The Inorganic Pollution of the Franschhoek River:

Sources and Solutions.

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A thesis submitted in fulfilment of the requirements for the degree Magister Scientiae, in the Department of Biodiversity and Conservation Biology, University of the Western Cape.

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Key words

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Eutrophication

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Salix babylonica.

Water quality

ABSTRACT

The Inorganic Pollution of the Franschhoek River: Sources and Solutions.

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The aim of the study was to quantify the extent of inorganic chemical pollution of the Franschhoek River and draw relationships between contaminants in water, sediment and plants. The invasive *Acacia mearnsii* and *Salix babylonica* and indigenous *Brabejum stellatifolium* species were chosen as biomonitors due to their wide spread distribution along the river and their apparent ability to accumulate heavy metals.

The sites chosen allowed for comparison of the river quality upstream with that of the river further down stream as it meandered through residential, agricultural and recreational areas, until it joined with the Berg River further downstream. The general aim of the study was to assess the degree of inorganic pollution in the Franschhoek River to evaluate its contribution to pollution of the Berg River, of which it is an important tributary. Also understanding the sources of the pollution would contribute to the ability to reduce pollution.

Plant and water samples were collected once a month for 12 months at 10 predetermined research sites along the Franschhoek River. In order to determine the major inorganic nutrients; water, plant and soil samples were collected at each site. Plant and water samples were collected once a month for 12 months with one summer (April 2009) and one winter (August 2009) soil collection. Dissolved oxygen, temperature and electrical conductivity were determined *in situ*.

The results indicated that majority of the concentrations of the elements in plants were higher than those in the sediment and in turn displayed higher concentrations than those in the water and that majority of the surface water contamination was noted downstream of the waste water treatment facility. The invasive *Acacia mearnsii* and *Salix babylonica* bioaccumulated more metals than the endemic tree *Brabejum stellatifolium.*

DECLARATION

I declare that *The Inorganic Pollution of the Franschhoek River: Sources and Solutions* is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Kim Marie Adams November 2011

Signed: ………………………………..

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CHAPTER 1

Literature Review

1.1 Background

This research was driven by a need to increase the knowledge base of surface waters by evaluating the inorganic chemical content of the water, soil and vegetation.

Freshwater biodiversity could possibly be one of the greatest conservation concerns globally (Brooks *et al*, 2006; Clarke 2009). Freshwater is likely to be one of the most valuable natural resources available; however it seems to be experiencing the greatest decline in biodiversity globally as the demand for freshwater increases exponentially (Dudgeon *et al.* 2006). In addition to the over exploitation by humans and industry of this rare commodity, other threats include invasive species, habitat destruction and degradation, water pollution and flow modification (Dudgeon *et al.* 2006). Freshwater is an invaluable resource as it provides us with an array of goods and services which contribute to global and local economic productivity. Biologically it harbours genetic information as well as supporting the provision of ecosystem services. South Africa is no exception when it comes to freshwater scarcity. However, as a third world developing country, social and economic growth takes priority over conservation (Alylward and Barbier,1992).

According to Tyson (1987), the South African climate is unpredictable with a highly seasonal distribution of rainfall, with evapo-transpiration rates exceeding rainfall resulting in a semi-arid to arid conditions. South Africa has very few natural lakes with rivers being variable between seasons (DWAF, 1996a). Those rivers in drier regions of the country tend to be characterized by seasonal and ephemeral flows while those occurring in wetter regions tend to be perennial (Midgley *et al.* 1994). Rivers in South Africa provide water for agricultural, industrial, recreational and domestic uses with 85 % of all water used being sourced from rivers while the remaining 15 % being sourced from groundwater (Ashton, 2007). Almost all of South African rivers are under threat from agrochemicals as industrial and domestic effluents from surrounding cities are discharged into adjacent rivers.

The protection of freshwater biodiversity locally as well as globally could fundamentally be the greatest conservation challenge faced to date due to the complex nature of these fragile ecosystems (van Wilgen, 1997). The prerequisites which facilitate effective management of freshwater ecosystems should include an array of management strategies, such as the sustainable use of freshwater, the preservation of freshwater biodiversity as well as key indicator species. Due to the lack of knowledge surrounding rivers in South Africa and the impending water crises it is crucial that researchers, stakeholders and policy makers make concerted efforts to protect freshwater biodiversity.

1.2 Aim

The aim of this study is to quantify the extent of inorganic chemical pollution of the Franschhoek River and draw relationships between contaminants in the plants, water and soil and where possible provide recommendations to improve the river quality. To evaluate the relative importance of contamination from the Franschhoek River as it flows into the Berg River.

1.3 Objectives

- Attempt to determine which are the main inorganic chemical pollutants of the Franschhoek River?

- By using riparian trees namely *Brabejum stellatifolium, Acacia mearnsii* and *Salix babylonica* determine which are the best biomonitors?

- Ascertain which practices are diminishing the water quality of the Franschhoek River?

1.4 River structure and function.

- **1.4.1 The river continuum concept.**

The river continuum concept (RCC), (Fig.1) is a model for describing and classifying flowing water from the source to its discharge area (Vannote *et al*. 1980), and encompasses physical parameters such as width, depth, sediment load, velocity, turbidity and chemical composition (Harper *et al*, 2008; Murray *et al*, 2009). The RCC makes two predictions: 1) biological communities change down the longitudinal dimensions of a river as it interacts with terrestrial communities (Vannote, 1980; Webster, 2007 and Thorp *et al*. 2010), which is brought about by a continuous gradient of physical conditions observable from the source to its discharge point (Vannote *et al*. 1980 and Murray *et al*. 2009).

1.4.2 Sources and zonations.

Rivers within the Western Cape of South Africa can more often than not be divided into five distinct zones namely; 1) the mountain stream zone 2) the foothill zone 3) the transitional zone 4) the lowland zone and 5) estuaries.

Mountain and cliff waterfalls are often the sources of rivers within Cape Town and are associated with acidic soils rich in organic matter (King *et al*. 1987). This zone, has high velocity flow regimes with the water either being clear or peat stained with high oxygen concentrations (Blob *et al*. 2008).

Defining characteristics include, steep gradients with narrow channels, a river bed made up of large boulders, bedrock and smaller cobbles, fast flowing waters and an average summer temperature of 20^{o}C. Riparian vegetation is mostly large trees which may or may not form a canopy. The foothill zone, unlike the mountain stream zone, is characterized by wider channels, decreasing gradients and decreased flow with a mean summer temperature above 20^{o}C (Vannote *et al*, 1980 and Davies and Day, 1998).

Transitional zones may be single zones as well as the confluence point of river channels. The area has dense riparian vegetation and a narrow band of riparian trees; the flow regime is variable and mean temperatures are above 20^{o}C (Davies and Day, 1998). The low land zone can be characterised by shallow gradients with vegetation changing from dense trees and shrubs to reed beds, the water is turbid and temperatures are well over 20^{o}C (Davies and Day, 1998; Schalmz and Fohrer 2009). The estuary is the zone where the river meets the ocean; this area is characterized by a zone of intermediate salinity and is often influenced by tidal action. Temperatures are variable as they are controlled by atmospheric temperatures and the interacting marine environment (Davies and Day 1998).

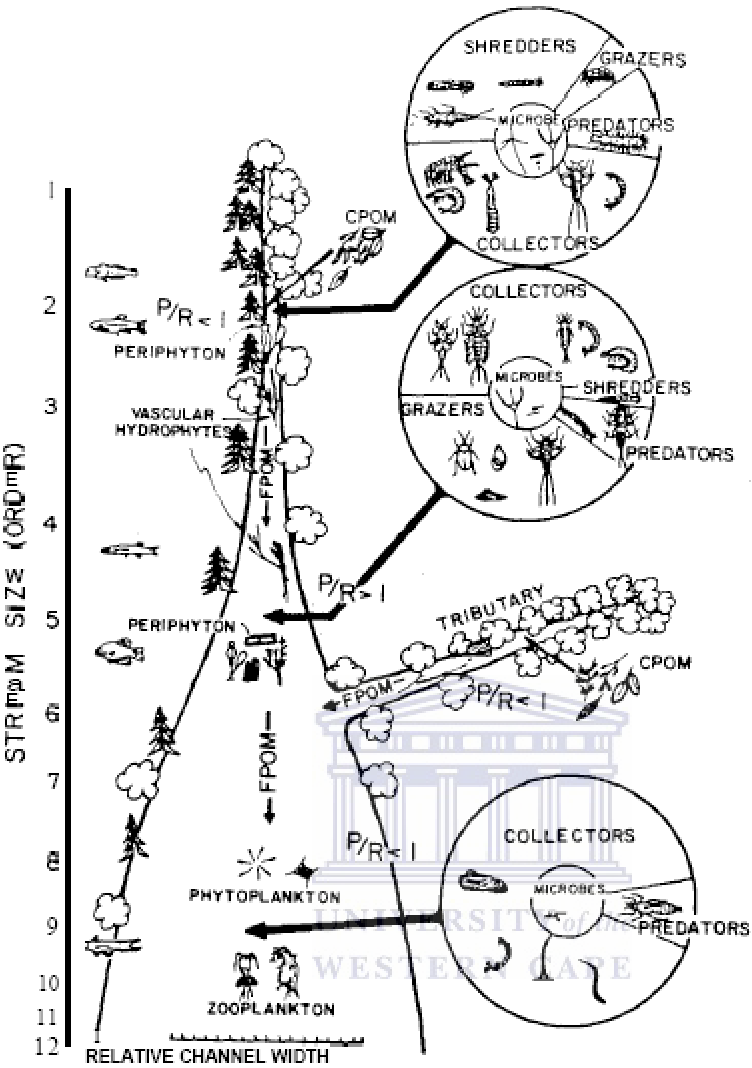


Figure 1.1 Vannote *et al.'s* longitudinal relationship between stream size and ecological structure and function (reproduced from Vannote *et al.* 1980).

- **1.4.3 River flow regimes.**

River flow regimes are a combination of timing, size and duration of flow events and are the key drivers of river ecosystems (Brown and King, 2002). River flow regimes regulate five critical ecological processes such as “the magnitude, frequency, duration, timing and rate of change of hydrologic conditions” (Poff and Ward, 1989). The magnitude of a flow regime may be defined as the amount of water moving past a fixed point per unit time and the greater the flood or drought the more severe the physiological impact. Frequency refers to the amount of floods or droughts of a given magnitude per time interval as well as its duration. The time of the year would affect flow regimes, e.g. in the Western Cape, one could expect dry summers resulting in reduced flow and wet winters resulting in increased flow (Poff *et al*. 1997; Lytle and Poff, 2004).

According to Nilsson and Renofalt (2008), varying flow regimes such as floods versus low flows affect ecosystem services and environmental processes, such as fish spawning (Brown and King, 2002), which may arise due to seasonal reversal which alters hydraulic and thermal conditions which are the cures for fish spawning.

1.5Heavy metal pollution in aquatic environments.

Heavy metals are among the most abundant environmental pollutants and pose threats to both aquatic and terrestrial biota (Klavins *et al*. 2000). The presence of these metals may be attributed to industrial and agricultural effluent, non-point source pollution, precipitation as well as the natural weathering of minerals. By determining the heavy metal content of plants, water and sediment one is able to gain insight into the chemical composition as well as the levels of toxicity of the surrounding environment (Akcay *et al*. 2003).

Metals such as aluminium, cadmium and lead exhibit high levels of toxicity even at trace quantities with Al being of major concern due to the health risks associated with it (Fatoki *et al*. 2002). In addition to Al; Zn, Cu, Pb and Cd are common pollutants found in aquatic ecosystems. According to Spear (1981), Zn has a low toxicity to humans; but it is lethal to fish. Copper; despite being essential to the livelihoods of many organisms it remains extremely toxic at low concentrations in aquatic environments. Cd in aquatic systems is toxic to fish and other aquatic organisms and when absorbed and accumulated in humans, Cd causes kidney failure as well as joint and arthritic disorders (Spear, 1981). According to Fischer (1987), Cd has carcinogenic as well as mutagenic characteristics. The United States Environmental Protection Agency (USEPA) has listed lead as one of the potentially most hazardous metals, as it is detrimental to aquatic organisms, benthic bacteria as well as freshwater and riverine plants (Fatoki *et al*. 2002).

A study conducted by Jackson *et al* (2007), over a one year period investigated the degree of metal contamination in the Berg River, Western Cape. By utilizing the nitric acid digestion technique they were able to extract metals from the water, soil and biofilm at various sites along the river. Site A being an agricultural area, Site B an informal settlement and Site C a pumping station. Once the metals had been extracted there concentrations were determined using inductive coupled plasma atomic emission spectrometry. The metals giving the highest means in the water were Al, Fe and Mn; in the sediment Al and Fe and in the biofilm Al and Fe. Their study indicated that the increase in Al and Fe concentrations in the water could be attributed to leaching of metals from waste and household detergents from the surrounding informal settlement. The agricultural area had the highest concentration of heavy metal contamination. In both the biofilm and the sediment the concentrations of Al and Fe were significantly higher ($p<0.05$) than Cu, Zn, Pb, Ni and Mn. From the results obtained it became evident that the pollution originated from agricultural and industrial waste discharge and that the level of heavy metals that were presented may have been detrimental to aquatic organisms.

To date the knowledge surrounding the speciation and concentration of heavy metals in South African riverine systems remains unsatisfactory.

1.6Point and non point source pollution.

Pollution is by far one of the most obvious problems associated with South African rivers and inland waters. Water pollution is far more complex than common litter such as plastic bags and tin cans; even natural occurring minerals may become pollutants under certain conditions. There has been a marked influx in fresh water and coastal pollutants over the years resulting in the degradation of vital water sources as well as the loss of species, biodiversity, goods and services (Carpenter *et al*. 1998).

Chemical inputs into aquatic ecosystems can either be classified as point or non point source pollutants. Point source pollutants are often easy to monitor and show minimal variation over time; these sources include effluent discharged from pipes and storm water drains (Davies and Day, 1998; Camargo and Alonso, 2006).

Non point pollutants have no discrete discharge points, hence making it difficult to monitor and regulate. Chemical inputs include agricultural run-off, precipitation, urban run-off from unsewered areas, logging and the construction of water ways (Carpenter *et al.* 1998; Davies and Day, 1998). In addition, they are the dominant contributors of phosphorus and nitrogen to river systems and often result in eutrophication (Herzog *et al.* 2008).

The concentrations of NO_2^- is directly proportional to the pH of the water, hence as the pH increases so does the NO_2^- concentrations. A study conducted by Camargo and Alonso (2006) highlighted the three major environmental concerns associated with inorganic nitrogen.

1) Inorganic nitrogen has the ability of increasing the hydrogen ion concentration resulting in acidification of fresh water systems.

2) Inorganic nitrogen can promote the development and production of many primary producers resulting in eutrophication of lakes and rivers and other fresh water systems.

3) When toxic concentrations are reached nitrogen becomes fatal to aquatic animals and impairs growth and reproduction, moreover it has adverse effects on the well beings of humans as well as the economy (Camargo and Alonso, 2006).

1.7Nutrients and eutrophication.

Nutrients are chemicals or elements which can be used directly by algae and macrophytic organisms for growth (Herzog *et al*. 2008). In relation to eutrophication, nutrients are mostly inorganic elements which are assimilated by plants and together with photosynthesis are used to manufacture and accumulate organic material in freshwater systems (Walmsley, 2000). Algae and aquatic macrophytes require approximately 20 different elements for normal growth and reproduction (Davies and Day, 1998; Walmsley, 2000). According to Walmsley, growth of aquatic macrophytes is dependant on the amount and rations of nutrients present in the water; hence "plant growth may be limited by the concentration of that nutrient that is present in the least quantities relative to the growth needs of the plant." This is known as the limiting nutrient concept. Due to the high demand for nutrients in aquatic systems it has been observed that the most frequent limiting nutrients are phosphorus (P) and nitrogen (N) (Javie *et al*. 2005).

Nitrogen occurs abundantly in nature and it is an essential constituent of many biomolecules (Camargo and Alonso, 2006). Anthropogenic factors coupled with population growth rates have altered natural landscapes, hydrological cycles as well as those essential nutrients required for plant growth (Rabalais, 2002). In aquatic systems N may be present as nitrates, nitrites and ammonia. Nitrate is of little concern in aquatic systems as it is often chemically reduced and converted to atmospheric nitrogen by microbes. Nitrite however is toxic at even low concentrations while ammonia remains a common pollutant associated with run-off effluent and may be present as either un-ionised NH_3 or as NH^{4-} ions (Davies and Day, 1998).

Phosphorus is present in nature in its inorganic form (Davies and Day, 1998) and occurs as orthophosphates, polyphosphates and organic phosphates. Phosphorus is an important nutrient for aquatic plant growth and when absent aquatic plants will not grow despite nitrogen availability. Run-off from agricultural land is a source of phosphorus in water and can pollute surface water and may cause algal blooms as well as promoting invasive plant growth (Walmsley, 2000). Excess nitrogen and phosphorus in both fresh water and marine ecosystems are far more detrimental than just the proliferation of invasive plants (Smith, 2003). Eutrophication is a major problem associated with degrading aquatic ecosystems.

According to Walmsley (2000), eutrophication can best be described as the process by which an aquatic or marine system becomes enriched with plant nutrients, more often than not nitrogen and phosphorus. Enrichment increases the amount of biological activity which ultimately leads to severe changes in the aquatic food web (Smith *et al.* 1999; Smith *et al*. 2006) due to the exponential growth of an ecosystems primary productivity (Khan and Ansari, 2006). In addition eutrophication promotes plant growth and then decomposition of primary producers with nuisance plants especially reaping the benefits. Eutrophication enhances the growth and proliferation of phytoplankton which results in a lack of oxygen which is required for aquatic organisms to survive. The water body eventually becomes aesthetically displeasing with human health related problems surfacing, moreover severe changes in water quality and ecosystem functioning (Khan and Ansari, 2005).

A study conducted by Smith (2003) on the eutrophication of freshwater and coastal marine ecosystems showed a predictable increase in the biomass of algae in freshwater and marine ecosystems. Those systems which received constant nutrient inputs had increased levels of cyanobacteria and profound detrimental effects on water quality.

1.8Plants as bio – indicators.

The degradation of fresh water ecosystems is increasing exponentially due to industrialization and urbanization (Testi *et al*. 2008). Heavy metals may enter river systems via effluent which is subsequently deposited in the surface soil as well as via air borne pollutants (Landberg and Greger, 1996). Research indicates that pollutants such as heavy metals can be accumulated by vascular plants and aquatic biota such as fish (Nussey *et al*. 2006). Bioaccumulation refers to the absorption and storing of pollutants in the organs or tissues of aquatic biota. However, it has been well documented that bioaccumulation can only occur if the rate of absorption by the organism exceeds the rate at which the contaminant is released (Nussey, 2006). The utilization of plants as bioindicators is useful as they provide valuable information concerning the environment integrated over time, past and present whereas chemical analysis provides more prompt and current information (Testi *et al*. 2008).

Plants are capable of absorbing heavy metals via their roots and mycorrhizae which in turn are accumulated and stored in the leaves. Utilizing plants as biomonitors is advantageous as it allows one to detect and measure low concentrations of heavy metals which may not have been detected by chemical extraction techniques (Madejon *et al.* 2004).

Plants may either be “excluders”, “accumulators” or “indicators” (Madejon *et al*, 2004). Those macrophytes having accumulator type responses are capable of tolerating high concentrations of heavy metals in there tissues, whereas those plants having indicator type responses have a relatively constant heavy metal uptake through there roots over a wide gradient. Madejon *et al.* (2004) suggests that these macrophytes show a linear relationship between the concentration in the plant tissue and in the soil. Hence plants having indicator or accumulator type characteristics could possibly be used as biomonitors. Madejon *et al.* (2004) investigated the effectiveness of using the white poplar (*Populas alba*) as a biomonitor for trace elements in a contaminated riparian forest. They surveyed eight heavy metals namely; As, Cd, Cu, Fe, Mn, Ni, Pb, Al and Zn in the leaves and stems of *Populas alba*. Twenty five trees were selected in a contaminated soil area and 10 trees in a non contaminated site. There research indicated that the contaminated *P. alba* trees had significantly higher Zn, Pb, Cu, Cd and As concentrations oppose to the non - contaminated site. Their results clearly indicated that the concentrations of heavy metals in poplar leaves were positively correlated with the amount of Cd and Zn present in the soil, indicating that the white poplar, *P. alba* could be used as an effective biomonitor for soil pollution.

1.9 The effect of invasive vegetation on riparian ecosystems.

The success of any riparian ecosystem is strongly dependant on the surrounding riparian vegetation (Castelli *et al*. 2000). Riparian vegetation maintains ecosystem functioning by reducing soil erosion, regulating soil and water nutrient levels as well as aiding in river bank stability (Castelli *et al*. 2000).

Riparian vegetation of the Western Cape has been greatly invaded by invasive plant species (Reinecke *et al*, 2008; Blanchard and Holmes, 2008). The most heavily impacted areas are the Fynbos biome with woody species and large perennial shrubs out competing indigenous plant species (Blanchard and Holmes, 2008). The most common invaders in the Western Cape include the Australian woody trees within the genus *Acacia* as well as *Eucalyptus sp* and *S. babylonica* from the Northern Hemisphere (Blanchard and Holmes, 2008).

Invasive species thrive in riverine ecosystems due to the hydrological regime of rivers and because the river is used as a transportation medium for propogules (Blanchard and Holmes, 2008; Castelli *et al*. 2000). Invasive vegetation degrades the natural vegetation structure, reduce species richness and diversity, in addition significantly decreasing water availability and altering soil and water nutrient levels. Invasive vegetation ultimately decreases ecosystem functioning and services.

1.10 Conclusion.

As can be seen, many factors affect the quality of freshwater ecosystems, many of which being anthropogenically induced. Protection of freshwater biodiversity could possibly be one of the more challenging tasks facing conservationists and researchers as it requires a thorough understanding of the surrounding riparian vegetation, migrating fauna and flow regimes of the river. Indicator plant species are important resources available to freshwater ecologists as they provide accurate and prompt information regarding water quality; however it is the implementation of effective management strategies which will ultimately aid in the protection of our freshwater systems.

CHAPTER 2

METHODOLOGY

2.1 Introduction

South Africa's average rainfall is approximately 450 mm per annum, which is about half the world's average of 860 mm/year. The driest part of South Africa; the Northern Province receives less than 200 mm/year of rainfall and the wettest regions; the eastern Provinces of Mpumalanga, Free state, Gauteng and Kwa Zulu Natal, receive in excess of 2500 mm per annum (Bennie and Hensley, 2001). As there are few natural lakes in South Africa, water supply is dependant on rivers, dams and ground water. Approximately 75 % of water flowing from South Africa into the sea does so along the eastern and southern seaboards where many short rivers occur (Bennie and Hensley, 2001). The Gariep River, the largest river in South Africa, flows from east to west and drains most of the countries water, with majority of the water being sourced in the Drakensberg and Maluti mountains and draining into the Atlantic Ocean on the west coast of Southern Africa (Swanevelder, 2004).

Industrial and agricultural pollutants common in South Africa include agricultural fertilizer, silt, toxic metals, litter, hot water and pesticides. Some of the most common pollutants come from informal settlements which lack adequate sewage and water purification facilities. The resulting pollution contributes to serious health problems e.g., typhoid, cholera and gastroenteritis which are transmitted by water contaminated with untreated sewage. Gastroenteritis is one of the three main causes of death among South African children under the age of five (van der Westhuizen, 2010).

2.2 Study area and study sites

2.2.1 Study area – Description of the Franschhoek valley

The Franschhoek River is located in the Western Cape Province, South Africa. This river rises in the Franschhoek Mountains at an altitude of 1500 m above sea level. The Franschhoek valley receives approximately 1412 mm of rain per annum with a mean annual evaporation rate of 1495 mm. The flows of the Franschhoek River are seasonal with natural high flows being observed during the winter months due to increased precipitation and low flows being observed in summer months. The area is characterized as having a Mediterranean climate as it receives most of its rainfall during winter. It receives the lowest rainfall during February and the highest during the winter month of June. The average midday temperatures for Franschhoek range from 14.6°C in July to 25.3°C in February. The region is the coldest during the evenings of July when the mercury readings decrease to 5.2°C on average and in the mornings just before sunrise (South African Weather Services, 2009).

The river flows along a defined channel for approximately 30 km before it reaches its Berg River confluence further downstream. The catchment geology is that of the Table Mountain Group (TMG) and is composed of quartzitic sandstone and sandy sediments. The river flows through high-density agricultural land, predominantly vineyards with the lower reaches flowing through low-density residential suburbs. The cultivation of grapes and deciduous fruits are the major economic drivers of the Franschhoek area, which has resulted in the removal of the original Fynbos vegetation, which is synonymous with the South Western Cape. The removal of Fynbos has resulted in the proliferation of invasive plant species such as *Acacia mearnsii, Myriophyllium* and *Salix babylonica*, occurring almost throughout the entire river.

2.2.2 Study sites and Plants selected

Ten sites were sampled along the length of the Franschhoek River (Fig. 2.1). For this study, I will refer to the locations on the Franschhoek River as sites (FR1, FR2, FR3….FR10). The plants selected for the study were *Brabejum stellatifolium* (Fig. 2.2), *Acacia mearnsii* (Fig. 2.3) and *Salix babylonica* (Fig. 2.4). Table 2.1 shows the distribution over the study sites.

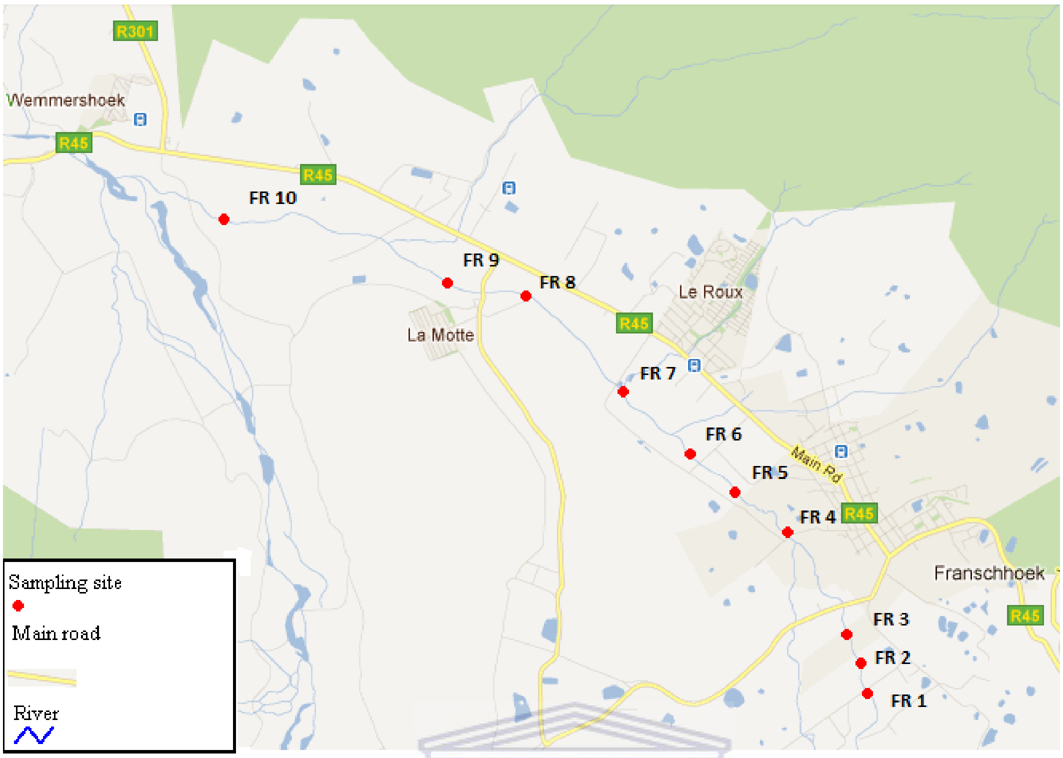


Figure 2.1 Geographical representation of sampling sites along the Franschhoek River, adapted from Google earth.

Location 1 – FR1

Site 1 is located, in the midst of a trout fish farm and is nearest the source of the Franschhoek River. The riparian vegetation is natural and undisturbed with *Brabejum stellatifolium* dominating. The riverbed consisted mainly of boulders and cobble. There were no indications of invasive fauna or flora.

Figure 2.2 fruiting *Brabejum stellatifolium,* commonly referred to as the Wild Almond.

Location 2, 3, 4, 5 and 6 – FR2, FR3, FR4, FR5 and FR6

All of the above locations were situated 3 km – 8.5 km from FR1 respectively. Each of the above mentioned sites was heavily invaded with alien vegetation, mainly *Acacia mearnsii* and *Salix babylonica*. The riverbed at these locations consisted mainly of boulders, cobble and sand. All five of these sampling locations ran adjacent to active vineyards or olive orchards.

Figure 2.3 the invasive tree *Acacia mearnsii.*

Figure 2.4 *Salix babylonica.* commonly referred to as the Weeping willow.

Location 7 – FR7

Site 7 is located approximately 10 km from FR1. This sampling site is located below the outfall (Fig. 2.5) of the municipal Waste Water Treatment Facility. *Acacia mearnsii*, *Salix babylonica* and other invasive macrophytes dominate this area. It too flows adjacent to an active vineyard. The water is murky and slow flowing (Fig. 2.6).

Figure 2.5 Municipal Treated Effluent Outlet located at FR7.

Figure 2.6 Murky River water at FR7

Location 8, 9 and 10 – FR 8, FR9 and FR10.

The sites mentioned above occur 11 km – 15 km from FR1 respectively. Site 8 (Fig. 2.7) has high algal growth with *A. mearnsii* and *S. babylonica*, occurring high up on the riverbank terrace. The riverbank is severely degraded at all of the above sites and with the exception of FR10, which has *B. stellatifolium*; the two remaining sampling locations (FR9 and FR10) show no evidence of indigenous vegetation apart from the *B. stellatifolium* at FR 10.

Figure 2.7 FR8 shows high algal growth, it is situated 1km downstream from the treated effluent outlet.

Table 2.1 Indicates all sites along the Franschhoek River, their geographical co ordinates and the various species of leaves collected per study site.

Site	Research sites	Location	Plant leaves collected.
1	Three streams	S:33º 95019 E:019º 10548	*B. stellatifolium*
2	Boekenhoudskloof	S:33º.94046 E:019º.11024	*B. stellatifolium ; A. mearnsii*
3	La Dauphine	S:33º.93025 E:019º.11317	*S. babylonica ; A. mearnsii*
4	La Corrone	S:33º.92046 E:019º.11205	*S. babylonica* *S. babylonica ; A. mearnsii*
5	Cabrierre estate	S:33º.91251 E:019º.11169	*S. babylonica ; A. mearnsii*
6	Kleindassenberg	S:33º.90583 E:019º.10223	*S. babylonica ; A. mearnsii; B.stellatifolium*
7	Rickety Bridge	S:33º.89935 E:019º.09305	*S. babylonica ; A. mearnsii*
8	Below the Roberstvlei tributary	S:33 º.89093 E:019 º.07903	*S. babylonica ; A. mearnsii*
9	Moreson	S:33º.88623 E:019º.076679	*S. babylonica ; A. mearnsii*
10	Franschhoek River confluence point.	S:33º.88309 E:019º.04754	*S. babylonica ; A. mearnsii ; B.stellatifolium*

2.3 Methodology

2.3.1 Sampling design

In this study, an inorganic chemical analysis of plant, water and sediment fractions along the Franschhoek River was conducted in order to obtain baseline data. Plant and water samples were collected from 10 predetermined sites along the Franschhoek River once a month for 12 months. Sampling commenced in February 2009 and continued for the duration of a year until April 2010. Un foreseen circumstances and road closures prevented sampling in May and November of 2009, sampling therefore continued for an additional two months until April 2010. Soil was collected twice; once in the summer of April 2009 and the winter of August 2009. The chosen sampling design allows for 1) heavy metal and nutrient comparison in plants, water and soil along the length of the Franschhoek River 2) assessing which medium (plants, water or sediment) is best for determining heavy metal and nutrient status of the river.

2.3.2 Data collection.

2.3.2a – Water

Water samples were collected at each site and stored in 300 ml plastic bottles. Dissolved oxygen content (% and $mg.l^{-1}$) and temperature were determined *in situ* using a hand held YSL model 55 and electrical conductivity was determined using a portable conductivity meter (Metrohm 644 conductivity, Switzerland). Water samples were stored in a refrigerator at 4 $^{\circ}$C to prevent algal growth.

2.3.2b – Leaves

Leaves from the indigenous tree *Brabejum stellatifolium* and the invasive trees *Acacia mearnsii* and *Salix babylonica,* were collected at the respective sites (Table 2.1). Healthy mature leaves were collected from four branches of the same shrub and tree and transferred to brown paper bags for transport to the laboratory.

2.3.2c – Sediment

Sediment was obtained from the soil-water interface of the river with a hand held garden shovel to a depth of 10 cm. The collected sediment was immediately stored in a zip lock bag and taken to the lab for further analysis. Soil samples were air dried and passed through a 2 mm sieve to remove excess debris and larger earth particles and stored in a cool dry area until further analysis.

2.4. Laboratory procedures.

2.4.1 Pretreatment of samples.

- **Water**

Water samples were filtered and pH was determined using a pH meter (PHM 64 Research meter, Radiometer Copenhagen), in order to measure H^+ ion activity. Nitrate, nitrite and ammonia levels were determined utilizing Aquamerck Reagent Kits. The ammonia, nitrate and nitrite tests were determined reflectrometrically using the Aquamerck ®11 117 Ammonium Tests, the Aquamerck ® 8032 Nitrate and the Aquamerck ® 8025 Nitrate MERCK test strips.

