An analysis of the performance of Constructed Wetlands in the treatment of Domestic Wastewater in the Western Cape, South Africa

Vanessa Lakay

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Supervised by: Dr Kevin Winter

DECLARATION

I know the meaning of plagiarism and declare that all of the work in the dissertation save for that which is properly acknowledged, is my own.

Signature: _____

Date: _____

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Abstract

Constructed wetlands (CWs) are being introduced in many parts of the world to treat wastewater. CWs offer several advantages over conventional treatment, most notably to save costs and energy. By contrast there are several limitations associated with the use of CWs, such as variability and unpredictability in treatment performance. However the literature focuses largely on the advantages of the CWs with little attention being given to the limitations and impacts on the receiving environment. In South Africa, there are a few studies concerned with the application and performance of CWs, but as yet there are no guidelines for the design and construction of these systems. The aim of this research is to determine the performance of three CWs situated on the periphery of Cape Town, Western Cape, with the intention of contributing to knowledge on the South African CWs performance in general. The research interest was to purposely shift attention to an analysis of the performance of CW systems that could be measured *in-situ* as opposed to laboratory-based studies where certain variables could be contained or controlled. In this study the focus is on determining the impact that these systems might have on the surrounding environment by analysing the impact from these CWs on surrounding or receiving water bodies. Samples of influent and effluent were collected from various points within the CW and from the surrounding water bodies every two weeks during the winter season when biological activity is least productive. Performance was determined by considering the mean percentage change from influent to effluent, the significance of the difference between influent and effluent and by comparing resultant effluent quality to the Department of Water Affairs' discharge standards. The results of the study indicate a range of performance both within and between systems, but overall the performance was poor, with the exception of NH_3 (96%) and *E. coli* (see below) that was removed at one of the sites, namely, at De Goede Hoop. While PO_4^{3-} was adsorbed, it was very low at all three sites; 3.8%, 7% and 20% at De Goede Hoop, Wolwedans and Babylonstoren respectively. Furthermore, DWA's effluent standards of 10 mg/l for PO_4^{3-} could not be met at all the sites. Poor PO_4^{3-} removal can be explained either by low O_2 concentrations or the choice of substrate that was used in the constuction. When O_2 concentrations are low, solubilisation of minerals and subsequent release of dissolved of phosphorus occurs. Mean *E. coli* removal percentages were considerably lower

compared to other studies undertaken elsewhere. *E. coli* removal was 85% at De Goede Hoop, 39% at Wolwedans and 65% at Babylonstoren. In general, the results indicate that more research on CW systems is required to improve our understanding of these systems. A better understanding of these systems will lead to enhanced design and thus assist in improved treatment performance so as to reduce the impact of CWs on the environment.

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BOD	Biological oxygen demand
COD	Chemical oxygen demand
CFU	Colony forming unit
CW	Constructed wetland
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
HF	Horizontal flow
HLR	Hydraulic loading rate
HRT	Hydraulic residence time
MA	Millennium Ecosystem Assessment
P.E	Person equivalent
SSF	Subsurface flow
TP	Total phosphorus
VF	Vertical flow

1 INTRODUCTION

Decentralised approaches[1] to wastewater treatment are increasingly being implemented because of the high costs of construction and operation of conventional, centralised wastewater treatment systems, especially in areas that are sparsely populated. In developing countries, funding for centralised systems as well as technical expertise to manage and operate these systems is limited (Massoud *et al*., 2009). However, Bradley *et al*. (2002) argue against an approach of selecting the simplest, least cost and least monitored decentralised systems as this may result in inadequate protection of human health and the environment.

Decentralised wastewater treatment systems, such as constructed wetlands (CWs), are increasingly being introduced in many parts of the world (Hoffmann *et al*., 2010). CWs are engineered systems designed and constructed to mimic natural wetlands and make use of the same processes that occur in natural wetlands, although often in a more controlled environment (Vymazal, 2005). CWs mimic ecosystem services provided by natural wetlands, namely in the ability of biomass to treat various types of wastewater through biological, physical and chemical processes. Werker *et al*. (2002) state that similar to naturally occurring counterparts, ecologically engineered systems provide ecosystem services that have high value but at low cost because these systems are also fuelled directly by solar radiation, are self-adaptive and require relatively little maintenance. CWs offer several advantages over conventional systems, such as financial and energy savings as well as a range of ancillary benefits, including recreation, education and wildlife habitat (Knight et al., 2001 cited in Lee et al., 2009 and Lee et al., 2009).

[1] Decentralised approaches to wastewater treatment focuses on the collection and treatment of wastewater near the point of generation

1.1 Present status

CWs are widely used for wastewater treatment in developed countries such as the USA, Australia and European countries (Gopal, 2003). In Europe alone, more than 5000 CWs systems have been constructed (Kadlec & Knight, 1996; Vymazal, 1998 cited in Kivaisi, 2001). As a result of the wide use of CWs in developed countries, a large portion of literature has been published in these countries over the past decade (see Kadlec & Knight, 1996, 2001; Mulamoottil *et al.,* 1998; Vymazal *et al.,* 1998; Mander & Jensen, 2002 cited in Gopal, 2003).

In developing countries, CWs could be beneficial, but still have to gain wider acceptance (Mohamed, 2004; Heers, 2006; Kamau, 2009 cited in Hoffmann *et al*., 2010). Nevertheless, Kivaisi (2001) is confident that there is potential for the development of CWs in developing countries. The author attributes this to the location of many developing countries in warm tropical and sub-tropical climates, which are conducive to higher biological activity and productivity, and hence better treatment performance.

In South Africa, the initial use of CWs was motivated by the need to remove nutrients from secondary treatment of domestic and industrial effluent (Wood, 1999). According to Wood (1999), there are approximately 70 CWs that are being used for the treatment of domestic and industrial wastewaters, mine drainage and agricultural and urban run-off (Wood, 1999). Wood (1999) notes that while full scale systems were being implemented in South Africa, research was only in its infancy such as at Free State and Wits Universities (Wrigley, 1988 and Rodgers, 1985 cited in Wood, 1999) and a research project at the Centre for Scientific and Industrial Research (CSIR) (Wood, 1998 and Batchelor, 1994 cited in Wood, 1999).

In terms of CW treatment performance research, a wealth of literature exists, however the current literature mainly focuses on laboratory experiments and field studies undertaken in North American and European countries, with very few studies being undertaken in developing countries. In the South African context, there have been a few studies dealing with the application and performance of CWs in the country (e.g. Wood, 1990; Wood, 1993; Wood, 1999) and engineering and design considerations (Wood, 1990). In terms of the CW performance literature, the Water Research Commission (WRC) research project identified a number of

established CWs with alternative configurations and operational approaches to provide an overview of performance. These included the following wetlands receiving secondary wastewaters: Mpophomeni vertical flow soil wetland for polishing biofilter effluent of phosphate, the Lethlabile meandering channel surface flow wetland for polishing biofilter and stabilisation pond effluent and the Ladybrand vertical flow gravel bed wetlands for polishing aerated lagoon and stabilisation pond effluent (Wood, 1999). From the study, Wood (1999) identified the key problems which limit CW treatment performance. These included the ability to control hydraulic residence time (HRT) so as to meet treatment objectives, permeability problems and short-circuiting of systems.

Although countries such as the USA, Denmark and Australia have guidelines for CW design, South Africa does not have any and thus many CWs in South Africa are based on reports extracted from international systems and rule-of-thumb assumptions (Wood, 1999). There also seems to be a lack of control of these systems in South Africa, e.g. permitting and monitoring of adherence to effluent discharge standards. The need to monitor these systems to determine performance of CWs in South Africa is becoming increasingly relevant while routine monitoring is essential for managing CW systems. Data obtained from long term monitoring are useful in predicting problems within the system and enabling operators to select appropriate actions and find solutions (Davis, 1995).

This thesis builds onto the existing CW treatment performance literature in South Africa and focuses on determining the performance of selected CW systems currently operating in the Western Cape. The study seeks to ascertain whether CWs that are designed to deal with relatively low volumes of domestic wastewater, were efficient and effective in reducing pollutants within acceptable water quality standards. The research also focused on the impacts of these systems on receiving waters by determining water quality and comparing these to the South African water quality guidelines and standards. Along with a general increase worldwide in the use of CWs, there appears to be a lack of concern regarding the impact of these systems on the environment. This assumption is based on the general lack of evidence found in the literature on this subject. Poorly performing CW systems have the potential to elevate ecological and human health risks.

1.2 Aims and objectives

The aim of the research project was to determine the performance of CWs in treating domestic wastewater (greywater and black water) in peri-urban / rural settings of the Western Cape, South Africa, where there is sufficient space to locate this type of treatment plant. The research sought to measure the ability of purpose built CWs to reduce or limit the throughput of excessive nutrients and to reduce the bacterial load so as to meet the Department of Water Affairs (DWA's) - formerly the Department of Water Affairs and Forestry (DWAF), wastewater discharge standards.

This research provided insight into the performance of these systems by focusing on three sites in the Western Cape, South Africa, where CWs were being used to treat domestic wastewater at a secondary level.

The research focused on the performance of the CW system, as well as the potential impact that these systems had on receiving waters. The study was done during the cooler months so as to determine the performance during non-ideal conditions. The objectives of the study were:

1. To determine the quality of wastewater before and after entering a CW system
2. To determine whether the wastewater quality exiting the CW met (DWA's) wastewater effluent discharge standards
3. To determine the quality of treated wastewater at the point of discharge into nearby vleis (lakes), streams and drainage channels.

The aim and objectives were selected to measure the performance of CW systems and determine whether there was a change in water quality after passing through the systems and whether water quality standards were being met. The research could contribute to a better understanding of CW systems and their performance in the South African context. Furthermore, as there is concern that decentralised systems such as CWs may be problematic in terms of treatment performance, it is

essential that more knowledge of the science regarding these systems be understood if they are to become credible and acceptable.

1.3 Overview of research methods and study design

The focus of this research was to determine the performance of selected CWs currently operating in the Western Cape. The research design focused on monitoring CWs at different sites as opposed to laboratory- based studies; however, there are also limitations which compromise the scientific potential of the study because there is no control over variables operating within the system, e.g. flow rate, wastewater strength.

A list of sites was selected from a consulting landscape architectural practice responsible for the design and installation of over 25 CWs located in the Western Cape. Since the focus of the research was restricted to sub-surface flow (SSF) in the treatment of domestic wastewater at a secondary level, site selection was narrowed down to three sites. The performance of the selected CWs was then determined by collecting and analysing water samples from the following: a single house at De Goede Hoop Estate, Noordhoek (De Goede Hoop); Wolwedans Farm, Stellenbosch (Wolwedans); and Babylonstoren Farm, Simondium (Babylonstoren). It should be noted however that the CWs at the three sites were all designed differently i.e. each with different flow rates, wastewater volumes and area sizes. It was thus not the intention of the researcher to compare these sights to each other but to analyse the general performance of each system. Sampling was undertaken the cooler months so as to provide insight into how the CWs performed under non-ideal conditions.

The CWs at Wolwedans and Babylonstoren were installed by the private property owner in the absence of municipal services for wastewater treatment services and infrastructure to these areas. The landowners thus installed these systems in order to manage wastewater in a cost effective manner. In contrast, the landowner on the De Goede Hoop Estate had access to municipal wastewater services, but chose to install a natural, biological system to treat domestic wastewater.

The CWs all formed part of a wastewater treatment train in which primary treatment occurred in the form of septic tanks and/or sedimentation settlement followed by aeration prior to wastewater entering the CWs. The treatment systems were designed and constructed by the same private water treatment engineering consulting company (See Appendix 1).

Field studies were conducted to collect water samples. The variables used in this study included selected nutrients and *E. coli*. The nutrients selected included ammonia (NH_3, ionised and un-ionised forms), nitrate (NO_3^-), nitrite (NO_2^-) and orthophosphate (PO_4^{3-}). The variables were chosen primarily as a result of the concern of high levels and loads of nutrients that enter freshwater and groundwater, as well as possible impacts of pathogens on downstream users.

The sampling points selected included influent and effluent points. This enabled a direct comparison between the influent and effluent concentrations, which then provided information about performance. The significance of the difference between the influent and effluent was also determined using regression analysis. Performance of the system was also analysed by comparing the effluent concentration to the DWA's effluent discharge standards, to determine how often effluent quality standards were being met.

Finally, the impact of CWs on surrounding water bodies, such as vleis (lakes), streams and drainage channels, were sampled to determine water quality and compared to the South African Water Quality guidelines.

1.4 Assumptions and limitations

As a result of limited resources, not all the variables could be compared to the standards and guidelines directly. For example, *E. coli* is not included in the discharge limit standards. The discharge standards contain faecal coliforms, of which *E. coli* is a large component. Similarly when comparing effluent quality with the South African Water Quality guidelines, these contain inorganic phosphorus, whereas the research was limited to orthophosphate (PO_4^{3-}).

A further limitation is that baseline data of surrounding water was not collected prior to the CWs being installed at the sites and thus it was not possible to compare a change in water quality resulting from the installation of these CWs.

1.5 Structure of the report

Chapter Two of this report discusses the literature review by focusing attention on the use of CWs as an alternative method to conventional wastewater treatment, and also considers how ecosystem service are capable of mimicking nature to solve human problems of waste. The chapter gives a detailed discussion about competing definitions, classifications and the valuation of ecosystem services, as well as an overview of the concept, historical perspective, classification and application of CWs. In closing, this chapter also outlines the components, design, pollutant removal mechanisms and treatment efficiencies of these systems. Chapter Three discusses the research methods and study design, and includes descriptions of the study areas, and details on the sampling method and laboratory analysis. Chapter Four presents the results and analysis, while Chapter Five concludes the study.

2 LITERATURE REVIEW

According to the United States Environmental Protection Agency's (USEPA) study findings, decentralized wastewater systems are appropriate for low-density communities are more cost-effective than centralized systems (Massoud et al., 2008). Thus more attention is being given to the benefits of decentralised wastewater treatment systems which treat wastewater at their source (Campbell and Ogden, 1999). A decentralised approach to treating sewage (point source treatment), potentially with CWs, may also provide a more efficient means to treat wastewater and may have value to both new developments as well as being retrofitted into existing developments (Campbell and Ogden, 1999).

One approach to decentralised wastewater treatment is making use of the constructed wetlands (CWs) to treat wastewater. Wetland systems provide ecosystem services such as water purification / treatment, fish, fibre, water supply, flood regulation and recreation opportunities (IWMI, 2006), offering a wide range of services that also meet humans needs (Turner, 1991). There is increasing interest in constructed wetlands to simulate natural wetland functions so as to provide services for human benefit (Hammer & Bastian, 1989). Constructed wetlands (CWs) for wastewater treatment are being designed and built to mimic the treatment processes that occur in natural wetlands by using a combination of naturally occurring biological, chemical and physical processes and interactions with plants, microbiota and substrate to treat wastewater. This literature review builds on this discussion by considering CWs as an alternative means of treating wastewater.

Studies show that CWs are capable of mimicking natural wetland processes and removing a large percentage of contaminants (e.g. Decamp & Warren, 2000; Ayaz & Akca, 2001). Furthermore, CWs designed for wastewater treatment may also provide a range of other ecosystem services that go beyond the primary aim of the construction (Ghermandi *et al.*, 2009). These ancillary benefits may include the provision of wildlife habitat (Lee *et al.*, 2009) as well as opportunities

for environmental education, recreation and water re-use (Knight *et al.,* 2001). CWs can also be designed to form a visually pleasing and functional landscape (Shutes, 2001).

Although CWs are considered to be relatively simple in terms of construction and operation, ecosystems are biologically complex, with numerous components that interact non-linearly (Banzhaf & Boyd, 2012). It should be noted that these systems behave as natural systems and thus require a better understanding if they are to operate effectively. Further to this, there appears to be inflated expectations regarding the capability of these systems to manage human waste. Gopal (1999) argues that the capability of wetlands to perform certain functions has been over estimated. The author also states that the over-generalisation of laboratory and short-term field experiments' results have been exaggerated to present a more promising account of the performance of CWs.

2.1 Ecosystem services

Ecosystem services are the benefits that households, communities and the economy receive from nature (Boyd & Banzhaf, 2007). Throughout human history, people have related well-being is related to the proper functioning of ecosystems (Brauman *et al*., 2007). In many cases, the realisation of the importance of ecosystem services came about when ecosystems were being degraded and thereby failed to provide these services (Brauman *et al.,* 2007). For example, the ancient Greeks were aware of the importance of soil retention; this knowledge however only came about after deforestation resulting in soil thinning (Fisher *et al*., 2009).

The origins of the modern history of ecosystem services are found in the 1970's and begin with the utilitarian framing of ecosystem functions as services to increase public interest and conservation efforts (Westman, 1977; Ehrlich & Ehrlich, 1981; de Groot, 1987 cited in Gomez-Baggethun *et al*., 2010). The interest in ecosystem services continued into the 1990s (e.g. Costanza *et al*., 1997 and Daily, 1997). Daily's (1997) book entitled *Natures Services: Societal Dependence on Natural Ecosystems,* is one of the first rigorous attempts to try and identify the range of ecosystem services and the economic value of these services (Salzman, 1997). Costanza *et al.* (1997) attempted to assign a monetary value to ecosystem services. Costanza *et al.* (1997)

used published studies as well as original calculations to estimate the value of 17 ecosystem services for 16 biomes. The authors concluded that ecosystems provide at least US$ 33 trillion worth of services annually. Furthermore, the Millennium Ecosystem Assessment (MA) (MA, 2003) contributed to placing ecosystem services on the policy agenda. Since the release of this assessment, the literature on ecosystem services has grown exponentially (Fisher *et al*., 2009).

2.1.1 **Defining ecosystem services**

Several definitions of ecosystem services are found within the wealth of ecosystem services literature. According to Boyd & Banzhaf (2006), ecologists and economists have failed to standardise the definition of ecosystem services, and this has resulted in competing meanings of the term. The following definitions are the three most common definitions cited in the literature, and although they have the same general idea, there are differences between them.

1. The MA (2003) provides a broad definition of ecosystem services as that which entails the "*benefits people obtain from ecosystems*". It draws on two earlier definitions offered by Daily (1997, p.3), who defines ecosystem services as the "conditions and processes through which natural ecosystems and the species from which they comprise, sustain and fulfil human life" and Costanza *et al*., (1997, p.1), who refer to ecosystem services as "the benefits that human populations derive, directly or indirectly, from ecosystem functions". In Daily (1997), the definition states that ecosystem services are "conditions and processes" as well as the actual life-support functions, whereas in Costanza *et al.* (1997), ecosystem services are the goods and services derived from the functions which are used by humanity (Fisher *et al*., 2009).

2. Boyd & Banzhaf (2007) argue that ecosystem services need to be defined in a way that is methodologically and economically consistent with the definition of goods and services used in conventional income accounts. The authors suggest that "*ecosystem services are components of nature, directly enjoyed, consumed or used to yield human well-being*" (p.619). In this definition emphasis is placed on ecosystem services as end products of nature and the fact that ecosystem services are components of nature rather than functions or processes (Boyd & Banzhaf, 2007). For example, water purification would not be

considered an ecosystem service, as stated by other authors (e.g. Daily, 1997), but rather a function of certain land cover types that help to produce clean water, which is considered the final ecosystem service. Other examples of ecosystem services provided by the authors include: surface water, vegetation types and species populations. According to Boyd & Banzhaf (2007), ecosystem service functions and processes are not end products in themselves and therefore are not services. Instead they offer functions and processes that are intermediates in the production of final services (Boyd & Banzhaf, 2007). The distinction between intermediate and end products is fundamental to welfare accounting (Boyd & Banzhaf, 2006). The authors state that if a distinction is not made between intermediate and end products, then the value of intermediate goods will be double counted since intermediate goods are already embodied in the value of final goods.

3. There is a counter argument to Boyd and Banzhaf (2006) as offered by Fisher *et al*. (2009, p.645) who suggest that "ecosystem services are the aspects of ecosystems utilised (actively or passively) to produce human well-being". In this definition, ecosystem services include ecosystem structure as well as processes and/or functions if they are consumed or utilised directly or indirectly by humanity (Fisher *et al*., 2009). Thus, functions and processes become services if humans benefit from them (Fisher *et al.*, 2009).

For the purpose of this research, the definition provided by the MA (2003), as well as the similar definitions provided by Daily (1997) and Costanza et al. (1997), are preferable. This is because these definitions regard water treatment as the actual ecosystem service. Using water purification as an example, according to the MA (2003) classification (Table 1), water purification would be considered a regulating ecosystem service. In contrast, the definition offered by Boyd & Banzhaf (2007) does not consider water purification an ecosystem service, but rather an ecosystem function or process. The authors argue that water purification is a function of certain land cover types (in the case of this research, wetlands / constructed wetlands) that help produce clean water. The clean water, which is the end product, would then be considered the ecosystem service.

2.1.2 Classifying ecosystem services

Ecosystem services do not only have multiple definitions but also different ways to classify them. The MA (2005) classifies ecosystem services in terms of the provision of services, regulating services, cultural services and supporting services. Table 1 provides examples of each type of service.

Table 1: Categories of ecosystem services and related services (Adapted from Millennium Ecosystem Assessment (2005)

Service classification	Service
Provisioning services	❖ Food ❖ Fibre ❖ Genetic resources ❖ Bio-chemicals, natural medicines, etc. ❖ Ornamental resources ❖ Fresh water
Regulating services	❖ Air quality regulation ❖ Climate regulation ❖ Water regulation ❖ Erosion regulation ❖ Disease regulation ❖ Pest regulation ❖ Pollination
Cultural services	❖ Cultural diversity ❖ Spiritual and religious values ❖ Recreation and ecotourism ❖ Aesthetic values ❖ Knowledge systems ❖ Educational values
Supporting services	❖ Soil formation ❖ Photosynthesis ❖ Primary production ❖ Nutrient cycling ❖ Water cycling

Source: Wallace (2007)

Wallace (2007) argues that the classification of ecosystem services by the MA (2005) as well as by other authors such as Costanza *et al.* (1997), de Groot *et al*. (2002) and Farber *et al.* (2002) use mixed processes (means) for achieving services and the services (ends) themselves within the same service category. The author argues, for example, that pollination, water regulation, photosynthesis and soil formation are not end products, but rather processes to achieve services such as food production and potable water. He states that water regulation, in its own right, is not

a service sought by humans, but is a process to achieve potable water. Hein *et al.* (2006) do not distinguish a category for supporting services, which represents ecological processes that underlie the functioning of the ecosystem. Instead they provide the following three categories: production services, regulation services and cultural services. They argue that the MA's inclusion of supporting services may lead to double counting as their value is already reflected in the other three service categories. However this does not imply that intermediate services are not services, e.g. tyres are sold either directly to consumers or sold to car companies and are intermediate products sold to consumers as part of cars (Costanza, 2008). Costanza (2008) concludes by stating that the goal for classification should not be a single, consistent system, as implied by Wallace (2007), but rather a pluralism of typologies that will each be useful for different purposes.

2.1.3 **Valuation of ecosystem services**

In the last 30 years, valuation of ecosystem services has become one of the most significant and fastest evolving research areas in environmental and ecological economics (Turner *et al.*, 2003). The interest in the valuation of ecosystem services came about as a result of an increasing awareness that the benefits provided by natural and semi-natural ecosystems were often underestimated in decision making (Helliwell, 1969; Odum & Odum, 1972 cited in Hein *et al*., 2006).

Ecosystem valuation is the process for expressing values for ecosystem goods for scientific observation and measurement (Farber *et al*., 2002). The motivation for valuation studies is to generate more comprehensive information for policy formulation and decision taking (Turner *et al.*, 2003). According to Randall (2002), and Hanley & Shogren (2002) cited in Turner *et al*. (2003), the purpose of ecosystem service valuation is not to put a price tag on the environment, but to express the effect of marginal change in ecosystem services provision in terms of a rate of trade off against other things people value.

The value of ecosystems comprises use and non-use[2] elements. An example of direct use values is cranberries or crabs that are exchanged in markets and easily priced (direct market uses) (Salzman, 1997). Recreational activities such as fishing (direct non-market) as well as more intangible existence values[3] and option values[4] are not exchanged in markets (non-market, non-use) (Salzman, 1997).

Some ecosystem products, such as timber and fish, are commodities valued in the marketplace. Goods that are typically bought in markets are 'excludable' and 'rival'. To be rival means that the use of a good by one person precludes its use by another; and excludable means that one person can prevent another person from using a certain good (Fisher *et al*., 2009). There are however many other services that are public goods and are free to any user. As people often do not pay for these services and because they can be used without diminishing their value, there is no direct measurement of demand and willingness to pay which makes it difficult to determine their value (Heal, 2000 cited in Brauman *et al*. 2007). A variety of methods for the economic valuation of ecosystem services exist. A brief summary of each of these ecosystem service valuation types is provided in Table 2.

[2] Derived from simply knowing that ecosystems exist.

[3] The value attached to the knowledge that species, natural environments and ecosystem services exist.

[4] The willingness to pay a certain sum today for the future use of an asset.

Table 2: Ecosystem service economic valuation methods and examples

Valuation method	Explanation	Example
Direct market valuation	This is the exchange value that ecosystem services have in trade (de Groot *et al.*, (2002).	Timber or produce.
Indirect market valuation		
❖ **Avoided cost**	Estimates the value of ecosystem services based on the costs of avoiding damages in the absence of those services (de Groot *et al*., 2002).	Flood control, which avoids property damages (de Groot *et al*., 2002
❖ **Replacement cost**	Estimates a value based on the cost to replace the ecosystem function or service (UNEP-WCMC, 2011).	Natural wastewater treatment by marshes which can be (partly) replaced with costly artificial treatment systems (de Groot *et al.*, 2002)
❖ **Factor income**	Services provided for the enhancement of incomes (Farber *et al.*, 2002).	Natural water quality improvements that increase commercial fisheries catch and thereby incomes of fishermen (de Groot *et al*., 2002).
❖ **Travel cost**	Determines the value of an ecosystem based on the amount of money spent to reach that particular destination	Used to value sites which are used for recreation purposes (Carson & Bergstrom, 2003).
❖ **Hedonic pricing**	Service demand may be reflected in the prices people will pay for associated goods (Farber *et al*., 2002).	Housing prices at beaches exceed prices of inland homes (Farber *et al*., 2002).
Contingent valuation	Service demand elicited by posing hypothetical scenarios that involve some valuation of alternatives (Farber *et al*., 2002).	People would be willing to pay for increased fish catch (Farber *et al*., 2002).

Ecosystem services valuation has also been applied to ecosystem services generated by constructed ecosystems. A discussion follows to determine whether CWs provide the same level of values for certain ecosystem services as natural wetlands.

2.1.4 **Valuation of constructed ecosystem services**

Constructed ecosystems are similar to natural ecosystems, but differ in the following aspects: in constructed ecosystems there is an enhancement of certain services with the decline of most other services; furthermore, higher direct use values than indirect use values are estimated for constructed ecosystems as compared to natural ecosystems (Yang *et al.,* 2008). Wetlands are considered one of the most valuable resources per unit area, providing a number of ecosystem services such as water supply, raw material, food, recreation (Costanza *et al*., 1997; Zedler, 2000) as well as water quality improvements.

A number of studies focus on CW valuation. For example, Ghermandi *et al.* (2009) set out to determine whether CWs provide the same level of values for certain ecosystem services. The services that were given attention included flood protection, water quality improvement, and water storage and supply. The findings led to the conclusion that constructed wetlands were highly valued for flood control, storm buffering and water quality improvement as well as for the provision of habitat and biodiversity, all of which were not the primary goals for the construction of these ecosystems.

In another study on monetary valuation, Chen *et al.,* (2009) undertook a valuation study by exploring net ecosystem values for constructed, human-interfered and natural wetlands. The results obtained are presented in Table 3 and indicate that the CW (Beijing wetland) has the largest net services value.

Table 3: Ecosystem services valuation of the Beijing wetland, norm wetland and Sanyang wetland

Item	Beijing wetland		Norm wetland		Sanyang wetland	
	($/ha/yr)	Proportion (%)	($/ha/yr)	Proportion (%)	($/ha/yr)	Proportion (%)
Waste treatment	131 948	63.82	4 902	31.34	-854	-121.58
Food and material production	40	0.02	425	2.72	895	127.47
Water supply	74 706	36.14	4 460	28.51	207	29.50
Gas regulation	-238	-0.11	156	1.00	48	6.88
Disturbance and water regulation	249	0.12	5 344	34.16	278	39.52
Habitat and refugia	35	0.02	357	2.28	128	18.21
Total	206 740	100.00	15 643	100.00	702	100.00

Where the Beijing wetland represents a constructed wetland; the norm wetland is a wetland in its average status as a representative of a natural wetland and the Sanyang wetland is an example of a human-interfered wetland (Source: Chen *et al*., 2009)

Similarly, Yang *et al*. (2008) valued a CW located in the Hangzhou Botanical Garden in Beijing. The authors valued the CW at 66.67 yuan (approximately RSA R90) m^{-2} per year, using the contingent valuation method and 1920 yuan (approximately RSA R2670) m^{-2} per year using a shadow project approach[5]. In comparison, (He *et al.*, 2005 cited in Yang, 2008), valued a natural wetland at 1.06 yuan (approximately RSA R1.50) m^{-2} per year, a 131 yuan (approximately RSA R180) m^{-2} per year for farmland and 1.88 yuan (approximately RSA R2.60) m^{-2} per year for a forest area as the average economic value of terrestrial ecosystems in China.

[5] Applied to assess the value of an ecosystem service by how much it costs to replace or restore it after it has been damaged

2.2 Constructed wetlands for wastewater treatment

The use of wetlands to improve water quality is not a new innovation. Natural wetlands have been used as convenient wastewater disposal sites for many years (Hoffmann *et al*., 2010). Wastewater is usually discharged, directly or indirectly into depressions in the landscape (Brix, 1994b) and thus if a wetland is not already present at the site, the wastewater will lead to the formation of one (Cooper & Boon, 1987 cited in Brix, 1994b). CWs have been used for over forty years in almost all the regions of the world (Hoffmann *et al.*, 2010).

The foundation for CW technology was laid by two German scientists, Dr Käthe Seidel and Dr Reinhold Kickuth (Lee *et al*., 2009). In the early 1950s, Dr Käthe Seidel was responsible for undertaking the first experiments aimed at the possibility of using wetland plants for wastewater treatment. The work of Dr Seidel stimulated other institutions in Germany to become involved in the study of wetlands for wastewater treatment (Seidel *et al.*, 1978 cited in Brix, 1994b). Dr Kickuth was responsible for the development of the Root Zone Method in the mid 1960s (Brix, 1994b). The Root Zone Method comprises a rectangular planted bed in selected soils that may contain calcium, iron or aluminium to improve the phosphorus precipitation capacity and in which water flows horizontally through the rhizosphere of the reeds (Brix, 1994b).

In North America, during the 1970s, experimentation with different designs of CWs took place (Spangler *et al*., 1976; Wolverton, 1982 cited in Brix, 1994b). Most of the initial work was related to the use of natural wetlands for wastewater treatment (Nichols, 1983), however it soon became apparent that the application of wastewater to natural wetlands was likely to result in changes to species composition, community structure and function, and therefore the overall value of a wetland (Brix, 1994b). It was realized that CWs have a greater potential for application as it allows the optimisation of control over the treatment process and CWs do not interfere with the values of natural wetlands (Reed & Bastian, 1985 cited in Brix, 1994b).

The earlier work in Europe influenced the development of CW technology in the United States (Brix, 1994b). The favourable results of these earlier projects prompted the construction of other key projects in the 1980s (Gearheart *et al*., 1989 cited in Brix, 1994b). In the 1990s a major

increase in the number of CWs took place as the application expanded to different kinds of wastewater (Hoffmann *et al.*, 2010).

Increasing worldwide interest in CWs is because of the potential for these systems to provide a range of benefits compared to conventional methods, including financial savings in electricity, human labour, lower construction and maintenance costs, and cost savings of chemicals, fuel, services and plant operation (Rousseau *et al.*, 2008; Lee *et al.*, 2009). The basic operation and maintenance costs for competing concrete and steel technologies are higher by a factor of 2 to 10 times when compared to CWs (Vymazal & Kröpfelová, 2008; Kadlec & Wallace, 2009). Table 4 compares the differences between the cost and pollutant removal efficiency of CWs and conventional treatment plants. The table shows that the construction cost is lower for CWs, while the management costs are substantially lower. The table also indicates higher removal efficiencies for CWs with regard to suspended solids (SS), total nitrogen (TN) and total phosphorus (TP). The table (compiled by Lee *et al.*, 2009) does not provide any detail in terms of the context of how these figures were derived, although it does provide a general overview of some of the advantages of CW treatment systems.

Table 4: Comparison of characteristics between CWs and conventional treatment plants

	Financial considerations			**Removal efficiency (%)**				
Treatment system	Construction cost ($)	Management cost ($/year)	Facility capacity (m^3)	BOD	SS	TN	TP	Remarks
Constructed wetland	220 000	300	800	80-90	80-90	40-50	50-60	Remove some heavy metals, *E. coli*
Conventional treatment plant	300 000	2000	450	80-99	70-80	20-30	<20	-

Source: Lee *et al. 2009* (BOD -Biological Oxygen Demand, SS – suspended solids, TN – total nitrogen, TP – total phosphorus)

Although CWs offer some advantages, as mentioned above, there are also limitations to using these systems. Gopal (1999) notes that CWs require a larger land area compared to conventional systems. Davis (1995) notes that the biological components of CWs are sensitive to toxic substances, such as ammonia and pesticides, and that wetland plants require a minimum amount of water in order to survive. Furthermore, their relatively slow rate of operation compared to conventional wastewater treatment techniques is a disadvantage (Shutes, 2001).

The use of CWs for wastewater treatment presents various other challenges. Werker *et al*. (2002) notes that although progress has been made in improving the design of CWs, there are still gaps in the understanding of these systems that limit the ability to achieve predictable and sustained levels of water quality treatment (Werker *et al*., 2002). Research has shown that CWs are more complex than conventional systems due to diffusive flow as well as a large number of other processes involved in the treatment (Hoddinott, 2006). Furthermore, it is also difficult to know how well a design will perform until it is completed and allowed time to mature (Werker *et al*., 2002).

Similar to conventional systems, CWs also demonstrate variability in performance in the removal of pollutants (Crites & Tchobanoglous, 1998 cited in Werker *et al.*, 2002). This is because although CWs are living, ecological systems that mimic natural wetlands (U.S. EPA, 2000) and their performance may be influenced by varying hydraulics and the internal wetland environment (Kadlec & Reddy, 2001; Hoddinott, 2006). Buchberger & Shaw (1995) note that because CWs cover a large surface area, and that the performance may be affected by factors such as rainfall, evapotranspiration and temperature. Kadlec (1989) notes that as these factors vary over time, and that CWs do not operate within a steady-state. Thus, very different performance levels may be experienced within a system over time. For example, the results obtained by Masi & Martinuzzi (2007) Table 5 shows large variability in removal efficiencies within and between various types of CW systems in Mediterranean countries. Ammonia removal efficiencies for horizontal flow in CWs range between from 18% to 76% and for nitrogen, the removal efficiencies range between 23% and 67%.

Table 5: Performance of CWs in Mediterranean countries

Type of CW	Organic content (%)	Nitrogen removal (%)	Ammonia removal (%)	Total solids (%)	Pathogens (%)
Horizontal flow	73-99	23-67	18-76	59-96	94-99.999
Vertical flow	52-95	--	78-99	48-98	96-99.9
Free water surface	11-63	21-76	15-82	36-67	90-99.999
Hybrid systems	86-99	43-89	85-96	72-84	98-99.9995
Vertical flow, raw wastewater	82-99.7	66-98	85	95-99.8	--

Source: Masi and Martinuzzi (2007)

Bavor *et al*. (1995) note that pollutant removal performance range between 0% and more than 90% for most of the pollutants that were tested. The authors note that these reports often include data for establishing systems, overloaded systems, and inappropriately designed or unmanaged systems. They argue that in the absence of comprehensive and widely accepted design criteria, this information could be misinterpreted to mean that CWs are unreliable and unpredictable as a wastewater treatment method.

2.2.1 Classification of constructed wetlands

CWs are classified according to the life form of the dominant macrophyte[6] in the system (Vymazal, 2005). CWs may either comprise free-floating macrophyte-based systems, submerged macrophyte-based systems or rooted emergent macrophyte-based systems (Brix, 1993). Vymazal *et al*. (1998) notes that different types of macrophyte-based systems may be combined with each other or with conventional treatment methods to exploit the advantages of the various systems.

2.2.1.1 Free-floating macrophyte-based systems

The development of free-floating macrophyte-based systems has been prompted by the desire for nutrient removal and to improve the performance of conventional stabilization ponds (Brix, 1994b). Free-floating habitats are diverse in form, habit and size (Vymazal *et al*., 1998). Examples of plants used in these systems include water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*) and duckweed (*Lemnaceae*) [Brix & Schierup, 1989 cited in Vymazal

[6] Macrophytes are macroscopic aquatic plants that are either emergent, submergent or floating

et al., 1998]. A schematic representation of a free-floating macrophyte-based system is shown below (Figure 1).

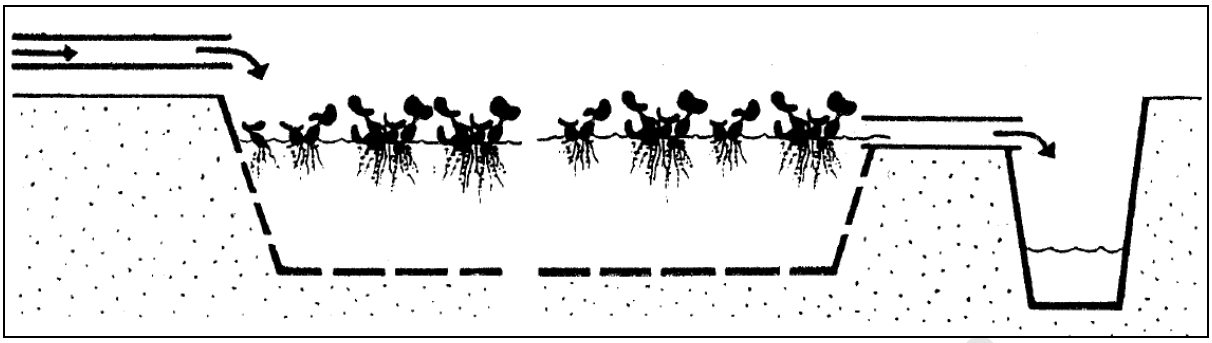

Figure 1: Representation of a free-floating macrophyte-based system. Source: Brix, 1993

*2.2.1.2 **Submerged macrophyte-based systems***

Submerged macrophytes have their photosynthetic tissue entirely submerged (Vymazal *et al*., 1998). Experiments have shown that minerals can be taken directly by the shoots of submerged plants (Vymazal *et al*., 1998); the roots of these plants are also responsible for nutrient uptake (Vymazal, 1995). Examples of submerged macrophytes include water weeds (*Elodea spp*.), quillworts (*Isoetes spp.*) and pond weeds (*Potamogeton spp*.) [Vymazal & Kröpfelová, 2008]. A schematic representation of a free-floating macrophyte-based system is shown in Figure 2.

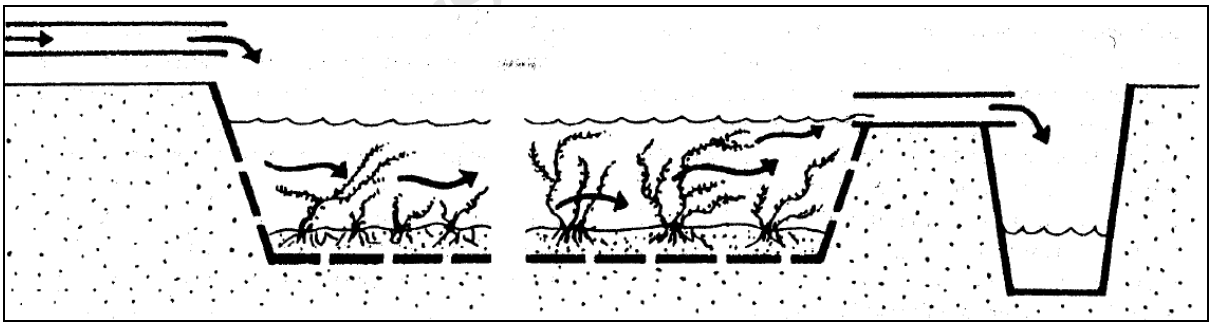

Figure 2: Representation of a submerged macrophyte-based system
Source: Brix, 1993

*2.2.1.3 **Emergent macrophyte-based systems***

Emergent macrophytes have their roots in the sediment and emergent stems and leaves, examples include reeds (*Phragmites*) and bulrush *(Typha)* (Greenway, 2000). Emergent macrophyte-based systems can be categorized according to the following flow regimes:

2.2.1.3.1 Surface flow systems

Surface flow systems comprise open areas of water with floating vegetation and emergent plants (Kadlec & Wallace, 2009). These systems are often densely vegetated (Department of Planning and Local Government, 2010). As these wetlands closely mimic natural wetlands, they attract wildlife, such as molluscs, amphibians, insects, birds, mammals, reptiles and fish (NADB database, 1993; Kadlec & Knight, 1996 cited in Kadlec & Wallace, 2009). The wetland base may be permeable, thus allowing water to exfiltrate (Lee *et al*., 2009). Wastewater passes over the support medium, between the stems of plants and through the surface debris (Lee *et al*., 2009). The shallow water depth of these systems along with the low flow velocity, regulate the water flow and ensure plug-flow conditions (Reed *et al*., 1998 cited in Vymazal *et al*., 1998). The most common application for surface flow systems is for advanced treatment of effluent from secondary or tertiary treatment processes (Kadlec & Wallace, 2009). A schematic representation of an emergent macrophyte-based system with surface flow is shown in Figure 3.

