4 (-0.78). In this matrix DO showed a strong positive correlation with pH (0.98), NO_3 (0.99), DIN (0.99), and negative correlation with T° (-0.89), and NH_4 (-0.81). Chloride was positively correlated with DO (0.73), pH (0.80), NO_3 (0.74), and DIN (0.73), and negatively correlated with T°(-0.74) and NH_4(-0.68).

Table 5. Spearman correlation among environmental variables (based on data of Fisho wetland data; n=6)

	EC	TP	PO_4	TN	BOD	DO	PH	T°	NO_3	NH4	DIN	Cl
EC	1.00											
TP	**0.84**	1.00										
PO4	-0.39	-0.13	1.00									
TN	0.2	0.55	**0.66**	1.00								
BOD	**0.66**	0.41	**-0.65**	-0.03	1.00							
DO	**-0.77**	**-0.81**	0.13	**-0.6**	**-0.71**	1.00						
PH	**0.66**	0.32	-0.39	-0.14	0.54	-0.26	1.00					
Tø	**-0.67**	**-0.71**	**0.66**	-0.12	**-0.78**	**0.75**	-0.23	1.00				
NO3	-0.46	-0.1	0.13	0.29	-0.06	-0.12	**-0.81**	-0.22	1.00			
NH4	0.03	0.46	0.13	**0.66**	0.14	-0.43	-0.14	-0.46	**0.55**	1.00		
DIN	**-0.54**	-0.17	0.13	0.26	-0.09	-0.03	**-0.77**	-0.17	**0.98**	**0.60**	1.00	
Cl	0.37	0.55	**-0.65**	-0.09	0.37	-0.37	-0.08	**-0.84**	0.35	0.43	0.31	1.00

Electrical conductivity was positively correlated with TP (0.84), BOD (0.66), and pH (0.66), and negatively correlated with DO (-0.77), T°(-0.67), and DIN (-0.54). TP showed negative correlation with DO (-0.81) and T° (-0.71), and positive correlation with Cl (0.55). Like the first two matrixes PO_4 was positively correlated with TN (0.66) but here the strength of correlation is lower. Chloride showed strong negative correlation with T° (-0.84).

4.2 **Spatial variation in environmental variables**

The three wetland sites were compared based on Mann Whitney Wilcoxon test. This test makes a pairwise comparison (compares two groups at a time) (Logan, 2010). As presented in table 6, the mean value of most of environmental parameters revealed a significant difference among wetlands.

Based on the output, Temperature (T°), Potential of hydrogen (pH), and electrical conductivity (EC) were significantly higher in the wetland located in urban downstream than those wetlands located in urban upstream of urban area (Kitto and Fisho wetlands). The concentration of total phosphorus (TP), orthophosphate (PO_4), and ammonia (NH_4) were also significantly higher in Boye wetland compared to Kitto and Fisho wetlands. There was no significant difference in environmental variables, except for BOD, between the two upstream wetlands (Kitto and Fisho wetlands) (see Table 7).

Table 6. Spatial variation of physicochemical parameters among wetland sites (Wilcoxon Rank sum test) mean ± standard deviation

	Urban Downstream wetland (Boye)	Urban Upstream wetland 1 (Kitto)	Urban Upstream wetland 2 (Fisho)
Parameters	n=8	n=6	n=6
pH***	8.51(0.10)a	7.72(0.17)b	8.12(0.18)b
EC (µs/cm)**	417 (116)a	141 (25)b	185 (10)b
TUR (NTU)	35.1 (6.3)	26.3 (2.8)	19.7 (6.9)
TP (mg/l)*	0.96(0.58)a	0.03(0.01)b	0.06(0.02)b
PO_4 (mg/l)**	0.67(0.52)a	0.001(0.001)b	0.003(0.002)b
TN (mg/l)	9.39(5.02)a	1.71(0.38)b	2.04(1.16)b
NO_3-N (mg/l)	0.53(0.31)	0.26(0.2)	0.56(0.32)
NH_4-N (mg/l)*	2.29(0.70)a	0.14(0.02)b	0.07(0.03)b
DO (mg/l)	3.12(1.24)ab	4.30(0.79)b	2.11(0.93)a
BOD (mg/l)***	38.5(18.3)ab	20.3(3.7)b	57(0.1)a
Cl^- (mg/l)	2.62(0.2)	10.25(2.55)	26.83(12.18)

"*" shows significant difference where, "*" = P < 0.05, "**"= P < 0.005, "***"= P < 0.001 (Wilcoxon rank-sum test). "a" and "b" indicate significance difference among sites.

The variation in the above mentioned environmental variables indicated the presence of high pollution gradient in Boye wetland. This might be due to the difference in the effluent quality or in the extent of catchment disturbance. It can be attributed to the location of the wetlands, since Boye wetland is located downstream of residential and commercial areas (as mentioned in wetland description). Since there is no liquid waste treatment plant in the town, the streams

Figure 4. A picture taken adjacent to Boye wetland showing the site which is considered as a waste disposal site for the nearby residents (A) and vehicle washing activity (B) (Photo credit: Adey Sileshi)

passing through residential and commercial areas are the receivers of domestic liquid waste. One of the incoming water which joins Boye wetland is Awaitu River, which is known to be the major carrier of the town's liquid waste. The other possible reason can be the difference in the extent of catchment disturbance. Although there are disturbances in the three wetlands the extent of anthropogenic activities which result in catchment disturbance is different. For example as shown in Figure 4, car washing and waste disposal are among the major disturbances in Boye wetland.

Table 7. Statistical summary for the spatial variation of physicochemical parameters between wetland sites based on Wilcoxon rank sum test. “P-corrected” is the p value after applying Bonferroni correction**.**

Boye ~ Kitto	W	P-value	P-corrected
TP	47	0.004	0.04
TN	40	0.004	0.04
PO4-P	47	0.003	0.03
NO3-N	24.5	0.1	1
NH4-N	47	0.001	0.01
BOD	26	0.84	8.4
DO	12	0.14	1.4
T°	32.5	0.3	3
Cl	7	0.03	0.3
TUR	30	0.49	4.9
pH	48	0.001	0.01
EC	46	0.003	0.03
Boye ~ Fisho			
TP	43	0.001	0.01
TN	40	0.005	0.05
PO4-P	45	0.001	0.01
NO3-N	32	0.33	3.3
NH4-N	47	0.001	0.01
BOD	12	0.14	1.4
DO	33.5	0.24	2.4
T	38.5	0.07	0.7
Cl	12.5	0.15	1.5
TUR	38	0.08	0.8
EC	44	0.001	0.01
pH	40	0.04	0.4

Fisho ~ Kitto			
TP	8	0.13	1.3
TN	22	0.59	5.9
PO_4-N	12	0.29	2.9
NO_3 -N	22	0.57	5.7
NH_4-N	14	0.59	5.9
BOD	5	0.004	0.04
DO	31	0.004	0.04
T°	27.5	0.15	1.5
Cl	16	0.82	8.2
TUR	26	0.24	2.4
EC	95	0.20	2
pH	11	0.31	3.1

Boxplots are powerful representations of location (mean value), variability, and data distribution of a small sample size. In this study, the boxplots also showed an increased concentration of nutrients (total phosphorus, orthophosphate, dissolved inorganic nitrogen, and total nitrogen) in the downstream wetland site. These plots also confirmed the non-normal distribution of data in each group. In all of the cases the box plot of downstream wetland (Dw) presented a higher discrepancy of values from the mean. This can be due to the difference in site (sampling point) characteristics (quality) within the same wetland. Ambelu et al. (2012) also indicated the presence of high pollution gradient in Boye wetland.