- **Plants**

The harvested leaves of *S. babylonica, A. mearnsii* and *B. stellatifolium,* were rinsed in distilled water and placed in an oven at 70°C to dry over a minimum of 24 hours and maximum of 36 hours. The dried leaves were milled utilizing a Wiley Mill and stored till further analysis. A 0.4 g sample of the milled leaves was weighed out and placed in cigarette paper; the samples were then digested using a sulphuric peroxide solution.

- **Sediment**

Sediment samples were air dried and passed through a 2 mm sieve to remove excess debris and larger earth particles. A 1 g sample of air dried sediment was weighed out, placed in cigarette paper, and digested using the conventional 3HCl:1HNO_3 aqua regia.

2.4.2 Acid digestion and Atomic Absorption Spectrophotometry (A.A.S)

2.4.2.1 Methodology – Sulphuric peroxide solution.

- **Leaves**

The digestion mixture of sulfuric acid and hydrogen peroxide was prepared as in (Allen *et al*, 1986). Due to the exothermic nature of the reactions, the mixture was prepared on ice. A sample of 0.4 g of dried milled leaves was added to digestible

cigarette filter paper and placed in an appropriately labelled digestion tube flask, to which 4.4 ml of sulphuric peroxide solution was added (Allen *et al*, 1986).
The samples were then placed in a digestion block with an initial temperature of 150°C. The temperature was increased every hour with an additional 50°C until a final temperature of 350°C was reached. The digestion was complete when a cloudy to colourless solution was obtained. The mixture was left to cool and filtered through Whatman no 42 paper and diluted with distilled water to volume in a 100 ml flask. Blank solutions were prepared following the same procedures. The digested plant material was analysed for Ca, Cd Cu, Fe, Pb, Mg, K, Na and Zn which was carried out utilizing a Unicam Solaar M Series Atomic Absorption Spectrophotometer.

Phosphorus was determined commercially by Elsenburg, Department of Agriculture, using ICP (Thermo Spectrometer, iCAP 6000 series). Nitrogen was determined utilizing a BUCHI Kjaldahl distillation unit K-350 and titration.

2.4.2.2 Methodology – HCl: HNO3 aqua regia

- **Sediment**

A 3:1 HCl: HNO_3 aqua regia digestion was used to digest sediment fractions. A 1.00g sample of sediment was placed in an appropriately labelled digestion tube to which 12 ml of aqua regia was added. The sample was then placed in a heating block for 3 h at 110°C. After evaporation, just before a precipitate was formed the sample was diluted with 20 ml of 2% (v/v with H_2O) nitric acid. The samples were filtered through Whatman no. 42 paper and diluted to 100 ml volume with distilled water. Blank solutions were prepared following the same procedures without the 1.00g of sediment. Sediment solutions were analyzed for Ca, Cd Cu, Fe, Pb, Mg, K, Na, Zn, using a Unicam Solaar M Series Atomic Absorption Spectrometer (AAS) with an air/acetylene flame system.

The nitrogen percentage present was calculated as follows:

%N = (Vol. HCl – Controls average ml HCL) * NHCl * 14007 / sample mass (mg)

Phosphorus was determined commercially by Elsenburg, Department of Agriculture, using ICP (Thermo Spectrometer, iCAP 6000 series). Nitrogen was determined according to Bremner and Mulvaney (1982) utilizing a BUCHI Kjaldahl distillation unit K-350 and titration.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 WATER

3.1.1 Electrical conductivity

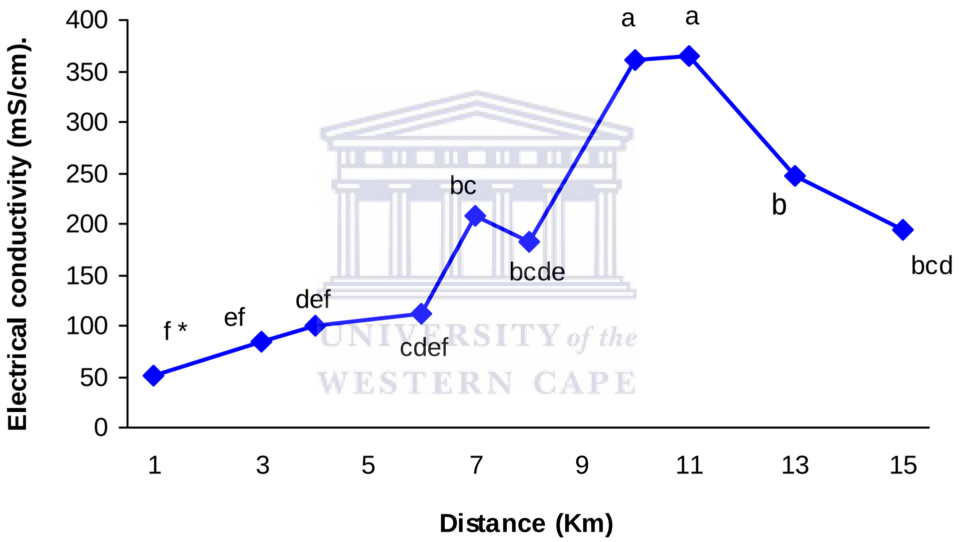
Electrical conductivity (EC) is a useful parameter for estimating the salinity of water, as it is often used to measure mineral concentration in an aqueous solution (Ekelemu and Zelibe, 2006). Various factors affect EC such as water temperature, the concentration of ions present in the water body (the higher the dissolved material in the water, the higher the EC), as well as the nature of the ions (Hayashi, 2004).

Electrical conductivity fluctuated throughout the length of the river (Fig3.1.1A) ranging between 51.92 mS.cm^{-1} – 365.25 mS.cm^{-1}. The lowest mean EC was recorded at a distance of 1km and the highest at 10km respectively; this coincides with the temperatures observed in Fig 3.1.3A (as temperature increases so does electrical conductivity). According to Ogbeibu and Egborge (1995), electrical conductivity is a measure of an aqueous solutions total ionic content. A value below 100 mS.cm^{-1} indicates a fresh water body.

Figure 3.1.1B shows the seasonal variation of electrical conductivity throughout the duration of the study. Electrical conductivity ranged between 83.7 0 mS/cm – 334.60 mS/cm. The highest EC was observed during March 2009 (day 32) and lowest EC in October of 2009 (day 252), rainfall throughout the months of September and October 2009 in the Franschhoek Valley, may be the cause of the drop in EC. A second spike in EC is observed at day 378 (summer, February 2010), this coincides with the seasonal temperature results observed in Fig 3.1.3B (As temperature increases, electrical conductivity increases). From the results obtained, EC decreased during the wet winter months and increased as summer approached.

The South African Water Quality Guideline for electrical conductivity in water to be used for domestic water supply is 0.7 $mS.cm^{-1}$ (DWAF, 1996 a & e).

A



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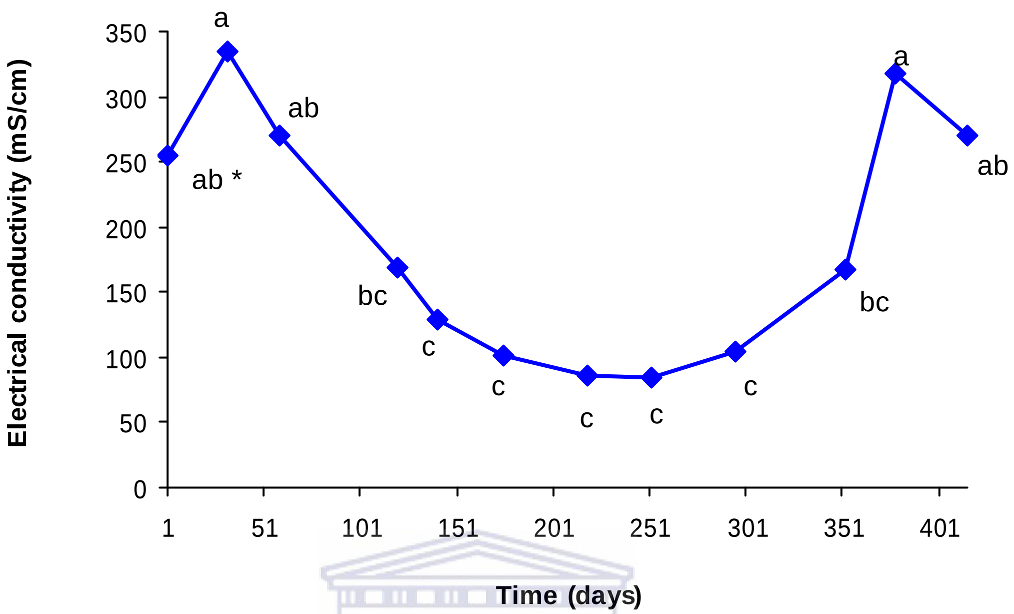


Figure 3.1.1 Means* with the same letters are not significantly different at p=0.05. (A) Electrical conductivity (mS.cm^{-1}) along the length of the Franschhoek River over the sampling period (2009 - 2010). (B) Seasonal variation of electrical conductivity (mS.cm^{-1}) in the Franschhoek River, for the duration of the study period

3.1.2 pH

The pH of an aqueous solution is an important indicator of its quality (Begum *et al.*, 2009). The pH is a measure of the acidity of a medium, based on its H^+ ion concentration. H^+ ions are inversely proportional to pH, hence as the H^+ ions increase pH decreases and as the H^+ ions decrease, pH increases. (Begum *et al.*, 2009).

The pH ranged 6.07 – 7.00 throughout the catchment, which is well within the livable range for aquatic organisms (Abowei, 2009). Sampling site 1 (1km) had the lowest mean pH of 6.07; this site has the purest water quality with virtually no inorganic pollution, 10km downstream from site 1, had the highest pH of 7.00 (Fig 3.1.2 A). This sampling site has a small yet steady stream of treated effluent being discharged into it; increased inorganic pollution often increases H^+ ion activity.

Figure 3.1.2 B indicates the seasonal variation of pH throughout the study period. The seasonal variation in pH ranges between 6.00 – 7.00. The lowest pH was observed at day 218 (September). This value coincides with the month which received excessive rainfall, which resulted in increased flow regimes and may have diluted the river system, flushing out many of the pollutants which contribute to a decreasing pH. The highest pH was observed at day 120. Increased agricultural run-off from surrounding vineyards, due to early autumn rains could be the cause of the increase in pH, as inorganic pollutants often increase H^+ ion concentrations.

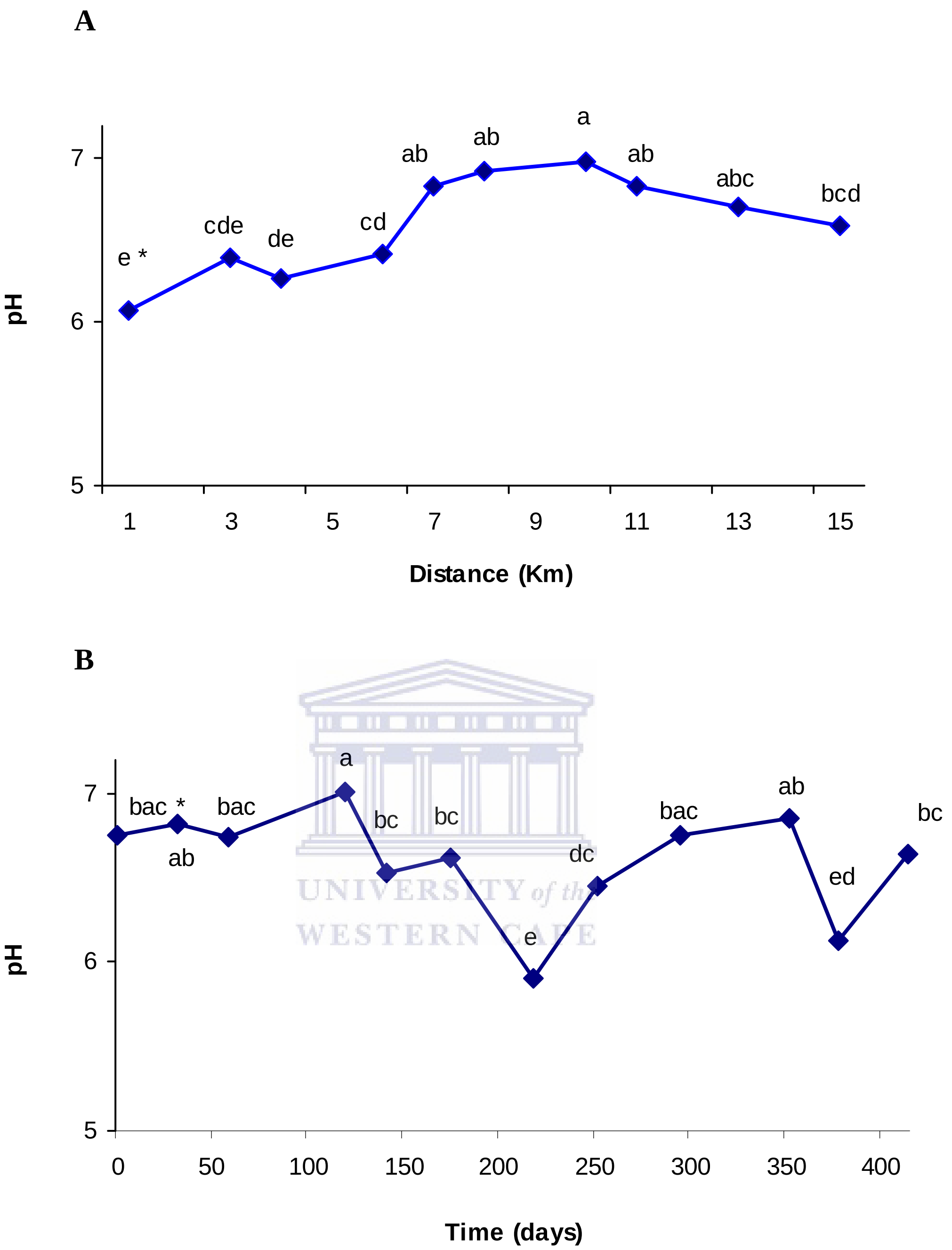


Figure 3.1.2 Means* with the same letters are not significantly different at p=0.05. (A) The pH along the length of the Franschhoek River over the sampling period (2009 - 2010). (B) Seasonal variation in pH in the Franschhoek River, for the duration of the study period.

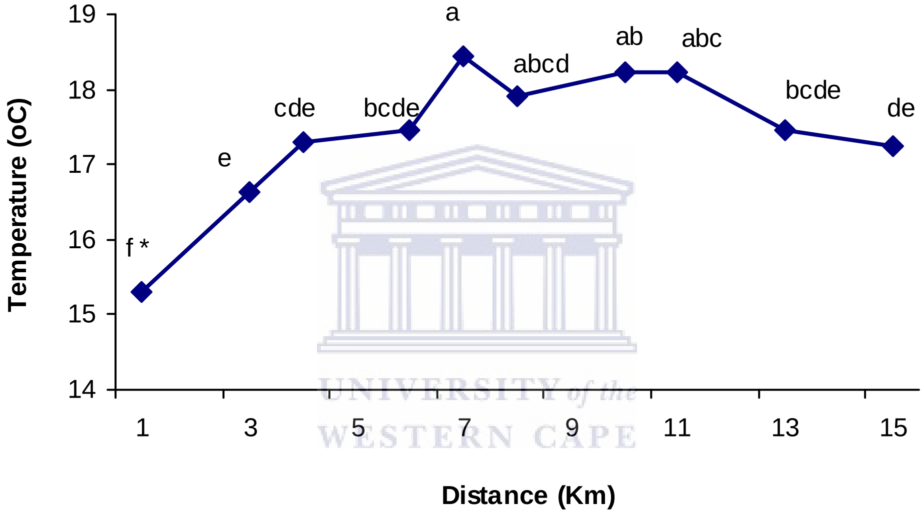
3.1.3 Surface water temperature.

Temperature plays an important role in aquatic ecosystems as it affects the rate of chemical reactions and hence the metabolic rate of organisms. The temperatures of inland riverine waters usually range 5 ^{o} C – 30 ^{o} C and is often dependant on an array of factors such as; the source of the water, rate of flow, climatic factors as well as structural characteristics of the catchment (DWAF, 1996).

Water temperature ranged between 15.3 $^{\circ}$C and 18.5 ºC along the length of the river (Fig 3.1.3A). The highest mean temperature of 18.5 $^{\circ}$C could be attributed to low canopy cover and slow flow rates. Ten km downstream (site 7) has equally high water temperatures and this could be attributed to the treated effluent being discharged at this site from the Stiebieul Waste Water Treatment Facility (WWTF). The lowest mean temperature of 15.3°C was observed at 1 km. This coincides with previously observed pH and EC readings of the same site. Upstream water temperatures, are significantly lower than those further downstream.

Figure 3.1.3B indicates the seasonal fluctuations of the water temperature. The mean seasonal water temperature ranged between 13.5 $^{\circ}$C and 21.7 $^{\circ}$C. The two highest mean temperatures recorded, occurred during the summer months of February 2009 (1d) and February 2010 (378d). Water temperature decreased steadily from day 1 until day 141(wet season) and increased from day 175 until day 378 (dry season). The lowest mean temperature of 13.5 °C was recorded during the winter month of June 2009. Results indicate that increased solar radiation during the summer months increase water temperatures of shallow waters, which in turn affects water temperature. The summer months displayed seasonal temperatures which were significantly different to the wet winter months.

A



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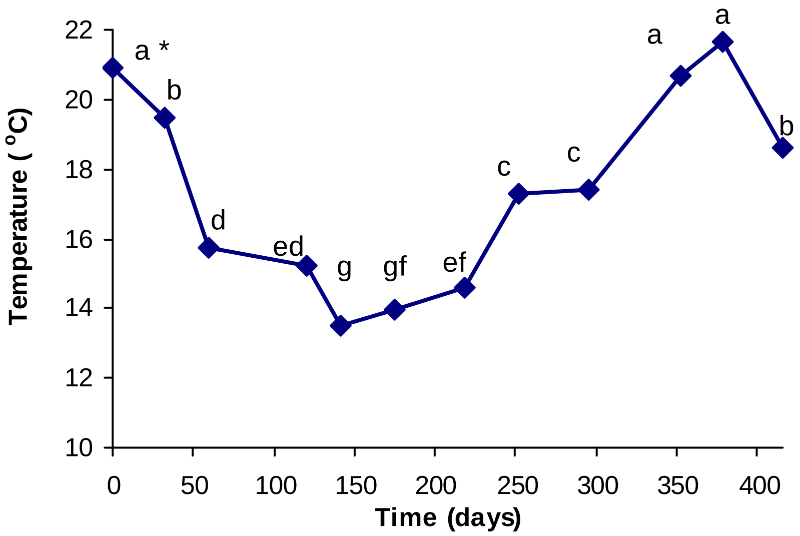


Figure 3.1.3 Means* with the same letters are not significantly different at p=0.05. (A) Surface water temperature (°C) along the length of the Franschhoek River over the duration of the sampling period (2009 - 2010). (B) Seasonal variation in surface water temperature (°C) in the Franschhoek River, for the duration of the study period.

3.1.4 Oxygen concentration

Oxygen is required for the survival of all aquatic organisms and is among one of the most important parameters for determining water quality (Araoye, 2009). In aquatic environments oxygen enters the water via the atmosphere or via phytoplankton (Diaz 2001) and is regulated by the rate of photosynthesis, light, abundance of plants and animals, water temperature and turbidity (Araoye, 2007).

Dissolved oxygen (DO) reflects the balance between oxygen consumption and oxygen production and can be used to determine the spatial and temporal distribution of many aquatic organisms. When oxygen supply does not meet oxygen demand and the levels of oxygen decline to a point that can no longer sustain life, a condition referred to as hypoxia sets in. Hypoxia occurs when water stratification and an excess of organic decomposition occur in concert. The accepted DO levels for aquatic environments is > 5mg/l (ANZECC, 2005), when DO decreases to 5mg/l mobile organisms may move to areas of higher DO concentration. When levels drop to <5mg/l severe stress and possible death may ensue (ANZECC, 2005). Low DO promotes the release of phosphorus and heavy metals and prevents the detoxification of ammonia by nitrification (Diaz 2001 and Araoye, 2009).

The mean range of dissolved oxygen along the length of the Franschhoek River was 4.77 – 8.31 mg/l (Fig 3.1.4A). Minimum and maximum means were obtained at sampling site 7 (10 km) and 10 (15 km) respectively. There was no inter study site variation between sampling sites 1 – 6 ($p \leq 0.05$). The low oxygen content at the WWTF (10 Km) may be attributed to decomposition of organic material, chemical breakdown of pollutants, decreased turbidity, increased microorganism activity and factors previously mentioned which all contribute to low dissolved oxygen content. It is well documented that aerobic microorganisms in chemical effluent reduce O_2 levels (Vega *et al*. 1998). In addition this sampling site was inundated with invasive plant species. The low oxygen content at this location corresponds with increased EC (Fig 3.1.1A), increased pH (Fig 3.1.2A) as well as elevated temperatures (Fig 3.1.3A). Higher temperatures reduce the solubility of dissolved oxygen in water, decreasing its concentration and thus its availability to aquatic organisms (Solheim, 2010).The observed oxygen content at sampling site 7 (10km) is below the accepted levels for aquatic environments (<5mg/l). Based on this parameter, sampling location 7 (10km) is not suitable for sustaining aquatic life.

Oxygen concentrations fluctuated seasonally with mean oxygen concentrations ranging 2.50 mg/l – 9.16 mg/l. Maximum oxygen concentrations was obtained at 218d and minimum concentrations at 415d (Fig 3.1.4B). Maximum concentrations were obtained in autumn, winter and early spring and lower concentrations were recorded in the dry summer season. Oxygen content and temperature are highly correlated, this can be observed at 415d (Fig 3.1.3 B), as temperature increases the oxygen content decreases. In addition, during summer months the consumption of oxygen by sediment as well as water column respiration which may exceed re-supply via photosynthesis, often leads to increased respiration resulting in a seasonal decline of oxygen. The increase in O_2 in the wet winter seasons could be attributed to the increased rainfall which increased stream flow, turbidity and mixing of the water column.

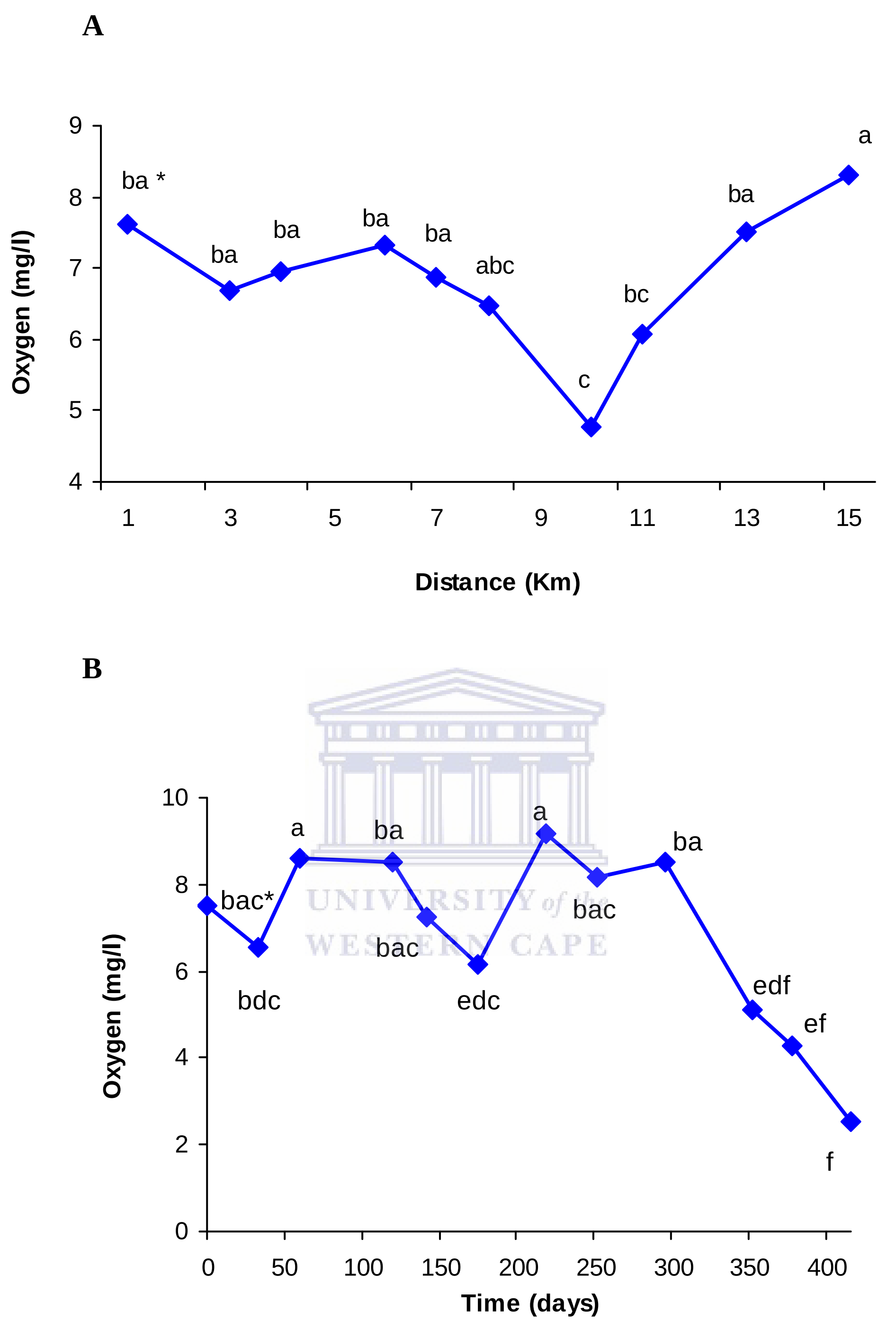


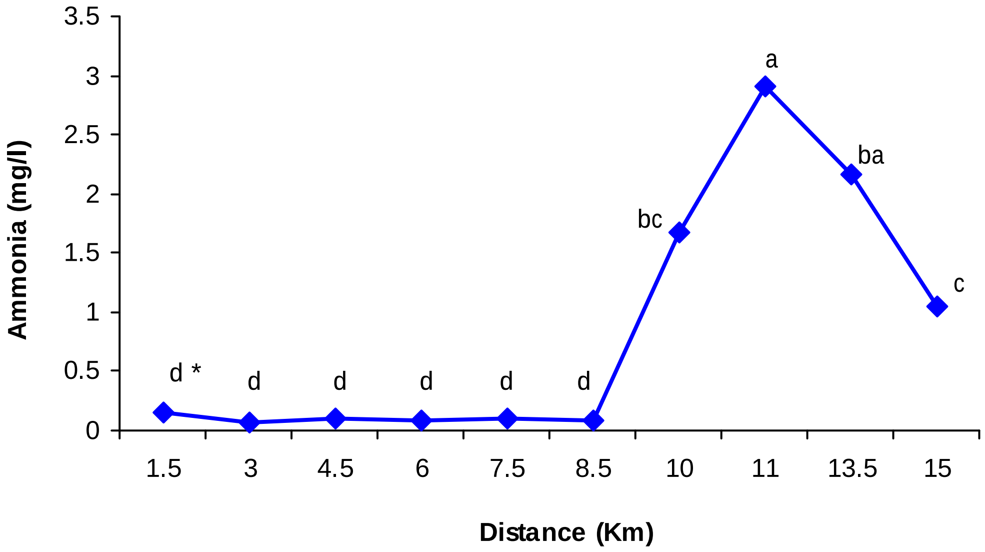
Figure 3.1.4 Means* with the same letters are not significantly different at p=0.05. (A) Oxygen (mg/l) along the length of the Franschhoek River over the duration of the sampling period (2009- 2010). (B) Seasonal variation in oxygen (mg/l) in the Franschhoek River, for the duration of the study period.

3.1.5 Ammonia concentrations

Ammonia concentrations in the study area were between 0.07 mg/l – 2.90 mg/l (Figure 3.1.5A). No significant variation was observed between sampling sites 1 – 6. The lowest mean ammonia concentration was recorded at sampling location 2 and the highest mean ammonia at site 8. The high levels of ammonia at this location could be due to a breakdown of organic material from the previous site just 1 km upstream, were the WWTF is located. High levels of ammonia may originate from the municipal wastewater discharge, run-off from surrounding agricultural land and vineyards. Downstream ammonia concentrations are significantly higher ($p=0.05$), than those upstream of sampling site 7.

Seasonal ammonia concentrations ranged of 0.05 mg/l – 1.87 mg/l (Figure 3.1.5B). The lowest mean ammonia concentration was recorded at day 141 (June 2009) and the highest at day 352 (January 2010). Ammonia levels were at their highest during the summer months (32, 352, 378 and 415 days) this may be attributed to decreased flow regimes, increased temperature and decreased oxygen content of the water column during the dry summer months, which resulted in an accumulation of organic material.

A



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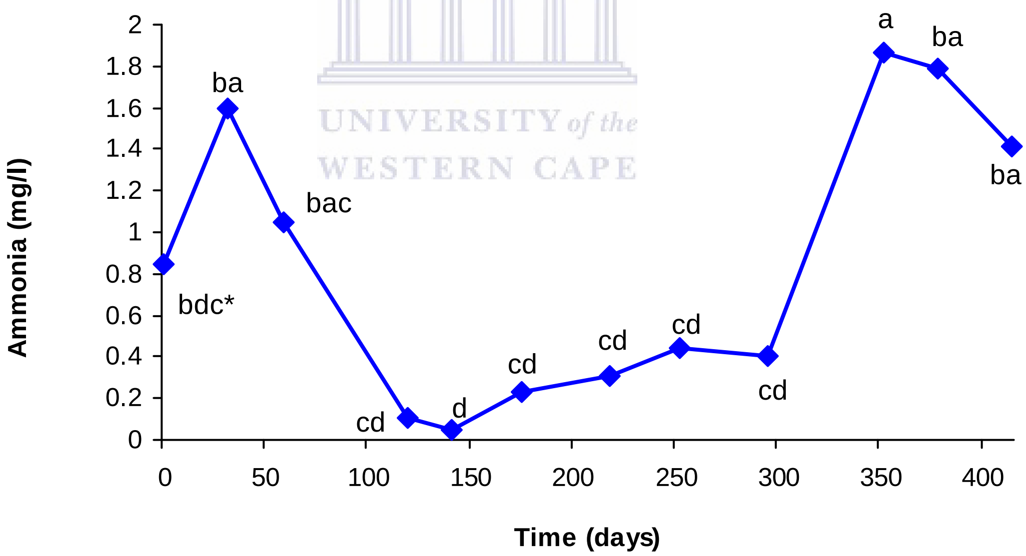


Figure 3.1.5 Means* with the same letters are not significantly different at p=0.05. (A) Ammonia (mg/l) along the length of on the Franschhoek River over the duration of the sampling period (2009 - 2010). (B) Seasonal variation in ammonia (mg/l) in the Franschhoek River for the duration of the study period.

3.1.6 Nitrate

Ammonia (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-) are the most common forms of dissolved inorganic nitrogen present in aquatic environments. Nitrogen may enter aquatic environments via atmospheric deposition, surface and groundwater run-off, the dissolution of nitrogen rich geological deposits and nitrogen fixation by certain prokaryotes (Camargo and Alonso, 2007).

The presence of these ions can be both spatial and temporal. Humans have altered the nitrogen cycle (Carpenter *et al.* 1998), thereby increasing the availability and mobility of global nitrogen, resulting in increased point and non point source pollution. Increased NH_4^+, NO_3^- and NO_2^- concentrations can enhance the proliferation of primary producers resulting in eutrophication of aquatic ecosystems leading to overproduction of organic matter with the resulting decomposition resulting in decreased DO concentrations. Eutrophication degrades water sources making it unsuitable for uses such as irrigation, industry, agriculture and recreation (Carpenter *et al*. 1998).

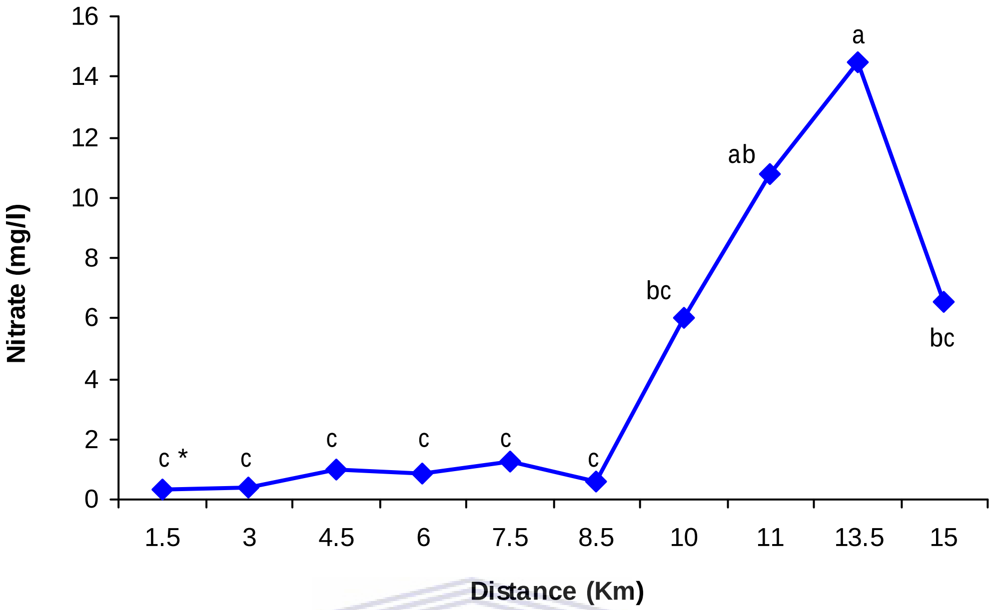
Ionized NH_4^+ is dependent on pH and water temperature and is highly toxic in its unionized form. Nitrite and nitrate toxicity in fish occurs when the ions oxidize iron atoms, converting them from oxygen carrying hemoglobin to methemoglobin; which cannot transport oxygen to body tissue. The accepted nitrate level for aquatic environments is 2 mg NO_3^-N/l.

