ENVIRONMENTAL IMPACTS OF EFFLUENT CONTAINING EDTA FROM DAIRY PROCESSING PLANTS

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ABSTRACT

Ethylenediaminetetraacetic acid (EDTA) is a well-known chelating agent, and has numerous applications in industries, for example in dairy industry to improve the cleaning efficiency of plant and equipment.

As EDTA is water-soluble and not volatile, it is eventually released into the environment with wastewater effluent. In general, EDTA has a low toxic impact for both humans and natural environments. There are some concerns, however, about its poor biodegradation in conventional wastewater treatment plants and natural environments, and its effect in mobilizing heavy metals from solid phases to pose a risk to groundwater.

In the late 1980's the environmental impact of EDTA was scrutinized in Europe. Since then, treatment and discharge of wastewater containing EDTA is increasingly required as environmental regulations become more stringent. This is the first investigation into the effects of EDTA in New Zealand.

In the New Zealand dairy industry, EDTA has been used as an additive alongside caustic agents to improve cleaning efficiency within dairy processing plants and to minimize dairy wastewater discharge into the environment. There are two main disposal methods of dairy wastes; direct discharge into the local stream after treatment, and spray irrigation onto pasture land. The primary aim of this research is to identify whether EDTA is detectable in the environment after the release of dairy wastes containing EDTA into that environment.

For the first time in New Zealand, an analytical method using reversed–phase ion-pair liquid chromatography, was established to determine EDTA present in dairy wastewater, and then applied to surface water, soils and groundwater with appropriate modifications. Method detection limits were 5 µg/L for dairy wastewater, 1 µg/L for surface water, 0.15 mg/kg (dry weight) for soils, and 2 µg/L for groundwater.

Significant concentrations of EDTA, as high as 83 mg/L, were observed in wastewater from dairy processing plants, when EDTA had been used alongside alkaline cleaning agents. The analyses have shown that approximate 93 % of EDTA was removed in the existing biological treatment process, which is an extended aeration activated sludge process, operated under alkaline pH 8.0–8.2 with a 3-week sludge retention time.

For surface water receiving the dairy effluent, 1 – 2.7 µg/L of EDTA were found, and no particular concerns were suggested about the associated heavy metals.

A quasi one-dimension vertical mixing model and a two-dimension (depth-averaged) 3DD hydrodynamic model were applied to simulate EDTA dispersion in the river. The modelling results for 'a worst case scenario' of high EDTA release combined with a low river flow, suggest that the dairy effluent discharge at the Fonterra Waitoa dairy site will not lead to a significant effect on the Waitoa River in terms of EDTA concentration.

Investigation of EDTA and heavy metal concentrations in pastoral topsoil and groundwater following the land application of dairy biomass concludes that there are no specific concerns. In contrast, the analyses suggest that heavy metals may be built up over long periods of irrigation with dairy effluent in soils, and then transported to the groundwater in the presence of EDTA. However, more research would be required to clarify this matter.

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1.0 CHAPTER ONE: THE VALUE AND ISSUES OF EDTA IN THE DAIRY INDUSTRY

Ethylenediaminetetraacetatic acid (EDTA) is a potential environmental contaminant for New Zealand. EDTA was recently reported at higher concentrations in surface waters than any other identified anthropogenic organic compounds in several European countries (Nowack and VanBriesen, 2005; Reemtsma et al., 2006). Indeed, EDTA has been severely restricted in some countries (The Australian Environmental Labelling Association Inc., 2004), or is carefully controlled in many other countries. For example, a target value of 10 µg/L EDTA was proposed for surface waters in Austria (Conrad, 2000; Fuerhacker et al., 2003). The international working group of water companies in the Rhine catchment area (IAWR) set out IAWR quality requirements as 5 µg/L concentration for well-degradable complexing agents and 1 µg/L for poorly degradable compounds. To date, EDTA is the only chelating agent identified as a problem by the International Commission for the Protection of the Rhine (IKSR) (Knepper, 2003).

EDTA, a powerful and cheap hexadentate chelating agent, is widely used as a sequestering agent to bind and mask undesirable metal ions in many industrial applications, such as in the cleaning process for the dairy industry (Knepper, 2003). As EDTA is highly water soluble, nearly all of these applications will eventually result in the release of EDTA into the aquatic environment via wastewater (Wolf and Gillbert, 1992; Conrad, 2000).

In general, EDTA has a low toxic impact for both humans and natural environments. However, there are several concerns associated with the use and application of EDTA. The main concern is that EDTA is poorly biodegradable and rather persistent in the environment (Bucheli-Witschel and Egli, 2001; Oviedo and Rodíguez, 2003; Sillanpää, 2005). The presence of EDTA at high concentrations in wastewater and surface waters has the potential to perturb the natural speciation of metals to cause harm to the organisms in the waterways (Xue

et al., 1995; Nowack, 2002; European Chemicals Bureau, 2004; Schmidt et al., 2004). Thus, EDTA has been included in the group of recognised polar, persistent pollutants.

It was recommended by Knepper (2003) that all industrial processes and productions related to poorly degradable chelating agents and compounds, such as EDTA, should be used as little as possible, and the emission into the aquatic environment should be as low as possible. As a result of these concerns, considerable effort had been made to reduce or substitute the production and use of EDTA between 1985 and 1999. In Europe, a voluntary declaration and subsequent voluntary agreements were achieved to significantly reduce EDTA release into the environment (Conrad, 2000).

Fonterra is a major multi-national dairy company seeking a "clean and green" image. Some 95% of its dairy products are exported to 140 countries around the world. The intent of its environmental group policy was stated as:

"Fonterra shall demonstrate a global commitment to protecting the environment. Sustainability, good environmental practice and environmental improvement are cornerstones of Fonterra's environment" (Fonterra Environmental Group Policy, 2006).

On a global basis, the mounting environmental pressure associated with the application of EDTA is likely to continue until the potential environmental effects of the production system are identified and addressed. Accordingly, Fonterra decided to investigate whether EDTA could be identified in natural environments following its use in dairy manufacturing plants. This is the essential focus of this thesis.

1.1 CLEANING PROCEDURES IN THE DAIRY INDUSTRY

Cleaning operations in food industries are essential to ensure the quality of products. This is referred to as a clean-in-place (CIP) system in the dairy industry

(Eide et al., 2003). The standard CIP cycle in the dairy industry is comprised of the following steps (Bylund, 1995):

(i) recovery of product residues by scraping, drainage and expulsion with water or compressed air;
(ii) pre-rinsing with water to remove loose dirt;
(iii) cleaning with alkaline detergents, usually NaOH, to clean off the protein and fat;
(iv) rinsing with clean water;
(v) disinfection by heating or with acid agents (HNO_3) to remove mineral; and
(vi) rinsing with clean water.

The prime objective of an efficient cleaning process in the dairy industry is to reduce the amount of cleaning chemicals and to use easily degradable chemicals during conventional wastewater treatment processes. It has been demonstrated by Bylund (1995) that using multi-chemicals is more likely to attain an effective cleaning result with lower chemical concentrations than merely containing the basic component of sodium hydroxide. This is applicable in particular for an effective complexing agent included in alkaline detergents.

Complexing agents are substances that can bind and mask metal ions to form highly stable and soluble compounds, and then lose their original chemical characteristics. For instance, in dairy industries, they are used for complexing highly insoluble Ca^{2+}, Mg^{2+} ions or other minerals to facilitate the elimination and prevention of the formation of milk-stone linings (Wolf and Gillbert, 1992). Ring complexes formed by the reaction of a multiple-charged metal ion and an organic complexing agent are called chelates. The complexing agents capable of forming such rings are called chelating agents or “chela” (Greek = Claw of Crab) (Knepper, 2003). At present, EDTA is still the most common and suitable complexing compound for many technical purposes and large quantities are used in a broad range of industrial applications, as well as in consumer products (Nörtemann, 1999; Reemtsma et al., 2006).

In the New Zealand dairy industry, EDTA has been used as an additive alongside caustic agents to improve cleaning efficiency within the dairy processing plants.

The role of a chelating agent ('claw of crab') illustrated in Figure 1.1 is to complex with metal ions and form metal-chelates. For instance, EDTA is used to facilitate the elimination of milk-stone linings due to the precipitation of calcium and magnesium on the surface of machinery within the manufacturing processes. A comparison of a standard with two-stages and the alternative using EDTA as a one-stage CIP procedure is shown in Figure 1.2. It can be seen that the main advantage of using EDTA is in reducing the time required for the CIP process, which leads to an increase of production and reduction in other relevant costs, such as wastewater treatment.

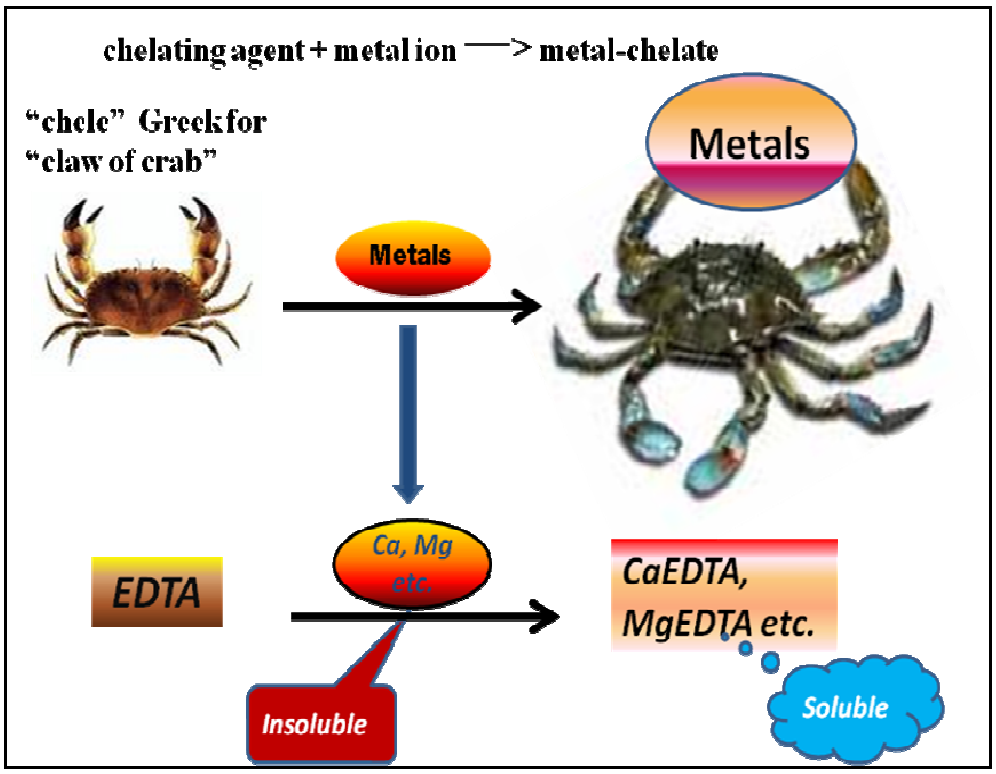

Figure 1.1 Chelating agents are used for a wide variety of industrial applications to bind undesirable metal ions. For example EDTA is used in the removal of precipitated calcium (Ca) and magnesium (Mg) on the surface of machinery in the cleaning process of dairy manufacturing plants.

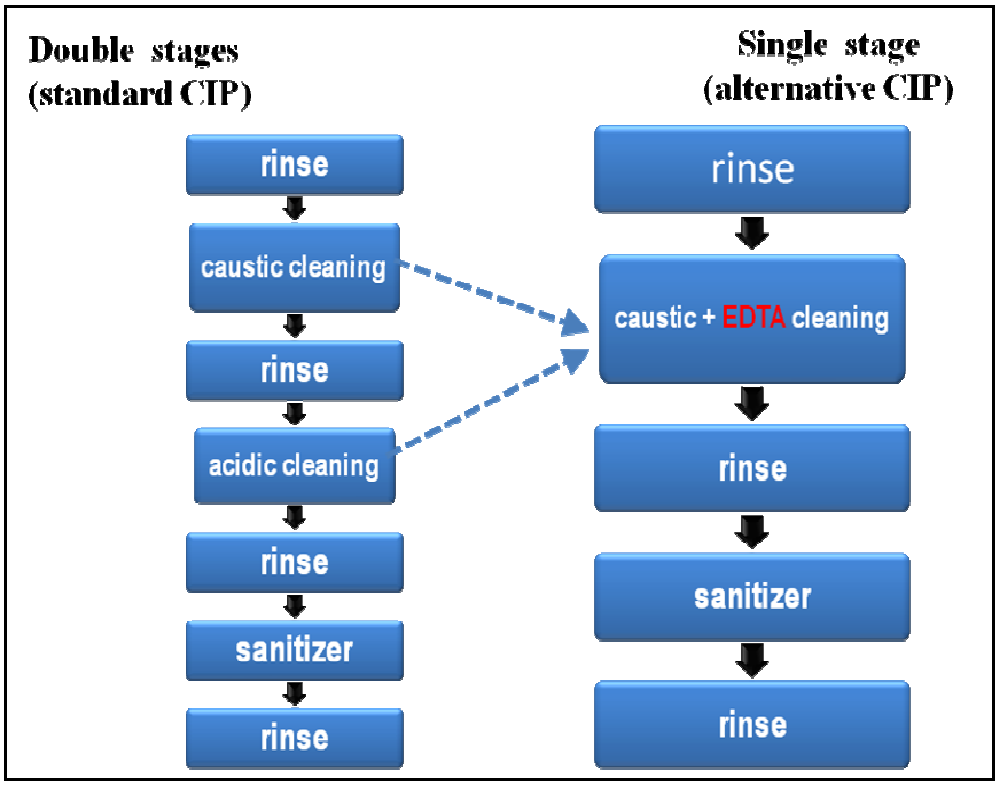

Figure 1.2 A comparison of a standard two-stage, and the alternative using EDTA as a one-stage cleaning procedure of the CIP system applied in manufacturing plants in the New Zealand dairy industry.

1.2 CURRENT METHODS OF DAIRY EFFLUENT DISPOSAL

Waste waters from dairy processing plants contain milk and milk product residues, as well as some additives from the specific dairy products and cleaning agents. They generally comprise high concentrations of organic materials such as proteins, carbohydrates and lipids, high concentrations of suspended solids, high biological oxygen demand (BOD) and chemical oxygen demand (COD), high nitrogen concentrations, high suspended oil and/or grease contents, and large variations in pH (Britz et al., 2004).

There are two commonly used methods for the disposal of dairy wastes in New Zealand. One method is to directly discharge wastewater into local waterways. This requires consents from the environmental managing authorities, the Regional

Councils in New Zealand. This is the method used by the Fonterra Waitoa dairy site. An alternative disposal method is to apply the dairy effluent to pasture land via spray irrigation (Figure 1.3), as is used at the Fonterra Kauri dairy site in Northland. This process is referred to as a land treatment system (Degens et al., 2000; Sparling et al., 2001).

Figure 1.3 *Dairy effluent applied onto pastureland via spray irrigation nearby the Fonterra Kauri dairy site in Northland, New Zealand.*

At the Fonterra Waitoa dairy site, wastewater from the manufacturing factory is pumped to a nearby wastewater treatment plant (WWTP) for treatment, after which it is discharged into the adjacent local waterway (the Waitoa River), within environmental stipulations of the regional council, in this case - Environment Waikato (Figure 1.4).

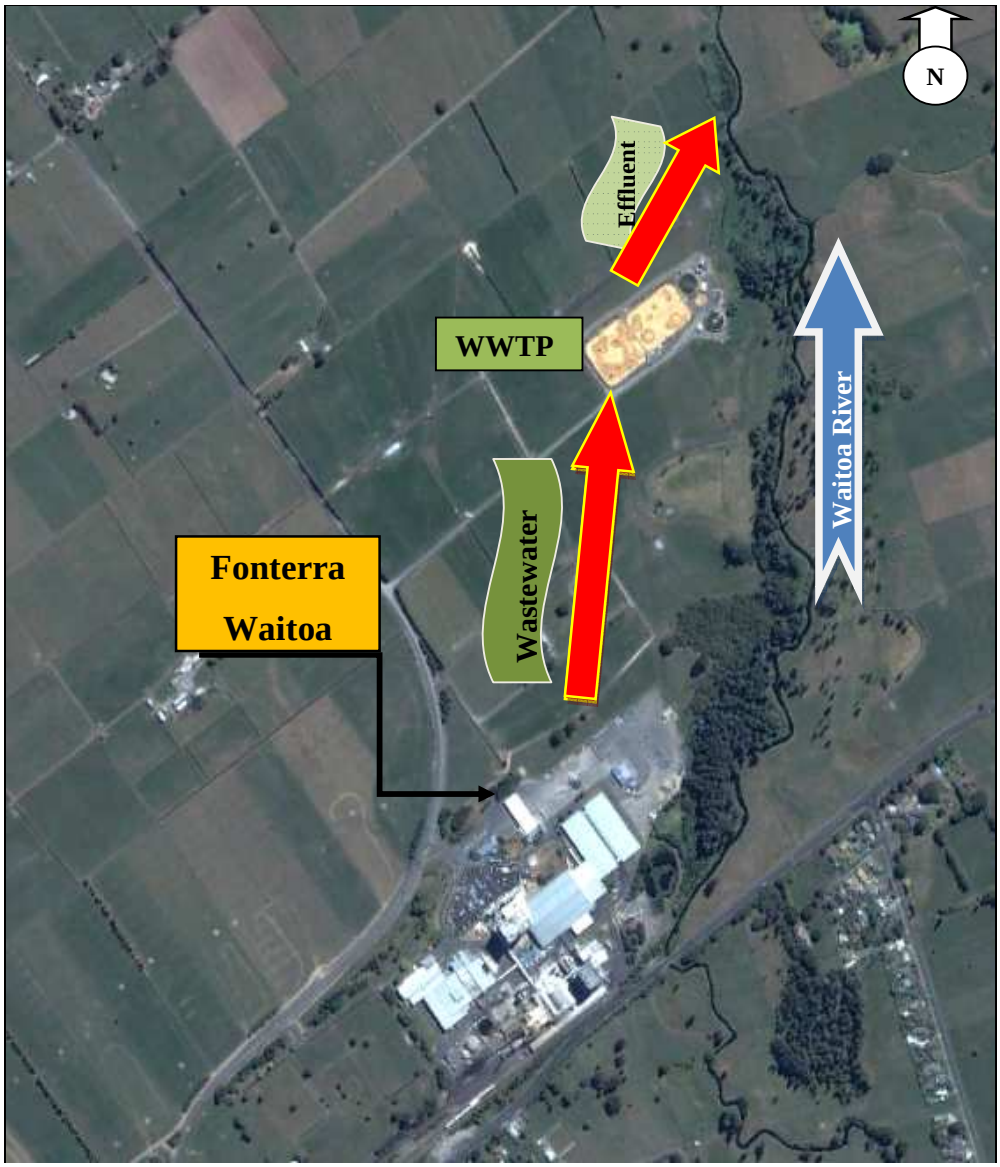

Figure 1.4 *Dairy effluent discharges into the adjacent waterway of the Waitoa River after treatment at the Fonterra Waitoa dairy site, New Zealand. WWTP is the wastewater treatment plant.*
(Source: Google Earth)

A study of the land treatment system for dairy effluent disposal indicated that a major disadvantage is the potential accumulation of immobile heavy metals in soils (Angin et al. 2005). Long term irrigation can induce changes in the quality of soil, and trace metal inputs sustained over long periods could pose a risk to groundwater (Haruvy et al., 1999; Friedal et al., 2000; Friedly et al., 2002). Nonetheless, the land treatment system for dairy effluent presents as an important and common option in New Zealand (Degens et al., 2000; Sparling et al., 2001),

where effluent from the dairy industry is regularly applied to nearby pastureland via spray irrigation such as of the Fonterra Kauri dairy site (Figure 1.3).

1.3 RESEARCH AIM

The primary aim of this research is to ascertain whether EDTA can be identified at NZ dairy plants subsequent to its use in the cleaning process of dairy manufacturers, and to ascertain its concentration range in the natural environment. The research approach is illustrated in Figure 1.5.

The major objectives of the research are to:

1) review the nature, use and concerns of EDTA especially as it relates to the dairy industry;
2) develop an analytical method with appropriate sensitivity to detect and quantify EDTA levels in different matrices of environmental samples;
3) quantify concentrations of EDTA in dairy wastewaters from processing plants, and discharged as effluent to the environment;
4) reveal EDTA removal efficiency by the existing wastewater treatment plants under normal operations;
5) investigate the presence of EDTA and any associated heavy metals in the local adjacent waterway, in this case – the Waitoa River;
6) conduct a dispersal simulation of EDTA in the local surface water by the 3D numerical model, and determine the dispersal paths and concentrations of EDTA in the river; and
7) determine EDTA in soils and ground waters following its application of dairy waste via a land treatment system onto pasture land; analyse levels of heavy metals in the associated soils and ground waters, and assess any significant transportation of heavy metals by EDTA;
8) interpret results to provide recommendations for measures to mitigate any potential effects of EDTA on the natural environment.

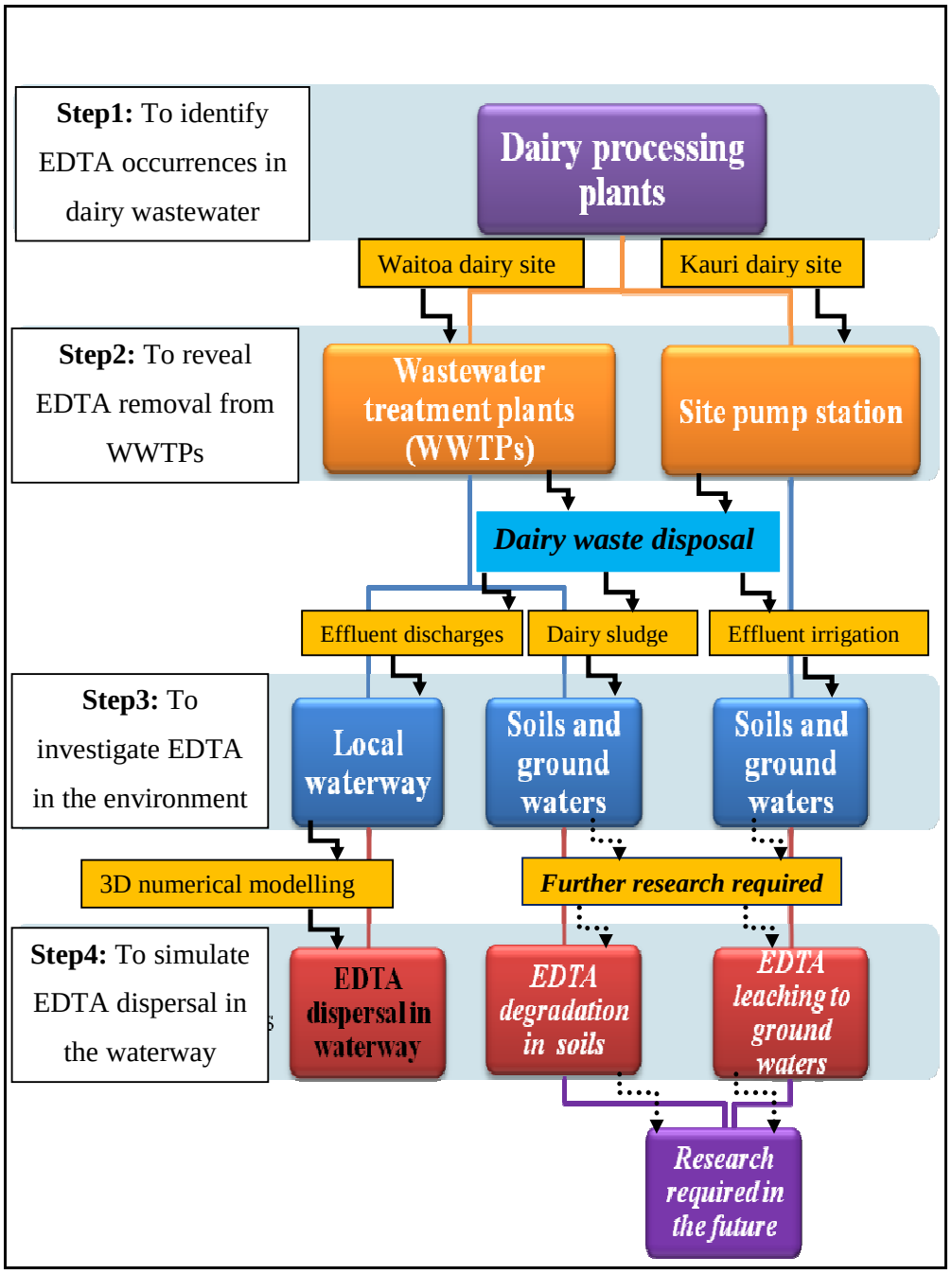

Figure 1.5 *The research approach includes four major steps to (i) identify EDTA occurrences in drainage of dairy processing plants, (ii) ascertain EDTA removal efficiency from wastewater treatment plants, (iii) investigate EDTA presence in the natural environment, and (iv) forecast EDTA potential environmental effects on waterways subsequent to the dairy wastewater discharge.*

This study primarily relates to the Waitoa and Kauri dairy sites of Fonterra Co-operative Group Limited in the North Island of New Zealand. Locations are shown in Figure 1.6. One of the worst case scenarios is when large volumes of wastewater containing high EDTA concentrations are discharged into a relatively

small waterway, and where EDTA is not effectively removed in the wastewater treatment plant (European Chemicals Bureau, 2004; Schmidt et al. 2004; Grundler et al. 2005). The Fonterra Waitoa dairy site is likely to be one of these cases, in which significant amounts of EDTA have been used in the cleaning process of manufacturing plants, EDTA removal efficiency by the existing wastewater treatment plants is unknown, and large volumes of dairy effluent are discharged into the relatively small Waitoa River. The Fonterra Kauri dairy site is chosen as a case study of a land treatment system for dairy effluent containing EDTA, to investigate the potential risk to ground waters when the dairy effluent disposal has been applied for a period of time.

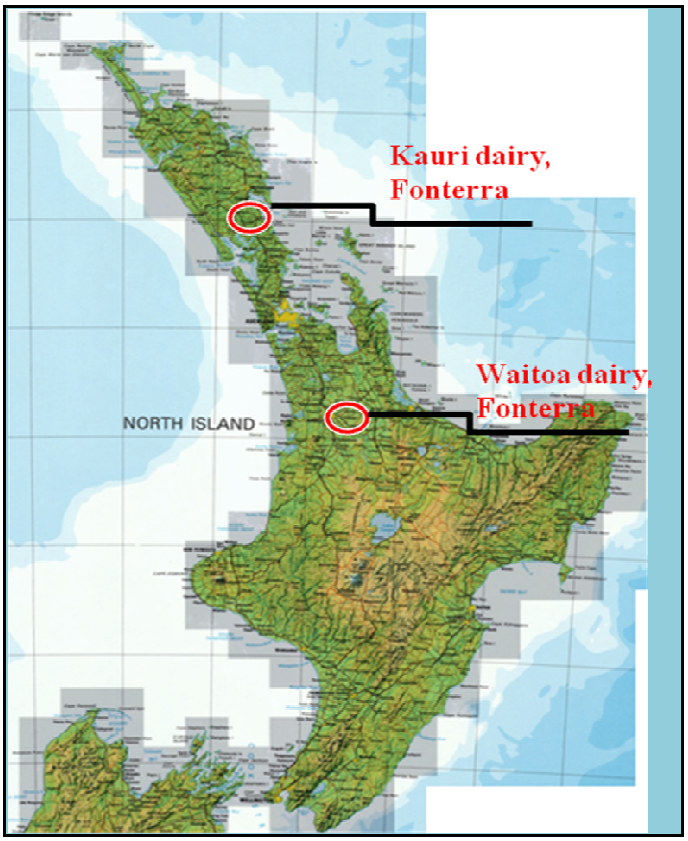

Figure 1.6 *Location of the Fonterra Waitoa and Kauri dairy sites, North Island, New Zealand.*
(Source: Map Toaster Topo/NZ)

1.4 LAYOUT OF THESIS

In order to address and achieve the objectives listed above, the thesis is structured as follows:

Chapter 2 – The Nature, Use and Concerns of EDTA. This chapter reviews the available literature pertinent to this study in terms of EDTA characteristics, application, behaviour, occurrences and risk in the eco-environment. It outlines the research questions associated with the current practice. A peer-reviewed paper entitled "EDTA in the environment: with special reference to the dairy industry" was published in the *International Journal of Environment and Waste Management,* pp.351-36, Vol. 1, No 4, 2007.

Chapter 3 – Methodology for Measuring EDTA. This chapter reviews the determination of EDTA with different matrices of samples, and presents a study of the development and validation of the method to determine EDTA in dairy wastewater using HPLC – UV. A peer-reviewed paper entitled "Determination of EDTA in dairy wastewater and the adjacent surface water" was published in P*roceedings of World Academy of Science, Engineering and Technology (WASET),* pp. 50-54, Vol. 34, Oct. 2008.

Chapter 4 – EDTA in Dairy Wastewater and Removal Efficiency. This chapter describes a case study of EDTA in the dairy industry, including application in the dairy cleaning process, concentration range occurring in the wastewater discharged from dairy manufacturing plants, EDTA removal efficiency by the existing site wastewater treatment plants under normal operations, and concentrations of EDTA in dairy effluent discharged into an adjacent waterway. A paper entitled "EDTA removal from the dairy wastewater treatment plant – a case study" has been submitted to the *International Journal of Environment and Sustainable Development.*

Chapter 5 - EDTA and Associated Heavy Metals in the Waitoa River. This chapter establishes a profile of EDTA and heavy metals in the local adjacent waterway of the Waitoa River. It explains the bioavailability of heavy metals, and demonstrates the possibility of influencing the natural speciation of metals due to the occurrence of EDTA in the Waitoa River.

Chapter 6 – Simulation of EDTA Dispersal within the Waitoa River. Two approaches namely (i) approximate calculations using quasi one-dimension vertical mixing model; and (ii) a numerical simulation of the hydrodynamic processes and effluent mixing in two-dimensions (depth-averaged) were undertaken to enhance the understanding of the fundamental aspects of the transport of EDTA within the Waitoa River.