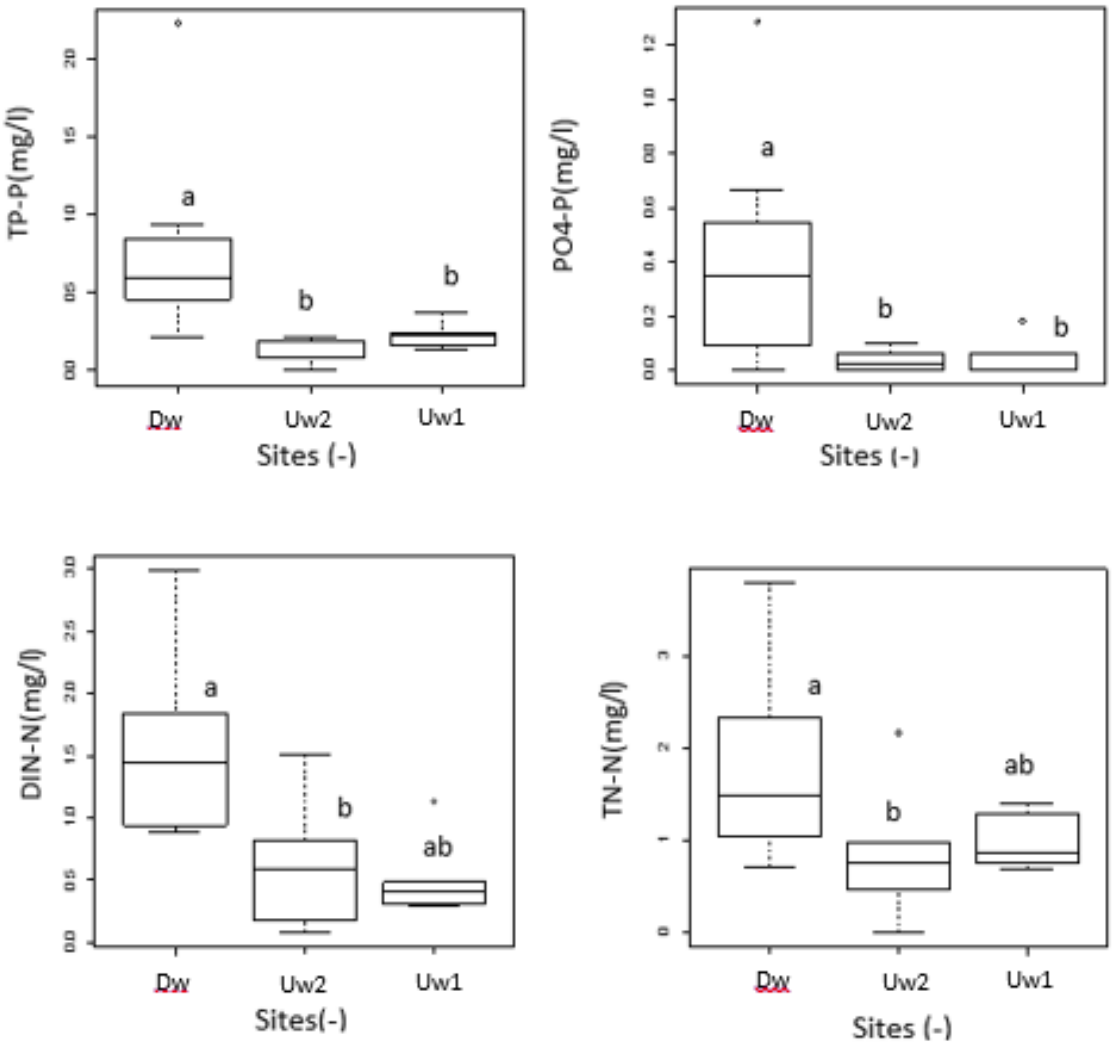


Figure 5 Variation in nutrient concentration among wetlands. DW = urban downstream wetland (Boye); UW2 = urban upstream wetland 2 (Fisho); UW1 = urban upstream wetland (Kitto)

4.3 **Water quality gradient among different zones**

(Different zones represent Sites before joining the wetland, within the wetland, and after passing through the wetland)

In order to assess the presence of significant retention or reduction of nutrients, and organic pollutants, statistical comparison was done by grouping wetland sites as those before confluence

of the wetland (streams); within the wetland and after passing through the wetland (See Figure 6 and Table 8).

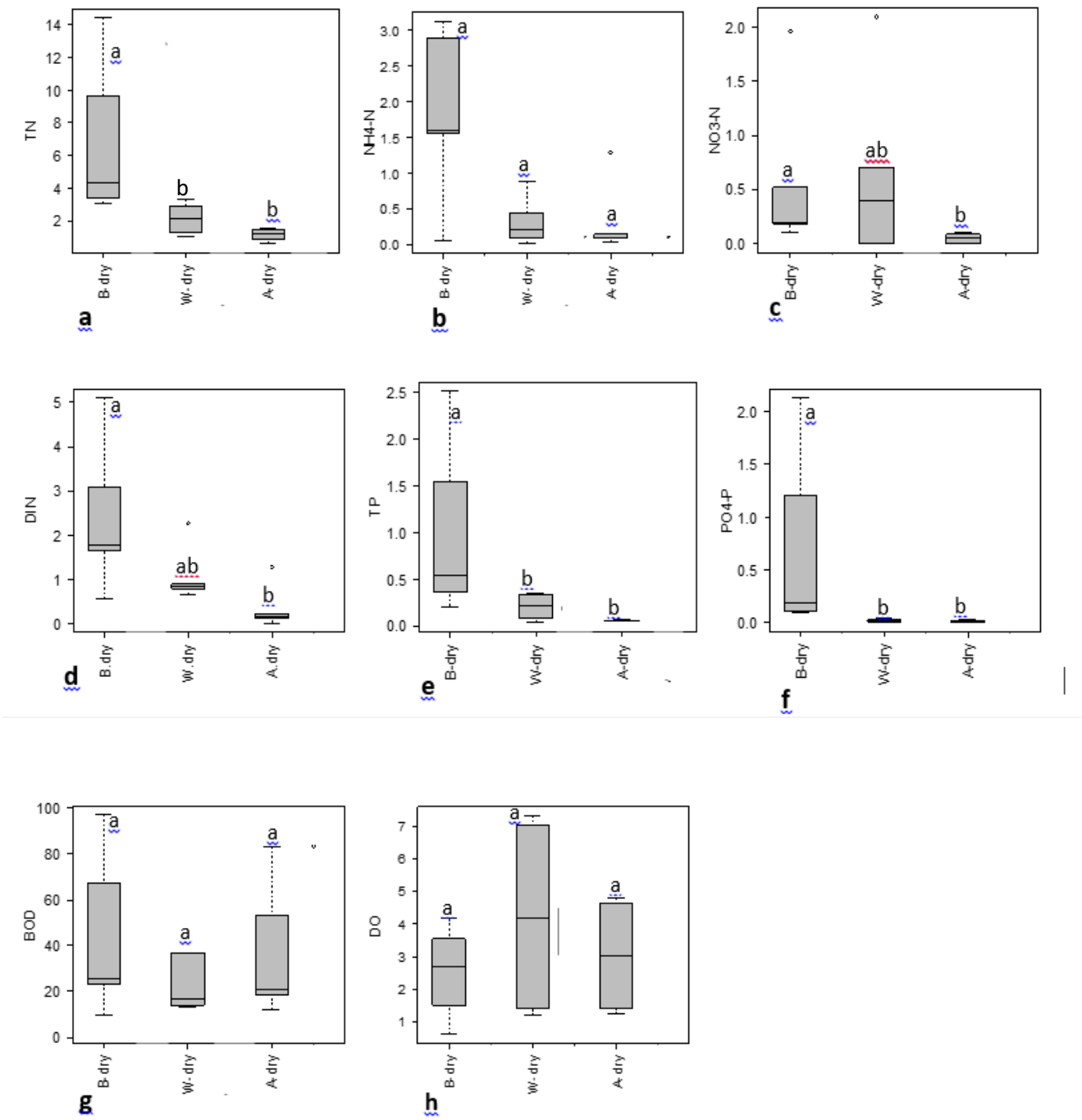


Figure 6. Nutrients (a, b, c, d, e, f), biochemical oxygen demand (g), and dissolved oxygen (h) gradient among the different zones. Pooled value of the three wetlands; B, W, and A are before

joining the wetland, within the wetland, and after passing through the wetland respectively. “a and b”, indicates significant difference among different sampling zones

A significantly lower concentration of TN (P = 0.03) and DIN (P = 0.02), and a considerably lower NH_4-N were observed in sites after passing through the wetland compared to those sites before joining the wetland (Fig. 6, a, b and d). This can be due to the presence of well-oxygenated environment within the wetlands (see Figure 6, h) which in turn facilitate the nitrification process, or uptake by plants (Metcalf and Eddy, 1991). The presence of high dissolved oxygen suppresses the process of denitrification (Gómez *et al*., 2002), since denitrification takes place in the anaerobic environment. There was a slight increase in the concentration of NO_3-N in sites located within the wetlands (See Figure 6. c). This might be due to the conversion of ammonia to NO_3-N or limited denitrification. However, there was a significant reduction in the effluent compared to the influent (p = 0.01).