Mean nitrate concentrations ranged 0.333 mg/l – 14.5 mg/l (Fig 3.16 A). The lowest mean nitrate concentration was recorded at sampling location 1 (1km). This low concentration was expected as this is the least polluted site, located nearest the origin of the Franschhoek River. Sampling location 9 displayed the highest mean nitrate concentrations of 14.50 mg/l. The nitrate concentrations varied considerably between upstream and downstream locations.

Sampling locations 1 – 6 (1 km – 8.5 km) displayed values < 2mg/l, whereas sampling locations 7 – 10 (10 km – 15 km) displayed concentrations ranging 6 mg/l– 14.50 mg/l. These two extremes suggest that nitrate was significantly correlated with the proportion of the downstream area that was occupied by productive vineyards and waste water treatment effluent. Sampling location 9 had high algal growth and invasive plant species which may explain the high concentration of nitrates. In addition, the inputs from the WWTF and afore mentioned factors all acting in concert may be the explanation as to the elevated nitrate concentrations from sampling locations 7 – 10 (10 km – 15 km). There is no significant variation between locations 1-6 ($p \leq 0.05$). These sites fall within the accepted nitrate levels of 2 mg NO_3^- N/l. The remaining sites display nitrate levels unsuitable for aquatic life.

Seasonal nitrate concentrations ranged 0.10 mg/l – 13.80 mg/l, with no significant difference in concentrations being observed between day 0 and 350 (Fig 3.1.6 B). The lowest mean nitrate was recorded at day 295 (summer, 2009) and the highest reading was recorded at day 378 (summer, 2010).

A



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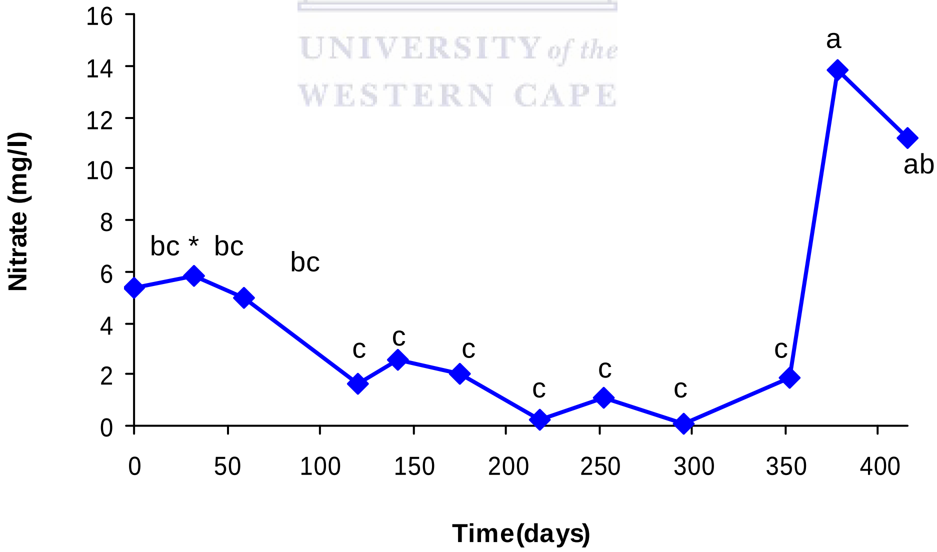


Figure 3.1.6 Means* with the same letters are not significantly different at p=0.05. (A) Nitrate (mg/l) along the length of the Franschhoek River over the duration of the sampling period (2009 - 2010). (B) Seasonal variation in nitrate (mg/l) in the Franschhoek River, for the duration of the study period.

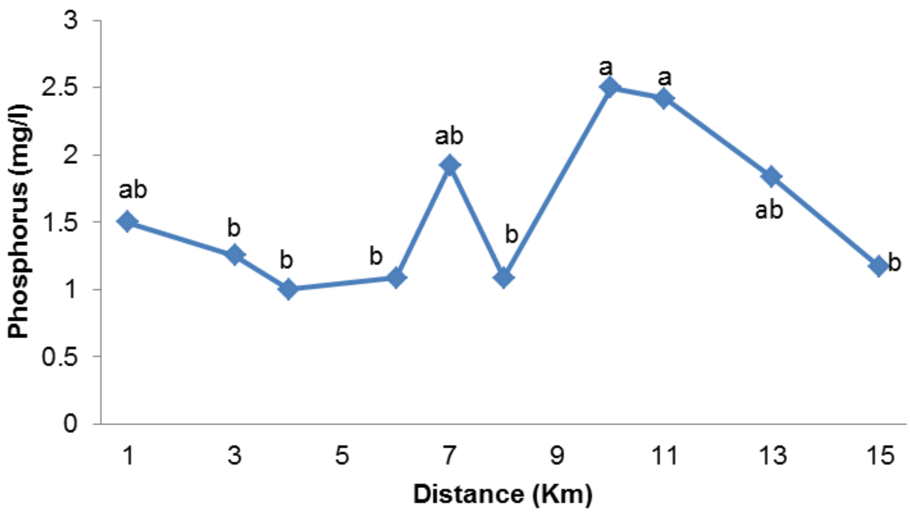
3.1.7 Phosphorus

Agricultural activities are a major source of phosphorus (P) in riverine ecosystems (Carpenter *et al.* 1998). Nutrient enrichment by P is often deleterious to aquatic ecosystems as it is the primary cause of eutrophication, decreases oxygen levels and contributes to toxic algal blooms. The Franschhoek Valley has been transformed to agricultural land and is therefore subjected to P inputs from fertilizers and manure. When these inputs exceed P outputs, bioaccumulation of P in the soil will occur, resulting in constant leaching into surrounding surface waters (Smith *et al.* 1999).

Mean phosphorus levels ranged 1.08 mg/l – 2.50 mg/l (Fig 3.1.7A), throughout the length of the river. The lowest mean P concentration was recorded at 6 km and 8.5 km respectively and the highest values at the WWTF (10 Km). It has been well documented in the literature that point source pollution from municipal sewage effluents is a major anthropogenic source of P (Vitosek *et al.* 1997; Carpenter *et al*, 1998; Howarth *et al*, 2000; Galloway and Cowling, 2002). P levels are higher post the WWTF this could be attributed to an increase in P load and encroachment by invasive plant species.

Mean seasonal P concentrations ranged 1 mg/l – 2.4 mg/l respectively. Phosphorus shows similar trends to ammonia, with an increase in concentration being observed in the dry summer months and decreased concentrations in the wet winter months. The lowest values were recorded from day 141 to 252 and the highest at day 49 and 415. Phosphorus levels were at their highest during the summer, and may be attributed to decreased flow regimes and accumulation of organic material.

A



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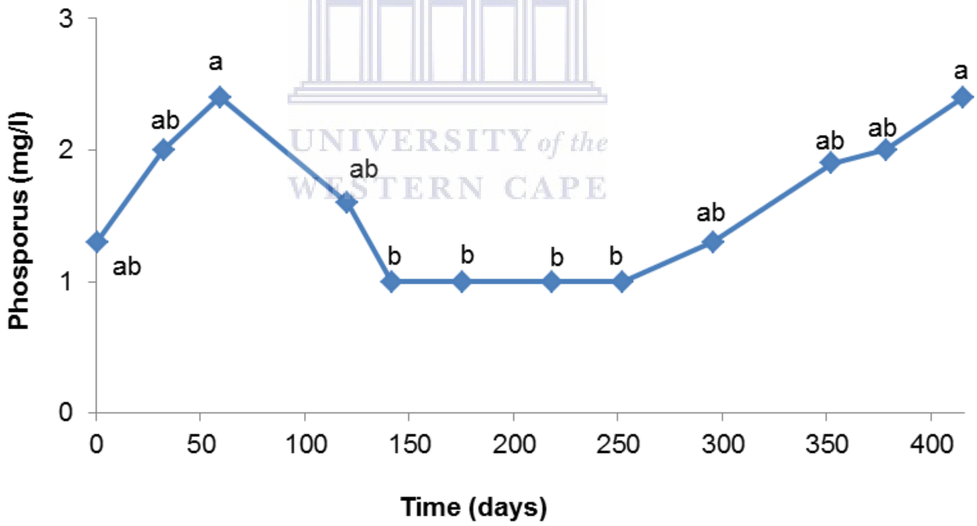


Figure 3.1.7 Means with the same letters are not significantly different at p=0.05. (A) Phosphorus (mg/l) along the length of on the Franschhoek River over the duration of the sampling period (2009 - 2010). (B) Seasonal variation in phosphorus (mg/l) in the Franschhoek River, for the duration of the study period.

3.1.8 Cadmium

Cadmium (Cd) is a chemical toxic to all living organisms because of its bioaccumulation abilities. Cd has a low solubility at alkaline and neutral pH and a high solubility under acidic conditions. Cd is chemically very similar to zinc and together with selenium often interact together. According to DWAF (1996b), Cd concentrations should be interpreted in conjunction with Zn concentrations. In nature, the Zn: Cd ratio is usually 300:1 (DWAF 1996b; WHO 1993).

Mean Cd concentrations ranged between 0.0012 mg/l – 0.26 mg/l. The lowest recorded value was noted at 3 km and the highest Cd concentrations were noted at 11 km. A significant difference in Cd concentrations was noted between the minimum and maximum concentrations (Fig 3.1.8 A). Sampling site 8 (11 km), is located 1 km downstream from a Waste Water Treatment Facility (WWTF), which releases treated effluent. Cadmium concentrations increased from 10 km – 11 km, before decreasing. Cadmium levels appeared lower upstream (before the WWTF) (10 km), and higher downs (after the WWTF).

Cadmium concentrations for the duration of the study period ranged between 0.0016 mg/l – 0.55 mg/l (Fig 3.1.8 B). Maximum Cd uptake was noted during summer at day 32 and showed a significant variation in concentration when compared with the remainder of the study. No additional significant variation was noted.

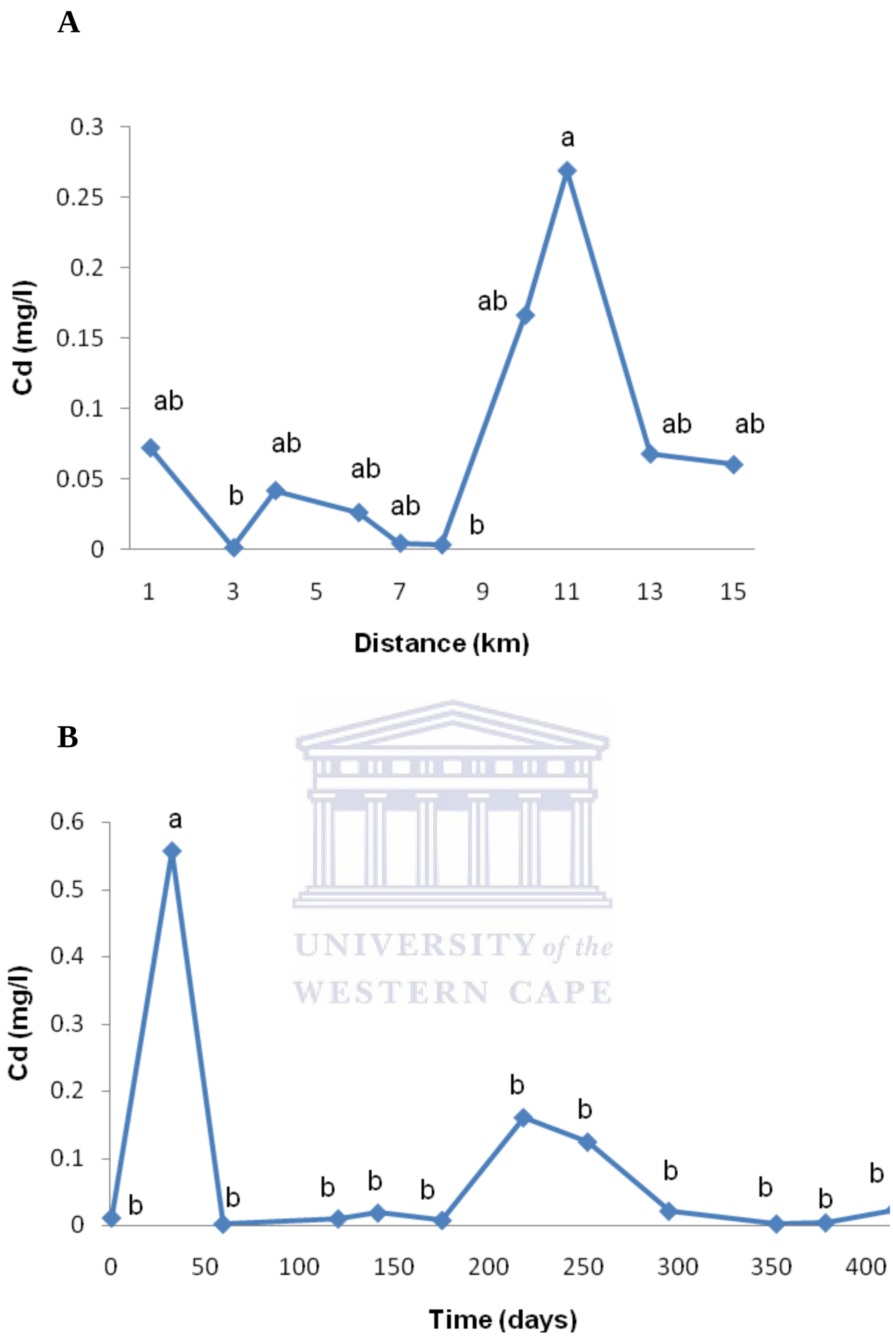


Figure 3.1.8 Means with the same letters are not significantly different at p=0.05. (A) Cadmium (mg/l) along the length of on the Franschhoek River over the duration of the sampling period (2009 - 2010). (B) Seasonal variation in Cadmium (mg/l) in the Franschhoek River, for the duration of the study period.

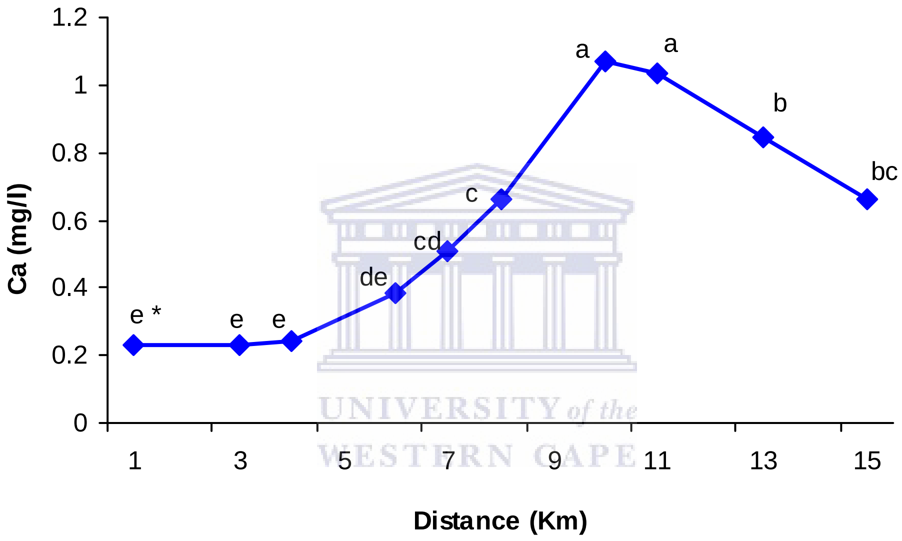
3.1.9 Calcium

Calcium (Ca) is an alkali earth metal which occurs naturally in water and is an essential element for all living organisms (Dallas and Day, 2004). Sources of calcium include mineral deposits such as calcium carbonate, phosphate and sulfates. The solubility of calcium in water is strongly governed by pH and temperature and often influences the absorption and toxicity of certain heavy metals (Orzepowski and Pulikowski, 2008). Unpolluted surface waters typically display calcium concentrations of 15mg/l (DWAF, 1996b).

The mean Ca concentration in the surface water varied significantly as it ranged between 0.23 mg/l – 1.07 mg/l (Fig 3.1.9A). The highest recorded value was obtained at sampling location 7 at the Waste Water Treatment Facility (WWTF). It is noteworthy that the values obtained here were 2 – 3 fold higher than that of the sites located upstream of the WWTF (Fig 3.1.9A). Ca is the primary flocculent ingredient in the treatment of effluent, and could be the explanation for the elevated levels of Ca at this sampling location. A secondary explanation as to the elevated Ca levels could be ascribed to pH levels. According to van Lonn (2007), it is the acidic pH which is an important factor of Ca content, hence processes increasing pH often result in increased Ca levels. This is corroborated by the pH values obtained for the study (Fig 3.1.2A). Sampling sites displaying high pH values displayed high Ca values and sampling sites displaying low pH values had a lower Ca content. Based on the SA Water Guideline parameters, Ca does not pose a threat to this riverine ecosystem as concentrations are well below 15 mg/l.

During the study period mean Ca values ranged between 0.40 mg/l – 0.87 mg/l (Fig3.1.9B). The highest concentration was measured at day 59 and 415 (summer) and the lowest Ca content was observed at day 120 - 295 (winter). From the results obtained there was significant variation between minimum and maximum seasonal Ca concentrations.

A



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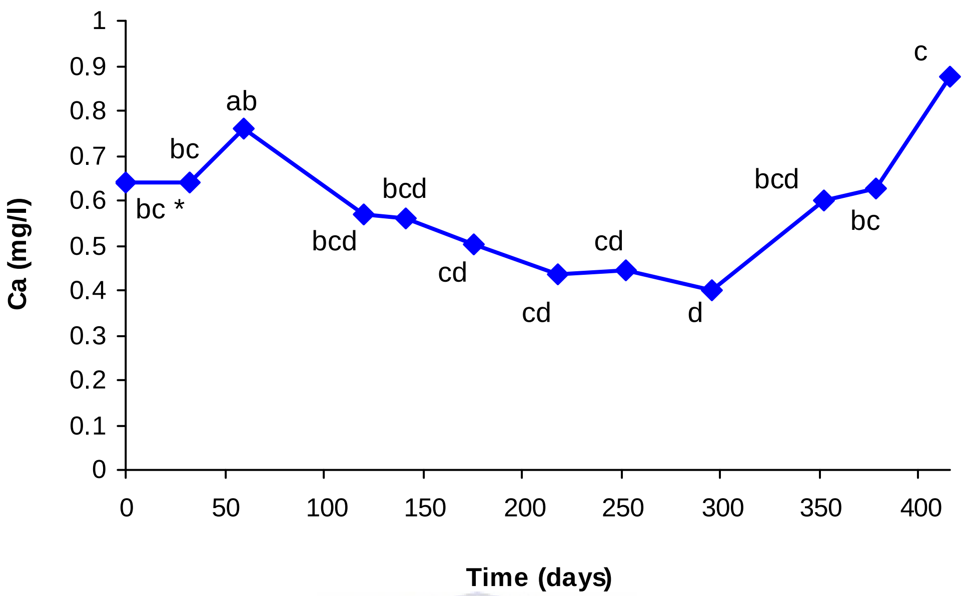


Figure 3.1.9 Means* with the same letters are not significantly different at p=0.05. (A) Calcium concentrations (mg/l) at the sampling sites on the Franschhoek River over the sampling period (2009 - 2010). (B) Seasonal variation of calcium in the Franschhoek River, for the duration of the study period.

3.1.10 Copper concentrations

The main sources of copper (Cu) in aquatic ecosystems includes weathering of geological structures, effluent from sewage treatment facilities, fertilizers and Cu based fungicides (DWAF, 1996d and Calvino *et al*. 2008). According to the South African Water Quality Guidelines, the Target Water Quality Range for copper in aquatic ecosystems is less than 0.3 µg/l in soft water and below 0.8 µg/l in medium soft water.

The mean Cu concentrations ranged between 0.00039 mg/l – 0.0038 mg/l (Fig 3.1.10A). With the exception of sampling location 10, no significant variation in Cu concentrations was observed along the length of the Franschhoek River. The highest reading of 0.0038 mg/l was observed at site 10 (15 km). The sudden increase in Cu may be attributed to the use of fertilizers and manure which increase Cu concentrations especially in cultivated areas.

Copper displayed minimal seasonal variation ranging from 0.01 mg/l – 0.10 mg/l (Fig 3.1.10B). Copper concentrations remained relatively stable and displayed minimal variation ($p = 0.05$), throughout the duration of the study period (2009 – 2010). The increase in Cu at 352d may be a result of the heavy rainfall experienced that month, which may have resulted in increased run-off from surrounding agricultural land. With the exception of the Cu concentration recorded at 352 d, no significant variation in the seasonal Cu concentration were recorded (Fig 3.1.10B).

According to Kabata-Pendias and Pendias (2001), rivers in close proximity to cultivated areas often display elevated copper concentrations as copper can reach water bodies in their soluble form and through erosion in colloid bound forms which accumulate in the soil below the water body. The copper concentrations in the water samples did not exceed the SA Water Quality Guideline of 0.8 µg/l (DWAF, 1996 b), copper therefore does not pose a threat to this riverine ecosystem.

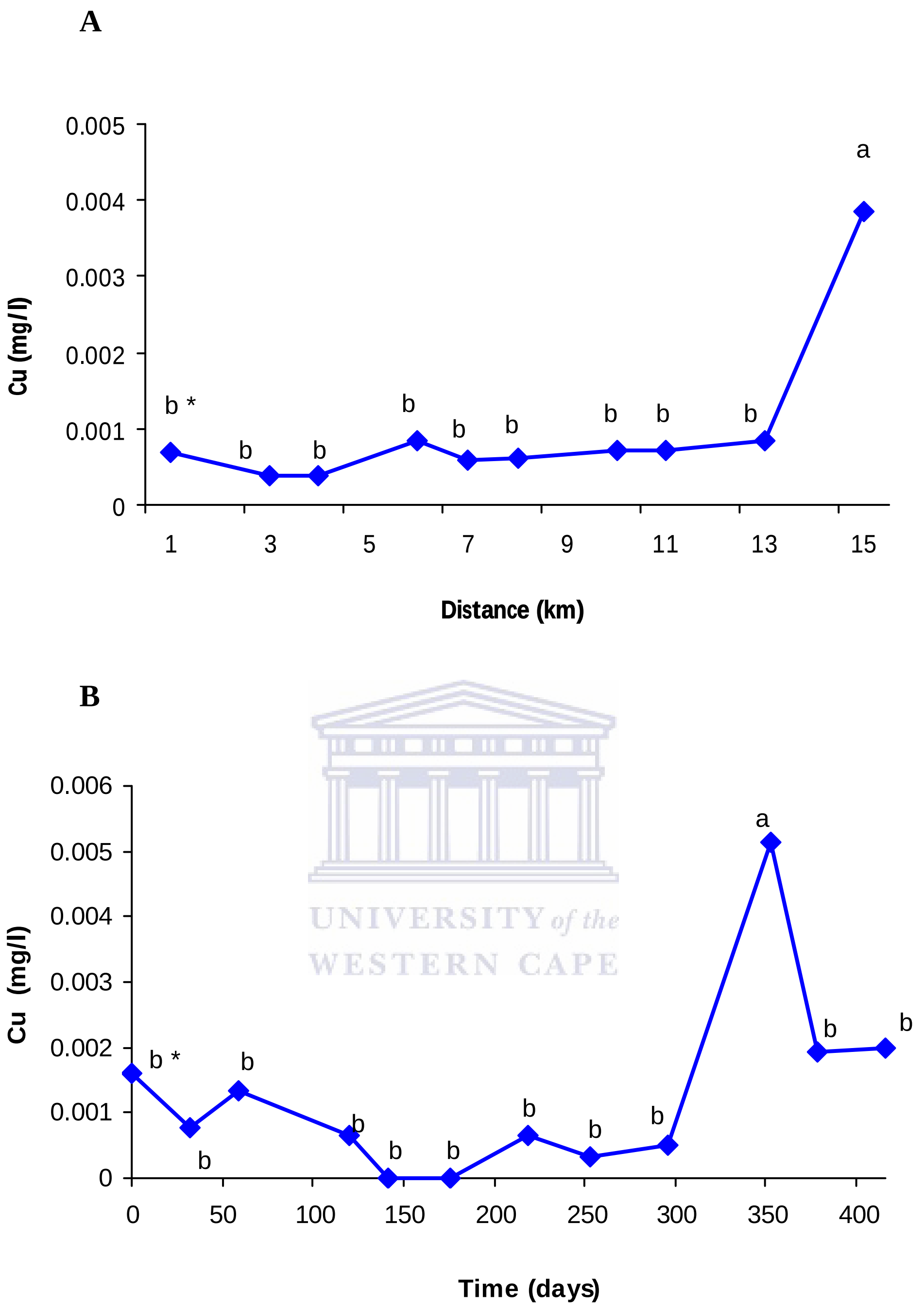


Figure 3.1.10 Means* with the same letters are not significantly different at p=0.05. (A) Copper concentrations (mg/l) along the length of the Franschhoek River over the sampling period (2009 - 2010). (B) Seasonal variation of copper in the Franschhoek River, for the duration of the study period.

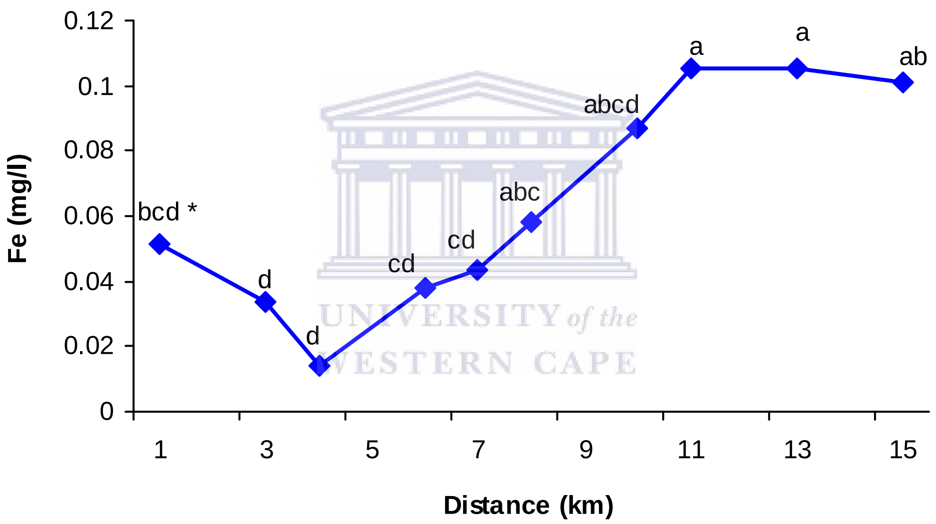
3.1.11 Iron concentrations

Iron (Fe) is an essential micronutrient and is required by all living organisms. Fe can be present in water as dissolved ferric iron, ferrous ion or suspended as ferrous hydroxides and is the 5th most abundant element in nature. Research indicates a relationship between pH and Fe concentrations. Water having neutral or alkaline pH usually has a dissolved Fe concentration within the 0.001 mg/l – 0.50 mg/l range, whereas water having a pH < 3.5 usually displays extremely elevated Fe levels. Various factors affect iron concentrations in aquatic environments, these include; pH, redox potential, turbidity, suspended matter and aluminum concentrations. Excessive intake of iron may result in haemochromatosis, damaging body tissue. Unpolluted surface waters typically display iron concentrations ranging between 0.001 mg/l – 0.50 mg/l (DWAF, 1996b) and 0 mg/l – 0.1 mg/l for domestic use (DWAF, 1996 a & e).

During the period of investigation the mean Fe concentrations in the surface water ranged between 0.01 mg/l – 0.10 mg/l (Fig 3.1.11A). The minimum Fe concentration was recorded at 3.5 km and the maximum Fe concentration was recorded between 11 km – 15 km. The lowest mean concentration of 0.01 mg/l was recorded upstream of the WWTF and the highest Fe concentrations of 0.10 mg/l being recorded downstream of the WWTF. The results indicated significant variation between minimum and maximum Fe concentrations. From the results obtained, seventy percent (70%) of the iron concentrations in the water samples were found to be within the accepted range for domestic use of 0-0.1 mg/l.

Seasonal mean Fe concentrations fluctuated and showed minimal trends (Fig 3.1.11B). Seasonal iron concentrations ranged between 0.032 mg/l – 0.11 mg/l. Fe concentrations were lowest during the wet winter seasons at days 141 - 295, heavy rainfall may have diluted Fe concentrations resulting in decreased concentrations and began increasing as the summer months approached, with maximum concentrations being observed at days 120 and 415.

A



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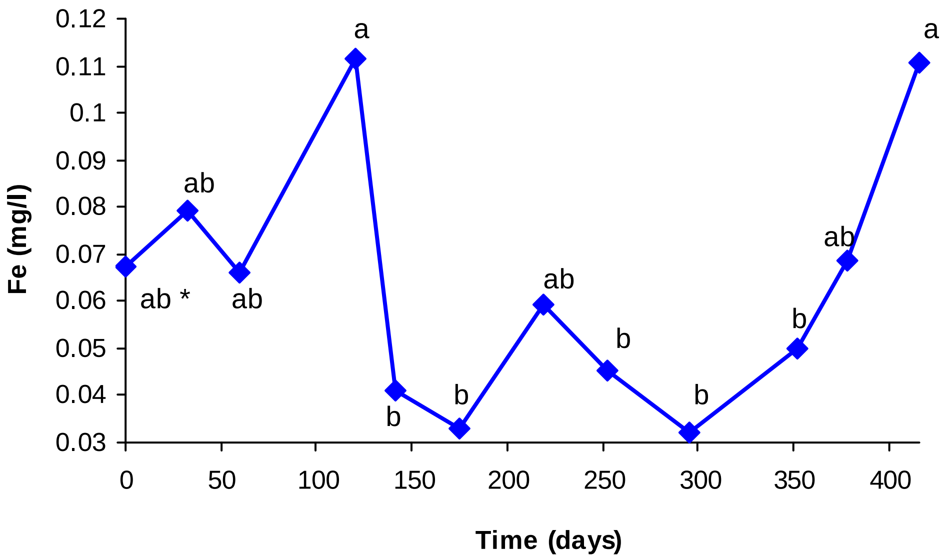


Figure 3.1.11 Means* with the same letters are not significantly different at p=0.05. (A) Iron concentrations (mg/l) along the length of the Franschhoek River over the sampling period (2009 - 2010). (B) Seasonal variation of iron in the Franschhoek River, for the duration of the study period.

3.1.12 Lead concentrations

Lead is considered one of the most problematic toxic heavy metals (Lau, 1994), and is readily available in many aquatic environments. The major sources of lead in aquatic ecosystems include street run-off, industrial and municipal waste water discharge, the weathering of sulphide ores and precipitation (DWAF, 1996d). Lead is potentially hazardous as it affects the blood, brains, nervous and renal systems and reproductive systems (Jaunakais *et al.* 2010).

The accepted concentration of lead for agricultural use according to the South African Water Quality Guideline is 0.2 mg/l and for livestock watering 0 – 0.5 mg/l (DWAF 1996 b, c, e).

The mean values of lead ranged from 0.00098 mg/l – 0.01 mg/l (Fig 3.1.12A). The highest mean concentration was recorded at site 10, while the lowest concentration was recorded at site 1 despite this observation; no significant variation between lead concentrations along the length of the Franschhoek River was noted. The lead concentration varied erratically and did not display the expected increase at the WWTF as many of the other elements did.

Seasonal lead concentrations ranged from 0.00027 mg/l – 0.04 mg/l (Fig 3.1.12B). The lowest readings were observed during the winter months and a sudden increase was observed in the summer month at day 415.

The SA Water Quality Guidelines for aquatic ecosystems states that the Target Water Quality Range for lead in medium soft water is 0 – 0.5µg/L and that 90% of all dissolved lead should be within this range. From the results obtained 100% of lead values fell within the required guidelines, indicating that lead does not currently pose a threat in this riverine ecosystem.