Chapter 7 – Investigation of EDTA and Heavy Metals in Soils and Groundwater. This chapter investigates the presence of EDTA and heavy metals in soils and ground waters where a long-term land treatment system of dairy effluent or waste sludge has been applied. The potential risk to ground waters with current practices of dairy effluent is evaluated for the Kauri dairy site.

Chapter 8 – Conclusions and Discussion. This chapter presents to interpret the major findings on environmental concerns of dairy effluent containing EDTA studied in this research, and provides suggestions for future research. A paper has been drafted and submitted to the *International Journal of Environment and Waste Management.*

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2.0 CHAPTER TWO: THE NATURE, USE AND CONCERNS OF EDTA

2.1 INTRODUCTION

The available literature pertinent to the study of EDTA characteristics, use, occurrence, behaviour, fate and risk in the environment are reviewed in this chapter.

- Section 2.2 describes the EDTA characteristics, application and environmental occurrence;
- Section 2.3 reviews the elimination and degradation of EDTA in the natural environment;
- Section 2.4 presents available knowledge on the environmental fate and risk of EDTA, including concern about the potential remobilization of heavy metals;
- Section 2.5 outlines known impacts in the natural environment, ecosystems, and for human health (toxicity);
- Section 2.6 discusses both natural and anthropogenically derived metals in soils, effects of EDTA, and soil remediation; and
- Section 2.7 discusses implication of EDTA in the dairy industry

2.2 EDTA AND ITS OCCURRENCE IN THE ENVIRONMENT

2.2.1 EDTA and APCAs

EDTA is the abbreviation for **E**thylene**D**iamine**T**etraacetatic **A**cid, which was patented in Germany in 1935 by F. Munz (Oviedo and Rodriguez, 2003), and industrially manufactured in 1939 (Knepper, 2003). Its molecular weight is 292 and empirical formula is $C_{10}H_{16}N_2O_8$. The molecule is a substituted diamine (Figure 2.1), usually marketed as sodium salts. EDTA belongs to the group of

aminopolycarboxylic acids (APCAs) (Nowack and Van Briesen 2005), which have the ability to form stable, water-soluble 1:1 complexes with di- and trivalent metal ions. The other important representatives of APCAs are nitrilotriacetate (NTA), diethylenetriaminepentaacetic acid (DTPA) etc. (Figure 2.2).

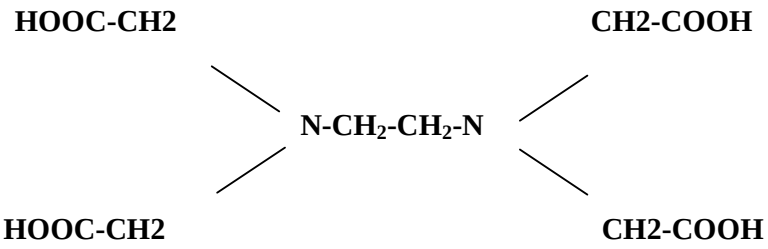


Figure 2.1 Molecular structure of EDTA.

(Source: Oviedo and Rodriguez 2003)

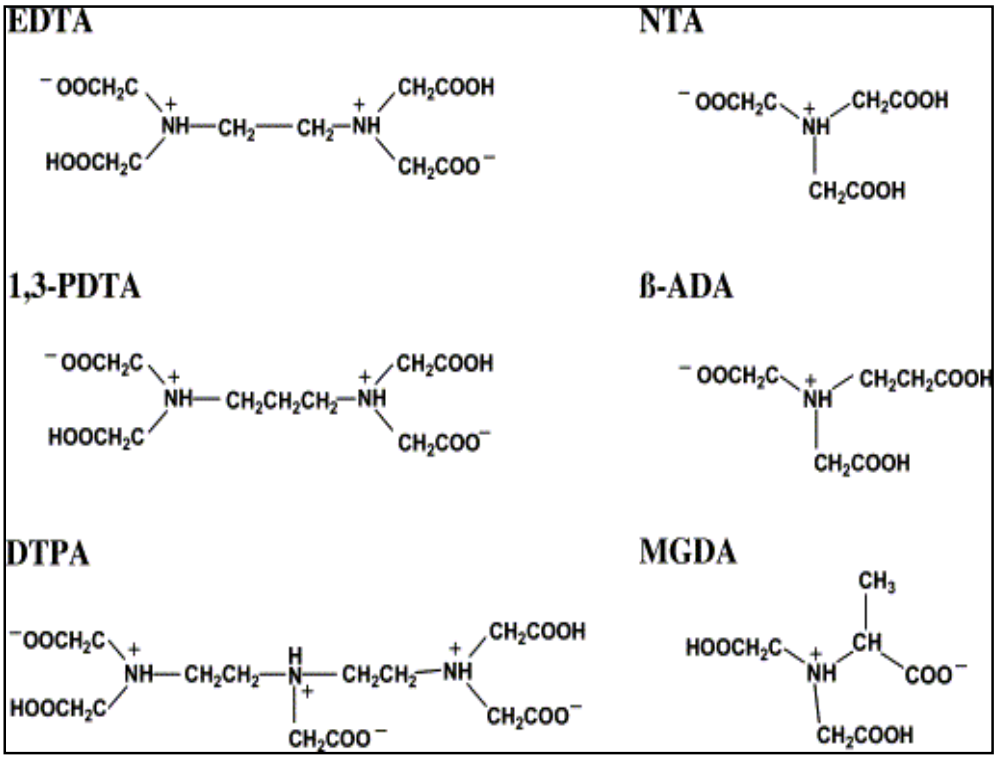


Figure 2.2 *Structural formulae of important aminopolycarboxylates EDTA = EthyleneDiamineTetraacetic Acid, NTA = NitriloTriacetatic Acid, 1,3 – PDTA = 1,3 – PropyleneDiamineTetraacetic Acid, β – ADA = β – AlanineDiaacetic Acid, MGDA = MethyGlycineDiacetic Acid DTPA = DiethyleneTriaminePentaacetic Acid*

(Source: Schmidt et al. 2004)

Worldwide, EDTA is the most powerful and common chelating (complexing or sequestering) agent used to bind and mask metal ions that could otherwise cause

undesired reactions since 1939 (Conrad, 2000; Knepper, 2003). EDTA has many favourable properties when used in chemical engineering processes. For example, formation of high stability metal-EDTA complexes loses the original chemical characteristics of the metal ions, and they become soluble in water and insoluble in organic solvents. Therefore, EDTA is widely used for solubilisation and/or transport of metal cations in the following applications:

(i) water treatment for scale and corrosion control in boiler and cooling waters;
(ii) foods to prevent degradation of flavour, colour, texture and appearance;
(iii) household, industrial and institutional cleaners to help to dissolve soap scum and hard water scale, such as in the dairy industry to facilitate the elimination of milk-stone linings;
(iv) agricultural application to improve plant uptake of micronutrients needed to correct trace metal deficiencies ; and
(v) a variety of other uses such as for paper-making, metal-working, pharmaceutical and cosmetics and environmental cleanup applications.

2.2.2 Production and Consumption of EDTA

EDTA is mainly produced and used as an acid (H_4EDTA) or a salt (Na_4EDTA). Only small amounts of other salts or metal complexes are produced and used. All production and use volumes are given here as H_4EDTA equivalents for an environmental risk assessment. Na_4EDTA is usually synthesised by cyanomethylation of ethylene diamine with sodium cyanide and formaldehyde, and H_4EDTA is produced by acidification with sulphuric acid and precipitation from aqueous solution from its salt (European Chemicals Bureau, 2004). There are only a few companies in the world which manufacture complexing agents. For instance, in 1999 about 90% of all aminopolycarboxylic acids were produced by only four companies (Akzo Nobel, BASF, Dow and Solutia) in the US and Europe (Knepper, 2003).

The European Union Risk Assessment Report (2004) indicated that 53,900 tonnes per annum (calculated as H_4EDTA) were produced in 1999 in Europe, of which 34,546 tonnes were consumed by European Union countries and the rest exported. The breakdown of usage in different applications is given in Table 2.1. The global

consumption of EDTA was estimated roughly as 100,000 tons/annum in 2001 (Schmidt et al., 2004).

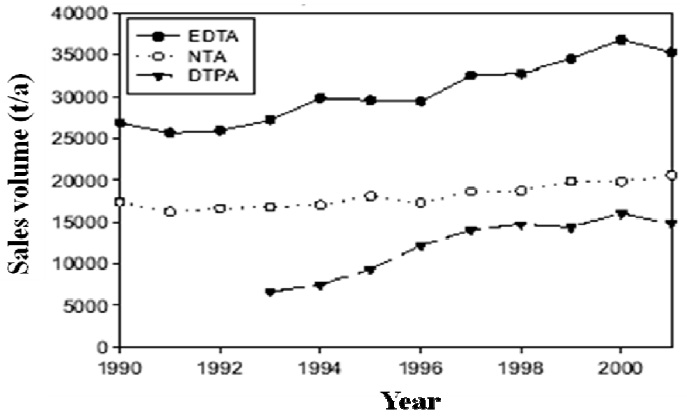
Table 2.1 Use of EDTA in Europe for the year 1999.

Use	Marketed amount (t/a)	Percentage (%)
Household detergents	2619	7.6
Industrial and institutional detergents	10,685	31
Photochemicals	4,191	12
Textiles	639	1.8
Pulp and paper	4,002	12
Metal plating	470	1.4
Agriculture	5,821	17
Cosmetic	756	2.2
Fuel gas cleaning	595	1.7
Polymer and rubber processing	469	1.4
Exports	1143	3.3
Others	2971	8.6
Total	**34546**	**100**

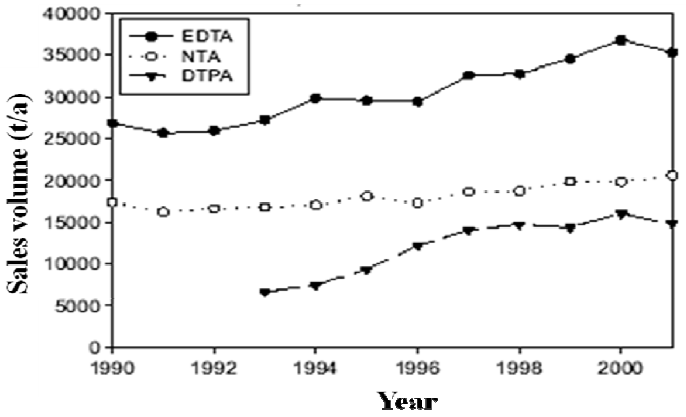
(Source: European Chemicals Bureau, 2004)

Table 2.1 shows that the total consumption of industrial and institutional detergents in Europe was 10685 tons/year in 1999, for which the dairy and beverage industries were reported as major consumers accounting for about 50% of the total (European Chemicals Bureau, 2004). Most of those industries consumed less than one ton per year, and less than 5% of them used more than 10 tons/year, of which over 50% had their own wastewater treatment plant (WWTP). However, about 800 customers throughout Europe were in the above industries. It has been recorded that 180 kg/day of EDTA was consumed at a dairy site producing whey proteins in Germany (European Chemicals Bureau, 2004).

EDTA consumption has been steadily increased during the last decade in Western Europe (Schmidt et al., 2004). However, the trend of EDTA sales in Germany has been generally declining, even though a slight increase can be observed again from 1998 onwards (Figure 2.3a & b). In 1991, the German authorities established a voluntary agreement with the German chemical industry for a 50% reduction in EDTA in German surface waters, due to the perception of EDTA as an environmentally hazardous substance (Conrad, 2000).



a. Western Europe



b. Germany

Figure 2.3 Trend of sales for the aminopolycarboxylates in Western Europe (a) and Germany (b) from 1990-2001. EDTA = EthyleneDiamineteTraacetic Acid, NTA = NitriloTriAcetate, DTPA = DiethyleneTriaminePentaacetic Acid from Schmidt et al. 2004

2.2.3 Occurrences of EDTA in the Environment

In almost all applications of EDTA conducted in aqueous medium, EDTA is subsequently released into the environment through wastewater. As an example, the sale of EDTA was reported as 3894 tons in 1999 in Germany, and the amount of EDTA being introduced into the aquatic environment, via wastewater, was calculated to be about 860 tons (Knepper, 2003). The presence of EDTA in soils may be due to agrichemical application, or to the disposal of wastes containing EDTA. EDTA is highly unlikely to be detected in the air because of its very low vapour pressure.

1. Surface waters

Both widespread use of EDTA in industrial and domestic applications and its slow removal under many environmental conditions have led to recognition that it likely comprised the highest concentration of anthropogenic compounds in many surface waters in Europe, and possibly the world (Sillanpää, 1997). Table 2.2 shows some of the concentration ranges of EDTA found in natural waters. The highest value of 2460 μg/L was found in lakes in Spain, and the second highest value of 1120 μg/L was in English rivers.

The International Association of Waterworks in the Rhine catchments area (IAWR) set out quality requirements for well-degradable complexing agents of 5 μg/L, and for poorly degradable compounds as 1 μg/L. The International Commission for the Protection of the Rhine (IKSR) has classified only EDTA as a relevant chelating agent in the Rhine catchment (Knepper, 2003).

Table 2.2 Occurrence of EDTA in surface waters.

Range of Concentration (μg/L)	Type of fresh water	Location
10 ~ 184	River	Australia
14 ~ 1120	River	England
158	River	France
2.0 ~ 104	River	Germany
6 ~ 60	River	Great Britain
900	River	Jordan
5 ~ 30	River	Mississippi (USA)
2.4 ~ 13	River	Santa Ana (USA)
2.0 ~ 45	River	Switzerland
1.7 ~ 44.0	Lake	Finland
2.9	Lake	Germany
0.52	Lake	Greece
599 ~ 2460	Lake	Spain
1 ~ 735	Lake	Swedish
1.2 ~ 4.0	Lake	Theiß (Romania)
2.6 ~ 29.2	Surface	Netherlands

(Data extracted from Bucheli-Witschel and Egli, 2001; Fuerhacker et al., 2003; Oviedo and Rodriguez, 2003; Schemidt et al., 2004; Nowack and VanBriesen, 2005)

2. Drinking water and groundwater

EDTA has also been reported in drinking water and groundwater as EDTA behaves as a persistent substance, evidently derived from EDTA-contaminated surface waters (Nowack et al. 1997). EDTA concentrations in U.S. groundwater receiving wastewater effluent recharge were reported at 1-72 μg/L, and EDTA was found to be a conservative tracer, with higher concentrations of EDTA corresponding to a greater percentage of reclaimed water in drinking water production wells (Nowack and VanBriesen, 2005). Research by Schmidt et al. (2004) demonstrated that raw waters were polluted by EDTA at concentrations of between 1.1–11 μg/L, and the investigated drinking waters were regularly polluted with EDTA at concentrations of up to 7 μg/L. In Germany EDTA in groundwater samples recorded between 15 - 30 μg/L (Fuerhacker et al., 2003). In Swiss groundwater, EDTA concentrations of 0.1 to 15 μg/L were found (Bucheli-Witschel and Egli, 2001).

3. Domestic and industrial wastewaters

The concentration of EDTA in all municipal wastewater treatment plant (WWTP) effluent and industrial WWTPs has been found to vary widely depending upon the type of industry, the amount applied, and any specific wastewater treatment procedure (Knepper, 2003). Some typical EDTA concentrations of effluents from industrial WWTPs were reported at between 100-20,000 μg/L in Germany, the highest concentration reaching 400,000 μg/L (Schmidt et al., 2004). According to an evaluation by the Europe Union (2004), local EDTA concentration in the receiving water in the immediate vicinity of industrial wastewater discharge points were predicted in a worst-case scenario to be as high as 12 mg/L.

For the dairy and beverage industry, the research by Schmidt et al. (2004) showed that:

- the possible EDTA concentrations in effluent were measured between 2500 – 25,000 μg/L; and

- local EDTA concentrations in the receiving water in the immediate vicinity of effluent discharge points were estimated to be in the range of 350 – 2600 μg/L as the worst-case scenario.

2.3 DEGRADABILITY AND ELIMINATION OF EDTA

In recent years, the degradability of EDTA has been increasingly scrutinized and investigated from different points of view (Nörtemann, 1999). Apparently, EDTA is not toxic to mammals at the occurred level in the aquatic environment, but there has been some concern about its potential to remobilize heavy metals out of river sediments and sewage sludge, which would lead to possible contamination of surface and groundwater (Alder et al., 1990; Xue et al., 1995; Kari and Giger, 1996; Nowack et al., 1997). Another reason to investigate EDTA degradability is the fact that it can be used for the remediation of sites contaminated with heavy metals or radionuclides (Nörtemann, 1999).

EDTA occurs in natural waters predominantly in the form of metal complexes due to its strong complexing characteristics and high stability. Basically, there are two different ways, including biological and non-biological pathways, to eliminate and degrade EDTA from the environment.

2.3.1 Biodegradation of EDTA

EDTA has been widely reported to either resist degradation or undergo slow biodegradation based upon the EDTA concentration detected from the corresponding influent and effluent (Alder et al., 1990; Allard, 1996; Kari & Giger, 1996; Hinck et al., 1997; Sillanpää, 1997; Eklund, 2002; Fuerhacker et al., 2003). Influent is generally referred to as untreated wastewater before it flows into a treatment plant, and effluent is treated wastewater to be discharged into environments. For instance, an Organization for Economic Co-operation and Development (OECD) screening test indicated 10% degradation of municipal

wastewater after 19 days when EDTA concentration was between 7 and 50 mg/l (European Chemicals Bureau, 2004).

In biodegradation tests, Eklund et al. (2002) demonstrated that EDTA was slowly biodegradable under aerobic conditions. The rate of biodegradation may vary strongly with the bacterial population present in the particular ecosystem. EDTA, especially in the form of the EDTA-iron-chelate, is readily decomposed on exposure to sunlight and yields biodegradable products. Nevertheless, the information collected to date strongly suggests that ready and ultimate biodegradability is essential for a reliable and quick elimination of the EDTA in the environment. The degradation mechanism and elimination of EDTA from the environment is discussed below.

1. Effects of bacteria and chemical speciation

Microbial degradation of aminopolycarboxylic acids, including EDTA, was reviewed by Bucheli-Witschel and Egli (2001). As a result of 40 years of study, three pure cultures of EDTA-degrading bacteria have been isolated, namely the genus *Agrobacterium* which is able to degrade the Fe(III)-EDTA complex; the strain *BNC1* - a gram-negative bacterium that is able to degrade Mg-EDTA, Ca-EDTA, Mn-EDTA, and Zn-EDTA and *DSM 9103* - also a gram-negative bacterium which was assigned to *Proteobacteria* (Satroutdinov et al., 2003; Nörtemann, 2005; Satroutdinov et al., 2005).

The first report of biodegradation (Belly et al., 1975) demonstrated decomposition of EDTA by microbial populations from an aerated lagoon receiving industrial effluents containing EDTA (Bucheli Witschel and Egli, 2001). The authors followed [^{14}C] CO_2 formation from an Fe(III) complex of radioactively labelled EDTA, which was incubated in the dark to prevent photodegradation. After an incubation period of 5 days, about 90% of the initially present EDTA had disappeared. 27% of the initial radioactivity of the acetate-labelled and 31% of the ethylene-labelled EDTA was recovered as $^{14}CO_2$, indicating that both the ethylene backbone and the acetyl groups were attacked. Optimum conditions were at pH between 7 and 8 (Sillanpää and Pirkanniemi, 2001).

Biodegradation of EDTA by a mixed bacterial culture, taken from sewage, was studied by Nörtemann (1992) and Henneken et al. (1995). The mixed culture used EDTA as the source of carbon and nitrogen. The chemical speciation was observed to have no influence (Nörtemann, 1992). However, it was later reported that uncomplexed EDTA interacted negatively with the cell walls of the bacteria and completely inhibited the bacterial growth, whereas Mg-EDTA and Ca-EDTA supported, and Fe-EDTA remained inert (Henneken et al., 1995). It has been suggested that the thermodynamic and biological stability of metal-EDTA complexes correlate (Henneken et al., 1998; Egli, 2001). The slow biodegradation of uncomplexed EDTA was suggested to be due to the chelation of essential trace metals from the medium (Thomas et al., 1998).

Several laboratory-scale EDTA degradation experiments have been published either (i) as closed bottle or batch culture experiments (Henneken et al., 1995; van Ginkel et al., 1997; van Ginkel et al., 1999; Satroutdinov et al., 2000), (ii) as Semicontinuous Activated Sludge (SCAS), (iii) as Continuous Activated Sludge (CAS) experiments (van Ginkel et al., 1997; Henneken et al., 1998; Kaluza et al., 1998), or (iv) as gas-lift bioreactor experiments (Henneken et al., 1998; Thomas et al., 1998). Pure cultures were used in some of the studies, whereas some used mixed cultures.

A pure culture of *Agrobacterium sp.* mineralised Fe(III)-EDTA, which was the sole carbon source for the isolate. At a substrate concentration of approximately 10 g/L, 90 % of Fe(III)-EDTA was degraded in three days, although photogradation could have played some role. Contrary to this, uncomplexed EDTA, Ni-EDTA and Cu-EDTA did not support bacterial growth (Sillanpää and Pirkanniemi, 2001). In contrast to other studies discussed below in detail, the degradation rate was found to be higher at low pH values (initial pH 6.2 and 7.4) and the pH had to be maintained below 8 for degradation of Fe(III)-EDTA to occur.

Work by Klüner et al. (1998), Nörtemann (1999), and Willelett and Rittmann (2003) demonstrated that a gram-negative, rod-shaped bacterium strain BNC1 (DSM 6780) was found to degrade EDTA and some of its metal complexes. When

strain BNC1 was used, the degradability of metal-EDTA complexes depended strictly on their thermodynamic stability. Metal complexes with a stability constant over 10^{12}, such as Fe(III)-, Co-, Cd-, Pb-, Ni-, and Cu-EDTA, were not metabolized. Ba-, Mg-, Mn-, Ca-, and Zn-EDTA, which have a stability constant below 10^{12}, were degraded.

The gram-negative bacteria strain DSM9103 is able to grow with EDTA as the sole source of carbon, nitrogen and energy (Witschel et al., 1997; Bohuslavek et al., 2001). Satroutdinov et al. (2003) used cell suspension of the bacterial strain DSM 9103 in the degradation experiments of EDTA. In their study, the metal speciation proved an important factor. The metal-EDTA (Me-EDTA) complexes studied could be divided into three groups according to their degradability. EDTA complexes with stability constant K below 10^{16} (log K<16), such as Mg-EDTA, Ca-EDTA, and Mn-EDTA, as well as uncomplexed EDTA, were degraded by the cell suspension at a constant rate to completion within 5-10 h of incubation. Me-EDTA complexes with log K above 16 (Zn-EDTA, Co-EDTA, Pb-EDTA, and Cu-EDTA) were not completely degraded during a 24-h incubation, which was possibly due to the toxic effect of the metal ions released. No degradation of Cd-EDTA or Fe(III)-EDTA by cell suspensions of strain DSM 9103 was observed under the conditions studied.

Additionally, Pitter and Sýkora (2001) also observed that biological degradability of ethylenediamine derivatives depended on the type and the number of the substituents (mono-, di-, tri- and tetrasubstituted derivatives). They found that the biodegradability of ethylenediamine derivatives depended on the type and number of substituents. The susceptibility to biodegradation decreased in the sequence of substituents $-COCH_3$, $-CH_3$, $-C_2H_5$, $-CH_2CH_2COOH$ and with polysubstitution. The biodegradability depended also on the kind and number of nitrogen atoms (Pitter and Sýkora, 2001; Sýkora et al., 2001).

In summary, the biodegradability of EDTA in the environment strongly depends upon the bacteria, EDTA species, and natural environmental factors, such as pH.

2. Effect of pH and sludge retention time (SRT)

Several studies have been conducted to investigate the effect of pH on the degradation of EDTA (van Ginkel et al., 1997 and 1999; Ek et al., 1999). The optimal pH for the degradation of EDTA by a mixed microbial population was found to be between 9 and 9.5. Complete breakdown of the target molecule was obtained at a hydraulic retention time (HRT) of 1.5 h. EDTA was mineralised as the only carbon source (Sillanpää and Pirkanniemi, 2001).

Biodegradation of EDTA was found to be strongly pH dependent in a semi continuous activated-sludge facility (van Ginkel et al., 1997; European Chemicals Bureau, 2004; van Ginkel and Geerts, 2005). EDTA was effectively removed at pH 8.5, in contrast to pH 6.5, suggesting that microorganisms use EDTA as a carbon and energy source only under alkaline conditions (van Ginkel et al., 1997; Sillanpää and Pirkanniemi, 2001). One of the investigations into the removal of EDTA was conducted in a full-scale activated sludge plant operated at pH between 7.5 and 8.5, and 20 days of sludge retention time with dairy wastewater containing ~30 mg/l EDTA. Approximately 90% removal of EDTA was observed from the analysis of influent, effluent and sludge concentrations of EDTA. However, no biodegradation took place at pH of 6.7 (van Ginkel et al., 1997). Table 2.3 demonstrates the removal of EDTA at five activated sludge plants treating only or predominantly wastewater from the dairy and beer industries (van Ginkel and Geerts, 2005).

Table 2.3 EDTA removal from 5 full-scale activated sludge plants treating wastewater from the dairy and beer industry.

Plant	Wastewater	pH	SRT* (days)	Removal (%)
I	Dairy	7.5-8.1	~20	~90
II	Beer	7.3-7.7	~23	~50
III	Dairy	7.8-8.4	~9	~30
IV (5˚C)	dairy and domestic	7.5-7.8	~40	~35
IV (20˚C)	dairy and domestic	7.8-8.0	~40	~95
V	dairy and domestic	6.9-7.1	~20	0
VI	Dairy	8.7-8.9	20	95[a]

[a] measured in a laboratory-scale activated sludge unit
*SRT – sludge retention time
(Source: van Ginkel and Geerts, 2005)

In addition to pH (Figure 2.4), sludge retention time seems to have a significant impact on EDTA degradation (Figure 2.5). It has been suggested that several organic compounds, such as EDTA, being considered recalcitrant to microbial attack, can be degraded at certain sludge retention time in laboratory mixed culture systems (Kaluza et al., 1998; Sillanpää and Pirkanniemi, 2001). In the dairy wastewater containing EDTA, sludge retention time above 20 days was found to be necessary (van Ginkel and Geerts, 2005). Another report revealed that pH had little impact on the removal rate of EDTA (pH 7 and 8.5), but the sludge retention time was far more significant (Sillanpää and Pirkanniemi, 2001).

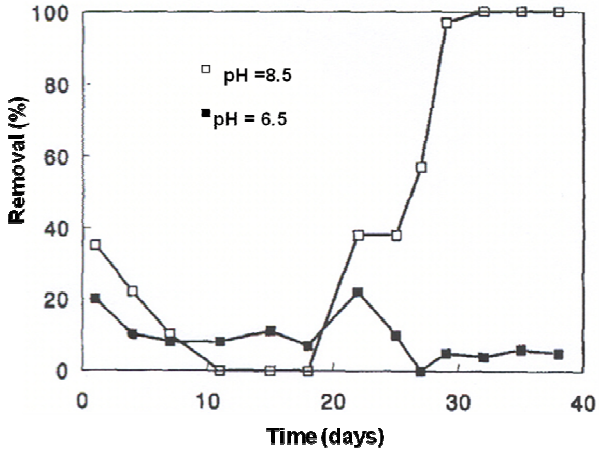


Figure 2.4 EDTA Removal efficiency of dairy wastewater at pH 6.5 ▪ and 8.5 ▫.

(Source: van Ginkel et al., 1997)

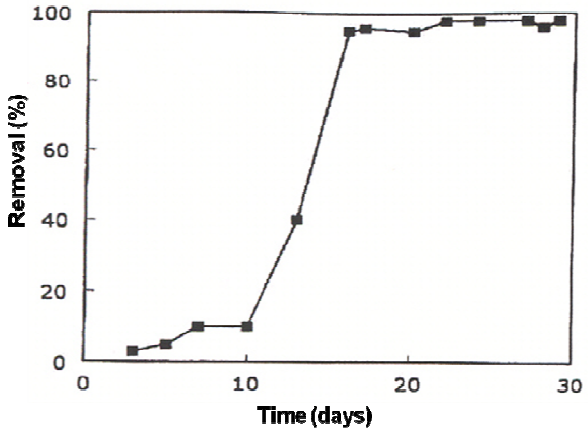


Figure 2.5 EDTA removal efficiency of dairy wastewater associated with the sludge retention time at pH value of 8.7 - 8.9

(Source: van Ginkel and Geerts, 2005)

Accelerating EDTA degradation is likely through the mechanism of biological oxidation. The other reasonable explanation for the phenomena could be the transition of EDTA-metal complexes as the alkalinity increases, especially for the more stable Fe(III)-EDTA and alkaline earth metal complexes. Alkaline earth metals can therefore compete successfully with trace metals to form EDTA complexes that are more biodegradable (van Ginkel et al., 1997). The relatively high sludge retention time required for the degradation of EDTA could be due to the slow kinetics of these reactions (Sillanpää and Pirkanniemi, 2001).

3. Pathway of EDTA degradation

It has been demonstrated that EDTA is able to be biodegraded in the presence of suitable microbial populations (Henneken et al., 1998; Nörtemann, 1999; Bucheli-Witschel and Egli, 2001; Egli, 2001 Sillanpää and Pirkanniemi, 2001; Willelett and Rittmann, 2003). The catabolism of EDTA by microbes involves:

i. Bacteria take up EDTA as a source of carbon and nitrogen via an energy-dependent carrier (Klüner et al., 1998);
ii. The EDTA, present as metal-EDTA complexes or free EDTA, is then oxidized under aerobic conditions. The intermediate products of oxidization are mainly ethylenediaminetriacetate (ED3A) and iminodiacetate (IDA) (Klüner et al., 1998);
iii. The final biodegradation products of EDTA are CO_2, and inorganic nitrates and ammonia (Kaluza et al., 1998; Van Ginkel et al. 1999).