Concerning phosphorus, a retention of TP and PO_4-P was revealed by the reduction in sites within the wetland and after passing through the wetland. These results are inline with a review done by Fisher and Acreman (2004) on the potential of natural wetlands to remove nutrients. The reduction in PO_4-P might be due to uptake by aquatic plants while the TP could be bound to particulate matter and sedimented as the residence time in the water is longer. The presence of vegetation and relatively flat topography of wetlands contribute to the higher residence time of the water in it. A combination of such processes reduce the nutrient load to the receiving water body, thereby prevent the accelerated rate of eutrophication.

Table 8. Statistical summary for comparision of nutrient, DO, and BOD among sites Before joining the wetland (B), within the wetlands (W), and after passing through the wetlands (A). P value was calculated with a 95% confidence interval (Kruskal-Wallis followed by Mann-Whitney Wilcoxon). B (n = 8); W (n=6), and A (n=5). “W” is the Wilcoxon statistics.

Parameters	Comparison groups	P-value	W
NH4-N	B*w	0.09	21
	B*A	0.09	4
	W*A	0.92	11.5
NO3-N	B*w	1	13
	B*A	0.01	0

	W*A	0.39	8
DIN	B*w	0.31	18
	B*A	0.02	1
	W*A	0.09	4
TN	B*w	0.06	15
	B*A	0.03	0
	W*A	0.2	3
PO4-P	B*w	0.3	16
	B*A	0.03	0
	W*A	0.55	5.5
TP	B*w	0.04	15.5
	B*A	0.03	0
	W*A	0.3	4
DO	B*w	0.69	6
	B*A	0.69	10
	W*A	0.69	6
BOD	B*w	0.55	16
	B*A	0.69	10
	W*A	0.55	16

4.4 Percent reduction of nutrients and organic pollutants

The percent reduction of nutrients and organic pollutants differs among wetlands (see Figure 7). The variation in percent reduction among wetlands can be attributed to the difference in influent quality, and extent of catchment disturbance. Wastewater, especially domestic waste waters, are the major source of ammonia and phosphates (Resende *et al*., 2010).

Among the three study wetlands, the concentration of nutrients and electrical conductivity were significantly higher in sites associated with Boye (urban downstream wetland) than Kitto and Fisho wetlands (located urban upstreams) (Table 6). This is because the streams associated with Boye (b1-b4) are the major carriers of Jimma town's liquid waste (Ambelu *et al*., 2012).

This study revealed that the natural riverine wetlands of Jimma town exhibited a potential to retain nutrients (TN, NH_4-N, TP, and PO_4-P) and organic pollutants (measured as BOD). However there is a difference in reduction capacity among the three wetlands.

It seems that Boye wetland showed the highest reduction of TP (95%), TN (91.4%), PO_4 (97.3%), DIN (76.42), and EC (65.5%). But it doesn't mean that Boye has a higher purifying capacity since the load of nutrients and level of EC in the incoming streams of the three wetlands is different. The load of nutrients, and electrical conductivity was higher in Boye wetland as indicated in Table 6 and Figure 5. This might magnify the % reduction value. Kitto wetland reduced DIN by 72.8% but didn't show a reduction in TN. The reduction in DIN can be associated with the nitrification-denitrification processes which remove NH_4 and NO_3 from the system (Gómez *et al*., 2002). The increase in the level of TN in Kitto wetland might be associated with organic nitrogen since total nitrogen is the sum of nitrate, nitrite, organic nitrogen and ammonia.

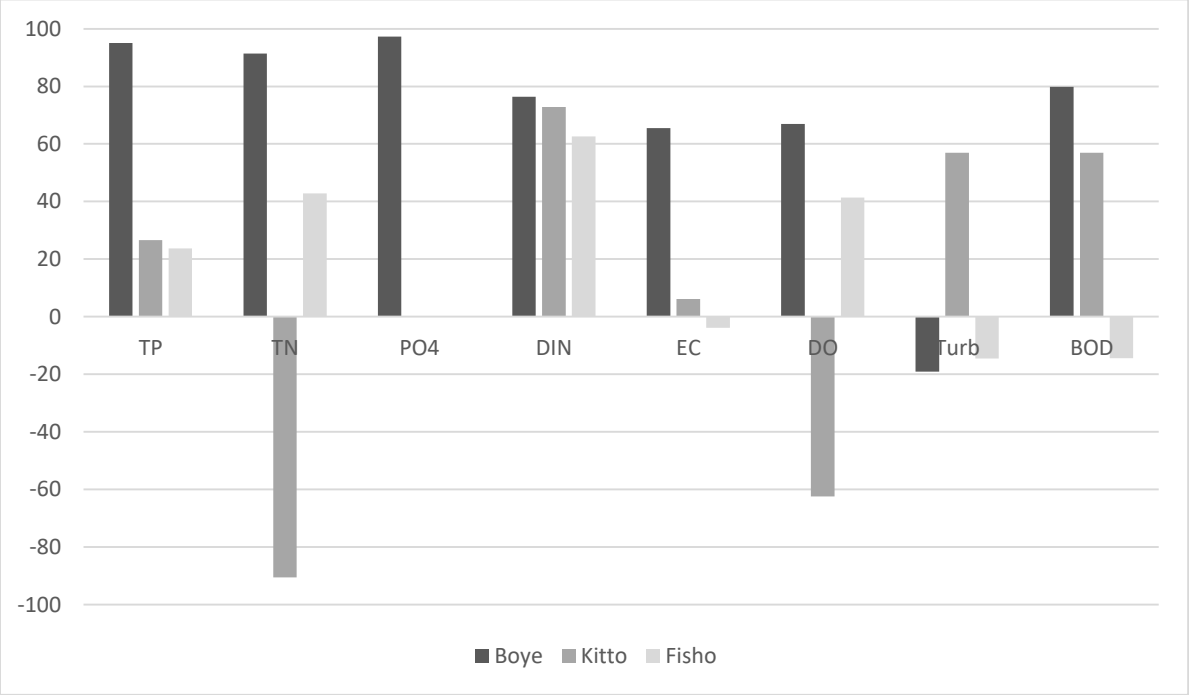


Figure 7. Percent reduction of nutrients and biochemical oxygen demand (BOD), and percent improvement of dissolved oxygen (DO) by natural riverine wetlands

Kitto wetland presented 56.9 % reduction in BOD, 62.4% reduction in the level of DO. A reduction in the level of BOD implies the degradation of organic matter mainly by aerobic biological activity (Bateganya et al., 2016). Through this process of degradation, the microbes used up the dissolved oxygen present in water and this explained the decrease in the level of DO observed in Kitto wetland. Boye wetland also exhibited 79.86% reduction of BOD, and 67% improvement in DO.

Normally the expectation was to see a reduction in the level of DO since there is a process which consumes DO but it was not the case in Boye wetland. This discrepancy might be due the release of oxygen from the photosynthesis process of vegetation within the wetlands however, it still needs further investigation.

All the three wetlands reduced TP (Boye= 95.4%; Kitto=26.5% and Fisho= 23.6%) and DIN (Boye= 76.4%; Kitto= 72.8 and Fisho=62.5%). These results are comparable with a study done in Uganda on the buffering capacity of urban natural wetlands (Bateganya et al., 2016).

4.5 The relation between sites and environmental variables: PCA model

A principal component analysis (PCA) model which was built by a forward selection procedure (permutation, n = 999) showed an overall model significance with a p-value of 0.005 (Monte Carlo permutation test, n = 999).

Since we have included eight environmental variables, the output of PCA analysis generated eight principal components. The principal components with the higher Eigen value explained the higher proportion of the variance (site-environment relation). In this model, the first axis explained 41.8% (PC1 and the second axis explained 24.1%. the cumulative proportion explained by the first two principal components is 65% (Table 9).