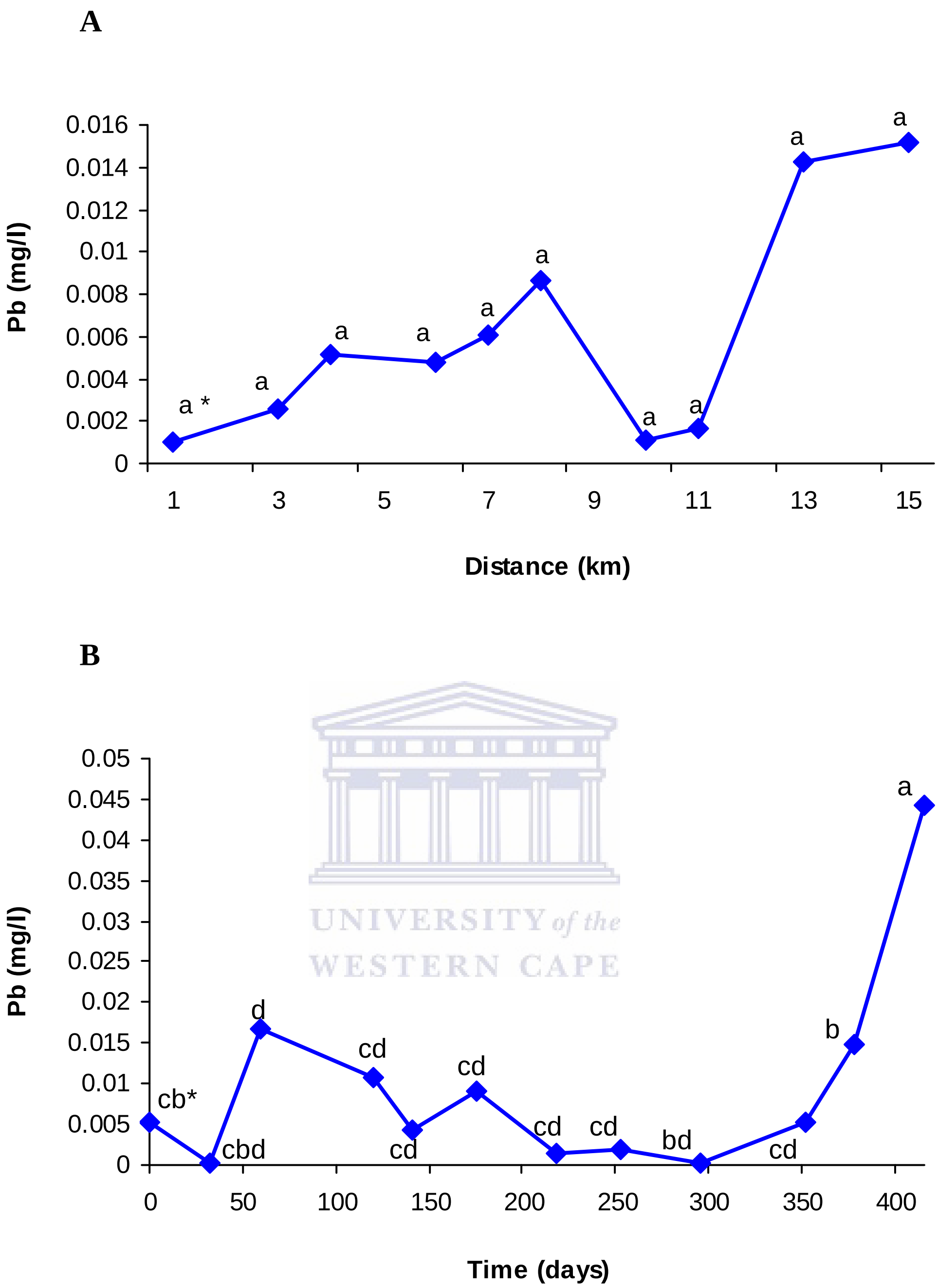


Figure 3.1.12 Means* with the same letters are not significantly different at p=0.05. (A) Lead concentrations (mg/l) along the length of the Franschhoek River over the sampling period (2009 - 2010). (B) Seasonal variation of lead in the Franschhoek River, throughout the duration of the study period.

3.1.13 Magnesium

Magnesium (Mg) is an alkaline earth metal and a common constituent of water. The solubility of Mg in water is determined by the carbonate/bicarbonate equilibrium and therefore the pH. Magnesium is an essential element for plants as it is an important component of enzyme co-factors. Magnesium hydroxide is soluble at pH 7 but solubility decreases as pH increases. Unpolluted surface waters typically display magnesium concentrations ranging from 4 mg/l - 10 mg/l (DWAF, 1996b and Bowen, 1979).

The mean values for Mg along the length of the river ranged between 0.07 mg/l – 0.25 mg/l (Fig 3.1.13A). The lowest mean Mg was recorded at sampling site 1 and the highest at the WWTF (10 km). Magnesium concentrations were lower upstream (before the WWTF) and higher post the WWTF. Despite the observed increase after the WWTF, Mg concentrations were still well below the SA Water Quality Guidelines of 4 mg/l – 10 mg/l and hence did not pose any threat to the riverine environment.

Seasonal magnesium concentrations ranged from 0.14 mg/l – 0.20 mg/l (Fig 3.1.13B). Magnesium concentrations decreased as winter approached and began to increase with the onset of summer. The highest concentrations were obtained in the summer months (1, 32; 59, 378 and 415 days) and the lowest concentrations were observed in winter. Despite the significant variation between minimum and maximum concentrations, Mg does not pose a threat to this riverine ecosystem.

A

B

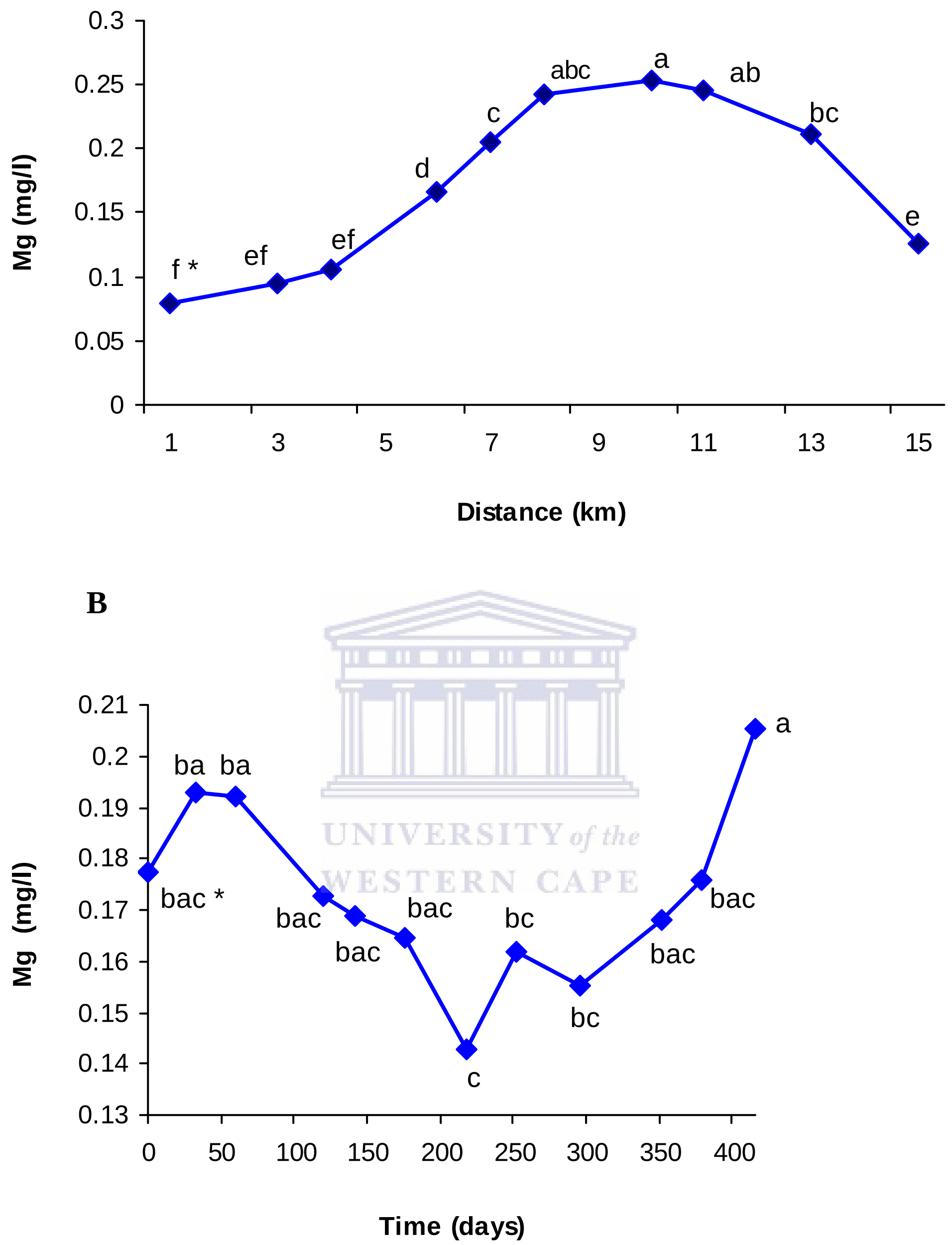


Figure 3.1.13 Means* with the same letters are not significantly different at p=0.05. (A) Magnesium concentrations (mg/l) along the length of the Franschhoek River over the sampling period (2009 - 2010). (B) Seasonal variation of magnesium in the Franschhoek River, for the duration of the study period.

3.1.14 Potassium

Potassium (K) is an alkali earth metal and forms positively charged potassium ions when it reacts with water. Potassium salts are highly soluble in water and does not precipitate easily; resulting in high levels of K in aquatic environments. Run-off from irrigation and fertilizers often elevate K levels. Unpolluted surface waters typically display potassium concentrations ranging from 2 mg/l – 5 mg/l (DWAF, 1996b).

Potassium concentrations showed no clear trends. Mean K concentrations ranged between 2.30 mg/l – 6.51 mg/l (Fig 3.1.14A). The K values obtained upstream of the WWTF, when compared to those values received downstreamof the WWTF, did not correspond with previously observed results (Figs 3.1.6A; 3.1.8A; 3.1.9A; 3.1.11A and 3.1.13A), which indicate lower metal concentrations before the WWTF. From the results obtained some K values are above the SA Water Quality Guideline regulations of 2 mg/l – 5 mg/l and therefore pose a threat to this riverine ecosystem.

Mean seasonal K concentrations ranged between 1.30 mg/l – 6.15 mg/l (Fig 3.1.14B). The first three months of sampling (1; 32 and 59d) displayed minimal seasonal significance in K concentrations in the surface water. As the wet season approached (120; 141; 175 and 218d) K levels began to increase, this may be due to seasonal overland run-off as previously mentioned. No clear seasonal trends were observed for potassium.

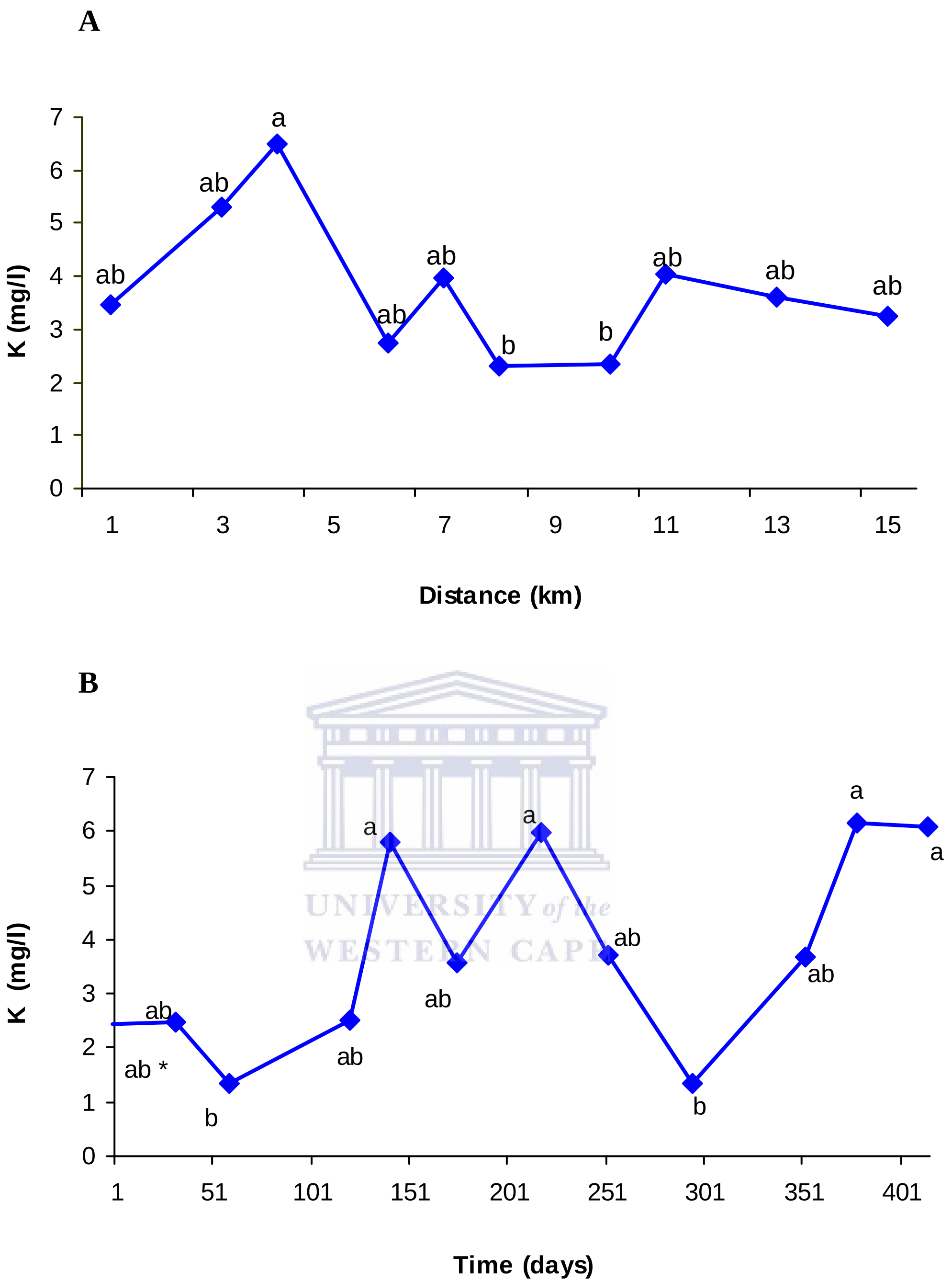


Figure 3.1.14 Means* with the same letters are not significantly different at p=0.05. (A) Potassium concentrations (mg/l) along the length of the Franschhoek River over the sampling period (2009 - 2010). (B) Seasonal variation of potassium in the Franschhoek River, for the duration of the study period.

3.1.15 odium concentrations

Sodium (Na) originated in aquatic environments by weathering of rocks, soils and erosion of salt deposits. Seawater contains approximately 11,000 ppm sodium compared with freshwater which contains 9 ppm. Na contents vary in freshwater environments depending on geological conditions and waste water contamination.

The mean Na concentrations ranged from 0.67 mg/l – 2.37 mg/l (Fig 3.1.15A). The lowest Na concentration was observed upstream of the WWTF and the highest mean value was recorded at the WWTF (sampling site 7). The highest value of 2.37 mg/l was approximately three fold higher than that recorded at location 2, indicating a significant difference between mean minimum and maximum concentrations. The high Na values at sites 7 – 10 may be due to the role that sodium plays in the waste water purification process as it is used in water purification to neutralize acids and by industry to prevent clogging of sewer pipes (DWAF, 1996b).

Seasonal mean Na concentrations ranged between 0.88 mg/l – 2.84 mg/l (Fig 3.1.15B). The lowest values were obtained in the wet season and the highest values were recorded during the summer months. Low flows coupled with decreased turbidity may have resulted in the increased concentration of sodium in the Franschhoek River catchment, during the dry season.

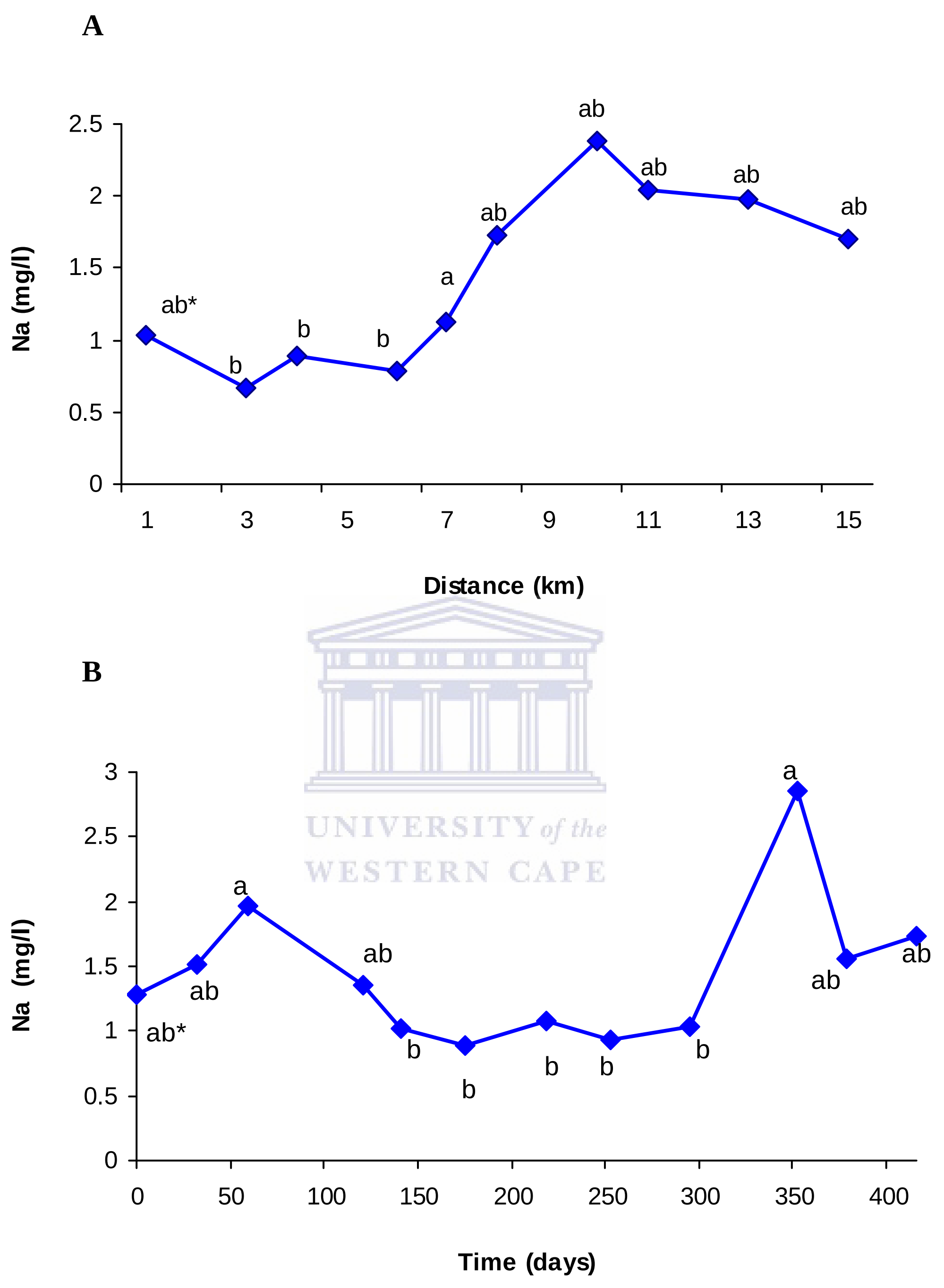


Figure 3.1.15 Means* with the same letters are not significantly different at p=0.05. (A) Sodium concentrations (mg/l) along the length of the Franschhoek River over the sampling period (2009 - 2010). (B) Seasonal variation of sodium in the Franschhoek River, for the duration of the study period.

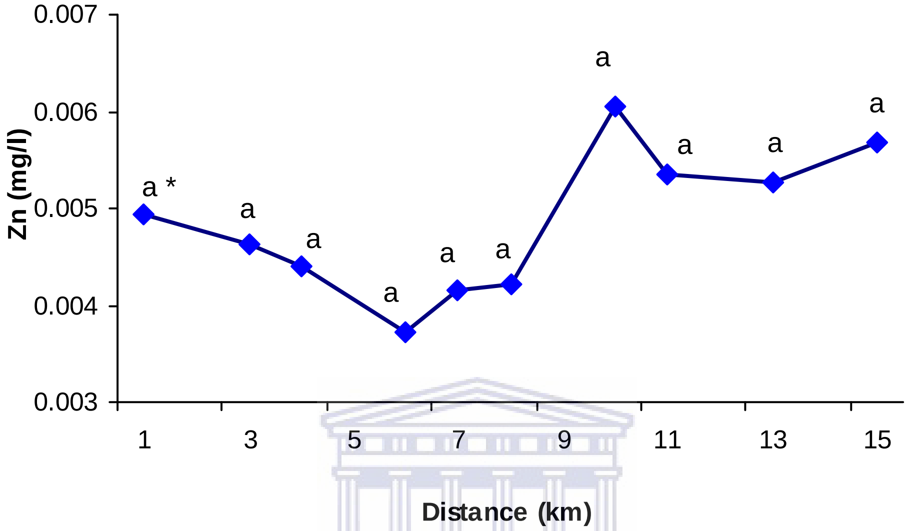
3.1.16 Zinc concentrations

The accumulation of zinc (Zn) in aquatic environments can be harmful to humans and the ecosystem when toxic levels are reached. Zinc is a common pollutant, and is mainly the result of weathering minerals, soil, atmospheric deposition, industrial effluents and urban run-off (DWAF, 1996b). Zinc, as with many other elements is readily available in the environment; due to the fact that it cannot be destroyed biologically only transformed from one oxidative state to another (Murugen *et al*. 2008). Zn is an essential element and is needed for growth of freshwater animals, over accumulation however is hazardous when consumed directly or indirectly (Murugen *et al*., 2008). The Target Water Quality Range (TWQR), for aquatic ecosystems is 2µg/l (DWAF, 1996b).

The mean Zn concentrations ranged from 0.003 mg/l – 0.006 mg/l (Figure 3.1.16A). The lowest mean Zn concentration was recorded at sampling location 4 and the highest was recorded at the Waste Water Treatment Facility (WWTF) at sampling site 7 (10km), despite these observations, no significant variation in Zn concentrations were observed along the length of the river. These values are well below the TWQR of 2µg/l and pose no threat to the environment.

Seasonal mean Zn concentrations ranged between 0.002 mg/l – 0.008 mg/l (Fig 3.1.16B). The lowest mean values were recorded at days 1 and 415 (summer) and the highest at day 252 (spring). The increased Zn concentrations could be due to the heavy rains experienced in that month, which may have resulted in agricultural run-off. Zn levels appear lower during the summer months and higher in the winter months.

A



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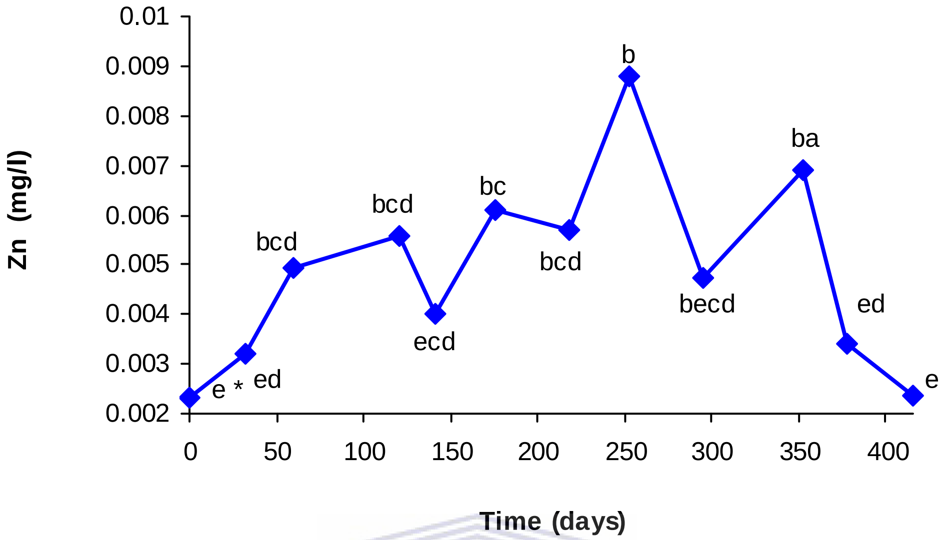


Figure 3.1.16 Means* with the same letters are not significantly different at p=0.05. (A) Zinc concentrations (mg/l) along the length of the Franschhoek River over the sampling period (2009 - 2010). (B) Seasonal variation of zinc in the Franschhoek River, for the duration of the study period.

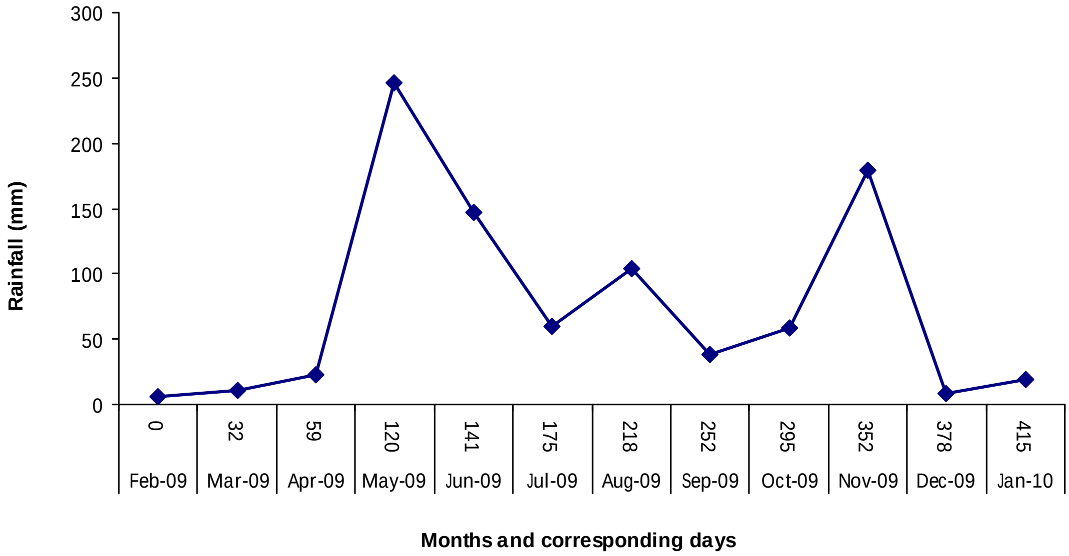


Figure 3.1.17 Rainfall (mm) for the duration of the study period, indicating the days of the year sampling was conducted with the corresponding month.

3.1.17 CONCLUSION

The present study is an attempt to detect changes in water quality characteristics within the river system with respect to nutrients and heavy metals. The study reveals that there are additions of large quantities of agricultural and waste water effluents particularly downstream of the Franschhoek River. Among the analyzed parameters, K, Na, Ca, nitrate and ammonia were the most abundant. Seasonal trends of Fe, Mg, Pb, Na, Ca, nitrate and ammonia indicate higher concentrations during the dry summer seasons and lower concentrations during the wet winter seasons. Dilution of the heavy metals during the wet winter season and slow seasonal flow regimes coupled with decreased turbidity and accumulation of the treated waste water during summer may have resulted in the increased concentrations of these heavy metals in the Franschhoek River catchment, during the dry seasons.

3.2 Seasonal variation in the chemical composition of the soil in the Franschhoek River.

Soil has the ability to accumulate contaminants and allows for the detection of pollutants which may be absent or in low concentrations in the water column (Shomar *et al.* 2005) and is therefore an important tool for assessing the quality of aquatic ecosystems (Silva and Rezende, 2002). The presence of heavy metals in the soil column can often indicate high levels of anthropogenic induced pollution opposed to natural weathering of geological structures (Binning and Bard, 2001), and depending on the river morphology and hydrological regimes, contaminants will often settle in the bottom sediment (Milenkovic *et al*. 2005).

The surface area of sediment particles is important as it acts as a binding site for metals and organic contaminants. Fine sediment particles therefore typically display increased sorption of contaminants as they have a higher relative surface area (Batley *et al*. 2005).

It is important when analyzing for contaminants of riverine ecosystems to consider, not only the surface water but sediments as well. The water column represents significant variation in its spatial and temporal metal contents and is hence problematic for obtaining a fair representation of the riverine system under study. Sediments however, represent contaminants over time and are useful as they provide contamination data of a spatial nature and a pollution history of a given system (Lord and Thompson 1988; Davies *et al*. 1991; Binning and Bard 2001).

Soils and sediment may contain a wide range of heavy metals depending on the geological nature of the environment as well as the role of anthropogenic activities in the area (Dube *et al*. 2000). Various factors affect heavy metal transportation and uptake by soil and sediment. These include among others the physiochemical properties of the metals, as well as the physical and chemical properties of the soil (Dube *et al*. 2000). According to Soares *et al.* 1999, those pollutants which settle in the sediment are often not available to aquatic organisms, however changing physiochemical characteristics of the water column may often aid in the release of the metals back into the aqueous phase, turning them into pollutants. Research indicates that seasonal accumulation of heavy metals in aquatic ecosystems are good indicator of environmental water quality (Goncalves and Boaventura, 1991 and Goncalves *et al*. 1992).

Table 3.2 indicates the seasonal concentration of soil fractions of metals (Zn, Cu, Ca, Pb, Mg, Na, K, Fe and Cd), during winter of August 2009 and the summer of April 2010.

The seasonal metal concentration of the analysed soil is presented in Table 3.2. It was found that the elements were arranged in the following order of concentration Fe > Ca > Na > K > Zn > Mg > Cu > Pb > Cd in the winter of 2009 and Fe > K > Ca > Zn > Na > Mg > Cu > Pb > Cd in summer of 2009. To date, no soil quality standards exists for South African soils, the following are sludge guidelines (mg/kg) according the DWA, (2006).

Table 3.2 indicates the seasonal concentration (mg/kg) of soil fractions of metals (Cd, Ca, Cu, Fe, Pb, Mg, K, Na, Zn), during winter of August 2009 and the summer of April 2009.

	Winter, August 2009				Summer, April 2009			
Metal	Min	Max	Mean	Stdev	Min	Max	Mean	Stdev
Cd	0.01	0.13	0.041	0.041	0.01	0.08	0.03	0.02
Ca	40.80	189.23	79.47	42.26	41.30	171.96	91.94	40.69
Cu	0.19	1.43	0.66	0.39	0.73	11.96	2.80	3.33
Fe	843.93	2365.60	1442.50	534.05	805.44	5925.44	2737.47	1870.29
Pb	0.00	0.31	0.04	0.10	0.00	0.31	0.04	0.10
Mg	12.44	52.51	22.28	11.65	9.94	108.37	36.34	35.70
K	0.00	258.17	32.21	81.89	0	684.1	138.31	243.13
Na	21.59	39.46	32.71	5.12	28.01	43.66	36.82	4.85
Zn	4.20	40.59	23.05	9.29	90.10	253.63	47.30	75.04

Table 3.3 South African sludge limits (mg/kg).

Sludge guidelines	Cd	Cu	Pb	Zn
Old *DWAF (pre 2006)	15.7	50	50.5	353.5
New *DWAF (2006)	40	1500	300	2800
Health	20	750	400	2750
Agriculture	20	1200	1200	3000

*DWAF: Department of Water Affairs and Forestry.

It is well documented that agricultural run-off, pesticides, household and sewer sludge can result in heavy metal contamination of urban and agricultural soils (Brady and Weil, 1999). Among the two seasons investigated, Zn displayed higher concentrations during the summer of 2010. This was mainly due to the fact that all farmers irrigated their land with groundwater during the dry summer months of October – April. The irrigation of crops and vineyards between these periods occurred for approximately 8 hours per day and 7days per week, resulting in agricultural run-off into the adjacent Franschhoek River. Apart from agricultural run-off, point source pollution from the Waste Water Treatment Facility (WWTF) is aggravated during the dry summer months. This is attributed to the absence of heavy rainfalls, decreased flow regimes and turbidity, which occurs during the wet winter season, which would dilute the riverine ecosystem and "flush out" pollutants. Similar results were observed by Shomar *et al*. (2004).

According to Kabata – Pendias and Pendias (1995) there is a strong relationship between Cd and Zn and the geochemistry of these two elements are closely related (Alloway, 1995), due to the similarity in their ionic structures and electro-negativities. Despite the reported antagonistic and synergistic nature of Cd and Zn (Wallace *et al*. 1980; Kitagishi and Vamane 1981; and Kabata – Pendias and Pendias, 1995), these heavy metals display similar downward movements through the soil profile. Mean Cd concentrations in winter ranged from 0.01 mg/kg – 0.13 mg/kg and 0.01 mg/kg – 0.08 mg/kg in summer; with mean winter Zn ranging between 4.20 mg/kg– 40.59 mg/kg and mean summer ranging from 90.1 mg/kg – 253.63 mg/kg. It was observed that as Zn concentrations increased Cd concentrations decreased. Summer Zn concentrations of the sediment were significantly higher than the winter concentrations, which may be attributed to the use of Zn based pesticides and Zn containing fertilizers, which percolate through the soil profile during the intensive summer irrigation periods of October – April.

Summer Ca concentrations ranged from 41.30 mg/kg – 171.96 mg/kg with a mean of 91.944 mg/kg and winter concentrations ranged between 40.80 mg/kg – 189.23 mg/kg with a mean of 79.470 mg/kg. The elevated Ca in summer may be due to the waste water treatment facility as Ca is a primary flocculant ingredient in treated waste water. Surface water which was rich in Ca may have percolated and settled into the sediment during summer which may have been aided by slow flow regimes and decreased turbidity.

The availability of Cu in soil is strongly governed by the presence of organic matter and the humic substances present in the soil (Wu *et al.* 2002). Copper toxicity, however, is dependent on soil organic matter, pH and organo - metallic complexes, all acting in concert (Valladares *et al*. 2009). In the present study mean seasonal Cu concentrations were 0.667 mg/kg in winter and 2.803 mg/kg in summer. Copper concentrations did not pose a threat to the aquatic environment. These findings are supported by the copper concentrations of the surface water.