2.2.1.3.2 Systems with subsurface flow

These are the most widely used type of CW in Europe and South Africa (Lee *et al*., 2009) and are referred to as reed bed treatments systems (Hofmann *et al*., 2010) or reed beds. Systems with subsurface flow often contain a ditch or a bed, sealed by an impermeable substance and media to support the growth of emergent plants (Lee *et al.*, 2009). This media is often composed of rock or crushed gravel and different soils, or in various combinations (Reed *et al*., 1995 & Kaseva, 2004 cited in Lee *et al*., 2009). The water surface is maintained below the gravel media, to reduce risk of odour, insect vectors or public exposure (Reed & Brown, 1995).

Subsurface flow systems are categorized into either horizontal flow (HF) or vertical flow (VF) CWs depending on the water flow through the system. Hybrid systems, which combine horizontal and vertical flow systems, have been used to improve treatment performance (Lee *et al.*, 2009).

2.2.1.3.2.1 Horizontal flow (HF) systems:

In HF systems, wastewater flows horizontally through a porous medium planted with emergent vegetation (Knowles *et al.*, 2011). As the wastewater flows through the rhizosphere, the wastewater is cleaned by microbiological degradation, chemical and physical processes (Brix 1987, Cooper *et al*., 1996 cited in Vymazal *et al*., 1998). These systems are often used for secondary treatment of single-family homes or small cluster systems (Wallace & Knight, 2006 cited in Kadlec & Wallace, 2009) or for small communities (Cooper *et al*., 1996 cited in Kadlec &Wallace, 2009). A schematic representation of an emergent macrophyte-based system with HF is shown in Figure 3.

2.2.1.3.2.2 Vertical flow (VF) systems:

The pollutant elimination principles of VF systems are similar to those for the HF systems (Vymazal *et al*., 1998). With reference to vertical flow systems, water is piped into the plant and percolates through the medium (Vymazal *et al*., 1998). VF systems have relatively small space requirements and provide good aeration conditions (McBride & Tanner, 2000). A schematic representation of an emergent macrophyte-based system with VF is shown in Figure 3.

2.2.1.3.2.3 Hybrid systems:

Various types of CWs can be combined in order to achieve higher treatment efficiency (Vymazal, 2005). In hybrid systems, different cells are designed for different types of reactions (Davis, 1995). The most common type of hybrid system is vertical flow systems and horizontal flow systems arranged in a staged manner (Vymazal, 2005). In combined horizontal and vertical flow systems, the benefits of each system can be combined to complement each other (Vymazal, 2005).

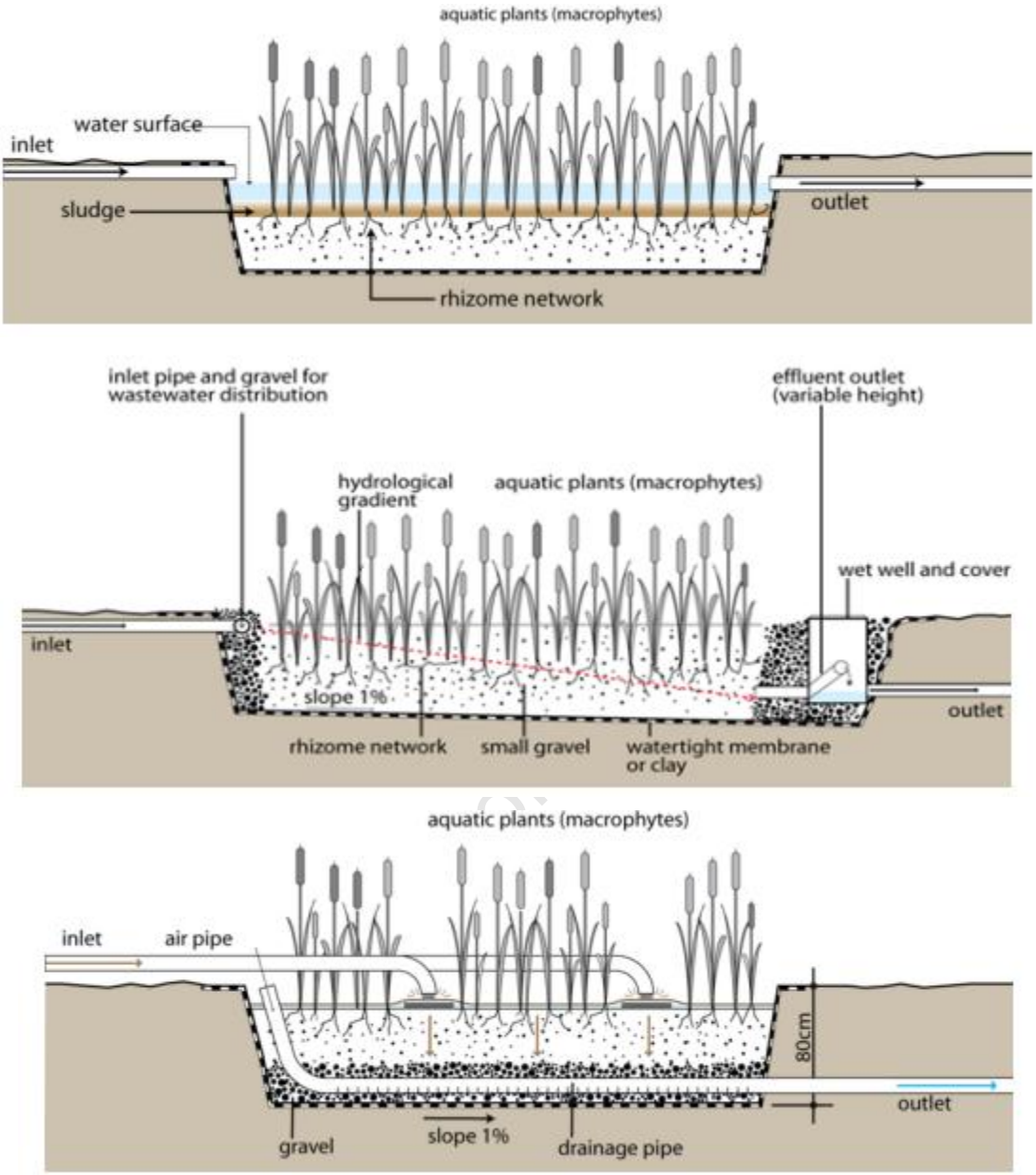

Figure 3: Representation of emergent macrophyte – based systems
Surface flow system (a), emergent macrophyte – based system with horizontal flow (b) emergent macrophyte – based system with vertical flow (c). (Source: Nilsson *et al*., 2012)

As mentioned above, there are various types of CWs; however the research will literature will only focus on HF CWs with emergent vegetation. Thus the rest of the literature review will mainly focus on these types of systems.

Figure Figure 4 provides a summary of the classification of CWs.

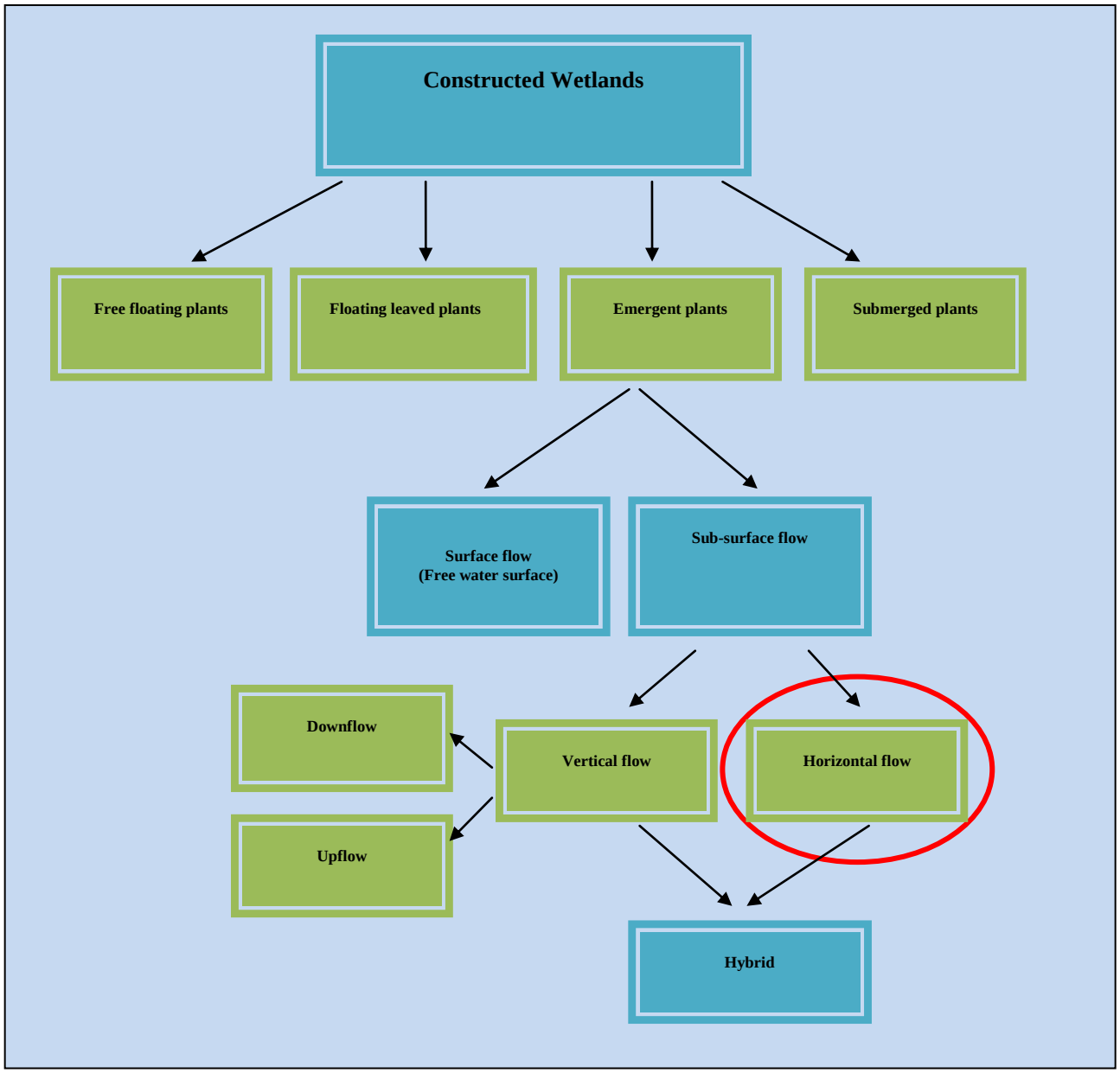

Figure 4: Classification of constructed wetlands for wastewater treatment systems
The red circle illustrates the focus area of the research, i.e. subsurface horizontal flow constructed wetlands with emergent vegetation. (Source: redrawn from Vymazal, 2001 in Vymazal, 2007)

2.2.2 Applications of CWs and present status

The use of CWs spans a wide range of applications such as mine drainage; urban stormwater; rivers, lakes and reservoirs; agricultural run-off; livestock wastewater; industrial wastewater, landfill leachate and sludge drying.

Sub-surface flow wetlands are the predominant wetland type used for wastewater treatment in Europe (Hoffmann *et al*., 2010), where most of these systems are soil or gravel based horizontal flow systems, planted with *Phragmites australis* (Haberl *et al*., 1995). Most of the systems used in Europe are used for secondary treatment for village sized communities of up to 1000 people (Brix, 1997). This is in contrast to North America, where surface flow wetlands are the dominant wetland type used mostly for tertiary wastewater treatment for larger communities (Brix, 1997).

2.2.3 Components of constructed wetlands

CWs comprise macrophytes, water, substrate and microbiota. These components all play a role in pollutant removal.

2.2.3.1 Macrophytes

2.2.3.1.1 Macrophyte adaptations

Wetland plants need adaptations to the shortage of oxygen in the root zone of CWs (Verhoeven & Sorrell, 2010). Waterlogged conditions in wetlands create a dynamic soil environment with on average, lower oxygen concentrations compared to unsaturated soil (Verhoeven & Sorrell, 2010). Wetland plants are morphologically adapted to living in water - saturated conditions as is evident by their large internal air spaces for the transportation of oxygen from their aerial organs to their roots and rhizomes (Brix, 1994b). The internal movement of oxygen down the plant serves the respiratory needs of the buried tissues and supplies the rhizosphere with oxygen via leakage from the roots (Brix, 1994b). The oxygen leakage from the roots creates oxidized conditions in the otherwise anoxic substrate and stimulates aerobic decomposition of organic matter and the growth of nitrifying bacteria (Brix, 1994b).

2.2.3.1.2 Role of macrophytes in CWs

Brix (1997) notes that because the most important removal mechanisms in most treatment systems (including conventional systems) are physical and microbial, and that the role of macrophytes in CWs treating wastewater has therefore been questioned. It has been shown however that planted wetlands remove larger quantities of nitrate than unplanted beds (Zhu & Sikora, 1995; Lin *et al.,* 2002 cited in Bastviken *et al.*, 2005). The mechanisms by which plants influence the treatment process are described below:

2.2.3.1.3 Nutrient uptake

Wetland plants require nutrients for growth and production (Brix, 1997). As wetland plants are highly productive, a considerable amount of nutrients can be bound in the biomass (Brix, 1997). Vymazal *et al.* (1998), note that if wetlands are not harvested, large amounts of nutrients incorporated into the plant tissue will be returned back to the water by decomposition.

2.2.3.1.4 Physical effects

Wetland plants stabilize substrate and limit channelized flow and slow water velocities, allowing suspended material to settle (Davis, 1995; Brix, 1997)

2.2.3.1.5 Oxygen leakage to the roots

Macrophytes transport oxygen from their aerial organs to their aerial organs to their roots and rhizomes (Brix, 1994b) that may eventually diffuse into the sediment and water (Reddy *et al.*, 1989; Vretare, 2001 cited in Bastviken, unpub.).

2.2.3.1.6 Influence on soil hydraulic conductivity

In subsurface CWs, the flow of water in the bed is intended to be largely subsurface through channels created by living and dead roots and rhizomes and soil pores (Brix, 1994b). As roots and rhizomes grow, they loosen and disturb the soil (Brix, 1997). When roots and rhizomes die and decay, they may leave behind tubular pores and channels, which are thought by some to increase and stabilize the hydraulic conductivity of the soil (Vymazal *et al*., 1998).

2.2.3.1.7 Provision of surface area for microbial growth

The roots and rhizomes buried in wetland soil provide a substrate for attached growth of microorganisms (Hoffmann, 1986 cited in Vymazal *et al.,* 1998). The attachment of microorganisms to submersed solid surfaces, such as macrophytes, results in the formation of biofilms (Bastviken, unpub.). Furthermore, attached bacteria are more active and more abundant than free-living bacteria in aquatic systems (Hamilton, 1987 cited in Bastviken, unpub.).

2.2.3.1.8 Energy source

Macrophytes use solar energy to assimilate inorganic carbon from the atmosphere to produce organic matter (Vymazal *et al*, 1998). This organic matter provides an energy source for heterotrophic bacteria such as denitrifying bacteria (Bastviken, unpub.).

2.2.3.1.9 Aesthetics and habitat provision

Plants support wildlife and create an aesthetically pleasing appearance (Brix, 1997).

*2.2.3.2 **Water (Hydrology and Hydraulics)***

According to Koskiaho (2006), the driving force behind the existence and function of CWs is closely linked to hydrology and hydraulics. Hydrology describes the quantity and temporal distribution of flow into a CW, whereas hydraulics relate to the patterns and velocities of water movement within a CW (Koskiaho, 2006). Hydrology is driven by weather phenomena (Koskiaho, 2006). Small changes in hydrology can have significant effects on a wetland and its effectiveness at treating wastewater (Davis, 1995).

The design of conventional systems is normally based on hydraulic residence time (HRT); some wetland treatment systems however show a more consistent correlation with hydraulic loading rate than with HRT (R Kadlec, pers. comm. cited in Davis, 1995). Hydrologic considerations in wetland design include climate and weather, hydroperiod, HRT, hydraulic loading rate (HLR), groundwater exchanges, evapotranspiration and water balances (Davis, 1995).

2.2.3.2.1 Climate and weather

Rainfall, snowmelt, drought and temperature can affect wetland treatment (Davis, 1995). For example, high flows caused by heavy rains decrease the efficiency of treatment wetlands as it results in increased flow velocities and reduced HRT (Davis, 1995). High flows may dilute some dissolved pollutants, while increasing the amount of suspended material. Minimum temperatures limit the ability of wetlands to treat some pollutants (Davis, 1995).

2.2.3.2.2 Hydroperiod

A hydroperiod is a cycle of flooded and dry conditions that a wetland experiences (Jackson & Myers, 2002). The hydroperiod of a wetland is as a result of the balance of inflow, outflow and storage (Davis, 1995). A hydroperiod is influenced by evapotranspiration and the HRT (Jackson & Myers, 2002).

2.2.3.2.3 Hydraulic residence time (HRT)

HRT is defined as the average amount of time that a parcel of water remains in the wetland prior to exiting (Ahlers, unpub.). The HRT is the key design criterion for functions such as sediment / toxicant removal and nutrient transformations/removal (Ahlers, unpub.). Variables affecting HRT are soil porosity (hydraulic conductivity), water depth and plant density (Jackson & Myers, 2002). HRT is defined as:

$T=V/Q$

Where T = HRT (days); V = volume (cm^3) and Q = average flow rate (cm^3/day)

2.2.3.2.4 Hydraulic loading rate (HLR)

Hydraulic loading rate (HLR) refers to the flow rate per unit area (ITRC, 2003). It is defined as:

$q = Q/A$

Where q = hydraulic loading rate; Q = average flow rate (cm^3/day); A = wetland area (cm^2)

Some wetlands are operated with intermittent feed, especially vertical flow wetlands (Kadlec & Wallace, 2009). Under these conditions HLR refers to the time average flow rate (Kadlec & Wallace, 2009).

2.2.3.2.5 Groundwater exchanges

If the wetland is properly sealed (lined), groundwater infiltration will be negligible (Davis, 1995).

2.2.3.2.6 Evapotranspiration

In subsurface CWs, evapotranspiration occurs when water is lost to the atmosphere from subsurface water surfaces (evaporation) and through emergent plants (transpiration) (Kadlec & Wallace, 2009). Many macrophytes do not conserve water during hot, dry weather, and can therefore transfer large amounts of water from the wetland into the atmosphere in summer (Davis, 1995). If evapotranspiration rates exceed inflow rates, pollutants may be concentrated to toxic levels and thus supplemental water is required (Davis, 1995).

2.2.3.2.7 Water balance

Water balance is an account of the inflow, outflow and storage (Davis, 1995). During design and operation, the water balance is essential in determining the conformance with the desired limits for HLR, HRT, hydroperiod range and mass balances (Davis, 1995).

2.2.3.2.8 Clogging of subsurface constructed wetlands

Clogging of the porous media and improper hydraulic design can cause flooding of subsurface CWs (Kadlec & Wallace, 2009). Clogging is especially relevant in wetlands using soil for the bed medium (Kadlec & Wallace, 2009). The cumulative physical, chemical and biological treatment processes may cause gradual clogging of the porous media (Knowles *et al*., 2011). Hoffmann *et al*. (2010) note that clogging in horizontal flow beds mostly occurs as obstruction of the inlet area by suspended solids or sludge accumulation. Clogging may result in decreased treatment performance or hydraulic malfunctions such as ponding of wastewater on the surface of the system and bypass of untreated water (Knowles *et al*., 2011). Hoffmann *et al*. (2010) note

that the best way to treat clogging is to control the efficiency of primary treatment. Clogging is also minimized by using larger gravel at the inlet (Hoddinott, 2006)

2.2.3.3 ***Substrate***

Soil, sand, rock, artificial media (Shutes, 2001) and gravel (Davis, 1995) are used as substrates in CWs. Substrates support living organisms in wetlands and provide storage for pollutants (Davis, 1995). Many chemical and biological transformations take place within substrates and the permeability of the substrate affects the flow of water through the wetland (Davis, 1995).

2.2.3.4 ***Microorganisms***

According to Wetzel (1993) in Davis (1995), a fundamental characteristic of wetlands is that their functions are largely regulated by microorganisms and their metabolisms. Microbial biomass is thus an important sink for organic carbon and nutrients (Davis, 1995). Microorganisms grow on the surface of soil particles and roots, where they create a highly active biofilm (Hoffmann *et al*, 2010).

Some microbial transformations occur under aerobic conditions and others under anaerobic conditions. Many species of bacteria can however function under both anaerobic and aerobic conditions .i.e. facultative bacteria in response to changing environmental conditions (Davis, 1995). Subsurface flow CWs are designed for aerobic and facultative wastewater treatment (Hoffmann *et al*., 2010). Facultative processes can occur under temporary oxygen limited / deprived conditions whereas aerobic processes always require the presence of oxygen (Hoffmann *et al*, 2010). The roles of microorganisms in CWs include the transformation of inorganic and organic substances into innocuous or insoluble substances; altering of the redox conditions of the substrate, which thus affects the processing capacity of the wetland; and assisting in the recycling of nutrients (Davis, 1995).

2.2.4 **Design parameters**

The design parameters for CWs are described below.

2.2.4.1 ***Pre-treatment***

CWs are used mainly for secondary treatment (Hoffmann *et al*., 2010). Suspended solids, larger particles and some organic matter need to be removed before wastewater can be treated in subsurface flow CWs (Hoffmann *et al.,* 2010). High concentrations of suspended solids may cause filtration bed clogging and subsequent surface flow (Vymazal, 2002; Hoffmann *et al.*, 2010).

Pre-treatment in small systems usually consists of a septic or settling tank (Vymazal, 2002). Septic tanks are widely used due to their simple construction (Hoffmann *et al*., 2010). Septic tanks are designed and constructed to receive domestic wastewater, in which two processes take place, namely, settling of solids and the decomposition of the accumulated solids by anaerobic digestion (Nguyen *et al*., 2007).

2.2.4.2 ***Surface area***

There are various methods used to size CWs. Examples include simple rule of thumb methods and computer modelling. According to Vymazal (2001) cited in Vymazal (2002), most CWs are sized for sufficient organic matter and suspended solids removal. Kickuth (1977) cited in Vymazal (2002) first proposed the following equation, which has been widely used for the sizing of CWs for domestic and municipal wastewater treatment:

$$A_h = Q_d (\ln C_i - \ln C_o) / K_{BOD}$$

Where A_h = surface area of the bed (m^2); Q_d = average flow (m^3d^{-1}); C_i = influent BOD_5 (mg/l); C_o = effluent BOD_5, and K_{BOD} = rate constant (md^{-1}). Kickuth (1977) proposed a K_{BOD} value of 0.19. Results from full-scale systems in Denmark and the United Kingdom however show that making use of this value for K_{BOD} resulted in systems with insufficient surface area to meet the required effluent parameters (Vymazal, 2002). The K_{BOD} rate thus varies for different CWs as a

result of influence or biodegradability of the influent and the type of media used (Frazer-Williams, 2010).

The rule of thumb method is a simple method for sizing CWs. In the Czech Republic, an area for CWs of 5 m^2 per person equivalent (P.E) is used as a rule of thumb (Vymazal, 2002). The area required per person however on its own is not sufficient for sizing CWs, this parameter can however be used in order to obtain a first indication of area requirements (Hoffmann *et al*., 2010).

2.2.4.3 ***Aspect ratio***

The aspect ratio (length: width ratio) of the CW is calculated using Darcy's Law (Hoddinott, 2006).

$$A_c = Q_s / [K_f (DH / ds)]$$

Where A_c, cross-sectional area of the bed (m^2); Q_s, average flow (ms^{-1}); K_f, hydraulic activity of the media (ms^{-1}); dH / ds, slope (mm^{-1}).

According to Kadlec & Wallace (2009), theoretically a CW with a higher aspect ratio is not better than one with a lower aspect ratio, as long as the water flow is distributed effectively. It has however been speculated that long, narrow flow paths are closer to plug flow conditions when compared to shorter, wider flow paths (Kadlec & Wallace, 2009). Many CWs are built with a low aspect ratio (less than 2) and many have an aspect ratio less than 1 (Vymazal, 1998a, b cited in Vymazal, 2002); the reason for using a low aspect ratio is to distribute wastewater to as wide a profile as possible so as to avoid clogging in the inlet zone (Vymazal, 2002).

2.2.4.4 ***Substrate / filtration media***

The provision of a suitably permeable substrate in relation to hydraulic and organic loading is the most essential design parameter for subsurface flow wetlands (Hoffmann *et al*., 2010). According to Vymazal (2002), the requirements for the filtration media are to: (1) facilitate macrophyte growth, (2) provide a high and sustainable filtration effect and (3) maintain high

hydraulic activity (flow). Vymazal (2002) notes that in the early 1970s and 1980s, CW systems used soil as a substrate, which only fulfilled the first two requirements and which then resulted in surface flow and lower treatment efficiency. He notes that in the late 1980's, coarse materials (gravel and gravel-sand) were introduced in the UK, which then resulted in all three requirements being met.

2.2.4.5 ***Vegetation***

Tanner (1996) lists the following requirements for plants that are used in CWs: (1) ecological acceptability (no significant weed or disease risks or dangers to ecological and genetic integrity of surrounding ecosystems; (2) tolerance of local climatic conditions, pests and diseases; (3) tolerance of polluted water and waterlogged conditions; (4) ready propagation and rapid establishment, spread and growth and (5) a high pollutant removal capacity, either directly or indirectly by enhancement of microbial transformations such as nitrification (root release of oxygen) and denitrification (production of carbon substrates).

Furthermore, Hoffmann *et al.* (2010), suggest that plants with extensive root and rhizome systems below ground be used as well as plants that are able to withstand shock loads and dry periods. Examples of plants that are used in subsurface CWs include common reed (*Phragmites australis*), broad-leaved cattail (*Typha latifolia*), papyrus sedge (*Cyperus papyrus*), canna lily (*Cannas spp*.) (Hoffmann *et al.*, 2010).

2.2.4.6 ***Sealing the bed***

According to Hoffmann *et al*. (2010), a plastic liner, clay layer or concrete base can be used to seal the bed at the base. Plastic liners include inter alia polyvinyl chloride (PVC), polyethylene (high density HDPE and linear low LLDPE) and polypropylene (PPE) (ITRC, 2003). When existing soils or clay that can provide adequate seal are available at the site, compacting these may be sufficient to line the wetland (Davis, 1995).

The purpose of lining the wetland is that it prevents exfiltration of wastewater from the wetland as well as the infiltration of groundwater into the system (Steiner & Watson, 1993). Exfiltration pollution may result in cases where an adequate water level is not assured for the maintenance of

wetland vegetation, whereas infiltration may result in the event that the retention time needed for wastewater treatment is reduced (Steiner & Watson, 1993).

2.2.5 Removal mechanisms in wetlands

The active reaction zone of CWs is the rhizosphere, as this is where the physiochemical and biological processes occur as a result of interactions between plants, microorganisms, the soil and pollutants (Stottmeister *et al*., 2003). CWs are able to remove a wide array of pollutants, including organic matter (measured as BOD and COD), suspended solids, nutrients, metals and pathogen. This literature review will however only focus on nutrients (nitrogen and phosphorus) and pathogen removal as these pollutants are the focus of the research.

2.2.5.1 Nitrogen removal processes

Nitrogen concentration is a concern as it has the potential to cause adverse impacts on receiving aquatic systems (Lee *et al*., 2009). In CWs, nitrogen removal is accomplished via physiochemical and biological treatment processes (Lee *et al*., 2009). The most important inorganic forms of nitrogen in wetlands include ammonia, nitrite, and nitrate, dinitrogen, nitrous oxide, (Kadlec & Wallace, 2009). Nitrogen removal processes are discussed below:

2.2.5.1.1 Ammonification

Ammonification is the biological conversion of organic nitrogen to ammonia (Lee *et al.,* 2009). The process occurs under both aerobic and anaerobic conditions (Kadlec & Wallace, 2009). Ammonia is converted from organic forms via a complex, energy-releasing biochemical process (Vymazal, 2007). Sometimes, microbes use the energy released for growth and the ammonia is incorporated into the microbial biomass (Vymazal, 2007).

Ammonification rates are fastest in the oxygenated zone and decrease as mineralization switches from aerobic to facultative anaerobic and obligate anaerobic bacteria (Vymazal *et al*., 1998). The rate of ammonification is dependent on temperature, pH, C / N ratio, available nutrients and soil structure (Reddy & Patrick, 1984). The optimal temperature for ammonification is between 40 and 60 ºC and the optimal pH is between 6.5 and 8.5 (Reddy & Patrick, 1984).

2.2.5.1.2 Nitrification

As a result of the decomposition process in wetlands, a significant portion of organic nitrogen is converted to ammonia (Mayo & Mutamba, 2004). Nitrification is defined as the biological oxidation of ammonium to nitrite and then nitrate (Reddy & Patrick, 1984). Nitrification, which is undertaken by nitrifiers such as *Nitrosomonas, Nitrospira* and *Nitrobacter,* followed by denitrification, is regarded as the most important pathway for ammonia removal in CW systems (Gersberg *et al*., 1985; Kadlec & Knight, 1996 cited in Lee *et al*., 2009). Nitrification is a chemoautotrophic process (Reddy & Patrick, 1984) in which nitrifiers obtain energy from the oxidation of ammonia and / or nitrite and use carbon dioxide as a carbon source (Vymazal, 2007). Keeney, 1973 and Paul & Clark, 1996 cited in Kadlec & Wallace (2009), note that although nitrification has typically been associated with chemoautotrophic bacteria, heterotrophic nitrification also occurs and can be of significance. Nitrification implies the oxidation of ammonia to nitrate under aerobic conditions and involves a two step process (Lee *et al.,* 2009). The first step in the process involves the oxidation of ammonium to nitrite and is undertaken by chemolithotrophic bacteria, which are entirely dependent on ammonia for the production of energy for growth (Vymazal *et al*., 1998). Lee *et al*. (2009), note that *Nitrosomonas* is the most recognized genus for ammonia oxidation. The second step in the nitrification process, which encompasses the oxidation of nitrate to nitrate is undertaken by facultative chemolithotrophic bacteria which are also able to use organic compounds, in addition to nitrate, for the production of energy for growth (Vymazal, 2007). *Nitrobacter* is the most recognized genus for nitrogen oxidation (Lee *et al*., 2009).

The rate of nitrification is influenced by temperature, alkalinity, pH, inorganic carbon source, moisture, microbial population and concentrations of ammonia-N and dissolved oxygen (Lee *et al*., 2009). The ideal temperature for nitrification is from 30 to 40 ºC and the pH from 7.5 to 8.6 (Vymazal *et al*., 1998)

2.2.5.1.3 Denitrification

Denitrification is a heterotrophic process whereby nitrate is converted to nitrogen gas by denitrifying organisms (Dincer & Kargi, 2000). Intermediate products in this process include nitrite, nitrogen oxide (NO) and nitrous oxide (N_2O) (Bashkin & Howarth, 2002).

Virtually all denitrifiers are chemoheterotrophs (Murray *et al*., 1990). Heterotrophic bacteria use an oxidized form of nitrogen as a terminal electron acceptor, and organic carbon as an electron donor (Lee *et al.,* 2009). The resultant free energy is then conserved in Adenosine triphosphate (ATP), following phosphorylation and is used by denitrifiers to support respiration (Vymazal *et al*., 1998).

Sufficient organic compound is also needed as an energy source for denitrifiers (Lee *et al.,* 2009). In CWs the carbon source is provided by organic pollutants of wastewater or microorganism cell materials (Lee *et al*., 2009). By supplementing organic-limited wastewater with a carbon source, the denitrification rate can be enhanced (Killingstad *et al*., 2002).

Many authors refer to denitrification as an anaerobic process (e.g. Verhoeven & Meuleman 1999). According to Hauck (1984) cited in Vymazal (2007), denitrification is undertaken mainly by facultative anaerobic heterotrophs that substitute oxidized N forms for O_2 as electron receptors in respiratory processes. This process follows aerobic biochemical routes (Hauck, 1984 cited in Vymazal, 2007). For the reasons above, according to Hauck (1984) cited in Vymazal (2007) it is misleading to refer to denitrification as an anaerobic process, as it takes place under anoxic conditions. Aerobic denitrification has also recently been discovered (Robertson *et al*., 1995 cited in Kadlec & Wallace, 2009).

According to Lee *et al*., (2009), the proportion of nitrogen removal by denitrification is typically from 60 to 95% compared to 1 to 34% taken up by plants and algae. Denitrification rates are influenced by nitrate concentration, microbes, type and quality of organic carbon source, hydroperiods, different plant species residues, the absence of oxygen, redox potential, soil moisture, temperature, pH value, and the presence of overlying water (Sirivedhin & Gray, 2006; Vymazal, 1995; Bastviken *et al*., 2005 cited in Lee *et al*., 2009). The optimum pH range is between 6 and 8 and the optimum temperature between 60 ºC and 75 ºC (Vymazal, 2007).

2.2.5.1.4 Anammox (anaerobic ammonium oxidation)

Lee *et al.* (2009) notes that anammox provides a potential alternative process for improving total nitrogen removal. According to Mulder *et al*. (1995), anammox is the anaerobic conversion of $NO_2^-$and NH_4^+ to N_2. In this process, which occurs under anaerobic conditions, ammonium is autotrophically oxidized to nitrogen gas with nitrite acting as an electron acceptor (Lee *et al*., 2009). According to van de Graaf *et al*. (1996), nitrate can also act as an electron acceptor. Anammox bacteria include *Candidatus brocadia anammoxidans, Planctomycetes spp*., *Thiobacillus senitrificans,* Thiomicrospira *denitrificans, Thiosphaera ponotropha, and Paracoccus denitrificans* (Lee *et al*., 2009).

2.2.5.1.5 Plant uptake and assimilation

According to Vymazal, 2007, the potential of emergent plants to take up nitrogen is quite low in CWs, but plant uptake is the major nitrogen removal process in CWs with free-floating macrophytes. Plants require nutrients for reproduction and growth. As wetland plants are highly productive, a considerable amount of nutrients can be bound in biomass (Brix, 1997). Nitrate and ammonium are readily taken up by plants at the root zone (California State University, 2009). This uptake of nutrients by plants converts inorganic forms of nitrogen into organic forms that can be used for cell and tissue growth (Vymazal, 1995). Various plant species differ in the preferred forms of nitrogen absorbed, depending on the available forms of nitrogen in the wetland (Lee *et al*., 2009). According to Atkin (1996), most plants are capable of absorbing any form of soluble nitrogen. Desirable traits for plants used in nutrient assimilation and storage is fast growth, high tissue nutrient content and the ability to obtain a high standing crop (Lee *et al.,* 2009). In order to remove nitrogen from wetlands, plant biomass is required to be harvested, as a vast majority of the nutrients incorporated into the plant tissue will be returned to the water by decomposition processes (Vymazal *et al*., 1998).

2.2.5.1.6 Ammonia adsorption

Ionised ammonia may be adsorbed from solution through cation exchange with detritus, inorganic sediments or soils (Vymazal, 2007). Adsorbed ammonia is bound loosely to substrates which allow it to be easily released when the chemistry conditions of the water change (Lee *et*

al., 2009). Nitrification reduces the concentration of ammonia in the water column; as a result some ammonia will be adsorbed to gain equilibrium with the new concentrations and thus if the ammonia concentration in the water column is increased, adsorbed ammonia would also increase (Vymazal, 2007). If the wetland substrates are exposed to oxygen, adsorbed ammonium may be oxidized to nitrate by periodic draining (Sun *et al.*, 2005; Sun *et al*., 2006; Kim *et al*., 2006 cited in Lee *et al.*, 2009).

2.2.5.1.7 Sedimentation

Most particulate organic nitrogen in CWs is removed by sedimentation (Taylor *et al*, 2005). Particulates may settle out on the wetland floor or adhere to plant stems (Lee *et al*., 2009).

2.2.5.2 ***Phosphorus removal***

Phosphorus in wetlands occurs as phosphate in organic and inorganic compounds (Reddy *et al*., 2005). Free orthophosphate is the only form of phosphorus that is used directly by algae and macrophytes and thus represents a major link between organic and inorganic phosphorus cycling in wetlands (Vymazal, 2007). Wetlands provide an environment suitable for the transformation of all forms of phosphorus (Vymazal, 2007). Phosphorus removal processes are discussed below:

2.2.5.2.1 Soil adsorption and precipitation

Adsorption refers to the movement of soluble inorganic phosphorus from soil pore water to soil mineral surfaces, where it then collects without penetrating the soil surface (Vymazal, 2007). Precipitation refers to the reaction of phosphate ions with cations Fe, Mg, Ca and Al (Vymazal, 2007). In acidic soils inorganic phosphorus is adsorbed on hydrous oxides of Fe and Al and may precipitate as insoluble Fe-phosphates and Al-phosphates (Reddy & D' Angelo, 1997). In alkaline soils, precipitation as insoluble Ca-phosphates occurs (Reddy & D' Angelo, 1997).

2.2.5.2.2 Plant uptake

Plants absorb phosphorus through their roots, which is then transported to their growing tissues Vymazal *et al.*, 1998). Plants and microbiota are able to use phosphorus rapidly because it is a limiting nutrient (Catts, unpub.). According to Boyd, 1969 & Vymazal, 1995 cited in Vymazal (2007), plant uptake of phosphorus is usually highest during the growing season. Catts (unpub.)

notes that emergent macrophytes typically have extensive rhizome and root systems, providing plants with the ability to store large amounts of phosphorus. Similarly to nitrogen, in order to remove phosphorus from wetlands, plant biomass is required to be harvested (Vymazal *et al*., 1998).

2.2.5.2.3 Microbiota uptake

Microbiota includes bacteria, fungi, algae and microinvertebrates. Microbiota have a much higher rate of phosphorus uptake compared to plants because they grow and multiply at higher rates (Catts, unpub.). Vymazal (2007) notes that the amount of phosphorus stored by microbiota is very small and that storage is only temporary as any phosphorus which has been taken up by microbiota is released back into the system after decay.

2.2.5.3 ***Pathogen removal***

Pathogens found in wastewater include bacteria (e.g. *E. coli, Salmonella typhi, Vibrio cholera, Shigella, Legionella, Leptospira, Yersinia),* protozoa *(e.g. Entamoeba, Giardia* and *Cryptosporidium),* helminths (intestinal worms) [e.g. *Ascaris, Enterobios, Taenia, Schistosoma, Trichuris, Fasciola*] and viruses e.g. *Adeno-, Entero-, Hepatitis A-, Polio-, Rota-, Norwalk Virus* (WHO, 2006 cited in Hoffmann *et al*., 2010).

CWs provide a suitable combination of biological, chemical and physical factors for the potential removal of pathogens. Physical factors include mechanical filtration, sedimentation (Vymazal *et al*., 1998) and absorption (Hoffman *et al*., 2010). Chemical removal mechanisms include oxidation, UV radiation and exposure to biocides excreted by some plants and adsorption to organic matter (Vymazal *et al.,* 1998). Biological removal includes antibiosis[7], predation and natural die-off (Seidel *et al*., 1978; Hyde & Ross, 1984; Gersberg *et al*., 1989; Cooper *et al*., 1986 cited in Vymazal *et al*., 1998).

[7] An association between two or more organisms that is detrimental to at least one of them

2.2.6 **Removal efficiencies**

2.2.6.1 ***Nitrogen removal***

Nitrogen removal percentages are mainly dependant on temperature, HRT and loading rate (Rousseau *et al.,* 2008; Lee *et al*., 2009). According to Lee *et al*. (2009), HRT is a critical factor for nitrogen removal, which often requires a longer retention time compared to COD and BOD removal. Studies by Huang *et al.* (2000) showed that ammonium and TKN concentrations decreased dramatically with an increase in HRT. Experiments undertaken by Akratos & Tsihrintzis (2007) showed that generally efficiency of CWs to treat wastewater decreases with an increase in HLR, as higher loading implies smaller HRT and thus lower removal efficiency.

Nitrogen removal may also be affected by coarseness of soils. According to Geller *et al*. (1990), nitrogen removal through adsorption is always more effective in fine-grained soils than in coarse-grained soils. Vymazal (2005) explains that this is because fine-grained soils have a higher cation exchange capacity. He also notes that fine-grained soils are often not used in horizontal flow systems as a result of their poor hydraulic conductivity and that adsorption capacity of the common media used is limited.