Table 9. Output of principal component analysis using “rda” function

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Eigen value	3.3458	1.9299	1.2141	0.67860	0.64057	0.15832	0.02774	0.005054
Proportion explained	0.4182	0.2412	0.1518	0.08482	0.08007	0.01979	0.00347	0.000630
Cumulative proportion	0.4182	0.6594	0.8112	0.97611	0.99590	0.99590	0.99937	1.000000

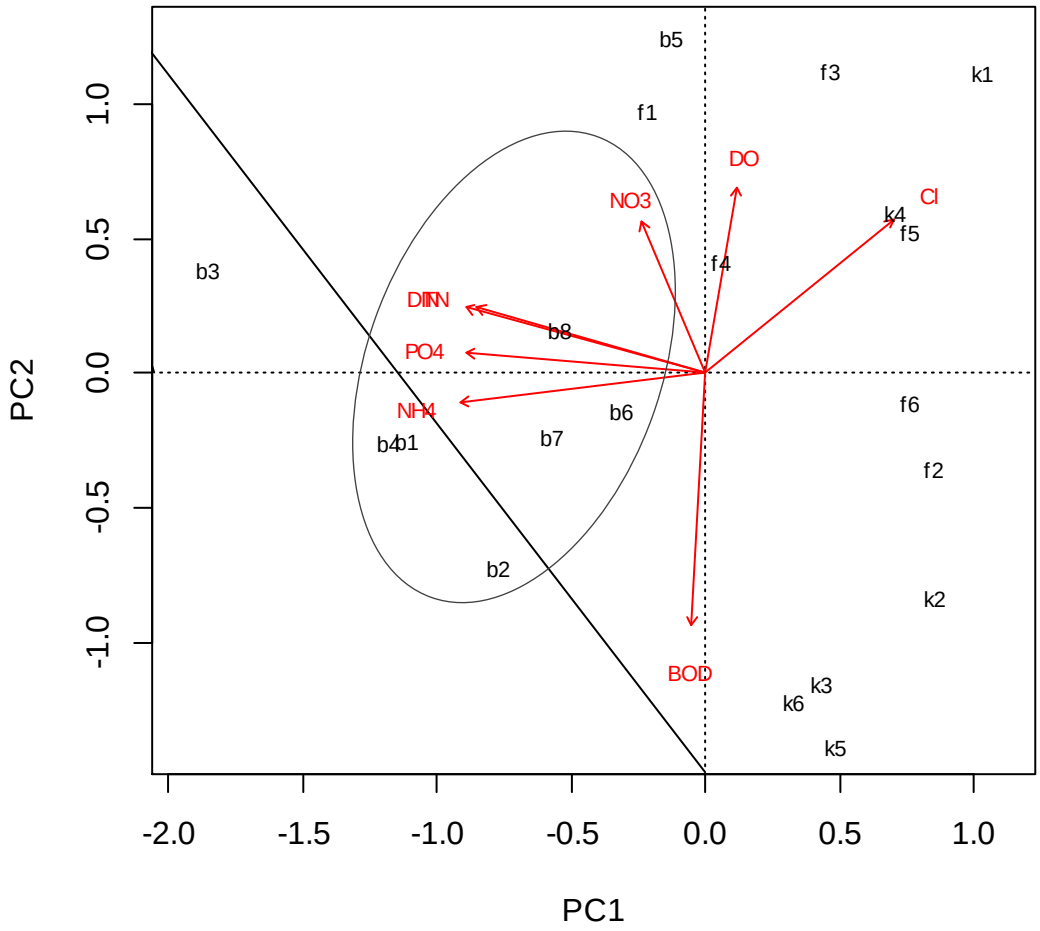


Figure 8. PCA biplot of environmental variables versus sites

The first axis of PCA (PC1) showed nutrient gradient and the second axis (PC2) showed pollution gradient. The first axis was positively correlated with DO and negatively correlated with BOD. Streams attributed to urban downstream wetland were characterized by high level of nutrients such as PO_4 , DIN, NH_4 and TN. In the negative first axis some sites of the upstream wetlands (Kitto wetland; k2, k3, k5, and k6,) were positively correlated with BOD and negatively correlated with DO since they are located in the same direction of BOD and opposite to DO. This indicated that these sites have higher BOD and low level of dissolved oxygen. Some sites of Fisho wetland (f2 and f6) revealed lower level of nutrients since they are directed a way from nutrients. One

site from kitto wetland (k4) which is located within the wetland, and in one of Fisho outlet (f5) were highly correlated with chloride.

Conclusions and recommendations

The gradient of nutrients, BOD, and DO before and after joining the wetlands showed retention of pollutants within the wetlands. Significantly lower concentration of TN, NH4-N, NO3-N, DIN, TP, and PO4-P was observed in sites after passing through the wetlands compared to those before joining the wetlands. Although BOD and DO didn't show a significant, a decrease in concentration of BOD and an increase in concentration of DO was observed in sites after joining the wetlands. In conclusion, this study proved the potential of natural riverine wetlands in Jimma, Ethiopia, to retain nutrients and organic pollutants. However, percent reduction of nutrients and organic pollutants varied among wetlands in relation to effluent water quality and catchment disturbance.

Based on the findings of this study its recommended that future integrated wetland management interventions should also target the incoming streams or rivers to reduce the nutrient and organic loading to wetlands.

Future studies should include different hydrological parameters such *as* hydraulic retention time, water depth, stream width etc. in order to identify the major processes which play a governing role in the process of nutrient removal.

References

Ambelu A, Mekonen S, G/Silassie A, Malu A and Kaliyaperumal K, 2013; Physicochemical and Biological Characteristics of two Ethiopian Wetlands.

American Public Health Association, A.W.W.A, Federation, T.W.a.E. 2005; *Standard methods for the examination of water and wastewater.* American Public Health Association (APHA), Washington, DC, USA.

Ansari, A. A., Gill, S. S., & Khan, F. A. (2010). Eutrophication: threat to aquatic ecosystems. In *Eutrophication: causes, consequences and control* (pp. 143-170). Springer Netherlands.

Babayemi, J.O., Ogundiran, M.B. & Osibanjo, O., 2016. Overview of Environmental Hazards And Health Effects Of Pollution In Developing Countries: A Case Study Of Nigeria. *J. Environmental Quality Management*, ***26*(1),** pp.51-71.

Bateganya, N.L., Nakalanzi, D., Babu, M. & Hein, T., 2015, Buffering Municipal Wastewater Pollution Using Urban Wetlands In Sub-Saharan Africa: A Case Of Masaka Municipality, Uganda. *J. Environ. Technol.*, ***36*(17),** pp.2149-2160.

Beuel, S., *et al*., 2016; A Rapid Assessment of Anthropogenic Disturbances In East African Wetlands. *J. Ecol. Indic.,* **67,** pp.684-692

Bodoque, J.M., et al., 2016; Recovering hydromorphological functionality to improve natural purification capacity of a highly human-modified wetland; *J. Ecological Engineering.*

Chapman, L. J., Chapman, C. A., Nordlie, F. G., & Rosenberger, A. E., 2001. Physiological refugia: Swamps, hypoxia tolerance and maintenance of fish diversity in the Lake Victoria region.

Chislock, M. F., Doster, E., Zitomer, R. a., & Wilson, A. E. (2013). Eutrophicati on : Causes , Consequences , and Controls in Aquati c Ecosystems. *Nature Education Knowledge*, (4), 1–8.

Clement,J. , Aquilina, L., Bour, O., Plaine, K., Burt, T., and Pinay, G., 2003; Hydrological flowpaths and nitrate removal rates within a riparian floodplain along a fourth-order stream in Brittany (France), *J. Hydrological processes.*

Crumpton, W.G., 2001. Using wetlands for water quality improvement in agricultural watersheds. The importances of a watershed scale approach. Water Sci Technol 44 (11 – 12): 559–564.

Davis, J., Horwitz, P., Norris, R., Chessman, B., McGurie, M., Sommer, B., Trayer, K., 1999. Wetland bioassessment manual for macroinveretebrates. National wetlands research and development program.

Devi, R., Tesfahune, E., Legesse, W., Deboch, B., Beyene, A., 2008. Assessment of siltation and nutrient enrichment of Gilgel Gibe dam, Southwest Ethiopia. Bioresource Technol. 99, 975–979

Dorgham, M. M. (2010). Eutrophication problem in Egypt. In *Eutrophication: causes, consequences and control* (pp. 171-194). Springer Netherlands.

Field, A., Miles, J., Field, Z., 2012; Discovering statistics using R

Fisher J. and Acerman M.C; 2004; wetlands nutrient removal; a review of the evidence.

Gebrselassie, H., Gashaw, T. & Mehari, A., 2014; Wetland Degradation In Ethiopia: Causes, Consequences, And Remedies. *J. Environment and Earth Science*, **4(11),** pp.40-48

Gómez, N., Sierra, M.V., Cortelezzi, A. and Capítulo, A.R., 2008; Effects Of Discharges From The Textile Industry On The Biotic Integrity Of Benthic Assemblages. *J. Ecotox. Environ. Safe*, ***69*(3),** pp.472-479.