Metal contamination by iron is evident in both winter and summer samples. Iron is a major constituent of many soils and is precipitated as hydroxides in alkaline soils, rendering them unavailable to plants (DWAF, 1996b). The high levels of iron in the sediment may be attributed to run-off from fertilizers, which settled in the bottom sediment. In addition high water temperatures often result in anoxic conditions resulting in Fe release (Shomar *et al*. 2004). In winter months, high flow regimes and increased turbidity elevate oxygen levels in aquatic environments, resulting in oxygen rich soils which contain Fe in its Fe (III) state. Anaerobic conditions which prevail in summer, result in Fe (III) being reduced to Fe(II), the resulting Fe(II) binds to phosphates forming a highly soluble Fe(III)-phosphate being released into the sediment in summer.

Seasonal mean Pb concentrations were recorded as 0.043 mg/kg in winter and 0.31 mg/kg in summer. Lead values were low and this may be attributed to the lack of mobility of this heavy metal in the sediment. According to Kalavrouziotis *et al*. (2009) lead is one of the least mobile elements and high levels of lead are typically only found in organically rich soils (Hughes *et al*. 1980 and Kalavrouziotis *et al*. 2009).

Sodium concentrations ranged from 21.59 mg/kg – 39.46 mg/kg in winter and 28.01 mg/kg – 43.66 mg/kg in summer. Sodium concentrations were considerably higher in the sediment than in the previously reported water data. This is expected as metals tend to percolate the water column and accumulate in the sediment (Kucuksezgin *et al*. 2007). Despite the significant variation in the metal concentrations of the water and sediment, seasonal concentrations correspond as decreased concentrations were recorded in winter and higher values were noted in summer, for both sampled media.

3.2.1 CONCLUSION

The concentrations of metals observed in the sediment were compared with the seasonal levels reported in the surface water. The overall results indicated that the sediment displayed higher concentrations of metals, primarily because sediments act as reservoirs for all contaminants and dead organic matter. Similar findings were reported by Saeed and Shaker, 2008 and Nguyena *et al*., 2005). As previously mentioned Fe> K> Na> Mg> Ca> Cu> Zn all displayed higher concentrations in the dry summer season with Zn being the only metal which showed significant variation between summer and winter accumulation in the above mentioned order of abundance.

3.3Spatial and seasonal variation of heavy metal concentration in vegetation.

Urban and rural rivers in the Western Province are typical of many in the rest of Southern Africa, in that they receive heavy metal and inorganic pollution from many anthropogenic sources such as treated and untreated waste water and run-off from urban and agricultural practices.

Heavy metals in sediments are often reflected in higher concentrations in aquatic flora (Buszewski *et al*. 2000) and the ability of certain riverine plants to absorb and accumulate heavy metals makes them useful indicators of environmental pollution and water quality (Agami *et al.* 1976; Kara, 2005). The chemical composition of the surrounding vegetation is considered a reflection of the heavy metals in the aquatic system (Dube *et al*. 2000; Presad, 2004). Metal bioaccumulation is dependent on a variety of biotic and abiotic factors which include temperature, pH and dissolved ions of the water column (Presad, 2004).

S. babylonica sp, is a deciduous tree and leaf abscission occurred during the winter month of August and the spring month of September 2009, sampling commenced on *S. babylonica* once leaf growth had re-occurred during October 2009.

3.3.1 Cadmium concentrations

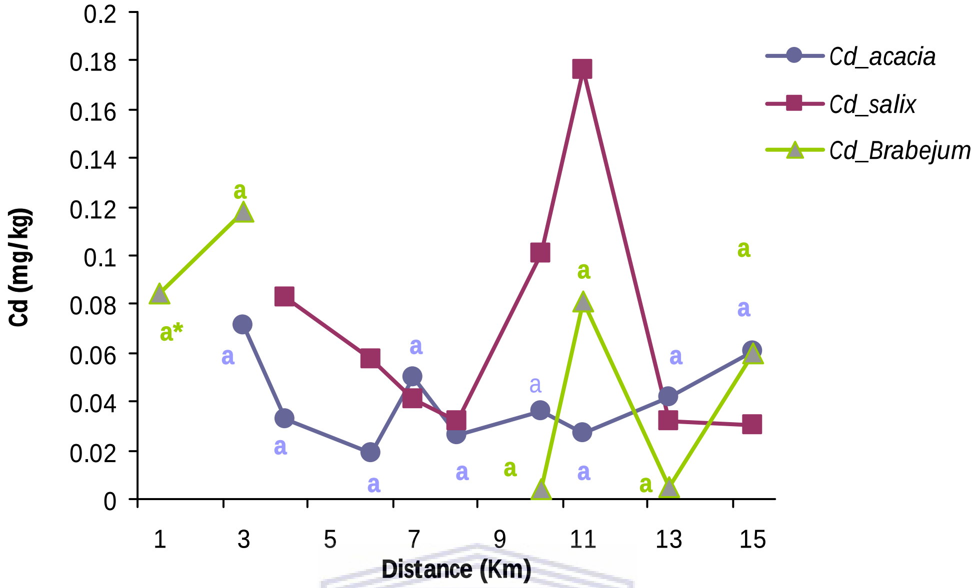
Cadmium is found at low levels in the environment and anthropogenic inputs include phosphate containing fertilizers and sewer sludge (Nriagu, 1980). Cadmium concentrations varied erratically along the length of the river and showed no significant variation (Fig 3.3.1 A). Maximum cadmium absorption was obtained by *S. babylonica* sp, with average minimum and maximum concentrations ranging between 0.0304 mg/kg – 0.176 mg/kg, with the maximum concentration being recorded at a distance of 11 km, 1km downstream of the Waste Water Treatment Facility (WWTF). *Acacia mearnsii* had an average range of 0.0187 mg/kg – 0.0603 mg/kg and *Brabejum stellatifolium* ranged from 0.0037 mg/kg – 0.118 mg/kg. Both *S. babylonica* and *B. stellatifolium* displayed higher concentrations at 11 km. There was no significant variation between the three species analyzed. The increased concentrations observed at the WWTF and at sampling site 8 (11km), may be attributed to the presence of sewer sludge and fertilizers which are considered possible inputs of Cd in aquatic environments (Nriagu, 1980).

Seasonal mean Cd concentrations for *B.stellatifolium* ranged from 0.007 mg/kg – 2.31 mg/kg. *S.babylonica* had a mean range of 0.009 mg/kg – 0.32 mg/kg and the invasive *A. mearnsii* had a mean range of 0.009 mg/kg – 0.18 mg/kg. All three species displayed similar trends (Fig 3.3.1 B). All the species increased in concentrations at day 295 one month after the heavy rainfall of the previous month. Cadmium samples were significantly higher in the analyzed plant samples than in the soil and the water. Of the three vegetation types analyzed *B. stellatifolium* was the most efficient at seasonal Cd accumulation.

El Bassam (1978), reported that cadmium concentrations of uncontaminated soils are typically below 0.5 mg/kg and that Cd concentrations in plants often range between 0.05 mg/kg – 0.2 mg/kg, with contaminated sites displaying slightly elevated concentrations in plants. From the results obtained all species displayed Cd uptake between the range of 0.05 mg/kg – 0.2 mg/kg along the length of the river and were thus within the typical limits.

Research conducted by Greger *et al.* (1995) provided evidence that when the salinity of the aquatic environment was high the bioaccumulation of Cd in plants decreased. However, increased sediment salinity resulted in increased Cd uptake. Greger *et al.* (1995) suggests that the former was due to $CdCl_x$ complexes being formed in the water which are difficult for plants to absorb, the latter, however, was possible with the addition of salts which release Cd bound in the surrounding sediment. Cadmium concentrations were low in all three media sampled. In addition to it being a metal rarely encountered in the environment, the input of nutrients often affect metal accumulation in plants, as other cations in the metal compete with the metal itself (Presad, 2004). Calcium is a nutrient which often affects metal uptake in plants; this was endorsed by research conducted by Greger and Berteh (1992), who reported that increased Ca concentrations resulted in decreased Cd accumulation in *S babylonica*.

A



0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Cd (mg/kg)
1
3
5
7
9
11
13
15
Distance (Km)
Cd_acacia
Cd_salix
Cd_Brabejum
a*
a

B

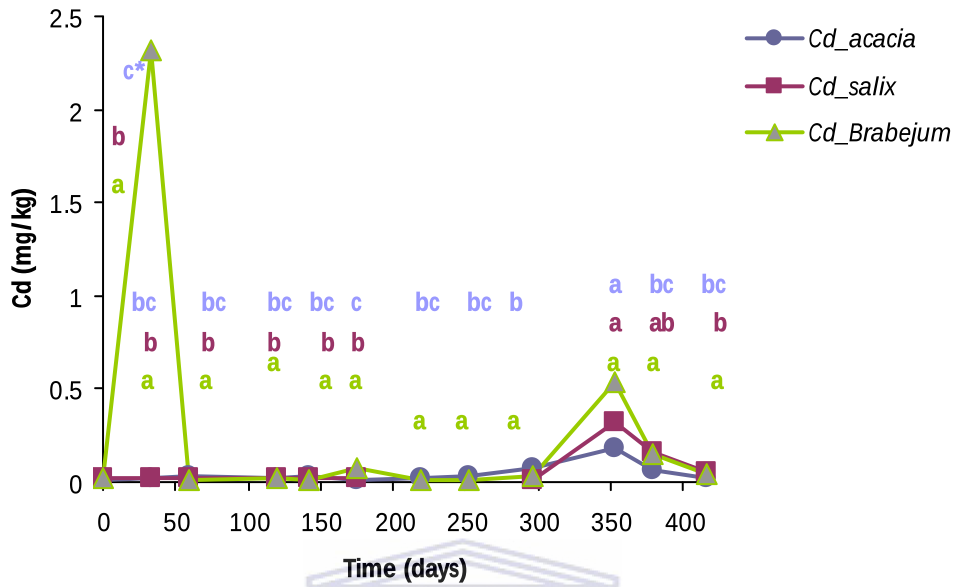


Figure 3.3.1 Means* with the same letter are not significantly different at p=0.05. (A) Foliar cadmium concentrations for *A. mearnsii, S. babylonica* and *B. stellatifolium* (mg/kg) along the length of the Franschhoek River over the sampling period (2009 - 2010). (B) Seasonal variation of cadmium concentration in the leaves of *A. mearnsii, S. babylonica* and *B. stellatifolium* along the Franschhoek River over the sampling period (2009 - 2010).

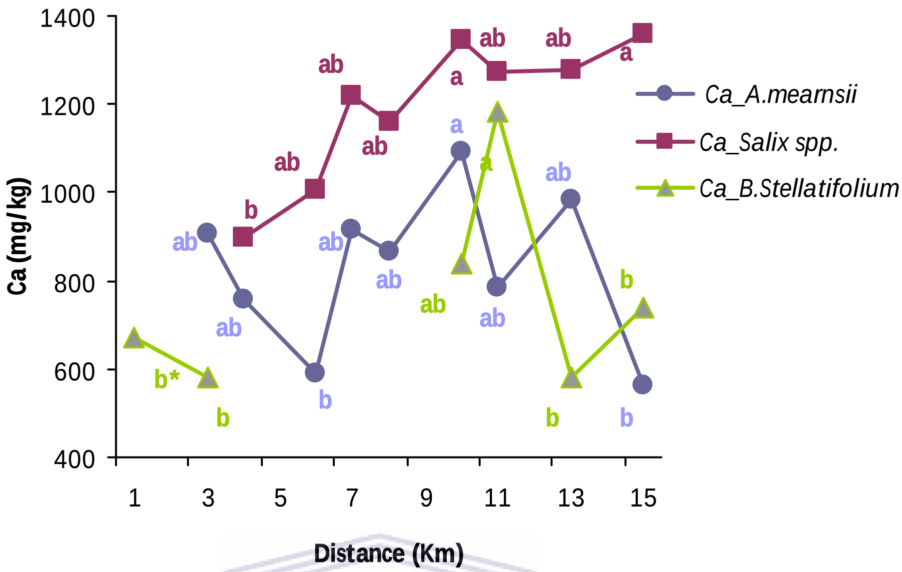
3.3.2 Calcium concentrations

Salix babylonica displayed the highest Ca absorption along the length of the river with average minimum and maximum concentrations ranging from 896.18 mg/kg – 1343.49 mg/kg. *Acacia mearnsii* displayed concentrations ranging between 562.60 mg/kg – 1091.90 mg/kg and *Brabejum stellatifolium* ranging from

580.20 mg/kg – 1180.80 mg/kg. Despite lower absorption by *B. stellatifolium* and *A. mearnsii* all three species experienced maximum concentrations at the WWTF (Fig 3.3.2 A). Calcium is a flocculent ingredient in the treatment of effluent, which could be the explanation for the elevated levels at this sampling location as it percolated the water column and settled in the bottom sediment.

Seasonal Ca concentrations varied between the three plant species analyzed. *S. babylonica* displayed a mean range of 200.28 mg/kg – 2020.5 mg/kg, *B. stellatifolium* ranged from 64.50 mg/kg – 1161.35mg/kg and *A. mearnsii* ranged between 5 mg/kg – 1488.2 mg/kg (Fig 3.3.2 B). All three species displayed a steady increase in Ca concentration with the onset of the summer months. Maximum absorption occurred at day 415 for all three species analyzed, with *A. mearnsii* and *B. stellatifolium* both displaying a decline in Ca concentration during the winter months. There was a significant difference between minimum (0days) and maximum (415 days) Ca concentrations in *S. babylonica* with minimal variation throughout the remainder of the year. A comparison of the invasive *S. babylonica* with the endemic *B. stellatifolium* indicates significant variation between Ca bioaccumulation. Comparing the amount of seasonal Ca in the plants with the water and sediment indicate concentrations in plants are higher than the sediment which in turn is higher than the water. Vegetation and water both display elevated Ca concentrations in the summer months and decreased in the winter months.

A



1400
1200
1000
800
600
400
Ca (mg/kg)
1
3
5
7
9
11
13
15
Distance (Km)
Ca_A.mearnsii
Ca_Salix spp.
Ca_B.Stellatifolium
ab
b
a
b*

B

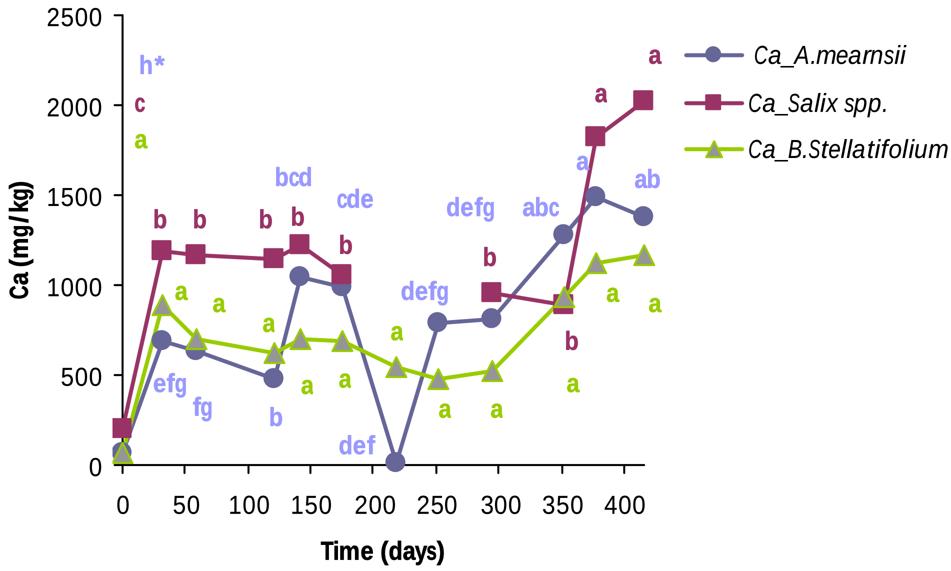


Figure 3.3.2 Means* with the same letter are not significantly different at p=0.05. (A) Foliar calcium concentrations for *A. mearnsii, Salix sp* and *B. stellatifolium* (mg/kg) along the length of the Franschhoek River over the sampling period (2009 - 2010). (B) Seasonal variation of calcium concentration in the leaves of *A. mearnsii, S. babylonica* and *B. stellatifolium* along the Franschhoek River over the sampling period (2009 - 2010).

3.3.3 Copper concentrations

Copper concentrations in unpolluted soils typically ranged from 10 mg/kg – 80 mg/kg (Mortvedt *et al.* 1972). According to Mengel and Kirby (1987), Cu organic complexes play a vital role in the regulation of Cu mobility in soil and sediment.

Copper concentrations along the length of the Franschhoek River showed minimal trends between the three species analyzed (Fig 3.3.3 A). Maximum uptake was recorded for A. *mearnsii* with concentrations ranging from 8.55 mg/kg – 23.03 mg/kg, followed by *S. babylonica* with a range of 7.29 mg/kg – 14.72 mg/kg. *Brabejum stellatifolium* displayed the least accumulation ranging between 3.06 mg/kg – 10.16 mg/kg. The maximum and most significant uptake occurred at 6 km in *A. mearnsii* and at 15km for *S. babylonica*. The trends of Cu accumulation in *S. babylonica* correspond to that of the previously reported water data.

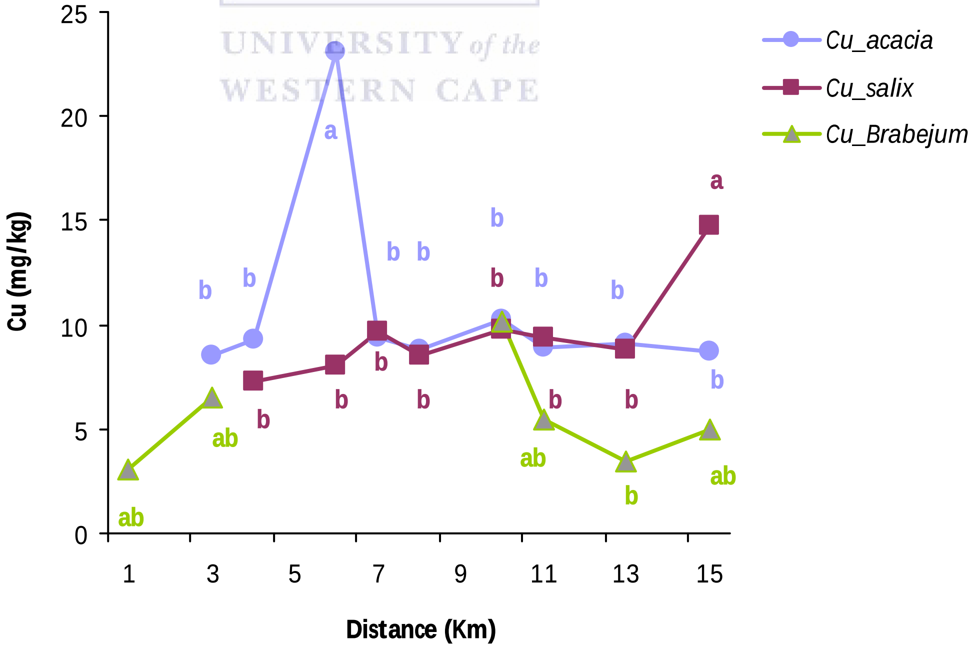
Copper is a trace metal necessary for the survival of plants. The presence of Cu in aquatic environments may be attributed to the treated sewer discharge and surface flows. The copper present in the previously reported water data, despite the negligible amounts eventually settled and accumulated in the soil and bottom sediment as it became associated with the particulate matter. Similar results were reported by (Jackson, 1998 and Cardwell *et al*. 2002).

Visible seasonal trends were observed for Cu accumulation in the leaves of the three analyzed plant species. *A. mearnsii* displayed Cu concentrations ranging from 5.47 mg/kg – 23.01 mg/kg (Fig 3.3.3 B). Maximum copper uptake occurred at 32days. This may be attributed to an accumulation of Cu in the sediment due to the absence of precipitation and decreased flow regimes. Similar trends were observed for *S. babylonica* with a mean range of 4.65 mg/kg – 19.03 mg/kg and *B. stellatifolium* displaying concentrations ranging from 2.88 mg/kg – 16.47

mg/kg. No significant variation in copper accumulation was observed between *A. mearnsii* and *S. babylonica* (Fig 3.3.3 B). The most significant variation in Cu accumulation was observed between *A. mearnsii* and *B. stellatifolium.* All three species displayed increased absorption during the dry summer months and decreased absorption in the wet winter season.

Copper accumulations in plants tend to be relatively low and lie at a range of 2 mg/kg – 20 mg/kg (Wallnofer and Engelhardt, 1984). Similar results were obtained in the present study with Cu accumulation in all three plant species ranging between 2.88 mg/kg – 23.01 mg/kg.

A



B

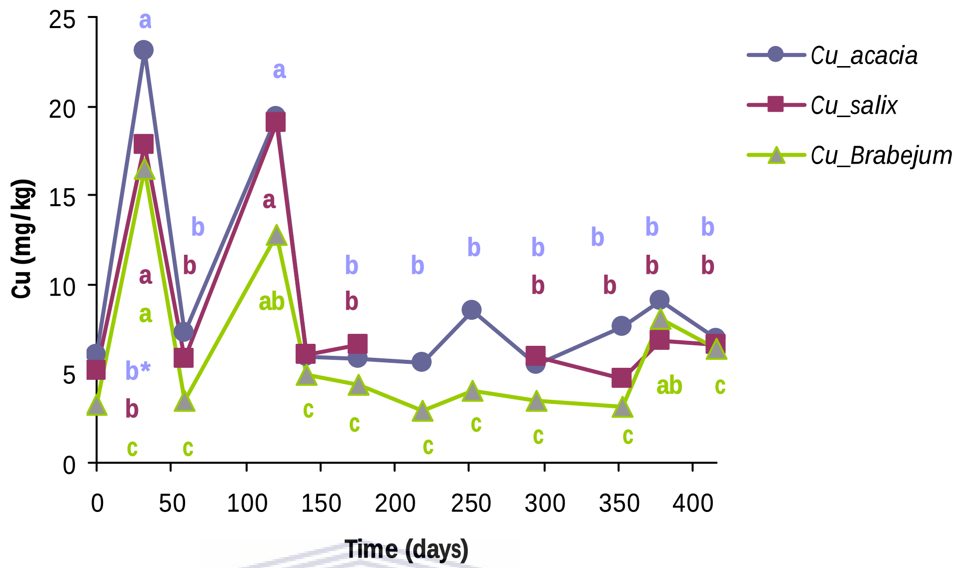


Figure 3.3.3 Means* with the same letter are not significantly different at p=0.05. (A) Foliar copper concentrations for *A. mearnsii, S. babylonic* and *B. stellatifolium* (mg/kg) along the length of the Franschhoek River over the sampling period (2009 - 2010). (B) Seasonal variation of copper concentration in the leaves of *A. mearnsii, S. babylonica* and *B. stellatifolium* along the Franschhoek River over the sampling period (2009 - 2010).

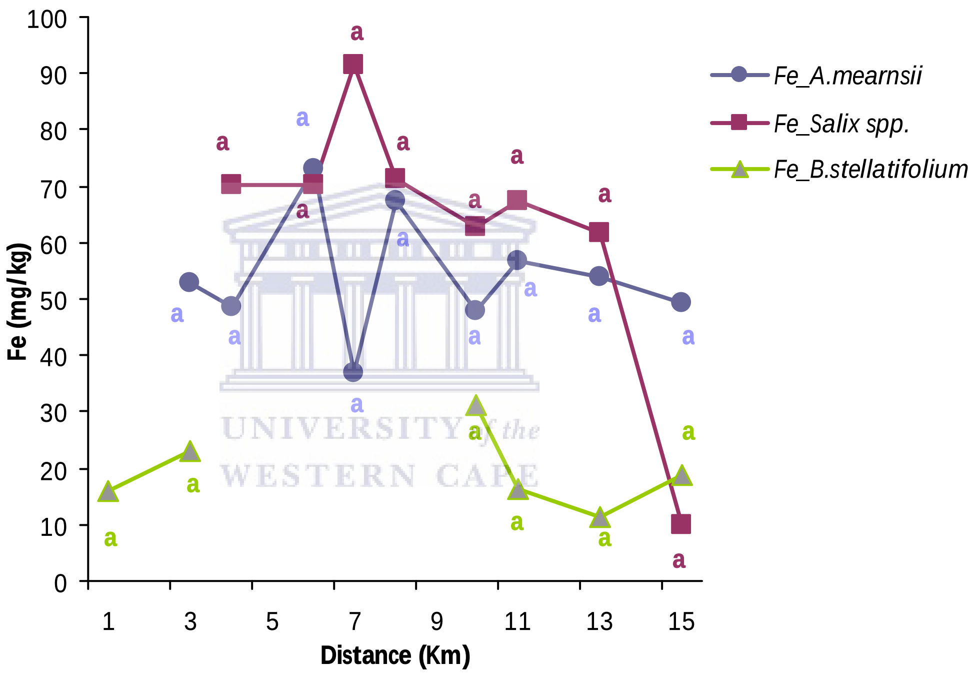
3.3.4 Iron concentrations

Iron is considered a micro nutrient essential for plant growth and survival. However, when the concentration limit is exceeded, Fe may be referred to as a heavy metal. Iron in plants is required for the functioning of an array of enzymes, especially those which take part in oxidation and reduction processes as well as electron transport in photosynthesis (photosystem I) (Rengel, 1997 ; Prasad, 2004).

Iron accumulation throughout the length of the river showed no significant variation between the three species analyzed. *S. babylonica* had a mean range of 10.10 mg/kg – 91.41 mg/kg. Maximum uptake occurred at 7.5 km followed by a sharp decline in concentration at 10km (Fig 3.3.4 A). *A. mearnsii* experienced Fe concentrations ranging between 36.74 mg/kg – 73.0 mg/kg. Maximum Fe uptake in *A. mearnsii* occurred at 6km with a sharp decline 1.5 km downstream. With the exception of sampling location 5 (7.5 km), no significant variation in Fe accumulation was observed between *A. mearnsii* and *S. babylonica.* Iron accumulation in *B. stellatifolium* showed similar trends as in Cu (Fig 3.3.3 A). Fe uptake in *B. stellatifolium* had a mean range of 11.36 mg/kg – 31.2 mg/kg. No variation in iron uptake was observed in *B. stellatifolium.* From the results obtained *S. babylonica* is best at Fe accumulation followed by *S. babylonica* and *B. stellatifolium.*

Seasonal iron uptake shows minimal changes amongst the three species analyzed (Fig 3.3.4 B) Maximum accumulation was observed for all three species at day 0 of the dry summer season, followed by a sharp decline until 120 days, whereby all three species show a slight increase in Fe uptake. Iron concentration began to increases at the onset of the dry summer seasons at 295 days and 352 days, respectively. All three species displayed significant variation between their minimum and maximum accumulation. *Acacia mearnsii* had a range of 0.85 mg/kg – 193.43 mg/kg, *S. babylonica* had a mean range of 1.116 mg/kg – 300.71 mg/kg, followed by *Brabejum stellatifolium* which displayed a mean absorption range of 0.36 mg/kg – 77.75 mg/kg (Fig 3.3.4 B). *S. babylonica* appears to be the most effective at accumulating Fe followed by *A. mearnsii* and *B. stellatifolium.*

A



B

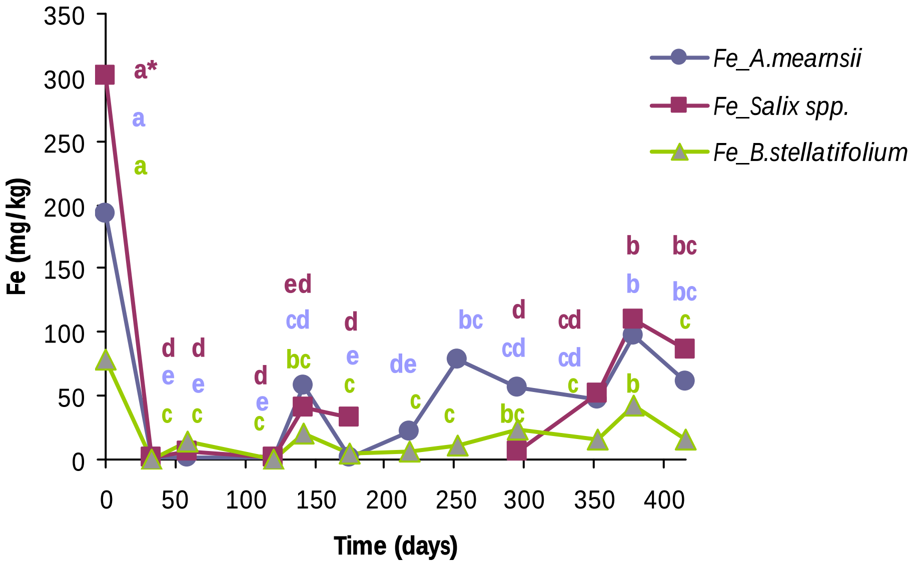


Figure 3.3.4 Means* with the same letter are not significantly different at p=0.05. (A) Foliar iron concentrations for *A. mearnsii, S. babylonica* and *B. stellatifolium* (mg/kg) along the length of the Franschhoek River over the sampling period (2009 - 2010). (B) Seasonal variation of iron concentration in the leaves of *A. mearnsii, S. babylonica* and *B. stellatifolium* along the Franschhoek River over the sampling period (2009 - 2010).

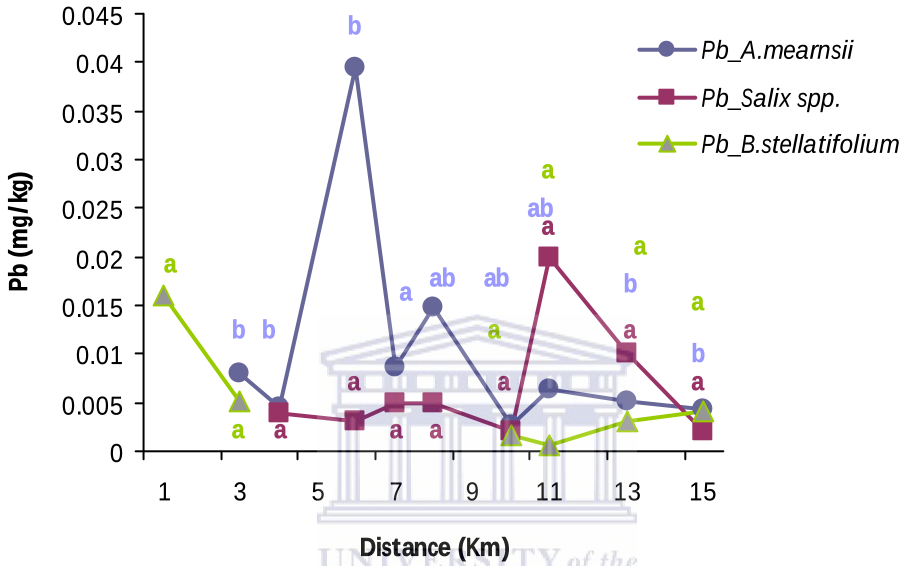
3.3.5 Lead concentrations

Lead concentrations along the length of the river indicated minimal trends between the three species analyzed (3.3.5 A). *A. mearnsii* displayed the highest overall Pb accumulation with a mean range of 0.00269 mg/kg – 0.0394 mg/kg. Maximum lead accumulation in *A. mearnsii* was observed at 6 km, followed by a significant decrease in Pb concentrations 1.5 km downstream. Minimum Pb concentrations were observed at a distance of 10 km at the WWTF.

S. babylonica concentrations ranged from 0.002 mg/kg – 0.02 mg/kg. Minimum accumulation was recorded at 10 km (sampling location 7, WWTF), similar to the previously mentioned *A. mearnsii*, with maximum accumulation in *S. babylonica* occuring a mere 1 km downstream at 11 km. No significant variation in Pb accumulation in *S. babylonica* was recorded. Of the three species analyzed, *B.stellatifolium* displayed the least overall Pb accumulation, with a mean range of 0.00054 mg/kg – 0.016 mg/kg. From the results obtained *A. mearnsii* was the best at Pb accumulation followed by *S. babylonica* and lastly *B. stellatifolium*. Seasonal lead concentrations indicate lower absorption in the dry summer season and slightly elevated concentrations during the wet winter season (3.3.5 B). *A. mearnsii* displayed seasonal trends ranging from 0 mg/kg – 0.0298 mg/kg. Maximum lead accumulation occurred at 120 days, this corresponds with the month which received the highest rainfall of 246 mm. Winter and early spring Pb concentrations were higher than those concentrations recorded during the dry summer months.

S. babylonica displayed seasonal Pb concentrations ranging between 0.001 mg/kg – 0.0764 mg/kg. Maximum accumulation was noted in winter at 175 days, 1 month prior to leaf abcission with concentrations remaining low throughout the remainder of the dry summer season. Results indicated significant variation between minimum and maximum Pb concentrations in *S. babylonica*. *Brabejum stellatifolium* displayed concentrations ranging from 0 mg/kg – 0.029 mg/kg. Maximum accumulation was noted at 218 and 252 days, with a reported monthly mean rainfall of 104 mm and 38.3 mm, respectively. Similar to *A. mearnsii* lower concentrations were noted in the dry summer season and higher seasonal Pb was noted in the wet winter season.