2.3.2 Photodegradation of EDTA

In the absence of evidence for the rapid biodegradation of EDTA, other mechanisms for its breakdown in the environment have been sought. One is photodegradation, i.e. degradation resulting from the absorption of sunlight. The susceptibility of a species to photodegradation depends upon its absorption spectrum; and the quantum efficiency of the photochemical reaction. The

absorption spectra of metal chelates reflect three types of electronic transitions (Wolf and Gilbert, 1992):

(i) transitions typical of the central metal ion, modified by the ligand field of the chelating molecule;
(ii) charge transfer transitions between metal ion and ligands; and
(iii) transitions typical of the ligand.

Of these, the charge transfer transitions are most likely to induce photochemical reactions, and typically involve absorption of radiation in the UV-visible wavelength range. Therefore, those species that exhibit strong absorbance bands in the region of the solar spectrum (wavelengths greater than 295 nm) are likely to be more susceptible to photodegradation. The process considered to be the most important for the elimination of EDTA from surface waters is direct photolysis, which results from the fraction of sunlight below 400 nm (Bucheli-Witschel and Egli, 2001). The study of Kari et al. (1995) for the Swiss River Glatt found that the reaction quantum yield of Fe(III)-EDTA was affected by the irradiation wavelength. With increase of wavelength, the quantum yield strongly decreased. If the solar irradiation was completely available, the half-life would range from 20 to 100 minutes (summer to winter), corresponding to flow distances of 0.6 to 3 km, respectively. The rapid photodegradation of Fe(III)EDTA results in a mean half-life of EDTA in river water of a few hours during summer and several days in winter. Degradation was slower in winter because of the bank vegetation shading the water while cloudiness also reduced the reaction rate.

Other environmentally relevant EDTA species (complexes with Mg^{2+}, Ca^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} and Hg^{2+}) will not photolyse (Kari et al., 1995; Kari and Giger, 1995 and 1996; Nowack and Baumann, 1998; Bohuslavek et al., 2001; Metsärinne et al., 2004). Complexes of EDTA with Co (III) and Mn (III) in aqueous solution were also found similarly unstable against photolysis, however, with lower reaction constants. The relative reaction rates of Fe (III), Co (III), and Mn (III) complex are in the ratio 1, 0.01 and 0.05. There is no significant contribution to the degradation of EDTA in the hydrosphere for the very low environmental concentrations of Co (III) and Mn (III) complexes and their relative low reaction constants (European Chemicals Bureau, 2004).

Photodegradation of Fe(III)EDTA is considered to be the main degradation of EDTA in the aqueous medium whereas microbial processes seem to be of little importance (Wolf and Gilbert, 1992; Kari et al., 1995; Kari and Giger, 1995; Klüner et al., 1998). Major photodegradation products were identified as carbon dioxide, formaldehyde and ED3A by Lockhart Jr. and Blakeley (1975). However, there seems to be very little information available about any further degradation of ED3A in the aqueous environment.

2.3.3 Physicochemical and Other Degradation of EDTA

Apart from the biodegradation and photodegradation of EDTA, physicochemical degradation may also play an important role in the elimination of EDTA.

The UV/H_2O_2 process, which is based on the photolysis of H_2O_2 (Madden et al., 1997; Davis and Green, 1999; Sillanpää and Pirkanniemi 2001; Rhoads and Davis 2004; Jiraroj et al. 2006), and the UV/ TiO_2 process, in which an electron vacancy is produced within TiO_2 when exposed to UV light (Ku et al., 1998; Krapfenbauer and Getoff, 1999; Rämö and Sillanpää, 2001; Pirkanniemi et al., 2003), have been used successfully to degrade EDTA in aqueous solution. The applicability of ozonation and an oxygen activation scheme (Chitra et al., 2004; Lee et al., 2004; Noradoun and Cheng, 2005) to remove EDTA have also been studied extensively.

EDTA is not expected to be significantly adsorbed onto solid matter as EDTA is a hydrophilic compound. On the other hand, it has been proposed that the elimination of EDTA from the water column by settling of EDTA-loaded particles might be a relevant process in the elimination of EDTA in lakes (Nowack et al., 1996a). This is supported by the occurrence of EDTA in lake sediments (Nowack et al., 1996b). Therefore EDTA adsorption onto sediments might be an important pathway for its elimination (Sillanpää et al., 1997).The adsorption of EDTA and its metal complexes, Cu, Fe(III), Hg, Mn and Ni onto lake sediment was studied by Sillanpää and Rämö (2001). The results revealed that EDTA and metal-EDTA complexes, even through being hydrophilic compounds, were indeed adsorbed

within one month of contact time (6.3-24.8%). The study shows that the adsorption of Fe(III)-EDTA complexes onto positively charged compounds, e.g., metal hydroxides, in solid matter might be another relevant process in some cases.

2.3.4 Degradation of EDTA in Soils and Sediments

A study of the biodegradability of EDTA in aquatic sediments and agricultural soils demonstrated slow but steady mineralization of all the EDTA carbon, suggesting that the degradation was accelerated by microbial action (Wolf and Gilbert, 1992). The biodegradation rates of free EDTA and its complex of Cu, Cd, Zn, Mg, Ca and Fe were comparable, while the NiEDTA showed lower biodegradability. No significant biodegradation was observed under anaerobic conditions (Bucheli-Witschel and Egli, 2001).

2.3.5 Summary

i. Complexing agents, such as EDTA, were once considered as stable and almost non-degradable compounds. In the light of later findings, it is evident that these substances often can be removed by favourable treatment processes.

ii. According to several studies referred to above, biodegradation of EDTA is possible if the reaction conditions are favourable, and if EDTA degrading bacteria species is present. However, long sludge retention time and elevated pH may limit the use of this method for the removal of EDTA in certain industrial situations.

iii. Photodegradation of Fe(III) complexes of EDTA has been suggested to be the major pathway for the removal of EDTA from the natural aquatic environment. Also, the adsorption of Fe(III)-EDTA complexes onto positively charged compounds in solid matter might be another relevant process in some cases.

iv. Advanced oxidation treatment processes discussed above have been shown to be promising tools for the degradation of recalcitrant organic pollutants.

These processes apply a combination of radiation, oxidants (ozone, hydrogen peroxide) and catalysts for degrading the target compounds.

v. Chemical species play a key role in the behaviour of chelating agents and influences their fate in the environment. It has been shown that adsorption as well as photochemical and biological degradations strongly depends upon the metal complexed by EDTA. Moreover, taking into account the excess of alkaline earth and transition metals, it is expected that no chelate is present in uncomplexed form in wastewaters.

vi. Contradictory results have been published concerning EDTA degradation in soil and sediments. Some groups found no biological EDTA breakdown, whereas others observed a slow microbial EDTA decomposition under aerobic conditions. No EDTA mineralization was found under anaerobic conditions.

2.4 ENVIRONMENTAL FATE AND RISK

EDTA has been used as an indicator of metal toxicity in a Toxicity Identification Evaluation Method by the U.S. Environmental Protection Agency (Hockett and Mount, 1996). Hence, the environmental fate of a chelating agent, such as EDTA, needs to be addressed with the presence of metals and how they interact with the chelates (Nowack, 2002). The environmental risk assessment is also related to the speciation under environmental conditions (Williams, 2005; Popov and Wanner, 2005).

2.4.1 EDTA metal complexes in the environment

1. Stability of EDTA complexes

The most important property of EDTA is to form complexes (usually 1:1-complexes) with multivalent metal ions. The metal ion is centred in the complexes to form a ring with a multi-charged metal ion and EDTA while being coordinately bound to nitrogen and oxygen atoms. Five or six-ring numbers formed of EDTA

leads to extremely stable complexes. The stability of those complexes, strongly depending upon the pH value, is usually described by the mass action law as:

$$K_{MeZ} = [MeZ^{(m-n)-}] / [Me^{n+}]*[Z^{m-}]$$

Where K_{MeZ} is the stability constant of the metal complex;

$[MeZ^{(m-n)-}]$ is the concentration of the metal complex;

$[Me^{n+}]$ is the concentration of the metal ion; and

$[Z^{m-}]$ is the concentration of the $EDTA^{4-}$ anion

EDTA is a strong complexing agent with a relatively weak affinity for Ca and Mg ions, but a high affinity for Fe, Mn, Cu, Cd, and Zn ions (Knepper, 2003). The stability constants for the most important metal ions and other APC complexing agents are given in Table 2.4.

Table 2.4 Stability constants of 1:1 complexes of NTA, EDTA and [s,s]-EDDS with di- and trivalent metal ions determined for an ionic strength of 0.1M at a temperature of 25˚C, or where indicated (+); at 20°C.

Metal ion	Log K Me NTA	Log Me EDTA	Log K Me EDDS
Mg^{2+}	5.47	8.83	5.82
Ca^{2+}	6.39	10.61	4.23
Mn^{2+}	7.46	13.81	8.95 (+)
Zn^{2+}	10.66	16.44	13.49(+)
Co^{2+}	10.38	16.26	14.06
Cu^{2+}	12.94	18.7	18.36
Pb^{2+}	11.34	17.88	12.7 (+)
Cd^{2+}	9.78	16.36	10.8 (+)
Al^{3+}	11.4	16.5	
Fe^{2+}	8.33(+)	14.27	
Fe^{3+}	15.9	25	22.0(+)
Ni^{2+}	11.5	18.52	16.79

(Source: Bucheli-Witschel and Egli, 2001)

2. Speciation of EDTA metal complexes

Generally, the speciation of EDTA in ecosystems is predicted by equilibrium calculations based on the stability constants. Two major difficulties, however, affect the prediction of EDTA speciation under environmental conditions. One is that other natural ligands, including organic and inorganic compounds, compete

with EDTA for the metals, and the other difficulty is that the speciation calculation assumes that chemical equilibrium has been reached (Bucheli-Witschel and Egli, 2001). In the case of EDTA, true speciation in the natural environment may differ considerably from the calculated equilibrium due to the slow kinetics of some metal exchange reactions (Sillanpää et al., 2001).

In natural waters where a large excess of Ca^{2+} and Mg^{2+} exists, exchanging reactions of EDTA complexes with metals have been shown to occur at slow rates with a time range from hours to days. Especially Fe(III)EDTA was observed to exchange rather slowly with other metals in river water (Xue et al., 1995; Nowack and Sigg, 1996). Besides, the species of EDTA complex in river water depends not only on the dissolved concentrations of the various cations and other ligands which determine the equilibrium speciation, but also on the initial specification of EDTA released from the wastewater (Sillanpää et al., 2001).

The speciation pattern of EDTA in a river system can be determined from a combination of measurements and equilibrium calculations (Nowack et al., 1997; Bucheli-Witschel and Egli, 2001; Stefano et al., 2003). The following distribution was predicted by Nowack et al. (1997): 31% Fe(III)EDTA, 30% ZnEDTA, 15% MnEDTA, 12% CaEDTA, 10%NiEDTA, 2% PbEDTA and 0.5% CuEDTA. More recently, investigations, based on a combination of measurement and equilibrium calculations, concluded that ZnEDTA (51%), Fe(III)EDTA (32%), CaEDTA (7%), MnEDTA (5%), MgEDTA (2%), PbEDTA (2%), CuEDTA (0.8%), and NiEDTA (<0.01%) in the natural water (Nowack, 2002; Schmidt and Brauch, 2004).

Sillanpää et al. (2001) modelled and simulated the speciation of EDTA and DTPA for the pulp and paper mill effluent, and subsequently in receiving waters. The results revealed that the main species were Mn and Ca complexes of EDTA and DTPA in pulp mill processing water; Fe(III) and Mn complexes of EDTA and DTPA in the wastewater; and Fe(III) and Zn complexes of EDTA and DTPA in the receiving water.

2.4.2 Exchange Reactions of Metal Complexes and Metal Remobilization

The effect of EDTA on metal mobility depends upon the speciation of EDTA complexes under the conditions of natural waters, which predict the metal ions are likely to be transported together with EDTA under environmental conditions (Xue et al., 1995). Metal ion exchange reactions occur in river waters as the released EDTA complexes are usually different from those estimated at thermodynamic equilibrium. Remobilization of metals from sediments by EDTA would depend on the competition between EDTA in solution and binding of metals to particular materials. The following discussion outlines the metal exchange, adsorption, dissolution, and remobilization processes.

1. Metal exchange

The equilibrium speciation of a metal complex system is established under the concentrations of all metals and ligands, and the stability constants of all complexes. A new equilibrium will be reached, if another metal-ligand complex or metal ion is added to the solution, when the kinetic of the reaction is not considered. Also, the re-equilibration of a natural system undergoing perturbations of metals or ligands cannot be expected to be rapid without the detailed kinetics knowledge (Sillanpää et al., 2001; Nowack, 2002). For instance, the exchange reactions of Fe(III)EDTA are notably slow in the natural water even if it is the most important released EDTA species (20 – 90%) with a very high stability constant (LogK of 25) (Kari et al., 1996; Nowack et al., 1996a; Nowack et al., 1997). Nonetheless, the study proved that exchange reactions did happen under natural aquatic conditions with an excess of Ca rather than trace metals (Sillanpää, 2005).

Additionally, the same mechanism of metal exchange has been applied to remove other heavy metal complexes with EDTA from soils (e.g. Pb, Cu, Zn etc.) (Kim and Ong, 1999; Ridge and Sedlak, 2004).

2. Adsorption and dissolution

EDTA has been developed to solubilize metals and keep them in the solution for many applications. It is most likely that EDTA decreases heavy metal adsorption by forming dissolved complexes, even if this only happens with very high concentration of EDTA (Stumm, 1995). Conversely, EDTA could significantly increase metal adsorption onto mineral surfaces at low concentration as EDTA complexes themselves are adsorbed onto the surface, which is similar to a ligand-exchange reaction to form complexes in the solution (Nowack, 2002).

The speciation of EDTA significantly affects the adsorption of EDTA complexes, as do the structure of EDTA complexes and the environmental conditions. Adsorption of metal-EDTA complexes to iron and aluminium oxides has been studied by several authors (Xue et al., 1995; Nowack et al., 1996; Nowack and Sigg, 1996; Nowack, 2002). The EDTA chelates of divalent metals Ca, Zn, Ni, Cu, Co(II) and Pb all showed the same ligand-like adsorption behaviour. Pb (II)-EDTA was adsorbed even more strongly. For trivalent metals, the EDTA chelates of LaEDTA and BiEDTA were adsorbed very strongly over the entire pH range, while Co (III)-EDTA was weakly adsorbed at low pH. Fe (III)-EDTA was also weakly adsorbed over the whole pH range (Nowack and Sigg, 1996). A much stronger adsorption of Co (II)-EDTA onto δ-Al_2O_3 occurred than for Co (III)-EDTA, which was attributed to the differences in the stereochemistry of these chelates (Nowack, 2002).

The dissolution of mineral phases by EDTA could be attributed to ligand exchange reactions (Stumm, 1997). The metal–oxygen bonds on the surface are weakened upon adsorption of the ligand, and the release of metal species from the surface into the adjacent solution is enhanced. Nowack and Sigg (1997) investigated systematically the influence of complexing on the dissolution of iron oxides by EDTA chelates. Conversely, the dissolution of iron oxides by uncomplexed EDTA was studied extensively by Nowack (2002), who found the rate of the ligand-promoted dissolution was related to the concentration of surface bound ligands. He noted particularly that, a change in the oxidation state of the coordinated metal ions could completely change the behaviour of the metal and the chelates (Nowack, 2002; Fisher et al., 2004). For example, the oxidation of Co (II) EDTA to Co (III) EDTA, the stability constant of Co (III) EDTA (LogK =

39.8) increases by a factor of 10^{20} relative to Co (II) EDTA (LogK = 16.3). Therefore, Co (III) EDTA is extremely stable and rather mobile in aquatic systems because of its exhibiting only very weak interaction with surfaces.

3. Metal remobilization

Metals adsorbed onto a solid can be remobilized by EDTA chelates (Nowack et al., 1996a; Sillanpää et al., 1997, Sillanpää and Romo, 2001; Ceremigna et al., 2005). Consequently, EDTA has been used for many years as an extractant for the metals from soils and sediments to characterize the plant available fraction. EDTA chelates have also been proposed as enhancers for the phytoremediation of heavy metals by plants and soil washing (Hong and Jiang, 2005; Juang and Wang, 2000; Manouchehri and Bermond, 2006; Manouchehri et al., 2006).

Remobilization of metals from sediments by EDTA depends upon the competition between EDTA in solution and binding of metals to particular matters, mostly by complexing with surface ligands (Knepper, 2003; Di Palma and Mecozzi, 2007). For example, Eklund et al. (2002) added EDTA to pulp mill effluent in the laboratory and compared it to wastewater without EDTA. The results showed that EDTA markedly increased the solubility of Zn, Cd, Pb, Fe, Ni and Cu, whereas the solubility of V, Mo, Tl, As, and Cr was not changed by the presence of EDTA.

High concentration of EDTA has the potential to significantly remobilize heavy metals from river sediments. Interestingly, the high concentration of EDTA does not automatically coincide with a high remobilization of heavy metals as the initial released speciation of EDTA is likely to be playing a key role that influences its potential to remobilize heavy metals (Kari and Giger, 1996). The importance of speciation of EDTA for the evaluation of remobilization process was also demonstrated by Nowack et al. (2001), who investigated the remobilization of metals from the surface of synthetic iron oxides and from river sediment by different EDTA chelates. They observed that (i) the order of the remobilization rate of Zn^{2+} from goethite was CaEDTA > Fe(III)EDTA, reflecting the slow exchange reaction of Fe(III)EDTA; (ii) the order of the remobilization rate of Pb^{2+} from goethite was found as Fe(III)EDTA > CaEDTA > ZnEDTA,

Fe(III) EDTA surprisingly illustrating the fastest exchange rate; and (iii) the remobilization rate of Zn^{2+} from a natural river sediment was CaEDTA > CuEDTA > Fe(III)EDTA.

In summary, the concentration of EDTA in solution and the initial released speciation of EDTA chelates are the key factors to evaluate the effect of EDTA on the remobilization of heavy metals from river sediments and treated sludge. Metal concentration, pH, nature of the sediment, and the interactions between EDTA chelates and metals also affect the remobilization process. It can be concluded that significant remobilisation processes are only likely to occur in extreme cases. For instance, high amount of EDTA release leads to an increase of metals with the high conditional complex-forming constants. There is no general rule to apply to all surface waters for the effect of EDTA on the heavy metal remobilization process.

2.4.3 Minimisation of EDTA Use and Substitutes

On a global scale EDTA is the most frequently used complexing agent in a variety of industrial applications, largely because it is the cheapest and most suitable complexing compound for many technical purposes (Nowack et al., 1997; Friedly et al., 2002). On the other hand, EDTA can be included in the group of polar persistent pollutants as it is not, or only slowly biodegradable. Consequently, the use of EDTA and its risk need to be minimised whenever possible (Sillanpää, 1997; Oviedo and Rodríguez, 2003; Fuerhacker et al., 2003; Knepper, 2003; Grundler et al., 2005). In order to limit the EDTA risk, efforts have been undertaken to reduce EDTA emission by substitution and changes in technical processes since the late 1980s (Conrad 2000).

Apart from EDTA, the most useful chelating agents are the aminopolycarboxycarboxylate group (Nowack and VanBriesen, 2005) including nitrilotriacetic acid (NTA), ethylene diamine disuccinate (EDDS) and diethylene triamine pentaacetic acid (DTPA) (Sillanpää, 1997; Fuerhacker, 2003; Cokesa Et al., 2004; Schmidt et al., 2004).

1. NTA

NTA contains four donor atoms and is a so-called quadridentate chelating ligand (Fig. 2.2). It forms 1:1 complexes with metal ions, and the stability of the NTA complex is several orders of magnitude lower than that of its EDTA complex, as can be expected from the lower chelating capacity of NTA (Table 2.4).

NTA is mainly eliminated in biological steps such as oxidation ponds and lagoons, activated sludge systems, or trickling filters (Alder et al., 1990; Kari and Giger, 1996; Egli, 2001; Nörtemann, 2005). For elimination efficiencies for NTA of different wastewater treatment plants was suggested over 90% and recommended that EDTA should be substituted by NTA wherever possible (Kari and Giger 1996; Madsen et al.; 2001). According to numerous laboratory studies, NTA is biologically degraded in fresh water (Egli, 2001). However, contradictory results have been reported on its degradation in marine and estuarine water samples (Hunter et al. 1986).

NTA is recommended as a substitute of EDTA within reasonable limits by the UBA (Umweltbundesamt, i.e. the German Federal Environmental Agency), whereas NTA use is not recommended or even restricted to special application in some other countries because of the assumed but not finally proven potential carcinogenicity (Conrad, 2000; Knepper, 2003). NTA is likely to have negative effects on heavy metal removal during wastewater treatment and on the mobilisation of metals from sediments in receiving waters (Perry et al., 1984; Madsen et al.; 2001).

2. EDDS

EDDS (Ethylene Diamine Di-succinic Acid) is a structural isomer of EDTA and a well biodegradable complexing agent with low toxicity. The field of possible application is therefore restricted due to the relatively low stability of their metal complexes (Table 2.4) (Nörtemann, 2005).

In 1999, Jaworska et al. completed an environmental risk assessment of [S, S]-EDDS (thereafter as EDDS) for a new, biodegradable, strong transition metal chelator. They concluded that completed biodegradation of EDDS was observed in all environmental matrices; and the predicted environmental concentration was around 1μg/L, which was well below the concentrations causing adverse effects towards the ecosystem. EDDS was evidently safe at the anticipated usage volumes. In some applications EDDS was suggested to replace the more poorly degradable EDTA (Schowanek et al., 1997; Bucheli-Witschel and Egli, 2001; Vandevivere et al., 2001; Tandy et al., 2006a). Furthermore, EDDS was suggested as a promising approach to reduce heavy metal contents in soils (Tandy et al., 2004; Hauser et al., 2005; Luo et al., 2005; Meers et al., 2005a; Tandy et al., 2006b & c). Hence, EDDS seems to be an appropriate substitute for EDTA with many areas of application, except it is much more expensive (Conrad, 2000).

3. DTPA

The biodegradation of DTPA has been shown to not occur, or only at extremely slow rates (Means et al., 1980; Hink et al., 1997; Alarcón et al., 2005) similar to EDTA. Regarding the application of DTPA, Conrad (2000) stated that the environmental problems of EDTA had not really been solved, but only shifted to another complexing agent.

4. Other APCs Chelating Agents

Apart from the above compounds, a few other chelating agents of amino polycarboxylates (APCs) could also be used as a substitute for EDTA. For instance, 1, 3-propylene diamine penta-acetic acid (PDTA) and β-alanine diacetic acid (β-ADA) are being used as oxidizing agents and as substitutes for EDTA, and methyl glycine diacetic acid (MGDA) (as well as NTA) are suggested as a replacement for the predominantly used EDTA in the dairy industry (Knepper, 2003).

2.5 ENVIRONMENTAL IMPACT OF EDTA

EDTA has been selected by the European Union authorities as one of the priority substances for extensive evaluation due to environmental concerns (European Commission, 2003). The extensive Technical Guidance Document (TGD) of the European Union was followed by the European risk assessment process. A comprehensive risk assessment of EDTA, which includes a critical review and discussion of about 250 validated references, was completed in 2004 (European Chemicals Bureau, 2004).

2.5.1 Risk of EDTA in the Natural Environment

1. Atmosphere

There is no concern about possible EDTA emission into the atmosphere because of its relatively low toxicity and physical properties. Likewise a risk to terrestrial organisms is not expected (European Chemicals Bureau, 2004; Grundler et al., 2005).

2. Aqueous environment

In the aqueous environment, the risk of EDTA depends upon the speciation of EDTA complexes occurring. The potential risk of EDTA to the aqueous environment is generally assessed by comparing the predicted no-effect concentration (PNEC) with the predicted environmental concentration (PEC) for the discharge from identified sources. A potential risk is indicated to the aqueous environment when the ratio of PEC/PNEC is >1 for an emission site (European Chemicals Bureau, 2004).

Toxicity

In the Risk Assessment Report of EDTA, relevant short-term and long-term ecotoxicity tests were evaluated (European Chemicals Bureau, 2004). Valid long-term tests are available on three different species: Fish, Daphnia and Algae (Sorvari and Sillanpää, 1996; Sillanpää and Oikari, 1996). Daphnia appeared to be the most sensitive species with 21-day NOEC (long-term no effect concentration) of 25 mg/L of Na_2H_2-EDTA which is comparable to 22 mg/L of H_4-EDTA (European Chemicals Bureau, 2004). The PNEC of 2.2 mg/L for EDTA in the aqueous environment was then determined as an assessment factor of 10 (Grundler et al., 2005).

In terms of the potential risk of EDTA, an extreme case is likely to occur in relatively small surface waterways experiencing large amounts of EDTA release. Thus, EDTA may pose a risk to the local aqueous environment in a situation where EDTA is used as an industrial cleaning agent to prevent precipitation of calcium, magnesium and heavy metals at large dairy and beverage plants. Grundler et al. (2005) reported that a high EDTA consumption of 10 tonnes per annum with no effective EDTA removal in the wastewater treatment plants (WWTPs) led to a PEC of 2.6 mg/L in the receiving surface water. Consequently, an appropriate risk reduction measure should be considered to prevent any potential risk (PEC >2.2 mg/L) for the local aqueous environment (European Chemicals Bureau, 2004).

Bioaccumulation

A highly polar, water soluble compounds such as EDTA would not be expected to bioaccumulate by partitioning into the lipid component of aquatic organisms (Madsen et al., 2001; Knepper, 2003). EDTA and other aminopolycarboxylates (APCs) will not, therefore, bioaccumulate in the aquatic food chain, which is supported by the fact that the concentration of EDTA in fish is no higher than in the surrounding water (Wolf and Gilbert, 1992; European Chemicals Bureau, 2004; Schmidt and Brauch, 2004).

Stimulation of Algal Growth

The stimulating effects of EDTA on the growth of algae and other organisms under laboratory conditions are well documented (Wolf and Gilbert, 1992; Eklund et al., 2002; Oviedo and Rodríguez, 2003). This phenomenon could be relevant, since the EDTA molecule contains approximately 10 % of nitrogen that could eventually be available to the aquatic microbiota. EDTA could also have an indirect effect when the exchange reactions of metal complexes occur in surface waters (Wolf and Gilbert, 1992; Sillanpää, 1997; Nirel et al., 1998). For instance, EDTA redissolves the calcic and ferric phosphates, releasing phosphorous or Fe^{3+} to stimulate the algal growth.

Based on the above discussion, EDTA may theoretically extract trace levels of essential metals from sludge and humic acids to make them more available for algae and other plants. However, the stimulation effect of EDTA appears to be negligible as the levels of EDTA and metals in surface waters are too low to make the nutrients available for the algal growth.

Heavy metal mobilisation

EDTA is able to mobilise heavy metals in the environment that is influenced by a series of factors. However, it appears that no conclusive experimental evidence of heavy metal mobilisation potential of EDTA at environmentally realistic concentrations is available (Knepper, 2003). This phenomenon can only occur in extreme cases of the high local concentration of EDTA (European Chemicals Bureau, 2004).

In summary, there appears to be little risk to the aqueous environment due to the influence of EDTA on the mobility of heavy metals, eutrophication and nutrient deficiency.

2.5.2 Mammalian Toxicology and Human Health of EDTA

In the aquatic environment, a large stoichiometric excess of calcium ions is likely to exist. The complex of CaEDTA should therefore be used for the safety

assessment. The occurrence of EDTA in drinking water is mainly via the oral route, though washing will also involve some dermal exposure.

1. Acute and long term toxicity

The published data indicate that conversion from the tetrasodium salt to the calcium disodium salt greatly reduces toxicity. In different studies after oral and dermal application, EDTA was evaluated to be no concern (Wolf and Gilbert, 1992; Grundler et al., 2005).

2. Teratology

Where studies involving EDTA have caused congenital abnormalities, there is a hypothesis that it is caused by the EDTA-induced zinc deficiency (Wolf and Gilbert, 1992). From a study on subcutaneous administration of CaEDTA, ZnEDTA and a mixture of the two to rats, it was concluded that CaEDTA is teratogenic in rats, and protection is afforded by incorporation of zinc in the chelate (European Chemicals Bureau, 2004).

3. Carcinogenicity

Based on the bioassay of Na_3EDTA for possible carcinogenicity on rats and mice, there was no specific data on kidney toxicity, and no tumours related to the treatment in either species. Thus, there is no concern in terms of carcinogenic potential of EDTA (European Chemicals Bureau, 2004; Grundler et al., 2005).

4. Acceptable daily intake for humans

Reflecting EDTA's low toxicity to humans, EDTA is permitted as an additive to a range of foodstuffs in the United States (U.S. Code of Federal Regulations, 2006), the Netherlands, the United Kingdom and Denmark (Wolf and Gilbert, 1992; Yuan and Van-Briesen, 1997).

The level causing no toxicological effect in rats was suggested as 5000 ppm in the diet, equivalent to 250 mg/kg. Based on this, the WHO level of acceptable daily intake for human is 0 to 2.5 mg/kg calculated as $CaNa_2EDTA$ (1/100 of no-effect) (Wolf and Gilbert, 1992). The levels of EDTA in rivers used for the preparation of drinking water are generally below 25 μg/L, and therefore pose no risk to human health. (European Chemicals Bureau, 2004)

Heimbach et al. (2000) reported a safety assessment of iron EDTA (sodium iron (III) ethylenediaminetetraacetic acid), including toxicological, fortification and exposure data. The data over the past 20 to 30 years has demonstrated that iron EDTA was safe and effective for iron fortification of food products and met the standards of "reasonable certainty of no harm".

2.6 METALS AND EDTA IN SOILS

2.6.1 Mineral and Trace Element of Milk and Dairy Products

The effluent from the dairy industry originates from the manufacturing process, utilities and services. The various sources generated from a dairy processing are spilled milk, spoiled milk, skimmed milk, whey, and wash water from milk equipment and floor washing (Bylund, 1995; Rajeshwari et al., 2000; Britz et al., 2004).