Hanasaki, N., *et al*., 2013; A Global Water Scarcity Assessment under Shared Socio-Economic Pathways. *J. Hydrol. Earth Syst. Sc.*, ***17*(7),** p.2393

Heimann, D. C., Roell, M.J., 2000. Sediment loads and accumulation in a small riparian wetland system in northern Missouri. Wetlands 20, 219–231.

Huang, W., Chen, Q., Ren, K. & Chen, K., 2015; Vertical Distribution and Retention Mechanism Of Nitrogen And Phosphorus In Soils With Different Macrophytes Of A Natural River Mouth Wetland. *J. Environmental monitoring and assessment*, **187(3),** p.97

Joy B. Zedler and Suzanne Kercher; 2005; WETLAND RESOURCES: Status, Trends, Ecosystem Services, and Restorability.

Keith Thompson; 2012; Hydrological assessments of ten wetlands in the Wellington region and recommendations for sustainable management: a holistic approach.

Kumar, D., Asolekar, S.R. & Sharma, S.K., 2015, Post-Treatment And Reuse Of Secondary Effluents Using Natural Treatment Systems: The Indian Practices. *J. Environmental monitoring and assessment*, **187(10),** p.612

Lehner, B.& Döll, P., 2004; Development And Validation Of A Global Database Of Lakes, Reservoirs, And Wetlands. *J. Hydrology*, ***296*****(1),** pp.1-22

Liquete, C., Udias, A., Conte, G., Grizzetti, B. & Masi, F., 2016, Integrated Valuation Of A Nature-Based Solution For Water Pollution Control. Highlighting Hidden Benefits. *J. Ecosyst Serv.*, **22**, pp.392-401.

Logan M., 2010; biostatistics design and analysis using R/ a practical guide; A John Wiley & Sons, Inc., Publication.

Mereta, S. T., *et al.,* 2012; Analysis of Environmental Factors Determining The Abundance And Diversity Of Macroinvertebrate Taxa In Natural Wetlands Of Southwest Ethiopia. *J. Ecol. Inform*., **7(1),** 52-61

Mitchell, S. A. (2013). The status of wetlands, threats and the predicted effect of global climate change: The situation in Sub-Saharan Africa. *Aquatic Sciences*, *75*(1), 95–112.

Moges, A., Beyene, A., Triest, L., Ambelu, A. & Kelbessa, E., 2016; Imbalance Of Ecosystem Services of Wetlands And The Perception Of The Local Community Towards Their Restoration And Management In Jimma Highlands, Southwestern Ethiopia. *J. Wetlands*, pp.1-15

Najib Lukooya Bateganya, Diana Nakalanzi, Mohammed Babu & Thomas Hein, 2015; Buffering municipal wastewater pollution using urban wetlands in sub-Saharan Africa: a case of Masaka municipality, Uganda, Environmental Technology, 36:17, 2149-2160,

Nyenje, P. M., Foppen, J. W., Uhlenbrook, S., Kulabako, R., & Muwanga, A. (2010). Eutrophication and nutrient release in urban areas of sub-Saharan Africa - A review. *Science of the Total Environment*, *408*(3), 447–455.

R development Core Team R, 2015: A Language and Environment For Statistical Computing. R Foundation For Statistical Computing, Vienna, Austria, Available at: http://www.R-project.org/.

Ramsar Convention Secretariat, 2013; The Ramsar Convention Manual: a guide to the Convention on Wetlands, Sixth edition. Ramsar Convention Secretariat, Gland, Switzerland.

Razali, N.M. & Wah, Y.B., 2011; Power Comparisons of Shapiro-Wilk, Kolmogorov-Smirnov, Lilliefors and Anderson-Darling Tests. *J. Stat. Model*, **2(1),** pp.21-33

Rebelo, L. M., McCartney, M. P., & Finlayson, C. M. (2010). Wetlands of Sub-Saharan Africa: Distribution and contribution of agriculture to livelihoods. *Wetlands Ecology and Management*, *18*(5), 557–572.

Reinelt, L.E., Homer, R.R., 1995. Pollutant removal from storm water runoff by palustrine wetlands based on comprehensive budgets. Ecol Eng 4, 77-97.

Resende, P.C., Resende, P., Pardal, M., Almeida, S. & Azeiteiro, U. Use Of Biological Indicators To Assess Water Quality Of The Ul River (Portugal). *J. Environmental monitoring and assessment,* **170(1),** pp.535-544 (2010).

Seleshi, Y. and Zanke, U. 2004; Recent Changes In Rainfall And Rainy Days In Ethiopia. *J. Climatology* **24(8),** 973-983

Seleshi, Y. and Zanke, U., 2004; Recent Changes In Rainfall And Rainy Days In Ethiopia. *J. Climatology* **24(8),** 973-983.

Strokal, M., *et al*., 2016; Alarming Nutrient Pollution of Chinese Rivers As A Result Of Agricultural Transitions. *J. Environ Res. Lett*., **11(2),** p.024014

Strokal, M., Kroeze, C., Wang, M. & Ma, L., 2017; Reducing Future River Export Of Nutrients To Coastal Waters Of China In Optimistic Scenarios. *J. Sci. Total Environ.,* **579,** pp.517-528

Teferi, E., Uhlenbrook, S., Bewket, W., Wenninger, J., Simane, B., 2010. The use of remote sensing to quantify wetland loss in the Choke mountain range, upper Blue Nile basin, Ethiopia. Hydrol Earth Syst Sc 14(12), 2415-2428.

Verhoeven, J.T., Arheimer, B., Yin, C. & Hefting, M.M., 2006; Regional and Global Concerns Over Wetlands And Water Quality. *J. Trends Ecol. Evol*., ***21*(2),** pp.96-103

Williams, G., 1993: Wetland surface water processes. WRP technical note HY-Ev-2.1. USA: Ramsar convention bureau.

Zhenlou, C., X. Shiyuan, X. Qixin, H. Xuefeng, and Y. Lizhong. 2002. Surface Water Pollution in the Yangtze river delta: Patterns and counter measures. Pedosphere 12(2): 111-120.