Temperature is a key environmental factor as it is important for the activities of nitrifying bacteria and the denitrification of CWs (Langergraber, 2007 cited in Lee *et al.,* 2009). Temperature affects both microbial activity and oxygen diffusion rates (Phipps & Crumpton, 1994 cited in Lee *et al.,* 2009). Studies by Akratos & Tsihrintzis (2007) showed an increase in removal efficiency of TKN and ammonia with an increase in temperature. Other factors include vegetation type, pH, dissolved oxygen concentrations and the hydroperiod of the wetland (Lee *et al.,* 2009).

Denitrification is considered to be the major removal process for nitrogen in most types of CWs (Vymazal, 2007). In wastewater, the concentrations of nitrate are usually low and therefore denitrification must be coupled with nitrification (Vymazal, 2007). As a result of their limited

oxygen transfer capacity, HF CWs do not provide good conditions for nitrification (Vymazal, 2007). According to Vymazal (2005), field measurements have shown that oxygenation of the rhizosphere in horizontal flow CWs is not sufficient, resulting in incomplete nitrification. According to Zhu & Sikora (1994), no obvious nitrification could be observed when dissolved oxygen concentration is lower than 0.5 mg/l. Denitrification however can be very efficient, even at low carbon to nitrogen ratios. In contrast to this, VF CWs provide good conditions for nitrification; however denitrification does not occur (Vymazal, 2005). There has thus been a growing increase in hybrid systems, in which the advantages of both systems can be combined to complement each other (Vymazal, 2005).

Vymazal (2002) compared the treatment efficiency of subsurface CWs to remove nitrogen in the Czech Republic with several other countries. He noted that total nitrogen removal efficiency averaged 41.6% compared to 42.9% in Denmark (Schierup *et al*., 1990b; Brix, 1994b, cited in Vymazal, 2002), 55.6% in North America (Kadlec & Knight, 1996 cited in Vymazal, 2002), 24.5% in Poland (Kowalik & Obarska-Pempkowiak, 1998) and 40.3% in Sweden (Sunblad, 1998). Keffala & Ghrabi (2005) set up a laboratory experiment to determine the nitrogen removal efficiency of various types of CWs. With regard to subsurface CWs, the following average removal efficiencies were reported; TKN, 2%; NH_4^+, 0% and NO_2^- / NO_3^-, 27%.

2.2.6.2 ***Phosphorus removal***

Subsurface flow in CWs often has a greater potential to remove nitrogen than phosphorus (Vymazal *et al*, 1998a in Arias *et al*., 2001). According to Arias & Brix (2004), the capacity of CWs to remove phosphorus is an issue that has not been satisfactorily solved.

Biota are not capable of removing significant amounts of phosphorus (Gray *et al.,* 2000). The potential for CWs to remove phosphorus is limited and highly dependent on the nature of the substrate, due to the phosphorus saturation of the media and consequently the reduction in phosphorus removal efficiency (Arias & Brix, 2004). Other factors that may reduce efficiency include the growth of biofilm attached to the media, which may reduce the contact time between the material and water, and the inhomogeneous nature of the media, which does not guarantee consistent performance of the system (Arias & Brix, 2004). According to Gray *et al*. (2000), it

has been found that the choice of substrate is important for maximizing phosphorus removal. According to Watson *et al*. (1989); Mann (1990) cited in Brooks *et al*. (2000), substrates are often selected based on local availability and particle size for reduced clogging, without consideration for their capacity to remove phosphorus. Vymazal (2004) argues that the limited ability for subsurface CWs can be attributed to the fact that the substrate used in these systems often does not contain adequate concentrations of Ca, Fe or Al. Numerous studies have been done to determine the ability of various media to remove phosphorus (e.g. Arias *et al*., 2001; Brooks *et al*., 2000; Brix *et al*., 2001; Arias & Brix, 2004).

The only sustainable phosphorus removal mechanism is plant uptake and harvesting (Lantzke *et al.*, 1998; Arias *et al*., 2001). The amount of phosphorus that can be removed by harvesting of plants only constitutes a small amount of the phosphorus loaded into the system (Brix, 1994a; 1997). Plants in subsurface wetlands can accumulate only less than 15% of the removed phosphorus at peak standing crop (Vymazal, 2001 cited in Vymazal, 2004). Furthermore, plants are usually not harvested, and therefore any phosphorus that has been taken up will be leached out during senescence[8] (Vymazal, 2004).

According to Richardson *et al* (1997), phosphorus removal mechanisms in subsurface wetlands (soil adsorption and precipitation, plant uptake, microbiota uptake) offer short term storage with a finite capacity. Once these short-term storage components have reached their capacity, they will no longer function effectively as storage areas (Richardson *et al*., 1997).

The poor phosphorus removal was illustrated in a gravel-based system used in Richmond, Australia in which the removal efficiency declined after only 1 - 2 years of operation (Mann & Bavor, 1993 cited in Xu *et al*., 2006). Similarly, Kadlec & Knight (1996) cited in Xu *et al*. (2006) showed that initial phosphorus removal rates from wetland systems in the United States are often above 90% but decline sharply after 4 - 5 years of cumulative phosphorus additions. A study by Vymazal (2004) examined phosphorus removal from horizontal sub-surface flow CWs in the Czech Republic from 1992 to 2001. The results of the study showed that the phosphorus

[8] Senescence is a time during the winter months when the reeds stop growing and die-back to the roots.

removal in horizontal flow CWs was generally low at less than 50%. The poor removal shown in these examples is attributed to the limited phosphorus sorption capacity of the substrate (Xu *et al*., 2006).

As a means to overcome phosphorus removal limitations, several alternatives have been suggested. These include construction of the whole system with chemically enriched media that is capable of removing phosphorus; pre-treatment in which chemical precipitation is undertaken and the removal of phosphorus in separate specific granular media filtration units (Arias & Brix, 2004).

2.2.6.3 ***Pathogen removal***

Faecal coliforms, including *E. coli*, are common indicator organisms used to assess water quality and to determine the presence of pathogens that may cause illness and disease in humans (Jamieson *et al*., 2004 cited in Boutilier *et al*., 2009). Subsurface flow CWs show removal efficiencies close to 100% for coliform and other bacteria (Barry *et al*., 2001 cited in Hoddinott, 2006). Decamp & Warren (2000) carried out experiments to investigate *E. coli* removal rates in various designs of pilot and laboratory - scale subsurface flow CWs. The following removal rates were reported for pilot scale systems; gravel based systems, 98.9% (planted) and 96.6% (unplanted); soil based system, 97.1% (planted) and 96.9% (unplanted). Removal rates for laboratory - scale systems were variable, ranging between 41% and 87%. The lower removal efficiency rates for laboratory - scale systems were attributed to a combination of lower retention time and less efficient microbial communities (Decamp & Warren, 2000). According to Netter (1993) cited in Decamp & Warren (2000), there is a direct relationship between *E. coli* removal and retention time in CWs. In terms of correlation between coliform removal and HLR, studies undertaken by Frazer – Williams (2010) determined that a weak relationship exists.

2.3 **Conclusion**

Increasing environmental concerns coupled with the need for efficient and low cost wastewater treatment, especially in isolated areas, has resulted in a greater interest in alternative wastewater treatment methods such as CWs. Research has shown that CWs are able to remove pollutants

such as nutrients and pathogens. Many successes have been reported. However, these results also show variable performances as well as underperformances in some instances. This may be attributed to the fact that some of these systems are overloaded, inappropriately designed or unmanaged, thus emphasising the need for comprehensive design criteria.

3 RESEARCH METHODS

The aim of the research project is to determine the performance of constructed wetlands (CWs) in treating domestic wastewater in peri-urban / rural settings of the Western Cape, South Africa. The research sought to measure the ability of purpose built CWs to reduce or limit the throughput of excessive nutrients and to reduce the bacterial load so as to meet the Department of Water Affairs (DWA's) wastewater discharge standards. This chapter discusses the research methods used in order to achieve the aim and associated objectives of the research.

The research design comprised the collection of primary and secondary data. Primary data were collected by undertaking field studies to obtain water quality information at selected sampling points in the period between July and October 2011. This data could then be used to determine the performance of constructed wetland (CW) systems. Secondary data were collected by way of interviews with the landowners and those responsible for the design and construction of the CW systems.

Field studies were conducted at selected sites to study the performance of CW systems in the treatment of domestic wastewater. The three sites selected are located in the Western Cape. The CWs that have been constructed at these sites are sub surface flow (SSF) CWs and are small scale systems (<125 people). These systems are being used to treat domestic wastewater (comprising black water and greywater) to a secondary level of treatment.

The field studies included water quality monitoring to determine the quality of water before and after entering the CWs. Water quality of the influent was compared with that of the effluent as an indication of the performance of each system. The effluent quality was also compared to DWA's effluent standards for discharge into a freshwater resource. Furthermore, since the treated effluent was eventually released into freshwater systems, it was necessary to determine whether the discharge from these treatment systems impacted on the receiving water bodies. It was therefore also necessary to monitor nearby culverts, streams and vleis (lakes) into which the

treated water was discharged. The water quality was then compared to the relevant South African water quality guidelines.

Information collected during the study included design information of each system that was gathered from meetings, site visits and e-mail correspondence with the developer and landscape architect. The design of these systems differs in terms of *inter alia* surface area and bed configuration, aspect ratio, depth of the bed, slope, flow rate, volume of water entering the system, plant species and plant density. Given these differences the study makes no attempt to compare these systems with each other.

Pilot studies were conducted to test the logistics and gather information prior to actual sampling and to improve the ability to monitor each of the selected systems.

The rest of the chapter will discuss the detailed method that was used to undertake the research. The chapter focuses on project initiation, sampling, laboratory analysis and data analysis.

3.1 Project initiation

3.1.1 Site selection

A site selection meeting was held with the landscape architect responsible for the planting of the CWs selected in this study and in other CWs in the Western Cape. A list of all these sites was provided and considered. The list included sites where CWs were being used to treat winery, agricultural and domestic wastewater. As the project only focused on the use of CWs for treating domestic wastewater, this list was narrowed down to three sites, namely De Goede Hoop Estate (Noordhoek), Wolwedans Farm (Stellenbosch) and Babylonstoren Farm (Simondium). Each of these sites will be explained in detail.

3.1.2 Secondary data collection

Once the sites were selected, information about the sites and CWs was collected. Information was gathered via e-mail correspondence and during meetings held with the landscape architect

and the engineer who designed the systems. They were able to provide a reasonable level of detail on the design of the systems as well as contact details of the landowners at the selected sites. The landowners / farm managers were then contacted and informal meetings were held with each of them. The purpose of these meetings was to obtain further information about each of the CWs, water consumption and previous water quality results that were available to them.

3.1.3 **Sample point selection**

All the sites were visited prior to selecting sampling points within each system. The landowners and engineer also provided input and advice in the selection of sampling points within each system. As a general rule it was decided that sampling points must include at least one point within the system where wastewater entered the CW; where the treated wastewater left the system; and at a stream / culvert / vlei into which treated effluent was released. Four or five sample points were selected for each site.

3.1.4 **Variable selection**

The variables chosen for sampling / analysis in this study included selected nutrients and *E. coli*. The nutrients selected included ammonia (NH_3 ionised and un-ionised forms), nitrate (NO_3^-), nitrite (NO_2^-) and orthophosphate (PO_4^{3-}). These nutrients were chosen primarily as a result of the concern regarding high levels of nutrients (especially nitrogen and phosphorus- containing compounds) that enter water sources and that may cause eutrophication. *E. coli* was used as a pathogen indicator and considered important to measure because of the concerns related to the possible impacts of pathogens on downstream water quality. These variables are also comparable with studies undertaken by other researchers. While chemical oxygen demand (COD) and biological oxygen demand (BOD) are also important parameters as they determine the amount of organic pollutants in the water, these did not form part of variable selection as a result of cost constraints associated with laboratory analysis.

Secondary variables that were measured included electrical conductivity, total dissolved solids, temperature and pH. These variables assisted in providing background information with regard to the physical properties of the water/wastewater (Appendix 2). Furthermore, the volume of water and flow rate of the water passing through the systems were also recorded. However, as

the water meter at Babylonstoren stopped working, the water volume and thus the flow rate could not be obtained for this site.

3.1.5 **Pilot studies**

Pilot studies were undertaken in which preliminary sampling was undertaken at the sites. This involved sampling of all of the points and undertaking laboratory analysis on the samples. The pilot studies assisted with identifying problems prior to the actual sampling and helped to determine where to select the sampling points.

3.2 **Study area**

The study area comprises three sites located on the periphery of Cape Town, Western Cape. These sites include a single household at De Goede Hoop Estate (GPS co-ordinates: 34^{o}05’21.58’’S, 18^{o} 22’12.94’’E) in Noordhoek, Wolwedans Farm in Stellenbosch (GPS co-ordinates: 33^{o}55’25.37”S, 18^{o}47’24.81”E) and Babylonstoren Farm in Simondium. (33^{o}49’24.12”S, 18^{o}56’8.67”E). A map shows the study sites in relation to each other (Figure 5

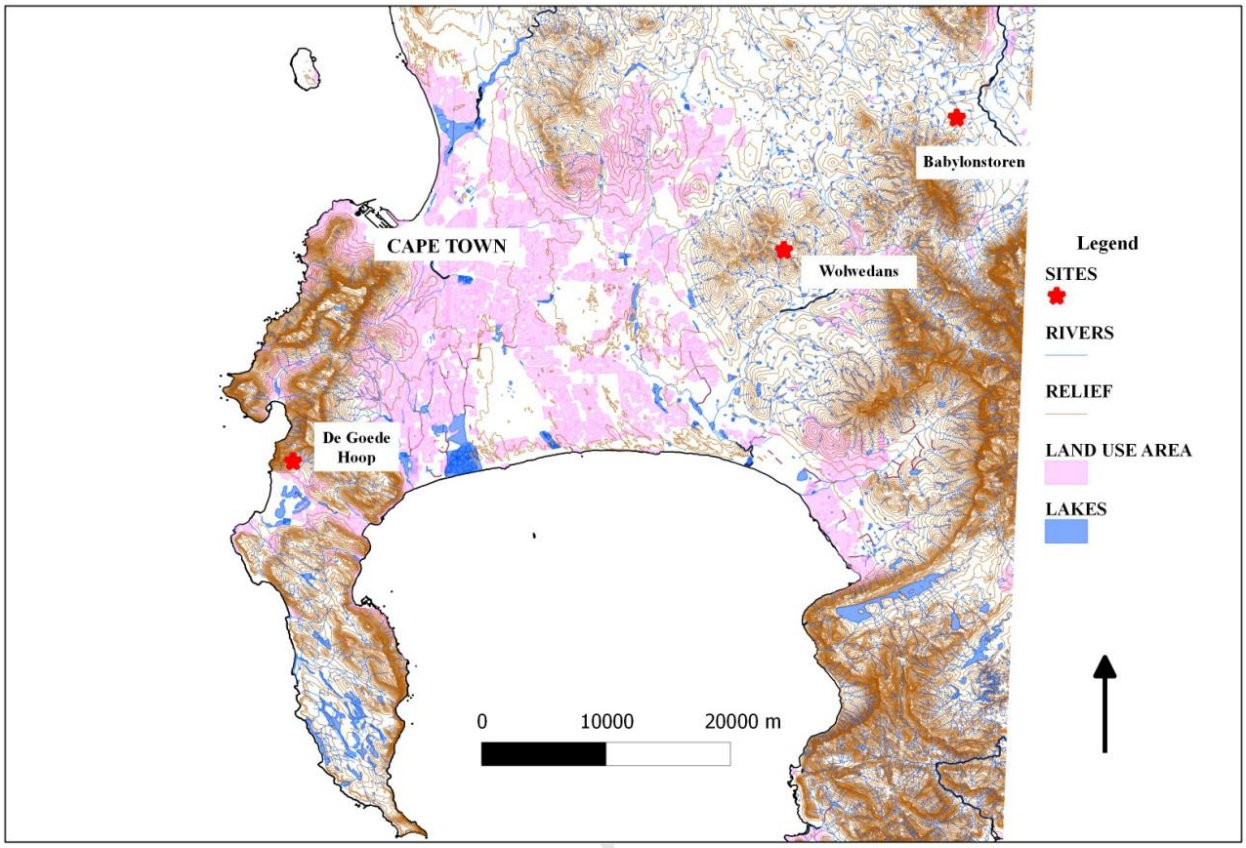

Figure 5: Locality map of the study area

3.2.1 De Goede Hoop Estate

De Goede Hoop is a security estate located in Noordhoek (Figure 6). The estate comprises a number of properties. This study however only included a single property within the estate. The CW located at the site treats domestic wastewater from one household, approximately 6-15 people (includes workers and guests). On average, 50 000 L of water is used per month. An Invensys water meter supplied by Balamanzi was installed to determine more accurately how much water passes through the CW system.

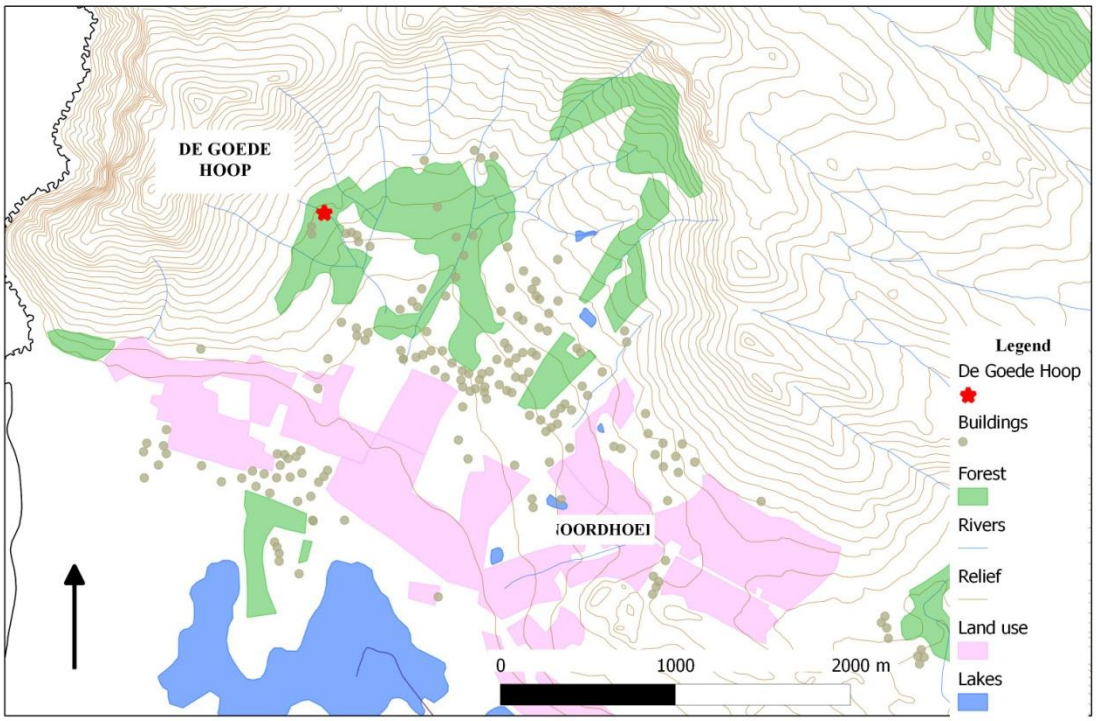

Figure 6: Location of De Goede Hoop Estate, Noordhoek

The wetland was constructed in September 2009 and planted with a variety of plant species. *Cyperus textilis* was planted in a snake-like pattern, and other plants planted around these (Figure 7Figure). Other plants used include *Schoenoplectus scirpoides, Cyperus dives, Cyperus papyrus, Cyperus thunbergii, Calopsis paniculata, Wachendorfia thyrsiflora, Pennisetum macrourum, Chondropetalum tectorum, Orphium frutescens, Juncus krausii, Elegia capensis, Psoralea pinnata, Juncus effuses, Carex clavata, Isolepis prolifer.*

The system was designed to have an average flow rate of 2 000 L per day and a peak flow rate of 4 000 L per day. The theoretical HRT is two days. The CW is rectangular in shape with an area of approximately 100 m^2. The CW is lined with clay with approximately 300 mm of recycled rubble (comprising 19 mm pieces of brick, ceramics, marble and granite) forming the substrate.

Sewage and greywater flows by gravity to a primary sedimentation tank, which comprises two chambers. Solid materials settles and are retained in a primary sedimentation tank. Solid-free

effluent then overflows from the primary sedimentation tank to a pump sump which in turn feeds a bioreactor. Whilst the bioreactor is filling, wastewater is continuously aerated. During the aeration cycle, the biomass is completely mixed and biodegradable COD degradation and nitrification occurs. At predetermined times, the aeration cycle ends and a period is then allowed for biomass settlement. From the bioreactor, wastewater is pumped into the CW. The water then enters a recirculation pond. Water from the recirculation pond is then recycled back into the CW. Some of the water from the recirculation pond is piped into a constructed pond approximately 5 m southwest of the CW. This activity occurred during the first four sampling sessions, thereafter the landowner reverted back to the previous closed system, in which all treated wastewater was circulated back into the CW. Treated effluent in the CW then either evaporates or enters the groundwater. For a graphical representation of water flow through the system, refer to Figure 12.

Figure 7: Constructed wetland at De Goede Hoop Estate (western view)

3.2.2 Wolwedans Farm

Wolwedans Farm, located in Stellenbosch (Figure 8) is a wine farm and consists of farm houses, a number of workers' cottages as well as other buildings. The CW (Figure 9) located at the site treats domestic wastewater from five households, an office (has one toilet, a washbasin and kitchen sink) and three storerooms (six toilets, two showers and nine washbasins in total). Workers cottages are not linked to the CW system.

Accurate information could not be obtained about water consumption. This is because the main source of the water used was extracted from a borehole which was not metered. On average, approximately 120 000 L of water is used per month during winter, this is lower compared to summer time when approximately between 150 000 – 180 000 L of water is used per month (these estimations were provided by the landowner). An Invensys water meter supplied by Balamanzi was used to determine the volume of water passing through the system.

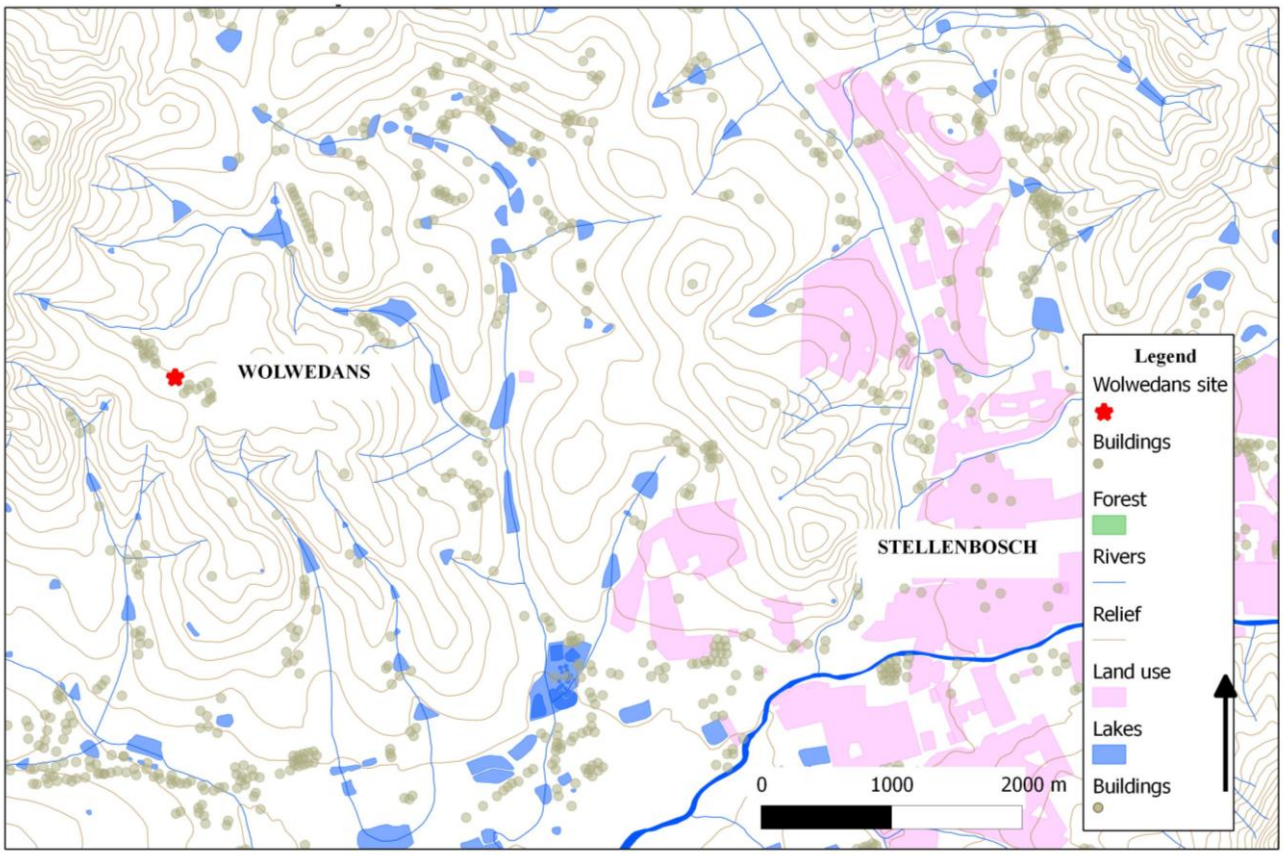

Figure 8: Location of Wolwedans Farm, Stellenbosch

The system was constructed in 2007 and planted with a variety of plant species. The plants used included *Elegia capensis, Cyperus textilis, Cyperus papyrus 'nana', Psoralea pinnata, Cyperus dives, Juncus krausii, Juncus effuses, Carex clavata, Scirpus nodosus, Pennisetum macrourum, Chondropetalum tectorum.* Rosemary was planted around the edge of the CW to act as a barrier to prevent people from accessing the CW area.

The system was designed to have an average flow rate of 1 100 L per day and a peak flow rate of 3 300 L per day. The theoretical HRT is two days. The CW is circular in shape with an area of approximately 110 m^2. The CW is lined with plastic. Approximately 1 m of recycled rubble (comprising 19 mm pieces of brick, ceramics, marble and granite) form the substrate.

Pre-treatment includes solids collection in septic tanks. Each household has a separate septic tank. Solids from the septic tank are treated with Sanitree's organic breakdown granules on a monthly basis. Overflow from the septic tanks are piped underground to a storage tank. From there wastewater is periodically pumped to an aeration tank. A quantity of this wastewater is then periodically drained to the CW. The wastewater then works its way through the CW. The treated water then works its way by gravity to the centre of the CW from where it is drained to a storage dam. During dry times water is pumped from the storage dam to the clean water dam to supplement the inflow from springs. The water from the clean water dam is used to irrigate the gardens and fill spraying equipment. About 800 m from the storage dam is a vlei which receives the storage dam water overflow. It should be noted that additional septic tank water (i.e. no CW treatment) from workers' cottages also enters this vlei area. However, prior to flowing into the vlei area, this water is captured in a French drain, and then overflows into a grassed trench. For a graphical representation of water flow through the system, refer to Figure 13.

Figure 9: Constructed wetland at Wolwedans Farm (southern view)

3.2.3 **Babylonstoren Farm**

Babylonstoren Farm, located in Simondium (Figure 10) is a fruit and wine farm which comprises work houses as well as several guesthouses for visitors. The CW (Figure 11) located at the site treats domestic wastewater from 25 work houses and 12 guest houses. On average, 319 000 L of water is used per month (based on January – December 2010 period). For two months of the year (February and March), wastewater from the wine cellar is also treated in the CW.

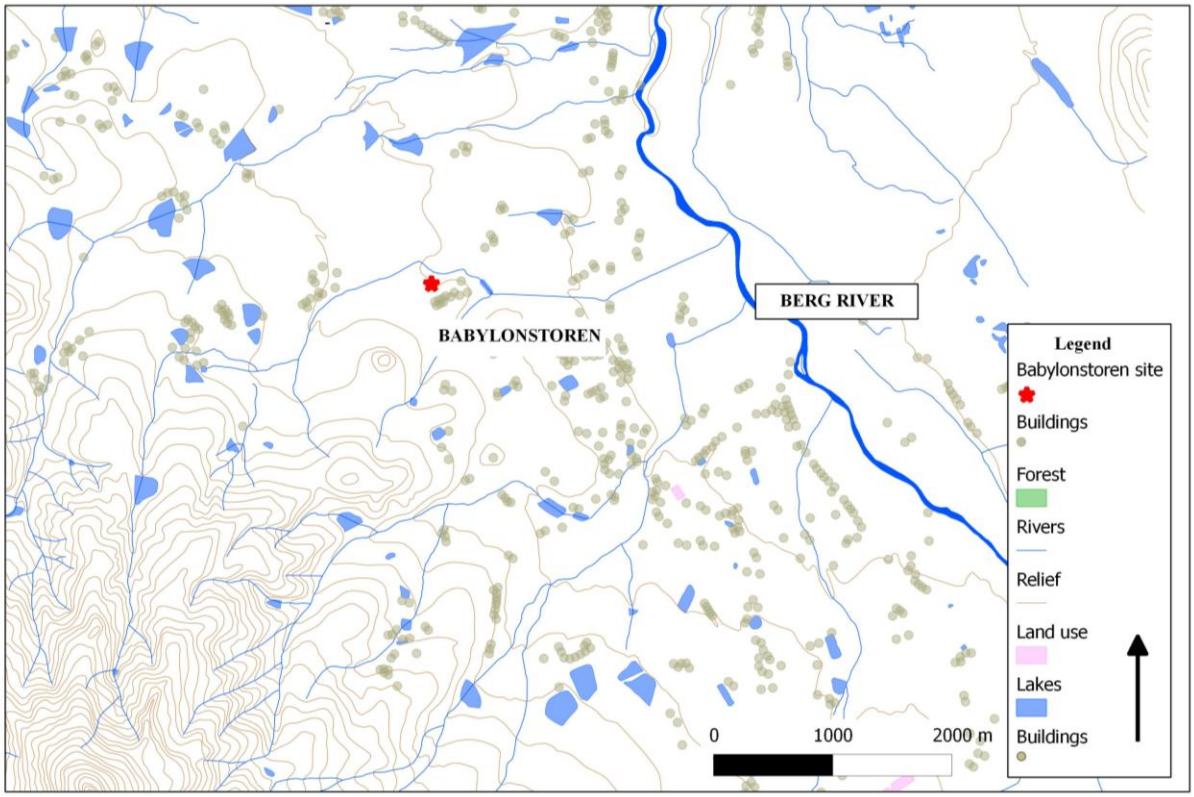

Figure 10: Location of Babylonstoren Farm, Simondium.

The system was constructed in February 2009 and planted with a variety of plant species. The plant species used include *Wachendorfia thyrsoides*, *Calopsis paniculata*, *Carex clavata*, *Juncus krausii*, *Juncus effuses*, *Orphium frutescens*, *Cyperus papyrus*, *Cyperus textiles*, *Cyperus dives*, *Psoralea pinnata*, *Chondropetalum tectorum*, *Schoenoplectus scirpoides*, *Scirpus nodosus*, *Isolepis prolifer*. Rosemary was planted around the edge of the CW to separate it from a plum plantation.

The system was designed to have a design peak flow rate of 50 000 L per day (sewage flow, 36 000 L and cellar flow, 14 000 L). The theoretical HRT is four days. The CW has a square shape; however the water flows only along the perimeter of the square in an area of approximately 140 m^2. The CW is lined with clay with a layer of recycled rubble (comprising 19 mm pieces of brick, ceramics, marble and granite) forming the substrate.

Wastewater from a septic tank is discharged by gravity to a two chambered primary sedimentation tank. Solid materials then settle and are retained in a primary sedimentation tank. Solid-free effluent is then pumped into a bioreactor. During the aeration cycle, the biomass is completely mixed and biodegradable COD degradation and nitrification occur. At the end of the aeration cycle, a period is then allowed for biomass settlement. Treated effluent is then pumped from the bioreactor to the start of the CW. The treated effluent then works its way through the CW and exits via a subsurface pipe, which leads to the adjacent culvert. Water from the culvert eventually leads into the Berg River approximately 2.2 km away. For a graphical representation of water flow through the system, refer to Figure 15

Figure 11: Constructed wetland at Babylonstoren Farm (southeastern view)

A comparison of the three study sites is provided in Table 6:

Table 6: Comparison of the study sites

Site	De Goede Hoop Estate	Wolwedans Farm	Babylonstoren Farm
Location	Noordhoek	Stellenbosch	Simondium
Construction date	September 2009	2007	February 2009
CW size	Approx 100 m^2	Approx. 110 m^2	Approx. 140 m^2
CW depth	Approx. 300 mm	Approx. 1 m	Approx. 300 mm
CW shape	Rectangular	Circular	Square (water flows along the perimeter of the square)
Number of households	1	5 households, an office (has one toilet, a washbasin and kitchen sink) and three storerooms	25 work houses and 12 guest houses
Retention time	Two days	Two days	Four days
Peak flow rate	4 000 L per day	3 500 L per day	50 000 L per day (sewage flow, 36 000 L per day and cellar flow, 14000 L per day)
Lining	Clay-lined	Plastic - lined	Clay – lined

3.3 Sampling

Sampling was undertaken between the cooler months of July and October 2011. This was done so as to provide insight into how the CWs performed under non-ideal conditions. Each site was sampled every two weeks at several sampling points. The sampling points for each site are as follows:

3.3.1 De Goede Hoop Estate

Samples were taken at five locations (Figure 12 and Figure 13) along the system, these included:

- Influent (bioreactor)
- Effluent (recirculation pond)
- Pond
- Groundwater tank
- Stream

The bioreactor was selected as it represents the influent prior to entering the CW. As the system is based on a recirculating design, the recirculation pond was sampled to represent the effluent concentration after treatment. The pond, located southwest of the system was also sampled, as it received treated water from the recirculation pond. Although the pond received treated effluent only for the duration of four sampling sessions, it was sampled throughout the sampling period. Although it could not be determined whether treated effluent entered the groundwater, groundwater samples were taken to determine whether it was being impacted by the system (groundwater samples were analysed to determine nutrient and *E. coli levels*). A nearby stream, flowing west of the CW was also sampled. Although, this stream did not form part of the treatment system, it was sampled anyway to determine whether it was impacted on in any way by the CW system.

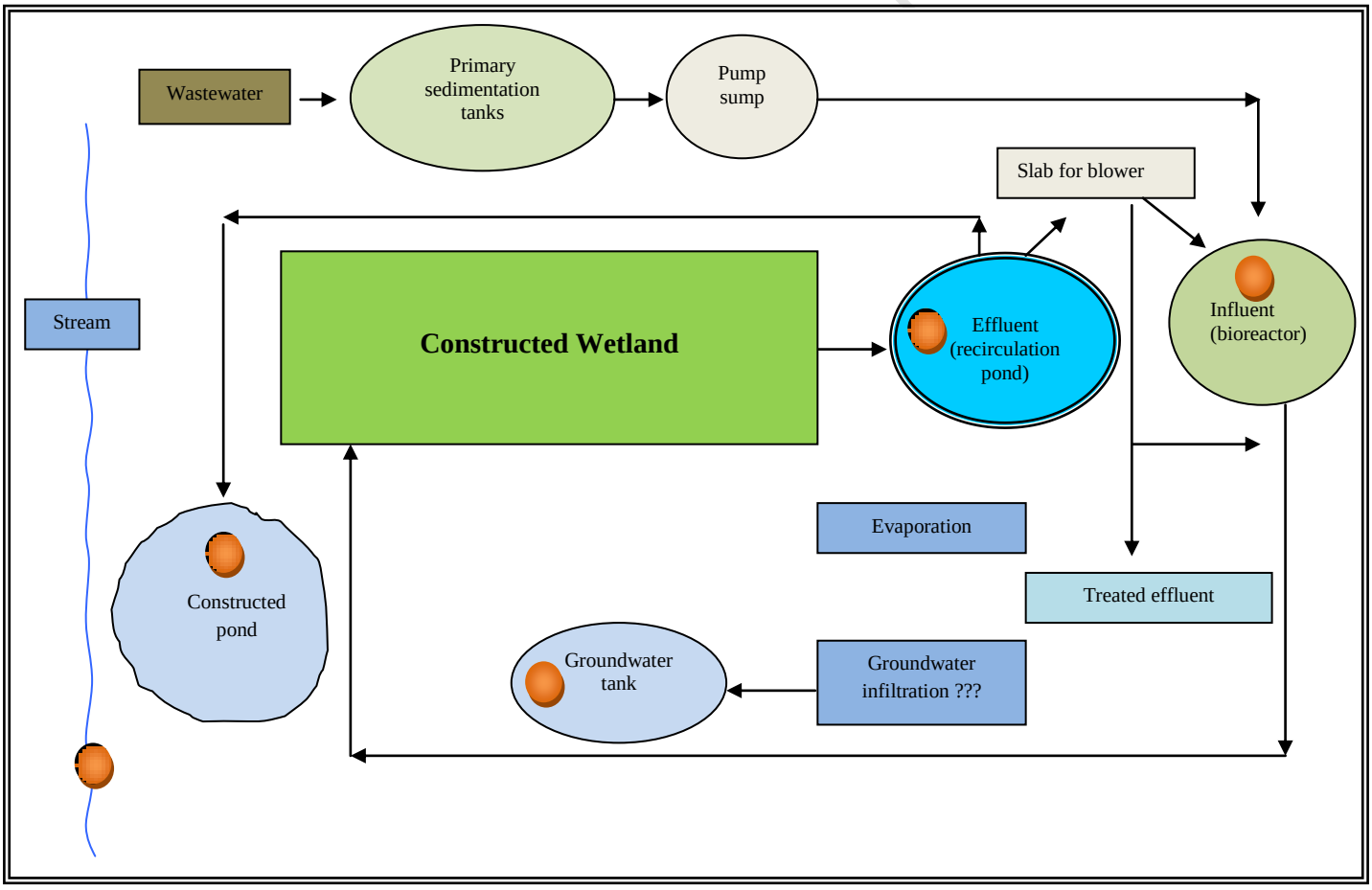

Figure 12: De Goede Hoop sampling points (diagram)
(Sampling points indicated by orange dots)

Figure 13: Sampling points at De Goede Hoop Estate
Bioreactor (a), recirculation pond (b), groundwater tanks (c) and stream (d)

3.3.2 **Wolwedans Farm**

Samples were taken at four locations (Figure 14 and Figure 15) along the system, these included:

- Influent (aeration tank)
- Effluent
- French drain overflow
- Vlei

The aeration tank was selected as it represents the wastewater prior to entering the CW. The outlet pipe at the end of the CW provided an indication of the effluent quality after treatment. As the vlei receives combined treated effluent from the CW and the French drain overflow, it was also sampled to determine the impact that these wastewater treatment measures have on the system. Since no data exist for the water quality of the vlei prior it to receiving treated wastewater, a spring, which formed the control, was used to compare the surrounding water quality with that of the vlei.

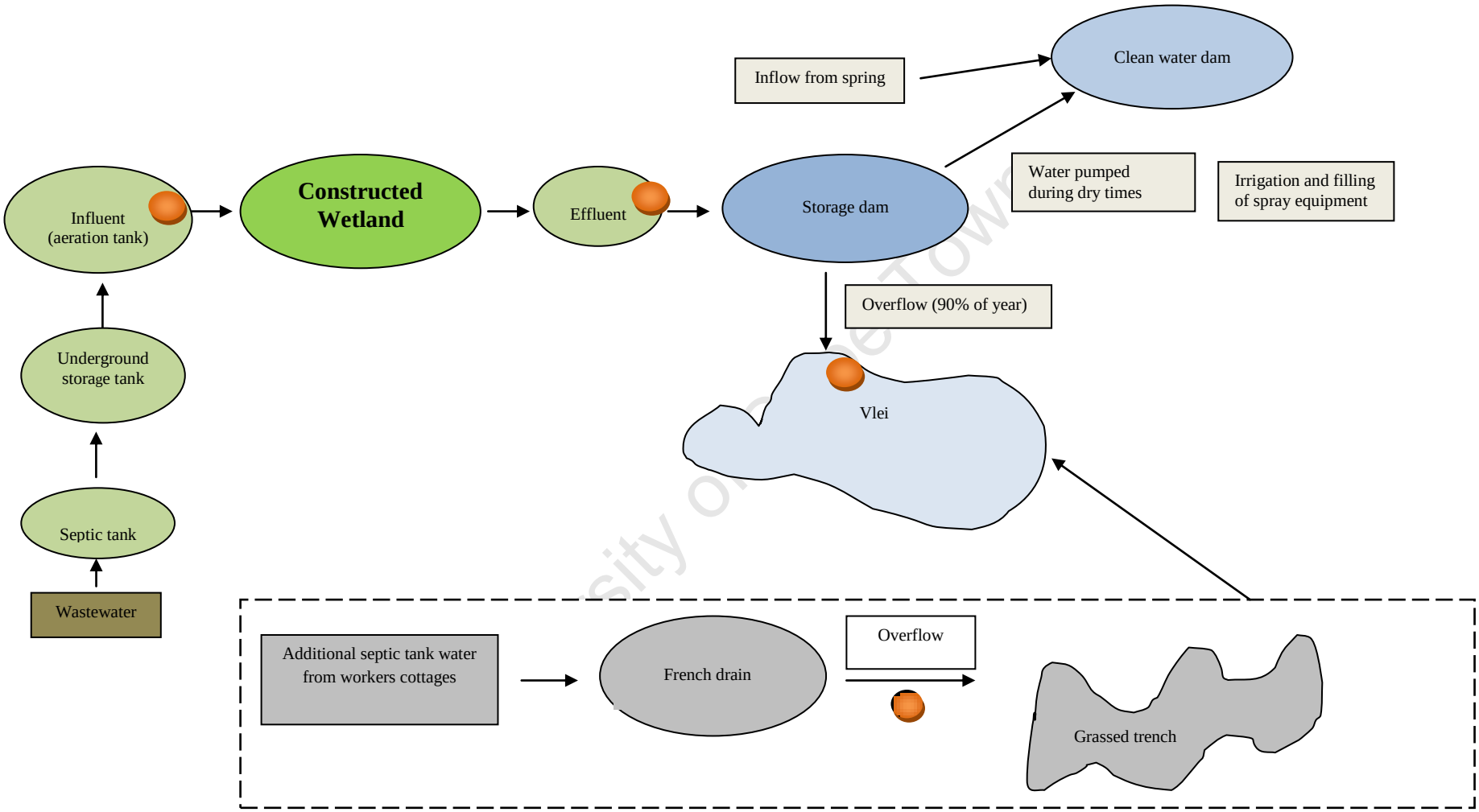

Figure 14: Wolwedans sampling points (diagram)
(Sampling points indicated by orange dots**)**

Figure 15: Sampling points at Wolwedans Farm
Aeration tank (a), effluent manhole b), French drain overflow (c) and vlei (d)

3.3.3 Babylonstoren

Samples were taken at four locations (Figure 16 and Figure 17) along the system, these included:

- Upper section of culvert (upper culvert)
- Influent (bioreactor)
- Effluent
- Lower section of culvert (lower culvert)

The bioreactor was selected as it represents the influent i.e. the wastewater prior to entering the treatment system. The CW output pipe provided information regarding the effluent concentration after treatment. Sampling at the upper and lower sections of the culvert provided an indication of the impact of the system on the surrounding environment as the upper culvert represented a section of the culvert before receiving discharged wastewater and the lower culvert presented a section of the culvert which received wastewater. The water

quality of the upper and lower culvert could then directly be compared with each other to determine the difference in pollutant concentrations.