The minor and trace element contents in milk and dairy products have been well documented (Coni et al., 1994, 1995 and 1996; Lindmark-Månsson et al., 2003; García et al., 2006). The levels of trace elements are correlated with animal feeding, year period of sample collection, environmental conditions and manufacturing processes (Coni et al. 1995; García et al. 2006). Selected trace elements were analyzed by Coni et al. (1996) in sheep and goats milk as well as for typical cheese. Also, concentration of major (Ca, Mg, P, K, Na and S) and trace (Fe, Mn, Cu, Zn and Al) in commercial goat fluid milk, evaporated, powdered, yogurt, and cheese products manufactured in the US were evaluated for

compositional differences by Park (2000). Lindmark-Månsson et al. (2003) chose Na, K, Cl, Ca, Mg, P, Fe, Cu, Zn, Mn, Cr and I as the minerals and trace elements to investigate the composition of Swedish dairy milk affected by geographic and seasonal changes. Lante et al. (2006) also contributed to the study of the characterisation of the mineral profile of dairy products (Crescenza and Sequacquerone cheeses), together with the corresponding milk. Macroelements (Ca, P, Na, Mg, K and S) and microelements (Al, Fe, Cu, Zn, Pb, Se and Cd) were analysed by inductively coupled plasma optical emission spectrometer (ICP-OES).

In summary, the minerals and trace metals of dairy milk could differ due to the diet, seasonal and geographic changes, and generally they are Ca, Mg, K, Na, Fe, Zn, Al, Cu, Mn, Cd and Se.

2.6.2 Metals in Soil

The use of wastewater for irrigation of agricultural land is a world-wide practice, which offers an economic alternative to disposal into surface waters and it contributes to nutrient cycling (Haruvy et al., 1999; Friedel et al., 2000; Angin et al., 2005). Furthermore, long term irrigation can induce changes in the quality of soil, especially as trace element inputs are sustained over long periods and may lead to the risk of groundwater contamination (Stuart and Milne, 2001; Silveira et al., 2003; Sinha et al., 2006).

Where wastewater is being used for the irrigation, there are various reports that describe the resulting effects on the physical and chemical properties of the soils (Shahalam et al., 1998; Friedel et al., 2000; Zhou et al., 2003; Angin et al., 2005; Dawes and Goonetilleke, 2006), plant or crop growing (Shahalam et al., 1998; Angin et al., 2005; Sinha et al., 2006), environment (Shahalam et al., 1998) and even birds (Laposata and Dunson, 2000). The study of Angin et al. (2005) suggested that the major disadvantage of the wastewater irrigation was the accumulation of immobile heavy metals in soils.

Effluent from milk processing to dairy products by dairy factories is commonly irrigated onto land in New Zealand (Degens et al., 2000; Sparling et al., 2001). There are not many studies about heavy metals in soil relevant to the dairy effluent. However, the suitability of dairy plant sludge for fertilisation of Cambisolic soil (soils that are either young or, depending on the climate, processes of soil formation occur slowly.) was investigated in northwest Spain by López-Mosquera et al. (2000). Soil properties (pH, electrical conductivity, organic matter content, N P, Ca, Mg, Na, K and Al) and soil and plant tissue heavy metal contents (Hg, Pb, Cd, Cu, Zn, Ni, and Cr) were determined in 12 grassland plots fertilised over a 1-4 year period with dairy sludge and conventional fertiliser (cattle slurry and mineral fertilisers), and in six meadows fertilised with conventional fertilisers only. Heavy metal contents were also determined in plant tissues from different plots. This study suggested that (i) application of dairy-plant sludge to grassland on a Cambisolic soil did not lead to harmful accumulation of heavy metals in the short- or medium-term (4 years). (However, the dairy sludge was a source of heavy metals for the soil); and (ii) long-term sludge application would eventually lead to a build-up of heavy metals in soils and plants.

2.6.3 EDTA and Soil Remediation

Metal contamination of soils by natural processes or human activities is one of the most serious ecotoxicological problems across the globe. Heavy metals may be adsorbed via specific or non-specific adsorption reactions in soils (Sauvé et al., 2000; Mulligan et al., 2001; Luo et al., 2003; Silveira et al.; 2003; Tandy et al. 2004). Ion oxides and organic matter are the most important soil constituents retaining heavy metal (Li and Shuman, 1996; Mulligan et al., 2001; Gyliené et al., 2004; Lim et al., 2005).

EDTA extraction

Heavy metals including lead, chromium, arsenic, zinc, cadmium, copper and mercury can cause significant damage to the environment and human health. The cleanup of the soils contaminated with the heavy metals has been one of the most difficult tasks for environmental engineering (Sun et al. 2001). Manouchehri et al.

(2006) studied the thermodynamic equilibrium (in 24 hours) and kinetics about major and trace metal extraction from soil by EDTA. The study of Hong and Jiang (2005) highlighted several factors relevant to selecting suitable chelating agents, including the chelating agent strength and selectiveness toward the target metal as well as its viability of recovery and reuse.

Various in-situ and ex-situ remediation techniques have been employed (Manouchehri and Bermond, 2006). A particularly promising technique is ex-situ soil washing with chelating agents (Peters, 1999; Juang et al., 2000; Gyliené et al., 2004; Lim et al., 2005). The advantage of the method is the high potential extraction efficiency and the specificity for heavy metals with low cost.

Due to the similarities between soil and sediment mineralogy, it can be applied with minor modification to contaminated sediment remediation (Ceremigna et al. 2005; Di Palma and Mecozzi, 2007).

EDTA for Phytoextraction

Besides the extraction of chelating agents, phytoextraction has been used as an alternative remediation technology for soils contaminated with heavy metals. Natural phytoextraction is generally conceived as too slow working. Subsequently, chemically enhanced phytoextraction has been developed to accelerate this processing (Afyuni and Rezaeinejad, 2006; Lombi et al., 2001; Madyiwa et al., 2003; do Nascimento et al., 2006). Several chelating agents, such as EDTA, have been studied for their ability to mobilize metals in soils and increase metal accumulation in plants (Wong et al., 2004; Meers et al., 2005a and 2005b; Ruley et al. 2006). This approach makes use of high-biomass crops that are induced to take up large amounts of metals when their mobility in soil is enhanced by chemical treatments, like EDTA.

Despite the success of this remediation technology, some concerns have been shown about the enhanced mobility of metals in soil and their potential risk of leaching to ground water (Cooper et al., 1999; Tandy et al., 2006a). Also a strategy for managing leaching losses needs to be considered as a part of EDTA-

enhanced phytoremediation plan (Thayalakumaran et al., 2003). Uncustomized additions of chelates may result in unsuccessful phytoremediation, and subsequently cause negative effects on the eco-environment (Song et al., 2005).

2.7 IMPLICATION OF EDTA IN THE DAIRY INDUSTRY

EDTA has been used as an additive to caustic (usually sodium hydroxide) cleaning solutions to facilitate removal of Ca, Mg and minerals during the cleaning of dairy industry processing plants (Wolf and Gilbert, 1992; European Chemicals Bureau, 2004; Schmidt et al., 2004). Significant amounts of EDTA have been applied in the dairy industry, in particular during the high milk production season, and due to the frequent specification change of milk products. The literature indicates that significant use of EDTA leads to high EDTA concentrations in the plant wastewater (Kari and Giger, 1996; European Chemicals Bureau, 2004). Subsequent discharges of the effluent is likely to cause a high local concentration of EDTA in receiving waters, and causes further environmental issues relating to heavy metals (Oviedo and Rodriguez, 2003; Nowack and Van Briesen, 2005). In particular, the effluents from a large dairy factory (Figure 2.6) discharged to a relatively small stream have the potential to pose a risk to aquatic environments.

Figure 2.6 A large-scale dairy factory of New Zealand in a rural setting.

The minerals and trace metals in milk and dairy products have been well documented as Ca, Mg, K, Na, Fe, Zn, Al, Cu, Mn and Cd (Lindmark-Månsson et al., 2003; García, 2006). EDTA complexes not only with Ca and Mg, but also with trace metals of milk and added minerals in the dairy industry.

For dairy factories with their own wastewater treatment plants, EDTA can be significantly removed under favourable conditions. This process is evidently dependant on the speciation of EDTA and wastewater treatment procedure (Bucheli-Witschel and Egli, 2001; Sillanpää and Pirkanniemi, 2001; Van Ginkel and Geerts, 2005). The study of Van Ginkel and Geerts (2005) for dairy effluents revealed that the elevated pH value evidently accelerated the degradation of EDTA during the wastewater treatment processing. In terms of the elevated pH accelerating EDTA degradation, it could possibly be through the mechanism of biological oxidation. The other reasonable explanation for the phenomena could be the transition of EDTA-metal complexes as the alkalinity increases, in particular for the most stable Fe(III)-EDTA and alkaline earth metal complexes. Alkaline earth metals can therefore compete successfully with trace metals to form EDTA complexes that are more biodegradable (van Ginkel et al., 1997). In addition to pH, sludge retention time seemed to have a significant impact on EDTA chelate removal from dairy effluents (Sillanpää and Pirkanniemi, 2001; Van Ginkel and Geerts, 2005). The relatively high sludge retention time required for the degradation of EDTA could be due to the slow reaction rate.

Study of land treatment systems for dairy effluents indicates that the major disadvantage of the wastewater irrigation is the accumulation of metals, particularly sodium, in soils. Long term irrigation can induce changes in the quality of soil. Complexation and mobilization of divalent metals such as Ca and Mg by EDTA may further degrade soil structure. Sustained inputs of trace elements may induce a risk to ground water due to EDTA chelates (Haruvy et al., 1999; Friedel et al., 2000). There are not many studies about heavy metals in soil in relation to the dairy effluents. However, the study of López-Mosquera et al. (2000) suggested the dairy sludge was a source of heavy metals for the soils.

2.8 CONCLUSIONS

(i) EDTA is the most powerful and widely used chelating agent to complex undesirable cations in many industrial areas. Almost all applications of

EDTA are conducted in aqueous media and it is subsequently released into the environment through wastewater. Hence EDTA is likely to be present in the highest concentration of all anthropogenic compounds in many surface waters, and also has been detected in drinking water and groundwater.

(ii) EDTA generally has a low toxic impact for both humans and natural environments. However, there are some concerns about its poor biodegradation in conventional wastewater treatment plants and natural environments, and its remobilisation of heavy metals. As a chelating agent EDTA was once considered as a stable and almost non-degradable compound. In the light of later findings, it is evident that EDTA can be removed by favourable treatment processes, including biodegradation, photodegradation of Fe(III)EDTA and advanced oxidation treatment processes. Contradictory results have been published concerning EDTA degradation in soil and sediments.

(iii) Chemical species plays a key role in the behaviour of EDTA and influences its fate in the environment. One of the main concerns about using EDTA is the significant remobilisation of heavy metals. However, this is only likely to occur in rare cases where high concentrations of EDTA, heavy metals, and unfavourable environmental conditions are present.

(iv) In the New Zealand dairy industry, EDTA has been used as an additive into caustic cleaning solutions to facilitate the removal of Ca, Mg and minerals during the cleaning process. Significant use of EDTA may lead to high concentrations in wastewater from processing plants', subsequent discharges of effluent is likely to cause a high concentration of EDTA in local receiving water, and to create further environmental issues relating to heavy metals. This is the first investigation into the effects of EDTA in New Zealand.

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3.0 CHAPTER THREE: METHODOLOGY FOR MEASURING EDTA

Measuring EDTA is a critical component of this research. To date there is no international standard method available. In this chapter, relevant analytical techniques to quantify EDTA in multi-media samples are reviewed, and an HPLC-UV analytical method developed that is based on the nature of dairy wastewater and potential interference. A paper titled 'Determination of EDTA in dairy wastewater and adjacent surface waters' by Xie, C. Z.; Healy, T.; Robinson, P.; Stewart, K. has been peer-reviewed and published in P*roceedings of World Academy of Science, Engineering and Technology (WASET),*Vol. 34, pp. 50-54, Oct. 2008.

3.1 OVERVIEW OF PRESENT ANALYTICAL TECHNIQUES FOR EDTA

A number of techniques exist for the analytical determination of EDTA. Relevant assays include gas and liquid chromatography, capillary electrophoresis, and the recently developed method of ion chromatography. The method to be selected depends upon the individual problem.

Most of the methods currently employed for the analysis of mixtures containing EDTA are based on gas chromatography (GC) or high performance liquid chromatography (HPLC).

3.1.1 Gas Chromatography

The high sensitivity of GC enables low level determinations of EDTA to be detected. The EDTA must firstly be isolated from its aqueous matrix in a pre-concentration step. This is generally by ion exchange- solid phase extraction.

After elutriation, the EDTA is derivatised to produce a volatile species for GC determination.

The sample preparation of EDTA involves their conversion to readily volatile alkyl ester derivatives, like methyl, ethyl or 1-propyl (Lee et al., 1996; Sillanpää et al., 1998). Detailed studies have demonstrated that often the derivation to propyl or butyl esters yields better results than derivatization to methyl esters (Wolf and Gilbert, 1992; Lee et al., 1996; Schmidt and Brauch, 2005). The derivatization step makes sample preparation tedious and time-consuming. Particularly, when EDTA exists as metal complexes, their derivatization is more difficult as metal complexes must be decomposed prior to derivatization by decreasing the pH value and causing a conversion of EDTA complexes into their free acid forms. GC methods are therefore not suitable for the determination of individual EDTA-metal species and determine only the integral content (Sillanpää, 1996).

In most cases, derivatization is preceded by a concentration step to increase the sensitivity of the overall process. In principle, two approaches exist:
(i). concentration of EDTA as anion on an anion exchanger (e. g. SAX) at pH 2-3, elution with formic acid and evaporation of the elute at 100-110^{0}C to dryness (Sillanpää et al., 1996; Lee et al., 1996; Schmidt and Brauch, 2005); and
(ii). simple evaporation of the acidified aqueous sample to dryness at 100-115^{0}C (Nishikawa and Okumuta, 1995; Fuerhacker et al., 2003).

More detailed studies have demonstrated that simple evaporation yields better results than the first approach. Both approaches require the complete removal of water traces for the following esterification. After derivatization followed by water addition, the resulting EDTA acid alkyl esters can be extracted from the aqueous phase and purified by liquid-liquid extraction with an organic solvent such as hexane (Fuerhacker et al., 2003), toluene (Sillanpää et al., 1998) or methylene chloride (Schmidt and Brauch, 2005).

While detection in earlier studies often involved the use of flame ionization detectors (FID) (Wolf and Gilbert, 1992; Fuerhacker et al., 2003), they have been

almost completely replaced in current studies by mass-selective (MSD) (Lee et al., 1996) or nitrogen-sensitive (NPD) detectors (Lee et al., 1996; Sillanpää et al., 1996 and 1998; Fuerhacker et al., 2003) that have become state-of-the-art due to their increased sensitivity. In the case of GC-MSD or GC-NPD detection, the detection limits are usually in the low μg/L range. Most published applications involved the analysis of all types of aqueous solutions (drinking water, surface water, ground water and wastewater), but also of food, sediments and fish (Schmidt and Brauch, 2005). In 1995, Nishikawa and Okumura treated their samples with a boron trifluoride-methanol mixture, after evaporation to dryness. The resulting methyl ester derivatives were determined by capillary GC-MS (Mass Spectrometry) with selective-ion monitoring. EDTA could be determined in the ranges 3.9-11.8 ng/ml in water.

The advantage of the GC method is high sensitivity which enables the detection of low concentrations of EDTA. The disadvantage is the time-consuming and labour intensive sample preparation. Consequently, the liquid chromatography method was developed to resolve this.

3.1.2 High Performance Liquid Chromatography (HPLC)

EDTA has been determined in wide variety of sample matrices by liquid chromatography (LC). This includes drinking water, surface water (Bedsworth and Sedlak, 1999 and 2001; Loyaux-Lawniczak et al., 1999), ground water (Ammann, 2002), wastewater (Sillanpää et al., 1995; Sillanpää, 1996; Nirel et al., 1998; Dodi and Monnier, 2004), medical products (Lin et al., 2003), sediments and soils (Nowack et al., 1996), fertilizers and micronutrients (Hernandez-Apaolaza, 1997) and others (Cagnasso et al, 2007). In recent years, high performance liquid chromatography (HPLC) has represented the most common approach for the determination of EDTA complex and trace metal separations. Compared to GC, it offers the major benefits that no extraction of the aqueous samples is required, and that no derivatization of the analytes for increase

volatility is necessary. Basically, the sample can be directly applied to the separating column.

The LC separation of EDTA is performed either on reversed-phased (RP) columns (Sillanpää et al., 1995; Nowack et al., 1996; Yuan and VanBriesen, 1997; Lin et al., 2003; Dodi and Monnier, 2004; Schmidt and Brauch, 2005) or by ion chromatography (IC) on an anion exchange column (Nirel et al., 1998; Bedsworth and Sedlak, 1999). The reversed-phase technique is usually based on ion-pair chromatography (IPC) that ion-pair reagent is added to the mobile phase to convert the target compounds into neutral components.

Detection is normally performed with a UV detector. For this purpose, by addition of excess of metal ions prior to, during, or after the chromatographic run (Schmidt and Brauch, 2005), the EDTA to be analysed is converted into a highly stable defined metal complex with favourable UV absorption characteristics. Most frequently Fe(III) (Nowack et al., 1996; Nirel et al., 1998; Bedsworth and Sedlak, 1999) and Cu(II) (Yuan and VanBriesen, 1997; Lin et al., 2003; Metsärinne et al., 2005) are used. It is also claimed that the methods using the Cu(II) EDTA complex are superior because the Fe(III) EDTA complex is photochemically unstable (Wolf and Gilbert, 1992). In addition to UV detection, some studies were performed using mass-spectrometric detection (Dodi and Monnier, 2004), fluorescence (Yuan and VanBriesen, 1997), electrochemical and also ICP-MS coupling (Ammann, 2002; Schmidt and Brauch, 2005).

The typical detection limits of LC are in the low mg/L range (Wolf and Gilbert, 1992; Schmidt and Brauch, 2005). Without the enrichment step, it is unlikely that LC is suitable for natural waters in which the concentrations are expected to be below detection limits. Examples of typical chromatograms of both GC and HPLC analysis are presented in Figure 3.1. LC method has been used as a standard method for determination of nitrilotriacetric acid (NTA), ethylenedinitrilotetraacetic acid (EDTA) and diethylenetrinitrilopentaacetic acid (DTPA) in water, wastewater and sludge in Germany (DIN 38413-8, 2000), in which clearly demonstrated that the major problem related to the liquid-

chromatographic determination of aminopolycarboxylic acid was poor sensitivity with working range of 0.1-20 mg/L.

Standard approaches to increase sensitivity include sample pre-concentration by evaporation, large injection volumes and the use of sensitive detectors. Some methods taking these aspects into account are managing to achieve lower detection limits down to 1 μg/L (Sillanpää and Sihvonen, 1997; Ammann, 2002; Dodi and Monnier, 2004; Quintana and Reemtsma, 2007).

Interestingly, the speciation study of metal complexes has received attention, which is of importance for estimating their environmental fate and ecotoxicological effects (Sillanpää, 1997). The HPLC method was described by Bedsworth and Sedlak (2001) for determination of the Cd(II), Co(II), Cu(II), Pb(II), and Zn(II) complexes of EDTA in municipal wastewaters and surface waters. The method involved separation by ion-exchange chromatography on a reversed-phase C_{18} column coated with ion-pair reagent, followed by post-column conversion to $FeEDTA^-$ and subsequent detection by UV absorbance. The method detection limit was $6\text{-}8x10^{-8}$ M (5-7ng) EDTA. Currently, a promising method for the determination of EDTA speciation at trace level has been published. It is based on the coupling of ion chromatography with ICP-MS involving on-line sample enrichment via column switching. This method allows the determination of nM levels of EDTA metal complexes in surface waters (Ammann, 2005). However, some relatively simple means are available for the determination of $Ni(II)\text{-}EDTA^-$ and Fe(III)EDTA species in water samples. The Fe(III) portion of the total EDTA speciation can be determined by illumination of the water sample, resulting in the complete photochemical decomposition of Fe(III)EDTA (Nowack et al., 1996).

HPLC: 1.2 mg/L EDTA **GC: 0.72 mg/L**

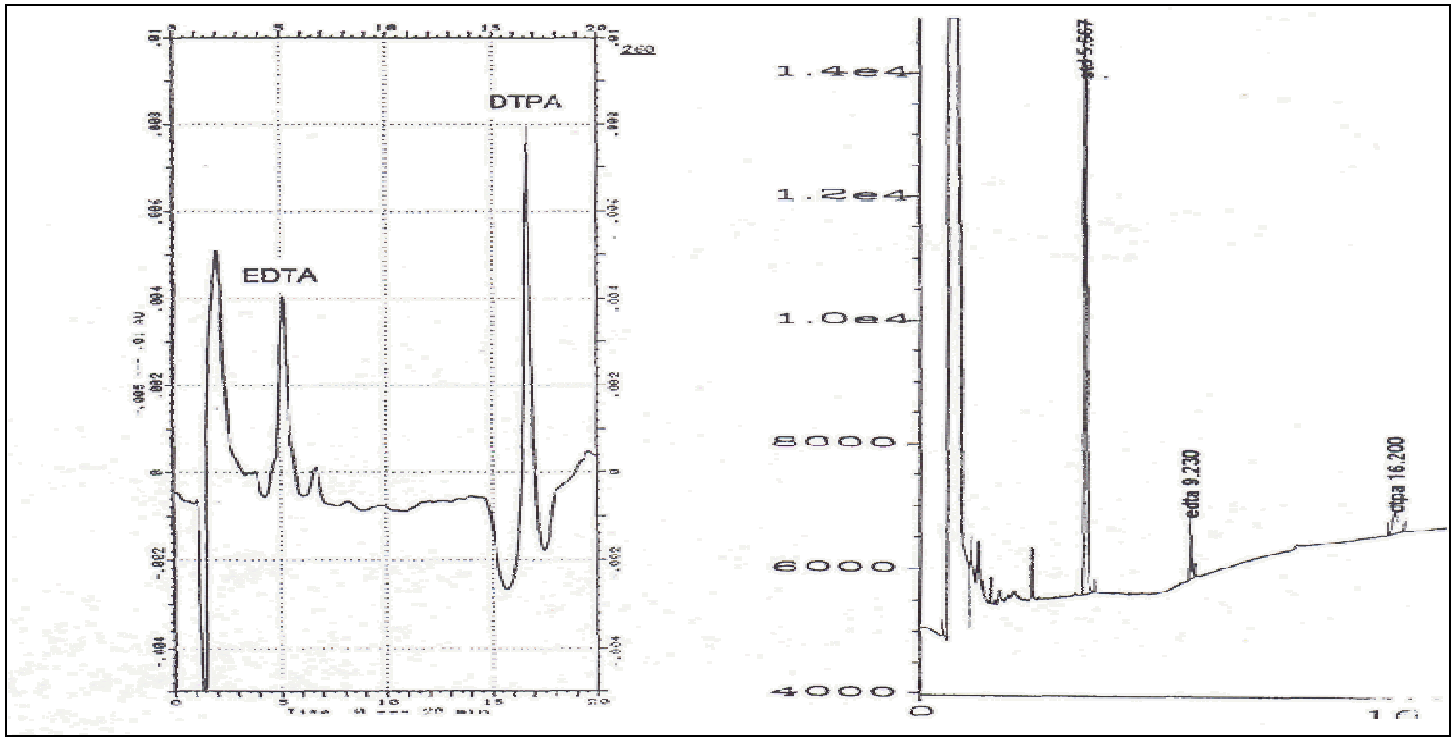

Figure 3.1 Typical chromatograms of both Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC) analysis

(Source: Sillanpää, 1996)

3.1.3 Capillary Electrophoresis

In recent years, capillary electrophoresis (CE) has evolved to an alternative separation technique besides HPLC allowing the determination of the speciation of complexing agents (Pozdniakova et al. 1999; Owens et al., 2000; Schmidt and Brauch, 2005). CE is a highly efficient separation technique that has been used for the analysis of inorganic and organic ions (Soga and Imaizumi, 2001; Brooks 2005). The principle of CE is based on the different migration speeds of electrically charged particles in an electric field (Wang and Li, 1995; Timerbaev et al., 2002); its particular benefit is its high separation efficiency at short analysis times (Baraj et al, 1995; Blatný et al, 1997; Padarauskas and Schwedt, 1997). Complexing agents are traditionally used in particular as an electrolyte additive for the modification of the ion mobility of the metal cations (Haumann and Bachmann, 1995; Conradi et al, 1996; Bürgisser and Stone, 1997; Fukushi et al., 1997). The first direct assay has been published by Òkeefe et al. in 1995. This method has proved useful for the direct determination of organic chelates and their metal complexes (Brooks, 2005). So far, the analysis of free EDTA and their metal complexes has been predominantly performed by capillary zone electrophoresis (CZE) techniques (Padarauskas and Sahwedt, 1997), but also the application of micellar electrokinetic capillary chromatography (MEKC) has been described (Harvey, 1996).

Electrophoretic assays for EDTA include all possible variations like the free acids (Zhang et al, 2005), the determination following conversion of all existing complex species into a single defined metal complex and also the differentiated determination of individual metal complex species (Conradi et al., 1996; Fukushi et al., 1997). Published application ranges include drugs (Pálmaesdóttir and Edholm, 1995), wastewater (Baraj et al., 1995), plating baths solutions and cosmetic products (Padarauskas and Sahwedt, 1997; Katata et al., 2006), surface water (Blatný et al., 1997), radioactive waste solution (Bürgisser and Stone, 1997), human plasma and urine (Sheppard and Henion, 1997), mayonnaise, and Vegetables (Fukushi et al., 1997), and drinking water (Zhang et al., 2005).
Compared with GC, LC, CE has the advantages of higher efficiency, simpler chemistry, faster separation time, ease of automation, smaller sample and reagent

requirements, but with poor detection limits. The poor concentration limit of detection is primarily caused by a consequence of short optical path length within the detection cell and the extremely small sample volume that can be introduced into the CE capillary. In 1999, He and Lee used large-volume sample stacking in acidic buffer to analyse small organic and inorganic anions by CE. Recently, a novel sample injection technique-large volume stacking using the EOF (electroosmotic flow) pump (LVSEP)-has been developed and applied for the first time in detection of EDTA in drinking water by capillary electrophoresis(Zhu et al., 2002; Zhang et al., 2005). The detection limit of the method was as low as 0.2 μg/L or 2.0 μg/L, with or without 10-fold pre-concentration procedure, respectively.

3.1.4 Ion Chromatography

Ion-Chromatography was introduced by Lucy and Ye (1995) as a new reagent system for determination of hexadentate aminopolycarboxylic acids. This system is based on the fluorescent ternary complex formed between lutetium, hexadentate aminopolycarboxylic acids, and 8-hydroxyquinoline-5-sulfonic acid (HQS) (Lucy and Ye, 1995). The formation of the lutetium, trans-1, 2-diaminocyclohexane-N, N, N', N'-tetraacetic acid, and 8-hydroxyquinoline-5-sulfonic acid (Lu-CDTA-HQS) fluorescent ternary complex was also used to determine chelating ligands (Ye and Lucy, 1996). The detection limit obtained was $2.5x10^{-8}$ M (ca. 0.5 ng) for EDTA. Additionally, the existence of ten-fold excess alkaline earth metal and the transition metal ions did not interfere with the determination of these chelating ligands with a metal-exchange sample pre-treatment step.

The coupling of ion chromatography with electrospray mass spectrometry (IC-MS) is a simple, sensitive and quick method for the determination of polar organic traces in water samples (Charies and Pépin, 1998; Bauer et al., 1999). Analysis of EDTA in aqueous samples, including wastewater, was completed by IC-MS on an anion exchange column after simple sample preparation steps. The detection limit was down to a concentration level of 1 μg/L (Knepper et al., 2005).

3.1.5 Other Methods

Except for the analytical methods discussed above, there are a few other methods which have been applied to analyse EDTA and metal-EDTA complexes, for example, electrochemical methods (Sillanpää and Sihvonen, 1997), spectrophotometry, atomic absorption spectrometry (AAS) (Güclü et al., 2000; Baytak and Türker, 2006) and titration method. Schmidt and Brauch (2005) have published an excellent review article on this matter.

3.1.6 Summary

EDTA can be determined in different samples by GC, LC, IC, CE and electrochemical techniques etc. GC (GC-MS and GC-NPD) allows the sensitive and reliable identification of the complexing agents. However, it involves cumbersome sample preparation and does not allow the determination of EDTA speciation in the sample. GC can be applied to drinking waters, surface waters and wastewaters. Alternatively, the determination in these matrices can be performed by liquid chromatography (LC). LC techniques also allow the separation and quantification of individual EDTA-metal species. However, the detection limit is too high for surface water analysis (i.e. low μg/L-range). Nevertheless, the coupling of ion chromatography with electrospray mass spectrometry (IC-MS) can resolve this matter. A number of EDTA –metal species can also be differentiated by capillary electrophoresis (CE); the sensitivity required for drinking water is achieved by LVSEP. The other methods, e.g. spectrophotometry, AAS etc. are prone to interferences, in particular in the case of complex matrices. As these techniques demonstrate relatively poor selectivity, they are usually only suitable for the determination of the general complexing capacity of samples.

3.2 METHOD DEVELOPMENT FOR ANALYSIS OF EDTA USING HPLC – UV

Taking into account the equipment availability and running cost, HPLC-UV seemed to be the most appropriate analytical technique for this project. The primary prime objective of the method development was to establish a robust and sensitive analytical technique with minimum interference to apply to multi-media samples, such as dairy wastewater. In view of the highly diversity of the dairy industry relating to their products and processes, this method development is based upon the dairy wastewater from Fonterra Co-operative Group Limited, Waitoa.

3.2.1 Initial Experiment Design

Before commencing the method development, it was necessary to review the nature of samples and the goal of HPLC separations.

1. Analysis of sample features

In general, wastewater from dairy processing plants contains high concentrations of organic material such as proteins, carbohydrates and lipids, high concentrations of inorganic compounds such as NO_3^- and large variations in pH (Britz et al., 2004).