A



B

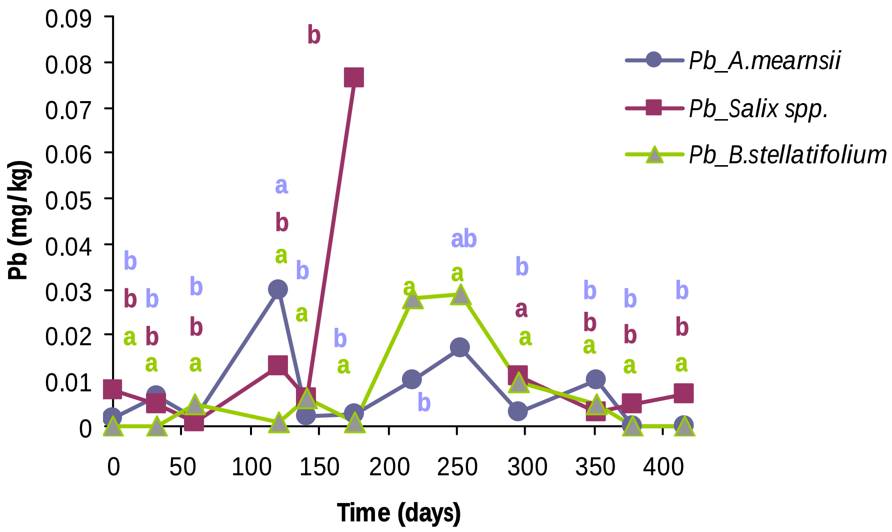


Figure 3.3.5 Means* with the same letter are not significantly different at p=0.05. (A) Foliar lead concentrations for *A. mearnsii, S. babylonica* and *B. stellatifolium* (mg/kg) along the length of the Franschhoek River over the sampling period (2009 - 2010). (B) Seasonal variation of lead concentration in the leaves of *A. mearnsii, S. babylonica* and *B. stellatifolium* along the Franschhoek River over the sampling period (2009 - 2010).

3.3.6 Magnesium concentrations

Minimal trends were observed amongst the three species analyzed (3.3.6 A). *Salix babylonica* displayed the highest overall magnesium accumulation with a mean range of 226.95 mg/kg – 540.43 mg/kg. No significant variation in Mg accumulation was observed along the length of the Franschhoek River in *S. babylonica*. Concentrations remained relatively stable with the exception of sampling location 10 (15 km), which displayed maximum accumulation of 540.43

mg/kg. No significant variation in Mg accumulation in *S. babylonica* was observed between upstream and downstream sampling sites. *Acacia mearnsii* displayed Mg accumulation ranging between 126 mg/kg – 238 mg/kg. Maximum accumulation was noted at the WWTF and minimum absorption occurred downstream at 15 km. Magnesium concentrations upstream of the Waste Water Treatment Facility (10km) displayed concentrations higher than those sampling sites occuring after the WWTF in *A. mearnsii*.

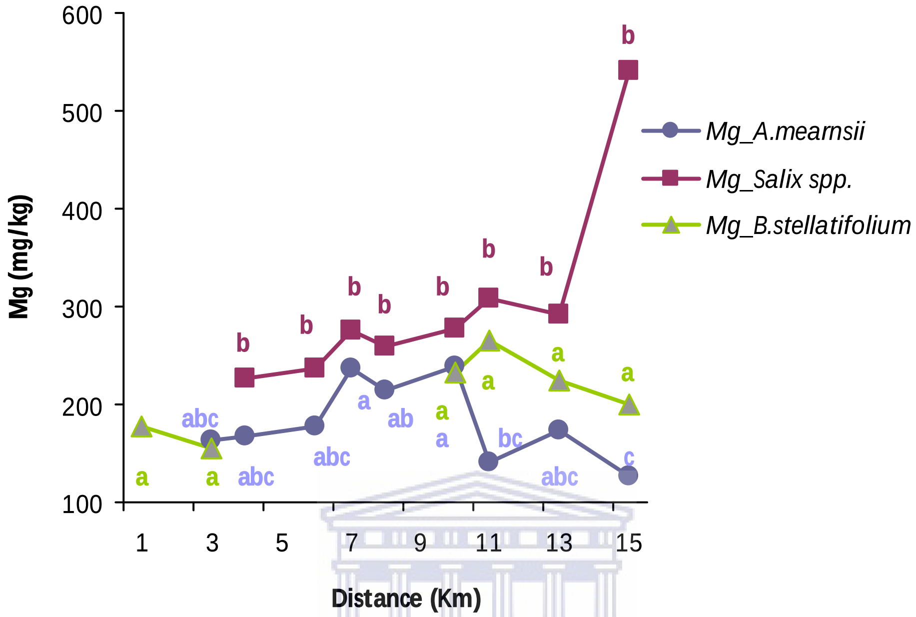
Magnesium uptake in *B. stellatifolium* displayed no significant variation between sampling sites. *B. stellatifolium* concentrations ranged from 155.57 mg/kg – 265.33 mg/kg. Maximum accumulaton occurred 1 km downstream of the WWTF at 11 km. Those sampling sites occuring post the WWTF displayed higher Mg concentrations when compared to the two sampling sites upstream of the WWTF. From the results obtained *S. babylonica* appears to be best at magnesium accumulation.

Seasonal magnesium concentrations indicated similar trends between *A. mearnsii* and *B. stellatifolium* (3.3.6 B). All three species, however, displayed a sharp increase in concentrations at 32 days. *A. mearnsii* displayed a mean range of 22.71 mg/kg – 313.29 mg/kg. Maximum Mg uptake occurred in June 2009 at 141 days, concentrations decreased steadily throughout the winter season until the onset of summer in Novermber 2009 at 295 days, when concentrations began to increase.

The sudden increase at 141 days may be attributed to increased run-off from neighbouring vineyards due to heavy rainfall, which may have resulted in the deposition of Mg in the sediment. Despite the observed increase in Mg; concentrations began to decrease throughout the remainder of the winter seasons. This may be due to the envrionment becoming diluted due to the rainfall experienced throughout the winter. Concentrations began to increase at the onset of summer as sediments begin to settle due to a decrease in turbidity and reduced flow regimes.

Similar trends were observed in *B. stellatifolium* which had a mean range of 23 mg/kg – 261. 68 mg/kg. Maximum accumulation was recorded during the dry summer seasons at 32 days and 415 days respectively. Concentrations began to decrease at the onset of winter at 120 days until the commencement of the summer seasons at 352 days where concentrations began to increase. *S. babylonica* displayed maximum Mg accumulation with a mean range of 39.28 mg/kg – 362.68 mg/kg. A significant variation in Mg concentrations of all the three species analyzed was observed at the commencement of the study at 0 days in February 2009 and the conclusion of the study at day 415.

A



Mg (mg/kg)
600
500
400
300
200
100
1
3
5
7
9
11
13
15
Distance (Km)
Mg_A.mearnsii
Mg_Salix spp.
Mg_B.stellatifolium

B

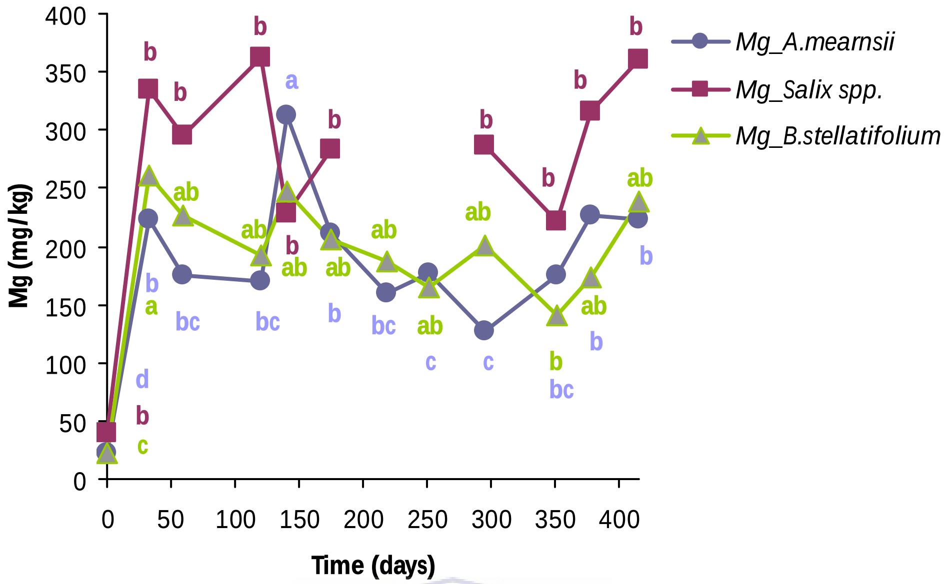


Figure 3.3.6 Means* with the same letter are not significantly different at p=0.05. (A) Foliar magnesium concentrations for *A. mearnsii, S. babylonica* and *B. stellatifolium* (mg/kg) along the length of the Franschhoek River over the sampling period (2009 - 2010). (B) Seasonal variation of magnesium concentration in the leaves of *A. mearnsii, S. babylonica* and *B. stellatifolium* along the Franschhoek River over the sampling period (2009 - 2010).

3.3.7 Potassium concentrations

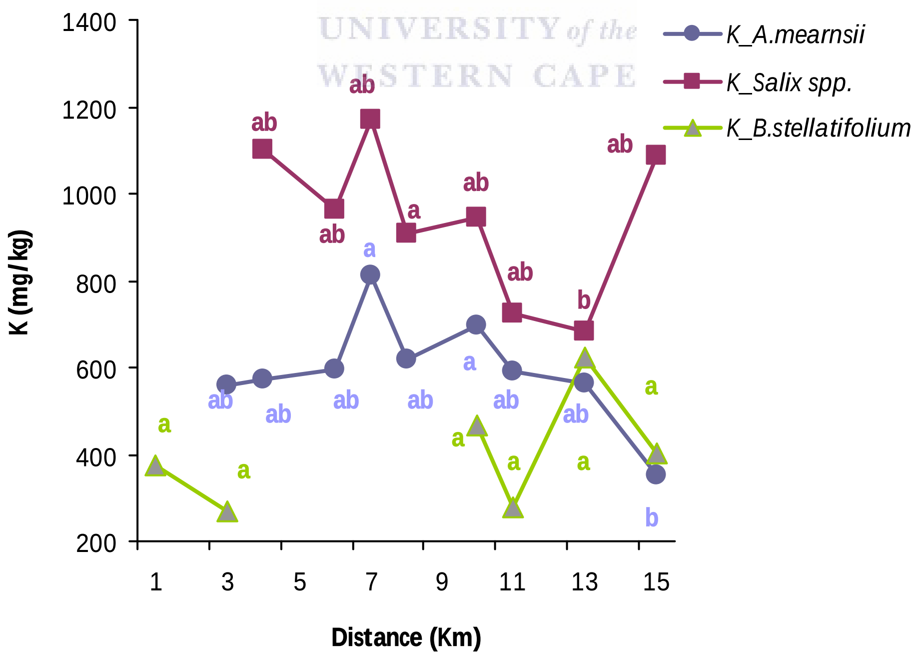
Potassium accumulation along the lengh of the Frasnchhoek River displayed minimal significant variation between the three species analyzed (3.3.7 A). *S. babylonica* displayed concentrations ranging from 640 mg/kg – 1088.2 mg/kg followed by *A. mearnsii* with a mean range of 349.50 mg/kg – 812.70 mg/kg and *B. stellatifolium* which displayed the least K accumulation with a mean range of 270.83 mg/kg – 624.0 mg/kg.

Maximum foliar K accumulation in *S. babylonica* occurred upstream of the WWTF at 7.5 km and a second increase was observed at the WWTF (10 km), followed by a steady decrease in K concentrations. *Acacia mearnsii* displayed similar trends as in *S. babylonica*. Maximum foliar K accumulation in *A. mearnsii* was observed at 7.5 km and 10 km respectively, followed by a steady decrease in K concentrations. With the exception of *B. stellatifolium*; *A.mearnsii* and *S. babylonica* both display lower K concentrations after the WWTF. From the results obtained *S. babylonica* sp is best at K accumulation followed by *Acacia mearnsii* and lastly *Brabejum stellatifolium*.

Visible seasonal trends were observed amongst the three species analyzed (3.3.7 B). *A. mearnsii* displayed seasonal K concentrations ranging from 79.0 mg/kg – 781 mg/kg. *S.babylonica* displayed the most significant variation between minimum and maximum accumulation with a mean range of 115.40 mg/kg – 6994.40 mg/kg, followed by *B. stellatifolium* with a mean range of 34 mg/kg – 4720 mg/kg.

Pottasium concentrations in *S. babylonica* were lowest during the dry summer seasons and highest during the wet winter seasons. The steep increse at 141 days corresponds to the winter month which recieved the highest rainfall of 246.40 mm (Fig 3.1.17). Concentrations decreased steadily as summer approached. Similar trends were observed for *B.stellatifolium*, with an increase in concentration being observed at 141 days followed by a significant decrease until 175 days, whereby concentrations began to increase. No significant variation in the accumulation of K in *A.mearnsii* was observed. From the results obtained *S. babylonica* followed by *A. mearnsii* were best at seasonal K accumulation.

A



B

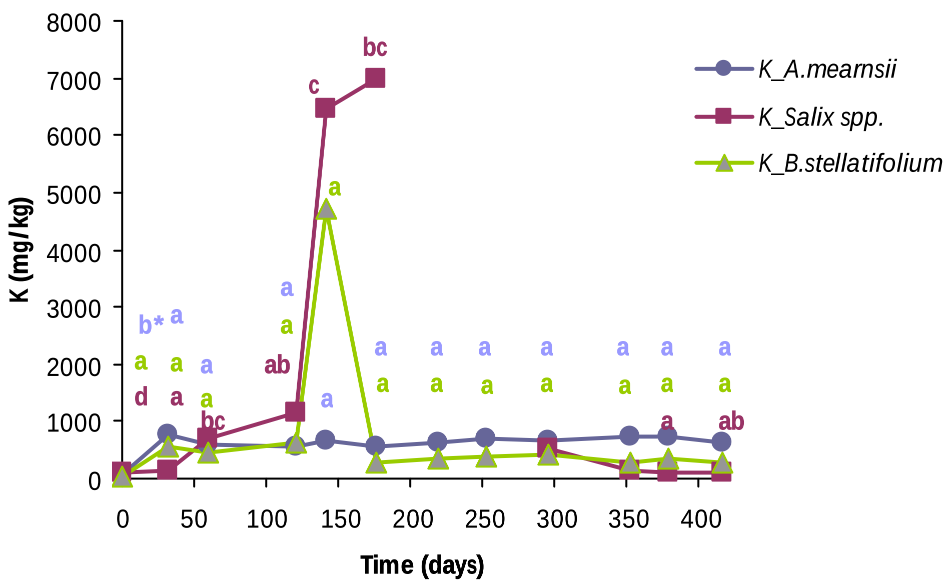


Figure 3.3.7 Means* with the same letter are not significantly different at p=0.05. (A) Foliar potassium concentrations for *A. mearnsii, S. babylonica* and *B. stellatifolium* (mg/kg) along the length of the Franschhoek River over the sampling period (2009 - 2010). (B) Seasonal variation of potassium concentration in the leaves of *A. mearnsii, S. babylonica* and *B. stellatifolium* along the Franschhoek River over the sampling period (2009 - 2010).

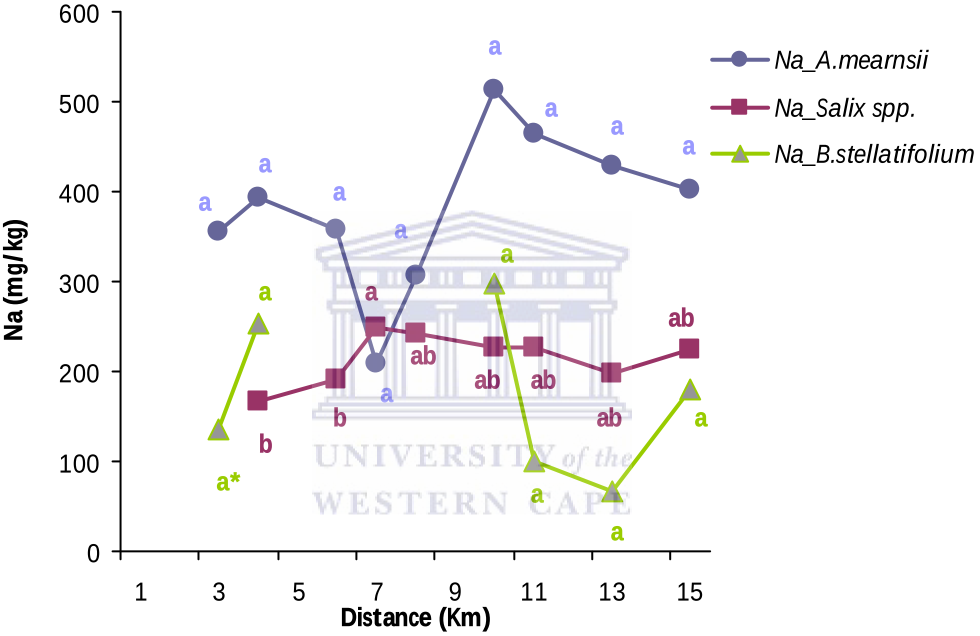
3.3.8 Sodium concentrations

Sodium concentrations along the length of the Franschhoek River displayed minimal trends among the three species analyzed (3.3.8 A). *A. mearnsii* displayed maximum Na accumulation between the three species with a mean range of

208.70 mg/kg – 513.80 mg/kg. Minimum significant inter study site variation was obseved among *S. babylonica* with concentrations ranging between 166.19 mg/kg – 249.20 mg/kg. *B. stellatifolium* displayed on average the least Na accumulation with a mean range of 65.90 mg/kg – 298.0 mg/kg. *A.mearnsii* displayed a sharp increase in Na concentrations at 8.5 km and 10 km respectively. Maximum Na uptake was recorded at the WWTF (10 km). All sampling locations located before the WWTF displayed lower concentrations than those sampling sites located after the WWTF. It can therefore be assumed that majority of the Na input was due to the waste water treatment process. The results obtained for *S. babylonica* do not display the same trends as observed for *A. mearnsii*. Concentrations increased slightly from 4.5 km – 7.5 km and remained relatively stable throughout the remainder of the river. *B. stellatifolium,* similarily to *A. mearnsii* displayed maximum accumulation at 10 km. However, no significant variation in accumulation was recorded between sampling sites for *B. stellatifolium.* From the results obtained *A. meansii* is more suited to Na bioaccumulation followed by *S. babylonica* and lastly *B. stellatifolium*. Similar results were obtained for the water analysis with maximum Na accumulation occuring at the WWTF.

Seasonal Na concentrations indicated lower concentrations in the wet winter season and slightly elevated concentrations during the dry summer seasons (3.3.8 B). *A. mearnsii* had a mean range of 127.30 mg/kg – 340.70 mg/kg, followed by *S. babylonica* which displayed a mean range of 119.27 mg/kg – 478.71 mg/kg and *B. stellatifolium* ranging from 65.33 mg/kg – 619.25 mg/kg. *Acacia mearnsii* displayed an obvious decline in concentrations at the onset of winter, followed by a slight rise in foliar accumulation as summer apprached. No significant variation in the Na bioaccumulation abilities of *A. mearnsii* was observed.

A



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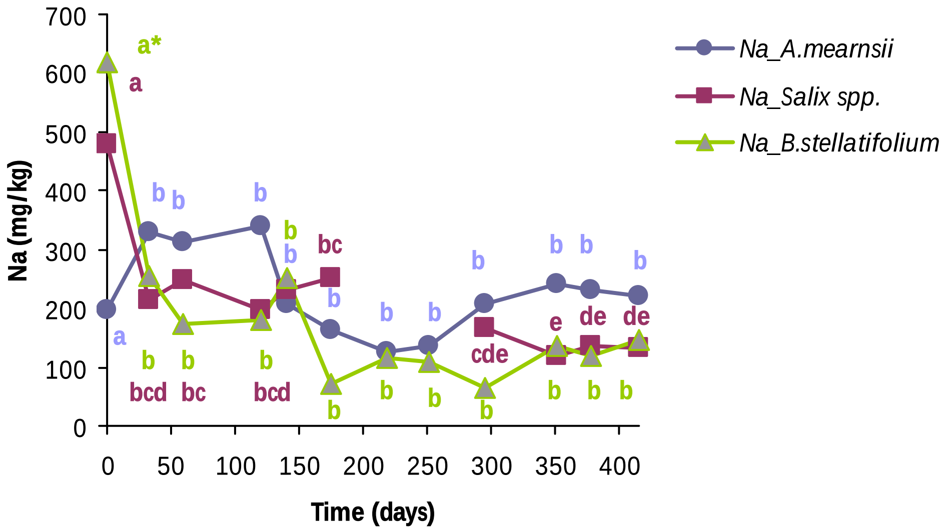


Figure 3.3.8 Means* with the same letter are not significantly different at p=0.05. (A) Foliar sodium concentrations for *A. mearnsii, S. babylonica* and *B. stellatifolium* (mg/kg) along the length of the Franschhoek River over the sampling period (2009 - 2010). (B) Seasonal variation of sodium concentration in the leaves of *A. mearnsii, S. babylonica* and *B. stellatifolium* along the Franschhoek River over the sampling period (2009 - 2010).

3.3.9 Zinc concentrations

Zinc accumulation along the length of the river showed minimal variation among the three species analyzed (Fig 3.3.9 A). *S. babylonica* displayed the highest Zn accumulation with a mean range of 48.024 mg/kg – 134.185 mg/kg, followed by

A. mearnsii which displayed a mean range of 45.06 mg/kg – 124.19 mg/kg and *B. stellatifolium* which had a mean range of 22.73 mg/kg – 105.12 mg/kg.

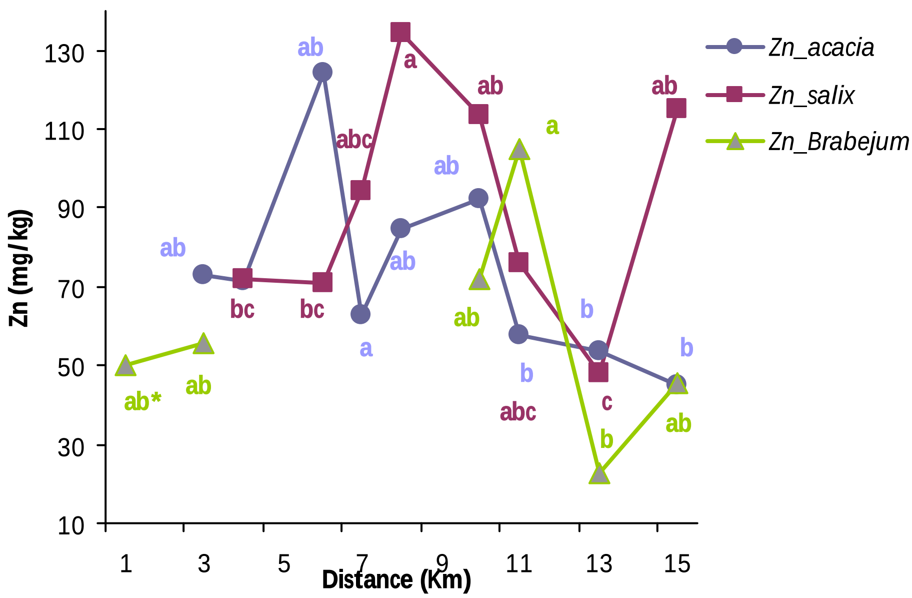
Zinc concentrations in *S. babylonica* increased steadily until 8.5 km, 1 km upstream of the WWTF, followed by a subsequent decrease in concentrations until 13.5 km whereby concentrations increased at 15km. *Acacia mearnsii* displayed erratic Zn concentrations with maximum accumulation occuring at 6km (124.19 mg/kg), followed by an additional spike downstream at 10 km (92.05 mg/kg). Zinc accumulation in the leaves of *A. mearnsii* are lower post the WWTF. No significant variation in Zn concentrations were observed in the leaves of

B. stellatifolium. When comparing *B. stellatifolium* Zn concentrations with *S. babylonica* and *A. mearnsii* no trends were observed. From the results obtained, *S. babylonica* and *A. mearnsii* were best at Zn accumulation.

Visible seasonal trends were observed between the three species analyzed (3.3.9 B). *S. babylonica* displayed a mean ragne of 41.137 mg/kg – 172.197 mg/kg, followed by .*A mearnsii* which had a mean range of 14.74 mg/kg – 145.61 mg/kg and *B. stellatifolium* which had a range of 7.917 mg/kg – 141.39 mg/kg. Maximum zinc accumulation in *S. babylonica* occurred during the dry summer season at 32 days and minimum accumulation occurred during the wet winter season at 175 days. There was a significant difference between minimum and maximum seasonal Zn concentrations in *S. babylonica*. Similar trends were observed for *A. mearnsii* with maximum accumulation occuring at 32 days and miniumum accumulation occuring during the wet season at 252 days. *B. stellatifolium* displayed clear variation in seasoanl Zn accumulation with maximum accumulation in summer and decreased accumulation occuring in the wet seasons. All three species displayed lower concentrations in the wet winter season and elavated concentrations in the dry summer season.

Zinc concentrations in soil typically range from 10 mg/kg – 300 mg/kg. However Zn concentrations in plants once accumulated ranged from 15 mg/kg – 100mg/kg (El – Bassam, 1978). Similar results were observed in the present study for the analysis conducted along the length of the Franschhoek River as well for seasonal Zn accumulation.

A



B

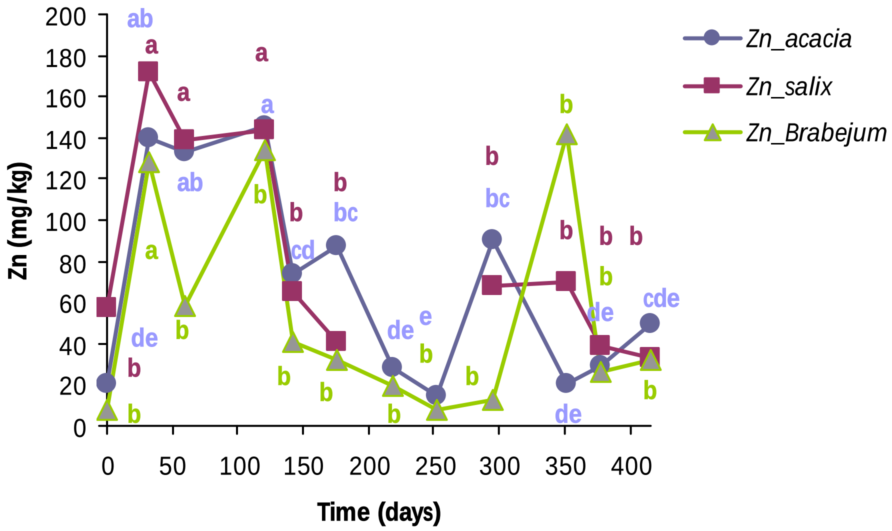


Figure 3.3.9 Means* with the same letter are not significantly different at p=0.05. (A) Foliar zinc concentrations for *A. mearnsii, S. babylonica* and *B. stellatifolium* (mg/kg) along the length of the Franschhoek River over the sampling period (2009 - 2010). (B) Seasonal variation of zinc concentration in the leaves of *A. mearnsii, S. babylonica* and *B. stellatifolium* along the Franschhoek River over the sampling period (2009 - 2010).

3.3.10 Nitrogen

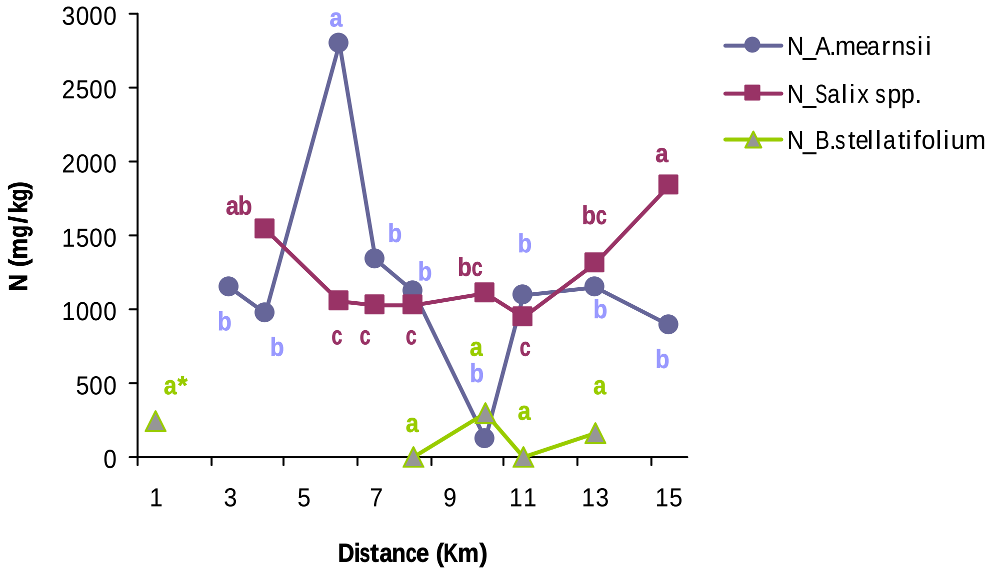
Nitrogen is a common constituent in many soils, especially in areas exposed to agricultural practices. Nitrogen tends to accumulates in soils and sediments when N inputs exceeds the demands and plants are no longer able to absorb it (Nosengo, 2003). Photosynthesis and many other metabolic processes depends on a certain amount of N as it promotes plant growth by being a primary constituent of chlorophyll, protein amino acids and photosynthetic activity (Gairola, 2009). Nitrogen reduction has been reported to occur in the leaves of plants and that the nitrate reductase enzyme is most active in plant leaves and juvinile leaves exposed to maximum irradiance (Wright and Davison, 1964).

Nitrogen concentrations varied drastically between the three species analyzed (3.3.10 A). *A. mearnsii* displayed a mean ragne of 116.70 mg/kg – 2801 mg/kg. Maximum N accumulation occurred at 6 km, followed by a significant decrease in concentrations at the WWTF (10km). Nitrogen concentrations increased steadily until 11 km and remained relatively constant throughout the remainder of the Franschhoek River. *S. babylonica* displayed equally high N concentrations, however, conflicting N uptake was observed when comparing accumulatiom between species at each study site. Maximum N uptake in *S. babylonica* was observed at 15 km. Nitrogen accumulation remained relatively stable along the length of the river with no significant variation being observed from sampling sites 4 – 9 (6 km – 13.5 km). *Brabejum stellatifolium* displayed the least N accumulationwith a mean concentration ranging between 2.7 mg/kg – 300.6 mg/kg. Maximum accumulation was noted at 10 km with concentrations remaining stable throughout the remainder of the river yet significantly lower than the invasive *A. mearnsii* and *S. babylonica*.

Seasonal concentrations varied among the three species analyzed (3.3.10 B). Maximum N accumulation occurred in *A. mearnsii* with a mean range of 327.10 mg/kg – 1762.50 mg/kg. The highest N uptake was noted in the dry summer season at 32 ; 352 and 415 days respectively, with the lowest concentrations being recorded during the winter months at 120 days – 295 days. *S. babylonica* nitrogen concentrations ranged from 154.80 mg/kg – 1580.79 mg/kg. Concentrations peaked at 120 days and decreased until leaf abcision occurred, when sampling recommenced during late spring, early summer at 295 days concentrations began to increase until it peaked at 378 days. Decreased soil moisture and increased irradiance during the dry summer seasons may have contributed to an increase in N in the summer months.

Nitrogen concentrations in *B. stellatifolium* remained relatively constant. Nitrogen concentration ranged from 156.80 mg/kg – 744.90 mg/kg. Maximum uptake occurred at 32days (summer, 2009). No significant variation in uptake in *B.stellatifolium* was noted for the reaminder of the study period. From the results obtained *A. mearnsii* and *S. babylonica* accumulated higher concentrations of N during the dry summer season opposed to the wet winter seasons when the riverine system becomes diluted with excess rainfall.

A



N (mg/kg)
3000
2500
2000
1500
1000
500
0
1
3
5
7
9
11
13
15
Distance (Km)
N_A.mearnsii
N_Salix spp.
N_B.stellatifolium
a
b
b
b
b
b
b
b
b
ab
c
c
c
bc
c
bc
a
a*
a
a
a
a

B

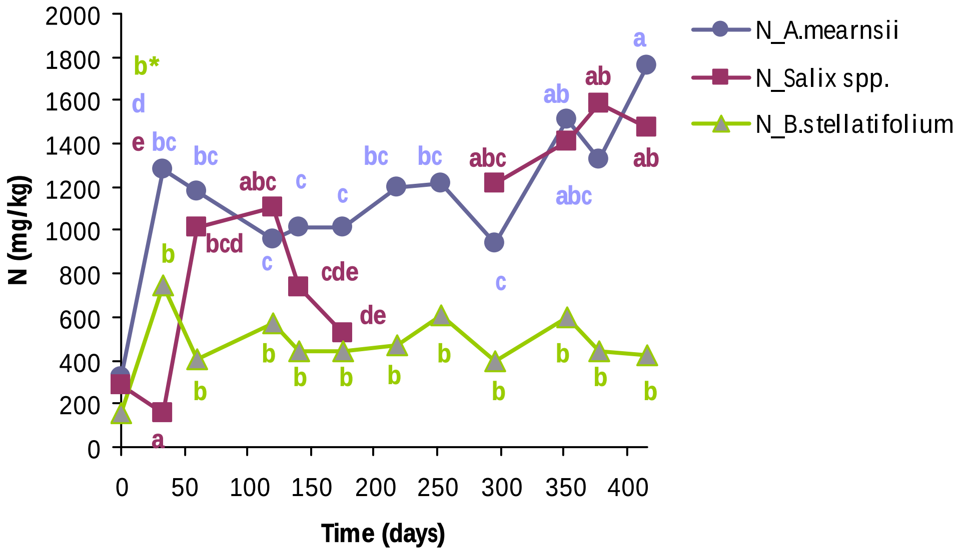


Figure 3.3.10 Means* with the same letter are not significantly different at p=0.05. (A) Foliar nitrogen concentrations for *A. mearnsii, S. babylonica* and *B. stellatifolium* (mg/kg) along the length of the Franschhoek River over the sampling period (2009 - 2010). (B) Seasonal variation of nitrogen concentration in the leaves of *A. mearnsii, S. babylonica* and *B. stellatifolium* along the Franschhoek River over the sampling period (2009 - 2010).