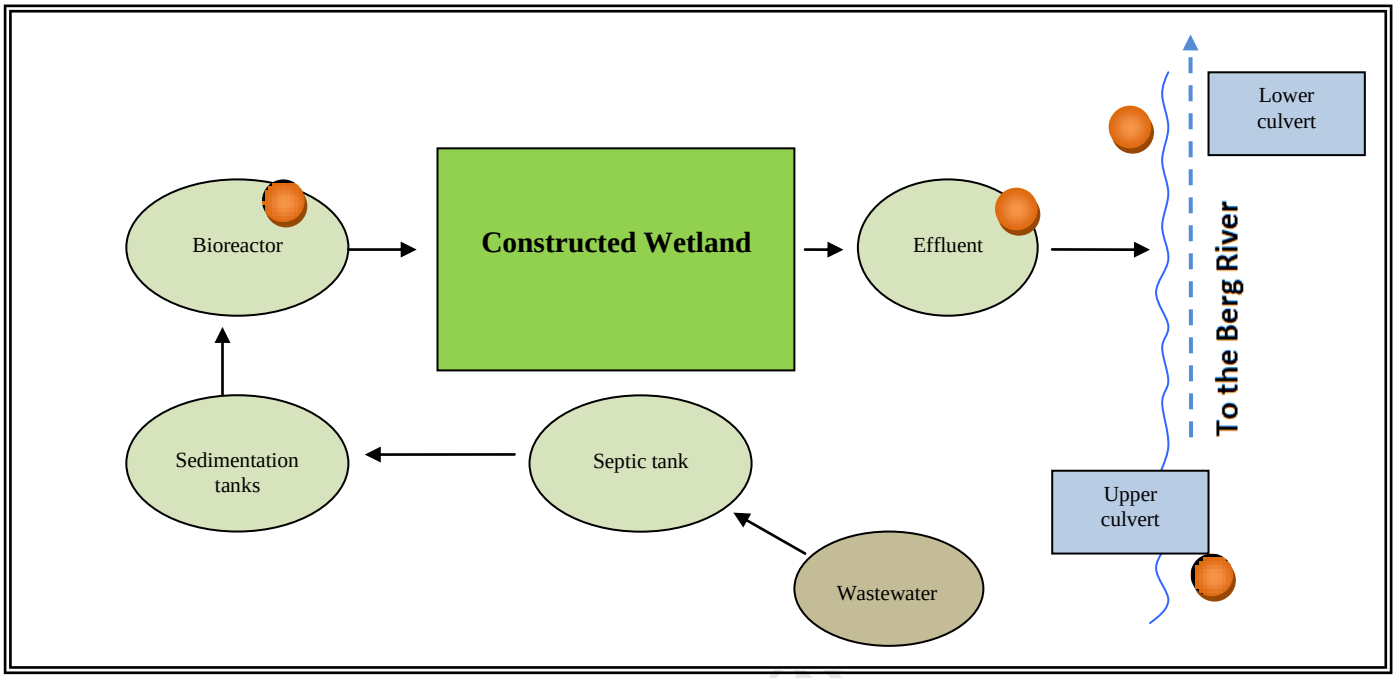

Figure 16: Babylonstoren sampling points (diagram)
(Sampling points indicated by orange dots)

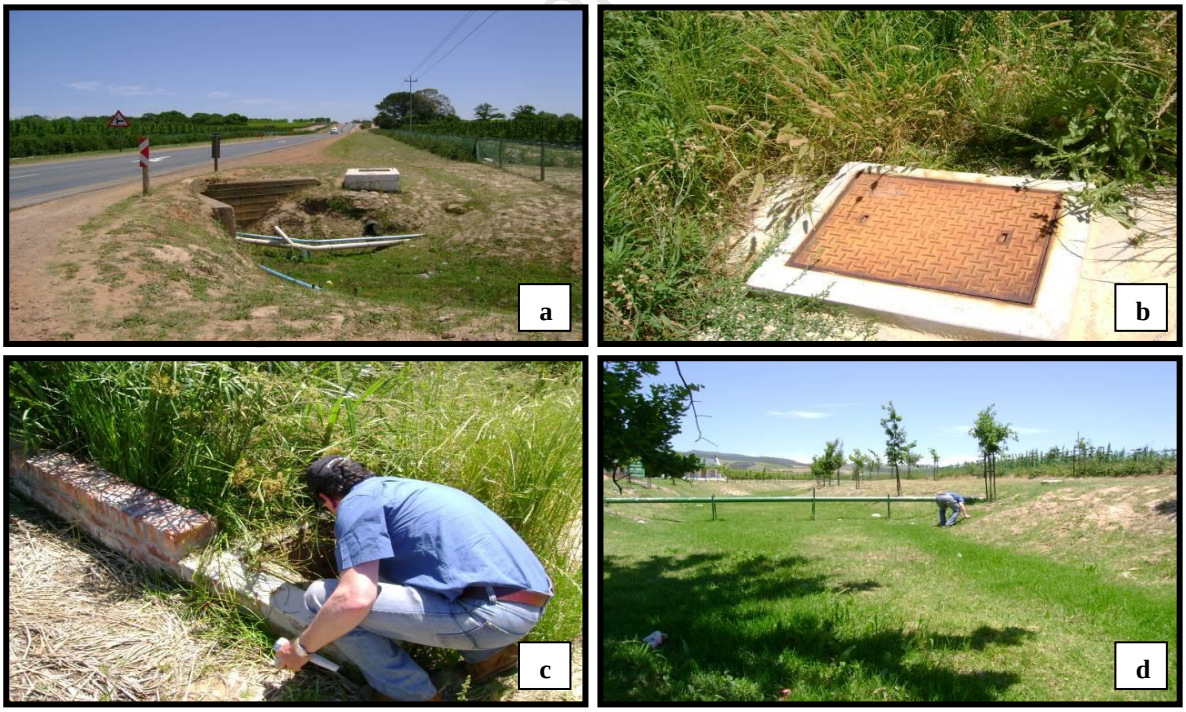

Figure 17: Sampling points at Babylonstoren Farm

3.3.4 Measurement of variables

Variables such as temperature, pH, total dissolved solids and electrical conductivity were measured on site using hand held meters. These variables were tested at each sample point and recorded. The instruments used included a pH scan WP1, 2 pH meter (*Eutech Instruments*) and an EC 59 electrical conductivity meter (*Martini Instruments*), which measured electrical conductivity, total dissolved solids and temperature. These instruments were regularly calibrated during the collection process.

The remaining variables, which could not be measured on site, were measured at the water analysis laboratory at the University of Cape Town. Samples were collected at each point and appropriately labelled. The samples were kept on ice to slow down bacterial growth prior to laboratory testing.

3.4 Laboratory methods

Laboratory testing was done to determine the concentrations of NH_3, NO_3^-, NO_2^-, and PO_4^{3-} and the number of *E. coli* colony forming units (CFUs). A description of the methods used to undertake these tests is provided below:

3.4.1 Chemical analysis

Ammonia: (NH_3 ionised and unionised)

The method used to determine the concentration of nitrate was the Salicylate Method (Hach Method 8155). A sample cell was filled with 10 ml of sample. To calibrate the spectrophotometer at zero, a blank control was prepared by filling a second sample cell with 10 ml deionized water. The contents of one Ammonia Salicylate Powder Pillow were added to each sample cell. The cells were stoppered and shaken to dissolve the reagent. A reaction time of three minutes was then allowed. The contents of one Ammonia Cyanurate Reagent Powder Pillow were then added to each cell. The cells were then stoppered and shaken to dissolve the reagent. A reaction time of 15 minutes was allowed (the presence of ammonia-nitrogen was indicated by a green colour).

As the DR 2700 portable spectrophotometer has a number of pre-installed programmes for various tests, it allows you to choose a test and it will automatically adjust the wavelength for the required test. For the ammonia test, the wavelength was automatically set at 655 nm. After the blank was used to zero the spectrophotometer, the sample was then inserted and the concentration read. The spectrophotometer provided the results in mg/l.

Nitrate (NO_3^-):

The method used to determine the concentration of nitrate was the Cadmium Reduction Method (Hach Method 8039). A sample cell was filled with 10 ml of sample. The contents of one NitriVer® 5 Nitrate Reagent Powder Pillow were added to this. The sample cell was stoppered and shaken vigorously for one minute. A reaction time of five minutes was then allowed (the presence of nitrate was indicated by an amber colour). As the DR 2700 portable spectrophotometer has a number of pre-installed programmes for various tests, it allows you to choose a test and it will automatically adjust the wavelength for the required test. For the nitrate test, the wavelength was automatically set at 500 nm.

To calibrate the spectrophotometer at zero, a blank control was prepared by filling a second sample cell with 10 ml sample but no NitriVer® 5 Nitrate Reagent. The sample was then inserted into the spectrophotometer and the concentration read. The spectrophotometer provided the results in mg/l.

Nitrite (NO_2^-):

The method used to determine the concentration of nitrite was the Diazotization Method (Hach Method 8507). A sample cell was filled with 10 ml of sample. The contents of one NitriVer® 3 Nitrite Reagent Powder Pillow were added to this and the sample cell swirled to dissolve the reagent in the mixture (the presence of nitrite was indicated by a pink colour). A reaction time of 20 minutes was allowed. As the DR 2700 portable spectrophotometer has a number of pre-installed programmes for various tests, it allows you to choose a test and it will automatically adjust the wavelength for the required test. For the nitrite test, the wavelength was automatically set at 507 nm.

To calibrate the spectrophotometer at zero, a blank control was prepared by filling a second sample cell with 10 ml sample but no NitriVer® 3 Nitrite Reagent. The sample was then

inserted into the spectrophotometer and the concentration read. The spectrophotometer provided the results in mg/l.

Orthophosphate (PO_4^{3-}):
The method used to determine the concentration of orthophosphate was the PhosVer 3 (Ascorbic Acid) Method (Hach Method 8048). A sample cell was filled with 10 ml of sample. The contents of one PhosVer® 3 Phosphate Powder Pillow were added to this. The sample cell was immediately stoppered and shaken vigorously for 30 seconds (the presence of orthophosphate was indicated by a blue colour). A reaction time of two minutes was allowed.

As the DR 2700 portable spectrophotometer has a number of pre-installed programmes for various tests, it allows you to choose a test and it will automatically adjust the wavelength for the required test. For the orthophosphate test, the wavelength was automatically set at 880 nm.

To calibrate the spectrophotometer at zero, a blank control was prepared by filling a second sample cell with 10 ml sample but no PhosVer® 3 Phosphate Reagent. The sample was then inserted into the spectrophotometer and the concentration read. The spectrophotometer provided the results in mg/l.

3.4.2 Testing for *E .coli*

M-endo agar was used as the media for growing *E. coli*. The method selected for preparing the agar was as per the method provided by Difco Manual (1953).

Preparation of the agar plates:
Reagents used for the preparation of the agar plates were obtained from Merck. Peptone powder (10 g), di-potassium hydrogen phosphate (2.5 g), lactose (10 g), sodium sulphite anhydrous (3.3 g), fuchsin (0.3 g) and agar powder (12.5 g) were added to a glass bottle. Distilled water was then added to the 1 litre mark and the mixture shaken. The pH of the solution was then adjusted to 7.4 ± 0.2 at 25 ^{o}C. The solution was then autoclaved for 30 minutes at 121 ^{o}C and 15 psi. Once the solution cooled down sufficiently, the solution was poured out into petri dishes and allowed to set.

Sample preparation and spreading of plates:

The water samples were diluted 1/10, ½, 1/100 and 1/10 000. 100 *u*l water sample of each dilution (including undiluted sample) was pipetted onto the prepared agar plates. A duplicate plate for each dilution was also prepared. Plates were incubated at 35 ^{o}C for 24-48 hrs.

Counting the *E .coli* colony forming units (CFUs):

The presence of *E. coli* is indicated by red colonies with a green metallic sheen. The number of CFUs at each concentration was then counted and recorded as the number of CFUs/100ml.

3.5 **Data analysis**

Statistical analysis of the laboratory results from data collected was undertaken using Statistica. Descriptive statistics were generated, which described and summarised the data. Regression analysis was then used to model the relationship between the pollutant variable and the explanatory variable i.e. location (influent and effluent) and flow rate. It could thus be determined whether there was a relationship between a specific pollutant and location (i.e. influent versus effluent) or flow rate. A more detailed discussion on the data analysis is provided in the following chapter.

4 RESULTS AND DISCUSSION

In this study constructed wetland (CW) treatment performance was determined by analysing removal efficiencies of the selected pollutants. However, this measurement of performance can be misleading as high removal efficiencies may indicate high influent concentrations with a much reduced effluent concentration, similarly, low removal efficiencies do not necessarily indicate poor performance but could indicate low influent concentrations (ITRC, 2003). Another indicator of performance used in this study was to compare treated effluent concentrations with the Department of Water Affairs' (DWA's) discharge standards as set out in the National Water Act (Act 36 of 1998) (South Africa, 1998) (Appendix 3). Water quality was also compared to the South African water quality guidelines (Appendix 3) to examine the possible consequences of the impact of CWs on the receiving water bodies.

As flow rate is one of the most significant operational variables that influence CW performance (Lee et al., 2009), the relationship between flow rate and the pollutant concentrations were also determined. It was initially assumed that there would be a significant relationship between pollutant concentrations and flow rate. Flow rate is important as it can influence hydraulic as well as pollutant loading (Lee *et al*., 2009). If the flow rate is too great and the contact time between the medium and wastewater is not adequate, then treatment performance will be negatively affected (Dairy Australia, 2008).

According to the regression analysis for the modelling of the relationships between the pollutant variables and explanatory variables, i.e. location (influent and effluent) and flow rate, for both De Goede Hoop and Wolwedans, the flow rate did not have a significant effect on the pollutant concentrations. The reason for this could be that the influent concentration may have been a more important factor, especially since the average daily flow rate (measured every two weeks) did not seem to change too drastically. Furthermore, the method of determining the flow rate, which included measuring the volume of wastewater passing through the system over a two week period and then calculating the flow rate from there may not have provided an accurate account of the flow rate. This is because perturbations in the flow resulting from peaks during certain parts of the day could not be presented using this method.

The data collected during sampling are presented in Table 13, Table 15, and Table 16. Statistical analysis was used to analyse the performance of the CW systems. This included the generation of descriptive statistics (Appendix 4), in which substituted values were established for data that were missing, e.g., as a result of the tank or stream running dry. If data were distributed normally, then the mean was used as a substitute for the missing value; if skewed, then the median was used as a substitute. The means provided an informative comparison between sampling points.

Linear regression was done to determine whether location and flow rate had a significant effect on the concentration of pollutants. Since no flow rate was recorded for the Babylonstoren site, two separate regression analyses were performed per pollutant variable. The first analysis included all three sites in which the location was considered; the second analysis included only De Goede Hoop and Wolwedans in which the location and flow rate were considered.

The dependent variable (i.e. the pollutant concentration variable) was examined to determine whether or not it was normally distributed. If not, data were logged to obtain a more normal distribution prior to proceeding with the analysis. The flow rate was centred (by subtracting the flow rate from the mean flow rate) to improve the interpretation of the regression equations. Binary numbers were allocated to the site and location variables since these were categorical. In the case of the location variable, the influent was coded as 0 and effluent was coded as 1. De Goede Hoop was assigned as the baseline site to which Wolwedans and Babylonstoren were compared.

A number of regression models were undertaken to determine the preferred model. The initial model included the location and site variables. This model was then expanded to include the interaction between location and site (e.g. Influent X Wolwedans and Influent X Babylonstoren). The preferred model between the two was then selected and other variables were systematically added and compared to determine whether the previous or subsequent model (which included more variables) was preferable. Other variables which were added systematically included flow rate, location X flow rate and site X flow rate. The preferred

model was determined by comparing the adjusted R^2, Akaike information criterion (AIC)[9] and Bayesian information criterion (BIC)[10] values. The AIC and BIC values were obtained by undertaking a 'goodness of fit' test. The model with the higher adjusted R^2 and lower AIC and BIC values was used to identify the preferred model. Once a model was selected, the *p*-value was used to determine whether an explanatory variable was significant at the 5% and 10% levels.

Residual analysis was undertaken to examine how well the models represented the data (i.e. how closely the model reflected what was actually happening). Residual plots were generated as part of the residual analysis. These included normality plots of the standardized residuals (used to determine whether the distributions of the standard residuals were sufficiently normal), and the plot of the predicted versus standardized residual scores. Outliers that were determined to be influential (this was done by considering the Cook's distance estimate) were removed and the regression models were repeated based on the new data set.

4.1 Regression analysis

Regression models were performed to determine the significance of treatment performance of the systems for the selected variables and to determine whether the flow rate impacted on performance. The flow rate could not be established for Babylonstoren and was not included in the analysis. A data subset that included site, location and flow rate was used only to compare De Goede Hoop and Wolwedans. The detailed results from the regression analysis are shown in Appendix 5. A table and summary of the results are provided in this chapter for each variable.

The following abbreviations are used in the regression equations:

I = influent
W = Wolwedans
B = Babylonstoren

[9] A measure of the relative goodness of fit of a statistical model.

[10] A measure of the relative goodness of fit of a statistical model, which adjusts for the number of parameters estimated as well as for the given amount of data.

4.1.1 Nitrogen (NH_3, NO_3^- and NO_2^-)

Ammonia (NH_3):

- De Goede Hoop vs. Wolwedans vs. Babylonstoren

  Two models were performed, these were:

  1. Log (NH_3) = I + W + B
  2. Log (NH_3) = I + W + B + I*W + I*B

- De Goede Hoop vs. Wolwedans

  Three models were performed, these were:

  1. Log (NH_3) = I + W
  2. Log (NH_3) = I + W + I*W
  3. Log (NH_3) = I + W + I*W + centred flow rate

Summary: Location has a significant effect on NH_3 concentration for all sites. For De Goede Hoop, the average influent concentration is 25.18 mg/l and the average effluent concentration is 1.92 mg/l. There is thus a decrease in the average NH_3 concentration of 23.26 mg/l at De Goede Hoop. The average influent concentration for Wolwedans is 61.8 mg/l compared to an average effluent concentration of 36.81 mg/l. There is thus an average decrease in the NH_3 concentration of 24.99 mg/l. For Babylonstoren, the average influent concentration is 4.61 mg/l compared to an average effluent concentration of 10.09 mg/l. There is thus an average increase in the NH_3 concentration of 5.48 mg/l at Babylonstoren. Furthermore, the models indicate that flow rate do not have an effect on NH_3 concentration.

The models indicate that there is no significant relationship between flow rate and NH_3 concentration at De Goede Hoop and Wolwedans.

Table 7 provides a summary of the regression results for NH_3.

Table 7: Ammonia (NH_3) regression results summary table

		De Goede Hoop	Wolwedans	Babylonstoren
Ammonia (NH_3): Log NH_3 = 0.284 + (1.117) I + (1.282) W + (0.722) B + (-0.889) I*W + (-1.459) I*B				
Location Significance: Significant difference in the value of log (NH_3) at the influent versus the effluent (influent p-value equals 0)	Effluent	Intercept 0.284 = 1.92 mg/l	Intercept + W 0.284 + 1.282 = 1.566 = 36.81 mg/l	Intercept + B 0.284 + 0.722 = 1.004 = 10.09 mg/l
	Influent	Intercept + I 0.284 + 1.117 = 1.401 = 25.18 mg/l	Intercept + W + I + I*W 0.284 + 1.282 + 1.117 - 0.889 = 1.791 = 61.8 mg/l	Intercept + B + I + I*B 0.284 + 0.722 + 1.117 – 1.459 = 0.664 = 4.61 mg/l
Flow rate Significance: N/A		N/A	N/A	N/A
Log (NH_3) = 0.284 + (1.117) I + (1.282)W + (-0.889)I*W				
Flow rate Significance: N/A		No relationship	No relationship	N/A

Nitrate (NO_3^-):

- De Goede Hoop vs. Wolwedans vs. Babylonstoren

  Two models were performed, these were:

  1. Log (NO_3^-) = I + W + B
  2. Log (NO_3^-) = I + W + B + I*W + I*B

- De Goede Hoop vs. Wolwedans

  Four models were performed, these were:

  1. Log (NO_3^-) = I + W
  2. Log (NO_3^-) = I + W + I*W
  3. Log (NO_3^-) = I + W + centred flow rate
  4. Log (NO_3^-) = I + W + centred flow rate + (W*centred flow rate)

Summary: Location has a significant effect on NO_3^- concentration for all sites. For De Goede Hoop, the average influent concentration is 5.47 mg/l and the average effluent concentration is 13.37 mg/l. There is thus an average increase in the NO_3^-concentration of 7.9 mg/l at De Goede Hoop. The average influent concentration for Wolwedans is 10.62 mg/l compared to an average effluent concentration of 19.59 mg/l. There is thus an average increase in the NO_3^- concentration of 8.97 mg/l. For Babylonstoren, the average influent concentration is 6.68 mg/l compared to an average effluent concentration of 5.32 mg/l. There is thus an average increase in the NO_3^- concentration of 1.2 mg/l at Babylonstoren.

The models indicate that there is a significant relationship between flow rate and NO3- concentration for De Goede Hoop and Wolwedans, a 1 unit change in flow rate, with a constant location, will result in a change in the average NO3- concentration by 1 mg/l (10^-0.002).

Table 8 provides a summary of the regression results for NO_3^-.

Table 8: Regression results summary for nitrate (NO_3^-)

		De Goede Hoop	Wolwedans	Babylonstoren
Nitrate(NO_3^-): Log NO_3- = 1.126 + (-0.388) I + (0.166) W + (-0.4) B + (0.122) I*W + (0.487) I*B				
Location Significance: Significant difference in the value of log (NO_3-) at the influent versus the effluent (influent p-value is 0.02).	Effluent	Intercept 1.126 = 13.37 mg/l	Intercept + W 1.126 + 0.166 = 1.292 = 19.59 mg/l	Intercept + B 1.126 – 0.4 = 0.726 = 5.32 mg/l
	Influent	Intercept + I 1.126 – 0.388 = 0.738 = 5.47 mg/l	Intercept + W + I + I*W 1.126 + 0.166 -0.388 + 0.122 = 1.026 = 10.62 mg/l	Intercept + B + I + I*B = 1.126 - 0.4 -0.388 + 0.487 = 0.825 = 6.68 mg/l
Flow rate Significance: N/A		N/A	N/A	N/A
Log (NO_3-) = 1.098 + (-0.333) (I) + (0.228) (W) + (-0.002) centred flow rate + (0.004) (W*centred flow rate)				
Flow rate Significance: Flow rate does not have a significant effect on NO_3-concentration (p-value is 0.3.03)	Effluent (change in flow rate of 1 unit)	Intercept + flow rate 1.098 – 0.002 = 1.096 = 12.47 mg/l	Intercept + flow rate + W + W*centred flow rate 1.098 – 0.002 + 0.228 + 0.004 = 1.328 = 21.28 mg/l	N/A
	Influent (change in flow rate of 1 unit)	Intercept + flow rate + I 1.122 - 0.002 - 0.372 = 0.752 = 5.65 mg/l	Intercept + flow rate + W + W*centred flow rate + I 1.098 – 0.002 + 0.228 + 0.004 - 0.333= 0.995 = 9.89 mg/l	N/A

Nitrite (NO_2^-):

- De Goede Hoop vs. Wolwedans vs. Babylonstoren

  Two models were performed, these were:

  1. Log (NO_2^-) = I + W + B
  2. Log (NO_2^-) = I + W + B + I*W + I*B

- De Goede Hoop vs. Wolwedans

  Four models were performed, these were:

  1. Log (NO_2^-) = I + W
  2. Log (NO_2^-) = I + W + I*W
  3. Log (NO_2^-) = I + W + centred flow rate
  4. Log (NO_2^-) = I + W + centred flow rate + (W*centred flow rate)

Summary: Location has a significant effect on NO_2^- concentration for all sites. For De Goede Hoop, the average influent concentration is 0.03 mg/l and the average effluent concentration is 0.08 mg/l. There is thus an average increase in the NO_2^-concentration of 0.05 mg/l at De Goede Hoop. The average influent concentration for Wolwedans is 0.38 mg/l compared to an average effluent concentration of 0.87 mg/l. There is thus an average increase in the NO_2^- concentration of 0.49 mg/l. For Babylonstoren, the average influent concentration is 0.05 mg/l compared to an average effluent concentration of 0.11 mg/l. There is thus an average increase in the NO_2^- concentration of 0.06 mg/l at Babylonstoren.

The models indicate that flow rate does not have a significant effect on NO_2^- concentration for De Goede Hoop and Wolwedans. A 1 unit change in flow rate, with a constant location, will result in a change in the average NO_2^- concentration by 1 mg/l (10^-0.002).

Table 9 provides the regression summary results for NO_2^-.

Table 9: Regression results summary for nitrite (NO_2^-)

		De Goede Hoop	Wolwedans	Babylonstoren
Nitrite (NO_2^-): Log (NO_2-) = -1.124 + (-0.356)(I) + 1.065 (W) + 0.173 (B)				
Location Significance: Significant difference in the value of log (NO_2^-) at the influent versus the effluent (influent p-value is 0.084)	Effluent	Intercept -1.124 = 0.08 mg/l	Intercept + W -1.124 + 1.065 = -0.059 = 0.87 mg/l	Intercept + B -1.124 + 0.173 = -0.951 = 0.11 mg/l
	Influent	Intercept + I -1.124 – 0.356 = -1.48 = 0.03 mg/l	Intercept + W + I -1.124 + 1.065 – 0.356 = -0.415 = 0.38 mg/l	Intercept + B + I + I*B = -1.124 + 0.173 -0.356 + 0.173 = -1.307 = 0.05 mg/l
Flow rate Significance: N/A		N/A	N/A	N/A
Log (NO_2^-) = -1.101 + (-0.4) (I) + (1.064) (W) + (-0.001) (centred flow rate) + (0.008) (W*centred flow rate)				
Flow rate Significance: Flow rate does not have a significant effect on NO_2^- concentration (p-value is 0.666)	Effluent (change in flow rate of 1 unit)	Intercept + flow rate -1.101 - 0.001 = -1.102 = 0.09 mg/l	Intercept + flow rate + W +W*flow rate -1.101 – 0.001 + 1.064 + 0.008 = -0.03 = 0.93 mg/l	N/A
	Influent (change in flow rate of 1 unit)	Intercept + flow rate + I -1.101 – 0.001 – 0.4 = -1.502= 0.3 mg/l	Intercept + flow rate + W +W*flow rate + I -1.101 – 0.001 + 1.064 + 0.008 - 0.4 = -0.43 = 0.37 mg/l	N/A

4.1.2 **Orthophosphate (PO_4^{3-})**

- De Goede Hoop vs. Wolwedans vs. Babylonstoren

  Two models were performed, these were:

  1. $PO_4^{3-} = I + W + B$
  2. $PO_4^{3-} = I + W + B + I*W + I*B$

- De Goede Hoop vs. Wolwedans

  Three models were performed, these were:

  1. $\text{Log } (PO_4^{3-}) = I + W$
  2. $\text{Log } (PO_4^{3-}) = I + W + I*W$
  3. $\text{Log } (PO_4^{3-}) = I + W + \text{centred flow rate}$

Summary: Location does not have a significant effect on PO_4^{3-} concentration for all sites. For De Goede Hoop, the average influent concentration is 31.79 mg/l and the average effluent concentration is 28.6 mg/l. There is thus an average decrease in the PO_4^{3-} concentration of 3.19 mg/l at De Goede Hoop. The average influent concentration for Wolwedans is 33.42 mg/l compared to an average effluent concentration of 30.23 mg/l. There is thus an average decrease in the PO_4^{3-} concentration of 3.19 mg/l. For Babylonstoren, the average influent concentration is 21.66 mg/l compared to an average effluent concentration of 18.48 mg/l. There is thus an average decrease in the PO_4^{3-}concentration of 3.18 mg/l at Babylonstoren. Furthermore, the models indicate that flow rate does not have an effect on PO_4^{3-} concentration.

The models indicate that there is no significant relationship between flow rate and PO_4^{3-} concentration at De Goede Hoop and Wolwedans.

Table 10 provides the regression results summary for PO_4^{3-}.

Table 10: Regression summary results for orthophosphate (PO_4^{3-})

		De Goede Hoop	Wolwedans	Babylonstoren
<u>Orthophosphate (PO_4^{3-}):</u> PO_4^{3-} = 28.603 + (3.184)I + (1.631)W + (-10.128)B				
<u>Location</u> Significance: No significant difference in the value of PO43- at the influent versus the effluent (influent p-value is 0.424)	Effluent	Intercept 28.6 mg/l	Intercept + W 28.603 + 1.631 = 30.23 mg/l	Intercept + B 28.603 - 10.128 = 18.48 mg/l
	Influent	Intercept + I 28.603 + 3.184 = 31.79 mg/l	Intercept + W + I 28.603 + 1.631 + 3.184 = 33.42 mg/l	Intercept + B + I 28.603 -10.128 + 3.184 = 21.66 mg/l
<u>Flow rate</u> N/A		N/A	N/A	N/A
Log (PO43-) = 1.427 + (0.049)I + (0.039)W				
<u>Flow rate</u> N/A		No relationship	No relationship	N/A

4.1.3 ***E. coli***

- De Goede Hoop vs. Wolwedans vs. Babylonstoren

  Two models were run, these included:

  1. Log *E. coli* = I + W + B
  2. Log *E. coli* = I + W + B + I*W + I*B

- De Goede Hoop vs. Wolwedans

  Four models were run, these included:

  1. Log *E. coli* = I + W
  2. Log *E. coli* = I + W + I*W
  3. Log *E. coli* = I + W + I*W + centred flow rate
  4. Log *E. coli* = I + W + centred flow rate + (I*centred flow rate)

Summary: Location has a significant effect on *E. coli* concentration for all sites. For De Goede Hoop, the average influent concentration is 397 192 CFUs/100ml and the average effluent concentration is 36 813 CFUs/100ml. There is thus an average decrease in the *E. coli* concentration of 360 379 CFUs/100ml at De Goede Hoop. The average influent concentration for Wolwedans is 486 407 CFUs/100ml compared to an average effluent concentration of 332 660 CFUs/100ml. There is thus an average decrease in the *E. coli* concentration of 153 747 CFUs/100ml. For Babylonstoren, the average influent concentration is 979 490 CFUs/100ml compared to an average effluent concentration of 403 645 CFUs/100ml. There is thus an average decrease in the *E. coli* concentration of 575845 CFUs/100ml at Babylonstoren.

The models indicate that flow rate does not have a significant effect on *E. coli* concentration for De Goede Hoop and Wolwedans. A 1 unit change in flow rate, with a constant location, will result in a change in the average *E. coli* concentration by 1 CFU/100ml (10^-0.002).

Table 11 presents the regression results summary for *E. coli*.

Table 11: Regression summary results for *E. coli*

		De Goede Hoop	**Wolwedans**	**Babylonstoren**
E coli: Log *E. coli* = 4.566 + (0.933) I + (0.956)W + (1.043)B + (-0.768)I*W + (-0.551)I*B				
Location Significance: Significant difference in the value of log *E. coli* at the influent versus the effluent at (influent p-value is 0.001)	Effluent	Intercept 4.566 = 36 813 CFUs/100ml	Intercept + W 4.566 + 0.956 = 5.522 = 332 660 CFUs/100ml	Intercept + B 4.566 + 1.043 = 5.606 = 403 645 CFUs/100ml
	Influent	Intercept + I 4.566 + 0.933 = 5.599 = 397 192 CFUs/100ml	Intercept + W + I + I*W 4.566 + 0.956 + 0.933 – 0.768 = 5.687 = 486 407 CFUs/100ml	Intercept + B + I + I*B 4.566 + 1.043 + 0.933 – 0.551 = 5.991 = 979 490 CFUs/100ml
Flow rate Significance: N/A		N/A	N/A	N/A
Log *E. coli* = 4.566 + (0.933) I + (0.956) W + (-0.768) I*W + (-0.002) centred flow rate				
Flow rate Significance: Flow rate does not have a significant effect on *E .coli* concentration (p- is 0.175)	Effluent (change in flow rate of 1 unit)	Intercept + flow rate 4.566 -0.002 = 4.564 = 36 644 CFUs/100ml	Intercept + flow rate + W 4.566 – 0.002 + 0.956 = 5.52 = 331 131 CFUs/100ml	N/A
	Influent (change in flow rate of 1 unit)	Intercept + flow rate + I 4.566 – 0.002 + 0.933 = 5.497 = 314 050 CFUs/100ml	Intercept + flow rate + W + I 4.566 – 0.002 + 0.956 + 0.933 = 6.453 = 2 837 919 CFUs/100ml	N/A

4.2 Treatment performance

This section makes use of the results obtained from the regression analysis as well as South African water quality guidelines to determine CW performance at the three sites. The section is divided into nitrogen removal efficiency, which includes NH_3 (includes ionised and un-ionised forms), NO_3^- and NO_2^-, followed by PO_4^{3-} and *E. coli* removal efficiencies.

4.2.1 Nitrogen removal (NH_3, NO_3^-, NO_2^-)

Nitrification and denitrification are the main processes for nitrogen reduction, together with some assimilation by biota. As mentioned in Chapter 2, nitrification is the oxidation of ammonium to NO_2^- and then NO_3^- under aerobic conditions, whereas denitrification is the reduction of NO_3^- to nitrogen gas under anoxic conditions. Thus for NH_3 removal, there has to be sufficient nitrification taking place whereas for NO_2^-/NO_3^- removal, sufficient denitrification has to occur.

The removal of NH_3 varied greatly between the three sites with no NH_3 removal occurring at Babylonstoren. The situation was similar for NO_3^-, in which there was variability in the removal percentages between the sites, however, NO_3^- removal only occurred at Babylonstoren. A more detailed and site specific discussion of the performance of the CWs at each site is presented in the sections following Table 12 and Table 13.

Table 12: Summary of removal percentages obtained for pollutants at all three sites

	De Goede Hoop			Wolwedans			Babylonstoren		
	Influent concentration	**Effluent concentration**	**Removal efficiency (%)**	**Influent concentration**	**Effluent concentration**	**Removal efficiency (%)**	**Influent concentration**	**Effluent concentration**	**Removal efficiency (%)**
NH_3 (mg/l)									
Mean	30.37	1.34	96%	63.14	41.07	35%	5	10.45	-109%
Standard deviation	21.69	1.88	-	17.16	25.74	-	4.75	6.22	-
Maximum	70	5.4	-	87.2	88.27	-	13.2	21.33	-
Minimum	6.73	0	-	42.93	17.33	-	0.97	4	-
NO_3^- (mg/l)									
Mean	6.08	14.05	-131%	14.28	22.01	-54%	8.18	5.78	29%
Standard deviation	5.64	4.75	-	10.76	10.89	-	4.38	2.55	-
Maximum	17.07	21.67	-	30	35	-	14	10.27	-
Minimum	0.33	8.13	-	3.33	10.33	-	1.47	2.67	-
NO_2^- (mg/l)									
Mean	0.05	0.08	-60%	2.09	1.81	13%	0.09	0.13	-44%
Standard deviation	0.03	0.03	-	2.69	1.86	-	0.09	0.08	-
Maximum	0.09	0.13	-	5.53	5.06	-	0.23	0.22	-
Minimum	0.01	0.04	-	0.01	0.13	-	0.01	0.02	-
PO_4^{3-} (mg/l)									
Mean	30.78	29.61	3.8%	35.44	28.21	20%	20.72	19.23	7%
Standard deviation	15.71	8.97	-	6.52	7.87	-	17.96	13.03	-
Maximum	64.67	40.87	-	43.53	42.53	-	50.4	43.2	-
Minimum	18.25	14.05	-	26.96	20.73	-	2.3	4.46	-
***E. coli* (CFUs/100 ml)**									
Mean	489 714	74 786	85%	739 833	455 000	39%	1 213 333	426 667	65%
Standard deviation	293 886	67 576	-	691 075	320 172	-	773 193	135 154	-
Maximum	740 000	200 000	-	2 000 000	1 000 000	-	2 200 000	590 000	-
Minimum	23 000	3 000	-	79 000	50 000	-	390 000	220 000	-

Table 13: Ammonia, nitrate (NO_3^-) and nitrite (NO_2^-) concentrations recorded at the sampling sites (grey shading indicates substituted data)

Sampling Date (2011)	11.07	26.07	10.08	23.08	06.09	20.09	04.10		19.07	02.08	16.08	30.08	13.09	27.09		19.07	02.08	16.08	30.08	13.09	27.09
De Goede Hoop								**Wolwedans**							**Babylonstoren**						
NH_3 (mg/l)																					
Influent (bioreactor)	35	13.07	6.73	23.2	19.8	70	44.8	**Influent (aeration tank)**	61.6	70.93	71.73	87.2	44.47	42.93	**Upper culvert**	1.33	0.36	0.07	1.1	0.88	0.35
Effluent (recirculation pond)	1.7	0.23	0.3	0.95	0	5.4	0.8	**Effluent**	88.27	31.46	29.33	52	28	17.33	**Influent (bioreactor)**	13.2	7.44	1.2	5.2	0.97	2
Constructed pond	35	6.3	10.07	11.5	1.4	10.07	10.07	**French drain overflow**	28.56	70.4	69.6	84.8	72.74	74.8	**Effluent**	10.27	5.07	21.33	9.6	12.4	4
Groundwater tank	0.02	0.02	0.02	0.02	1.56	0	0.01	**Vlei**	0.47	0.3	0.43	0.1	0.61	0.33	**Lower culvert**	1.6	3.53	0.2	0.2	0.83	0.2
Stream	0.02	0.02	0.13	0.2	0	0.05	0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NO_3^- (mg/l)																					
Influent (bioreactor)	7.1	17.07	8.67	3.2	0.33	1.87	4.33	**Influent (aeration tank)**	30	14	24	3.33	4.33	10	**Upper culvert**	2.3	4.53	2	3.9	2.53	2.53
Effluent (recirculation pond)	13.5	12.06	12	8.13	19.33	11.67	21.67	**Effluent**	31.7	35	28.33	10.33	12	14.67	**Influent (bioreactor)**	1.47	6.27	6.67	11.33	14	9.33
Constructed pond	10.7	8.83	14.67	1.87	3.33	8.83	8.83	**French drain overflow**	0	9.67	7	5.33	4.4	5.33	**Effluent**	10.27	5.07	6.67	2.67	5.33	4.67
Groundwater tank	2.47	0.53	2.47	2.47	3.73	1.2	4	**Vlei**	1.03	2.4	4.93	3.2	2	1.6	**Lower culvert**	1.6	2.8	10.67	3.73	1.87	0.83
Stream	0	0.9	5.33	0	0.8	1.07	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NO_2^- (mg/l)																					
Influent (bioreactor)	0.087	0.06	0.08	0.01	0.006	0.026	0.062	**Influent (aeration tank)**	5.52	5.53	1.115	0.012	0.012	0.373	**Upper culvert**	0.005	0.01	0.014	0	0.026	0.002
Effluent (recirculation pond)	0.049	0.05	0.09	0.08	0.127	0.09	0.074	**Effluent**	1.71	2.72	5.057	0.129	0.269	0.968	**Influent (bioreactor)**	0.01	0.158	0.03	0.23	0.038	0.068
Constructed pond	0.134	0.07	0.14	0.16	0	0.134	0.134	**French drain overflow**	0.008	0.093	0.093	0.012	0.065	0.101	**Effluent**	0.018	0.058	0.13	0.16	0.213	0.22
Groundwater tank	0.005	0.02	0.005	0.005	0.005	0.005	0.004	**Vlei**	0.008	0.015	0.069	0.041	0.007	0.009	**Lower culvert**	0.051	0.054	0.05	0.08	0.038	0.039
Stream	0	0.02	0.05	0.002	0	0.007	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-

4.2.1.1 ***De Goede Hoop***

The average influent concentration was 25.18 mg/l and the average[11] effluent concentration was 1.92 mg/l. From the p-value, it can be determined that the difference between the average influent and effluent NH_3 concentrations is significant.