Wastewater from the Fonterra Waitoa dairy factory is composed of three parts:

- Processing water, which includes cooling water and heating processes with free pollutants, is discharged into the Waitoa river with storm water;
- Cleaning wastewater originates from the cleaning of the equipment, which has been in contact with milk or milk products, and chemicals applied to the Clean – In – Place (CIP) system, are collected in a sump and subsequently pumped to the treatment ponds for further treatment to certain standards, and then discharged to the Waitoa river; and

- Sanitary wastewater is piped into a separate treatment pond, and then discharged to the Waitoa River.

The method development of HPLC–UV was focused on the cleaning wastewater, in which chemicals containing EDTA were applied during the cleaning process.

2. Separation goals

The analyte of interest - EDTA, which has a molecular weight less than 2000 g/mol, exists in an ionic pattern as 1:1 aqueous – soluble metal complexes. Objectives of the experimental design were set as following:

- The analyte of interest – EDTA being separated from the myriad of individual compounds in samples;
- Sharp, symmetrical chromatographic peaks;
- Separation time less than 10 minutes for practical time considerations;
- Capacity/retention factor $2 < k' < 10$ and maximum column plate number for a quality chromatogram. The retention factor for the analyte of EDTA is defined as $k' = t_R - t_M / t_M$, where t_R is the time between sample injection and an analyte peak reaching a detector at the end of the column t_M is the time taken for the mobile phase to pass through the column ; and
- Minimum use and ease of disposal chemicals.

The aim of HPLC separations in this case was to ensure that the analytical component of $[Fe(III)EDTA]^-$ was completely separated from other compounds in dairy wastewater samples, with a practical separation time of less than 10 minutes, and to ensure that other metal – EDTA complexes were totally converted into $[Fe(III)EDTA]^-$ before analyses. The method was thus optimized for a dairy wastewater matrix, including checking potential interferences at levels found in dairy wastewater.

3. Apparatus

The HPLC system consisted of a Shimadzu LC–10 AT VP Liquid Chromatography (USA) with a 50 µl sample loop, a Shimadzu SPD–10A VP UV-

Vis detector set at 265 nm, a Hypersil C_{18} RP column (Phenomenex) of length 200 mm, diameter 4.6 mm and particle size 5µm, and a Phenomenex security guard column. The HPLC recording and integration software was PowerChrom (eDAQ Pty Ltd, Australia) attached to a Powerlab/8sp data recorder (ADInstrument). All water was obtained from an ELGAS TAT ® UHQII system and filtered through 0.45µm Nylon filters (Phenomenex). Degassing of the mobile phase was achieved by helium sparging. HPLC system components are shown in a flow chart as below (Figure 3.2).

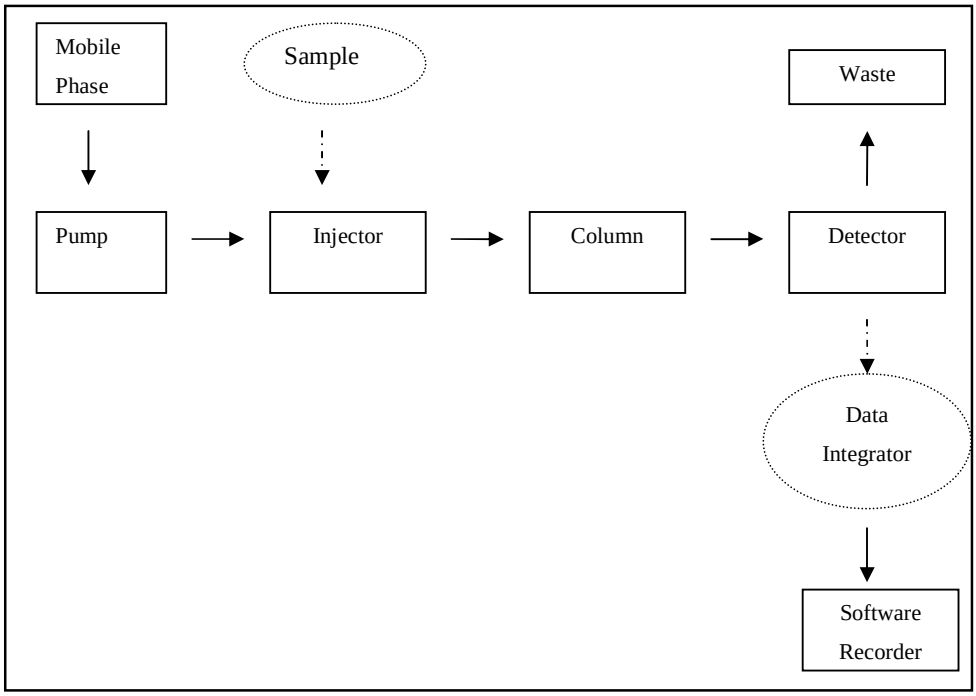

Figure 3.2 HPLC System Components for the determination of EDTA applied in this research.

4. ***Reagents, chemicals and solutions***

Reagents were all chromatographic analysis grade or reagent grade used without further purification. A sodium formate / formic acid buffer solution (pH 3.3) was prepared by dissolving 0.17g sodium formate (BDH) and 0.33 ml (Ajax Finchem) 90% formic acid in 1 L of water. An ion-pair reagent solution (15 mM TBABr) was prepared by dissolving 4.836g of tetra-n-butylammoniumbromide ($C_{16}H_{36}NBr$, 322.38 g/mol) (Merck) in 1L of pH = 3.3 buffer solution. A stock EDTA standard solution (0.1 g/L EDTA) was prepared by dissolving 0.1462 g

ethylenediaminetetraacetic acid iron sodium salt (MW = 421.10 g/mol) (Merck) in 1L of water, and stored in the refrigerator wrapped in tin foil. Standard solutions, ranging from 0–750 µg/L EDTA for calibration, were prepared daily from the stock solution. A Fe^{3+} solution (0.1941g/L or 3.47 mM) was prepared by dissolving 2.4203 g $FeCl_3 \cdot 6H_2O$ (Merck) and 0.144ml HCl (37% Merck) in 500 ml water. A nitrate solution (1 g/L) was prepared by dissolving 0.4077 g of KNO3 (Seelze–Hannover) in 250 ml water as a stock solution for further dilution. Calcium (0.1 g/L) and magnesium (0.1 g/L) ion solutions were prepared by dissolving 0.2732g of CaCl2·$6H_2O$ (BDH) and 0.1046 g of $MgCl_2 \cdot 6H_2O$ (BDH) in 500 ml water respectively for further dilution.

3.2.2 Optimizing Chromatographic Separations

A number of HPLC methods have been published to determine EDTA concentration in multi-media samples. A review of the literature indicated that the method of Loyaux-Lawniczak et al. (1999) was appropriate as a starting point as it was reported to be suitable for measuring EDTA in natural waters.

1. Buffer solution

In selecting a particular buffer, the buffer capacity and its UV absorbance should be taken into account. Buffer capacity is determined by pH, buffer pKa and buffer concentration. Generally, the effective pH control range is given by pKa ± 1.5. The pKa of formic acid/K-formate is 3.8, buffer range is 2.8 – 4.8 and the UV cutoff is 210 nm (10mM) (absorbance < 0.5) (Snyder et al., 1997).

The analyte of interest – EDTA complexed as $[Fe(III)EDTA]^-$ is a basic compound and its retention in the RP column is related to the pH value of the mobile phase. The pH value of 3.3 was chosen to prevent precipitation of iron as 99.2% of $[Fe(III)EDTA]^-$ exists in its deprotonated form when pH value is 3.3 (Loyaux-Lawniczak et al., 1999). Furthermore, this buffer solution was chosen due to there being no absorbance at the wavelength of 265 nm (Snyder et al., 1997).

2. Solvent of the mobile phase

In reversed phase (RP) separations, the sample retention can be controlled by varying the solvent strength of the mobile phase. This can be achieved either by using different solvents or varying the percent organic (% B) composition with the same solvent in the mobile phase. Both solvents of acetonitrile (ACN) (Nowack, et al., 1996; Cagnasso et al., 2007) and methanol (MeOH) (Loyaux-Lawniczak et al., 1999; Laine and Matilinen, 2005; Katata, et al., 2006) were investigated. A similar retention time was achieved using a lower percentage of ACN (1%) than MeOH (5%) if other parameters remained the same (Figure 3.3). The study of different % B compositions of MeOH showed that increasing % MeOH shortened the retention time (Figure 3.4). Buffer solution with 2% MeOH was selected for giving a practical retention time and a good separation ($2< k'<10$).

3. Ion–pairing reagent

An addition of the ion–pair reagent to the mobile phase can often improve peak shapes and large changes in separation selectivity for ionic samples (Snyder et al., 1997). The ion–pair reagent, tetrabutylammonium (TBA) bromide (TBABr) (Nowack et al., 1996; Loyaux-Lawniczak et al., 1999; Lin et al., 2003; Laine & Matilainen, 2005) / TBA hydroxide (Nirel et al., 1998; DIN 38413-8, 2000; Cagnasso, et al., 2007) and TBA hydrogen sulphate (Katata et a., 2006), is often used as TBA^+ is positively charged on its nitrogen and competes with anions, for instance, $[Fe(III)EDTA]^-$, NO_3^-, Cl^- to form an ion-pair. The varied concentrations of TBABr in the mobile phase were studied, and the results observed that the retention of $[Fe(III)EDTA]^-$ compound decreased when the concentration of TBABr was increased and other parameters remained the same, with a 100 µg/L EDTA standard solution (Figure 3.5 and Figure 3.6). The concentration of 15mM TBABr was selected for the determination of EDTA in dairy wastewater.

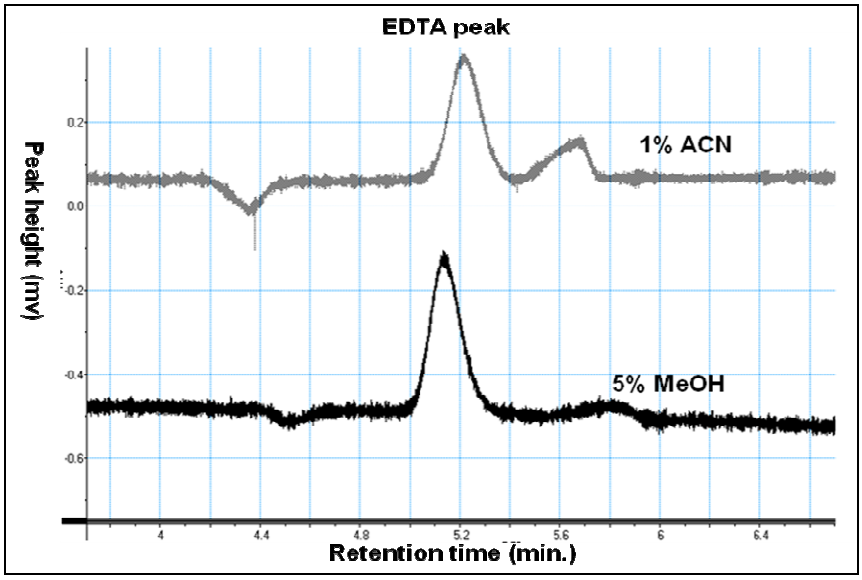

Figure 3.3 A similar $[Fe(III)EDTA]^-$ retention time obtained using a lower percentage of ACN (1%) than MeOH (5%)during chromatographic separation when other parameters remain the same.

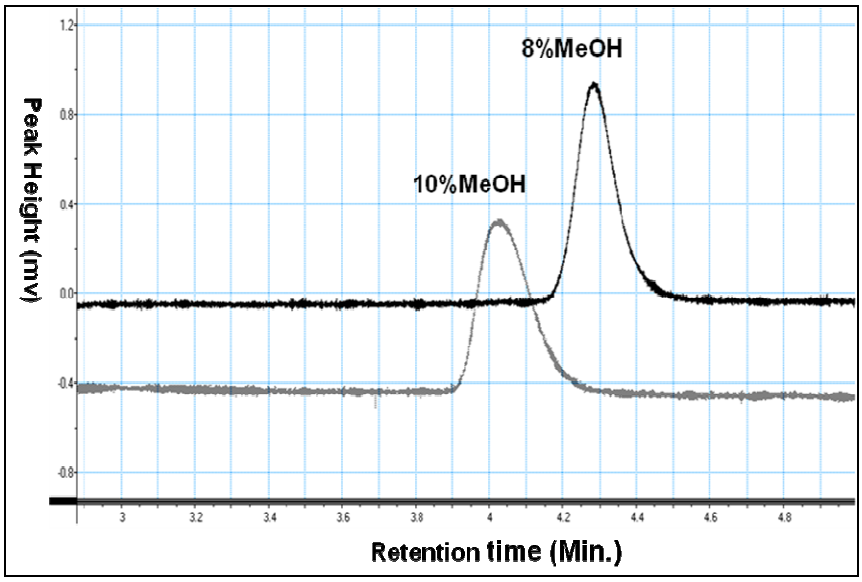

Figure 3.4 High composition of solvents shortened the $[Fe(III)EDTA]^-$ retention time during chromatographic separation when other parameters remain the same.

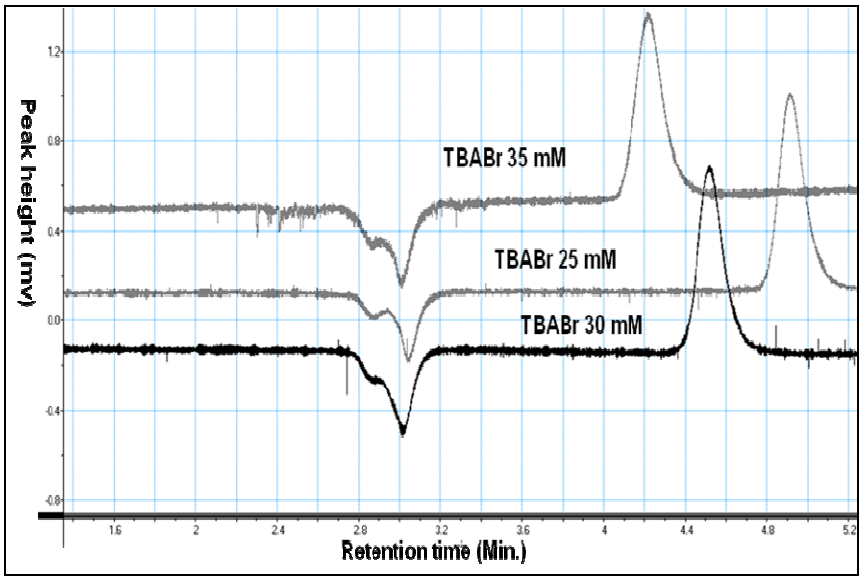

Figure 3.5 Retention time of $[Fe(III)EDTA]^-$ decreases with increasing the concentration of tetrabutylammonium bromide (TBABr) in the mobile phase during chromatographic separation when other parameters remain the same.

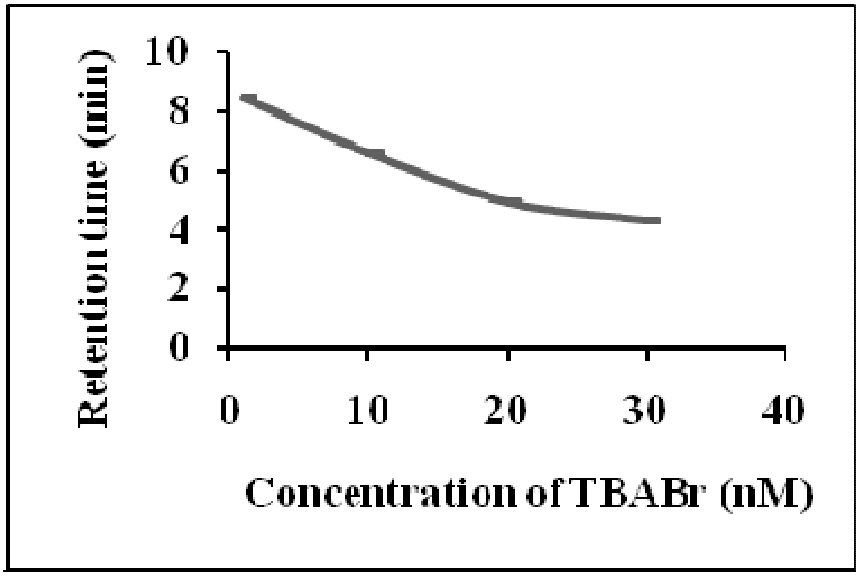

Figure 3.6 Effects of concentrations of tetrabutylammonium bromide (TBABr) in the mobile phase on the retention of $[Fe(III)EDTA]^-$ during chromatographic separation when other parameters remain the same.

4. Flow rate of mobile phase

In general, retention time increases with a lower flow rate, but the separation often improves (Figure 3.7). The flow rate was set at 0.9 ml/min for a better separation with a practical analysis time.

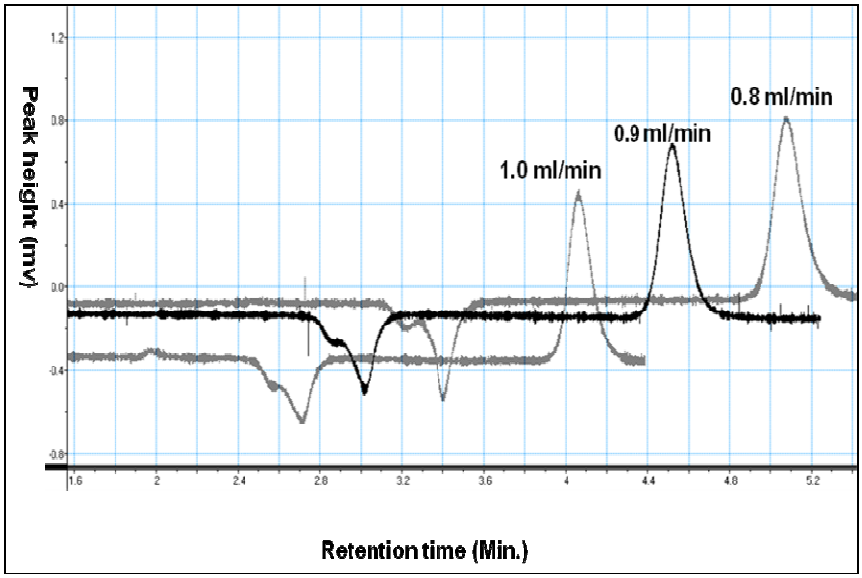

Figure 3.7 [Fe(III)EDTA]- is retained longer with a lower flow rate of the mobile phase during chromatographic separation when other parameters remain the same.

5. Resolution of HPLC separation

The HPLC separation for $[Fe(III)EDTA]^-$ of interest in the dairy wastewater sample is shown in Figure 3.8 by varying solvent strengths and ion-pair reagent TBABr concentrations with the optimized flow rate (0.9 ml/min). The optimal solvent strength and concentration of TBABr for peak resolution were 2% methanol and 15mM TBABr.

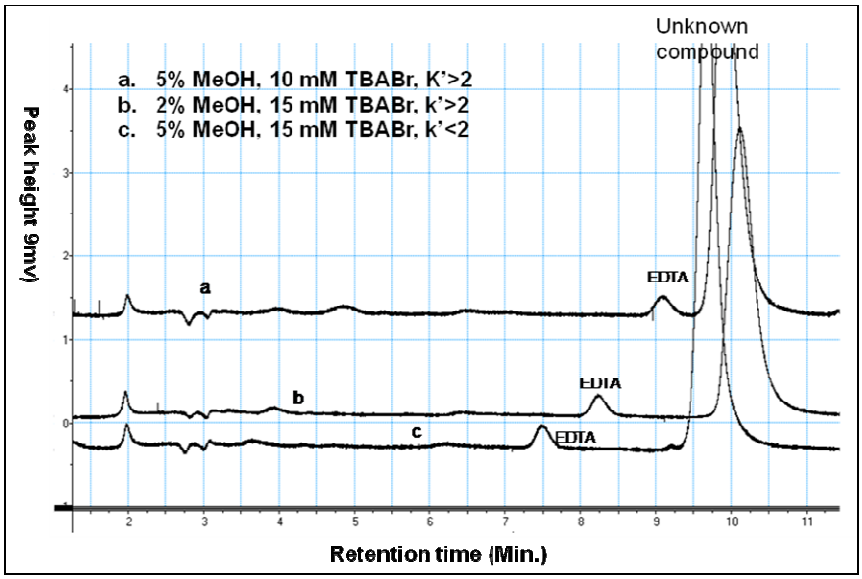

Figure 3.8 Overlay of chromatograms during chromatographic separations by varying composition of mobile phases to ensure that [Fe(III)EDTA]- is completely separated from other compounds for dairy wastewater samples

6. Wavelength of UV detector

The choice of wavelength of UV detector depends upon the analyte absorbance of interest, sample interference and the mobile phase absorbance. The detector signal (A) is proportional to the molar absorptivity (ε) of the compound of interest. In fact, the $[Fe(III)EDTA]^-$ has a large value of ε and can be detected at a higher wavelength (>210 nm) (Snyder et al., 1997). A wide range of wavelength between 240nm and 330 nm has been used for the UV detection (Loyaux-Lawniczal et al., 1999; DIN 38413-8, 2000; Laine and Matilainen, 2005; van Ginkel and Greerts, 2005; Katata et al., 2006). A wavelength of 265 nm was determined by wavelength scan using UV–Visible Recording Spectrophotometer UV–240 (Shimadzu) and Graphic Printer PR – 1. The scan was carried out with different concentrations of EDTA using the mobile phase as reference. The spectrophotogram is shown in Figure 3.9.

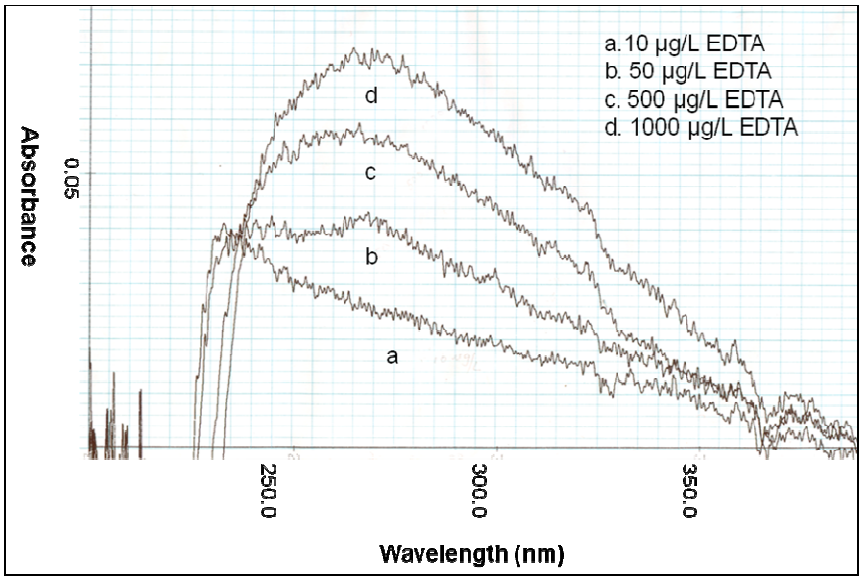

Figure 3.9 Wavelength scan spectrophotograms of $[Fe(III)EDTA]^-$ with different concentrations of EDTA standard solution

3.2.3 Compounds that potentially complicate analysis

Some inorganic compounds can complicate the determination of EDTA.

1. Nitrates

Some authors (Nowack et al., 1996; Loyaux-Lawniezak et al. 1999; DIN 38413 – 8, 2000) reported that the determination of EDTA could be under-estimated in the presence of high concentration of NO_3^- due to cross-sensitivities. Also, nitrate (NO_3^-) shows a minor absorption at the wavelength of 265 nm (the wavelength used to detect EDTA), which can cause over-estimation of EDTA (Snyder et al., 1997).

The concentration of nitrate in dairy wastewater samples varies depending upon manufacturing processes. Levels of nitrate found in dairy wastewater in this case were generally below 100 mg/L. An experiment was undertaken by adding different concentrations of $NaNO_3$ (10, 50 and 100 mg/L) to a 100 µg/L EDTA standard solution (Figure 3.10). This indicated that there is no apparent

interference to the [Fe(III) EDTA]$^-$ peaks at nitrate concentrations likely to be found in dairy wastewater.

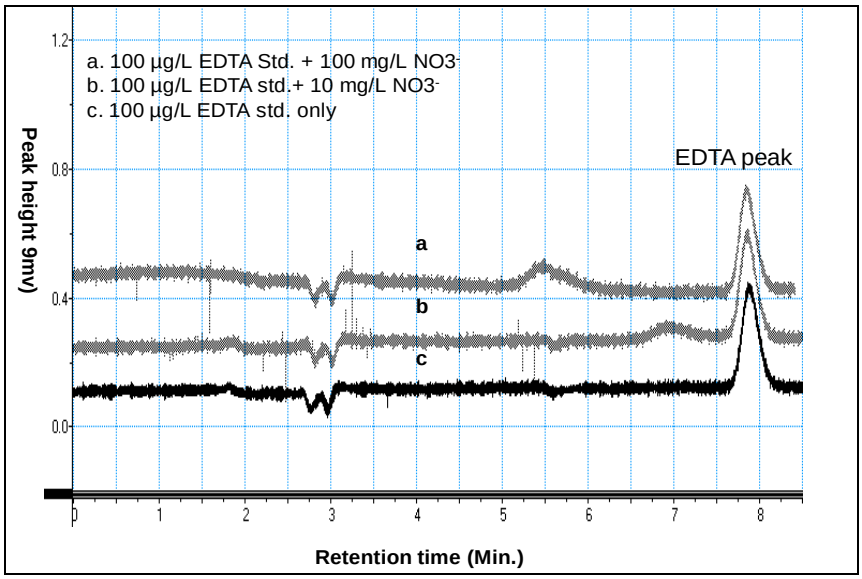

Figure 3.10 The effect of addition of nitrate on HPLC analysis of EDTA.

2. Metals

Nowack et al. (1996) stated that waters with high calcium and magnesium ions may influence the determination of EDTA due to a matrix effect. The content of calcium in the Waitoa dairy effluent was stated to be 8-10 times higher than would be expected in local clean water (Waitoa dairy factory fact sheet). To investigate possible interference, an experiment was carried out by spiking a 100µg/L EDTA standard solution with different concentrations (10, 25, 50 and 100 mg/L) of Ca^{2+} and Mg^{2+} (Figure 3.11 and Figure 3.12). The effect of a mixture of Ca^{2+} and Mg^{2+} at the approximate ratio of Ca^{2+} and Mg^{2+} in the dairy wastewater (4:1), was also studied (Figure 3.13). The overall results showed no apparent interference of these metals on the determination of EDTA by HPLC-UV at concentrations likely to be found in dairy waste water.

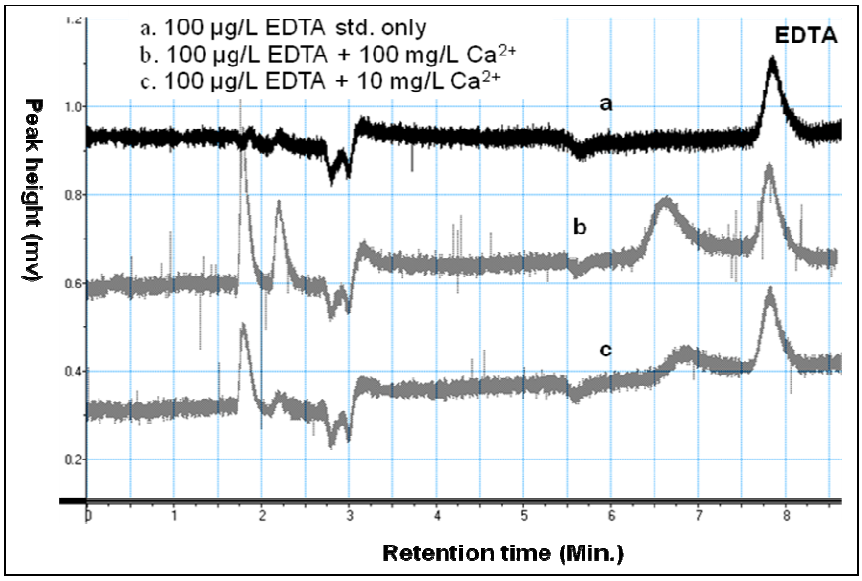

Figure 3.11 The effect of addition of calcium on HPLC analysis of EDTA.

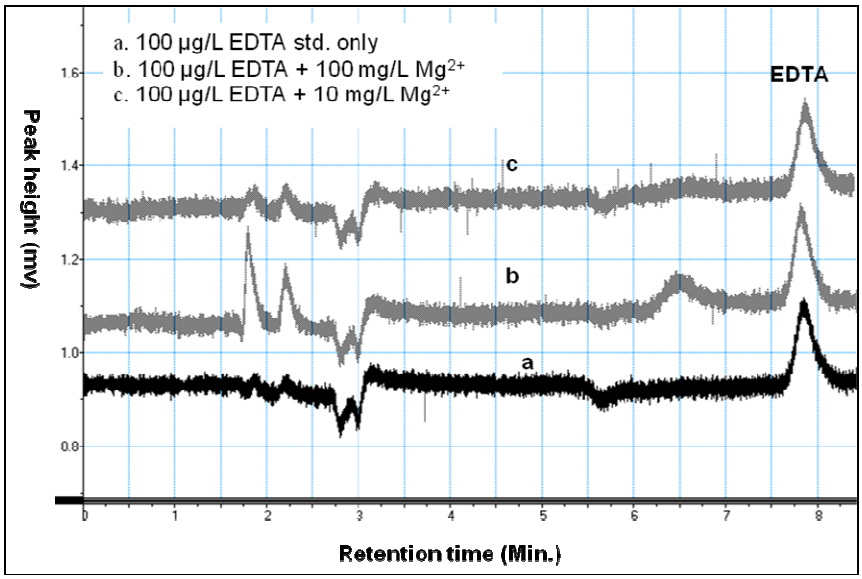

Figure 3.12 The effect of addition of magnesium on HPLC analysis of EDTA.