3.3.11 Phosphorus concentration

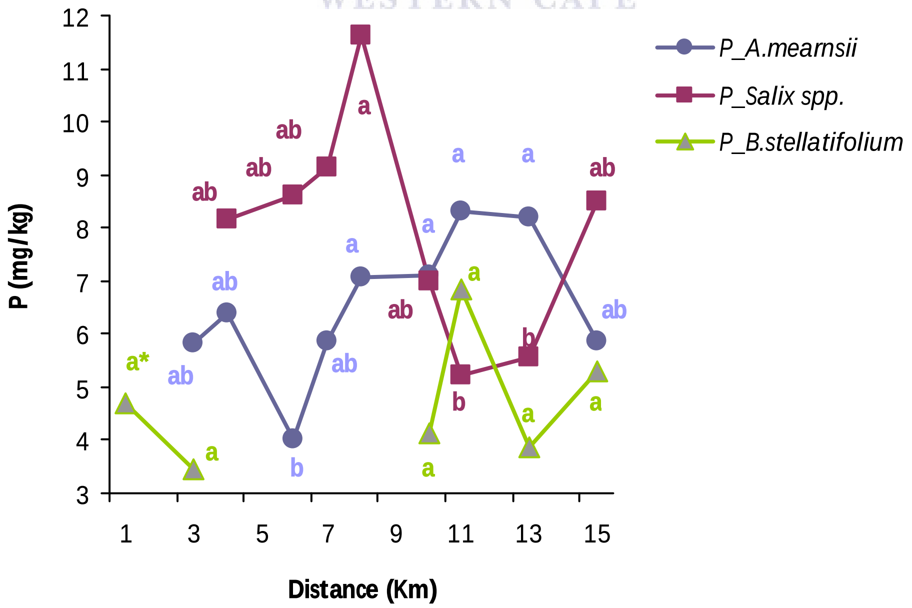
Phosphorus is a mineral nutrient essential for plant growth and survival. Plants have therefore seeked out mechanisms allowing them to aquire this mineral. According to Grennan (2008), phosphorus is considered a limiting mineral in part to the low P concentrations in soils and sediment and due to its low solubility and high sorption acting in concert. When the availability of P in sediment or soil becomes limiting, plants employ a variety of strategies to increase inorganic phosphorus (P_i) acquistion, these include, but are not limited to altering root structure and function as well as modifying rhizpheres (Franco-Zorilla *et al*. 2004 ; Ticconi and Abel, 2004).

Phosphorus concentrations varied among the three plant species analyzed. *S. babylonica* displayed P uptake ranging from 5.22 mg/kg – 11.62 mg/kg. with significant variation being observed between minimum and maximum concentrations. Phosphorus concentrations in *S. babylonica* increased gradually from 4.5 km – 8.5 km where maximum accumulation was recorded. Concentrations decreased steadily until 11 km where recorded concentrations were half that of the maximum P accumulated. Phosphorus uptake in *S. babylonica* increased steadily until 15 km and displayed overall higher concentrations post the WWTF.

Acacia mearnsii had a mean P range of 4 mg/kg – 8.3 mg/kg. Phosphorus accumulation in *A. mearnsii* displayed an inverse P uptake when compared with *S. babylonica*. Similar to *S. babylonica*, *Acacia mearnsii* displayed higher P concentrations post the WWTF. *Brabejum stellatifolium* displayed concentrations ranging from 3.45 mg/kg – 6.83 mg/kg. Similar to *A. mearnsii,* maximum accumulation occurred 1 km downstream of the WWTF, followed by a sharp decrease at 13.5 km.

Seasonal P concentrations varied drastically between the three species analyzed. *S. babylonica* displayed maximum accumulation with a mean range of 3.4 mg/kg – 10.71 mg/kg. *S. babylonica* concentration remained stable throughout the first six months of sampling, however once foliar growth recommenced at 352 days. P concentrations increased significantly. Minimal trends where observed between P uptake in *A. mearnsii*. Phosphorus accumulation in *A. mearnsii* ranged from 5 mg/kg – 8.83 mg/kg. There was a significant difference between minimum and maximum accumulation, however seasonal uptake remained erratic. The last four months of the dry summer season displayed increased P uptake, with similar results being observed by *S. babylonica*.

A



B

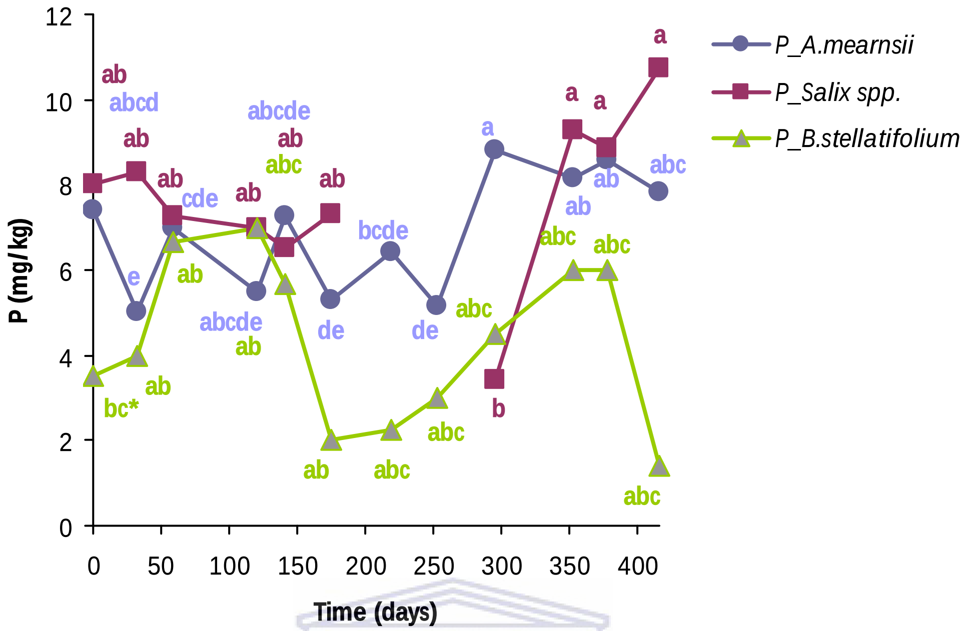


Figure 3.3.11 Means* with the same letter are not significantly different at p=0.05. (A) Foliar phosphorus concentrations for *A. mearnsii, S. babylonica* and *B. stellatifolium* (mg/kg) along the length of the Franschhoek River over the sampling period (2009 - 2010). (B) Seasonal variation of phosphorus concentrations in the leaves of *A. mearnsii, S. babylonica* and *B. stellatifolium* along the Franschhoek River over the sampling period (2009 - 2010).

3.1.12 Conclusion

- Metal accumulation among plant species.

The concentration of metals along the length of the Franschhoek River varied among the three species analyzed. The leaves of *S. babylonica* displayed higher concentrations for Cd, Ca, Mg, K, Zn and P when compared with *Acacia mearnsii* and *Brabejum stellatifolium* when leaves were present in the summer prior to abscission. It can therefore be assumed that *Salix babylonica* is best at metal uptake of said elements in the summer months when leaves are present. *Acacia mearnsii,* however, displayed higher concentrations of Cu, Fe, Pb, Na and N incomparison with *S. babylonica* and *B. stellatifolium*. *B. stellatifolium* displayed maximum seasonal foliar Cd uptake and *S. babylonica* displayed maximum seasonal foliar Fe uptake. The invasive riparian trees displayed an overall higher acquistion of metals than the endemic *B. stellatifolium*.

- Metal accumulation along the length of the Franschhoek River.

Higher mean concentrations of Cd, Ca, Mg, Zn and Pb were observed in *S.babylonica* downstream of the Franschhoek River or post the WWTF and Fe, Na, Ca and P in *A. mearnsii*. The high levels of these elements may be attributed to non point source run-off from the intensive agriculture and viticulture practices as well as storm water run-off and point source pollution originating from the treated waste water facility. Of all the metals analyzed among the three plant species, eight out of eleven elements displayed higher concentrations at the waste water treatment facility and / or after the WWTF.

- The effect of seasonality on metal accumulation.

Of all the elements analyzed, P, Zn and Mg displayed an obvious reduction in concentrations in the wet winter season in *B. stellatifolium* and Mg in *A. mearnsii*. An array of factors affect metal accumulation in plants. The dilution effect of the heavy rains, coupled with increase soil moisture may have contributed to a reduction in metal bio accumulation.

CHAPTER 4

SUMMARY AND RECOMMENDATIONS

4.1 Summary

The aim of this study was to quantify the extent of inorganic chemical pollution of the Franschhoek River and draw relationships between contaminants in the plants, water and soil and where possible provide recommendations to improve the river quality. The results indicated that the water quality of the Franschhoek River is facing severe chemical pollution due to inorganic chemical pollutants mainly K, Na, Ca, nitrate and ammonia along the length of the river. Seasonal trends reveal that Fe, Mg, Pb, Na, Ca, nitrate and ammonia all displayed elevated concentrations during the warmer summer months. The electrical conductivity, nitrate, iron and potassium concentrations are all above the South African Target Water Quality Guidelines and without adequate mitigation measures inplace, the health of the river and surrounding fauna and flora, will be adversly affected.

Depending on the rivers morphology and hydrological regimes, contaminants will often settle in the bottom sediment (Milenkovic *et al*, 2005), thereby making sediments a good reservoir for nutrients and heavy metals. According to Goncalves and Boaventura, (1991) and Goncalves *et al*, (1992), seasonal accumulation of heavy metals in aquatic ecosystems is a good indicator of environmental water quality as they represent contaminants over time and are useful as they provide contamination data of a spatial nature and temporal history of pollution of a given system. Increased concentrations of Fe, K, Na, Mg, Ca, Cu and Zn were recorded in the dry summer season. This may be attributed to decreased flow regimes and an increase in run-off from the irrigation regimes which occur throughout summer.

Of all the three plant species analyzed, *S. babylonica* displayed the highest acquisition of heavy metals in months when leaves were present. According to the accepted typical ranges for metals in plants, all three plant species were within the accepted ranges for Cd, Cu and Zn. The overall results indicated that Fe, K, Na, Ca, Mg, Cu, Pb, Zn and Cd, in plants and sediments were higher than those in the water. However, seasonal results indicate higher Fe and Pb accumulation in sediment. This suggests that most metals were accumulated by plants from sediment rather than the water.

The water quality of the Franschhoek River is greatly affected by agricultural and viticulture practices as well as urban and storm water run-off. The primary source of downstream water contamination was the treated effluent run-off from the Waster Water Treatment Facility.

4.2 Recommendations

An intergrated approach should be adopted when considering possible mitigation and remediation of riverine ecosystems. Despite the obvious water supply crisis being faced by sub saharan Africa, research and data surrounding water quality of riverine ecosystems remains scant. First and foremost continued holistic research, regarding water quality should be conducted, which assesses the quality of the entire riverine ecosystem.

The deletarious impact of urbanization is evident in the Franschhoek River. The river displays declining water qualilty, degradation of the stream channel, loss of ecosystem integrity and functioning due to inundation of invasive macrophytes as well as the loss of biodiversity.

The water quality of the Franschhoek River is greatly affected by agricultural and viticultural practices as well as urban and storm water run-off. The primary source of downstream water contamination was the treated effluent run-off from the waste water treatment facility. Those farmers who source water from the Franschhoek River should adequately treat the water before releasing it back into the river system.

Proposed recommendations with regards to the treated waste water effluent should include, but not be limited to:

1. Improving the quality of the waste water being discharged into the Franschhoek River.

2. Control the quantity of the waste water being discharged, ensuring that it is within permissable levels.

3. Ensure that the treatment plant can accommodate an increase in the effluent as urbanization continues to increase in Franschhoek.

4. Construct a secondary waste water treatment plant. Due to increased urbanization in the Franschhoek area, the current waste water treatment facility, is unable to accommodate the increased volume of effluent.

As the river is used primarily for agricultual practices (irrigation and livestock) and recreational purposes, Total Maximum Daily Loads (TMDL), for all receiving water including major and minor tributries should be considered, specific to each requirement.

Due to the high load of fertilizers being used, eutrophication remains a possibility according to DWAF 1996a, on farm management practices to mitigate nitrogen levels include, but are not limited too:

1. diluting the nitrogen rich source if an alternate water source is available.

2. utilizing the nitrogen rich source only during vegetative plant growth stages.

3. limit the groundwater leaching as to reduce the liklihood of groundwater nitrogen contamination.

4. Removing nuisance algae and water plants from irrigation water with screens and filters.

Plans should be developed in order to prevent further degradation of the Franschhoek River especially as it is a primary tributary to the Berg River. In addition to monitoring; management guidelines should be developed which encompass Best Management Practices (BMP), to reduce run-off, pollutant consitituents and contaminants from receiving waters as the area is predominatly farm land. BMP's should employ both operation and management procedures.

The Franschhoek River should be monitored on an on going basis and appropriate action taken when water quality deminishes. BMP's and mitigation measures should promote urban biodiversity, enhance amenity and the aesthetics of the river.

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APPENDICES

Appendix I: Raw data for sediment metal concentrations during summer and winter.

Date	Season	Location	Distance (km)	Ca	Cu	K	Na	Mg	Zn	Fe	Cd
3/8/2009	WET	F1	1.5	55.92	0.95	0.00	39.46	26.75	18.68	2343.16	0.05
3/8/2009	WET	F2	3	57.26	1.42	258.17	34.34	52.51	21.48	2365.60	0.10
3/8/2009	WET	F3	4.5	54.10	0.19	63.99	21.59	22.37	23.10	1240.20	0.04
3/8/2009	WET	F4	6	64.88	0.25	0.00	29.59	20.09	29.06	1492.59	0.02
3/8/2009	WET	F5	7.5	40.88	0.56	0.00	38.37	24.78	22.90	1414.96	0.13
3/8/2009	WET	F6	8.5	74.19	1.01	0.00	34.48	12.44	26.30	998.82	0.04
3/8/2009	WET	F7	10	103.32	0.75	0.00	31.44	17.69	26.82	1226.92	0.01
3/8/2009	WET	F8	11	189.23	0.82	0.00	30.70	18.76	17.41	1552.15	0.01
3/8/2009	WET	F9	13.5	81.60	0.34	0.00	31.07	13.54	40.59	946.74	0.01
3/8/2009	WET	F10	15	73.32	0.41	0.00	36.10	13.89	4.20	843.93	0.01
6/4/2010	DRY	F1	1.5	45.47	2.15	460.62	43.66	93.58	22.42	4133.75	0.01
6/4/2010	DRY	F2	3	171.96	3.93	684.10	43.01	108.37	20.87	5636.52	0.02
6/4/2010	DRY	F3	4.5	78.30	1.79	0.00	35.90	45.93	9.01	1530.92	0.04
6/4/2010	DRY	F4	6	109.88	1.56	205.07	37.31	24.79	19.94	2265.14	0.07
6/4/2010	DRY	F5	7.5	102.52	0.89	0.00	33.38	9.94	77.43	1883.70	0.03
6/4/2010	DRY	F6	8.5	112.71	1.38	33.39	35.82	17.93	20.90	1027.50	0.03
6/4/2010	DRY	F7	10	126.88	11.96	0.00	41.66	20.73	23.28	2805.58	0.02
6/4/2010	DRY	F8	11	71.32	1.79	0.00	33.43	16.61	9.68	1360.69	0.05
6/4/2010	DRY	F9	13.5	59.11	1.86	0.00	36.04	11.69	15.90	5925.44	0.01
6/4/2010	DRY	F10	15	41.30	0.73	0.00	28.01	13.84	253.63	805.44	0.08

Appendix II: Raw data for water nutrient and metal concentrations.

Site	Distance (km)	Oxygen (mg/l)	Oxygen (%)	pH	Temperature (C°)	Conductivity (mS)	Ammonia (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)
FR1	1.5	7.86	82.5	6.177	17.3	76	0	1	0.9
FR2	3	8.46	92.9	6.351	19.8	94	0	1	0.7
FR3	4.5	8.15	89.4	5.965	19.8	128	0	2	0.6
FR4	6	7.75	86.2	6.875	20.6	165	0	2	0.7
FR5	7.5	8.85	102.1	6.916	22.5	159	0	2	0.8
FR6	8.5	6.7	75.1	6.973	21.5	238	0.2	0.5	1
FR7	10	3.48	39.8	7.112	22.5	568	0.3	2	0.6
FR8	11	8.63	99.4	7.39	22.4	518	4.7	10	2.1
FR9	13.5	7.6	85.5	7.205	21.5	360	1.9	15	1.6
FR10	15	7.42	81.6	6.585	21.4	238	1.4	18	1
FR1	1.5	7.56	75.3	6.146	17	70	0	1	0.7
FR2	3	8.5	89.4	6.462	18.4	82	0	1	0.6
FR3	4.5	7.92	84	5.98	18.7	127	0	1	0.7
FR4	6	7.66	80.5	6.489	18.1	153	0	1	0.6
FR5	7.5	8.04	86.9	6.846	18.7	190	0	1	0.7
FR6	8.5	7.69	82.4	7.22	19	267	0	0	0.5
FR7	10	1.76	20.2	7.382	20.7	707	2.6	0	0.6
FR8	11	6.1	69.7	7.583	22.9	895	5.7	9	1.8
FR9	13.5	5.53	63.7	7.342	22.2	504	3.9	20	1.9
FR10	15	4.63	49.3	6.8	18.9	351	3.8	24	1.4
FR1	1.5	7.14	65.7	6.419	15	75	0	1	0

FR2	3	9.5	93.1	6.521	14.4	80	0.1	1	0
FR3	4.5	7.56	76.2	6.031	15.3	128	0	2	0
FR4	6	18.6	83.2	6.476	14.4	162	0	0	0
FR5	7.5	7.11	76.2	6.823	14.8	163	0	0	0
FR6	8.5	9.26	90.7	7.083	16.2	257	0	1	0
FR7	10	4.74	44.5	7.015	17.9	635	3.7	2	0
FR8	11	5.53	57.3	7.344	16.7	565	5	12	0
FR9	13.5	8.5	79.8	7.01	16.2	324	0.8	18	0
FR10	15	7.88	86	6.724	16.5	306	0.9	13	0
FR1	1.5	10.25	98.4	6.936	14.1	54	0	0	0
FR2	3	6.77	66.4	6.94	15.4	201	0	1	0
FR3	4.5	10.11	100.3	6.849	18.4	152	0	2	0
FR4	6	6.25	63.8	6.887	15.5	130	0	1	0
FR5	7.5	9.48	90.7	7.075	15.2	179	0.1	3	0
FR6	8.5	5.93	57.1	7.069	15.1	189	0	2	0
FR7	10	6.75	64.2	7.045	15	204	0.2	3	0
FR8	11	9.58	95.6	7.108	14.9	213	0.4	2	0
FR9	13.5	10.46	105.1	7.105	14.5	183	0.2	2	0
FR10	15	9.74	86.3	7.057	14.4	183	0.2	0.2	0
FR1	1.5	8.95	84.7	6.248	13.2	55	0	1	0
FR2	3	3.97	38.5	6.334	13.9	127	0	1	0
FR3	4.5	9.87	95.7	6.122	14	101	0	2	0
FR4	6	5.04	47.2	6.407	13.4	115	0	3	0
FR5	7.5	5.34	50.9	6.728	12.9	163	0	3	0
FR6	8.5	7.62	75.6	6.725	14.7	97	0	2	0
FR7	10	5.34	52.8	6.777	14.7	110	0	3	0
FR8	11	9.25	92.3	6.922	14.6	117	0.4	4	0
FR9	13.5	11.22	101.3	6.592	12	191	0	4	0
FR10	15	5.95	55.1	6.401	11.6	206	0.1	3	0
FR1	1.5	8.9	83.1	6.201	12.1	34	0	0	0

FR2	3	2.89	27.4	6.239	13	62	0	0	0
FR3	4.5	4.76	44.8	6.145	13	69	0	1	0
FR4	6	3	33	6.628	15.5	49	0	2	0
FR5	7.5	9.14	85.9	6.786	15	111	0	3	0
FR6	8.5	3.79	37.8	6.857	14.9	169	0	2	0
FR7	10	3.8	37.2	6.866	14.5	129	0.4	1	0
FR8	11	5.22	51.1	6.852	14	149	1.3	4	0
FR9	13.5	10.15	98.6	6.871	13.8	134	0.6	4	0
FR10	15	9.86	95.1	6.768	13.6	111	0	3	0
FR1	1.5	9.41	89.5	3.532	13.2	36	0.9	0	0
FR2	3	9.34	90.8	6.244	14	56	0	0	0
FR3	4.5	9.06	88.6	6.165	14.4	57	0.5	0	0
FR4	6	9.33	91.6	4.261	14.4	67	0.1	0	0
FR5	7.5	9.21	90.9	6.709	15.1	78	0	0	0
FR6	8.5	9.09	91	6.832	15.1	105	0.1	0	0
FR7	10	9.12	90.3	6.883	15.1	116	0.2	1	0
FR8	11	8.75	79.1	5.932	15.1	126	0.8	0	0
FR9	13.5	9	89	6.138	14.8	111	0.5	0	0
FR10	15	9.31	91.9	6.319	14.8	110	0	1	0
FR1	1.5	8.51	83.4	6.135	14.6	29	0.4	0	0
FR2	3	8.78	88.3	6.199	15.7	41	0.1	0	0
FR3	4.5	8.33	87.5	6.116	15.8	49	0.2	1	0
FR4	6	8.91	90.9	6.364	16.4	59	0.2	0	0
FR5	7.5	8.05	88.6	6.414	19.7	78	0.2	1	0
FR6	8.5	7.75	82.6	6.626	18.5	114	0.2	0	0
FR7	10	7.59	80.7	6.637	18.7	126	0.9	3	0
FR8	11	7.42	7.9	6.726	18	136	0.9	3	0
FR9	13.5	8.23	86.8	6.668	17.5	108	0.8	1	0
FR10	15	8.07	84.49	6.614	17.8	97	0.5	2	0
FR1	1.5	9.04	9.11	6.096	15.6	36	0.1	0	0

FR2	3	8.81	91	6.284	17.1	62	0.2	0	0
FR3	4.5	7.58	80	6.372	17.3	69	0.1	0	0
FR4	6	10.75	84.4	6.701	19.8	82	0.2	0	0
FR5	7.5	7.64	83.5	6.909	19.8	113	0.2	0	0
FR6	8.5	7.62	82.3	7.007	19	123	0.2	0	0
FR7	10	8.78	89.2	7.19	15.5	151	0.7	0	0
FR8	11	7.55	82.6	6.99	15.8	151	1	0	0
FR9	13.5	8.84	89.6	7.073	15.8	107	0.7	0	0
FR10	15	8.5	90.3	6.894	18.3	147	0.6	1	0
FR1	1.5	5.19	48.7	6.46	17.2	47	0	0	0
FR2	3	4.64	5.11	6.495	19.7	68	0	0	0
FR3	4.5	3.85	43.3	6.808	20.1	67	0	0	0
FR4	6	3.45	40	6.192	21	103	0	0	0
FR5	7.5	3.45	39.7	7.243	22.5	121	0	0	0
FR6	8.5	7.72	86.4	7.029	20.7	135	0	0	0
FR7	10	3.46	39.5	6.918	22.2	370	5	0	0
FR8	11	1.2	15	7.309	22.4	371	5.4	0	0
FR9	13.5	1.82	20.1	7.177	20.4	220	5.3	6	0
FR10	15	16.3	18.1	6.916	20.8	177	3	13	0
FR1	1.5	4.59	48.9	6.217	18.5	57	0.3	0	0
FR2	3	6.09	6.72	6.377	20.5	67	0.4	0	0
FR3	4.5	4.51	51.1	6.275	21.5	111	0.4	1	0
FR4	6	5.4	69.1	6.61	22	113	0.4	1	0
FR5	7.5	3.85	33.6	6.657	24.4	992	0.5	2	0
FR6	8.5	1.33	14.7	6.696	21	153	0.4	0	0
FR7	10	2.25	26.1	6.196	21.7	651	4.5	52	0
FR8	11	0.94	10	5.563	21.6	538	4	30	0
FR9	13.5	3.65	42.8	4.881	22.5	290	5.4	52	0
FR10	15	10.2	48.4	5.759	23	212	1.6	0	0
FR1	1.5	4.16	10.7	6.287	15.8	54	0.2	0	0

FR2	3	2.44	19.1	6.326	17.6	69	0.1	0	0
FR3	4.5	1.64	18	6.336	19.2	141	0.1	0	0
FR4	6	1.6	17.2	7.078	18.4	144	0.2	0	0
FR5	7.5	2.32	24.4	6.804	20.8	137	0.3	0	0
FR6	8.5	3.33	33.8	6.993	19.3	351	0	0	0
FR7	10	0.17	20	7.684	20.4	567	1.5	5	0
FR8	11	2.6	30.2	6.259	20.3	604	5.3	55	0
FR9	13.5	5.06	51.5	6.343	18.3	433	5.9	52	0
FR10	15	1.9	20	6.269	15.9	196	0.5	0	0

Site	**Distance (km)**	**Zn**	**Cu**	**Ca**	**Pb**	**Mg**	**Na**	**K**	**Fe**	**Cd**	**P**
FR1	1.5	-0.0019	0.0011	0.1222	-0.0229	0.0711	0.2382	3.7385	-0.0142	-0.01	1
FR2	3	-0.0011	0.0018	0.1325	-0.0046	0.0734	0.4573	1.6816	-0.0087	0.00	1
FR3	4.5	0.003	0.0014	0.2571	-0.0275	0.12	0.5503	0.2476	-0.0122	0.00	1
FR4	6	0.0002	0.0019	0.5808	-0.0183	0.179	0.8994	0.7238	0.1054	-0.02	1
FR5	7.5	0.0047	0.0019	0.492	-0.0206	0.2012	0.7288	9.9132	0.0919	-0.03	1
FR6	8.5	0.0021	0.0014	0.8702	0.0218	0.2705	1.0067	0.9041	0.0879	0.00	1
FR7	10	0.0053	0.0024	1.297	0.0284	0.2487	2.9168	1.3204	0.0882	-0.01	1
FR8	11	0.0029	0.001	1.3216	0.0327	0.2922	2.7903	1.5811	0.1775	-0.01	3
FR9	13.5	0.0051	0.0014	0.7614	0.024	0.2418	1.9947	1.79	0.0829	-0.01	2

FR10	15	0.0028	0.0017	0.5437	0.0393	0.0756	1.2636	2.3204	0.0732	-0.02	1
FR1	1.5	0.0002	0.0009	0.1341	0.0082	0.0741	-0.1033	2.5492	-0.0141	-0.05	1
FR2	3	-0.0017	-0.0004	0.0996	-0.002	0.1287	0.0712	2.1837	-0.0013	-0.02	1
FR3	4.5	0.0007	-0.0001	0.2214	-0.0082	0.1602	0.267	2.1061	0.0247	0.00	1
FR4	6	0.0018	0.0007	0.4313	0.0347	0.1988	0.5228	0.9656	0.1479	-0.03	1
FR5	7.5	0.0014	0.0012	0.5597	-0.0143	0.3027	0.7591	10.4931	0.0975	-0.04	1
FR6	8.5	0.0035	0.0008	0.9239	0.009	0.2412	1.2697	1.0893	0.1646	-0.07	1
FR7	10	0.007	0.0022	1.3445	-0.018	0.2918	4.2505	1.1452	0.0688	2.06	3
FR8	11	0.0066	0.0011	1.291	-0.027	0.2368	4.0215	1.1117	0.1663	3.02	5
FR9	13.5	0.0044	0.001	0.7676	0.0203	0.2284	2.3539	1.4738	0.0843	0.43	4
FR10	15	0.0082	0.0003	0.6122	0	0.0647	1.7879	1.7754	0.0531	0.27	2
FR1	1.5	0.0019	0.0014	0.1718	-0.0081	0.0709	2.5177	2.9698	0.0213	0.07	2
FR2	3	0.0049	0.001	0.1961	-0.004	0.1249	1.005	2.0953	0.0188	-0.02	2
FR3	4.5	0.0044	0.0004	0.3072	-0.0162	0.1636	0.9138	1.7348	0.0134	0.00	1
FR4	6	0.0026	0.0012	0.5087	-0.0162	0.2768	0.8968	0.6893	0.0303	0.01	1
FR5	7.5	0.0057	0.0004	0.8615	-0.0182	0.2664	1.0946	0.218	0.073	-0.01	2
FR6	8.5	0.0031	0.0025	0.9207	-0.0244	0.2623	1.7137	1.9251	0.2876	0.02	1
FR7	10	0.0106	0.0007	1.5969	-0.0285	0.2701	1.7405	0.9821	0.0505	0.00	3

FR8	11	0.0048	0.0011	1.3791	-0.0264	0.2071	4.2488	0.8581	0.0784	0.02	2
FR9	13.5	0.0062	0.0023	0.8608	-0.0102	0.2235	3.2885	1.0511	0.0595	0.02	1
FR10	15	0.0053	0.0023	0.797	-0.0142	0.0551	2.1963	1.0863	0.0289	-0.01	1
FR1	1.5	0.0059	0.0009	0.1618	0.0039	0.1096	2.2604	1.6241	0.002	-0.01	6
FR2	3	0.0059	0.0002	0.3669	0.0078	0.1102	0.9911	3.8934	0.0233	-0.02	3
FR3	4.5	0.0075	-0.0001	0.3585	-0.0059	0.1226	2.1872	4.1299	0.015	0.02	1
FR4	6	0.0076	0.0012	0.4058	0.0137	0.2054	0.9753	3.2088	0.0274	0.04	2
FR5	7.5	0.0058	0.0003	0.7129	0.0117	0.2225	0.9653	1.8727	0.0895	0.00	2
FR6	8.5	0.0018	0	0.712	0.0333	0.2406	1.1494	1.8695	0.1718	-0.03	2
FR7	10	0.0053	0.0015	0.7976	0.0229	0.2355	1.2239	0.2022	0.1912	-0.02	2
FR8	11	0.0053	-0.0001	0.8239	-0.0021	0.2165	1.2912	5.671	0.1723	-0.03	2
FR9	13.5	0.0059	0.0016	0.6694	0.0146	0.2111	1.3438	1.5297	0.2058	0.02	2
FR10	15	0.0048	0.001	0.6828	0.0083	0.0531	1.2195	0.9949	0.2152	0.01	2
FR1	1.5	0.0024	-0.0011	0.1693	0.0041	0.0749	1.177	1.0227	-0.0069	-0.03	1
FR2	3	0.0025	-0.001	0.2507	-0.002	0.0981	0.4441	1.0216	-0.0043	-0.01	1
FR3	4.5	0.0021	0.0003	0.2835	0.0061	0.1064	1.2515	43.0929	-0.012	-0.01	1
FR4	6	0.0027	-0.0002	0.3965	-0.0123	0.1851	0.7648	1.6229	-0.0038	-0.02	1
FR5	7.5	0.0044	-0.001	0.666	0	0.2073	0.8116	1.7903	0.0337	0.02	1
FR6	8.5	0.0039	0.0001	0.7039	-0.0063	0.2385	0.9992	3.3448	0.0694	-0.01	1

FR7	10	0.0057	0.0007	0.8455	-0.0167	0.2451	1.0985	2.6682	0.1207	-0.01	1
FR8	11	0.0099	-0.0005	0.8741	-0.0063	0.2198	1.2176	2.7201	0.0879	-0.04	1
FR9	13.5	0.002	0.0011	0.6753	-0.0083	0.2177	1.2775	0.0198	0.0758	-0.02	1
FR10	15	0.0047	-0.0004	0.7362	-0.0021	0.0966	1.1281	0.574	0.0508	-0.05	1
FR1	1.5	0.0167	0.0008	0.2572	0.0181	0.0789	1.1667	1.8528	-0.0134	-0.03	1
FR2	3	0.0029	-0.0008	0.2154	0.0101	0.0481	0.5836	1.0108	-0.0136	0.05	1
FR3	4.5	0.0061	0.0003	0.1857	0.0363	0.0648	0.6614	1.2054	-0.0334	0.02	1
FR4	6	0.0028	-0.0004	0.2357	0.0161	0.0908	0.3792	2.4293	-0.0175	-0.03	1
FR5	7.5	0.0043	-0.0003	0.3405	0.0101	0.1229	0.8122	2.5934	0.0364	-0.01	1
FR6	8.5	0.0039	-0.0011	0.4153	0	0.2305	0.7159	3.8406	-0.0135	0.04	1
FR7	10	0.0058	-0.0005	0.7285	-0.0017	0.2309	0.8297	6.7826	0.0282	-0.05	1
FR8	11	0.0067	-0.0003	0.7336	-0.0051	0.2668	1.0965	7.3385	0.1159	0.00	1
FR9	13.5	0.0057	0.0005	0.8643	-0.0017	0.2702	1.1915	4.7229	0.0743	-0.03	1
FR10	15	0.0061	0.0002	1.0304	0.0085	0.2409	1.3744	3.8922	0.1642	-0.04	1
FR1	1.5	0.0029	0.0003	0.713	-0.0333	0.1666	1.237	2.4429	0.3076	-0.01	1
FR2	3	0.0011	-0.0002	0.4988	-0.0216	0.0651	1.2825	22.7283	0.0675	0.00	1
FR3	4.5	0.0052	-0.0013	0.1025	-0.0039	0.0601	0.6613	1.5114	0.021	0.38	1