In terms of the removal percentage, there was a decrease in the mean NH_3 concentration of 96% over the sampling period (Table 12). The NH_3 concentrations recorded at the effluent met DWA's discharge standards of 3 mg/l, 86% of the time (Figure 18), with the mean NH_3 effluent concentration also meeting DWA's standard.

According to the regression analysis, the average influent NO_3^- concentration was 5.47 mg/l and the average effluent concentration was 13.37 mg/l. For NO_2^-, based on the regression models, the average influent was 0.03 mg/l and the average effluent was 0.08 mg/l. For both NO_3^- and NO_2^-, the difference between the average influent and effluent concentrations was significant.

Over the sampling period, there was an increase in the mean NO_3^- and NO_2^- concentrations of 131% and 60% respectively (Table 12). The combined NO_3^-/NO_2^- effluent concentrations recorded met DWA's discharge standards of 15 mg/l NO_3^-/NO_2^- 71% of the time (Figure 18) and the mean NO_3^-/NO_2^- concentration also met DWA's standards.

[11] This refers to the average as concentration as determine via regression analysis as opposed to the mean, which refers to the arithmetic mean of the data set

4.2.1.2 ***Wolwedans***

According to the p-value obtained from the regression analysis, there was a significant difference between the NH_3 concentration at the influent and effluent. The average influent concentration was 61.8 mg/l compared to an average effluent concentration of 36.81 mg/l.

There was an average decrease in NH_3 of 35% over the sampling period (Table 12). It should be noted however that even though there was a significant difference between influent and effluent concentrations, the effluent quality did not once meet DWA's discharge standards for NH_3 of 3 mg/l (Figure 18).

According to the regression analysis, NO_3^- and NO_2^-, the average influent concentration was 5.47 mg/l for NO_3^- and 0.38 mg/l for NO_2^-. The average effluent concentration was 13.37 mg/l for NO_3^- and 0.87 mg/l for NO_2^-. For both NO_3^- and NO_2^-, the difference between the average influent and effluent concentrations was significant.

There was an increase in the mean NO_3^- concentration of 54% from influent to effluent over the sampling period (Table 12). For NO_2^-, there was a decrease in the mean concentration of 13%[12] (Table 12). The resultant effluent quality only met DWA's discharge standards for NO_3^- / NO_2^- 33% (Figure 18) of the time and the mean NO_3^- / NO_2^- concentration did not meet DWA's standards.

4.2.1.3 ***Babylonstoren***

The regression analysis indicates that there was a significant difference between the average NH_3 concentration at the influent and effluent. The average influent concentration was 4.61 mg/l compared to an average effluent concentration of 10.09 mg/l.

The mean percentage change for NH_3 was an increase in concentration of 109% over the sampling period (Table 12). The effluent quality did not once meet DWA's discharge limit standards for NH_3 (Figure 18).

[12] According to the regression model, there is an increase in NO_2^-, however this discrepancy is due to the poor model fit of the data

According to the regression model, the average influent concentration for NO_3^- was 6.68 mg/l and the average effluent concentration was 5.32 mg/l. For NO_2^-, the average influent concentration was 0.05 mg/l and the average effluent concentration was 0.11 mg/l. The difference between the average influent and effluent concentrations for both NO_3^- and NO_2^- was considered significant.

In terms of removal percentage, for NO_3^-, there was a 29% decrease in the mean concentration from influent to effluent (Table 12). For NO_2^- however, there was an increase in the concentration from influent to effluent of 44% (Table 12). The effluent quality met DWA's discharge limit standards for NO_3^-/NO_2^- 100% of the time (Figure 18).

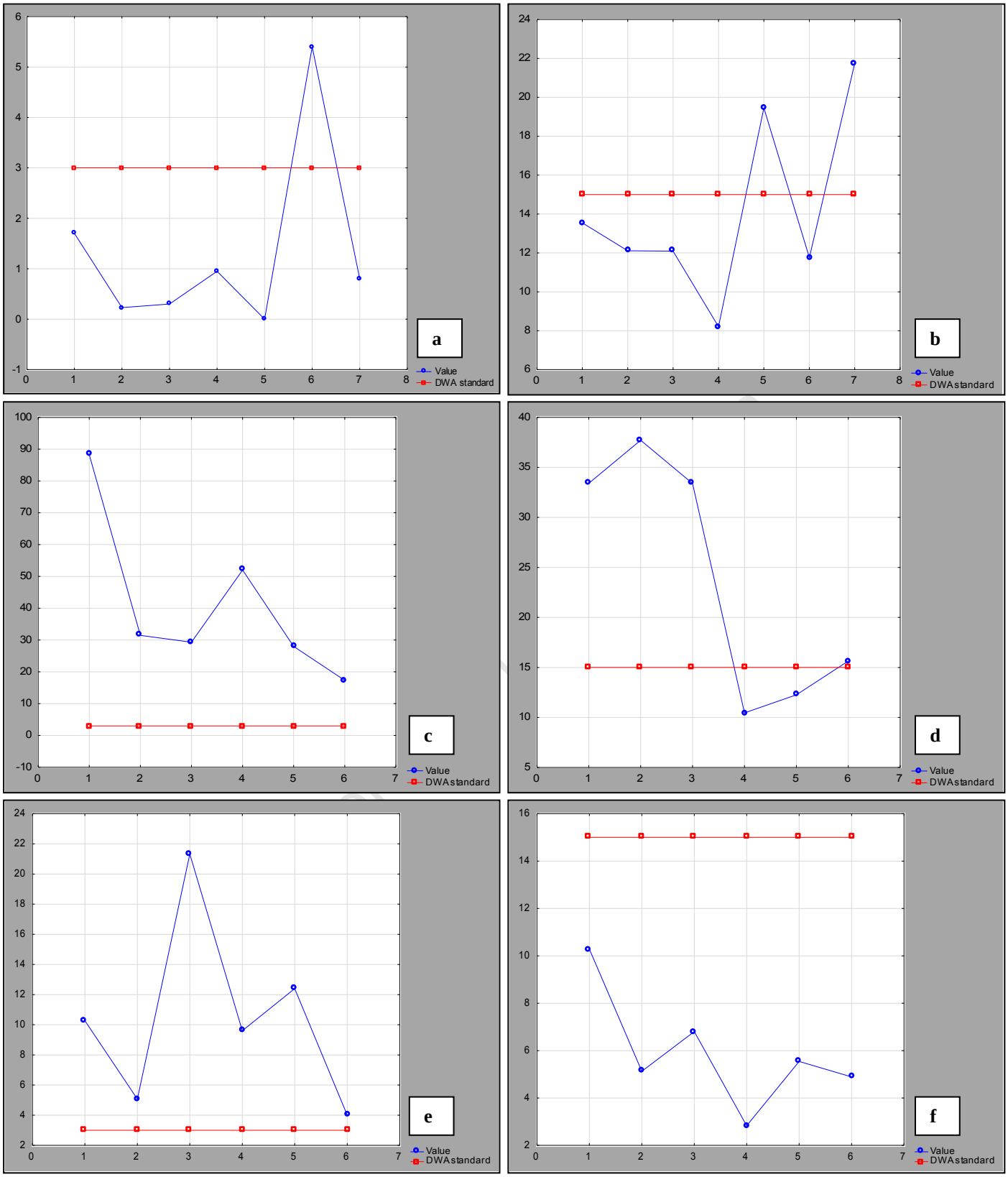

Figure 18: Comparison of effluent concentrations for NH_3 and NO_3^-/ NO_2^- against DWA's discharge limit standards

De Goede Hoop: NH_3 (a), NO_3^-/NO_2^- (b); Wolwedans: NH_3 (c), NO_3^-/NO_2^- (d) and Babylonstoren: NH_3 (e), NO_3^-/NO_2^- (f).

4.2.1.4 ***Nitrogen removal summary***

The negative NH_3 removal at Babylonstoren could be as a result of ammonification of organic nitrogen taking place but no nitrification, thus resulting in higher NH_3 concentrations. Limited nitrification can be attributed to a lack of oxygen within the system, this is because nitrification is an aerobic process and therefore oxygen is a limiting factor. Nitrification requires 4.3 g of O_2 per gram of NH_3 (Akratos & Tshirintzis, 2007). Akratos & Tshirintzis (2007) state that insufficient nitrification may occur during cooler periods and this may concur with the study findings because the CW was monitored during the cooler months between July and October. The researchers also note that during cooler time periods, plants have a decreased ability to provide sufficient oxygen for nitrification. This does not however explain the fact that nitrification did take place at the other two sites over the same sampling period.

The poor NH_3 removal performance of the Babylonstoren CW could also have resulted from factors causing ponding or flooding (Figure 19), which was noticed throughout most of the sampling period. According to Kadlec & Wallace (2009), flooding in CWs is normally caused by clogging of the porous media and improper hydraulic design. Clogging of the porous media may have resulted in a decrease in the performance of the CW (Knowles *et al*., 2011). See section 2.2.3.2.8 for more detail on clogging.

Figure 19: Flooding of the Babylonstoren constructed wetland

Short-circuiting caused by improper hydraulic design results in the rapid movement of water from the inlet to the outlet along a preferential path or paths (ITRC, 2003), and may cause the actual hydraulic residence time (HRT) to differ significantly from the theoretical HRT (Davis, 1995), which in turn hampers performance.

According to Akratos & Tshirintzis (2007), HRT plays an important role in removal efficiency. In a laboratory scale experiment undertaken by Akratos & Tshirintzis (2007), in most instances, the longer the HRT, the higher the removal efficiency (at 15^{o}C). For example in Table 14, with reference to a CW with a river bed cobble substrate (CO-R), and planted with common reed, the NH_3 removal increased from 25.6% at an HRT of six days to 67.8% at a residence time of 20 days.

Table 14: Ammonia removal statistics for HRT of 6, 8, 14 and 20 days

	MG-C				MG-R				MG-Z				FG-R				CO-R			
HRT (days)	6	8	14	20	6	8	14	20	6	8	14	20	6	8	14	20	6	8	14	20
Ammonia mean removal (%)	11.6	35.8	29	68.9	2.7	-24.2	15.1	32.6	-1.3	-7.9	15	4.6	55.3	65.9	66.3	70.7	25.6	49.6	44.7	67.8

MG – C represents medium gravel planted with cattail, MG – R represents medium gravel planted with common reed, MG-Z represents medium gravel unplanted; FG-R represents fine gravel planted with common reed and CO-R represents river bed cobbles planted with common reed.
(Source: Akratos & Tshirintzis, 2007)

In contrast to Babylonstoren, nitrification did occur at the De Goede Hoop and Wolwedans sites. The NH_3 removal rates at De Goede Hoop (96%) and Wolwedans (35%) were comparable with those obtained in a study undertaken by Knight *et al*. (1993) in Huang *et al*. (2000), in which removal rates averaged 35% and ranged between -4% and 94% for 12 CW systems.

Sufficient denitrification for the removal; of NO_3^- did not occur at De Goede Hoop and Wolwedans as both these systems obtained negative removal rates for NO_3^- and also had high NO_3^- effluent concentrations. However, even though Babylonstoren achieved a positive NO_3^- removal rate, it was relatively low at 29%, but comparable with a the NO_3^-/NO_2^- removal rate of 27% obtained during experiments carried out by Keffala & Ghrabi (2005).

Dentrification is a heterotrophic process, and organic carbon is required as a carbon source (e.g. plants). Denitrification is thus dependent on the quantity and quality of an organic source, which supports bacterial growth (Ingersoll & Baker, 1998 cited in Bastviken *et al*., 2005). Luederitz *et al*. (2001) argue that in relatively new wetlands, the development of a carbon-rich habitat for denitrifiers has not yet occurred. All of the wetlands in this study are still considered relatively new, with Wolwedans being constructed in 2007 and De Goede Hoop and Babylonstoren constructed in 2009, and thus could account for the minimal denitrification rates. Furthermore, the vegetation density at De Goede Hoop and Wolwedans was lower than that of Babylonstoren, with De Goede Hoop having very sparsely distributed vegetation. The sparse distribution of vegetation observed at De Goede Hoop could be attributed to the poor location of the CW that does not receive much sunlight; for Wolwedans the larger plants at the boundary of the CW may prevent sunlight from reaching smaller plants in the CW. Research has shown that plants are important for NO_3^- removal (e.g. Lin *et al.,* 2002; Weisner *et al*., 1994); it is thus assumed that sparsely vegetated CWs will not be as effective in removing NO_3^- as compared to more densely vegetated wetlands.

It is also worth noting that as sampling was done during the cooler months, decomposition during the warmer months may have depleted the pool of readily available degradable organic matter, which would result in reduced denitrification later in the year (Bastviken *et al*., 2005).

4.2.2 **Orthophosphate (PO_4^{3-})**

The main removal mechanisms for PO_4^{3-} in SSF CWs are adsorption onto porous media and plant uptake (Kadlec & Knight, 1996 cited in Akratos & Tsihrintzis, 2007). For all three sites, orthophosphate removal was positive but low and ranged between 3.8% and 20%. The removal percentages achieved at each site are discussed as following Table 15.

Table 15 provides a summary of the actual PO_4^{3-} values recorded on each sampling occasion per sampling point.

Table 15: Orthophosphate (PO_4^{3-}) concentrations (mg/l) recorded at the sampling sites

(Grey shading indicates substituted data)

De Goede Hoop							
Sampling Date (2011)	11.07	26.07	10.08	23.08	06.09	20.09	04.10
Influent (bioreactor)	18.25	31.2	20.47	27.06	23.33	64.67	30.47
Effluent (recirculation pond)	14.05	23.73	33.13	35.53	29.9	40.87	30.07
Constructed pond	23.55	28.13	26.53	35.8	30.1	28.13	28.13
Groundwater tank	1.16	0.58	1.16	1.16	2.07	1.41	0.91
Stream	0.08	0.28	4.17	2.53	5.97	2.92	2.04
Wolwedans							
Sampling Date (2011)	19.07	02.08	16.08	30.08	13.09	27.09	-
Influent (aeration tank)	31.7	26.96	31.06	38	43.53	41.4	-
Effluent	26.27	20.73	24.33	31.53	42.53	23.87	-
French drain overflow	23.9	29.07	22.4	30.47	36.6	27.93	-
Vlei	2.43	0.51	2.17	3.41	2.31	2.32	-
Babylonstoren							
Sampling Date (2011)	19.07	02.08	16.08	30.08	13.09	27.09	-
Upper culvert	14.05	23.73	33.13	35.53	29.9	40.87	-
Influent (bioreactor)	1.16	0.58	1.16	1.16	2.07	1.41	-
Effluent	0.08	0.28	4.17	2.53	5.97	2.92	-
Lower culvert	18.25	31.2	20.47	27.06	23.33	64.67	-

*4.2.2.1 **De Goede Hoop***

According to the regression model, the average PO_4^{3-} influent concentration was 31.79 mg/l and the average effluent concentration was 28.6 mg/l. The p-value indicated that the change in PO_4^{3-} from the average influent to effluent was not significant. In terms of percentage removal, the mean removal percent during the sampling period was 3.8% (Table 12). The PO_4^{3-} effluent concentrations however did not meet DWA's discharge limit standard of 10 mg/l (Figure 20).

4.2.2.2 *Wolwedans*

The p-value obtained during the regression analysis indicated that the change in the average concentration from influent to effluent was not significant. The average influent concentration was 33.42 mg/l and the average effluent concentration was 30.23 mg/l. The mean PO_4^{3-} removal percent over the sample period was 7% (Table 12). The PO_4^{3-} effluent concentrations did not meet DWA's discharge standards (Figure 20).

4.2.2.3 *Babylonstoren*

The p-value indicated that the change in the average PO_4^{3-} concentration from influent to effluent was not significant. The average influent concentration was 21.66 mg/l and the average effluent concentration was 18.48 mg/l. The mean PO_4^{3-} removal percent over the sample period was 20% (Table 12). The PO_4^{3-} effluent concentrations did not meet DWA's discharge standards (Figure 20).

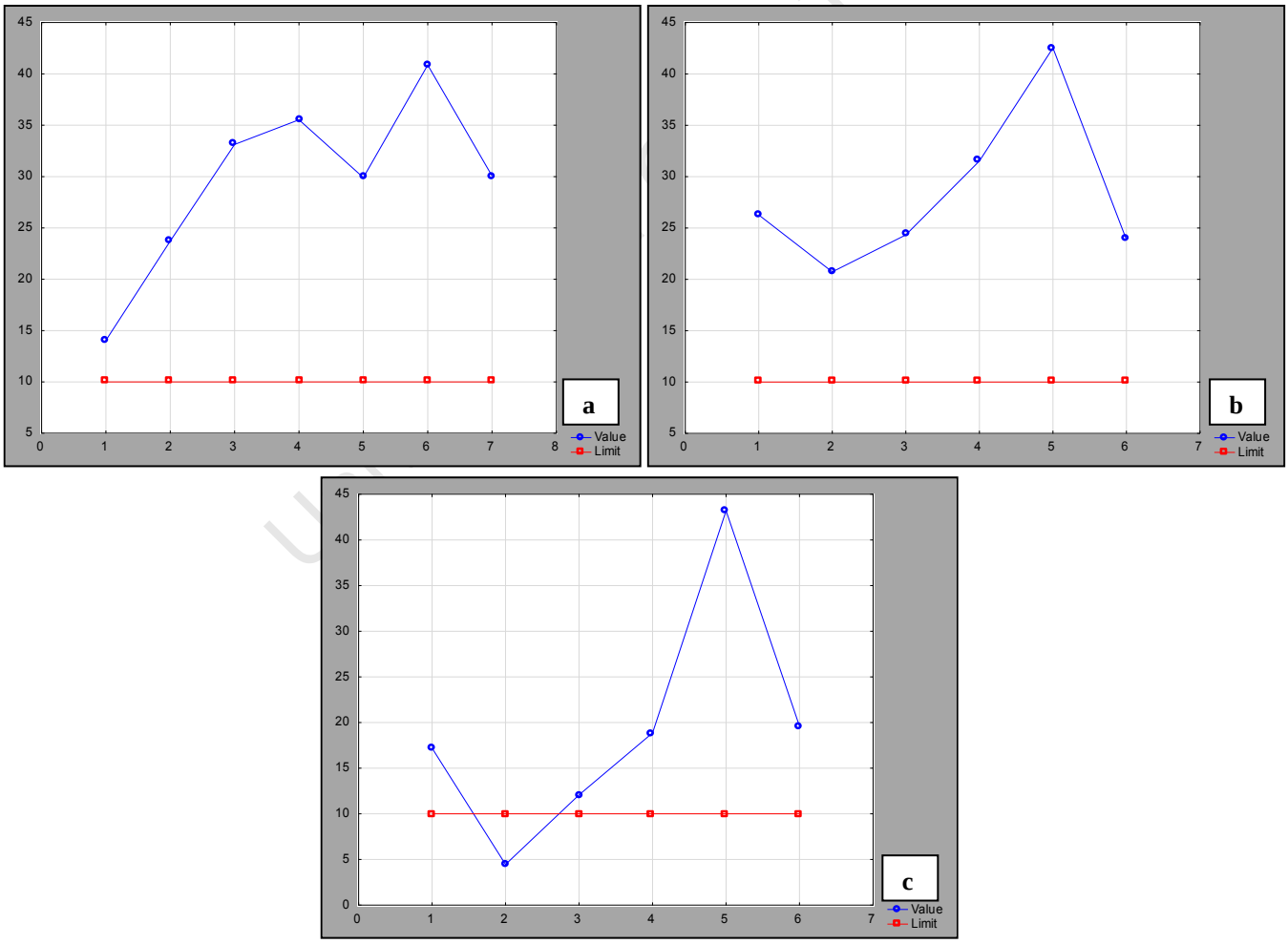

Figure 20: Comparison of effluent concentrations for PO_4^{3-} against DWA's discharge limit standards
De Goede Hoop (a), Wolwedans (b) and Babylonstoren (c)

4.2.2.4 ***Orthophosphate (PO_4^{3-}) removal summary***

The PO_4^{3-} removal percentage obtained over the sampling period is considerably lower compared to that obtained from laboratory - scale experiments undertaken by Akratos & Tsihrintzis (2007), in which removal rates ranged between 28% and 89%. The low PO_4^{3-} removal rates at the sites could either be attributed to low O_2 concentrations, which may lead to the solubilisation of minerals and the release of dissolved phosphorus (Akratos & Tsihrintzis, 2007) or the type of substrate used. The substrate used at all sites is recycled rubble, comprising 19 mm brick, ceramics, marble and granite. These materials may not contain sufficient quantities of Al, Fe or Ca to adsorb P. The adsorption of P that occurs in wetland soils is controlled by the interaction of redox potential, pH, Fe, Al and Ca minerals and the amount of native soil P (Faulkner & Richardson, 1989 cited in Vymazal, 2004). As the pH of the wastewater was always either approximately neutral or alkaline, the presence of Ca would be important. This is because, as mentioned in Chapter 2, under alkaline conditions, precipitation occurs as insoluble Ca-phosphates, whereas under acidic conditions, Fe and Al may precipitate as Fe-phosphates and Al-phosphates.

4.2.3 ***E. coli***

Pathogen removal in CWs is carried out through a combination of biological, chemical and physical factors. The *E. coli* removal was variable between the sites and ranged between 39% and 85%. More detail on the treatment performance is provided as following Table 16.

Table 16 provides a summary of the actual *E. coli* values recorded on each sampling occasion per sampling point.

Table 16: *E. coli* concentrations (CFUs/100ml) recorded at the sampling sites (Grey shading indicates substituted data)

De Goede Hoop							
Sampling Date (2011)	**11.07**	**26.07**	**10.08**	**23.08**	**06.09**	**20.09**	**04.10**
Influent (bioreactor)	625 000	110 000	590 000	660 000	740 000	23 000	680 000
Effluent (recirculation pond)	72 000	50 000	94 000	200 000	100 000	3 000	4 500
Constructed pond	30 000	35 000	33 000	130 000	14 000	33 000	33 000
Groundwater tank	0	0	0	0	0	0	0
Stream	0	0	0	0	0	0	10 000
Wolwedans							
Sampling Date (2011)	**19.07**	**02.08**	**16.08**	**30.08**	**13.09**	**27.09**	-
Influent (aeration tank)	1 000 000	79 000	400 000	330 000	2 000 000	630 000	-
Effluent	520 000	50 000	350 000	280 000	1 000 000	530 000	-
French drain overflow	1 800 000	1 300 000	1 400 000	2 400 000	2 800 000	2 700 000	-
Vlei	0	6000	8000	0	40000	0	-
Babylonstoren							
Sampling Date (2011)	**19.07**	**02.08**	**16.08**	**30.08**	**13.09**	**27.09**	-
Upper culvert	0	0	0	0	0	0	-
Influent (bioreactor)	2 000 000	990 000	400 000	2 200 000	1 300 000	390 000	-
Effluent	590 000	390 000	350 000	470 000	540 000	220 000	-
Lower culvert	280 000	330 000	56 000	196 000	430 000	150 000	-

4.2.3.1 ***De Goede Hoop***

The average influent *E. coli* concentration was 397 192 CFUs/100 ml and the average effluent concentration is 36 813 CFUs/100 ml. According to the p-value, there was a significant difference between the average influent and effluent *E. coli* concentrations. In terms of removal percentage, there was a decrease in the mean *E. coli* concentration of 85% over the sampling period (Table 12). The *E. coli* effluent concentrations did not meet DWA's discharge standards for faecal coliforms (Figure 21).

4.2.3.2 ***Wolwedans***

According to the p-value, there was a significant difference between the average influent and effluent concentrations. The average influent concentration was 486 407 CFUs/100 ml and the average effluent concentration was 332 660 CFUs/100 ml. In terms of removal percentage, there was a decrease in the *E. coli* concentration of 39% over the sampling period (Table 12). The *E. coli* effluent concentrations did not meet DWA's discharge standards for faecal coliforms (Figure 21).

4.2.3.3 ***Babylonstoren***

There was a significant difference between the average influent and effluent *E. coli* concentrations. The average influent concentration was 979 490 CFUs/100 ml and the average effluent concentration was 403 645 CFUs/100 ml. In terms of removal percentage, there was a decrease in the mean *E. coli* concentration of 65% over the sample period (Table 12). The *E. coli* effluent concentrations did not meet DWA's discharge standards for faecal coliforms (Figure 21).

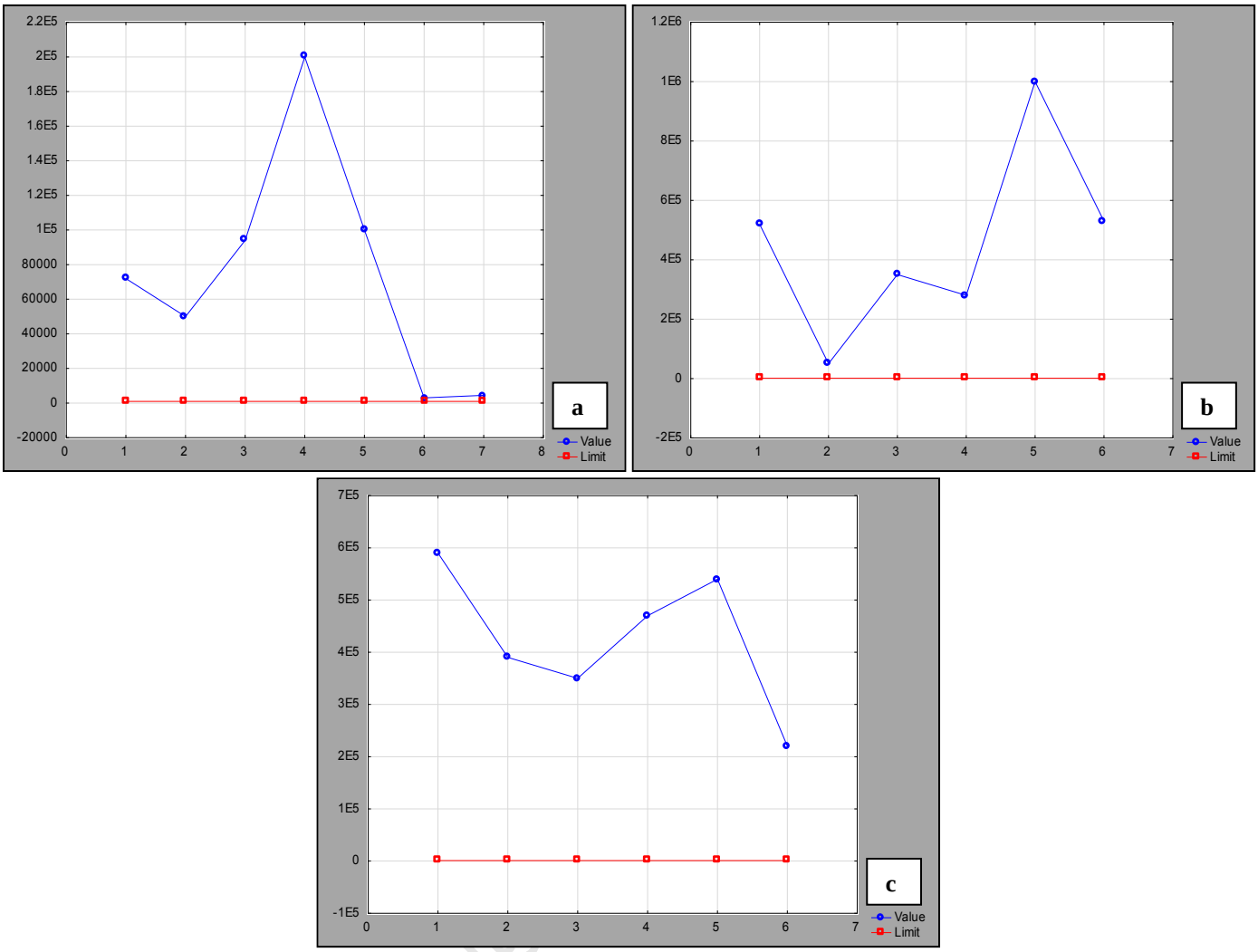

Figure 21: Comparison of effluent concentrations for *E. coli* against DWA's discharge limit standards
De Goede Hoop (a), Wolwedans (b) and Babylonstoren (c)

4.2.3.4 ***E. coli removal summary***

The *E. coli* removal rates obtained are considerably lower than those obtained by Decamp & Warren (2000) for four pilot-scale systems, in which very high removal rates between 96.6 and 98.9% were obtained. Keffala & Ghrabi (2005) also obtained a high *E. coli* removal of 90% in their laboratory-scale set-up.

It should be noted that a high removal percentage does not always indicate low bacteria numbers in the effluent. Vymazal (2010b) argues that the number of bacteria is more important than the reduction value. With regard to the DWA's discharge limit standards for pathogens, there is no standard provided for *E. coli*. However a standard is provided for faecal coliforms, of which *E. coli* is a component. It can be deduced that for all three sites, the

standard for faecal coliforms of 1000 CFUs/100 ml was not met. This is because *E .coli* was present at high levels in the effluent and exceeded this amount by far.

The lower *E. coli* removal at Wolwedans and Babylonstoren as compared to the literature could be a result of HRT. The longer the HRT, the longer the bacteria are exposed to unfavourable conditions (Vymazal, 2010b). Figure 22 illustrates the effect of HRT on bacterial removal.

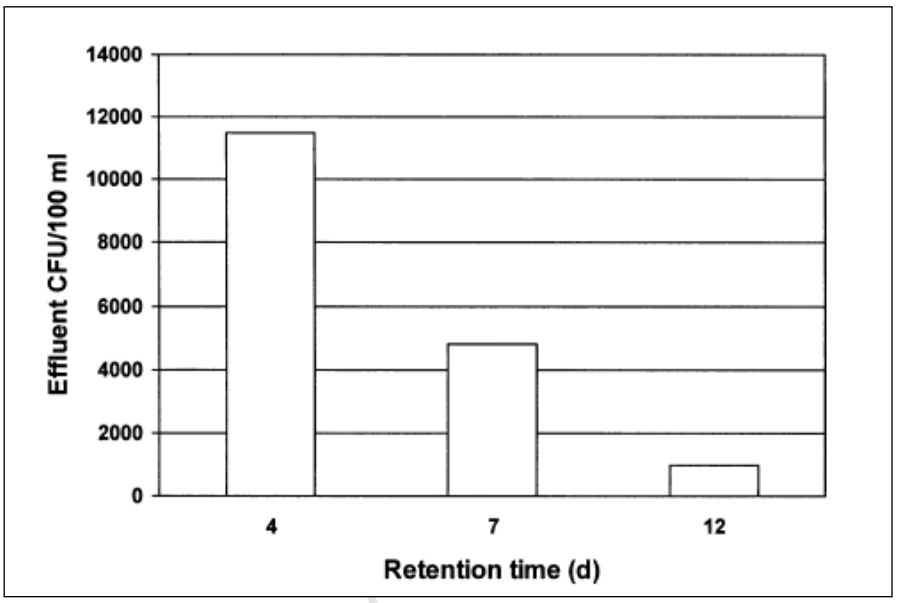

Figure 22: Faecal coliforms in the effluent of a FWS constructed wetland
(Source: Kurt & van Bruggen, 2000 cited in Vymazal, 2010b)

As mentioned before, the HRT at Babylonstoren may have been reduced as a result of short-circuiting or clogging of the system. The actual HRT of the CW at Wolwedans may also deviate from the theoretical HRT of two days because the depth of the constructed system does not match that of the intended design and is not able to hold the water long enough for effective treatment to take place.

4.3 Impact of CWs on surrounding water bodies

The impact of the treated effluent on surrounding water bodies was assessed by comparing the resultant water quality to the relevant South African water quality guidelines. These guidelines included DWA's discharge limit standards and South African water quality guidelines for aquatic ecosystems and domestic wastewater. The following is noted with regard to guidelines for aquatic ecosystems:

- The guideline refers to un-ionised NH_3 only as the toxicity to aquatic biota is directly related to the concentration of the un-ionised form (DWAF, 1996b). The percentage of un-ionised NH_3 was calculated using pH and temperature, as set out in the tables provided in DWAF (1996b) and Hach (1999).

- The guideline includes total inorganic N as a parameter which was determined by adding NH_3, NO_3^- and NO_2^-.

- Guidelines were not given for PO_4^{3-}, but for inorganic P, of which PO_4^{3-} is a component.

4.3.1 **De Goede Hoop**

The CW at the De Goede Hoop site was designed to be a closed system where water is constantly re-circulated within the system with evaporation and possible groundwater infiltration taking place (if clay liner not effective). Therefore flows from the CW were not expected to have an impact on a nearby stream flowing across the property; the stream was however monitored.

During the first four sampling sessions, the system did not operate as a closed system as the property owner diverted some of the treated water to the nearby constructed pond (from where the water either evaporated or infiltrated the ground). However, during the last three sampling sessions, the system was reverted back to a closed system. Influent concentrations were expected to be lower in a closed system as compared to an open system as the treated water which circulates through the system dilutes the concentration of the incoming wastewater. The treated wastewater retained in the constructed pond was monitored.

Groundwater on the property was also monitored. The property owner abstracted and pumped groundwater into groundwater tanks (for irrigation purposes); groundwater was thus easily available to monitor.

The constructed pond:

The effluent sample was compared to DWA's discharge limit standards (Table 17). DWA's effluent standard was met 14% of the time for NH_3, 100% of the time for NO_3^-/NO_2^-, 0% for both PO_4^{3-} and *E. coli*.

Table 17: Comparison of the constructed pond at De Goede Hoop with DWA's discharge limit standards

Contaminant	Constructed pond	DWA's discharge limit standards (RSA, 1999)
NH_3 (mg/l)	35 6.3 10.07 11.5 1.4 10.07 10.07	3
NO_3^- + NO_2^- (mg/l)	10.834 8.9 14.81 2.03 3.33 8.964 8.964	15
PO_4^{3-} (mg/l)	23.55 28.13 26.53 35.8 30.1 28.13 28.13	10
***E. coli* (CFUs/100 ml)**	30 000 35 000 33 000 130 000 14 000 33 000 33 000	1 000

Stream:

Water quality in the stream was compared to DWA's discharge standards as well as the guidelines for aquatic ecosystems (Table 18); the NH_3, NO_3^-/NO_2^- and PO_4^{3-} effluent concentrations met DWA's standard 100% of the time and *E. coli* met the standard 86 % of the time.

The NH_3 (un-ionised form) concentrations met the guideline for aquatic ecosystems of 7 mg/l, 100% of the time. The inorganic N effluent concentrations were within the mesotrophic[13] range 71% of the time. The PO_4^{3-} effluent concentrations were within the oligotrophic[14] range 86% of the time.

[13]Under mesotrophic conditions, the following effects may be experienced; high levels of species diversity, productive systems, nuisance growth of aquatic plants and blooms of blue-green algae and algal blooms (seldom toxic) [DWAF, 1996b].

14 Under oligotrophic conditions, the following may be experienced; usually moderate levels of species diversity; usually low productivity systems with rapid nutrient cycling; no nuisance growth of aquatic plants or the presence of blue-green algal blooms. [DWAF, 1996b].

Table 18: Comparison of the stream at De Goede Hoop with South African water quality standards

Contaminant	Stream	DWA's discharge limit standards (RSA, 1999)	South African water quality guidelines for aquatic ecosystems (DWAF, 1996b)			
NH_3 (mg/l)	0.02; 0.02; 0.13; 0.2; 0; 0.05; 0.67	3	N/A			
NH_3 (un-ionised ammonia only) [mg/l]	0.00001; 0.00001; 0.00004; 0.00005; 0; 0.00001; 0.00018	N/A	7			
NO_3^- + NO_2^- (mg/l)	0; 0.92; 5.38; 0.002; 0.8; 1.077; 0	15	N/A			
Inorganic N (NH_3 + NO_2^- + NO_3^-) [mg/l]	0.02; 0.94; 5.51; 0.202; 0.8; 1.127; 0.67	N/A	<0.5 mg/l, oligotrophic conditions	0.5-2.5 mg/l, mesotrophic conditions	2.5-10 mg/l , eutrophic conditions	>10 mg/l , hypertrophic conditions
PO_4^{3-} (mg/l)	0.08; 0.28; 4.17; 2.53; 5.97; 2.92; 2.04	10	<5 mg/l, oligotrophic conditions [15]	5-25 mg/l, mesotrophic conditions	25-250 mg/l, eutrophic conditions	>250 mg/l, hypertrophic conditions
***E. coli* (CFUs/100 ml)**	0; 0; 0; 0;0;0; 10 000	1 000	N/A			

[15] These values are for total inorganic phosphorus and not PO_4^{3-}

Groundwater tank:

The water obtained from the groundwater tank was compared to DWA's discharge standards as well as the guidelines for domestic wastewater (Table 19). The guideline for domestic wastewater includes water for gardening, which is the purpose for which the groundwater was being stored. In terms of the groundwater quality, all the parameters, NH_3, NO_3^-/NO_2^- and *E. coli* met DWA's discharge standards 100% of the time, indicating that groundwater is not being polluted by the CW.

For the guidelines for domestic water, the effluent concentrations of NH_3, NO_3^-/NO_2^- and *E. coli* were in the 'no health and aesthetic effects', 'no adverse health effects', and 'negligible risk of microbial infection' categories, respectively, for 100% of the time.

Table 19: Comparison of the groundwater tank at De Goede Hoop with South African water quality standards

	Groundwater Tank	DWA's Discharge limit standards (RSA, 1999)	South African water quality guidelines for domestic water (DWAF, 1996a) Target water quality range			
NH_3 (mg/l)	0.02; 0.02; 0.02; 0.02; 1.56; 0; 0.01	3	0-1 No health or aesthetic effects.	1-2 Possibility of taste and odour complaints from consumers.	2-10 Consumer complaints of objectionable taste and odours likely. Disinfection by chlorine can be compromised.	>10 Unacceptable in domestic water. Danger of formation of nitrite. Likelihood of fish deaths in aquaria. Chlorination is severely compromised.
NO_3^- + NO_2^- (mg/l)	2.47; 0.55; 2.47; 2.47; 3.735; 1.205; 4.004	15	0-6 No adverse health effects.	6-10 Rare instances of methaemo-globinaemia in infants; no effects in adults. Concentrations in this range generally well tolerated.	10-20 Methaemo-globinaemia may occur in infants. No effects in adults.	>20 Methaemo-globinaemia occurs in infants. Occurrence of mucous membrane irritation in adults.
PO_4^{3-} (mg/l)	1.16; 0.58; 1.16; 1.16; 2.07; 1.41; 0.91'	10		N/A	N/A	N/A
***E. coli* (CFUs/100 ml)**	0; 0; 0; 0; 0 0; 0	1000	0 Negligible risk of microbial infection.	0-10 Slight risk of microbial infection with continuous exposure.	10-20 Risk of infectious disease transmission with continuous exposure; slight risk with occasional exposure.	>20 Significant and increasing risk of infectious disease transmission. As faecal coliform levels increase, the amount of water ingested required to cause infection decreases.

4.3.2 **Wolwedans**

At this site, treated wastewater from the CW eventually enters a vlei about 800 m downstream. The vlei, was monitored over the sampling period. The vlei also received wastewater from an additional source; i.e. French drain overflow (which received treated wastewater from a separate septic tank receiving wastewater from work houses); sampling was undertaken at the French drain.

French drain:

As expected, the French drain overflow was highly contaminated with pollutants (Table 20) and meets DWA's standards for NH_3, PO_4^{3-} and *E. coli* 0% of the time. In contrast, the NO_3^- / NO_2^- effluent concentrations meet DWA's standards 100% of the time.

Table 20: Comparison of the French drain overflow at Wolwedans with DWA's discharge standards

Contaminant	**French drain overflow**	**DWA's discharge standards (RSA, 1999)**
NH_3 (mg/l)	28.56 70.4 69.6 84.8 72.74 74.8	3
NO_3^- + NO_2^- (mg/l)	0.08 9.763 7.036 5.342 4.465 5.431	15
PO_4^{3-} (mg/l)	23.9 29.07 22.4 30.47 36.6 27.93	10
***E. coli* (CFUs/100ml)**	1 800 000 1 300 000 1 400 000 2 400 000 2 800 000 2 700 000	1 000

Vlei:

The water quality of the vlei were compared to DWA's discharge standards and guidelines for aquatic ecosystems (Table 21). NH_3, NO_3^-/NO_2^- and PO_4^{3-} met DWA's standards 100% of the time. Meanwhile, *E. coli* levels were met 50% of the time.