Annex

Annex I. RECORDED DATA

Table 10. Environmental variables and their respective measured values

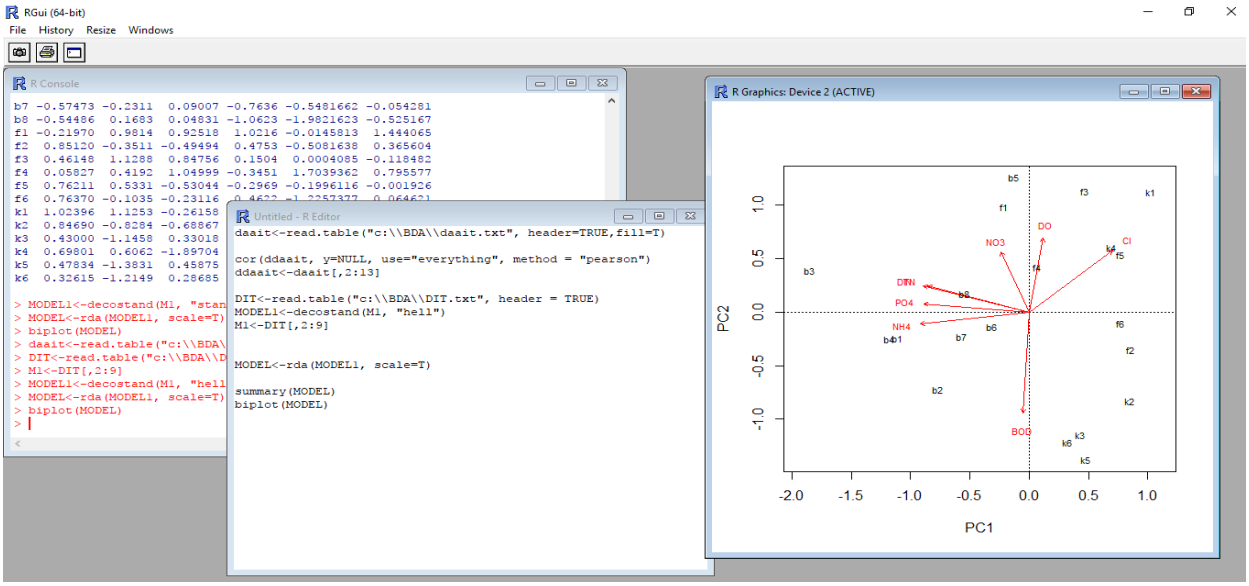
SITE	TUR	EC	TP	PO4	TN	BOD	DO	pH	T°	NO3	NH4	DIN	Cl
B1	15.4	495.0	0.38	0.20	6.35	30.0	1.4	8.76	25.1	0.21	3.11	3.32	2.50
B2	18.8	605.0	0.89	0.56	13.30	165.0	2.48	8.78	25.7	2.66	6.22	8.88	2.00
B3	28.3	1142.0	4.99	4.25	43.20	33.3	1.59	8.97	24.3	0.38	3.08	3.46	2.50
B4	25.0	244.0	0.57	0.19	4.83	23.3	0.43	8.3	22.1	0.19	2.88	3.07	3.00
B5	41.6	230.0	0.32	0.09	2.24	13.3	11.29	8.45	33.2	0.68	0.11	0.80	3.50
B6	67.8	192.4	0.35	0.01	2.31	17.3	1.95	8.18	27.5	0.08	0.78	0.86	3.25
B7	52.9	197.5	0.12	0.04	1.51	13.3	1.34	8.42	26.8	0.04	0.88	0.92	2.01
B8	30.7	231.0	0.05	0.03	1.40	12.0	4.47	8.2	22.7	0.00	1.29	1.29	2.25
F1	16.6	74.8	0.03	0.01	3.10	10.0	4.45	7.51	25	0.52	0.05	0.57	2.00
F2	22.2	225.0	0.04	0	1.13	21.3	2.32	7.95	24.3	0.00	0.00	0.01	8.50
F3	23.3	70.6	0.00	0	0.98	14.0	7.31	6.98	24.6	0.66	0.02	0.68	9.50
F4	27.9	166.7	0.04	0	2.64	36.7	2.15	7.83	23.3	2.07	0.21	2.28	13.50
F5	33.1	186.0	0.05	0	1.37	18.6	4.28	7.92	23.8	0.08	0.10	0.19	20.49
F6	34.6	125.4	0.01	0	1.05	20.9	5.29	8.15	24.6	0.00	0.03	0.03	7.50
K1	50.9	166.7	0.14	0	0.00	29.3	6.74	8.99	18	1.27	0.02	1.29	75.48
K2	8.6	199.0	0.02	0.01	1.19	101.3	0.76	7.93	22.1	0.00	0.10	0.10	41.49
K3	23.5	228.0	0.06	0.01	1.04	60.0	1.28	7.81	22.8	0.12	0.07	0.19	3.50
K4	9.8	178.0	0.03	0	7.75	16.0	1.04	8.01	25	0.00	0.09	0.09	36.49
K5	20.4	171.6	0.05	0	0.60	83.3	1.23	7.89	23.5	0.10	0.14	0.24	2.50
K6	5.2	171.8	0.06	0.01	1.67	53.3	1.59	8.09	24.1	0.05	0.09	0.14	1.50

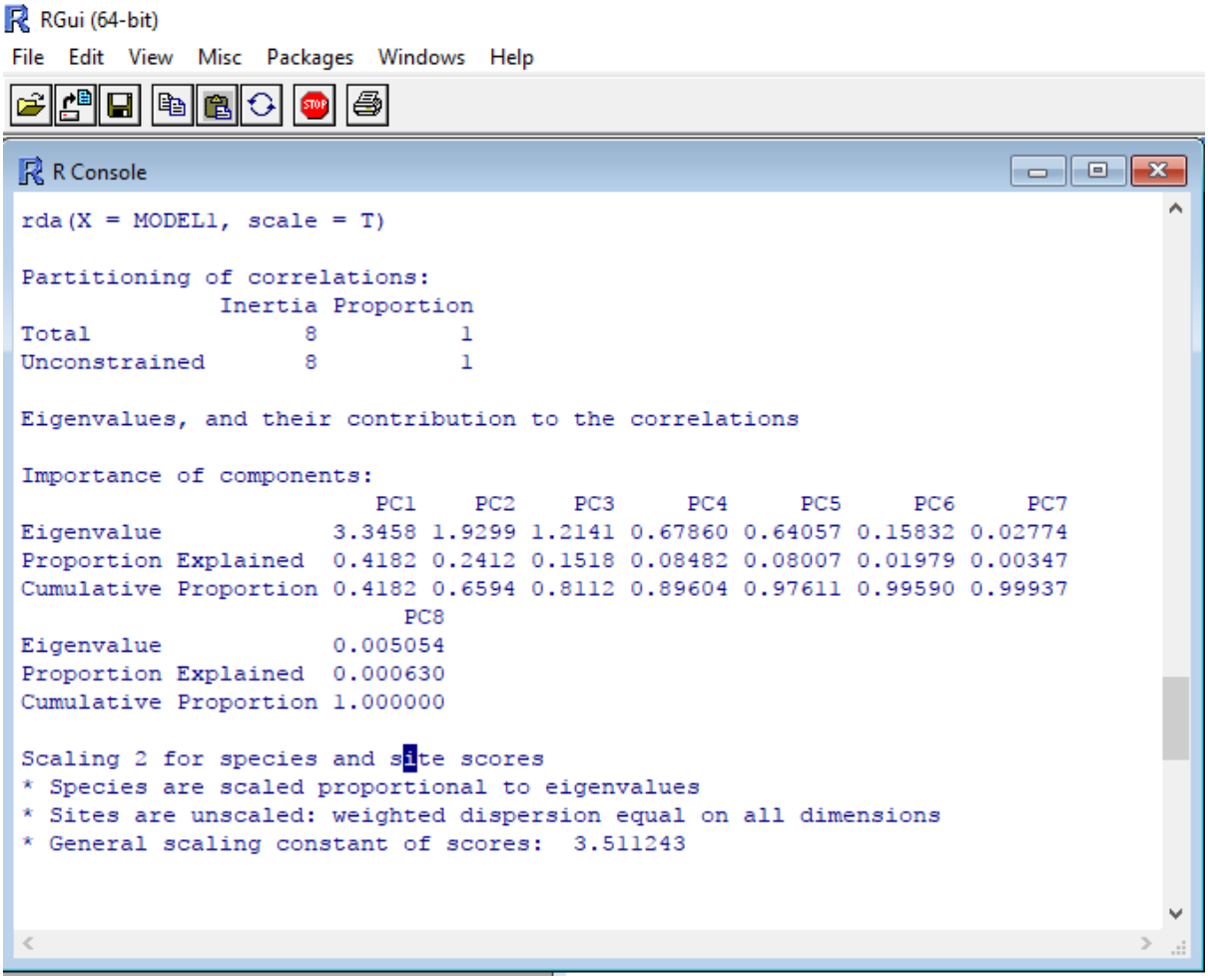
Annex II. Site codes

Table 11. Site codes and their respective full name, and associated wetland

Site code	local name	wetland	
B1	Kochie stream	Boye	Urban down stream
B2	Dololo stream	Boye	
B3	University stream	Boye	
B4	Awaitu river	Boye	
B5	Within the wetland 1	Boye	
B6	Within the wetland 2	Boye	
B7	Within the wetland 3	Boye	
B8	Outlet point	Boye	
F1	Degoye stream 1	Fisho	Urban upstream wetland 2
F2	Degoye stream 2	Fisho	
F3	Within the wetland 1	Fisho	
F4	Within the wetland 2	Fisho	
F5	Outlet point 1	Fisho	
F6	Outlet point 2	Fisho	
K1	Kitto stream 1	Kitto	Urban upstream wetland 1
K2	Kitto stream 2	Kitto	
K3	Within the wetland 1	Kitto	
K4	Within the wetland 2	Kitto	
K5	Outlet point 1	Kitto	
K6	Outlet point 2	Kitto	

Annex III. R sample out puts





Annex IV. Procedures for physicochemical analysis

1. Nitrogen (Ammonia): Nesslerisation method

Apparatus and equipment

a. Spectrophotometer: spectrophotometer having a range of 300 to 700nm.

b. Nessler tubes or 100mL capacity volumetric flasks.

Reagents and standards

a. Zinc sulphate: dissolve 10g ZnSO4.7H2O in distilled water and dilute to 100mL.

b. Sodium hydroxide, 6N: dissolve 24g NaOH and dilute to 100mL.

c. EDTA reagent: dissolve 50g EDTA in 60mL water containing 10g NaOH. Cool and dilute to 100mL.

d. Rochelle salt solution: dissolve 50g potassium sodium tartarate in 100mL. Remove ammonia by boiling off 30mL solution, cool and dilute to 100mL.

e. Nessler reagent: mix well 100g Hgl2 and 70g Kl. Dissolve in small quantity of water. Add this mixture to a cooled solution of 160g NaOH in 500mL water. Dilute to 1000mL. Keep overnight, store supernatant in coloured bottle.

f. Standard ammonium solution: dissolve 3.819g NH4Cl dried at 100°C in distilled water and dilute to 1000mL. Dilute 10mL of the solution to 1000mL. 1mL = 10硃g NH3.