FR4	6	0.0079	0.0002	0.1221	-0.0176	0.0715	0.7791	1.3838	0.0273	0.10	1
FR5	7.5	0.0038	-0.0007	0.1825	0.0186	0.0951	0.4926	1.374	0.0122	0.11	1
FR6	8.5	0.0041	-0.0016	0.2567	0.017	0.1661	0.8165	1.7207	0.0287	0.12	1
FR7	10	0.0086	-0.0021	0.5104	0.0077	0.1897	1.2068	2.1335	0.0145	0.11	1
FR8	11	0.0016	0.0003	0.5852	0.0248	0.2081	1.2634	11.0862	0.0131	0.27	1
FR9	13.5	0.0073	-0.0017	0.6519	0.0124	0.2092	1.49	9.8809	0.0409	0.33	1
FR10	15	0.0145	0.0002	0.735	0.01	0.1977	1.599	5.4519	0.061	0.18	1
FR1	1.5	0.0108	-0.001	0.0914	-0.005	0.0477	0.995	0.9867	0.0824	0.78	1
FR2	3	0.014	-0.0007	0.1262	-0.0067	0.0581	0.4617	2.635	0.0799	-0.02	1
FR3	4.5	0.005	-0.0006	0.1736	-0.017	0.0778	0.8514	3.416	0.0377	0.00	1
FR4	6	0.0073	-0.0002	0.2385	0	0.1077	0.7114	3.6565	0.031	0.09	1
FR5	7.5	0.0099	0.001	0.3434	0.0068	0.1986	0.7414	3.5208	0.0265	0.03	1
FR6	8.5	0.0095	-0.0013	0.576	0.0204	0.2139	0.9652	3.5611	0.0338	-0.06	1
FR7	10	0.0084	-0.0002	0.6605	-0.0187	0.2357	1.1631	4.5109	0.0484	-0.04	1
FR8	11	0.0065	-0.0008	0.7603	0.0085	0.251	1.2023	4.3108	0.0448	-0.02	1
FR9	13.5	0.0096	-0.001	0.8473	0.0256	0.2107	1.1499	5.5979	0.0475	0.07	1
FR10	15	0.0069	0.0014	0.6475	0.0051	0.2181	1.0414	4.9164	0.0228	0.40	1
FR1	1.5	0.0024	0.0003	0.4327	-0.018	0.0539	0.9175	4.8974	0.0377	0.14	1
FR2	3	0.0065	-0.001	0.1109	-0.0049	0.0755	0.4514	0.4228	0.0116	-0.04	1
FR3	4.5	0.0044	-0.0011	0.1169	0.0016	0.1047	0.7659	0.546	0.0167	0.03	1

FR4	6	0.0053	-0.001	0.2371	0	0.1823	0.7192	0.8709	0.0469	0.13	1
FR5	7.5	0.0026	-0.0009	0.274	-0.0279	0.1972	0.8396	0.9258	0.0097	-0.03	10
FR6	8.5	0.0054	-0.0008	0.1076	0.0082	0.2291	1.1309	0.961	0.0109	-0.01	1
FR7	10	0.0076	0.0001	0.5601	0.0065	0.2432	1.1642	1.3322	0.0049	0.01	1
FR8	11	0.0019	-0.0005	0.6964	-0.0082	0.2118	1.4938	0.9186	0.0196	0.01	1
FR9	13.5	0.0061	-0.0001	0.7857	0.0082	0.2124	1.5135	0.8485	0.1069	0.00	1
FR10	15	0.0052	0	0.6701	0.036	0.0434	1.2991	1.5562	0.0582	-0.04	1
FR1	1.5	0.0089	0.0009	0.1349	-0.0052	0.0489	0.4647	4.3648	0.0635	-0.01	1
FR2	3	0.0044	-0.0002	0.1425	0.0052	0.1125	0.7317	7.6483	0.0835	0.01	1
FR3	4.5	0.0086	0.0013	0.3778	0.0052	0.0882	0.7639	8.6314	0.0259	0.03	1
FR4	6	0.0056	0.0027	0.2754	-0.0115	0.1817	0.8405	6.2571	0.0072	0.01	1
FR5	7.5	0.0051	0.0013	0.5657	-0.0131	0.2344	19.103	3.5989	0.0039	-0.02	1
FR6	8.5	0.0105	0.0033	0.7208	0.0164	0.256	1.1268	0.6793	0.0122	-0.05	1
FR7	10	0.0057	0.0017	1.0557	0.0066	0.2744	1.3484	0.6936	0.0012	-0.04	2
FR8	11	0.0063	0.0027	1.264	0.0082	0.2114	1.4642	1.3324	0.0077	0.00	3
FR9	13.5	0.0065	0.0025	0.671	0.0266	0.2205	1.3151	1.6815	0.1543	0.00	1
FR10	15	0.0076	0.0352	0.7878	0.0149	0.0521	1.3271	1.8832	0.1421	-0.02	1
FR1	1.5	0.0053	0.001	0.1768	0.0232	0.083	0.5366	9.9374	0.1012	0.01	1
FR2	3	0.0144	0.0029	0.2544	0.0046	0.128	0.8914	6.3745	0.1027	-0.03	1
FR3	4.5	0.0034	0.0019	0.3136	-0.003	0.1332	1.0303	4.9675	0.0282	-.01	1

FR4	6	0.0003	0.003	0.4492	-0.0046	0.1583	0.956	5.1083	0.0033	0.00	1
FR5	7.5	0.0015	0.0028	0.4926	0.0106	0.2254	1.1821	5.4862	0.0186	0.00	1
FR6	8.5	0.002	0.0027	0.7163	-0.0061	0.2636	1.4439	2.6518	0.0845	0.01	1
FR7	10	-0.001	0.0001	1.5095	0.0298	0.2286	2.6797	1.7831	0.0065	0.01	6
FR8	11	0.0064	0.001	0.8723	0.0149	0.2882	2.9687	6.4209	0.0708	-0.03	2
FR9	13.5	0.0005	0.002	1.4569	0.0651	0.0475	2.0368	9.312	0.1927	0.00	5
FR10	15	0.0013	0.0018	0.0099	0.0149	0.2024	1.8194	9.4785	0.0781	0.01	1
FR1	1.5	0.0038	0.0027	0.2058	0.0964	0.0654	0.9781	4.9916	0.0518	0.01	1
FR2	3	0.0017	0.0031	0.3379	0.0495	0.1133	0.6952	11.8607	0.0451	0.07	1
FR3	4.5	0.0023	0.0023	0.2448	0.0443	0.0733	0.8489	6.5584	0.0425	0.03	1
FR4	6	0.0006	0.001	0.7272	0.0729	0.1611	0.9465	6.1441	0.05	0.04	1
FR5	7.5	0.0006	0.0009	0.6119	0.109	0.1915	0.9807	6.0545	0.0283	0.04	1
FR6	8.5	0.0009	0.0015	1.0101	0.014	0.3015	1.1108	5.1241	0.1049	-0.01	1
FR7	10	0.0036	0.002	1.9509	-0.0056	0.3498	1.1495	4.7393	0.0736	-0.02	8
FR8	11	0.0053	0.0036	1.793	0.0056	0.3302	1.4559	4.9721	0.3106	0.04	7
FR9	13.5	0.004	0.0004	1.1554	-0.0056	0.2529	4.8182	5.5754	0.1369	0.00	2
FR10	15	0.0007	0.0025	0.7149	0.0614	0.2155	4.2943	4.9628	0.2645	0.03	1

Appendix III: Raw data for vegetation, nutrient and metal concentrations.

	Location	Plant	Nitrogen	phosphorus	Ca	Cu	K	Na	Mg	Zn	Fe	Cd	Pb
	F1	Brabejum	324	3.00	62.00	8.00	37.00	83.00	17.00	10.00	82.00	0.0100	0.0000
	F2	Brabejum	79	3.00	63.00	1.00	24.00	1439.00	29.00	7.00	84.00	0.0000	0.0000
	F7	Brabejum	122	3.00	60.00	1.00	38.00	384.00	24.00	8.00	83.00	0.0000	0.0000
	F10	Brabejum	102	5.00	73.00	3.00	37.00	571.00	22.00	7.00	62.00	0.0000	0.0000
	F2	Acacia	349	6.00	87.00	10.00	10.00	2002.00	19.00	16.00	163.00	0.0000	0.0000
	F3	Acacia	329	5.00	12.00	9.00	67.00	3235.00	28.00	36.00	208.00	0.0200	0.0000
	F6	Acacia	340	5.00	88.00	3.00	96.00	1005.00	15.00	20.00	232.00	0.0000	0.0004
	F7	Acacia	312	6.00	104.00	4.00	87.00	1005.00	31.00	15.00	205.00	0.0300	0.0017
Collection 1	F8	Acacia	328	13.00	77.00	5.00	142.00	2790.00	25.00	20.00	168.00	0.0100	0.0000
	F9	Acacia	343	9.00	62.00	6.00	68.00	2417.00	23.00	21.00	162.00	0.0100	0.0008
	F10	Acacia	289	8.00	69.00	5.00	84.00	1448.00	18.00	12.00	216.00	0.0000	0.0095
	F3	Salix	247	10.00	275.00	3.00	74.00	431.00	23.00	43.00	301.00	0.0200	0.0063
	F4	Salix	322	9.00	188.00	6.00	134.00	441.00	30.00	25.00	405.00	0.0300	0.0032
	F5	Salix	342	11.00	187.00	7.00	182.00	647.00	32.00	57.00	438.00	0.0300	0.0174
	F6	Salix	282	22.00	199.00	7.00	125.00	452.00	52.00	80.00	261.00	0.0300	0.0105
	F7	Salix	331	1.00	101.00	6.00	113.00	614.00	43.00	22.00	278.00	0.0000	0.0079
	F8	Salix	281	1.00	163.00	3.00	96.00	473.00	54.00	124.00	303.00	0.0200	0.0000
	F9	Salix	217	2.00	289.00	4.00	89.00	293.00	41.00	51.00	119.00	0.0400	0.0118
	FR1	Brabejum	1301.38	3.00	754.54	13.01	7674.81	233.05	243.95	104.45	0.54	9.2305	0.0000
	FR2	Brabejum	242.70	6.00	473.66	12.58	2160.62	193.52	179.69	37.00	0.46	0.0155	0.0011
	FR7	Brabejum	945.47	4.00	1918.07	27.26	10671.77	359.00	440.48	193.01	1.35	0.0036	0.0033

	FR10	Brabejum	490.25	3.00	408.79	13.07	2270.17	227.65	181.24	177.58	0.41	0.0155	0.0000
	FR2	Acacia	1330.67	4.00	741.82	16.53	8173.73	313.61	242.58	37.00	1.69	0.0239	0.0279
	FR3	Acacia	1355.52	5.00	867.02	17.52	7778.61	245.53	226.76	127.65	1.20	0.0363	0.0046
	FR5	Acacia	1158.47	7.00	683.80	28.90	5771.34	303.67	202.43	123.82	1.18	0.0558	0.0033
Collection 2	FR6	Acacia	1260.63	5.00	677.15	14.96	7571.59	234.33	214.79	195.37	0.85	0.0177	0.0000
	FR7	Acacia	1435.72	5.00	969.15	30.65	11258.53	512.09	300.81	190.81	1.45	0.0078	0.0028
	FR10	Acacia	1120.56	4.00	170.28	29.93	6341.54	377.25	153.94	162.93	0.55	0.0187	0.0000
	FR3	Salix	1737.84	6.00	824.37	14.76	13903.73	90.51	372.42	159.78	1.19	0.0538	0.0000
	FR4	Salix	2387.16	7.00	897.94	15.62	13719.91	235.85	303.53	157.21	0.95	0.0186	0.0188
	FR5	Salix	1155.58	5.00	1045.02	15.80	11508.05	230.44	368.09	143.91	0.99	0.0177	0.0014
	FR6	Salix	1155.58	6.00	1073.11	15.02	11260.52	205.57	278.01	326.65	0.83	0.0072	0.0174
	FR7	Salix	1324.04	11.00	1497.78	24.98	20242.89	273.65	250.45	178.56	1.64	0.0116	0.0035
	FR8	Salix	1120.56	10.00	1307.15	14.58	6420.55	224.33	296.47	104.02	1.31	0.0092	0.0000
	FR10	Salix	1960.98	13.00	1665.74	23.74	12328.78	240.17	477.24	135.25	0.91	0.0448	0.0007
	FR1	Brabejum	280.14	15.00	384.16	12.06	8446.33	119.73	145.39	130.24	0.19	0.0053	0.0000
	FR2	Brabejum	105.05	3.00	575.17	20.12	2061.50	169.94	152.11	128.77	-0.02	0.0257	0.0000
	FR10	Brabejum	1330.67	3.00	907.90	6.03	8500.55	247.60	279.67	142.39	0.92	0.0319	0.0038
	FR2	Acacia	210.11	5.00	146.65	14.29	6965.15	272.71	138.18	112.81	0.86	0.0138	0.0107
	FR3	Acacia	245.12	5.00	258.00	11.44	3830.33	273.93	177.55	206.45	0.91	0.0344	0.0000
	FR5	Acacia	2801.40	4.00	590.24	23.04	5953.85	357.12	177.99	124.19	0.73	0.0188	0.0394
	FR6	Acacia	315.16	6.00	791.63	23.73	5981.79	394.49	219.86	151.57	0.91	0.0248	0.1443
Collection 3	FR7	Acacia	1190.60	6.00	675.59	25.69	7413.72	388.45	198.00	159.36	0.93	0.0044	0.0024
	FR8	Acacia	1225.61	8.00	496.95	24.40	4819.06	385.99	154.64	158.45	0.74	0.0109	0.0173
	FR9	Acacia	871.08	8.00	677.43	28.64	6319.71	324.79	137.32	163.54	1.08	0.0126	0.0134
	FR10	Acacia	840.42	2.00	224.01	3.78	3874.97	328.45	158.91	88.54	0.64	0.0333	0.0114
	FR3	Salix	595.30	8.00	313.25	14.36	9400.55	191.86	298.91	118.97	2.33	0.0040	0.0229
	FR4	Salix	1155.58	6.00	446.49	13.35	11257.85	156.10	273.92	184.38	1.66	0.0151	0.0012
	FR5	Salix	314.37	9.00	1760.50	24.55	16520.99	204.10	377.18	91.10	1.30	0.0169	0.0199
	FR6	Salix	1138.07	13.00	652.19	13.24	11533.92	123.94	316.16	192.78	1.62	0.0069	0.0126

	FR7	Salix	1365.68	10.00	1491.08	26.70	18115.35	203.53	200.39	128.81	1.75	0.0911	0.0025
	FR8	Salix	1082.84	3.00	1620.50	26.32	6440.48	239.79	493.99	196.49	0.59	0.0011	0.0224
	FR9	Salix	1470.74	3.00	1809.41	28.06	10104.64	235.10	337.27	140.63	2.15	0.0212	0.0187
	FR10	Salix	1715.86	4.00	1051.78	5.71	9436.25	210.62	603.63	94.72	1.17	0.0161	0.0044
	FR1	Brabejum	280.14	7.00	503.77	2.45	3072.86	183.38	186.98	72.87	0.38	0.0404	0.0044
	FR2	Brabejum	420.21	6.00	502.14	2.62	2167.86	159.09	146.30	93.56	0.05	0.0041	0.0105
	FR10	Brabejum	525.26	7.00	1089.78	5.28	8019.92	182.07	344.99	8.56	43.76	0.0046	0.0025
	FR2	Acacia	1428.57	8.00	610.64	6.54	5205.09	247.32	144.12	146.23	1.07	0.0173	0.0006
	FR3	Acacia	980.49	5.00	700.70	8.66	3940.48	281.24	139.90	199.95	1.04	0.0275	0.0012
	FR5	Acacia	1120.56	6.00	509.97	6.17	4446.50	324.98	170.95	107.01	3.22	0.1059	0.0056
	FR6	Acacia	700.35	9.00	508.65	6.41	4904.45	283.41	216.87	130.52	0.95	0.0110	0.0000
Collection 4	FR7	Acacia	1330.67	6.00	812.76	7.17	10939.75	393.06	228.50	198.22	1.15	0.0131	0.0015
	FR8	Acacia	1540.77	8.00	640.94	8.97	6050.85	355.87	147.72	14.21	2.30	0.0083	0.0015
	FR3	Salix	1715.86	6.00	333.90	2.52	699.34	127.80	113.29	105.13	0.23	0.0038	0.0000
	FR4	Salix	700.35	7.00	817.82	4.56	6689.21	192.08	277.20	119.30	0.99	0.0060	0.0030
	FR5	Salix	873.25	12.00	1430.77	6.32	10762.50	289.25	341.51	225.35	1.78	0.0086	0.0000
	FR6	Salix	980.49	5.00	1300.73	8.65	12513.13	361.22	384.50	132.68	1.68	0.0460	0.0000
	FR7	Salix	1117.77	11.00	1615.28	5.23	6312.64	225.60	459.77	201.35	2.16	0.0101	0.0045
	FR8	Salix	593.81	5.00	1844.26	6.98	5316.51	282.30	345.78	134.03	1.53	0.0381	0.0000
	FR9	Salix	1117.77	5.00	829.19	6.68	6237.05	252.17	145.46	50.92	30.53	0.0194	0.0000
	FR1	Brabejum	315.16	4.00	663.94	3.69	2082.81	257.24	208.36	18.17	0.79	0.0101	0.0034
	FR2	Brabejum	386.16	7.00	549.29	5.01	3481.44	256.35	158.82	28.21	3.03	0.0090	0.0040
	F10	Brabejum	630.32	6.00	882.58	6.18	8596.67	183.40	372.52	74.59	55.09	0.0022	0.0114
	F2	Acacia	595.30	8.00	604.39	3.28	2377.99	80.47	108.92	99.96	8.90	0.0114	0.0073
	F3	Acacia	631.89	7.00	1426.70	6.56	7730.01	258.84	387.65	49.69	66.56	0.0668	0.0000
Collection 5	F5	Acacia	1614.84	7.00	1062.51	6.45	14102.43	239.36	264.04	30.37	52.08	0.0571	0.0000
	F6	Acacia	838.32	8.00	946.57	12.86	6809.07	239.45	303.15	133.63	147.70	0.0199	0.0022
	F7	Acacia	1470.74	5.00	1217.28	4.34	2734.95	266.02	442.25	41.62	55.61	0.0166	0.0006
	F8	Acacia	525.26	10.00	699.48	5.33	6378.48	161.17	162.21	105.41	31.85	0.0229	0.0017

	F9	Acacia	1439.32	6.00	1368.86	3.03	6475.59	195.86	524.80	54.91	49.14	0.0229	0.0045
	F4	Salix	386.16	6.00	989.75	6.79	5552.70	256.10	118.85	72.14	51.74	0.0229	0.0000
	F6	Salix	1082.84	7.00	1465.60	5.38	7339.92	209.18	339.33	57.90	28.34	0.0212	0.0013
	F1	Brabejum	315.16	2.00	842.54	5.35	3093.74	91.46	228.10	25.85	5.55	0.0035	0.0025
	F2	Brabejum	386.16	3.00	622.60	3.63	2907.74	53.49	187.43	69.11	6.52	0.0154	0.0000
	F10	Brabejum	630.32	1.00	593.05	4.00	2337.36	66.33	204.80	0.33	0.11	0.0067	0.0006
	F2	Acacia	595.30	3.00	736.88	7.33	6406.92	168.99	143.44	291.60	1.05	0.0361	0.0000
	F3	Acacia	631.89	4.00	786.51	7.10	5785.77	166.68	135.42	33.23	1.70	0.0000	0.0055
	F5	Acacia	1614.84	4.00	927.37	5.36	4745.65	104.35	387.04	25.20	1.26	0.0000	0.0018
Collection 6	F6	Acacia	838.32	6.00	906.80	4.57	3518.56	186.68	251.53	80.14	0.50	0.0056	0.0009
	F7	Acacia	1470.74	7.00	2018.67	5.70	7596.96	272.14	234.84	90.83	1.07	0.0057	0.0050
	F8	Acacia	525.26	7.00	846.07	6.02	6401.76	121.21	188.76	13.15	1.64	0.0152	0.0022
	F9	Acacia	1439.32	6.00	715.96	4.89	5548.56	123.11	141.44	74.71	1.12	0.0045	0.0027
	F4	Salix	386.16	6.00	885.37	10.09	5337.67	259.28	230.40	42.30	30.48	0.0114	0.0022
	F7	Salix	385.19	8.00	1684.48	5.14	7822.47	213.95	359.50	37.98	33.83	0.0511	0.0058
	F8	Salix	805.40	8.00	593.95	4.65	7674.38	284.72	259.85	43.14	34.15	0.0047	0.2214
	F1	Brabejum	455.23	3.00	546.96	3.97	2957.71	114.43	186.72	17.97	5.92	0.0076	0.1036
	F2	Brabejum	245.12	2.00	388.09	1.99	1295.02	116.79	72.56	36.72	0.00	0.0235	0.0058
	F7	Brabejum	175.09	1.00	535.81	2.24	3235.30	150.93	232.90	14.96	9.24	0.0075	0.0017
	F10	Brabejum	1015.51	3.00	717.63	3.36	6047.27	78.08	259.10	7.85	12.42	0.0057	0.0035
Collection 7	F2	Acacia	1190.60	4.00	2208.89	8.32	7063.14	158.04	184.44	15.70	20.02	0.0098	0.0032
	F3	Acacia	1330.67	8.00	904.07	6.75	5721.44	156.78	133.39	8.39	27.57	0.0112	0.0045
	F5	Acacia	1260.63	5.00	525.83	8.22	7467.37	116.26	161.49	143.39	19.95	0.0072	0.0061
	F6	Acacia	1540.77	9.00	647.59	4.34	7644.71	175.84	192.50	10.52	28.84	0.0079	0.0128
	F8	Acacia	1260.63	6.00	630.74	4.91	7330.00	86.63	120.25	15.09	20.84	0.0097	0.0234
	F9	Acacia	1190.60	6.00	727.01	4.39	6265.05	143.14	159.79	3.99	29.58	0.0000	0.0173
	F10	Acacia	595.30	7.00	305.59	1.95	1506.05	54.56	168.55	2.13	5.06	0.0715	0.0036
	F1	Brabejum	420.21	4.00	442.02	3.15	4153.75	98.41	135.69	15.43	4.59	0.0347	0.0692
	F2	Brabejum	840.42	1.00	407.28	6.56	5215.33	150.04	148.93	2.03	24.81	0.0000	0.0103

	F10	Brabejum	735.37	4.00	571.98	2.36	2509.63	79.26	208.88	6.30	3.98	0.0076	0.0083
	F2	Acacia	1575.79	3.00	746.96	7.41	2798.79	56.85	163.50	21.22	253.70	0.0047	0.0087
Collection 8	F3	Acacia	1120.56	3.00	732.79	14.29	5045.65	60.58	115.84	13.10	31.93	0.0808	0.0066
	F5	Acacia	1229.88	3.00	654.73	7.11	9871.07	190.63	185.05	10.28	52.08	0.0943	0.0519
	F6	Acacia	1330.67	5.00	786.02	4.74	9069.30	259.33	268.71	16.64	46.48	0.0050	0.0136
	F8	Acacia	1330.67	9.00	825.53	7.69	6350.34	136.05	152.20	14.56	63.89	0.0021	0.0131
	F9	Acacia	700.35	8.00	992.41	9.93	8310.89	122.02	172.97	12.63	23.07	0.0016	0.0102
	F1	Brabejum	490.25	5.00	556.50	3.23	3133.59	66.25	203.16	1.43	15.95	0.0568	0.0054
	F2	Brabejum	595.30	4.00	296.45	4.61	4407.13	80.54	211.15	4.77	59.72	0.0495	0.0234
	F9	Brabejum	385.19	5.00	580.17	3.42	6240.28	65.90	225.28	22.73	11.36	0.0046	0.0036
	F10	Brabejum	105.05	4.00	644.12	2.48	2907.44	48.64	165.73	23.35	5.90	0.0216	0.0072
	F3	Acacia	1435.72	15.00	700.59	6.16	9741.66	262.80	164.65	27.71	92.61	0.0044	0.0011
Collection 9	F6	Acacia	875.44	9.00	694.02	6.97	9179.48	178.17	220.98	99.68	103.47	0.0541	0.0016
	F7	Acacia	805.40	11.00	898.54	4.20	5303.07	168.90	87.91	115.76	40.01	0.0928	0.0056
	F8	Acacia	980.49	6.00	905.39	4.97	7635.31	184.04	103.07	138.55	35.05	0.1263	0.0041
	F9	Acacia	910.46	11.00	878.99	5.20	5570.63	220.23	66.08	140.83	48.57	0.0699	0.0015
	F10	Acacia	628.74	1.00	768.31	5.33	3254.84	219.70	122.22	18.06	20.72	0.1049	0.0046
	F4	Salix	1470.74	1.00	852.56	7.43	4992.52	122.24	239.65	14.27	24.46	0.0271	0.0026
	F5	Salix	1715.86	3.00	803.74	3.12	2464.72	97.49	221.66	15.31	2.05	0.0050	0.0040
	F7	Salix	1190.60	5.00	1256.87	7.53	6615.50	155.37	273.18	238.05	3.20	0.0069	0.0000
	F8	Salix	665.33	3.00	934.38	7.10	5592.63	144.20	435.73	20.80	1.08	0.0027	0.0254
	F9	Salix	1050.53	5.00	942.42	4.52	7336.86	321.45	264.17	52.91	1.34	0.0037	0.0264
	F1	Brabejum	105.05	6.00	892.78	3.55	1962.48	138.82	150.06	178.66	14.58	0.5157	0.0036
	F2	Brabejum	350.18	8.00	884.18	2.90	3326.07	145.49	148.57	177.60	16.37	1.0696	0.0057
	F8	Brabejum	1362.28	6.00	1150.39	4.10	2468.20	98.11	163.15	160.78	10.15	0.0619	0.0011
	F10	Brabejum	665.33	7.00	803.68	2.18	3183.34	160.62	100.93	48.53	19.90	0.4952	0.0132
	F2	Acacia	1155.58	7.00	1533.72	5.52	5922.11	207.33	203.63	0.87	38.22	0.5399	0.0282
	F3	Acacia	1680.84	7.00	1185.43	5.81	7539.82	196.20	155.27	13.75	52.98	0.0489	0.0221
	F6	Acacia	2549.90	8.00	1342.96	5.61	7342.88	199.37	179.05	8.56	45.87	0.0822	0.0011

Collection 10	F7	Acacia	455.23	10.00	1308.91	6.11	10292.67	364.58	226.06	60.52	27.84	0.0725	0.0074
	F9	Acacia	1680.84	8.00	1210.70	17.23	8495.87	275.88	177.28	28.91	82.07	0.2578	0.0000
	F10	Acacia	1540.77	9.00	1097.97	5.47	4835.05	205.78	110.11	6.89	33.72	0.1027	0.0015
	F3	Salix	2486.24	9.00	624.32	3.02	21876.95	46.01	204.54	18.63	28.37	0.1026	0.0010
	F4	Salix	700.35	14.00	1004.04	4.52	21704.75	65.51	281.14	22.46	29.12	0.0377	0.0061
	F5	Salix	1187.63	14.00	750.10	4.70	20585.08	216.35	237.92	167.21	79.05	0.0683	0.0000
	F6	Salix	1117.77	14.00	1132.00	4.90	12449.80	154.87	204.87	139.95	74.91	0.0538	0.0000
	F7	Salix	1225.61	5.00	880.68	2.28	7828.14	122.11	173.07	143.49	20.22	0.5650	0.0000
	F8	Salix	1327.35	4.00	1305.74	7.20	10085.77	138.07	173.38	-0.38	56.05	1.4013	0.0000
	F9	Salix	1855.93	5.00	549.81	5.98	7249.45	95.17	277.94	0.72	71.25	0.0655	0.0142
	F1	Brabejum	702.11	6.00	1164.89	7.79	4616.95	126.57	198.42	18.88	50.60	0.1617	0.0000
	F2	Brabejum	490.25	6.00	1108.08	9.33	3632.14	106.65	212.67	51.43	61.78	0.1782	0.0000
	F10	Brabejum	140.07	6.00	1103.84	7.26	2294.64	119.37	108.01	8.36	12.96	0.1011	0.0000
	F2	Acacia	1324.04	7.00	1357.80	7.13	9820.46	200.60	164.11	24.97	51.84	0.0963	0.0005
	F5	Acacia	1190.60	8.00	1852.06	5.86	12001.67	143.42	317.49	28.17	101.73	0.0412	0.0000
	F6	Acacia	1330.67	7.00	1685.16	13.37	4669.16	289.08	292.87	31.88	151.23	0.0634	0.0000
Collection 11	F7	Acacia	1260.63	7.00	1379.89	6.37	9604.24	312.87	320.06	22.84	64.07	0.0810	0.0000
	F8	Acacia	1649.95	9.00	1595.37	14.29	5913.97	224.56	231.48	47.17	166.24	0.0557	0.0000
	F9	Acacia	1334.00	12.00	1243.86	6.67	4338.78	249.86	109.75	27.18	77.42	0.0339	0.0000
	F10	Acacia	1225.61	10.00	1303.25	9.50	4570.31	188.12	152.01	24.89	69.37	0.0913	0.0000
	F3	Salix	2661.33	8.00	2002.94	5.86	18207.57	152.64	301.58	31.00	83.92	0.3731	0.0000
	F4	Salix	1505.75	18.00	1690.33	6.49	14886.12	96.85	273.39	25.87	116.20	0.3762	0.0000
	F5	Salix	1050.53	10.00	1968.52	8.20	15502.15	122.01	322.75	23.40	125.53	0.0660	0.0014
	F6	Salix	1330.67	17.00	1700.35	8.44	5634.63	253.96	194.55	95.79	104.82	0.0593	0.0020
	F7	Salix	1610.81	2.00	1806.40	5.57	10883.75	127.99	342.86	54.65	99.77	0.0406	0.0005
	F8	Salix	1225.61	2.00	1583.93	6.37	7813.64	118.84	399.89	29.88	137.39	0.0920	0.0000
	F9	Salix	1680.84	5.00	2014.65	6.80	11516.20	82.50	375.79	8.01	99.45	0.0497	0.0000
	F1	Brabejum	455.23	5.00	1218.82	6.51	3841.48	117.95	214.62	9.63	11.29	0.0160	0.0000
	F2	Brabejum	2941.47	5.00	1114.70	8.01	2718.79	179.78	219.65	31.07	18.43	0.0297	0.0000

	F8	Brabejum	700.35	5.00	1211.25	6.82	3085.72	103.60	367.51	49.46	22.15	0.0998	0.0000
	F10	Brabejum	595.30	9.00	1100.64	4.15	1500.48	184.57	151.97	39.69	9.83	0.0233	0.0000
	F2	Acacia	2941.47	9.00	1222.01	7.73	6647.85	197.04	274.81	34.31	39.14	0.0358	0.0000
Collection 12	F5	Acacia	1505.75	7.00	1103.67	7.18	6607.78	247.27	209.25	35.57	62.45	0.0377	0.0000
	F6	Acacia	1505.75	8.00	1299.78	5.58	7430.95	231.89	195.59	138.84	50.82	0.0246	0.0000
	F7	Acacia	1435.72	8.00	1534.09	8.49	4443.43	241.96	319.81	25.50	79.83	0.0393	0.0000
	F8	Acacia	1575.79	7.00	1107.89	7.14	7860.81	192.12	125.21	50.16	76.25	0.0061	0.0000
	F9	Acacia	1610.81	8.00	1972.91	5.34	4822.76	205.94	215.87	10.93	63.23	0.0050	0.0000
	F3	Salix	1330.67	10.00	1899.54	7.56	13009.17	123.53	274.91	27.10	74.16	0.0267	0.0030
	F4	Salix	1575.79	12.00	2289.97	6.04	12052.54	96.16	342.82	47.38	43.05	0.0322	0.0000
	F5	Salix	1540.77	9.00	1817.91	7.91	16074.55	187.02	301.15	30.62	82.65	0.1231	0.0025
	F6	Salix	1155.58	9.00	1757.91	5.73	11944.97	171.37	301.59	47.74	97.49	0.0340	0.0000
	F7	Salix	1470.74	10.00	1757.87	4.91	6993.64	109.64	388.40	19.45	123.60	0.1416	0.0000
	F8	Salix	1400.70	11.00	2117.30	7.93	15547.48	136.85	306.58	30.13	72.07	0.0180	0.0000
	F9	Salix	1820.91	14.00	2503.04	5.91	5182.33	111.61	605.94	31.99	108.41	0.0269	0.0000