The effluent concentrations of NH_3 (un-ionised form) were well below the guideline 100% of the time. For inorganic N, the effluent concentrations were within the range of eutrophic conditions 67%[16] of the time. However, for PO_4^{3-}, the effluent concentration was within the range of oligotrophic conditions, 100% of the time.

[16]Eutrophication could result in low species diversity, highly productive systems, nuisance growth of aquatic plants and blooms of blue-green algae; algal blooms may include species, which are toxic to man, livestock and wildlife (DWAF, 1996b).

Table 21: Comparison of the vlei at Wolwedans with the South African water quality guidelines

	Vlei	DWA's discharge standards (RSA, 1999)	Guidelines for aquatic ecosystems (DWAF, 1996b)			
NH_3 (mg/l)	0.47; 0.3; 0.43; 0.1; 0.61; 0.33	3	N/A			
NH_3 (un-ionised ammonia only	0.0209; 0.0019; 0.0017; 0.0002; 0.0061; 0.0015	N/A	7			
NO_3^- /NO_2^- (mg/l)	1.038; 2.415; 4.999; 3.241; 2.007; 1.609	15	N/A			
Inorganic N (NH_3 + NO_2^- + NO_3^-)	1.508; 2.715 5.429; 3.341 2.617;1.939	N/A	<0.5 mg/l, oligotrophic conditions	0.5-2.5 mg/l, mesotrophic conditions	2.5-10 mg/l, eutrophic conditions	>10 mg/l, hypertrophic conditions
PO_4^{3-} (mg/l)	2.43; 0.51; 2.17; 3.41; 2.31;2.32	10	<5 mg/l, oligotrophic conditions	5-25 mg/l, mesotrophic conditions	25-250 mg/l, eutrophic conditions	>250 mg/l, hypertrophic conditions
***E. coli* (CFUs/100 ml)**	0; 6 000; 8 000; 0 40 000; 0	1 000	N/A			

4.3.3 **Babylonstoren**

Treated wastewater was discharged into a culvert which runs south of the CW. Although the culvert is not considered an aquatic system, it does eventually flow into the Berg River albeit approximately 2.2km from the CW. The pollutant concentrations for the lower culvert were compared to DWA's discharge limits (Table 22). Both the upper culvert and lower culvert were sampled, and the concentration of the pollutants was compared before and after the wastewater from the CW entered the culvert.

For the lower culvert, the discharge standards for NH_3 were met 83% of the time, for NO_3^- /NO_2^- 100% of the time and for PO_4^{3-} 67% of the time. *E. coli* concentrations did not once meet the standard. Furthermore, there is an increase in the concentration of all the pollutants from the upper to lower culvert, suggesting that the treated wastewater contributes pollutants to the system.

Table 22: Comparison of the lower and upper culvert with DWA's discharge limit standards

	Upper culvert	Lower culvert	DWA's discharge limit standards (RSA, 1999)
NH_3 (mg/l)	1.33 0.36 0.07 1.1 0.88 0.35	1.6 3.53 0.2 0.2 0.83 0.2	3
NO_3^- + NO_2^- (mg/l)	2.31 4.54 2.01 3.90 2.56 2.53	1.65 2.85 10.72 3.81 1.91 0.87	15
PO_4^{3-} (mg/l)	5.9 0.948 4.47 1.29 7.32 1.09	12.6 1.6 4.26 3.7 11.3 6.9	10
***E. coli* (CFUs/100 ml)**	0 0 0 0 0 0	280 000 330 000 56 000 196 000 430 000 150 000	1 000

4.4 Summary of overall performance

The CWs show variable performance both within and between systems. Variation in performance is consistent with the literature, for example, in Akratos & Tsihrintzis (2007), the removal efficiencies obtained for various systems had relatively large standard deviations for TKN, NH_3, TP and PO_4^{3-}, indicating large variations within the systems. Furthermore, as mentioned in Chapter 1, the results obtained by Masi & Martinuzzi (2001), also demonstrated a large variation in percentages of removal between different systems. In all three study sites, the variation in pollutant removal between the sites could not be compared, because it was known from the outset that each site differed in terms of design, structure and the volumes of wastewater passing through the system.

Overall the performance of the systems showed poor removal efficiencies, but with a few exceptions, e.g. NH_3 and *E. coli* removal percentages for De Goede Hoop. Furthermore, the systems did not always meet DWA's discharge standards. This is particularly the case for PO_4^{3-} and *E. coli,* in which standards were not met for all the sites. It must be reiterated that CWs might not have been operating at their peak levels of performance as sampling was undertaken during the cooler, wetter months when oxygen transport to the substrate is limited and when rain may have entered the systems, reducing HRT and thus negatively affecting treatment performance. Treatment performance was thus tested under the under less ideal, but realistic conditions because these cooler conditions need to be factored into the performance and expectations for CWs

According to Lee *et al.*, (2009), good performance is dependent on growth of macrophytes, wetland design, and operation and maintenance. The poor performance of systems could be attributed to inappropriate design as well as lack of maintenance of the systems. For example, the short HRT at Wolwedans and hydraulic short-circuiting at Babylonstoren are examples of how design issues may have negatively affected the performance of these systems. In terms of macrophyte growth, the vegetation was sparse at De Goede Hoop and Wolwedans, as compared to Babylonstoren, which may have impacted NO_3^- removal. Issues regarding lack of maintenance were also experienced at Wolwedans and Babylonstoren. Furthermore, regarding the impacts on surrounding water bodies, the CWs did not seem to have a large impact for all three sites, although *E. coli* levels were found to be high at the vlei and lower culvert at Wolwedans and Babylonstoren, respectively.

5 Conclusions

The aim of the research project was to determine the performance of constructed wetlands (CWs) in treating domestic wastewater in peri-urban / rural settings of the Western Cape, South Africa. CW systems are currently being used world-wide to treat various types of wastewater (e.g. domestic, industrial, agricultural, stormwater) and are considered a viable option for wastewater treatment. The research focused on the wastewater treatment by CWs as an ecosystem service. For the purpose of this study, the definition of ecosystem services from the MA (2003) was used, which defines ecosystem services as "*the benefits people obtain from ecosystems*".

CWs also provide a range of other ecosystem services such as visual enhancement, habitat provision and recreational opportunities. However, as these systems behave as natural systems, a better understanding is required if they are to operate effectively. The research literature generally lends support to the applicability and functionality of these systems; there is however a general failure to highlight the limitations or to emphasise the fact that these systems are biological systems which do not have unlimited capacity to treat wastewater. The fact that CWs are based on natural processes also means that unpredictability and variability in treatment performance should be expected. It has also been argued that the capability of wetlands to perform certain functions has been over estimated and that the over-generalisation of laboratory and short-term field experiments' results has been exaggerated to present a more promising account of the performance of CWs (Gopal, 1999). Furthermore, there does not seem to be much concern regarding the impact of (ineffective) CWs on the environment.

This research focused on the performance of three CWs on the periphery of Cape Town, Western Cape and aimed to contribute to knowledge on South African CWs performance in general. This was done by considering the percentage change in pollutants from the influent point to the effluent point and also by comparing effluent concentrations to effluent discharge standards. Moreover the research interest was to purposely shift attention to an analysis of the performance of CW systems *in situ* as opposed to laboratory-based studies where certain variables are contained or controlled. The focus was also on determining the potential impact

that these systems had on the surrounding environment by analysing the potential impact of the CWs on surrounding or receiving water bodies. This was done by determining the quality of the effluent and the water quality of the streams, culverts and vleis surrounding the CW systems.

The results of the study indicated variable performance within and between systems but overall the pollutant removal performance was poor, with the exception of NH_3 (96%) and *E. coli* (see below) removal at De Goede Hoop. The mean PO_4^{3-} removal was low at all three sites; 3.8%, 7% and 20% at De Goede Hoop, Wolwedans and Babylonstoren respectively. Furthermore DWA's effluent standards of 10 mg/l for PO_4^{3-} were not met at all the sites. Poor PO_4^{3-} removal can be explained either by low O_2 concentrations or by the choice of substrate used. When O_2 concentrations are low, solubilisation of minerals and subsequent release of dissolved phosphorus occurs. Substrates containing Al, Fe or Ca are able to absorb P better than substrates that do not contain these metals. Mean *E. coli* removal percentages were considerably lower compared to research undertaken by others, e.g. 96.6% - 98.8% *E. coli* removal was obtained by Decamp & Warren (2000) for four pilot-scale systems and 90% removal was obtained by Keffala & Ghrabi (2005) for a laboratory - scale system. *E. coli* removal was 85% at De Goede Hoop, 39% at Wolwedans and 65% at Babylonstoren and at all the sites Department of Water Affairs' (DWA's) effluent standards for faecal coliforms of 1000 CFUs/100ml were not met. Poor *E. coli* removal could be because of a short hydraulic retention time (HRT) in which insufficient time is allowed for pathogen removal through natural die-off, predation, sedimentation, filtration and adsorption.

Overall, it is likely that poor performance of the systems could be attributed to poor design (which caused surface flow and short HRT), and problems with vegetation growth and lack of maintenance. It should also be noted that the CWs were monitored during the cooler periods, when biological activity and productivity are lowest, perhaps suggesting that these systems need to be managed differently depending on the time of the year. Although the performance of the systems was poor, they did not have a significant impact on the surrounding environment as all the pollutants, except *E. coli*, were within the DWA's discharge standards as well as the South African water quality guideline limits for the majority of the time.

5.1 **Recommendations**

CWs systems behave as natural systems and thus require a better understanding if they are to operate effectively. The results indicate that more research is required in South Africa to obtain a better understanding of CWs so as to improve treatment performance. Future work on CW performance in South Africa should include monitoring over a longer time period; e.g. one year so that performance in both cooler and warmer months can be recorded. This study only considered the cooler periods and therefore provided performance data when biological activity and productivity were at the lowest. Furthermore a broader range of contaminants should be included such as chemical oxygen demand (COD), biological oxygen demand (BOD) and suspended solids. Additionally flow rates should be monitored more closely as obtaining flow rate data over a two week period did not provide information regarding perturbations in the flow from peaks occurring at certain parts of the day.

Improved performance of the selected CWs may be achieved by improving the management, operation and maintenance as well as the design of the systems. For design aspects, consideration should be given to increasing the HRT as a long HRT is an important factor for contaminant removal; the HRT can be increased by increasing the volume of the system. To minimise clogging of the systems, which may negatively affect performance, large diameter rocks should be placed at the inlet and outlet pipes. It should also be ensured that flow control structures, which are used to adjust water levels, are sized to handle the maximum design flows so as to minimise short–circuiting (Davis, 1995). In locations such as De Goede Hoop, where there is not much sunlight, shade – tolerant plant species should be selected (Steiner & Watson, 1993). Plantings should ideally occur during spring to early summer so that as much growth can occur prior to winter, thereby reducing winter plant mortality (Steiner & Watson, 1993).

In terms of management of the systems, education may play an important role in changing behaviour e.g. discouraging overloading of the CW and encouraging continuous monitoring and maintenance of the systems. Maintenance should include routinely monitoring the inlets and outlets, which should be cleaned of any debris as this may result in clogging (U. S. EPA, 2000). Influent suspended solids accumulated at the inlet will need removal over time as these accumulations may result in a reduced HRT (U. S. EPA, 2000). Washing or replacing of the substrate is also needed so as to maintain hydraulic conductivity (Ellis et al., 2003).

Changes in water levels affect HRT, oxygen diffusion and plant cover; any significant changes in water levels should thus be investigated immediately (U. S. EPA, 2000). Furthermore, the following should be routinely monitored; inflow and outflow rates, water quality, water levels and indicators of biological conditions (e.g. percent cover of dominant plant species and measurement of microbial populations) (U. S. EPA, 2000). Harvesting of plants and litter removal may be necessary depending on the design of the system (U. S. EPA, 2000). Annual removal of vegetation or replanting of vegetation may be needed to maintain flow patterns and treatment functions (U. S. EPA, 2000).

6 References

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APPENDIX 1: HWT COMPANY PROFILE

APPENDIX 2: BACKGROUND VARIABLES

Appendix 2a: Background variables recorded at De Goede Hoop (grey shading indicates substituted data)

	11.07.11	26.07.11	10.08.11	23.08.11	06.09.11	20.09.11	04.10.11
pH							
Influent (bioreactor)	7.6	7.4	7.4	7.6	7.5	7.6	7.5
Effluent (recirculation pond)	7.9	7.8	7.8	7	7.7	7.4	6.9
Constructed pond	7.6	7.6	7.4	7.6	6.7	7.6	7.6
Groundwater tank	5.8	5.8	5.7	5.8	5.7	6	5.9
Stream	6.3	6	5.6	5.6	5.7	6.2	6.2
Temperature (^{o}C)							
Influent (bioreactor)	16.7	14.4	13.6	13.4	15.5	15.1	16.7
Effluent (recirculation pond)	17.5	11.6	13.1	13.5	14.4	14.2	15.7
Constructed pond	16	10.6	13.1	12.6	15.8	13.1	13.1
Groundwater tank	17.1	17.1	15.8	17.1	18.2	17	17.3
Stream	16.4	14.5	14.8	14.5	14.7	14.7	15.6
Electrical conductivity (µS/cm)							
Influent (bioreactor)	806	860	568	791	584	889	1075
Effluent (recirculation pond)	902	838	744	860	768	889	942
Constructed pond	721	816	787	751	531	751	751
Groundwater tank	311	311	301	311	304	321	318
Stream	537	506	495	591	583	565	582
Total dissolved solids(ppm)							
Influent (bioreactor)	408	428	259	394	243	444	512
Effluent (recirculation pond)	438	419	372	431	385	442	471
Constructed pond	363	410	395	375	242	375	375
Groundwater tank	153	153	150	153	152	154	158
Stream	271	256	246	294	286	284	291

Appendix 2b: Background variables recorded at Wolwedans

	19.07.11	02.08.11	16.08.11	30.08.11	13.09.11	27.09.11
pH						
Influent (aeration tank)	8.2	7.7	8	7.4	7.5	7.4
Effluent	7.8	7.4	7	7.5	7.4	7.1
French drain overflow	7.8	8.1	7.8	7.9	7.6	7.6
Vlei	8.2	7.4	7.1	7	7.6	7.2
Temperature (^{o}C)						
Influent (aeration tank)	21	20.3	21.1	14.4	15.5	19
Effluent	16.2	17.2	16.8	13.2	14.2	18.4
French drain overflow	14.8	15.2	14.7	13.1	14.3	17.3
Vlei	16.3	14.6	13.5	12.7	13.8	15.6
Electrical conductivity (µS/cm)						
Influent (aeration tank)	1058	1034	953	1140	989	2668
Effluent	1026	1019	962	1030	985	3275
French drain overflow	1327	1303	1246	1157	1361	1324
Vlei	339	324	310	321	305	303
Total dissolved solids(ppm)						
Influent (aeration tank)	510	517	473	567	511	1334
Effluent	512	508	483	514	493	1634
French drain overflow	679	653	569	589	679	667
Vlei	171	163	140	160	152	155

Appendix 2c: Background variables recorded at Babylonstoren

	19.07.11	02.08.11	16.08.11	30.08.11	13.09.11	27.09.11
pH						
Upper culvert	8.5	8.4	7.5	7.2	7.1	7.2
Influent (bioreactor)	7.4	7.6	7.8	6.9	7	6.9
Effluent	7.7	7.8	7.8	7.2	7.5	7.2
Lower culvert	8	7.8	7.2	7.1	7	7.1
Temperature (^{o}C)						
Upper culvert	13.5	14.5	16	13.2	14.4	16.4
Influent (bioreactor)	16.7	16	19.1	15.5	17.1	18.8
Effluent	16.6	16.5	16.4	14.9	15.2	18
Lower culvert	14.5	15.1	13.9	12.7	13.9	14.9
Electrical conductivity (µS/cm)						
Upper culvert	375	402	417	405	372	356
Influent (bioreactor)	723	735	675	831	561	472
Effluent	1080	728	604	1012	789	645
Lower culvert	439	540	426	410	391	402
Total dissolved solids(ppm)						
Upper culvert	184	199	208	201	183	177
Influent (bioreactor)	361	366	338	419	279	242
Effluent	535	362	304	503	395	321
Lower culvert	216	270	211	205	196	191

APPENDIX 3: DISCHARGE STANDARDS AND WATER QUALITY GUIDELINES

Appendix 3a: Department of Water Affairs effluent discharge standards

DEPARTMENT OF WATER AFFAIRS – GENERAL AND SPECIAL AUTHORISATION

Discharge limits and conditions set out in the National Water Act, Government Gazette No. 20526, 8 October 1999

Wastewater limit values applicable to discharge of wastewater into a water resource

SUBSTANCE/PARAMETER	GENERAL LIMIT	SPECIAL LIMIT
Faecal Coliforms (per 100 ml)	1 000	0
Chemical Oxygen Demand (mg/l)	75*	30*
pH	5,5-9,5	5,5-7,5
Ammonia (ionised and un-ionised) as Nitrogen (mg/l)	3	2
Nitrate/Nitrite as Nitrogen (mg/l)	15	1,5
Chlorine as Free Chlorine (mg/l)	0,25	0
Suspended Solids (mg/l)	25	10
Electrical Conductivity (mS/m)	70 mS/m above intake to a maximum of 150 mS/m	50 mS/m above background receiving water, to a maximum of 100 mS/m
Ortho-Phosphate as phosphorous (mg/l)	10	1 (median) and 2,5 (maximum)
Fluoride (mg/l)	1	1
Soap, oil or grease (mg/l)	2,5	0
Dissolved Arsenic (mg/l)	0,02	0,01
Dissolved Cadmium (mg/l)	0,005	0,001
Dissolved Chromium (VI) (mg/l)	0,05	0,02
Dissolved Copper (mg/l)	0,01	0,002
Dissolved Cyanide (mg/l)	0,02	0,01
Dissolved Iron (mg/l)	0,3	0,3
Dissolved Lead (mg/l)	0,01	0,006
Dissolved Manganese (mg/l)	0,1	0,1
Mercury and its compounds (mg/l)	0,005	0,001
Dissolved Selenium (mg/l)	0,02	0,02
Dissolved Zinc (mg/l)	0,1	0,04
Boron (mg/l)	1	0,5

- **After removal of algae**

Appendix 3b: South African Water Quality Guidelines for domestic water use

Ammonia

Background Information

Introduction

Ammonia (NH_3), where the nitrogen atom is in the III oxidation state, can readily take up an additional (hydrogen ion) to form the ammonium ion (NH^+_4). In solution ammonia occurs in equilibrium with the ammonium ion and the position of equilibrium is governed by pH and temperature. Ammonia is not toxic to man at the concentrations likely to be found in drinking water but does exert other effects. For example, elevated concentrations of ammonia can compromise the disinfection of water and give rise to nitrite formation in distribution systems, which may result in taste and odour problems.

Occurrence

At high pH, ammonia exits predominantly as a gas in solution, and can be released to the atmosphere from water. At low and neutral pH, ammonia is found predominantly as the ammonium ion. Ammonia can also be microbiologically oxidised to nitrates.

Surface waters which are not contaminated with organic wastes, generally have a low ammonia nitrogen concentration, typically less than 0.2 mg/P. Concentrations exceeding 10 mg/P are found in raw untreated sewage; ammonia concentrations tend to be elevated in waters where organic decomposition under anaerobic conditions takes place. Ammonia is found in runoff from agricultural lands, where ammonium salts have been used for fertilizers.

Interactions

The chemical reactions and toxicity effects of ammonia are closely correlated to **pH**. Ammonia is more toxic under alkaline than neutral conditions, but has a very low toxicity under acidic conditions. Ammonia can also form complexes with many of the transition metals, notably **copper**.

Measurement

The criteria are based on the free ammonia nitrogen concentration. This is the sum of the NH_3 and NH_4 nitrogen concentrations, and is given in units of mg/P. The reference method for the determination of ammonia is the phenate colorimetric method, where an intensely blue compound, indophenol, is formed from the reaction of ammonia, phenol, and hypochlorite, under catalysis by Mn(II). Where other methods are used, their characteristics relative to the reference method should be known.

Data Interpretation

Mean values should be used to compare with the criteria given.

Treatment Options

At near-neutral pH, the non-toxic ammonium ion predominates while the toxic-free ammonia form predominates as the pH increases to approximately 11. The volatile nature of the free ammonia form provides a useful treatment technique to remove ammonia from water supplies. The pH of the water can be increased to greater than 11.0 by the addition of a strong alkali such as sodium hydroxide, to convert all the ammonia to the volatile free form.

Treatment involves spraying water in droplet form down through a vertical stripping tower, through which air is blown countercurrent to the water flow. This strips the volatile ammonia from solution into the atmosphere with the air stream, leaving ammonia residuals of less than 1.0 mg/P. Air stripping of ammonia may, however, cause a local smell nuisance

if the ammonia concentrations are significantly high.

The water is then made usable by re-adjusting the pH to approximately 7. Any residual ammonia is likely to exist as the non-toxic ammonium ion. If total removal of ammonia is required the water can be passed through commercially available ion exchange resins which have an affinity for ammonia.

Ammonia removal systems are not suited to treating domestic water on a household scale.

The Effects of Ammonia

Norms

The norms used in the guideline for ammonia are primarily based on aesthetic effects, although indirect health effects associated with the possible formation of nitrite in distribution systems have been taken into account.

Effects

The chemistry of ammonia is very complex, especially where transition metals are present in water, and while ammonia itself is of relatively low toxicity, this is not necessarily the case for some of its organometallic complexes.

Taste and odour complaints are likely to occur if the ammonia concentration exceeds 1.5 mg/P. High concentrations of ammonia can also give rise to nitrite, which is potentially toxic, especially to infants.

Mitigation

Investigate the causes of associated taste and odour problems and implement measures designed to control eutrophication.

Criteria

Effects of Ammonia on Aesthetics and Human Health

Ammonia Range (mg/PN)	Effects
Target Water Quality Range 0 - 1.0	***No health or aesthetic effects***
1.0 - 2.0	Possibility of taste and odour complaints from consumers
2.0 - 10.0	Consumer complaints of objectionable taste and odours likely. Disinfection by chlorine can be compromised
> 10.0	Unacceptable in domestic water. Danger of formation of nitrite. Likelihood of fish deaths in aquaria. Chlorination is severely compromised

Sources of Information

APHA 1989. *Standard Methods for the Examination of Water and Waste Water*, 17th Edition. American Public Health Association, American Water Works Association, Water Pollution Control Federation. Published by the American Public Health Association,

Indicator Organisms
Faecal Coliforms

Background Information

Introduction

Faecal coliforms, and more specifically *Escherichia coli (E.coli)*, are the most commonly used bacterial indicators of faecal pollution. This indicator group is used to evaluate the quality of wastewater effluents, river water, sea water at bathing beaches, raw water for drinking water supply, treated drinking water, water used for irrigation and aquaculture and recreational waters. The presence of *Escherichia coli* is used to confirm the presence of faecal pollution by warm-blooded animals (often interpreted as human faecal pollution). Some organisms detected as faecal coliforms may not be of human faecal origin but are almost definitely from warm-blooded animals.

Occurrence

Faecal coliforms have been shown to represent 93 % - 99 % of coliform bacteria in faeces from humans, poultry, cats, dogs and rodents. Some faecal coliform tests also enumerate *Klebsiella spp*., which can originate from non-faecal sources and a few other bacterial strains also of non-faecal origin. *Escherichia coli* usually comprises approximately 97 % of coliform bacteria in human faeces. The remainder include *Klebsiella spp*., *Enterobacter spp.* and *Citrobacter spp.*

Interactions

The activities of micro-organisms are dependent on all physical, chemical, and biological interactions of the aquatic environment, which determine their growth rate and survival. See **indicator organisms**.

Measurement

Faecal coliforms are usually enumerated as counts (number of colonies)/100 mPof water. Water samples must be refrigerated immediately after collection and should be analysed within six hours. Prior to analysis, domestic water samples containing residual chlorine must be dechlorinated, usually with sodium thiosulphate. Analysis may be by membrane filtration (0.45 Φm diameter pore size), pour plates or by multiple tube fermentation techniques. Faecal coliform bacteria are all bacteria which produce typical blue colonies on m-FC agar within 20 - 24 hours of incubation at 44.5 EC. *Escherichia coli* are considered to be all the faecal coliforms which test indole-positive at 44.5 EC.

Data Interpretation

Mean values should be used to compare with the criteria given and should be interpreted as maximal values, not to be exceeded. See **indicator organisms.**

Treatment Options

See **indicator organisms.**

The Effects of Faecal Coliforms

Norms

The norm used in the guideline for faecal coliforms is human health.

Effects

Faecal coliforms are primarily used to indicate the presence of bacterial pathogens such as *Salmonella spp*., *Shigella spp*. *Vibrio cholerae, Campylobacter jejuni, Campylobacter coli, Yersinia enterocolitica* and pathogenic *E. coli*. These organisms can be transmitted via the faecal/oral route by contaminated or poorly-treated drinking water and may cause

diseases such as gastroenteritis, salmonellosis, dysentery, cholera and typhoid fever.

The risk of being infected by microbial pathogens correlates with the level of contamination of the water and the amount of contaminated water consumed. Higher concentrations of faecal coliforms in water will indicate a higher risk of contracting waterborne disease, even if small amounts of water are consumed.

Mitigation

A person who is suspected of having contracted a water-related infectious disease should receive medical attention.

Criteria

Effects of Faecal Coliforms on Human Health

Faecal Coliform Range (counts/100 mℓ)	Effects
Target Water Quality Range 0	***Negligible risk of microbial infection***
0 - 10	Slight risk of microbial infection with continuous exposure; negligible effects with occasional or short-term exposure
10 - 20	Risk of infectious disease transmission with continuous exposure; slight risk with occasional exposure
> 20	Significant and increasing risk of infectious disease transmission. As faecal coliform levels increase, the amount of water ingested required to cause infection decreases

Sources of Information

See **indicator organisms.**

Nitrate

Background Information

Introduction

Nitrate is the end product of the oxidation of ammonia or nitrite. Nitrate (NO_3^-) and nitrite (NO_2^-) are the oxyanions of nitrogen in which nitrogen is found in the +V and +III oxidation states, respectively. Nitrates and nitrites occur together in the environment and interconversion readily occurs. Under oxidising conditions nitrite is converted to nitrate, which is the most stable *positive* oxidation state of nitrogen and far more common in the aquatic environment than nitrite.

Nitrate in drinking water is primarily a health concern in that it can be readily converted in the gastrointestinal tract to nitrite as a result of bacterial reduction.

Occurrence

Mineral deposits of nitrates are rare due to the high water solubility of nitrates, although large deposits of sodium nitrate (saltpetre) occur in the desert regions of Chile. Nitrates are ubiquitous in soils and in the aquatic environment, particularly in association with the breakdown of organic matter and eutrophic conditions.

Concentrations of nitrate in water are typically less than 5 mg/P of nitrate-nitrogen (or, alternatively, 22 mg/P nitrate). A significant source of nitrates in natural water results from the oxidation of vegetable and animal debris and of animal and human excrement. Treated sewage wastes also contain elevated concentrations of nitrate.

Nitrate tends to increase in shallow ground water sources in association with agricultural and urban runoff, especially in densely populated areas. Nitrate together with phosphates stimulate plant growth. In aquatic systems elevated concentrations generally give rise to the accelerated growth of algae and the occurrence of algal blooms. Algal blooms may subsequently cause problems associated with malodours and tastes in water and the possible occurrence of toxicity.

Interactions

Interactions with nitrate are present with all conditions associated with the presence or breakdown of organic matter. For example, enrichment of waters with **dissolved organic carbon** can increase the rate of denitrification by providing an energy source for the denitrifying bacteria. The processes of nitrification, denitrification and the active uptake of nitrate by algae and higher plants are regulated by temperature and **pH**.

Measurement

The criteria for nitrate are given in terms of the concentration of nitrate plus nitrite nitrogen in units of mg/P. The reference method for the determination of the sum of the nitrate and nitrite concentration is by cadmium reduction followed by diazotisation. Nitrite alone can be determined by diazotisation without prior reduction of the nitrate. Where other methods are used, their characteristics relative to the reference method should be known.

Data Interpretation

Single-sample maximal values should be used to compare with the criteria given and should be interpreted as non-exceedance limits for children under two years of age and as mean values for older children and adults.

Transient elevations of nitrate and nitrite concentrations above non-exceedance limits are of less importance than continuous elevated concentrations.

Where water is well-oxygenated, it can be assumed that the nitrate plus nitrite nitrogen concentrations are largely due to the presence of nitrate. Nitrite concentrations only become significant in deoxygenated systems.

Treatment Options

Nitrate is not readily removed from domestic water supplies. Some reduction of nitrate may be achieved using slow sand filtration, but the method is not reliable. Biological reduction of nitrate to nitrogen gas (denitrification) is feasible in the presence of a suitable carbon source, but the increase in carbonaceous matter is not compatible with a high quality water supply. Non-specific methods of removing nitrate include:

- Passing the water stream through an **ion exchange** column with a selective affinity for nitrates. The method is expensive because other anions will be removed at the same time, depending on the nature of the resin used. However, it may be attractive on a household scale where only water used for drinking purposes is treated.

- **Reverse osmosis**, which will remove nitrate effectively from water, along with high percentages of virtually all other ions and many organic compounds. A low-pressure home unit will conveniently treat small quantities of drinking water satisfactorily. The module is replaced when it begins to block through fouling or scaling.

On a commercial scale the processes described require competent operation, control and maintenance.

The Effects of Nitrate and Nitrite

Norms

The norm used in the guideline for nitrate and nitrite is human health. There are no direct aesthetic impacts.

Effects

Upon absorption, nitrite combines with the oxygen-carrying red blood pigment, haemoglobin, to form methaemoglobin, which is incapable of carrying oxygen. This condition is termed *methaemoglobinaemia*. The reaction of nitrite with haemoglobin can be particularly hazardous in infants under three months of age and is compounded when the intake of Vitamin C is inadequate.

Metabolically, nitrates may react with secondary and tertiary amines and amides, commonly derived from food, to form nitrosamines which are known carcinogens.

Mitigation

A diet, adequate in Vitamin C, partially protects against the adverse effects of nitrate/nitrite. Methaemoglobinaemia in infants can only be mitigated by blood transfusion.

Criteria **Effects of Nitrate/Nitrite on Human Health**

Nitrate/nitrite Range (as mg/PN)	Effects
Target Water Quality Range 0 - 6	***No adverse health effects***
6 - 10	Rare instances of methaemoglobinaemia in infants; no effects in adults. Concentrations in this range generally well tolerated
10 - 20	Methaemoglobinaemia may occur in infants. No effects in adults
> 20	Methaemoglobinaemia occurs in infants. Occurrence of mucous membrane irritation in adults

Appendix 3c: South African Water Quality Guidelines for aquatic ecosystems

Ammonia

Background Information

Introduction

Un-ionized ammonia (NH_3) is a colourless, acrid-smelling gas at ambient temperature and pressure. It is produced naturally by the biological degradation of nitrogenous matter and provides an essential link in the nitrogen cycle.

Ammonia may be present in the free, un-ionized form (NH_3) or in the ionized form as the ammonium ion (NH_4^+). Both are reduced forms of inorganic nitrogen derived mostly from aerobic and anaerobic decomposition of organic material. They exist either as ions, or can be adsorbed onto suspended organic and inorganic material.

The toxicity of ammonia is directly related to the concentration of the un-ionized form (NH_3), the ammonium ion (NH_4^+) having little or no toxicity to aquatic biota. The ammonium ion does, however, contribute to eutrophication. Modifying factors may alter the acute toxicity by altering the concentration of un-ionized ammonia in the water through changes in the ammonia-ammonium ion equilibrium, or may increase the toxicity of the un-ionized ammonia to organisms.

Occurrence

Ammonia is present in small amounts in air, soil and water, and in large amounts in decomposing organic matter. Natural sources of ammonia include gas exchange with the atmosphere; the chemical and biochemical transformation of nitrogenous organic and inorganic matter in the soil and water; the excretion of ammonia by living organisms; the nitrogen fixation processes whereby dissolved nitrogen gas enters the water and ground water. Ammonia, associated with clay minerals enters the aquatic environment through soil erosion. Bacteria in root nodules of legumes fix large amounts of nitrogen in the soil and this may be leached into surrounding waters.

Ammonia is a common pollutant and is one of the nutrients contributing to eutrophication. Commercial fertilizers contain highly soluble ammonia and ammonium salts. Following application of fertilizer, if the concentration of such compounds exceeds the immediate requirements of the plant, transport *via* the atmosphere or irrigation waters can carry these nitrogen compounds into aquatic systems. Other sources of ammonia include:

- fish-farm effluent (un-ionized ammonia);
- sewage discharge;
- discharge from industries that use ammonia or ammonium salts in their cleaning operations;
- manufacture of explosives and use of explosives in mining and construction; and
- atmospheric deposition of ammonia from distillation and combustion of coal, and the biological degradation of manure.

Interactions

The most significant factors that affect the proportion and toxicity of un-ionized ammonia in aquatic ecosystems are water temperature and pH. An increase in either results in an increase in the relative proportion of un-ionized ammonia in solution, and hence an increase in toxicity to aquatic organisms, as given in Table 1.

Table 1: Contribution of un-ionised NH_3 to Total Ammonia (expressed as a percentage), as a Function of pH Value and Water Temperature

pH	Water Temperature (°C)							
	0	5	10	15	20	25	30	35
6.0	0.0083	0.012	0.019	0.027	0.039	0.056	0.079	0.11
6.5	0.026	0.039	0.059	0.086	0.12	0.18	0.25	0.35
7.0	0.083	0.12	0.18	0.27	0.39	0.56	0.79	1.1
7.5	0.26	0.39	0.58	0.85	1.2	1.7	2.4	3.4
8.0	0.82	1.2	1.8	2.6	3.8	5.3	7.3	9.9
8.5	2.6	3.8	5.5	7.9	11	15	20	26
9.0	7.6	11	16	21	28	36	44	52
9.5	21	28	37	46	55	64	71	78

Ammonia toxicity is also affected by the concentrations of dissolved oxygen, carbon dioxide and total dissolved solids, and the presence of other toxicants, such as metal ions. The acute toxicity of ammonia to fish increases as dissolved oxygen decreases. Ammonia is oxidized to nitrate in well oxygenated waters. Ammonia may also be adsorbed onto suspended and bed sediments and to colloidal particles.

Measurement

Ammonia criteria for aquatic ecosystems are calculated from the total ammonia concentration, that is, the sum of the NH_3 and NH_4^+ concentrations. The reference method for the determination of total ammonia is the phenate hypochlorite method, followed by spectrophotometry or colorimetry. The concentration of free ammonia is estimated from Table 1. The most reliable results are obtained on fresh samples. However, if prompt analysis is not possible, samples should be preserved with H_2SO_4, stored at 4 EC, and neutralised with NaOH or KOH prior to analysis.

As with all determinations of nutrients, care must be taken to prevent contamination of water samples. Glass bottles/vials, suitably pre-cleaned to remove nitrogenous contaminants, are required. Samples should not be preserved with nitric acid and no head space in the bottle should be allowed.

Data Interpretation

Single measurements of ammonia are of limited use. Preferably, weekly ammonia concentrations, averaged over a period of at least 4 weeks, with the minimum and maximum values reported, should be compared with the Target Water Quality Range (TWQR).

Interpretation of the ammonia criteria is based on the free ammonia concentrations. The potential effect of ammonia on the aquatic environment is modified by the chemical species present, the relative proportions of each, and other factors such as pH, temperature and dissolved oxygen concentration.

Ninety percent (90 %) of all free ammonia estimates should be within the TWQR. All free ammonia estimates should be below the chronic effect value (CEV). In the case of accidental spills, chronic and acute toxicity effects will occur if ammonia estimates exceed the Acute Effect Value (AEV).

Effects and Criteria

Norms

The norms for assessing the effects of free ammonia on aquatic ecosystems are chronic and acute toxic effects of ammonia on aquatic organisms.

Effects

The toxicity of ammonia and ammonium salts to aquatic organisms is directly related to the amount of free ammonia in solution. At low to medium pH values, the ammonium ion dominates, but as pH increases ammonia is formed, the latter being considerably more toxic to aquatic organisms. Prior exposure or acclimation to ammonia increases the tolerance of fish to ammonia and enables them to withstand concentrations that would otherwise be acutely lethal.

Un-ionized ammonia affects the respiratory systems of many animals, either by inhibiting cellular metabolism or by decreasing oxygen permeability of cell membranes. Acute toxicity to fish may cause a loss of equilibrium, hyper-excitability, an increased breathing rate, an increased cardiac output and oxygen intake, and in extreme cases convulsions, coma and death.

Chronic effects include a reduction in hatching success, reduction in growth rate and morphological development, and pathological changes in tissue of gills, liver and kidneys. An increased ventilation of the gills following exposure to ammonia indicating a respiratory effect has been observed in mayfly larvae *Ecdyonurus dispar*.

Criteria

The TWQR and criteria for un-ionised ammonia in aquatic ecosystems are :

TWQR and Criteria	Un-ionised Ammonia Concentration (µg N/l)
Target Water Quality Range (TWQR)	***# 7***
Chronic Effect Value (CEV)	15
Acute Effect Value (AEV)	100

Note:

X The data available satisfied the minimum database requirements, therefore no safety factors were applied.

Modifications

In certain areas, or at certain sites, it may be necessary to modify the ammonia criteria provided in this guideline. Where any modification is anticipated, *the user of the guidelines must obtain expert advice*. All modifications must afford the same level of protection to aquatic ecosystems as stipulated by the criteria given in this guideline.

Nitrogen (Inorganic)

Background Information

Introduction

The term inorganic nitrogen includes all the major inorganic nitrogen components (NH_3 + NH_4^+ + NO_2^- + NO_3^-) present in water. Both the dissolved forms of inorganic nitrogen and those adsorbed onto suspended inorganic and organic material are included, since they are all available for uptake by algae and higher plants.

Ammonia (NH_3) and ammonium (NH_4^+) are reduced forms of inorganic nitrogen and their relative proportions are controlled by water temperature and pH. Both forms can exist as dissolved ions, or can be adsorbed onto suspended material.

Nitrite (NO_2^-) is the inorganic intermediate, and nitrate (NO_3^-) the end product, of the oxidation of organic nitrogen and ammonia. Nitrate is the more stable of the two forms and is usually far more abundant in the aquatic environment. In view of their co-occurrence and rapid inter-conversion, nitrite and nitrate are usually measured and considered together. Inter-conversions between the different forms of inorganic nitrogen are part of the nitrogen cycle in aquatic ecosystems.

Inorganic nitrogen is primarily of concern due to its stimulatory effect on aquatic plant growth and algae. Most aquatic organisms are sensitive to the toxic effects of ammonia. See guideline for ammonia.

Occurrence

Surface runoff from the surrounding catchment area, the discharge of effluent streams containing human and animal excrement, agricultural fertilizers and organic industrial wastes are the major sources of inorganic nitrogen which enters aquatic systems. In highly impacted catchments, the inorganic nitrogen arising from human activities can greatly exceed "natural" sources. In addition, many groups of bacteria are able to transform organic nitrogen to inorganic nitrogen during the decomposition of organic material.

Inorganic nitrogen is seldom present in high concentrations in unimpacted surface waters. This is because inorganic nitrogen is rapidly taken up by aquatic plants and converted into proteins and other organic forms of nitrogen in plant cells. In South Africa, inorganic nitrogen concentrations in unimpacted, aerobic surface waters are usually below 0.5 mg N/P but may increase to above 5 - 10 mg N/Pin highly enriched waters.

Oxidized forms of inorganic nitrogen (usually nitrate) can sometimes be present in very high concentrations (> 150 mg NO_3-N/P) in ground water. Such high concentrations can occur under natural conditions (e.g., mineral salts derived from rocks and soil, not due to man's activity), or due to seepage from sewage systems and leaching of organic and inorganic fertilizers from soil.

Interactions

The processes of ammonification, nitrification, denitrification, and the active uptake of nitrate by algae and higher plants, are regulated by water temperature, oxygen availability and pH. Changes to water temperature and pH affect the rates at which these processes occur and the concentration of inorganic nitrogen present in water.

Nitrite can be transformed rapidly to nitrate, and vice versa, by bacterial processes. Under aerobic conditions, nitrite is oxidized to nitrate by nitrifying bacteria. Conversely, under

anaerobic conditions, nitrate is reduced to nitrite (and then to molecular nitrogen) by denitrifying bacteria. Denitrification is the most important process whereby nitrate is lost from aquatic systems.

Enrichment of waters with dissolved organic carbon (e.g., through the discharge of treated sewage effluent) can increase rates of nitrogen loss via denitrification by providing an energy source for denitrifying bacteria.

Several chemical bonding processes, as well as the forces which control the charge on the surface of sediment particles, regulate the amount of inorganic nitrogen which may be adsorbed to, or desorbed from, suspended particulate material. Typically, these reactions are strongly influenced by water temperature and pH, as well as by bacterial activity.