Calibration Prepare a calibration curve using suitable aliquots of standard solution in the range of 5 to 120µg/100mL for reference following the same procedure as 1 to5 but using the standard solution in place of sample.

Procedure

a. Take 100mL of sample. Add 1 mL ZnSO 4 solution and 0.4 or 0.5 mL NaOH to obtain the pH of 10.5. Allow to settle and filter the supernatant through 42 No. Whatman filter paper.

b. Take suitable aliquot of sample

c. Add 3 drops of Rochelle salt solution or 1 drop of EDTA mix well. d. Add 3mL Nessler reagent if EDTA is used or 1mL if Rochelle salt solution is used. Make up to 100mL.

e. Mix well and read percent transmission after 10 minutes at 410nm using a blank prepared in the same way by taking distilled water instead of sample.

2**. NITRATE NITROGEN**
Phenoldisulfonic Acid Method

1. Determine the chloride content of the water sample and treat 100 mL with an equivalent amount of silver sulfate solution (1mL for l mg C1) to precipitate the chlorides.

2. Remove the precipitated chloride either by centrifugation or by filtration, coagulating the AgC1 by heat if necessary.

3. If the sample has color of more than 10 unit (on platinum cobalt scale), decolorize by adding 3 mL aluminum hydroxide suspension to 150 mL sample; stir very thoroughly; allow to stand for a few minutes; then filter, discarding the first portion of the filtrate.

4. Pipette a suitable quantity of the sample or the clarified filtrate into an evaporating dish and neutralize to approximately P H 7.

5. Evaporate to dryness over a hot water bath.

6. Add 2 mL phenoldisulfonic acid reagent and rub the residue thoroughly to insure dissolution of all solids. If needed heat on the water bath a short time to dissolve the entire reside.

7. Dilute with 20 mL of distilled water and add with stirring about 6 to 7 mL of NH4OH or about 5 to 6 mL KOH solution (12N) until maximum yellow color is developed.

8. Remove any resulting flocculent hydroxides by filtration or add the EDTA reagent drop wise with stirring until the turbidity re dissolves

9. Transfer the filtrate of clear solution to a 50-mL volumetric flask or graduated cylinder. Rinse the dish, glass rod and filter paper with distilled water, adding the rinsing to the flask or cylinder until all the colored solution has been transferred.

10. Dilute to the 50- mL mark with distilled water, and mix thoroughly

11. Measure the absorbance at a wave length of 410 nm against a blank prepared from the same volumes of reagents as used for the samples.

12. Construct a calibration curve in the range 0-2 mg/L NO3 – N by adding 0, 0.2, 0.5, 1.0, 3.0, 5.0, and 10 mL of standard nitrate solution to separate evaporating dishes and treating them in the same way as the sample.

13. Determine the μg of NO3- N in the sample by reference to the calibration curve.

14. Calculation: a) mg/L NO3-N = µg NO3-N mL sample b) mg/L NO3 = µg NO3- N X 4.427 mL sample
Note: Nitrite levels in excess of 0.2 mg/L erratically increase the apparent Nitrate concentration as it responds like nitrate. Hence, the nitrite must be converted to nitrate by a suitable oxidizing agent prior to the determination of nitrate.

3. PHOSPHATE
Stannous Chloride Method

A**) Determination of Orthophosphate**

1. Prepare the following series of phosphate standards by measuring the indicated volume of standard phosphate solution into separate 100 mL volumetric flasks (Or graduated cylinders).

2. To the sample, add 0.05 ml 1 drop) of phenolphthalein indicator solution. If the sample turns pink, add strong acid solution drop wise until the color is discharged

3. With a measuring pipette, add 4 mL acid- molybdate solution to each of the standards and sample

4. Mix thoroughly by inverting each flask four to six times.

5. With medicine dropper, add 0.5 mL (10 drops) of stannous chloride solution to each of the standards and sample.

6. Stopper and mix by inverting each flask four to six times

7. After 10 minutes, but before 12 minutes, measure the color photo metrically at 690 nm using distilled water as blank.

8. Construct a calibration curve using the standards and determine the amount of phosphate in µg present in the sample.

9. Calculation

Calculation a) mg/L PO_{43} = ∝g phosphate Ml of sample b) mg/L P =∝g PO_{43}-X 0.32614 Ml of sample C) mg/L P_2O_5 = ∝g PO_{43} x 1.4946 Ml of sample

B) **Determination of Total Phosphate**

1. Take a 50 mL sample in a 250 mL Erlenmeyer flask and dilute to 100 mL with distilled water

2. Add 1 drop (0.05 mL) of phenolphthalein indicator solution

3. If a pink color develops, add strong acid solution one drop at a time until the pink color disappears. Then add 1 mL extra of the acid solution.

4. Boil the acid- treated sample gently for 90 minutes, adding distilled water from time to time to keep the volume between 25 and 50 mL .

5. Cool to room temperature.

6. Stirring the sample constantly; add sodium hydroxide solution until a faint pink color reappears.

7. Transfer sample to a 100 mL volumetric flask or graduated cylinder

8. Rinse the flask, glass beads, and stirring rod with distilled water and add the wash to the flask/cylinder and dilute to the 100 mL mark with distilled water.

9. Complete the determination as described for orthophosphate starting with step 3.

10. Calculate the total phosphate using the formulae given for orthophosphate.

4. DISSOLVED OXYGEN (DO): The Azide Modification of the Winkler Method

1) Collection the sample in glass-stoppered BOD bottle of 250-300 mL capacity. Write down the volume of the bottle.

2) Remove the glass stopper from the sample bottle, using a measuring pipet, add 1 ml if manganous sulfate solution followed by 1 ml alkali-iodide-azide reagent. Place the tip of the pipet below the surface of the water so as to allow the heavy solution to flow in without contact with the air

3) Stopper carefully to exclude air bubbles and mix by inverting the bottle a few times

4) Allow the resulting precipitate to settle at least to one half the bottle volume to leave clear sup mate above the manganese hydroxide floc.

5) Remove the stopper again, and with measuring pipet, add 1ml conc. Sulphuric acid

6) Re stopper carefully to prevent air from entering the bottle Mix by inverting several times until the precipitate completely dissolves and the brown or yellow color is distributed uniformly.

7) Titrate with 0.025 N sodium thiosulfate solutions a volume corresponding to 200 ml original sample after correction for sample loss by displacement with reagents. Thus for a total of 2 ml of

reagents (1 ml each of MnSO4 and alkali-iodide- azide reagents) in a 300-ml, titrate 200x300 = 201 ml 298

8) Gradually add small portions of the sodium thiosulfate titrant while constantly swirling the liquid in the flask, until the sample changes to a pale yellow or straw color

9) Add a few drops of starch indicator solution and continue the titration to the first disappearance of the blue color.

10) Calculation mg/L DO=A x N x8000 Ml of sample Where: 28 28 A = ml sodium thiosulfate N= Normality of sodium thiosulfate

Note 1) if the end points is over run, add a measured volume of treated sample and titrate carefully to the proper end point. Correct for the amount of sample added.

2) Disregard subsequent re colorations.

5. BOD measurement: Titrimetric method

Equipment and apparatus

a. BOD bottles 300mL capacity (clean with a detergent, rinse thoroughly and drain before use) with a water seal.

b. Incubator or water-bath to be controlled at 20ºC or at any desired temperature 1ºC. Exclude all light to prevent photosynthetic production of DO.