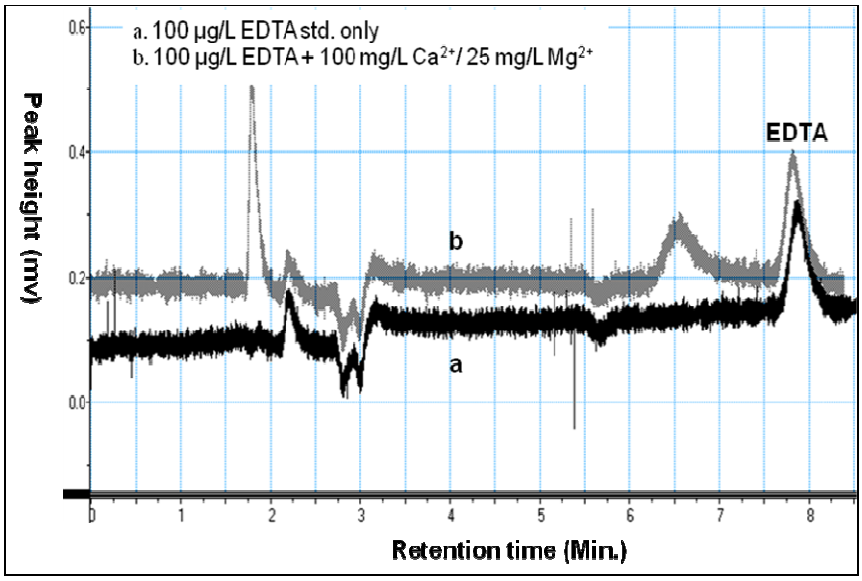

Figure 3.13 The effect of addition of a mixture of calcium and magnesium (ratio 4:1) on HPLC analysis of EDTA.

3. Addition of excess ferric ions

In dairy wastewater from processing plants, EDTA exists mainly in the form of Ca-EDTA and Mg-EDTA. These species have low pKa values and slow exchange kinetics (Nowack et al. 1996). Pre-treatment is thus needed to convert these species to Fe(III)EDTA for the analysis of total EDTA in a sample. A series of experiments were carried out which involved the addition of different molar ratios of Fe^{3+} ($1xFe^{3+}$, $1.5xFe^{3+}$, $2xFe^{3+}$, $5xFe^{3+}$, $10xFe^{3+}$ and $20xFe^{3+}$) to a 100 µg/L EDTA standard solution under different pre-treatment conditions. The procedures involved:

- Heating in 90^{o}C water bath for over 3 h (Nowack et al., 1996; Loyux-Lawniczak et al., 1999);
- Placing in a dark place over-night (van Ginkel et al. 1999; Katata et al. 2006); and
- Boiling for 1.5 h.

The experimental results revealed that (i) the addition of excess Fe^{3+} altered the baseline and shifted the peak retention time of the chromatogram at higher concentration of iron (Figure 3.14), but appeared not to affect the peak area and hence the determination of EDTA; and (ii) similar results were obtained with

different pre-treatment conditions. Consequently, as it was the most experimentally convenient, the overnight pre-treatment was applied for subsequent experiments.

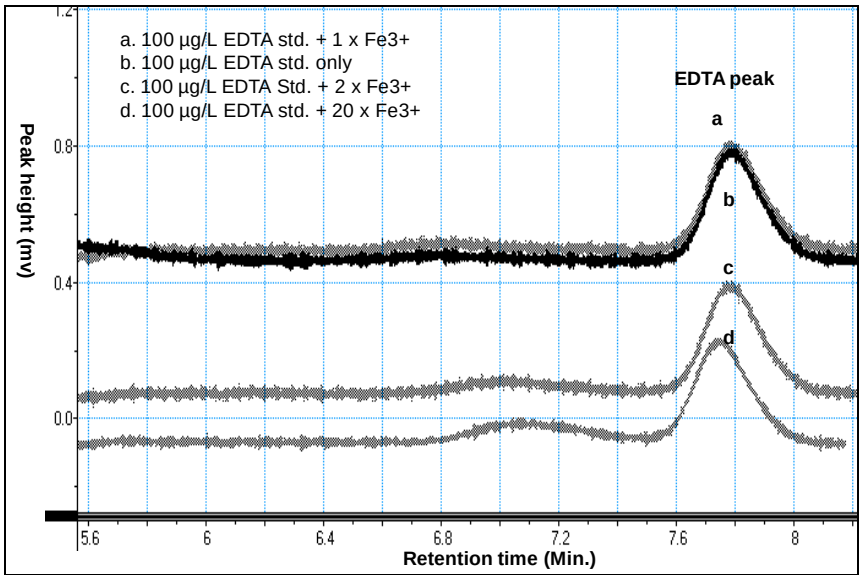

Figure 3.14 Overlay of EDTA chromatograms to determine the effect of addition of Fe^{3+} on HPLC analysis.

4. Exchange of metal complexes

The determination of EDTA may be under-estimated if some EDTA exists as other metal complexes, most likely to be Ca^{2+} in waters. The molar extinction coefficient of $[Ca(II)EDTA]^{2-}$ is much less than that of the $[Fe(III)EDTA]^{-}$, so smaller absorbance peaks would be expected to be observed (Loyux-Lawniczak et al., 1999). This was confirmed using 100 µg/L solutions of CaEDTA and FeEDTA (Figure 3.15). Hence, it is vital to ensure that all metal complexes, most likely $[Ca(II)EDTA]^{2-}$ in both dairy wastewater and natural waters, convert to $[Fe(III)EDTA]^{-}$ with the appropriate pre-treatment.

The experiment was established according to the method of Loyux-Lawniczak et al. (1999), and accomplished by adding 10 times equivalent ferric ion to a 100 µg/L EDTA standard solution as $[Fe(III)EDTA]^{-}$ and a 100 µg/L EDTA as $[Ca(II)EDTA]^{2-}$ to verify any losses of EDTA compared with 100 µg/L EDTA standard solution only with the pre-treatment. The test results (Figure 3.16)

showed that the same peak areas were observed within the three independent solutions and indicated the completed conversion of $[Ca(II)EDTA]^{2-}$ to $[Fe(III)EDTA]^{-}$, and no losses of EDTA from the pre-treatment.

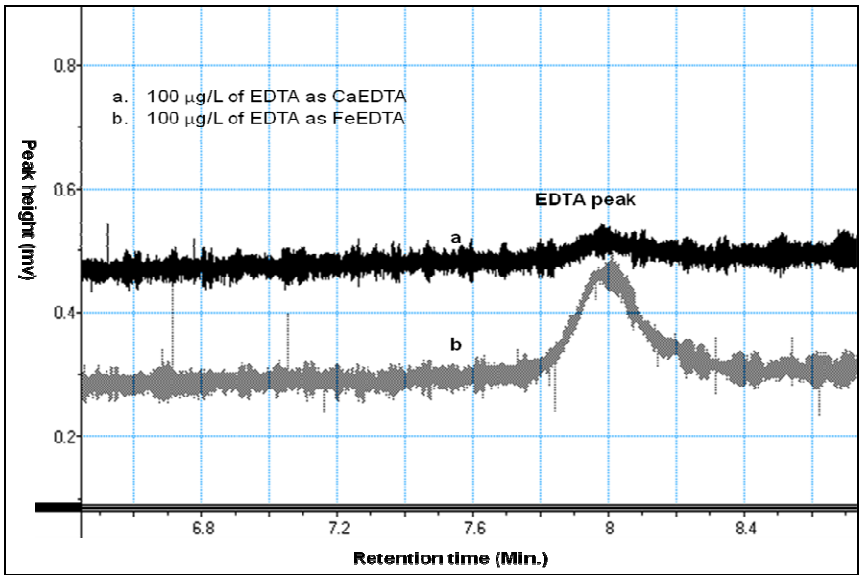

Figure 3.15 Absorbance comparison of 100 µg/L EDTA as $[Fe(III)EDTA]^{-}$ and $[Ca(II)EDTA]^{2-}$.

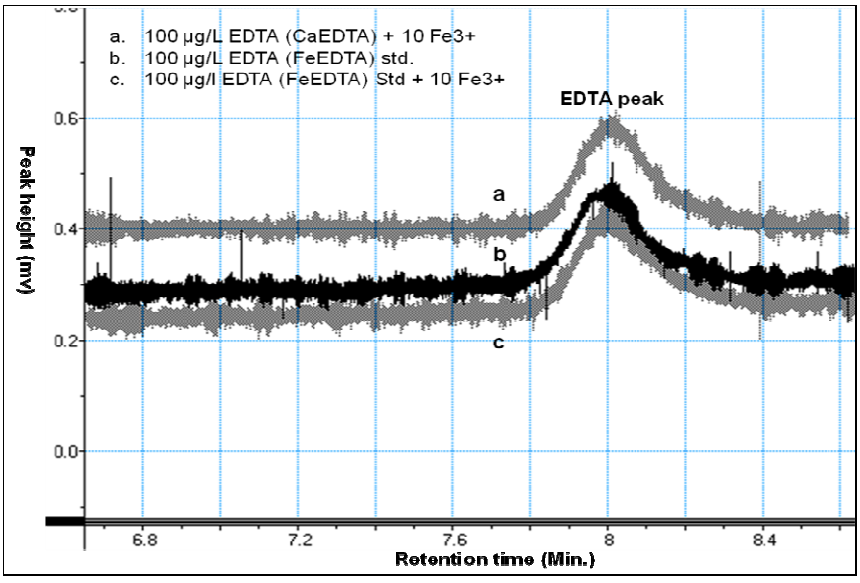

Figure 3.16 Overlay of chromatograms of 100 µg/L EDTA from independent solutions of standard EDTA as $[Fe(III)EDTA]^{-}$, converted $[Ca(II)EDTA]^{2-}$ and pre-treated standard as $[Fe(III)EDTA]^{-}$.

3.2.4 Method Accuracy, Precision and Detection Limit

There is no Certificated Reference Material (CRM) available for EDTA in waters. The method accuracy was checked using the analyte spike recovery, in which 100 µg/L EDTA of standard was added to dairy wastewater samples (Figure 3.17). The spike recovery was between 98 % and 102 % (n = 9). Additionally, the method accuracy was demonstrated by comparing peak areas of 1 mg/L of EDTA standard as NaFe(III)EDTA manufactured by different companies. The same peak areas were observed from the independent solutions, illustrated in Figure 3.18.

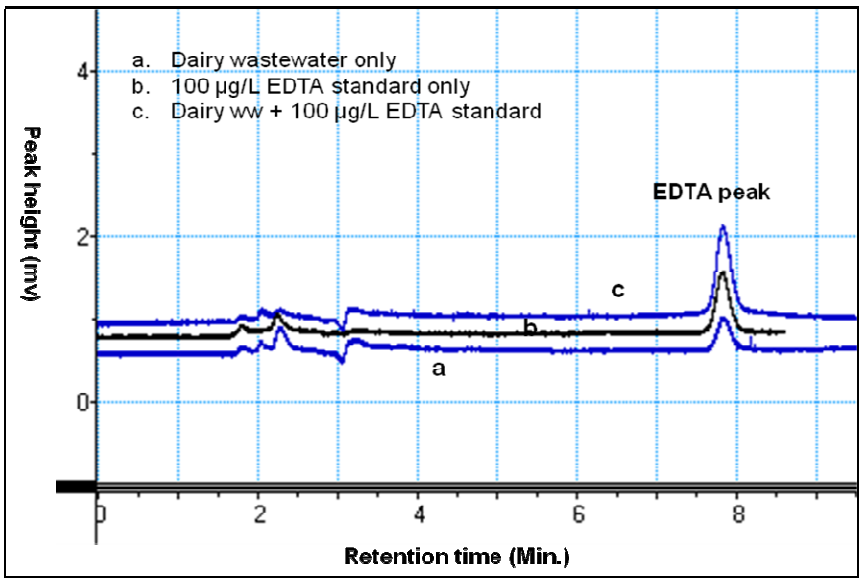

Figure 3.17 Overlay of chromatograms of a dairy wastewater sample only, 100 µg/L EDTA standard solution and dairy wastewater sample spiked by 100 µg/L EDTA with 101 % EDTA recovery achieved.

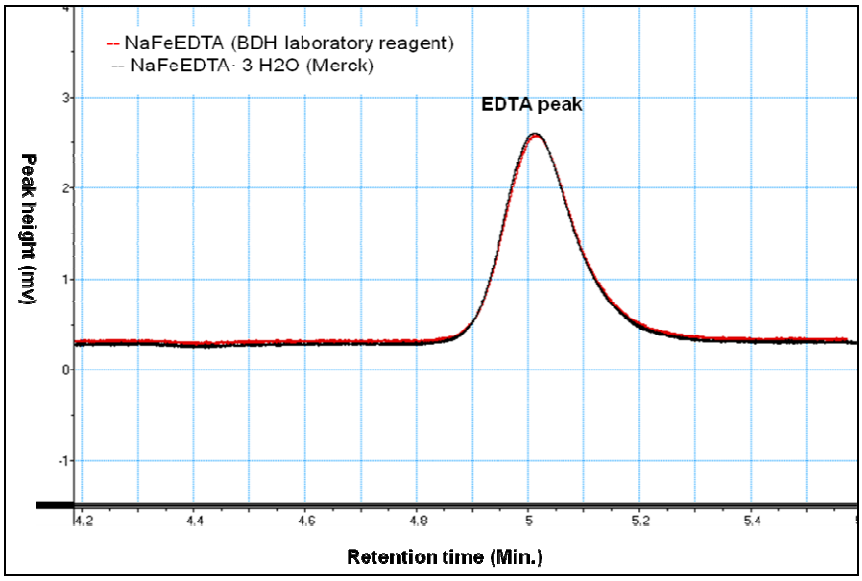

Figure 3.18 Overlay of chromatograms at the concentration of 1 mg/L EDTA as NaFeEDTA manufactured by different companies.

A daily calibration curve of concentration versus peak area was obtained with EDTA concentrations ranging from 0 to 1000 µg/L (0, 10, 50, 100, 200, 500 and 1000). Good linearity (0.99-0.999) was observed during the experiment.

Precision of the method was determined by analyzing individual sample 12 times. The repeatability, given as the relative standard deviation (RSD), was less than 1.5 %. The method detection limit (MDL) was calculated as three times the standard deviation of sample 1 (3x1.43 µg/L), giving 5 µg/L EDTA.

This developed method was applied to the determination of EDTA in multi-media environmental samples in the research that details were described in following chapters (Xie et al., 2008).

Table 3.1 Method repeatability test results from replicate dairy wastewater analyses.

Tests	Sample 1 (μgL^{-1})	Sample 2 (μgL^{-1})
1	107.2	1792.4
2	107.9	1759.3
3	107.1	1758.9
4	107.7	1780.3
5	107.5	1756.3
6	106.9	1737.5
7	106.2	1801.1
8	106.9	1751.0
9	107.7	1753.6
10	104.7	1765.7
11	107.0	1766.8
12	103.0	1759.3
Average	106.7	1765.2
STDEV	1.43	17.97
% RSD	1.34	1.01

3.3 CONCLUSIONS

Having reviewed available analytical methods for the determination of EDTA, an appropriate HPLC-UV method has been established for this research. The method accuracy was checked using the analyte spike recovery as there is no Certificated Reference Material (CRM) available for EDTA in waters. Precision of the method was determined by analyzing samples in replicate. The method detection limit was calculated as 5 µg/L of EDTA.

The next task is to apply the developed method to ascertain whether EDTA can be detected in dairy waste waters as well as in environments.

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Zhu, Z., Zhang, L., Marimuthu, A. & Yang, A. 2002. Large-Volume Sample Stacking for Analysis of EDTA by Capillary Electrophoresis. *Electrophoresis*, 23, 2880-2887.

4.0 CHAPTER FOUR: EDTA IN DAIRY WASTEWATER AND REMOVAL EFFICIENCY

EDTA is believed normally to be of low risk to human health and environments. But an impact from EDTA may be evident in some cases at sites where there is an output source (European Chemicals Bureau, 2004; Grundler et al., 2005). The research undertaken in this chapter aims to ascertain levels of EDTA in wastewater from dairy processing plants, EDTA removal efficiency from the existing wastewater treatment plants under normal operating conditions at a case study of the Waitoa dairy site, Fonterra Co-operative Group Limited (Fonterra Waitoa dairy site). The specific objective is to ascertain whether EDTA can be identified in the dairy waste waters.

Based on this chapter, a paper entitled 'EDTA in dairy wastewater and removal efficiency- a case study' by Xie, C. Z.; Healy, T.; Robinson, P.; Stewart, K. has been submitted to the *International Journal of Environment and Sustainable Development*.

4.1 APPLICATION OF EDTA IN DAIRY PROCESSING PLANTS AT THE FONTERRA WAITOA DAIRY SITE

The Waitoa dairy site, Fonterra Co-operative Group Limited (Figure 4.1) was established in 1902. There are approximately 500 staff processing up to 5.0 million litres of milk a day, and it is primarily a nutritional powder site. A cleaning compound containing EDTA, namely Eliminator or Eliminator LF, has been used as an additive to caustic cleaning agents during the CIP (clean-in-place) process in manufacturing plants. The primary drive for dairy industries using EDTA (also known as a single-stage cleaning) is to reduce CIP time and increase available production time. This is achieved by adding EDTA to the caustic step of CIP and dropping the use of the acid step (see Chapter 1). Single-stage cleaning

also reduces water consumption and hence wastewater generation and nitrate/phosphate emission to wastewater through lower use of nitric acid and phosphoric acid containing blends (Orica Chemnet, 2004).

Figure 4.1 Waitoa dairy site, Fonterra Co-operative Group Limited
(Photo provided by the Waitoa dairy factory)

At the time of this study, there were two processing plants manufacturing cheese and milk powder using Eliminator/ Eliminator LF alongside the caustic cleaning agents at the Waitoa dairy site. Eliminator is a strong liquid solution of organic acid salts and surfactants, which contains EDTA (34.0 to 36.0 %) with a pH around 14. The consumption of Eliminator associated with the CIP could vary depending on milk volumes and market demands for the specific dairy products. The ratio of EDTA in the caustic tank was generally controlled at 0.1 - 0.3% and the ratio of EDTA residue in discharge was controlled at less than 0.1%. The historical usage of EDTA at the site is tabulated in Table 4.1. The usage of Eliminator containing EDTA is, suggested by the factory, likely to be increased depending upon the market demand for dairy products in the future.

Table 4.1 Historical Usage of EDTA at the Waitoa dairy site, Fonterra Co-operative Group Limited, New Zealand.

Seasons	Usage of Eliminator/ Eliminator LF (Litres)	Specific Gravity (kg/L)	Na_4EDTA (kg)	35% EDTA (kg)
2003-2004		1.3	9990*	2650
2004-2005		1.3	24787*	6575
2005-2006	63190	1.3	82147	21791
2006-2007	46940	1.3	61022	16187
2007-2008	23610	1.3	30693	8142

(Source: Waitoa dairy factory and Orica Chemnet*)

4.2 DETERMINATION OF EDTA IN DAIRY WASTEWATER USING HPLC-UV

4.2.1 Cleaning Wastewater Discharge System at the Waitoa Dairy Site

The cleaning wastewater of 12-14 streams from all processing plants, which has been in contact with milk or milk products, and chemicals applied during the clean-in-pace (CIP) process, is collected in a sump on site and subsequently pumped to the wastewater treatment plants (WWTPs) through a 1 km long pipeline going across the company farm (Figure 4.2).

4.2.2 Wastewater Treatment Plants (WWTPs) at the Waitoa Dairy Site

Wastewater is generally referred to as "influent" as it enters WWTPs. The WWTP at the Waitoa dairy site includes two major ponds with a capacity of 46,000 m^3 operated in series and two clarifiers (settling tanks) operated in parallel (Figure 4.2 and Figure 4.3).

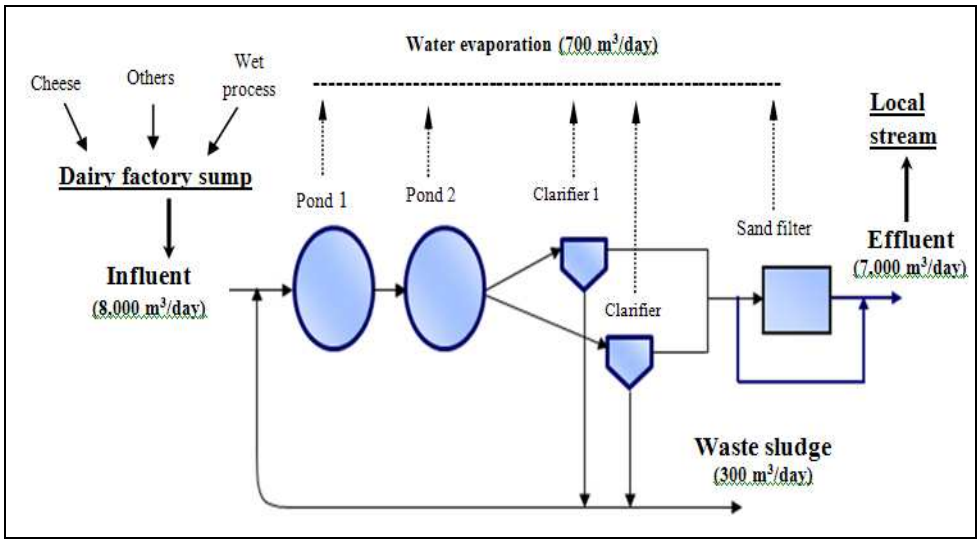

Figure 4.2 Schematic wastewater biological treatment flow diagram in an extended aeration sludge system at the Waitoa dairy site.

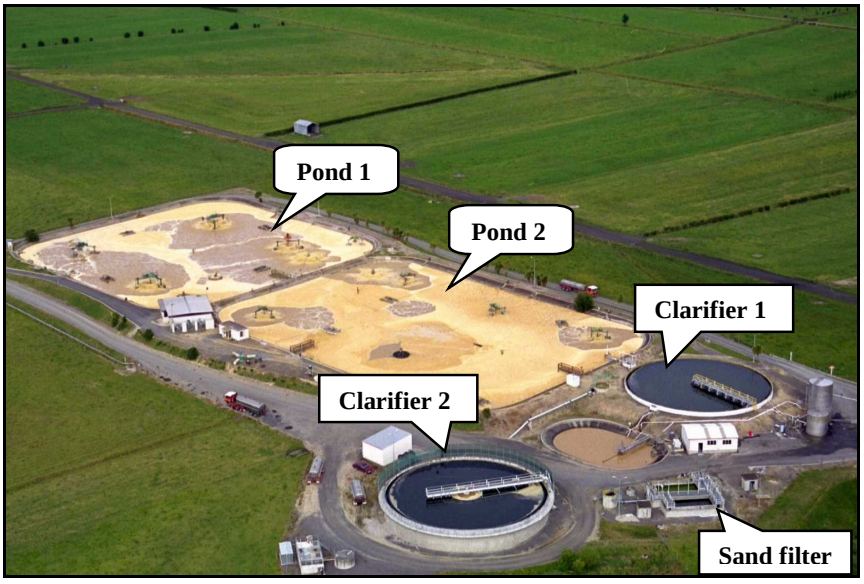

Figure 4.3 A layout of the wastewater treatment plant at the Waitoa dairy site.
(Source: Waitoa dairy factory)

Once at the ponds, the wastewater is mixed with activated sludge contained in the ponds. Activated sludge is a liquid mass containing floc, made up of bacteria and other micro-organisms. The activated sludge uses the wastewater as a food source, consuming oxygen in the process. Oxygen is added continuously to the system by the aerators to satisfy the micro-organisms. The level of oxygen in the ponds is measured continuously by a computer turning the aerators on or off to maintain

the oxygen concentration. This wastewater treatment processing is named as "an extended aeration sludge treatment" (see Figure 4.4).

Figure 4.4 An extended aeration sludge wastewater treatment pond, where the activated sludge uses dairy wastewater as a food source and consumes oxygen to satisfy the micro-organisms.

From the ponds the activated sludge mass flows into either of two clarifiers, in which the floc is separated from the water (Figure 4.5). Water, about 7000 m^3 per day on average, is clean enough to be discharged into the Waitoa River on a daily basis. Some of the water is directed through a sand filter to give additional cleaning when the turbidity exceeds 99 NTU (Nephelometric turbidity units). In this case the discharge is automatically stopped, and an alarm set off. The suspended solid matter is therefore the major monitoring parameter for the effluent.

The floc that settles to the bottom of the clarifiers (the sludge) (Figure 4.6) is returned to the ponds, which is generally pond 1. However, a certain amount of sludge needs to be disposed off daily to keep the system stable as the micro-organisms grow continuously.

Figure 4.5 Clarifiers where the floc is separated from the water by gravity at the Waitoa dairy wastewater treatment plant.

Figure 4.6 Some of the sludge from the bottom of clarifiers is pumped back to the treatment ponds or disposed off to retain the micro-organisms for the processing at the Waitoa wastewater treatment plant.

The disposed sludge (0.5% solid average) needs to be thickened (2.5% solid average) before being trucked away for a land treatment. The sludge thickening is achieved on gravity belts where polymer is dosed into the sludge to create larger flocs, excess water is drained off and pumped back to the ponds (Figure 4.7). The

thickened sludge is trucked away and spread onto nearby pasture land (see Chapter 7).The amount of disposed sludge is about 300 m³ per day on average.

The WWTP at the Waitoa dairy site is operated under aerobic conditions and remains highly efficient with COD removal of 99% (Rule, 1997). The operating pH value of the ponds is controlled at pH = 8.0 – 8.2, and the sludge age is 3 weeks average.

Figure 4.7 Gravity belts operate to thicken the sludge before being trucked away and spread onto nearby pasture land at the Waitoa dairy wastewater treatment plant.

4.2.3 Wastewater Sample Collection

Wastewater samples were collected at the following sites within the operating factory in order to determine firstly whether the EDTA can be detected within the system and if so, at what levels of EDTA at the Waitoa dairy site, Fonterra Co-operative Group Limited.

1. Sampling sites

Samples were collected at the sites illustrated in Figure 4.8 as the following:

a. Cheese drain, wet process flume from the processing plants at the Waitoa dairy site;
b. Influent into the wastewater treatment plant (WWTP);
c. pond 1 and pond 2 at the WWTP; and
d. Effluent discharged into the adjacent stream –the Waitoa River.

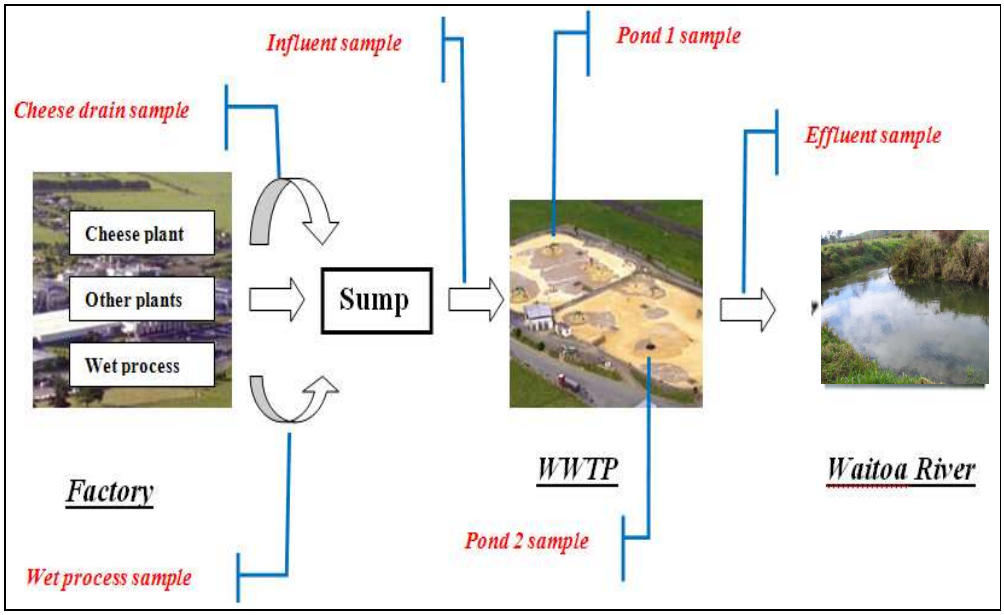

Figure 4.8 Flow diagram of wastewater and sample collecting sites at the Waitoa dairy site, Fonterra Co-operative Group Limited

2. Seasonal conditions

The operation of dairy industries is generally related to the milk production. Figure 4.9 demonstrates the operation status of dairy manufacturing plants. The seasonal effects of milk production and factory operation were therefore considered as below while collecting the wastewater samples at the Fonterra Waitoa dairy site.

a. Low factory operation due to the machinery down time for maintenances at low milk production: 28, 29 and 30 August 2007; and
b. Full factory operation at high milk production: 22 - 24 October 2007 (14 -16 November for cheese drain only) and 9 – 16 December 2007.

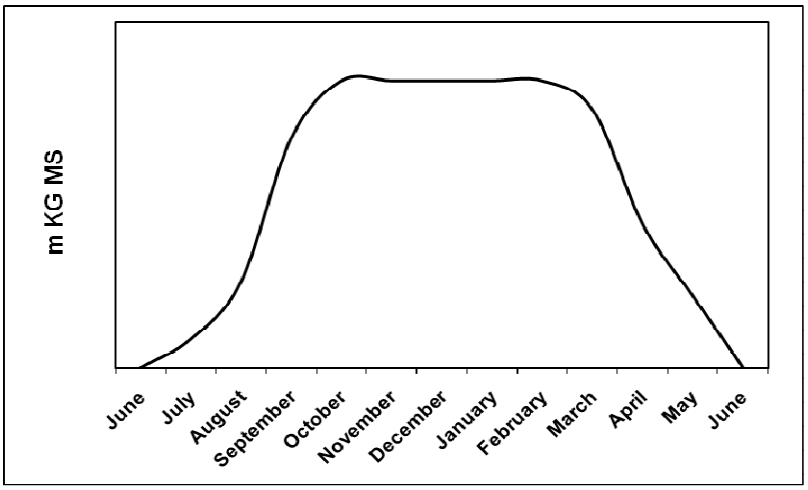

Figure 4.9 Indicative operation status of manufacturing plants during a year at the Waitoa dairy site, Fonterra Co-operative Group Limited.