When particulate material settles out of the water onto the sediments, bound inorganic nitrogen becomes trapped in the sediments. Some of the bound inorganic nitrogen can be released by diffusion into the overlying water; nitrate present in anaerobic sediments can be lost from the system via denitrification.

Measurement

The concentration of inorganic nitrogen species in water is obtained by adding together the individual concentrations of ammonia ($NH_3 + NH_4^+$), plus nitrite (NO_2^-) and nitrate (NO_3^-). No single analytical technique will provide a measure of inorganic nitrogen.

Nitrite plus nitrate is determined by the cadmium reduction method followed by diazotisation and spectrophotometry. Nitrite alone is determined by diazotisation without prior reduction of the nitrate present to nitrite. Total ammonia, the sum of the NH_3 and NH_4^+ concentrations, is determined by the phenate hypochlorite method, followed by spectrophotometry or colorimetry.

Where other analytical methods are used, their characteristics relative to the reference type methods should be known. Concentrations are usually expressed as milligrams of inorganic nitrogen per litre of water sample (mg N/P).

Prior filtration may be required where water samples are turbid. Considerable difficulty is experienced in the analysis of any inorganic nitrogen that is adsorbed onto the surface of suspended material or associated with bottom sediments.

Analysis of inorganic nitrogen in the laboratory should be started as soon as possible after sample collection to minimize the effects of bacterial transformation and pH changes. Water samples should preferably not be preserved with acid before analysis; rather, the samples should be kept at low temperature (< 4 EC).

Data Interpretation

Occasional increases in the inorganic nitrogen concentration above the Target Water Quality Range (TWQR) are less important than continuously high concentrations. Single measurements of inorganic nitrogen are a poor basis for assessment. Average summer inorganic nitrogen concentrations provide the best basis from which to estimate the likely biological consequences of inorganic nitrogen.

Weekly inorganic nitrogen concentrations, averaged over a period of at least 4 weeks, should be compared with the TWQR.

Any assessment of the influence of inorganic nitrogen concentrations should be coupled to an evaluation of the inorganic nitrogen to inorganic phosphorus ratio. Unimpacted systems

typically have an N:P ratio greater than 25-40 : 1, whilst most impacted (i.e., eutrophic or hypertrophic) systems have an N:P ratio of less than 10:1. At such low N:P ratios, nitrogen fixation is likely to occur; this will provide additional inorganic nitrogen to the system.

In unimpacted, well-oxygenated (dissolved oxygen concentration 80-120 % saturation) waters, most (> 80 %) of the inorganic nitrogen should be present as nitrate; typically, ammonia concentrations will be below 0.1 mg N/P, or less than 20 % of the inorganic nitrogen present.

Where effluent discharges containing high ammonia or nitrate concentrations have impacted on aerobic waters, background inorganic nitrogen concentrations rise. This will usually be accompanied by a decrease in the dissolved oxygen concentration and an increase in the BOD, COD and pH.

Effects and Criteria

Norms

Changes in the trophic status accompanied by the growth of algae and other aquatic plants in rivers, lakes and reservoirs, is the norm used to assess the effects of inorganic nitrogen on aquatic ecosystems.

Effects

Site-specific conditions, especially the availability of phosphorus, are critically important in modifying the influence of inorganic nitrogen on eutrophication. Inorganic nitrogen toxicity is not considered to be important for setting inorganic nitrogen water quality guidelines for protection of aquatic ecosystems.

Inorganic nitrogen concentrations below 0.5 mg N/P are considered to be sufficiently low that they can limit eutrophication and reduce the likelihood of nuisance growths of blue-green algae and other plants. However, in the presence of sufficient available phosphorus, nitrogen-fixing organisms will be able to fix atmospheric nitrogen, thereby compensating for any deficit caused by low inorganic nitrogen concentrations.

The information given in the table below illustrates typical symptoms associated with selected ranges of inorganic nitrogen concentrations, if all other nutrients and environmental conditions are within favourable ranges for the organisms concerned.

Average Summer Inorganic Nitrogen Concentration (mg/P)	Effects
< 0.5	Oligotrophic conditions; usually moderate levels of species diversity; usually low productivity systems with rapid nutrient cycling; no nuisance growth of aquatic plants or the presence of blue-green algal blooms.
0.5 - 2.5	Mesotrophic conditions; usually high levels of species diversity; usually productive systems; nuisance growth of aquatic plants and blooms of blue-green algae; algal blooms seldom toxic.
2.5 - 10	Eutrophic conditions; usually low levels of species diversity; usually highly productive systems, nuisance growth of aquatic plants and blooms of blue-green algae; algal blooms may include species which are toxic to man, livestock and wildlife.
> 10	Hypertrophic conditions; usually very low levels of species diversity; usually very highly productive systems; nuisance growth of aquatic plants and blooms of blue-green algae, often including species which are toxic to man, livestock and wildlife.

Criteria

The inorganic nitrogen concentration for a specific system must be based on the existing trophic status of the system. It is undesirable to allow inorganic nitrogen concentrations to rise to a level which will change the trophic status of the system. A Target Water Quality Range should be derived only after case- and site-specific studies.

Water Resource	*Target Water Quality Range*
All surface waters	X ***Inorganic nitrogen concentrations should not be changed by more than 15 % from that of the water body under local unimpacted conditions at any time of the year; and*** X ***The trophic status of the water body should not increase above its present level, though a decrease in trophic status is permissible (see Effects); and*** X ***The amplitude and frequency of natural cycles in inorganic nitrogen concentrations should not be changed.***

Modifications

The inorganic nitrogen criteria given in the above table should only be modified in the case of turbid systems. Limited light penetration or increased turbulence will reduce the extent of nuisance algal growths at a given inorganic nitrogen concentration. Nuisance growths of free-floating aquatic macrophytes (e.g. Water Hyacinth) will not be reduced in such cases.

Phosphorus (Inorganic)

Background Information

Introduction

Phosphorus can occur in numerous organic and inorganic forms, and may be present in waters as dissolved and particulate species. Elemental phosphorus does not occur in the natural environment. Orthophosphates, polyphosphates, metaphosphates, pyrophosphates and organically bound phosphates are found in natural waters. Of these, orthophosphate species H_2PO_4 and HPQ^{-2} are the only forms of soluble inorganic phosphorus directly utilizable by aquatic biota. Soluble Reactive Phosphate (SRP), or orthophosphate, is that phosphorus which is immediately available to aquatic biota which can be transformed into an available form by naturally occurring processes.

The forms of phosphorus in water are continually changing because of processes of decomposition and synthesis between organically bound forms and oxidised inorganic forms. The phosphorus cycle is influenced by the exchange of phosphorus between sedimentary and aqueous compartments. In turn this is affected by various physical, chemical and biological modifying factors such as mineral-water equilibria, water pH values, sorption processes, oxygen-dependent redox interactions, and the activities of living organisms.

Phosphorus is an essential macronutrient, and is accumulated by a variety of living organisms. It has a major role in the building of nucleic acids and in the storage and use of energy in cells. In unimpacted waters it is readily utilized by plants and converted into cell structures by photosynthetic action. Phosphorus is considered to be the principle nutrient controlling the degree of eutrophication in aquatic ecosystems.

Occurrence

Natural sources of phosphorus include the weathering of rocks and the subsequent leaching of phosphate salts into surface waters, in addition to the decomposition of organic matter. Spatial variation is high and is related to the characteristics of regional geology. Phosphorus levels are generally lowest in mountainous regions of crystalline rocks and levels increase in lowland waters derived from sedimentary deposits.

In South Africa, phosphorus is seldom present in high concentrations in unimpacted surface waters because it is actively taken up by plants. Concentrations between 10 and 50 Φg/Pare commonly found, although concentrations as low as 1 Φg/Pof soluble inorganic phosphorus may be found in "pristine" waters and as high as 200 mg/Pof total phosphorus in some enclosed saline waters.

Elevated levels of phosphorus may result from point-source discharges such as domestic and industrial effluents, and from diffuse sources (non-point sources) in which the phosphorus load is generated by surface and subsurface drainage. Non-point sources include atmospheric precipitation, urban runoff, and drainage from agricultural land, in particular from land on which fertilizers have been applied.

Interactions

Phosphate is extremely reactive under oxidizing conditions, and interacts with many cations (e.g. Al, Fe, Ca) to form relatively insoluble compounds that precipitate out of water. Availability is also reduced by adsorption of phosphate to inorganic colloids, organic compounds such as humics and particulate material (e.g. clays, carbonates, hydroxides).

The flow regime is a major factor in the mobility, availability and spatial distribution of phosphorus within a river. Settlement of particulate matter and biotic uptake result in the removal of phosphorus from the water column to the sediments, and during periods of low discharge, stream bed sediments act as a sink for phosphorus. During rainfall events, phosphorus levels may be elevated by runoff from the land, and by re-suspension and flushing of deposited material from the river bed to the water column.

Several chemical bonding processes regulate the amount of inorganic phosphorus which is bonded to iron, aluminium, calcium or organic polyphenols and adsorbed onto suspended particulate material. Adsorbed phosphorus may be released from the sediments under conditions of high flow and under anoxic conditions from both sediments and water. The form of phosphorus in natural surface waters and the equilibrium of the different forms, is influenced by pH.

Measurement

Phosphorus concentrations are usually determined as orthophosphates, total inorganic phosphate or total dissolved phosphorus (which includes organically bound phosphorus and all phosphates). The dissolved forms are measured after filtering the sample through a pre-washed 0.45 Φm filter. Concentrations of particulate phosphorus can be calculated from the difference between the concentrations of the total and dissolved fractions.

Chemical analysis of phosphorus usually centres around the reactivity of phosphates with molybdate ions. During enzymatic and acidic hydrolysis complexes of phosphorus are converted to phosphate species, which are then measured colorimetrically. Four operational categories of phosphates result; these are soluble reactive P, soluble unreactive P, particulate reactive P, and particulate unreactive P. The most commonly measured is Soluble Reactive Phosphate (SRP).

Analysis of phosphorus in the laboratory should be started as soon as possible after sample collection to minimize the possible effects of bacterial transformation and pH changes. Water samples should preferably not be preserved with acid before analysis; rather, the samples should be kept at low temperature (< 4 EC).

Data Interpretation

Occasional increases in the inorganic phosphorus concentration above the Target Water Quality Range (TWQR) are less important than continuously high concentrations. Single measurements of phosphorus are a poor basis for assessment. Average summer inorganic phosphorus concentrations provide the best basis from which to estimate the likely biological consequences of phosphorus. Weekly inorganic phosphorus concentrations, averaged over a period of at least 4 weeks, should be compared with the TWQR.

Any assessment of the influence of the inorganic phosphorus concentrations should be coupled with an evaluation of the ratio of inorganic nitrogen to inorganic phosphorus. Unimpacted streams typically have an N:P ratio greater than 25 - 40:1, whilst most impacted (i.e., eutrophic or hypertrophic) systems have an N:P ratio of less than 10:1.

Effects and Criteria

Norms

Changes in trophic status accompanied by the growth of algae and other aquatic plants in rivers, lakes and reservoirs, are the norms used to assess the effects of inorganic phosphorus on aquatic ecosystems.

Effects

The most significant effect of elevated phosphorus concentrations is its stimulation of the growth of aquatic plants. Both phosphorus and nitrogen limit plant growth, and of these, phosphorus is likely to be more limiting in fresh water. The effect is dependent on the form of phosphorus present in the water, since not all forms are available for uptake by plants. Other factors, such as water temperature, light and the availability of other nutrients, also play an important role in limiting plant growth.

Inorganic phosphorus concentrations of less than 5 Φg P/Pare considered to be sufficiently low to reduce the likelihood of algal and other plant growth.

The information given in the table below illustrates typical symptoms associated with selected ranges of inorganic phosphorus concentrations, if all other nutrients and environmental conditions are within favourable ranges for the organisms concerned.

Average Summer Inorganic Phosphorus Concentration (Φg/P)	Effects
< 5	Oligotrophic conditions; usually moderate levels of species diversity; usually low productivity systems with rapid nutrient cycling; no nuisance growth of aquatic plants or blue-green algae.
5 - 25	Mesotrophic conditions; usually high levels of species diversity; usually productive systems; nuisance growth of aquatic plants and blooms of blue-green algae; algal blooms seldom toxic.
25 - 250	Eutrophic conditions; usually low levels of species diversity; usually highly productive systems, with nuisance growth of aquatic plants and blooms of blue-green algae; algal blooms may include species which are toxic to man, livestock and wildlife.
> 250	Hypertrophic conditions; usually very low levels of species diversity; usually very highly productive systems; nuisance growth of aquatic plants and blooms of blue-green algae, often including species which are toxic to man, livestock and wildlife.

Criteria

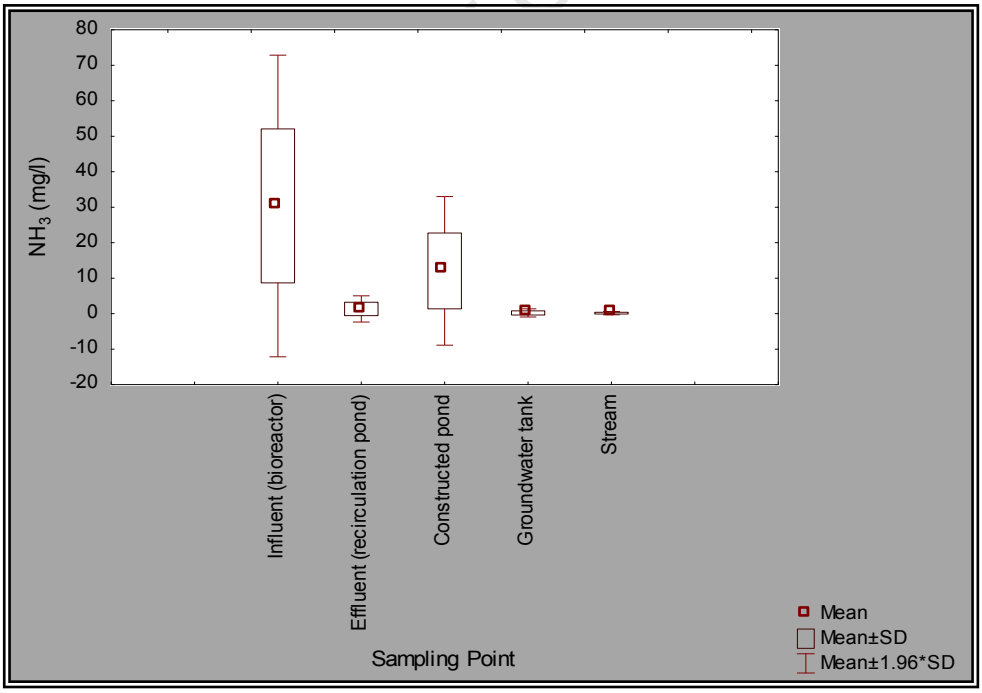
The inorganic phosphorus concentration for a specific system must be based on the existing trophic status of the system. It is undesirable to allow inorganic phosphorus concentrations to rise to a level which will change the trophic status of the system. A Target Water Quality Range should be derived only after case- or site-specific studies.

APPENDIX 4: DESCRIPTIVE STATISTICS

Appendix 4a: Descriptive statistics for NH_3 at De Goede Hoop

Location	Valid N	Mean	Median	Min	Max	25th Percentile	75th Percentile	Range	Std. dev.	CV (%)
Influent (bioreactor)	7	30.37	23.20	6.73	70.00	13.07	44.80	63.27	21.69	71.40
Effluent (recirculation pond)	7	1.34	0.80	0.00	5.40	0.23	1.70	5.40	1.88	140.17
Constructed pond	7	12.06	10.07	1.40	35.00	6.30	11.50	33.60	10.69	88.62
Groundwater tank	7	0.23	0.02	0.00	1.56	0.01	0.02	1.56	0.58	250.43
Stream	7	0.16	0.05	0.00	0.67	0.02	0.20	0.67	0.24	152.73

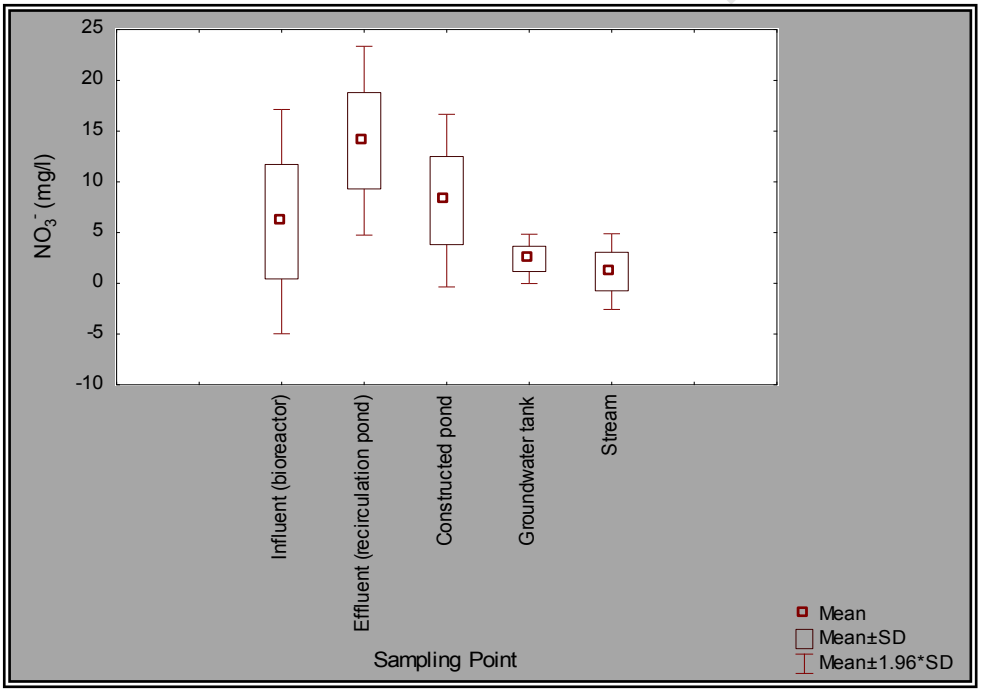
Appendix 4b: Box and whisker plot for NH_3 at De Goede Hoop



Appendix 4c: Descriptive statistics for NO_3^- at De Goede Hoop

Location	Valid N	Mean	Median	Min	Max	25th Percentile	75th Percentile	Range	Std. dev.	CV (%)
Influent (bioreactor)	7	6.08	4.33	0.33	17.07	1.87	8.67	16.74	5.64	92.70
Effluent (recirculation pond)	7	14.05	12.06	8.13	21.67	11.67	19.33	13.54	4.75	33.77
Constructed pond	7	8.15	8.83	1.87	14.67	3.33	10.70	12.80	4.34	53.26
Groundwater tank	7	2.41	2.47	0.53	4.00	1.20	3.73	3.47	1.24	51.64
Stream	7	1.16	0.80	0.00	5.33	0.00	1.07	5.33	1.90	164.09

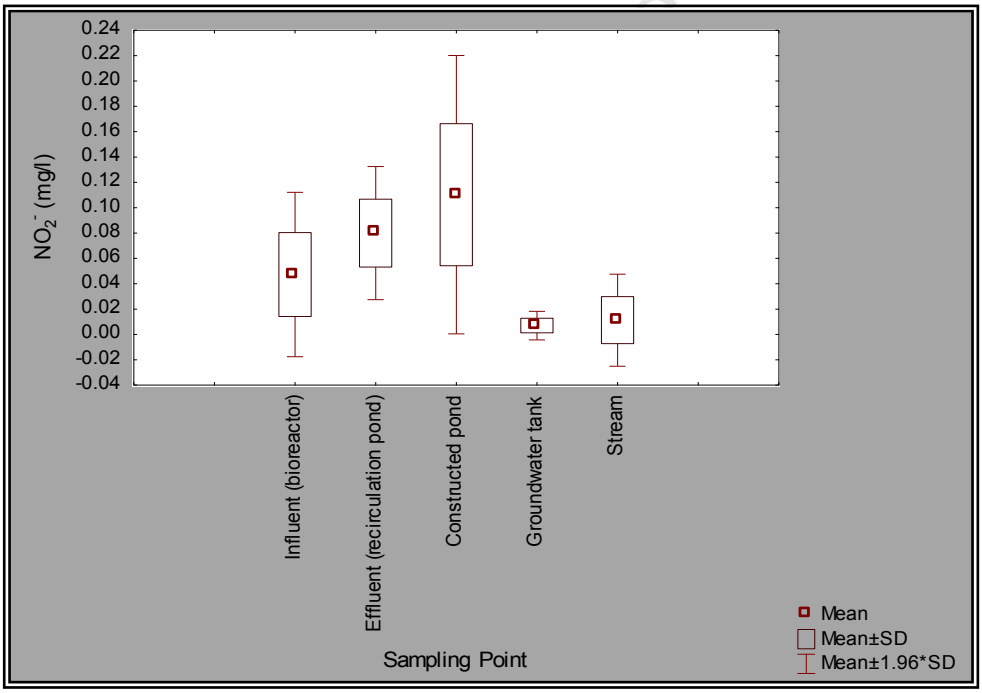
Appendix 4d: Box and whisker plot for NO_3^- at De Goede Hoop



Appendix 4e: Descriptive statistics for NO_2^- at De Goede Hoop

Location	Valid N	Mean	Median	Min	Max	25th Percentile	75th Percentile	Range	Std. dev.	CV (%)
Influent (bioreactor)	7	0.05	0.06	0.01	0.09	0.01	0.08	0.08	0.03	70
Effluent (recirculation pond)	7	0.08	0.08	0.04	0.13	0.05	0.09	0.08	0.03	33.49
Constructed pond	7	0.11	0.13	0	0.16	0.07	0.14	0.16	0.06	50.79
Groundwater tank	7	0.01	0.01	0	0.02	0.01	0.01	0.02	0.01	82.07
Stream	7	0.01	0	0	0.05	0	0.02	0.05	0.02	164.24

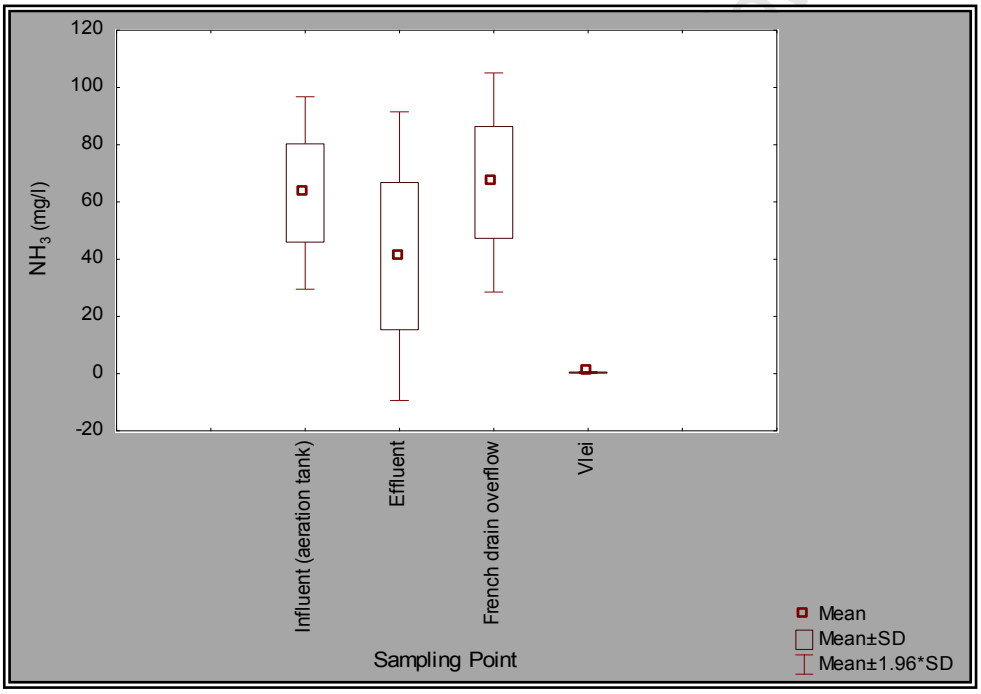
Appendix 4f: Box and whisker plot for NO_2^- at De Goede Hoop



Appendix 4g: Descriptive statistics for NH_3 at Wolwedans

Location	Valid N	Mean	Median	Min	Max	25th Percentile	75th Percentile	Range	Std. dev.	CV (%)
Influent (aeration tank)	6	63.14	66.27	42.93	87.20	44.47	71.73	44.3	17.16	27.18
Effluent	6	41.07	30.40	17.33	88.27	28.00	52.00	70.9	25.74	62.67
French drain overflow	6	66.82	71.57	28.56	84.80	69.60	74.80	56.2	19.53	29.22
Vlei	6	0.37	0.38	0.10	0.61	0.30	0.47	0.5	0.17	46.48

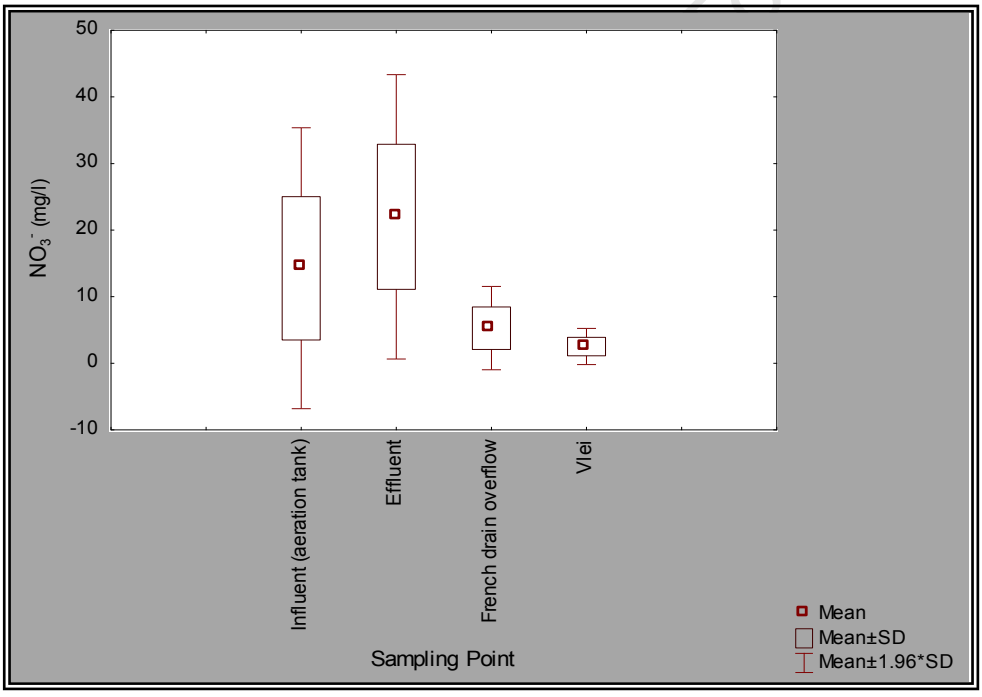
Appendix 4h: Box and whisker plot for NH_3 at Wolwedans



Appendix 4i: Descriptive statistics for NO_3^- at Wolwedans

Location	Valid N	Mean	Median	Min	Max	25th Percentile	75th Percentile	Range	Std. dev.	CV (%)
Influent (aeration tank)	6	14.28	12.00	3.33	30.00	4.33	24.00	26.7	10.76	75.37
Effluent	6	22.01	21.50	10.33	35.00	12.00	31.70	24.7	10.89	49.49
French drain overflow	6	5.29	5.33	0.00	9.67	4.40	7.00	9.7	3.19	60.33
Vlei	6	2.53	2.20	1.03	4.93	1.60	3.20	3.9	1.39	54.90

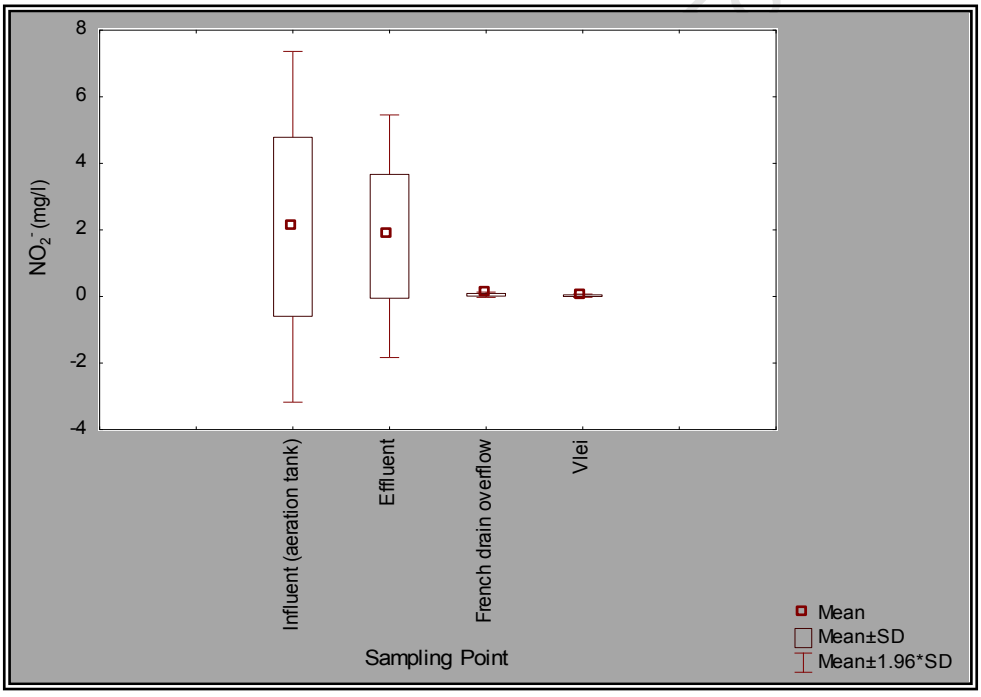
Appendix 4j: Box and whisker plot for NO_3^- at Wolwedans



Appendix 4k: Descriptive statistics for NO_2^- at Wolwedans

Location	Valid N	Mean	Median	Min	Max	25th Percentile	75th Percentile	Range	Std. dev.	CV (%)
Influent (aeration tank)	6	2.094	0.744	0.012	5.530	0.012	5.520	5.518	2.688	128.40
Effluent	6	1.809	1.339	0.129	5.057	0.269	2.720	4.928	1.860	102.81
French drain overflow	6	0.053	0.051	0.008	0.101	0.012	0.093	0.093	0.040	76.39
Vlei	6	0.025	0.012	0.007	0.069	0.008	0.041	0.062	0.025	101.25

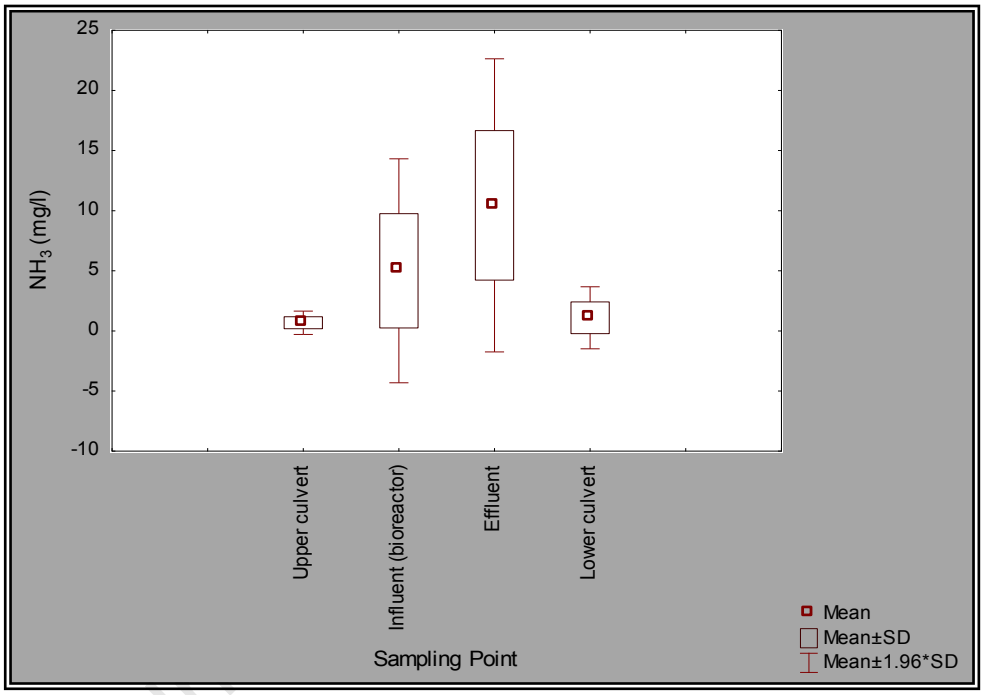
Appendix 4l: Box and whisker plot for NO_2^- at Wolwedans



Appendix 4m: Descriptive statistics for NH_3 at Babylonstoren

Location	Valid N	Mean	Median	Min	Max	25th Percentile	75th Percentile	Range	Std. dev.	CV (%)
Upper culvert	6	0.68	0.62	0.07	1.33	0.35	1.10	1.26	0.49	72.53
Influent (bioreactor)	6	5.00	3.60	0.97	13.20	1.20	7.44	12.23	4.75	95.01
Effluent	6	10.45	9.94	4.00	21.33	5.07	12.40	17.33	6.22	59.56
Lower culvert	6	1.09	0.52	0.20	3.53	0.20	1.60	3.33	1.32	120.35

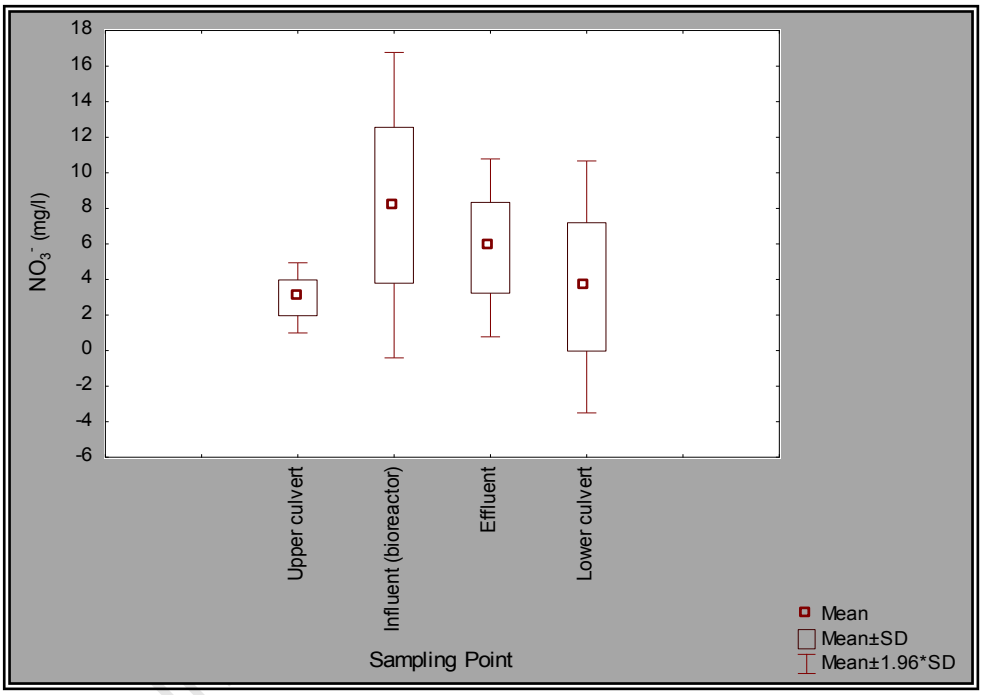
Appendix 4n: Box and whisker plot for NH_3 at Babylonstoren



Appendix 4o: Descriptive statistics for NO_3^- at Babylonstoren

Location	Valid N	Mean	Median	Min	Max	25th Percentile	75th Percentile	Range	Std. dev.	CV (%)
Upper culvert	6	2.97	2.53	2.00	4.53	2.30	3.90	2.53	1.01	33.98
Influent (bioreactor)	6	8.18	8.00	1.47	14.00	6.27	11.33	12.53	4.38	53.60
Effluent	6	5.78	5.20	2.67	10.27	4.67	6.67	7.60	2.55	44.15
Lower culvert	6	3.58	2.34	0.83	10.67	1.60	3.73	9.84	3.61	100.86

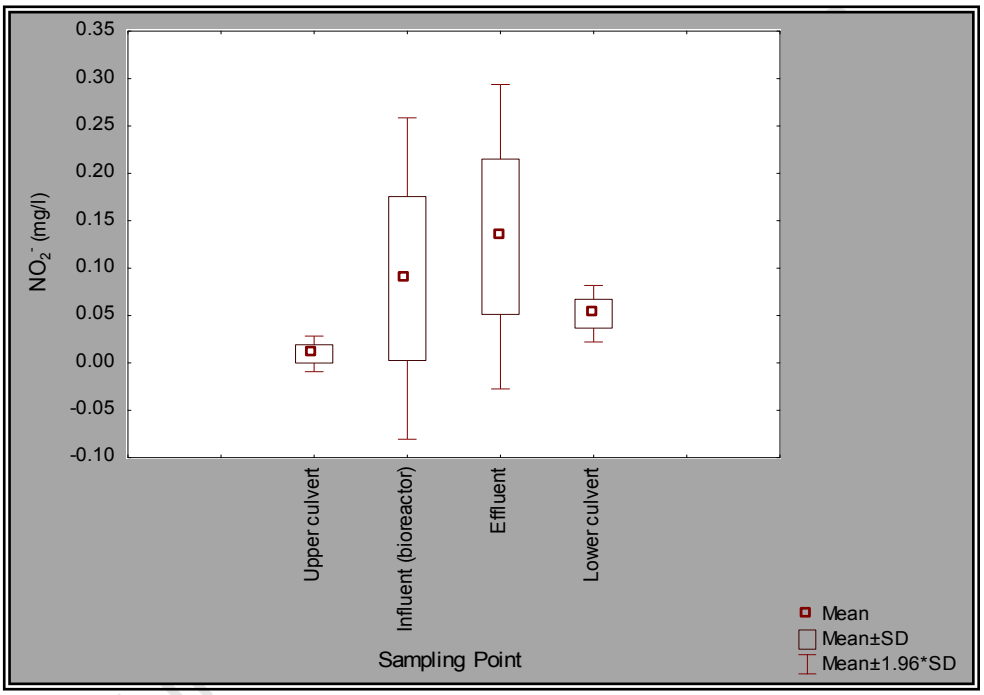
Appendix 4p: Box and whisker plot for NO_3^- at Babylonstoren



Appendix 4q: Descriptive statistics for NO_2^- at Babylonstoren

Location	Valid N	Mean	Median	Min	Max	25th Percentile	75th Percentile	Range	Std. dev.	CV (%)
Upper culvert	6	0.01	0.01	0.00	0.03	0.00	0.01	0.03	0.01	100.91
Influent (bioreactor)	6	0.09	0.05	0.01	0.23	0.03	0.16	0.22	0.09	97.18
Effluent	6	0.13	0.15	0.02	0.22	0.06	0.21	0.20	0.08	61.52
Lower culvert	6	0.05	0.05	0.04	0.08	0.04	0.05	0.04	0.02	29.27

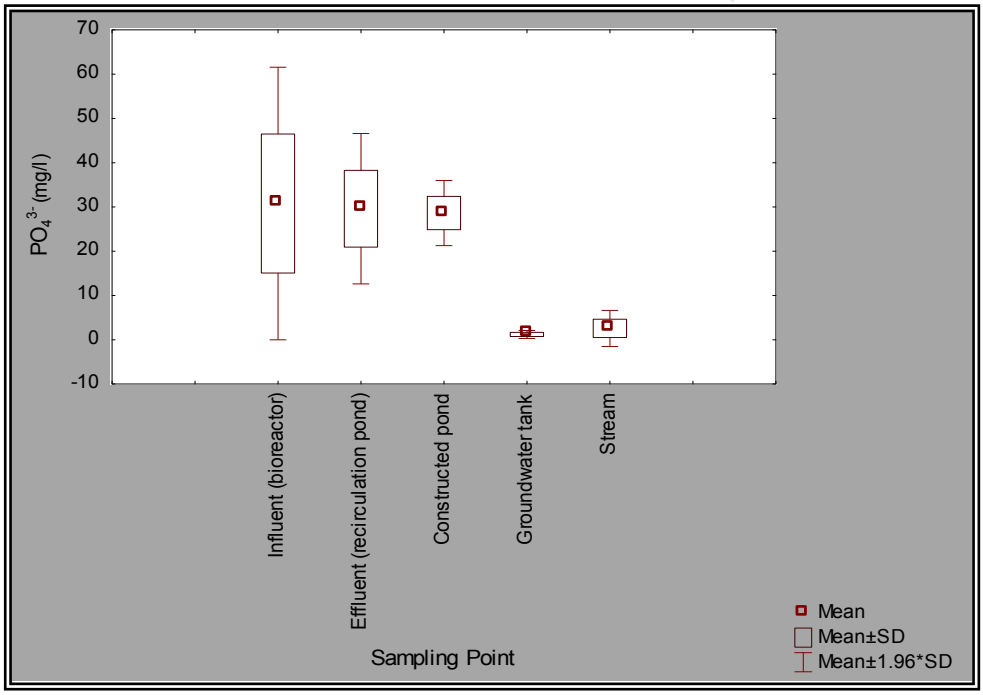
Appendix 4r: Box and whisker plot for NO_2^- at Babylonstoren