Reagents and standards

All reagents listed in DO estimation are used for BOD. In addition following reagents are required:

a. Phosphate buffer: Dissolve 8.5g KH2PO 4, 21.75g K2HPO 4, 33.5g Na2HPO 4.7H2O and 1.7g NH4C; in distilled water and dilute to 1000mL. The pH should be 7.2without further adjustment. Discard reagent if there is any sign of biological growth.

b. Magnesium sulphate: Dissolve 22.5g MgSO 4.7H2O in about 700mL of distilled water and dilute to 1 Litre.

c. Calcium chloride: Dissolve 27.5g anhydrous CaCl2 in about 7000mL of distilled water and dilute to 1 Litre.

d. Ferric chloride: Dissolve 0.25g FeCl3.6H2O in about 700mL of distilled water and dilute to 1 L.

e. Sodium sulphate solution 0.025N: Dissolve 1.575g Na2SO 3 in distilled water and dilute to 1000mL. Solution should be prepared daily.

f. Acid and Alkali solutions 1N: Prepare 1N H2SO4 and 1N NaOH or neutralization of caustic or acidic samples.

g. Nitrification inhibitor: 2-chloro-6-(trochloromethyl) pyridine [Nitrification inhibitor 2570-24 (2.2% TCMP), Hach Co. equivalent]

h. Glucose-glutamic acid solution: Dry reagent grade glucose and glutamic acid at 103ºC for 1h. Dissolve 150 mg glucose and 150mg glucose acid in distilled water and dilute to 1000mL. Prepare fresh immediately before use.

Procedure

Preparation of dilution water:

a. The source of dilution water may be distilled water, tap or receiving-stream water free of biodegradable organics and bio inhibitory substances such as chlorine or heavy metals.

b. Aerate the required volume of dilution water in a suitable bottle by bubbling clean-filtered compressed air for sufficient time to attain DO saturation at room temperature or at 20ºC/27ºC. Before use stabilize the water at 20ºC/27ºC.

c. Add 1mL each of phosphate buffer, magnesium sulphate, calcium chloride and ferric chloride solutions in that order for each Litre of dilution water. Mix well. Quality of dilution water may be checked by incubating a BOD bottle full of dilution water for 5 days at 20ºC for 3 days at 27ºC. DO uptake of dilution water should not be more than 0.2mg/L and preferable not more than 0.1mg/L.

d. For wastes which are not expected to have sufficient microbial population, seed is essential. Preferred seed is effluent from a biological treatment system. Where this is not available, supernatant from domestic wastewater (domestic sewage) settled at room temperature for at least 1h but not longer than 36hours is considered sufficient in the proportion 1-2mL/L of dilution water. Adopted microbial population can be obtained from the receiving water microbial

population can be obtained from the receiving water body preferably 3-8 km below the point of discharge. In the absence of such situation, develop an adapted seed in the laboratory.

e. Determine BOD of the seeding material. This is seed control. From the value of seed control determine seed DO uptake. The DO uptake of seeded dilution water should be between 0.6mg/L and 1mg/L.

Sample preparation:

a. Neutralise the sample to pH 7, if it is highly acidic or alkaline.

b. The sample should be free from residual chlorine. If it contains residual chlorine remove it by using Na2S 2O 3 solution as described below.

c. Take 50mL of the sample and acidify with addition of 10mL 1 + 1 acetic acid. Add about 1g Kl. Titrate with 0.025N Na2S 2O 3, using starch indicator. Calculate the volume of Na2S 2O 3 required per Litre of the sample and accordingly add to the sample to be tested for BOD.

d. Certain industrial wastes contain toxic metals, e.g. planting wastes. Such samples often require special study and treatment.

e. Bring samples to 20 ± 1ºC before making dilutions.

f. If nitrification inhibition is desired, add 3mg 2-chloro-6-(trichloromethyl) pyridine (TCMP) to each 300mL bottle before capping or add sufficient amount to the dilution water to make a final concentration of 30mg/L. Note the use of nitrogen inhibition in reporting results.

g. Samples having high DO contents, DO ≥ 9mg/L should be treated to reduce the DO content to saturation at 20ºC. Agitate or aerate with clean, filtered compressed air.

Dilution of sample: Dilutions that result in a residual DO of at least 1mg/L and DO uptake of at least 2mg/L produce reliable results. Make several dilutions of the pre-treated sample so as to obtain about 50% depletion of DO or DO uptake of 2mg/L. Prepare dilutions as follows:

Siphon out half the required volume of seeded dilution water in a graduated cylinder or volumetric flask without entraining air. Add the desired quantity of mixed sample and dilute to the appropriate volume by siphoning dilution water. Mix well with plunger type mixing rod to avoid entraining air.

Sample processing:

a. Siphon the diluted or undiluted sample in three labeled bottles and stopper immediately.

b. Keep 1 bottle for determination of the initial DO and incubate 2 bottles at 20ºC for 3days. See that the bottles have a water seal.

c. Prepare a blank in triplicate by siphoning plain dilution water (without seed) to measure the O 2 consumption in dilution water.

d. Also prepare a seed blank in triplicate to measures BOD of seed for correction of actual BOD.

e. Determine DO in a BOD test can in the blank on initial day and end of incubation period by Winkler method as described for DO measurement.

f. DO estimation in a BOD test can also be done by membrane electrodes. A DO probe with a stirrer is used to determine initial and final DO after incubation in BOD samples.

6. Chloride (Cl-)

Apparatus

a. Porcelain dish, 200mL

b. Pipettes

c. Burettes

d. Glass rod

Reagents and standards

a. Potassium chromate indicator: dissolve 50g K2Cr2O 7 in distilled water. Add AgNO 3 till definite red precipitate is formed. Allow to stand for 12hrs. Filter and dilute to 1000mL.

b. Silver nitrate, 0.0141N: Dissolve 2.395g AgNO 3 and dilute to 1000mL. Standardise against NaCl, 0.0141N; 1mL of 0.0141N AgNO3 = 0.5 mg Cl-.

c. Sodium chloride, 0.0141N: dissolve 824.1mg NaCl (dried at 40°C) and dilute to 1000mL; 1mL = 0.5 mg Cl-. 132

d. Special reagent to remove colour and turbidity: dissolve 125g AlK(SO4) 2.12H2O or AlNH4(SO 4) 2.12H2O and dilute to 1000mL. Warm to 60°C and add 55mL conc. NH4OH slowly. Let stand for 1 hour. Transfer to a large bottle and wash precipitate by successive addition with thorough mixing and decanting with distilled water until free from chloride. When freshly prepared, a suspension occupies a volume of approximately 1L.

Calibration The silver nitrate solution should be standardised against sodium chloride solution of 0.0141N. It gives the strength of silver nitrate solution 1mL = 0.5mg chlorides as Cl-.

Procedure

a. Take 50mL well mixed sample adjusted to pH 7.0-8.0and add 1.0 mL $K_2Cr_2O_7$.

b. Titrate with standard $AgNO_3$ solution till $AgCrO_4$ starts precipitating as pale red precipitate

c. Standardise $AgNO_3$ against standard NaCl

d. For better accuracy titrate distilled water (50mL) in the same way to establish reagent blank. A blank of 0.2 to 0.3mL is usual.

Annex V. Field Observation Checklist for Assessment of Wetlands

I. General information

1. Name of Wetland : _____ *Kebele* _____

2. Date of observation:_____; Season:_____

3. Weather condition (rainy, foggy, sunny) _____; Geographical location_____N. _____E

4. Wetland type / hydroperiod:

a) Permanently flooded,

b) Semi-permanently flooded

c) Seasonally flooded

d) Artificially drained

5. Size of the wetland (ha)_____

II. Hydrogeomorphological Assessment

6. Hydrological modification

a) Drainage _____ b) Storm water input_____ c) ditch inlet & outlet_____

d) Filling_____ e) Culvert_____ f) Others_____

7. Wetland geomorphic setting

a) Depressional type
b) Flood plain
c) Riverine
d) Lacustrine

III. Land use pattern

8. Land use pattern around the wetland

a) Grazing land_____
b) Agricultural land_____
c) Forest land_____
d) Native vegetation_____
e) Plantation_____
f) Mixed (artificial & natural) plants__
g) Residential area_____
h) Infrastructures (road, bus terminals, hotels, hospitals, Offices)_____
i) Recreational_____
j) Settlement_____
k) Industries (Garaj, Coffee processing plant)_____

9. Land use practices Within Wetland Upland/ Adjacent

Grazing _____ _____

Cultivation _____ _____

Tree removal _____ _____

Shrub clearing _____ _____

Grass cutting _____ _____

Car washing _____ _____

Waste damping _____ _____

Fishing _____ _____

Plantation _____ _____

Brick-making/Clay mining _____ _____

Water use & extraction _____ _____

Other potential threats: _____ _____

10. Conservation measures already taken for minimizing wetland degradation:

    a) Area closure
    b) Protecting the area by the community
    c) Catchment treatment using biological & physical measures
    d) Protecting waste damping into wetland
    e) Preventing siltation

11. Ecological status of the wetland

    A. Natural / referenced / least impacted site;
    B. Agricultural impacted
    C. Urban impact