(**m KG MS** – million kilograms of milk solid)

3. Frequency

Samples were collected as:

a. a 24–hour composite flow-proportional sample at the cheese drain, wet process plant, influent, and effluent sites (Figure 4.10); and
b. for the oxidation pond1 and 2 of the WWTP, each sample from morning and afternoon were combined into a composite sample for the day.

Figure 4.10 A 24–hour composite flow-proportional sample collection setting of wastewater from the cheese drain, wet process plant, influent, and effluent at the Waitoa dairy site, Fonterra Co-operative Group Limited.

4. Sample storage

Samples were collected in opaque PE bottles to avoid photolysis of the Fe(III)EDTA and frozen (-18^0C) until analysis.

4.2.4 Determination of EDTA in Dairy Wastewater

1. Analytical apparatus and reagents

Analytical apparatus and reagents involved were as listed in Chapter 3.

2. Sample pre-treatment

The wastewater sample pre-treatment involved following steps:

a. Taking 1 – 5 mL aliquots depending upon the expected concentration;
b. Adding appropriate (5 – 9 mL) Fe^{3+} solution at the concentration of 1.94 mg/L to the test tube;
c. Leaving overnight in the dark to allow complexing of Fe(III)EDTA;
d. Filtered through 0.45 µm cellulose nitrate filters (Phenomenex) using a syringe unit; and
e. Injecting 50 µL of sample into the HPLC system at ambient temperature.

3. Quality control

A calibration curve during the determination of EDTA was carried out daily, ranging from 0 – 1000 µg/L of EDTA. The linearity of the daily calibration defined as a correlation coefficient (r^2) varied between 0.9988 and 0.999 (Figure 4.11). One blank, duplicate every 10^{th} sample and EDTA standard spike recovery every 20^{th} sample were analyzed for each run. Samples with high concentration of EDTA were diluted to the calibration range. The chromatogram of a typical dairy

wastewater sample is shown in Figure 4.12. A daily work file is attached as Appendix 1.

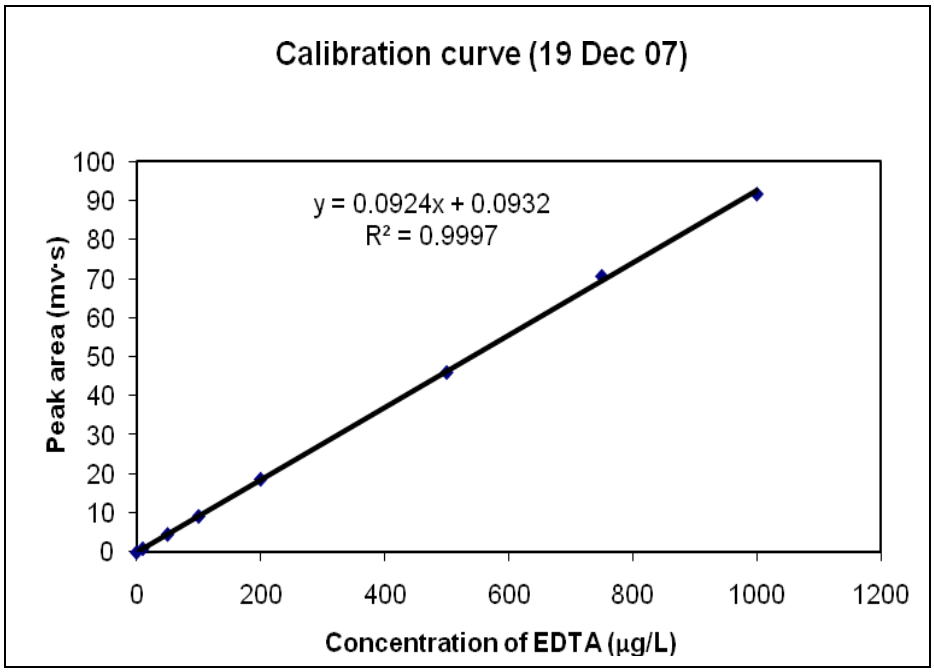

Figure 4.11 A daily EDTA calibration curve ranging from 0 - 1000 µg/L by HPLC-UV with freshly prepared EDTA solutions.

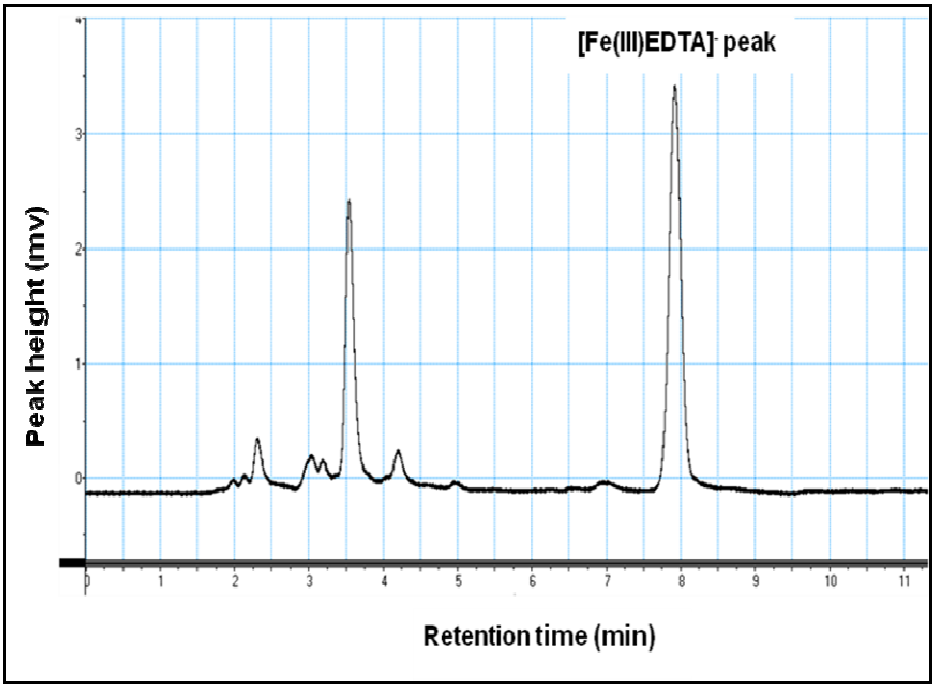

Figure 4.12 A chromatogram of a typical dairy wastewater sample of influent from the Waitoa dairy site, Fonterra Co-operative Group Limited.

4.3 ANALYTICAL RESULTS

EDTA is identified by the retention time (minutes) and the concentration of EDTA is quantified by the peak area (mv·s) of $[Fe(III)EDTA]^-$.

4.3.1 EDTA Occurrence in Dairy Processing Wastewater

EDTA concentration in wastewater samples of the wet process flume and cheese plant drain from the Waitoa dairy site are shown in Table 4.2. Figure 4.13 and Figure 4.14 indicate the number of data points of each group of samples.

Table 4.2 EDTA concentrations detected in dairy wastewater(wet process and cheese drain) from processing plants at the Waitoa dairy site, Fonterra Co-operative Group Limited, based on 13 24-hour composite samples in August, October (November) and December, 2007.

Milk Season Conditions	Sampling Date	Wet Process (μg/L)	Cheese Drain (μg/L)
Low	27 - 28 Aug. 2007	< 5	NO*
Low	28 - 29 Aug. 2007	< 5	NO
Low	29 - 30 Aug. 2007	< 5	NO
High	21 - 22 Oct. 2007	36430	-**
High	22 - 23 Oct. 2007	82743	-
High	23 - 24 Oct. 2007	25944	-
High	13 - 14 Nov. 2007	-	59619
High	14 - 15 Nov. 2007	-	76655
High	15 - 16 Nov. 2007	-	17183
High	9 - 10 Dec. 2007	14850	195
High	10 - 11 Dec. 2007	13554	19602
High	11 - 12 Dec. 2007	23756	33874
High	12 - 13 Dec. 2007	9903	1223
High	13 - 14 Dec. 2007	13876	296
High	14 - 15 Dec. 2007	6489	711
High	15 - 16 Dec. 2007	14720	10701

***** NO – not operating
** - no samples collected

Figure 4.13 indicates the variation of EDTA concentration in the wet process flume at the Waitoa dairy site, Fonterra Co-operative Group Limited. For samples of 27–30 August 2007, EDTA was not detected (< 5 µg/L) indicating no EDTA was being applied. However, during the sampling period of 21-24 October and 9-16 December 2007, EDTA was detected with concentrations as high as 82700 µg/L. The averaged concentration of EDTA detected from continuous sampling during the week of 9-16 Dec. 2007 was 13900 µg/L.

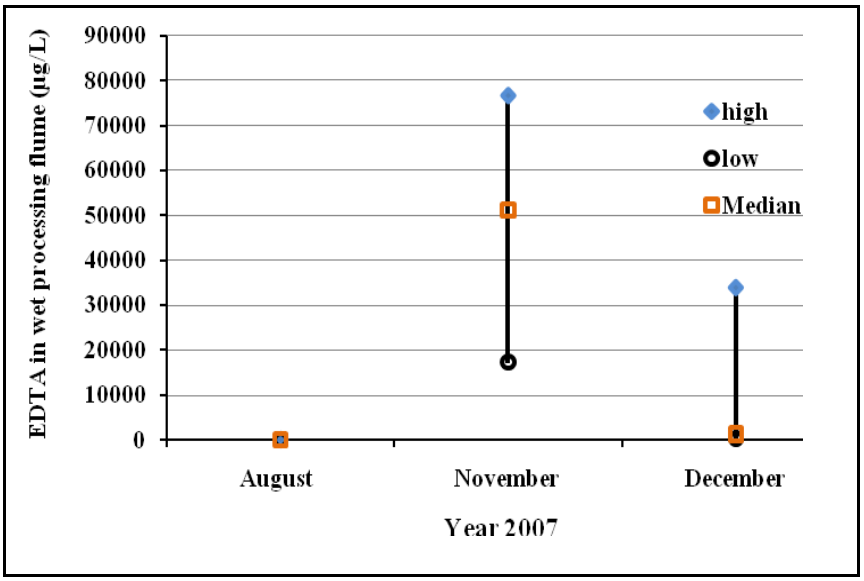

Figure 4.13 Variation of EDTA concentrations detected in the wastewater of the wet process flume at the Waitoa dairy site, based on 13 24-hour composite samples in August, November and December, 2007.

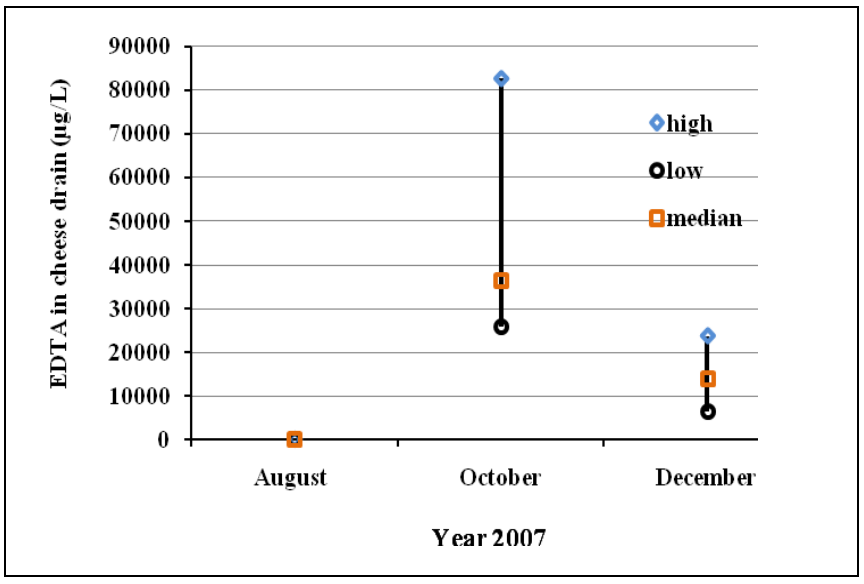

Figure 4.14 Variation of EDTA concentrations observed in wastewater of cheese drain at the Waitoa dairy site, based on 13 24-hour composite samples in August, October and December, 2007.

Figure 4.14 presents the observed EDTA concentrations in the cheese drain at the Waitoa dairy site. There were no wastewater samples from 27-30 August 2007 as the cheese plant was not operated due to machinery maintenance. For the sampling period of 13-16 November (samples were missed in October) and 9-16 December 2007, while the plant was routinely operating, the concentrations of EDTA detected were between 195 µg/L and 76700 µg/L. It is evident that concentrations of EDTA in the processing wastewater are closely related to the daily manufacturing schedule. The level of EDTA in the processing wastewater is normally controlled below 0.1 % by the manufacturing plants.

4.3.2 EDTA Reduction during the Waitoa Wastewater Treatment Process

Analytical results of EDTA concentrations from the influent to the effluent are tabulated in **Error! Reference source not found.** and illustrated in Figure 4.15, when the wastewater was treated by an extended aeration biological system at the Waitoa dairy site.

Table 4.3 Tested results of EDTA concentrations in the influent, treatment ponds and the effluent in an extended aeration system at the Waitoa wastewater treatment plant, Fonterra Co-operative Group Limited, based on based on 13 samples collected in August, October and December, 2007.

Milk Season Conditions	Sampling Date	Influent (µg/L)	Pond 1 (µg/L)	Pond 2 (µg/L)	Effluent (µg/L)
Low	27 - 28 Aug. 2007	301	428	299	152
Low	28 - 29 Aug. 2007	93	442	235	93
Low	29 - 30 Aug. 2007	226	293	179	161
High	21 - 22 Oct. 2007	1420	475	238	227
High	22 - 23 Oct. 2007	3170	405	255	261
High	23 - 24 Oct. 2007	1374	249	256	147
High	09-10 Dec. 2007	1997	122	64	98
High	10 - 11 Dec. 2007	1211	106	50	72
High	11 - 12 Dec. 2007	5194	295	263	78

High	12 - 13 Dec. 2007	1747	148	125	185
High	13 - 14 Dec. 2007	1625	105	166	147
High	14 - 15 Dec. 2007	1738	119	73	115
High	15 - 16 Dec. 2007	3505	304	166	130
	Average	**1813**	**269**	**182**	**144**
	STDEV	**1434**	**139**	**84**	**56**

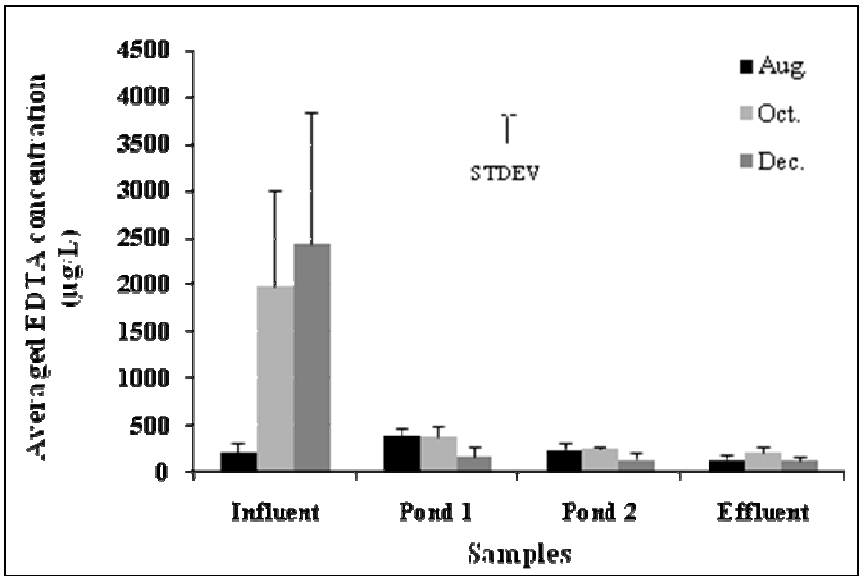

Figure 4.15 Detected EDTA concentrations with a standard deviation in the influent, treatment ponds and the effluent at the Waitoa dairy wastewater treatment plant based on 13 samples collected in August, October and December, 2007.

Table 4.3 shows changes of the EDTA concentrations in wastewater samples of the influent, treatment ponds – pond 1 and pond 2, and the effluent during the sampling period of August, October and December 2007, in which time the Waitoa dairy WWTP was under usual operating conditions.

1. Influent (wastewater to the treatment ponds)

It is evident firstly that EDTA was present in the influent when the cheese plant was not operating and EDTA was not detected in the wet process flume on 27-30 August 2007. This can be caused either by the partial CIP water recycling or by a residue of EDTA in the site sump. It is also possible that EDTA was applied in other production processes (Udabage et al. 2000). Secondly, the concentration of

EDTA in the influent to the WWTPs varied between 1400 µg/L and 5200 µg/L from the whole dairy factory in October and December 2007. In a continuously sampled week of December 2007, the averaged concentration of EDTA in influent from the factory was 2400 µg/L.

2. EDTA in treatment ponds

The Waitoa dairy wastewater treatment plants (Figure 4.2 and Figure 4.3) were operated under aerobic conditions and remained highly efficient with COD removal of 99%. The operating pH of the ponds was controlled at 8.0–8.2, and the sludge retention time was 3 weeks on average. Concentrations of EDTA with one standard deviation in pond1 and pond 2 are also illustrated in Figure 4.15. It can be seen that concentrations of EDTA in treatment ponds were relatively high in August and even in October 2007, ranging from 249–475 µg/L in pond 1 and 179-299 µg/L in pond 2. For pond 1, the averaged EDTA value was 388 µg/L in August and 376 µg/L in October. For pond 2, the averaged EDTA value was 238 µg/L in August and 250 µg/L in October. In particular for October 2007, one of aerators malfunctioned in pond 2 during when the wastewater sample collection. This indicates that the biodegradability of EDTA in ponds is strongly dependent upon the operation of the WWTPs, and it may not be fully operated due to the machinery down time for maintenance during the low milk production season.

3. Effluent (wastewater discharged into the environment)

The detected EDTA concentrations in the effluent discharged into the local waterway of the Waitoa River varied between 72 and 261 µg/L. The averaged EDTA concentration of the effluent was 135, 212 and 118 µg/L in August, October and December 2007, separately. These values were well below the Predicted No Effect Concentration (PNEC) for aquatic environments of 2.2 mg/L EDTA suggested by the European Union (European Chemicals Bureau, 2004).

4.3.3 EDTA Removal Efficiency by an Extended Aeration Activated Sludge at the Fonterra Waitoa Dairy Site

Figure 4.16 shows the overall averaged EDTA concentration with a standard deviation for wastewater samples from the influent, treatment ponds, and effluent at the Waitoa dairy WWTPs during sampling periods. The mass balance of EDTA during the treatment process is explained in Table 4.4, when the dairy WWTPs were under normal operation.

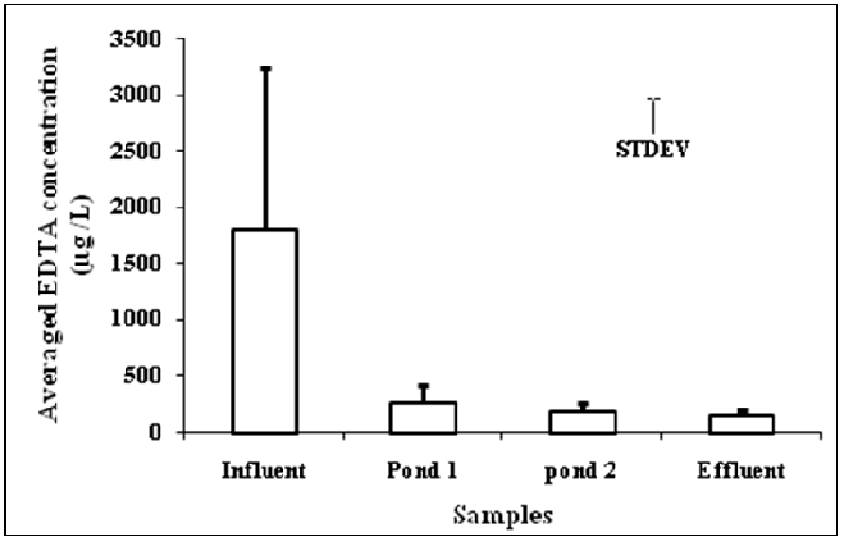

Figure 4.16 Overall calculated EDTA changes during an extended aeration activated sludge treatment process at the Waitoa dairy wastewater treatment plant, based on 13 samples collected in August, October and December, 2007.

Table 4.4 Mass balance of EDTA at the Waitoa dairy wastewater treatment plant, based on 13 days of August, October and December, 2007.

Sample	Influent			Effluent		
Date	Volume (m^3)	EDTA Con. (µg/L)	EDTA amount (g)	Volume (m^3)	EDTA Con. (µg/L)	EDTA amount (g)
27-28 Aug. 2007	9420	301	2835	6533	152	993
28-29 Aug. 2007	9963	93	927	7374	93	686
29-30 Aug. 2007	7056	226	1595	8343	161	1343
21-22 Oct. 2007	10374	1420	14731	7863	227	1785
22-23 Oct. 2007	11025	3170	34949	8598	261	2244
23-24 Oct. 2007	11549	1374	15868	7945	147	1168

09-10 Dec. 2007	5293	1997	10570	5954	98	583
10-11 Dec. 2007	4208	1211	5096	2986	72	215
11-12 Dec. 2007	5145	5194	26723	3234	78	252
12-13 Dec. 2007	6394	1714	10959	5098	185	943
13-14 Dec. 2007	5242	1625	8518	6942	147	1020
14-15 Dec. 2007	8468	1738	14717	6664	115	766
15-16 Dec. 2007	9620	3505	33718	7235	130	940
Average	**7981**	**1813**	**13939**	**6521**	**144**	**995**

It can be seen from Table 4.4 that

(i) there may have a significant difference between volumes of the influent and the effluent. On any one day, water level of the ponds can be allowed to rise or fall to keep the flow to the river constant. So one day the effluent discharge can be higher than the influent and the next it might be the opposite;

(ii) Evaporation of wastewater during the treatment can be quite high as there is over 3000 m^2 of surface area of the ponds. The actual area in contact with air is much larger due to the aeration. Evaporation of wastewater on average, as advised by the factory, is evidently 10 % of the wastewater volume of influent. In this case, there was no significant difference (4.5%) between the influent (7981 m^3) and effluent (6521 +798 + 300 = 7619 m^3).

The reduction of EDTA from the dairy WWTPs is defined as the mass difference of EDTA between the influent and the waste discharges, which include the sludge and effluent. The influent from the factory site took about 6-7 days to be discharged into environments due to large capacity of the ponds and clarifiers. Thus the reduction of EDTA from the dairy WWTPs cannot be assessed on a daily basis.

The removal of EDTA from the wastewater treatment processing was calculated as 93% based upon a mass difference between the overall averaged EDTA amounts of the influent and waste discharges (including the effluent and sludge) during the sampling period of August, October and December 2007 (Table 4.5).

Approximately 93 % of EDTA removal was therefore achieved by an extended aeration activated sludge treatment, operated at a pH value of 8.0 – 8.2 and sludge age of three weeks at the Waitoa dairy WWTPs.

Table 4.5 EDTA removal efficiency by an extended aeration activated sludge system operating under pH 8.0-8.2 and 3-week sludge age at the Waitoa dairy site, Fonterra Co-operative Group Limited, based on 13 sampled collected in August, October and December, 2007.

Items	EDTA mass (g/day)	Percentage (%)
Influent	13939	100
Effluent	995	7.1
Sludge	43	0.3
Breakdown	12901	92.6

4.4 DISCUSSION

4.4.1 Variation of EDTA Concentrations in the Industrial Wastewater

As EDTA is water-soluble and not volatile, it is eventually released into wastewater. The investigation of Schmidt et al. (2004) reported that variation of EDTA concentrations was detected between 100 and 20,000 μg/L in the industrial wastewater in Germany. Concentrations of EDTA in wastewater of the dairy and beverage industry were observed from 2,500 to 25,000 μg/L in Germany.

Varied concentrations, as high as 82,700 μg/L of EDTA, were detected in the dairy wastewater from processing plants at the Waitoa dairy site. During the high milk production season of October and December in 2007, in which time the factory was operating to capacity, concentrations of EDTA in the influent into watstewater treatment plants from the Waitoa dairy factory were tested ranging from 1,200 to 5,200 μg/L. Concentrations of EDT in the effluent discharged into the local waterway from the Waitoa wastewater treatment plant was 72- 260 μg/L of EDTA.

4.4.2 EDTA Removal Efficiency by an Activated Sludge Biological Treatment

EDTA is generally believed to either resist degradation or undergo slow biodegradation (Kari and Giger, 1995; European Chemicals Bureau, 2004). However, it can be degraded under favorable conditions, which depend on the speciation of EDTA, the bacterial population and operating conditions during the biological treatment processing (Egli, 2001; van Ginkel and Geerts, 2005).

1. Speciation of EDTA in the wastewater treatment ponds

The primary objective for the use of EDTA in the dairy industry is to prevent precipitation of calcium, magnesium or other minerals. Table 4.6 shows total metal monitoring results (sodium and potassium not included) in wastewater treatment ponds at the Waitoa wastewater treatment plant.

Table 4.6 Total metal monitoring results from 2004 and 2005 in wastewater treatment ponds at the Waitoa dairy site.

	Pond 1				Pond 2			
Tests	**T*Ca** (mg/L)	**TMg** (mg/L)	**TFe** (mg/L)	**TAl** (mg/L)	**TCa** (mg/L)	**TMg** (mg/L)	**TFe** (mg/L)	**TAl** (mg/L)
1	90.5	22.8	10.1	35.8	83.4	21.5	8.8	32.5
2					80.7	22.7	11.1	
3					95	23	8	
4	99.9	22.8	7.3	15.2	92.6	20.6	5.9	13.3
5					95.2	21.5	4.9	
6	95.3	22.4	5.3		92.6	21.6	5.3	
7	102	24.4	6.5	11.1	94.7	22.4	5.6	9.57
8					86.1	18.2	6.3	
9					93.1	22	10	
10	80.7	24.8	8.1		81.5	25.2	8.3	
Mean	**93.7**	**23.4**	**7.5**	**20.7**	**89.5**	**21.9**	**7.4**	**18.5**

T* - total

(Source: Waitoa dairy factory)

Based upon calculations of metal-EDTA stability constants as demonstrated in Table 4.7, the majority of the EDTA is likely to be in the form of an iron(III)

complex in the aerobic treatment ponds as the K_{FeEDTA}*[Fe] is so much higher than other metals.

Table 4.7 Calculation of EDTA speciation in the treatment ponds based on the EDTA complexing constant at the Waitoa dairy wastewater treatment plants.

Treatment ponds	Metals	Me. Con. (mg/L)	M/W (g/molar)	Me. Con. (mM)	LogKa	Ka*[Me]
Pond 1	**TMg**	23.44	24.3	0.96	8.83	7.0E+08
	TCa	93.68	40.1	2.34	10.61	9.0E+10
	TAl	20.7	26.98	0.77	16.5	3.0E+16
	TFe	7.46	55.8	0.13	25	1.0E+24
Pond 2	**TMg**	21.87	24.3	0.9	8.83	6.0E+08
	TCa	89.49	40.1	2.23	10.61	9.0E+10
	TAl	18.46	26.98	0.68	16.5	2.0E+16
	TFe	7.42	55.8	0.13	25	1.0E+24

However, the total iron concentration in the pond reflects Fe^{3+} (iron exists as Fe^{3+} due to the aerobic condition) in solution plus Fe^{3+} associated with particulate matter, such as dirt, protein, and fats which may have negative charges to which Fe^{3+} will be attracted. Fe^{3+} will be precipitated under alkaline conditions when pH is controlled at ~8.0 in treatment ponds via the following reaction:

$$Fe^{3+}(aq) + 3OH^{-}(aq) \leftarrow \rightarrow Fe(OH)_3(s)$$

The concentration of Fe^{3+} precipitated with OH^- will be $⅓x10^{-6}$M assuming pH is 8.0 in the treatment ponds. Meanwhile, Fe^{3+} complexes with the strong chelating agent EDTA to form a 1:1 complex via the following reaction:

$$Fe^{3+}(aq) + EDTA^{4-}(aq) \leftarrow \rightarrow [Fe(III)EDTA]^{-}$$

The complexing stability constant is Ka = $[Fe(III)EDTA]^-/[Fe^{3+}]*[EDTA^{4-}]$ (Ka = 10^{25} at 25^0C).

In treatment pond 1 the concentration of Fe^{3+} required for complete complexing with EDTA, is 0.4-1.7 μM which is the same as the EDTA concentration (**Error! Reference source not found.**). The actual total Fe concentration in pond 1 was 0.13 mM, which is three orders of magnitude higher than the EDTA concentration. This suggests that the majority, if not all, of the EDTA will be in the form of an

Fe(III) complex in the aerobic treatment ponds. Theoretically, the EDTA concentration could be as high as 37,440 µg/L if Fe(III) was completely complexed in treatment ponds.

2. Biodegradablity of EDTA in the activated sludge treatment process

The principle of a biological wastewater treatment of aerobic process, such as activated sludge system, is to use naturally contained micro-organisms in the sludge converting undesirable material into environmentally benign substances. EDTA has been reported to have no notable degradation with conventional wastewater treatment plants. Nonetheless, it has been proved that EDTA can be removed by activated sludge systems operated under alkaline conditions (van Ginkel et al., 1997; van Ginkel and Boelema, 1999). Removal of EDTA in activated sludge systems depends upon (i) sludge retention time, (ii) alkaline medium, and (iii) activated sludge with a wide range of micro-organisms (van Ginkel and Boelema, 1999).

van Gingle and Boelema (1999) demonstrated that the microbial population contained in dairy activated sludge is able to biodegrade EDTA during the wastewater treatment process. To achieve an effective EDTA removal by the microbiological degradation, it has been suggested that the pH of reaction mixtures should preferably remain at 7-9 with a sludge retention time of at least one week (van Ginkel et al., 1997; van Ginkel and Boelema, 1999). Other contaminants in the wastewater containing EDTA can be effectively purified at a pH of about 8-9 by using microorganisms in flocs without employing a special material to carry the microorganisms (van Ginkel and Boelema, 1999). The relatively high sludge retention time required for the degradation of EDTA could be due to the slow kinetics of the reactions (Sillanpää and Pirkanniemi, 2001). EDTA nitrogen has been converted into either nitrate or ammonia to evaporate during the process of EDTA biodegradation at the activated sludge wastewater treatment plant (van Ginkel and Boelema, 1999).