Appendix 4s: Descriptive statistics for PO_4^{3-} at De Goede Hoop

Location	Valid N	Mean	Median	Min	Max	25th Percentile	75th Percentile	Range	Std. dev.	CV (%)
Influent (bioreactor)	7	30.78	27.06	18.25	64.67	20.47	31.20	46.42	15.71	51.05
Effluent (recirculation pond)	7	29.61	30.07	14.05	40.87	23.73	35.53	26.82	8.67	29.29
Constructed pond	7	28.62	28.13	23.55	35.80	26.53	30.10	12.25	3.75	13.11
Groundwater tank	7	1.21	1.16	0.58	2.07	0.91	1.41	1.49	0.46	38.17
Stream	7	2.57	2.53	0.08	5.97	0.28	4.17	5.89	2.08	80.95

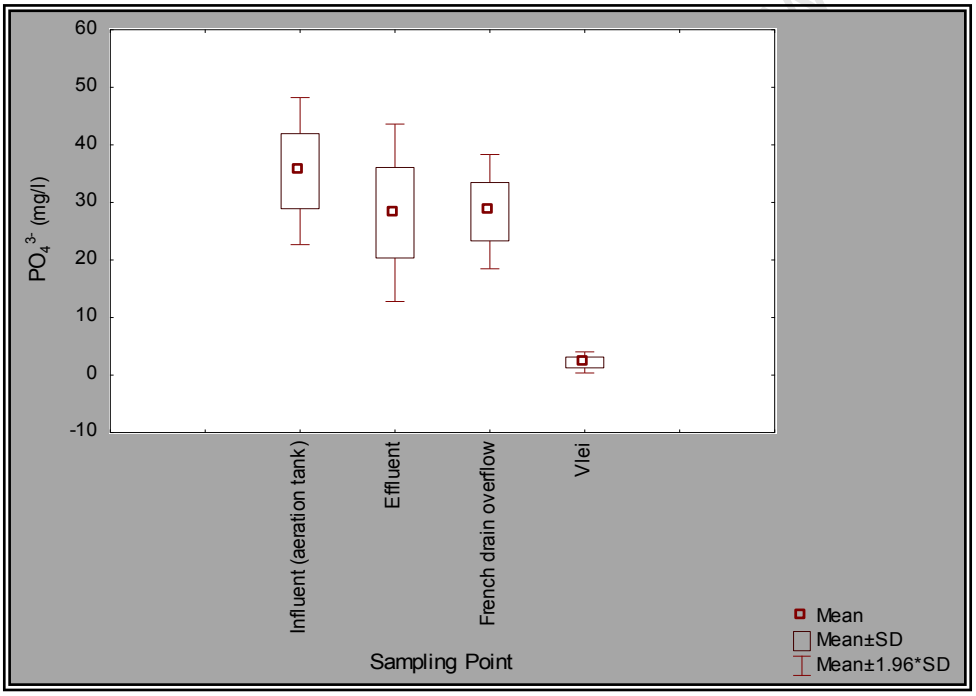
Appendix 4t: Box and whisker plot for PO_4^{3-} at De Goede Hoop



Appendix 4u: Descriptive statistics for PO_4^{3-} at Wolwedans

Location	Valid N	Mean	Median	Min	Max	25th Percentile	75th Percentile	Range	Std. dev.	CV (%)
Influent (aeration tank)	6	35.44	34.85	26.96	43.53	31.06	41.40	16.6	6.52	18.40
Effluent	6	28.21	25.30	20.73	42.53	23.87	31.53	21.8	7.87	27.90
French drain overflow	6	28.40	28.50	22.40	36.60	23.90	30.47	14.2	5.07	17.85
Vlei	6	2.19	2.32	0.51	3.41	2.17	2.43	2.9	0.94	42.80

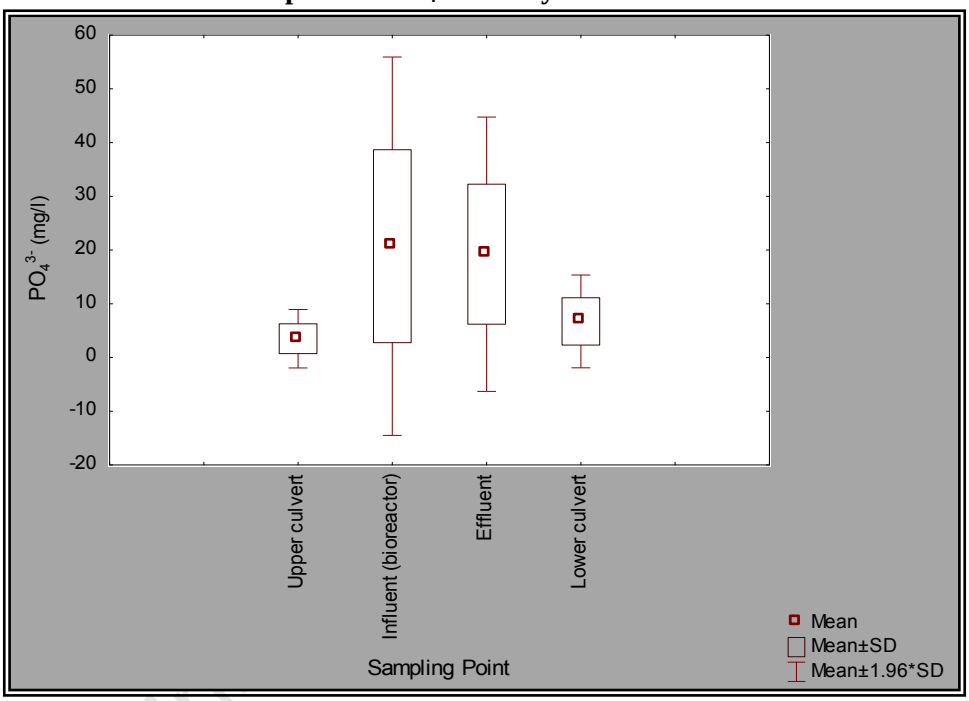
Appendix 4v: Box and whisker plot for PO_4^{3-} at Wolwedans



Appendix 4w: Descriptive statistics for PO_4^{3-} at Babylonstoren

Location	Valid N	Mean	Median	Min	Max	25th Percentile	75th Percentile	Range	Std. dev.	CV (%)
Upper culvert	6	3.50	2.88	0.95	7.32	1.09	5.90	6.37	2.77	79.21
Influent (bioreactor)	6	20.72	18.05	2.30	50.40	7.73	27.80	48.10	17.96	86.69
Effluent	6	19.23	18.04	4.46	43.20	12.07	19.60	38.74	13.03	67.73
Lower culvert	6	6.73	5.58	1.60	12.60	3.70	11.30	11.00	4.40	65.47

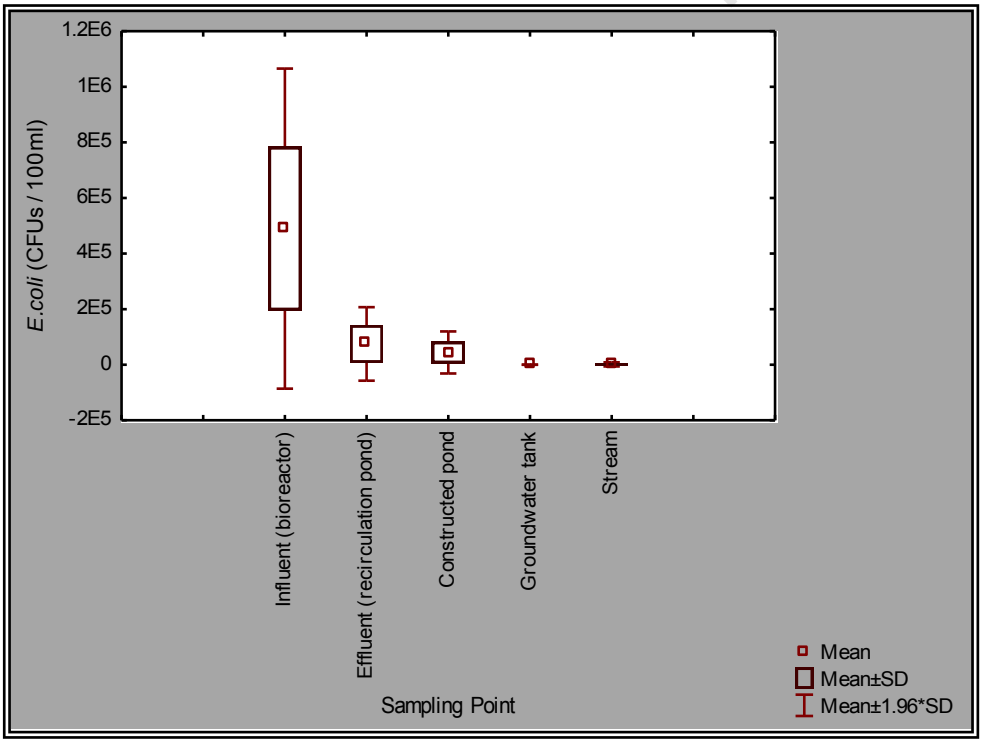
Appendix 4x: Box and whisker plot for PO_4^{3-} at Babylonstoren



Appendix 4y: Descriptive statistics for *E. coli* at De Goede Hoop

Location	Valid N	Mean	Median	Min	Max	25th Percentile	75th Percentile	Range	Std. dev.	CV (%)
Influent (bioreactor)	7	489714	625000	23000	740000	110000	680000	717000	293886	60.01
Effluent (recirculation pond)	7	74786	72000	3000	200000	4500	100000	197000	67576	90.36
Constructed pond	7	44000	33000	14000	130000	30000	35000	116000	38592	87.71
Groundwater tank	7	0	0	0	0	0	0	0	0	0
Stream	7	1429	0	0	10000	0	0	10000	3780	264.58

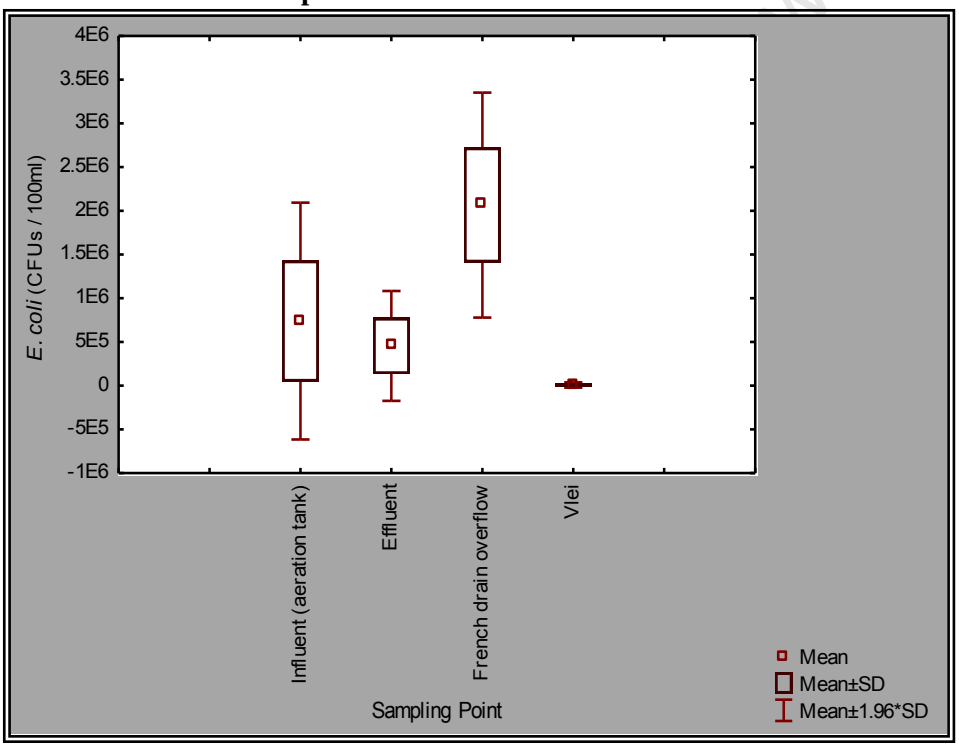
Appendix 4z: Box and whisker plot for *E. coli* at De Goede Hoop



Appendix 4aa: Descriptive statistics for *E. coli* at Wolwedans

Location	Valid N	Mean	Median	Min	Max	25th Percentile	75th Percentile	Range	Std. dev.	CV (%)
Influent (aeration tank)	6	739833	515000	79000	2000000	330000	1000000	1921000	691075	93.41
Effluent	6	455000	435000	50000	1000000	280000	530000	950000	320172	70.37
French drain overflow	6	2066667	2100000	1300000	2800000	1400000	2700000	1500000	656252	31.75
Vlei	6	9000	3000	0	40000	0	8000	40000	15582	173.13

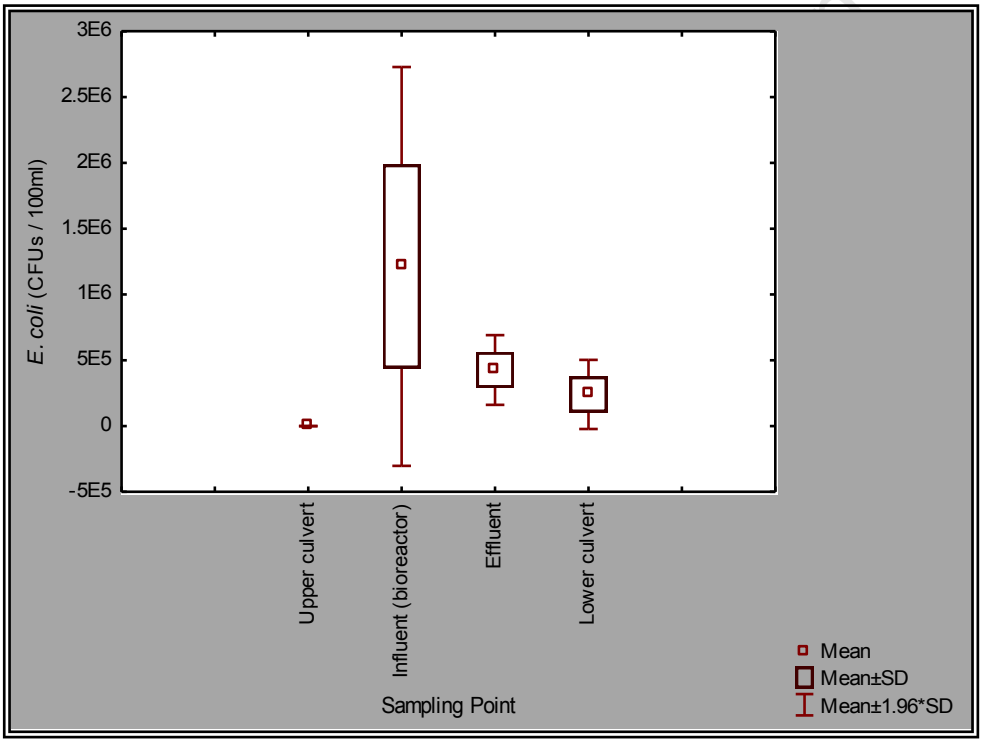
Appendix 4ab: Box and whisker plot for *E. coli* at Wolwedans



Appendix 4ac: Descriptive statistics for *E. coli* at Babylonstoren

Location	Valid N	Mean	Median	Min	Max	25th Percentile	75th Percentile	Range	Std. dev.	CV (%)
Upper culvert	6	0	0	0	0	0	0	0	0	0
Influent (bioreactor)	6	1213333	1145000	390000	2200000	400000	2000000	1810000	773193	63.72
Effluent	6	426667	430000	220000	590000	350000	540000	370000	135154	31.68
Lower culvert	6	240333	238000	56000	430000	150000	330000	374000	133934	55.73

Appendix 4ad: Box and whisker plot for *E. coli* at Babylonstoren



APPENDIX 5: REGRESSION ANALYSIS

The following abbreviations are used in the regression equations:

I = influent

W = Wolwedans

B = Babylonstoren

Nitrogen (NH_3, NO_3^-, NO_2^-)

Ammonia (NH_3):

- De Goede Hoop vs. Wolwedans vs. Babylonstoren

Two models were performed, these were:

1. Log (NH_3) = I + W + B
2. Log (NH_3) = I + W + B + I*W + I*B

The preferred model:

The preferred model that best describes the relationship between NH_3 concentration and the explanatory variables is model 2, i.e. log (NH_3) = I + W + B + I*W + I*B. The preferred model was obtained by comparing AIC, BIC and adjusted R^2 values. The adjusted R^2 value for model 2 is 0.795. Table 5a presents the regression summary for the preferred model.

Table 5a: Regression summary for log (NH_3) = I + W + B + I*W + I*B

	b*	Std err.	b	Std err.	t(32)	p-value
Intercept			0.284	0.101	2.797	0.009
Influent	0.956	0.123	1.117	0.144	7.785	0.000
Wolwedans	1.019	0.119	1.282	0.149	8.581	0.000
Babylonstoren	0.574	0.119	0.722	0.149	4.834	0.000
Interaction term (Influent*Wolwedans)	-0.555	0.132	-0.889	0.211	-4.210	0.000
Interaction term (Influent*Babylostoren)	-0.910	0.132	-1.459	0.211	-6.906	0.000

R= 0.907, R^2= 0.822, adjusted R^2= 0.795, F (5, 32) = 29.621, p<0, std error of estimate= 0.268

Table 5a shows that:

1. There was a significant difference in the value of log (NH_3) at the effluent tank between De Goede Hoop and Wolwedans (Wolwedans p-value = 0.0001 which is significant at 5% level) and De Goede Hoop and Babylostoren (Babylonstoren p-value = 0, which is significant at 5% level).

2. There was a significant difference in the value of log (NH_3) at the influent versus the effluent at De Goede Hoop (influent p-value = 0, which is significant at the 5% level)

3. When the baseline site was changed to Wolwedans, there was a significant difference between the influent and the effluent at Wolwedans (influent p-value equals 0, which is significant at the 5% level)

4. When the baseline site was changed to Babylonstoren, there was a significant difference between the influent and the effluent at Babylonstoren (influent p-value = 0, which is significant at the 5% level)

5. The change in the relationship between log(NH_3) and influent versus effluent for Wolwedans as compared to the De Goede Hoop was significant (Interaction term [Influent *Wolwedans] p-value = 0, which is significant at the 5% level)

6. The change in the relationship between log(NH_3) and influent versus effluent for Babylonstoren as compared to the De Goede Hoop was significant (Interaction term [Influent*Babylostoren] p-value = 0, which is significant at the 5% level)

Residual analysis:

The residual analysis showed that the assumptions for model 2 were valid. This is because the distribution of standard residuals is normal. Furthermore, the plot of predicted values versus residual scores indicates that the assumption of homogeneity[17] is valid.

[17] Occurs when the errors in the predictions from the regression behave in the same way across the dataset

Regression equation:

- Log NH_3 = 0.284 + (1.117) I + (1.282) W + (0.722) B + (-0.889) I*W + (-1.459) I*B

  De Goede Hoop:

  Influent: average log (NH_3) influent is 0.284 +1.117 = 1.401 = 25.18 mg/l

  Effluent: average log (NH_3) effluent is 0.284 = 1.92 mg/l

  Wolwedans:

  Influent: average log (NH_3) influent is 0.284 + 1.117 + 1.28 – 0.89 = 1.791 = 61.8 mg/l

  Effluent: average log (NH_3) effluent is 0.284 + 1.282 = 1.566 = 36.81 mg/l

  Babylonstoren:

  Influent: average log (NH_3) influent is 0.284 + 1.117 + 0.722 – 1.459 = 0.664 = 4.61 mg/l

  Effluent: average log (NH_3) effluent is 0.284 + 0.72 = 1.004 = 10.09 mg/l

- De Goede Hoop vs. Wolwedans

  Three models were performed, these were:

  1. Log (NH_3) = I + W
  2. Log (NH_3) = I + W + I*W
  3. Log (NH_3) = I + W + I*W + centred flow rate

The preferred model:

Model 2 i.e. Log (NH_3) = I + W + I*W is the preferred model. It can be concluded that there is no significant relationship between the NH_3 concentration and the flow rate.

Nitrate (NO_3^-):

- De Goede Hoop vs. Wolwedans vs. Babylonstoren

Two models were performed, these were:

1. Log (NO_3^-) = I + W + B
2. Log (NO_3^-) = I + W + B + I*W + I*B

The preferred model:

The preferred model that best describes the relationship between NO_3^- concentration and the explanatory variables is model 2, i.e. log (NO_3^-) = I + W + B + I*W + I*B. The preferred model was obtained by comparing AIC, BIC and adjusted R^2 values. The adjusted R^2 value for model 2 is 0.292. Table 5b provides the regression summary for the preferred model.

Table 5b: Regression summary for log (NO_3^-) = I + W + B + I*W + I*B

	b*	Std err.	b	Std err.	t(31)	p-value
Intercept			1.127	0.108	10.464	0.000
Influent	-0.580	0.237	-0.388	0.159	-2.447	0.020
Wolwedans	0.232	0.222	0.166	0.159	1.045	0.304
Babylonstoren	-0.560	0.222	-0.400	0.159	-2.522	0.017
Interaction term (Influent*Wolwedans)	0.135	0.252	0.122	0.229	0.535	0.597
Interaction term (Influent*Babylostoren)	0.538	0.252	0.487	0.229	2.132	0.041

R= 0.625, R^2= 0.390, adjusted R^2= 0.292, F (5, 31) = 3.9691 p 0< 0.007, std. error of estimate= 0.285

Table 5b shows that:

1. There was no significant difference in the value of log (NO_3^-) at the effluent tank between De Goede Hoop and Wolwedans (Wolwedans p-value is 0.304, which is not significant)

2. There was a significant difference in the value of log (NO_3^-) at the effluent tank between De Goede Hoop and Babylostoren (Babylonstoren p-value is 0.017, which is significant at the 5% level).

3. There was a significant difference in the value of log (NO_3^-) in the concentration of influent versus the effluent at De Goede Hoop (influent p-value is 0.02, which is significant at the 5% level).

4. When the baseline site is changed to Wolwedans, there was a significant difference between the influent and the effluent at Wolwedans (influent p-value is 0.02, which is significant at the 5% level)

5. When the baseline site was changed to Babylonstoren, there is a significant difference between the influent and the effluent at Babylonstoren (influent p-value = 0.02, which is significant at the 5% level)

6. The change in the relationship between log (NO_3^-) and influent versus effluent for Wolwedans compared to De Goede Hoop was not significant (interaction term [influent * Wolwedans] p-value = 0.597, which is not significant).

7. The change in the relationship between log (NO_3^-) and influent versus effluent for Babylonstoren compared to De Goede Hoop was significant (interaction term [influent * Babylonstoren] p-value = 0.041, which is significant at the 5% level).

Residual analysis:

The residual analysis showed that the assumptions for model 2 were valid because the distribution of standard residuals is relatively normal. Furthermore, the plot of the predicted values versus residual scores indicates that the model fit improves with the larger values.

Regression equation:

- Log NO_{3-} = 1.126 + (-0.388) I + (0.166) W + (-0.4) B + (0.122) I*W + (0.487) I*B

  De Goede Hoop:

  Influent: average log (NO_3^-) influent is 1.126 -0.388 = 0.738 = 5.47 mg/l

Effluent: average log (NO_3^-) effluent is 1.126 = 13.37 mg/l

Wolwedans:

Influent: average log (NO_3^-) influent is 1.126 -0.388 + 0.166 +0.122 = 1.026 = 10.62 mg/l

Effluent: average log (NO_3^-) effluent is 1.126 + 0.166 = 1.292 = 19.59 mg/l

Babylonstoren:

Influent: average log (NO_3^-) influent is 1.126 – 0.388 -0.4 + 0.487 = 0.825 = 6.68 mg/l

Effluent: average log (NO_3^-) effluent is 1.126 -0.4 = 0.726 = 5.32mg/l

- De Goede Hoop vs. Wolwedans

Four models were performed, these were:

1. Log (NO_3^-) = I + W
2. Log (NO_3^-) = I + W + I*W
3. Log (NO_3^-) = I + W + centred flow rate
4. Log (NO_3^-) = I + W + centred flow rate + (W*centred flow rate)

The preferred model:

The preferred model that best describes the relationship between NO_3^- concentration and the explanatory variables is model 4, i.e. log (NO_3^-) = I + W + centred flow rate + (W*centred flow rate). It can be concluded that there is a relationship between the NO_3^- and the flow rate; however this relationship is not significant (p-value = 0.303). The preferred model was obtained by comparing AIC, BIC and adjusted R^2 values. The adjusted R^2 value for model 4 is 0.471. Table 5c provides the regression summary for the preferred model.

Table 5c: Regression Summary for log (NO_3^-) = I + W + centred flow rate + (W*centred flow rate)

	b*	Std err.	b	Std err.	t(20)	p-value
Intercept			1.098	0.081	13.502	0.000
Influent	-0.506	0.149	-0.333	0.098	-3.407	0.003
Wolwedans	0.347	0.149	0.228	0.098	2.334	0.030
Centred flow rate	-0.464	0.439	-0.002	0.002	-1.056	0.303
Interaction term (Centred flow rate*Wolwedans)	0.862	0.439	0.004	0.002	1.964	0.064

R= 0.748, R^2= 0.559, adjusted R^2= 0.471, F(4,20)=6.349, p<0.002, std. error of estimate= 0.244

Regression equation:

- Log (NO_3^-) = 1.098 + -(0.333) (I) + (0.228) (W) + (-0.002) centred flow rate +(0.004) (W*centred flow rate)

  De Goede Hoop:

  Flow rate: the equation indicates that if the average flow rate increases by 1 unit, the log NO_3^- concentration would change by -0.002 (p-value for centred flow rate = 0.303, which is not significant).

  Wolwedans:

  Flow rate: the equation indicates that if the average flow rate increases by 1 unit, the log NO_3^- concentration would change by -0.002 (by changing the baseline site to Wolwedans, it was determined that the centred flow rate p-value for Wolwedans = 0.303, which is not significant).

Nitrite (NO_2^-):

- De Goede Hoop vs. Wolwedans vs. Babylonstoren

  Two models were performed, these were:

  1. Log (NO_2^-) = I + W + B
  2. Log (NO_2^-) = I + W + B + I*W + I*B

The preferred model:

The preferred model that best describes the relationship between NO_2^- concentration and the explanatory variables is model 1, i.e. log (NO_2^-) = I + W + B. It can thus be concluded that the relationship between NO_2^- and influent versus effluent does not change across the sites (as the preferred model does not contain interactions between influent and site variables). The preferred model was obtained by comparing AIC, BIC and adjusted R^2 values. The adjusted R^2 value for model 1 is 0.37. Table 5d provides the regression summary for the preferred model.

Table 5d: Regression summary for log (NO_2^-) = I + W + B, R= 0.649, R^2= 0.421

	b*	Std err.	b	Std err.	t(34)	p-value
Intercept			-1.124	0.192	-5.838	0.000
Influent	-0.233	0.131	-0.356	0.200	-1.782	0.084
Wolwedans	0.647	0.147	1.065	0.242	4.397	0.000
Babylonstoren	0.105	0.147	0.173	0.242	0.714	0.480

Adjusted R^2= 0.37, F (3, 34) = 8.235, p<0, std error of estimate= 0 .616

Table 5d shows that:

1. There was a significant difference in the value of log (NO_2^-) at the effluent tank between De Goede Hoop and Wolwedans (Wolwedans p-value = 0, which is significant at the 5% level)

2. There was no significant difference in the value of log (NO_2^-) at the effluent tank between De Goede Hoop and Babylostoren (Babylonstoren p-value = 0.48, which is not significant).

3. There was a significant difference in the value of log (NO_2^-) at the influent versus the effluent at De Goede Hoop (influent p-value = 0.084, which is significant at the 10% level).

4. When the baseline site is changed to Wolwedans, it can be determined that the there was a significant difference between the influent and the effluent at Wolwedans (influent p-value = 0.084, which is significant at the 10% level)

5. When the baseline site is changed to Babylonstoren, it can be determined that the there was a significant difference between the influent and the effluent at Babylonstoren (influent p-value = 0.084, which is significant at the 10% level)

6. As no interaction terms between influent and the sites are included in the model, this means that there was no significant change in the relationship between log NO_2^- and influent from De Goede Hoop to Wolwedans/Babylonstoren.

Residual analysis:

The residual analysis showed that the assumptions for model 1 are valid. This is because the distribution of standard residuals is relatively normal. Furthermore, the plot of predicted values versus residual scores indicates that the assumption of homogeneity is valid.

Regression equation:

- Log (NO_2^-) = -1.124 + (-0.356)(I) + 1.065 (W) + 0.173 (B)

  De Goede Hoop:

  Influent: average log (NO_2^-) influent is -1.124 -0.356 = -1.48 = 0.03 mg/l

  Effluent: average log (NO_2^-) effluent is -1.124 = 0.08 mg/l

Wolwedans:

Influent: average log (NO_2^-) influent is -1.124 - 0.356 + 1.065 = -0.415 = 0.38 mg/l

Effluent: average log (NO_2^-) effluent is -1.124 + 1.065 = -0.059 = 0.87 mg/l

Babylonstoren:

Influent: average log (NO_2^-) influent is -1.124 – 0.356 + 0.173 = -1.307 = 0.05 mg/l

Effluent: average log (NO_2^-) effluent is -1.124 + 0.173 = -0.951 = 0.11 mg/l

- De Goede Hoop vs. Wolwedans

Four models were performed, these were:

1. Log (NO_2^-) = I + W
2. Log (NO_2^-) = I + W + I*W
3. Log (NO_2^-) = I + W + centred flow rate
4. Log (NO_2^-) = I + W + centred flow rate + (W*centred flow rate)

Model 4 i.e. Log (NO_2^-) = I + W + centred flow rate + (W*centred flow rate) is the preferred model. It can thus be concluded that there is a relationship between the NO_2^- and the flow rate; however this relationship is not significant (p-value 0.666). The preferred model was obtained by comparing AIC, BIC and adjusted R^2 values. The adjusted R^2 value for model 4 is 0.634. Table 5e provides the regression summary for the preferred model.

Table 5e: Regression summary for log (NO_2^-) = I + W + centred flow rate + (W*centred flow rate)

	b*	Std err.	b	Std err.	t(21)	p-value
Intercept			-1.101	0.175	-6.29	0
Influent	-0.234	0.121	-0.4	0.207	-1.932	0.067
Wolwedans	0.62	0.121	1.064	0.208	5.125	0
Centred flow rate	-0.154	0.352	-0.001	0.004	-0.437	0.666
Interaction term (Centred flow rate*Wolwedans)	0.646	0.352	0.008	0.005	1.835	0.081

R= 0.832, R^2= 0.693, adjusted R^2= 0.634, F (4,21)=11.843, p< 0.00003, std. error of estimate = 0.528

Regression equation:

- Log (NO_2^-) = -1.101 + (-0.4) (I) + (1.064) (W) + (-0.001) (centred flow rate) + (0.008) (W*centred flow rate)

  De Goede Hoop:

  Flow rate: the equation indicates that if the average flow rate increases by 1 unit, the log NO_2^- concentration would change by - 0.001 (centred flow rate p-value = 0.666, which is not significant).

  Wolwedans:

  Flow rate: the equation indicates that if the average flow rate increases by 1 unit, the log NO_2^- concentration would change by - 0.001 + 0.008 = 0.007 (by changing the baseline site to Wolwedans, it was determined that the centred flow rate p-value for Wolwedans = 0.666, which is not significant).

Orthophosphate

- De Goede Hoop vs. Wolwedans vs. Babylonstoren

  Two models were performed, these were:

  1. PO_4^{3-} = I + W + B
  2. PO_4^{3-} = I + W + B + I*W + I*B

Model 1 i.e. PO_4^{3-} = I + W + B is the preferred model. It can thus be concluded that the relationship between PO_4^{3-} and influent versus effluent does not change across the sites. The preferred model was obtained by comparing AIC, BIC and adjusted R^2 values. The adjusted R^2 value for model 1 is 0.109. Table 5f provides the regression summary for the preferred model.

Table 5f: Regression summary for PO_4^{3-} = I + W + B

	b*	Std err.	b	Std err.	t(34)	p-value
Intercept			28.603	3.787	7.553	0.000
Influent	0.126	0.155	3.184	3.930	0.810	0.424
Wolwedans	0.060	0.175	1.631	4.765	0.342	0.734
Babylonstoren	-0.375	0.175	-10.218	4.765	-2.144	0.039

R= 0.425, R^2= 0.181, adjusted R^2= 0.109, F (3, 34) = 2.503, p< 0.076, std. error of estimate= 12.113

Table 5f shows that:

1. The p-value for Wolwedans was 0.734, which was not significant at 5% or 10% levels. This indicates that there was no significant difference in the value of PO_4^{3-} at the effluent tank between De Goede Hoop and Wolwedans.

2. There was a significant difference in the value of PO_4^{3-} at the effluent tank between De Goede Hoop and Babylostoren (Babylonstoren p-value is 0.039, which is significant at the 5% level).

3. The p-value for the influent is 0.424, which is not significant. This indicates that there was no significant difference in the value of PO_4^{3-} at the influent versus the effluent at De Goede Hoop.

4. When the baseline site was changed to Wolwedans, it could be determined that there was no significant difference between the influent and effluent at Wolwedans (influent p-value = 0.424, which is not significant)

5. When the baseline site was changed to Babylonstoren, it could be determined that there was no significant difference between the influent and effluent at Babylonstoren (influent p- value = 0.424, which is not significant)

6. As no interaction terms between influent and the sites were included in the model, this meant that there was no significant change in the relationship between PO_4^{3-} and influent at Wolwedans / Babylonstoren versus De Goede Hoop.

Residual analysis:
The residual analysis showed that the assumptions for model 2 are valid. This is because the distribution of standard residuals is fairly normal. Furthermore, the plot predicted values versus residual scores indicate that the assumption of homogeneity is valid.

Regression equation:

- $PO_4^{3-} = 28.603 + (3.184)I + (1.631)W + (-10.128)B$

  De Goede Hoop:
  Influent: average PO_4^{3-} influent = 28.603 + 3.184 = 31.79 mg/l
  Effluent: average PO_4^{3-} effluent = 28.6 mg/l

  Wolwedans:
  Influent: average PO_4^{3-} influent = 28.603 + 3.184 + 1.631 = 33.42 mg/l
  Effluent: average PO_4^{3-} effluent = 28.603 + 1.631 = 30.23 mg/l

  Babylonstoren:
  Influent: average PO_4^{3-} influent = 28.603 + 3.184 – 10.128 = 21.66 mg/l
  Effluent: average PO_4^{3-} effluent = 28.603 – 10.128 = 18.48 mg/l

- De Goede Hoop vs. Wolwedans

  Three models were performed, these were:
  1. Log (PO_4^{3-}) = I + W
  2. Log (PO_4^{3-}) = I + W + I*W
  3. Log (PO_4^{3-}) = I + W + centred flow rate

Model 1 i.e. Log (PO_4^{3-}) = I + W is the preferred model. It can thus be concluded that there is no relationship between the PO_4^{3-} concentration and the flow rate, and neither does the relationship between PO_4^{3-} and location (influent vs. effluent) differ between sites.

E. coli

- De Goede Hoop vs. Wolwedans vs. Babylonstoren

Two models were run, these included:

1. Log *E. coli* = I + W + B
2. Log *E. coli* = I + W + B + I*W + I*B

The preferred model:

The preferred model that best describes the relationship between *E. coli* concentration and the explanatory variables is model 2, i.e. log *E. coli* = I + W + B + I*W + I*B. The preferred model was obtained by comparing AIC, BIC and adjusted R^2 values. The adjusted R^2 value for model 2 is 0.498. Table 5g provides the regression summary for the preferred model.

Table 5g: Regression Summary for log *E. coli* = I + W + B + I*W + I*B

	b*	Std err.	b	Std err.	t(32)	p-value
Intercept			4.566	0.187	24.412	0.000
Influent	0.728	0.206	0.933	0.264	3.527	0.001
Wolwedans	0.694	0.200	0.956	0.275	3.472	0.002
Babylonstoren	0.757	0.200	1.043	0.275	3.789	0.001
Interaction term (Influent*Wolwedans)	-0.437	0.222	-0.768	0.389	-1.973	0.057
Interaction term (Influent*Babylostoren)	-0.313	0.222	-0.551	0.389	-1.414	0.167

R= 0.705, R^2= 0.498, Adjusted R^2= 0.419, F (5, 32) = 6.34, p< 0, std error of estimate = 0.495

Table 5g shows that:

1. There was a significant difference in the value of log *E. coli* at the effluent tank between De Goede Hoop and Wolwedans (Wolwedans p-value = 0.002, which is significant at the 5% level) and De Goede Hoop and Babylostoren (Babylonstoren p-value = 0.001, which is significant at the 5% level).

2. There was a significant difference in the value of log *E. coli* at the influent versus the effluent at De Goede Hoop (influent p-value = 0.001, which is significant at the 5% level)

3. When the baseline site was changed to Wolwedans, there was a significant difference in the value of log *E. coli* between the influent and the effluent at Wolwedans (influent p-value = 0.001, which is significant at the 5% level)

4. When the baseline site was changed to Babylonstoren, there was a significant difference between the influent and the effluent at Babylonstoren (influent p-value = 0.001, which is significant at the 5% level)

5. The change in the relationship between log *E. coli* and influent versus effluent for Wolwedans as compared to the De Goede Hoop was significant (Interaction term [Influent *Wolwedans] p-value = 0.057, which is significant at the 10 % level)

6. The change in the relationship between log *E. coli* and influent versus effluent for Babylonstoren as compared to the De Goede Hoop was not significant (Interaction term [Influent*Babylostoren] p-value is 0.167, which is not significant)

Residual analysis:

The residual analysis showed that the assumptions for model 2 are valid. This is because the distribution of standard residuals is fairly normal. Furthermore, the plot of predicted values versus residual scores indicates that the assumption of homogeneity is valid.

Regression equation

- Log *E. coli* = 4.566 + (0.933) I + (0.956)W + (1.043)B + (-0.768)I*W + (-0.551)I*B

De Goede Hoop:

Influent: average log *E. coli* influent is 4.566 + 0.933 = 5.599 = 397192 CFUs/100ml

Effluent: average log *E. coli* effluent is 4.566 = 36813 CFUs/100ml

Wolwedans:

Influent: average log *E. coli* influent is 4.566 + 0.933 + 0.956 -0.768= 5.687= 486407 CFUs/100ml

Effluent: average log *E. coli* effluent is 4.566 + 0.956 = 5.522= 332660 CFUs/100ml

Babylonstoren:

Influent: average log *E. coli* influent is 4.566 + 0.933 + 1.043 – 0.551 = 5.991= 979490 CFUs/100ml

Effluent: average log *E. coli* effluent 4.566 + 1.043 = 5.606 = 403 645 CFUs/100ml

- De Goede Hoop vs. Wolwedans

Four models were run, these included:

1. Log *E. coli* = I + W
2. Log *E. coli* = I + W + I*W
3. Log *E. coli* = I + W + I*W + centred flow rate
4. Log *E. coli* = I + W + centred flow rate + (I*centred flow rate)

Model 3 i.e. Log *E. coli* = I + W + I*W + centred flow rate is the preferred model. It can thus be concluded that there is a relationship between the *E. coli* concentration and the flow rate; however this relationship is not significant (p-value = 0.175). The preferred model was obtained by comparing AIC, BIC and adjusted R^2 values. The adjusted R^2 value for model 3 is 0.371. Table 5h provides the regression summary for the preferred model.

Table 5h: Regression Summary for log *E. coli* = I + W + I*W + centred flow rate

	b*	Std err.	b	Std err.	t(21)	p-value
Intercept			4.566	0.211	21.623	0.000
Influent	0.675	0.216	0.933	0.299	3.124	0.005
Wolwedans	0.690	0.224	0.956	0.311	3.076	0.006
Interaction term (Influent*Wolwedans)	-0.469	0.268	-0.768	0.440	-1.748	0.095
Centred flow rate	-0.222	0.159	-0.002	0.002	-1.403	0.175

$R = 0.687$, $R^2 = 0.472$, adjusted $R^2 = 0.371$, $F(4,21) = 4.69$, $p < 0.007$, std error of estimate= 0.559

Residual analysis:

The residual analysis showed that the assumptions for model 2 are valid. This is because the distribution of standard residuals is not normal and has a bimodal distribution. Furthermore, the plot of predicted values versus residual scores indicates that the assumption of homogeneity is valid.

Regression equation:

Log *E. coli* = 4.566 + (0.933) I + (0.956) W + (-0.768) I*W + (-0.002) centred flow rate

De Goede Hoop:

Flow rate: the equation indicates that if the average flow rate increases by 1 unit, the log *E. coli* concentration would change by - 0.002 (centred flow rate p-value = 0.175, which is not significant).

Wolwedans:

Flow rate: the equation indicates that if the average flow rate increases by 1 unit, the log *E. coli* concentration would change by - 0.002 (centred flow rate p-value = 0.175, which is not significant.