At the Waitoa wastewater treatment plant, wastewater from the dairy processing plants was treated by an extended aeration sludge treatment system operating at

pH of 8.0-8.2 and three-week sludge retention time. The EDTA removal efficiency compared with the international research is demonstrated in Table 4.8.

Table 4.8 EDTA removal efficiency at the Waitoa dairy wastewater treatment plant compared with the international research results under various pH and sludge retention time (SRT)

Wastewater type	Sludge type	pH	SRT (day)	EDTA Removal (%)	Sources
Dairy	***dairy***	***8.0-8.2***	***~20***	***~93***	***this study***
Dairy	dairy	7.5-8.1	~20	~ 90	van Ginkl and Geerts, 2005
Beer	beer	7.3-7.7	~23	~ 50	van Ginkl and Geerts, 2005
Dairy	dairy	7.8-8.4	~9	~ 30	van Ginkl and Geerts, 2005
Dairy+domestic	municipal	6.9-7.1	~20	0	van Ginkl and Geerts, 2005
Dairy+domestic	municipal	8.7-8.9	20	95	van Ginkl and Geerts, 2005
Municipal	municipal	8.0-9.0	>28	100	van Ginkl and Boelema, 1999
Municipal	municipal	8.0-9.0	>29	>89	van Ginkl and Boelema, 1999
Municipal	industrial	8.6-8.8	>49	100	van Ginkl and Boelema, 1999
Municipal	industrial	8.7-8.9	>49	72-100	van Ginkl and Boelema, 1999
Municipal	paper mill	8.5-9.0	10	~ 80	van Ginkl and Boelema, 1999

4.5 CONCLUSIONS

i. An HPLC-UV method was used to investigate occurrences of EDTA in dairy processing wastewater and EDTA removal efficiency through an activated sludge biological treatment process. The method has demonstrated a good linearity (r^2 0.9988–0.9998), duplicate limits (less than 6.3%, n=5) and EDTA standard spike recovery (100-102%, n=6). The method detection limit (MDL) was 5 µg/L of EDTA.

ii. Significant concentration of EDTA was observed in wastewater samples of the manufacturing plants, where the Eliminator or Eliminator II containing 34 – 36 % of EDTA was applied in the CIP system of the cleaning process. The highest concentrations of EDTA from the cheese

drain and the wet process were approximately 77000 µg/L and 83000 µg/L, respectively. However, those levels of EDTA were below the value of EDTA (0.1%) controlled by the process plant at the Waitoa dairy site, Fonterra Co-operative Group Limited.

iii. During the lower milk production season, EDTA was detected in influent when no Eliminator or Eliminator LF had been applied to either the cheese or wet process plants. This indicates that there may have some other EDTA sources, which may either involve the production process to change the product features or originate from the CIP system of other plants due to the recycling of cleaning water. Concentrations of EDTA in the influent into the wastewater treatment plant varied from 90 µg/L to 5200 µg/L were detected. The EDTA concentration of the influent also reflects the level of manufacturing activities.

iv. The analyses showed an effective reduction of 93% EDTA was achieved by the extended aeration activated sludge process from the dairy wastewater treatment plants, operated under alkaline conditions of pH 8.0–8.2 with 3-week sludge retention time.

v. The concentration of EDTA detected in the Waitoa dairy effluent discharged into the Waitoa River was 72-260 µg/L during the sampling period of August, October and December in 2007, which is well below the threshold value of 2.2 mg/L of predicted effect concentration for the aquatic environment, advised by the European Union

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5.0 CHAPTER FIVE: EDTA AND ASSOCIATED HEAVY METALS IN THE WAITOA RIVER

The widespread occurrence of EDTA and its poor biodegradability under many environmental conditions led to recognition that EDTA likely comprised the highest concentration of anthropogenic compounds in many surface waters in Europe, and perhaps even in the world (Chapter 2). The International Commission for the Protection of the Rhine identified EDTA as the only relevant chelating agent in the Rhine catchments (Knepper, 2003). The International Association of Waterworks in the Rhine catchment area recommended an EDTA target value of 5 μg/L for surface waters at 90-percentile of one year (Schmidt et al. 2004).

High concentration of EDTA in surface waters is possible to disturb the natural speciation of metals, to affect metal bioavailability, and consequently to affect organisms in the aquifer, or pose a risk to groundwater and drinking waters (Nowack, 2002). Presently, there is no regulation of EDTA concentration for surface waters in New Zealand, except that a maximum acceptable value of EDTA for drinking waters is set as 0.7 mg/L for health purposes (Ministry of Health, 2005).

The specific objective of this chapter is to investigate occurrences of EDTA and associated heavy metals in the Waitoa River, into which large volumes of wastewater containing EDTA are discharged from the Fonterra Waitoa dairy site. Purposes of this investigation are to identify EDTA concentrations in the Waitoa River subjected to the dairy effluent discharge from the Fonterra Waitoa site, and ascertain the potential of heavy metals remobilized from rive sediments due to the EDTA chelates in the receiving water of the Waitoa River.

5.1 OCCURRENCES OF EDTA IN THE WAITOA RIVER

5.1.1 Sample Collection

Surface water samples were collected at 2500 m (site 1) and 10 m (site 2) upstream, 10 m (site 3) and 60 m (site 4) downstream from the Waitoa dairy effluent discharge point in the Waitoa River on 28-30 August, 22–24 October, 2007, shown in Figure 5.1- Figure 5.5. Surface water samples included one morning and one afternoon sample which were combined with two morning and two afternoon sub-samples, respectively. All samples were collected in opaque PE bottles to avoid photolysis of the $[Fe(III)EDTA]^-$ and refrigerated at 4^0C until analysis. At time of sample collection the river was approximately 6-8 m wide and 1-2 m deep.

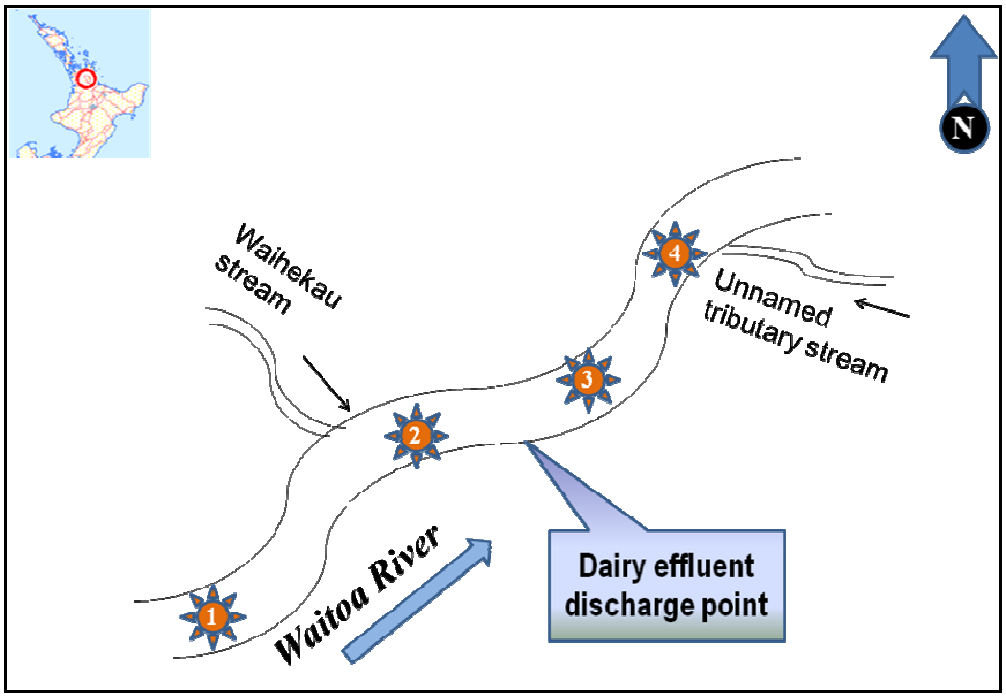

Figure 5.1 Locality sketch for surface water sampling sites, including 2 upstream and 2 downstream from the dairy effluent discharge point in the Waitoa River where large volumes of dairy effluents discharged from the Waitoa dairy wastewater treatment plants.

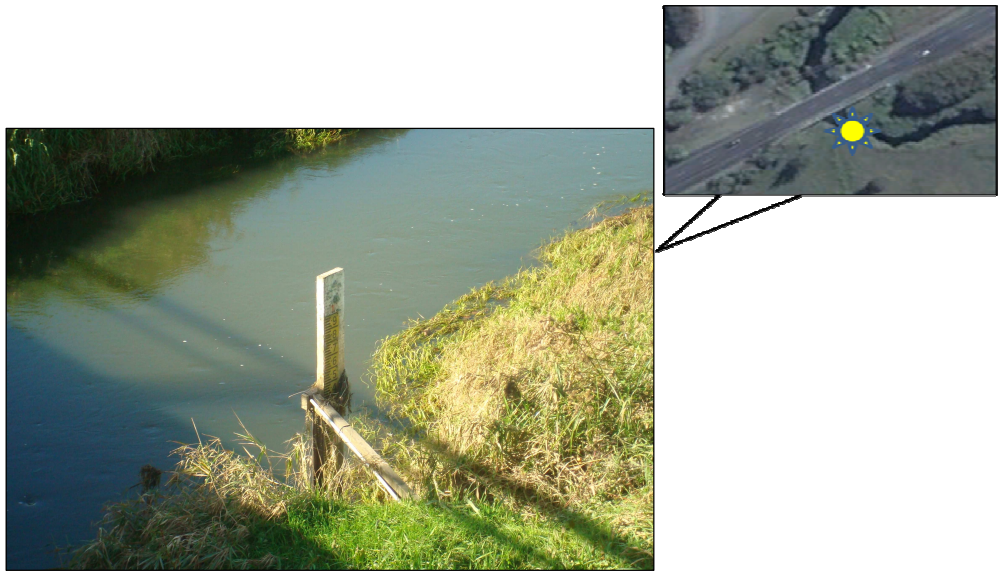

Figure 5.2 Surface water sampling site 1 at 2,500 m upstream from the Waitoa dairy effluent discharge point (SH 26 Bridge) in the Waitoa River

Figure 5.3 Surface water sampling site 2 at 10 m upstream from the Waitoa dairy effluent discharge point in the Waitoa River.

Figure 5.4 Surface water sampling site 3 at 10 m downstream from the Waitoa dairy effluent discharge point in the Waitoa River.

Figure 5.5 Surface water sampling site 4 at 60 m downstream from the Waitoa dairy effluent discharge point in the Waitoa River.

5.1.2 Determination of EDTA for Surface Water Samples

The established HPLC-UV analytical method in Chapter 3 needs to be modified for measuring EDTA in surface water samples due to their low concentrations. This is generally achieved by pre-concentrating surface water samples (Loyaux-

Lawniczak et al. 1999). However, the high calcium (Ca) and magnesium (Mg) with the pre-concentration process may affect the measurement (Nowack et al. 1996). It is necessary to obtain an appropriate pre-concentration factor for the determination of EDTA in surface waters, and to assure the pre-concentrated Ca and Mg do not affect the determination of EDTA.

1. Sample pre-treatment

Several experiments were designed not only with different pre-concentration factors to achieve the optimal pre-concentration factor (10*x*, 5*x*, 2*x* and 1*x*) (Figure 5.6), but also with a carboxylic acid (CBA) solid phase extraction (SPE) C18 clean cartridges (Phenomenex) to remove the interferences of Ca^{2+} and Mg^{2+} (Figure 5.7 and Figure 5.8). Pre-concentration of five times without a SPE cleaning cartridge was determined to detect the concentration of EDTA in surface waters.

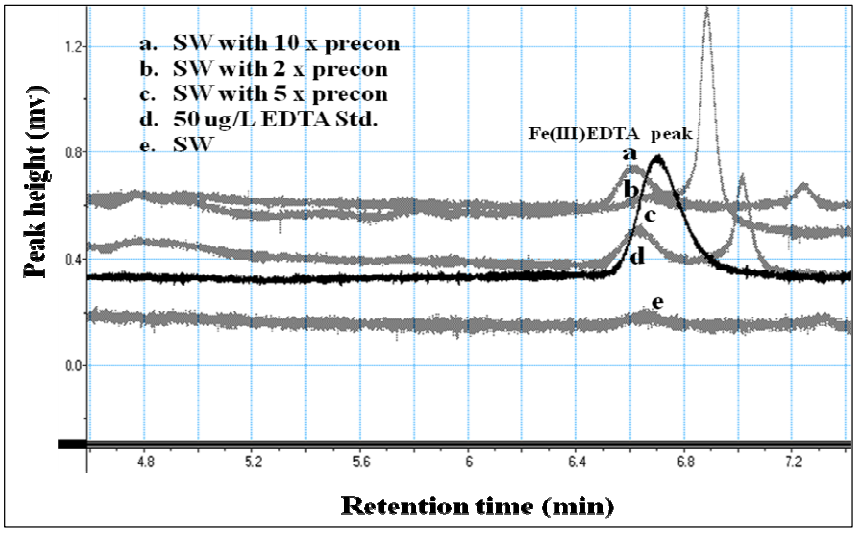

Figure 5.6 Overlay of chromatograms of surface water (SW) samples with x1, x2, x5 and x10 pre-concentration compared with a 50 µg/L EDTA standard solution.

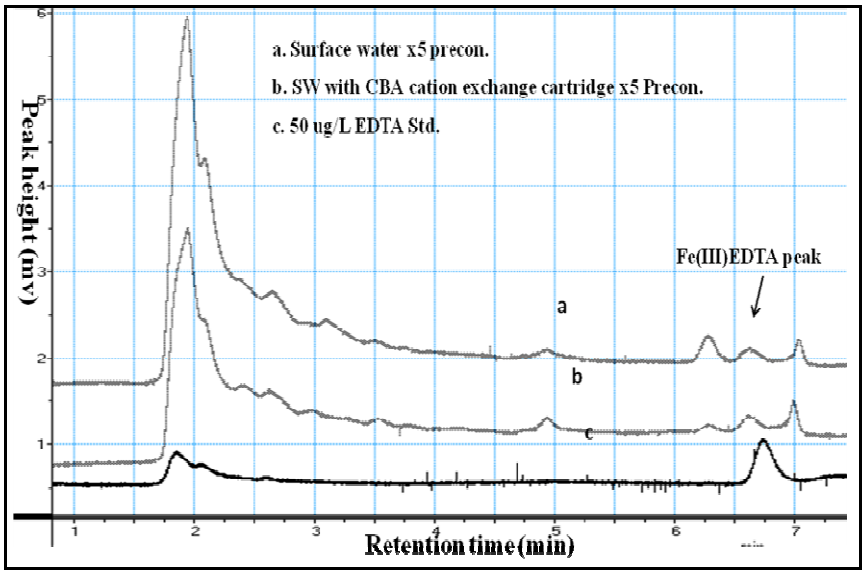

Figure 5.7 Overlay of chromatograms of the surface water sample with and without a CBA SPE clean cartridges, compared with a 50 µg/L EDTA standard solution.

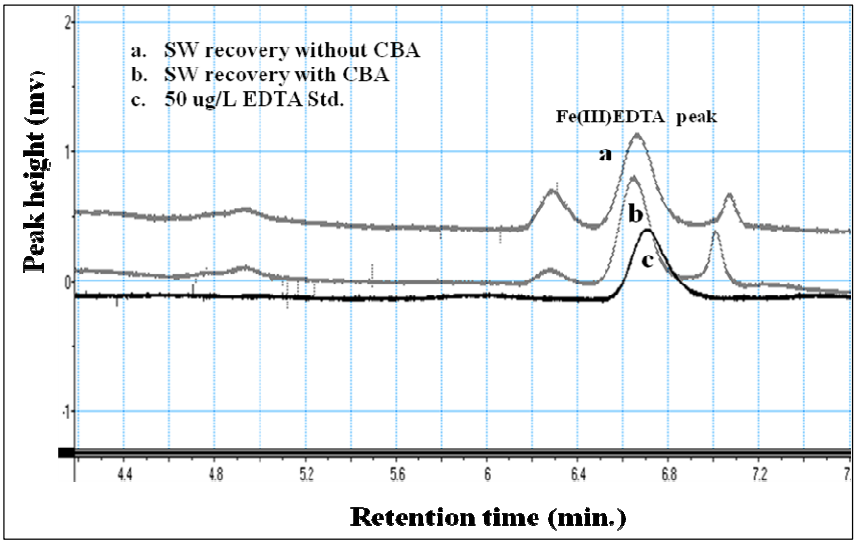

Figure 5.8 Overlay of chromatograms of the surface water spiked by a 50 µg/L EDTA standard with and without a CBA clean cartridge, compared with a 50 µg/L EDTA standard solution.

Sample pre-treatment involved the following steps:

- taking a 10 mL surface water sample into a 20 mL vial and heated to dryness in the 90^{o}C drying oven;

- adding 1.5 mL mobile phase and 0.5 mL 1.94 mg/L Fe^{3+} solution to the vial, leaving overnight in the dark to allow complexing of Fe(III)EDTA;
- filtering the sample through 0.45 µm cellulose nitrate filters (Phenomenex) to a test tube; and
- injecting a 50 µL sample into the HPLC system at ambient temperature.

This process gave a five-fold pre-concentration for determination of EDTA in surface waters. The detection limit was actually 1µg/L of EDTA calculated by the method detection limit of 5µg/L EDTA for HPLV-UV.

2. *EDTA Concentrations in the Waitoa River*

Forty eight surface water samples were analyzed by the HPLC-UV method with a pre-concentrated factor of 5. A calibration curve was established daily at the concentration of 0 - 150 µg/L (0, 10, 20, 50, 80, 100 and 150 µg/L). A blank, a duplicate every 10th sample and a spike recovery of 50 µg/L EDTA standards every 20th sample were undertaken per run for a quality control. The averaged duplicate variability was within 8.1 % (n=5) and the spiked recovery varied from 97 – 107% (n=3). A typical chromatogram of the surface water sample is shown in Figure 5.9. A daily work is attached as Appendix 2.

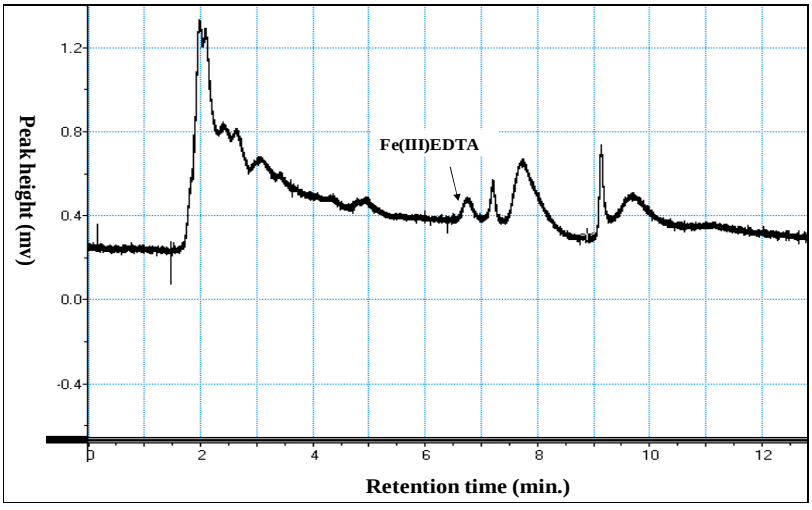

Figure 5.9 Chromatogram of a typical surface water sample from the Waitoa River.

Table 5.1 gives analytical results of EDTA upstream and downstream from the Waitoa dairy effluent discharge point in the Waitoa River during sample periods of August and October, 2007. Figure 5.10 illustrates median and highest concentrations of EDTA at the sampling sites in the Waitoa River. Figure 5.11 presents the overall averaged pH value, temperature and EDTA concentration at sampling sites during sampling periods,

It can be seen from Table 5.1 that both median and the highest concentrations of EDTA were slightly, but not significantly increased 60 m downstream of the dairy effluent discharge point. The highest concentration of EDTA detected at 60 m downstream was 2.7 µg/L during the sampling periods. This finding is also confirmed by the effects of pH and temperatures on the Waitoa River (Figure 5.11).

5.1.3 Analysis of Associate Metals in the Waitoa River

Associated metals were also analyzed for collected surface water samples by ICP-MS method. This was conducted by Hill Laboratories, Hamilton. The details were attached in Appendix 3.

5.1.4 Other EDTA Sources to the Waitoa River

Apart from the Fonterra Waitoa dairy effluent containing EDTA discharged into the Waitoa River, there is another source of EDTA, namely the Wallace Corporation Limited (Wallace). Wastewater, generated from a meat rendering plant, an abattoir, and a tannery, is treated in a site collective pond system and then discharged into the Waitoa River. The Wallace is located 3 km upstream of the Fonterra Waitoa dairy site (Figure 5.12). It has been suggested by Mr. S. Carter (personal communication), who is the environmental manager of the Wallace Corporation Limited, that small amounts of EDTA are applied in the tannery. In order to clarify this potential discharge of EDTA, samples were randomly collected on 30 May 2008 from the pond where wastewater was ready to be discharged, upstream and downstream of the company boundaries in the Waitoa River. Analytical results of EDTA are shown in Table 5.2. EDTA is

clearly present in the wastewater of the Wallace. Moreover, the EDTA contribution of the Wallace is also demonstrated by the difference in EDTA concentrations upstream and downstream of the company boundaries in the Waitoa River.

Table 5.1 Tested concentrations of EDTA in the Waitoa River, August and October, 2007.

Date	Sample	River Flow	River Height	EDTA concentration (µg/L)				pH				Temperature (^{0}C)			
				US*[1] 2500 m	US 10 m	DS 10 m	DS*[1] 60 m	US 2500 m	US 10 m	D1 10 m	DS 60 m	US 2500 m	US 10 m	DS 10 m	DS 60 m
		(m^3/s)	(m)												
28 Aug.	am	2.28	13.85	1.3	<1*[2]	1.0	<1	8.8	9.4	9.3	9.2	13.1	13.2	13.2	13.3
2007	pm			1.9	<1	1.1	2.7	9.2	9.3	9.2	9.2	13.4	13.4	1.3	13.7
29 Aug.	am	2.8	13.95	8.0*[3]	1.0	1.0	2.5	8.3	8.2	8.2	8.6	13.2	13.2	13.1	14.4
2007	pm			<1	<1	<1	1.5	8.4	8.5	8.2	8.6	15.6	14.4	14.1	14.4
30 Aug.	am	2.80	13.95	1.1	2.5	1.1	1.0	8.6	8.7	8.7	8.6	13.3	14.8	12.8	12.9
2007	pm			1.4	1.0	2.4	1.5	8.9	8.6	8.6	8.8	13	14.9	13.3	15.7
22. Oct.	am	6.76	13.95	1.2	<1	<1	<1	6.7	7	7.1	6.9	13.8	13.8	13.8	13.9
2007	pm			<1	<1	<1	1.8	6.9	7.1	7	6.9	15.1	14.6	14.3	14.4
23. Oct.	am	3.78	13.82	<1	<1	<1	1.2	6.9	6.9	6.8	6.9	15.3	15.1	15.2	15
2007	pm			<1	<1	<1	1.0	6.8	6.9	7	6.9	18.5	16.3	16.3	16.3
24. Oct.	am	3.19	13.78	2.2	2.0	1.7	1.8	6.9	6.8	6.8	6.9	16	16	16.2	16.1
2007	pm			<1	1.8	<1	1.1	6.9	6.8	6.9	6.9	18	17.9	17.9	18
Mean		**3.60**	**13.89**	**1.3**	**1.3**	**1.2**	**1.5**	**7.8**	**7.9**	**7.8**	**7.9**	**14.8**	**14.8**	**14.5**	**14.8**

*[1] US – upstream, DS – downstream
*[2] EDTA concentration below the method detection limit
*[3] not included for the mean value calculation

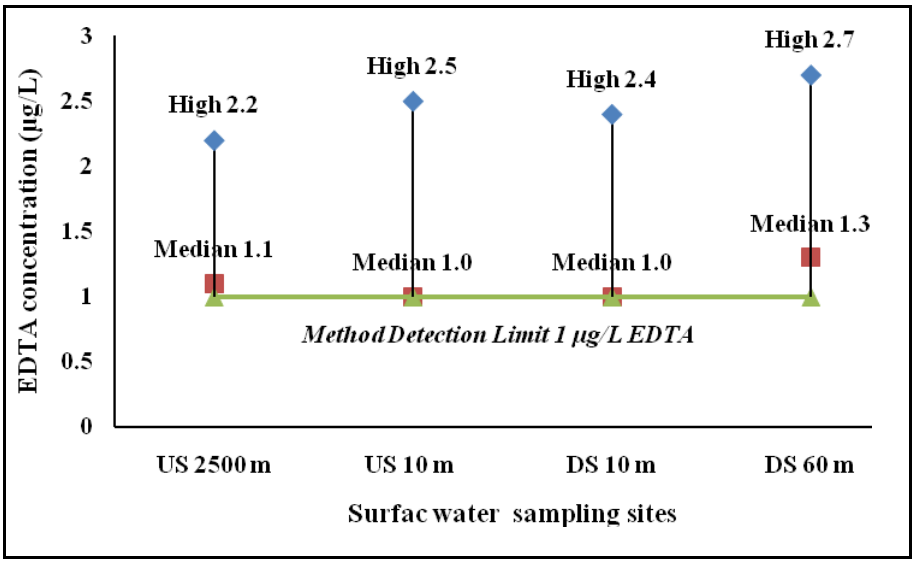

Figure 5.10 Median and highest EDTA concentrations of 12 surface water samples collected upstream (US) and downstream (DS) from the Waitoa dairy effluent discharge point in the Waitoa River in August and October, 2007.

(Source: Xie et al. 2008)

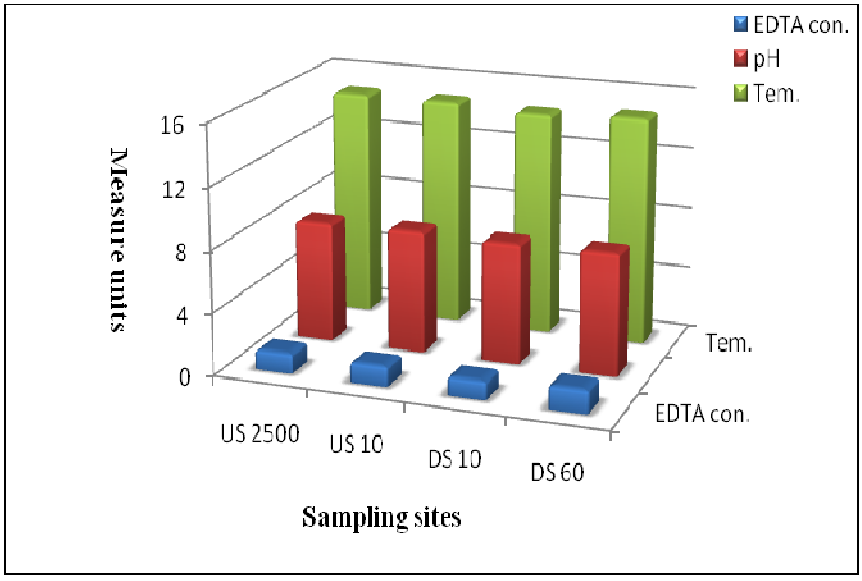

Figure 5.11 Averaged pH, temperature and EDTA concentrations of 12 surface water samples collected upstream (US) and downstream (DS) from the Waitoa dairy effluent discharge point in the Waitoa River in August and October, 2007.

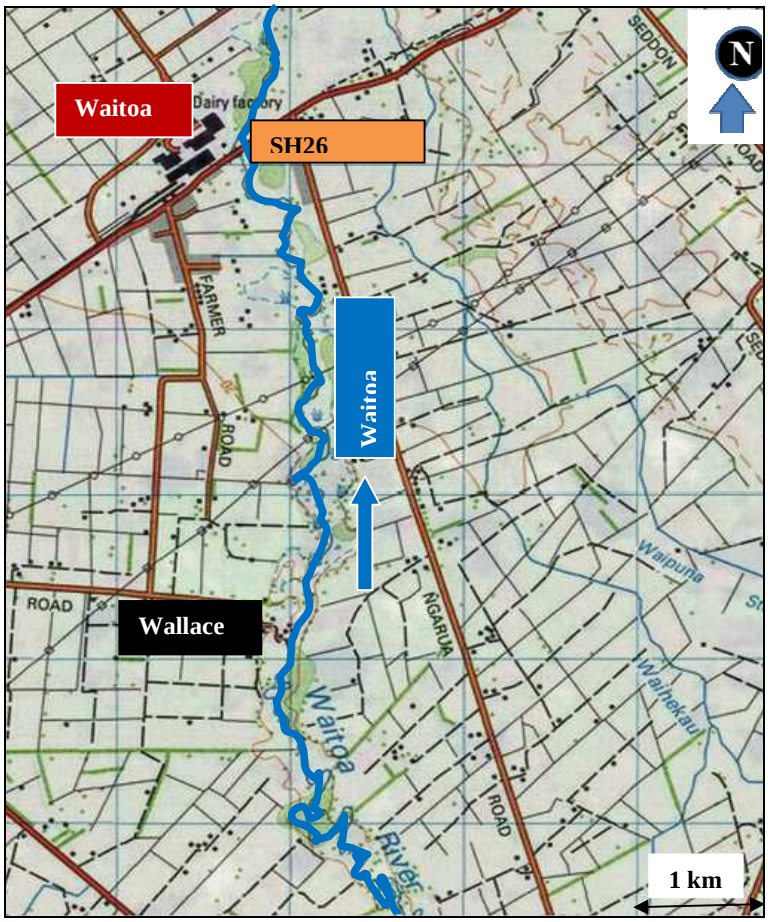

Figure 5.12 Effluent containing EDTA discharged into the Waitoa River from the Wallace Corporation Limited, located 3 km upstream of the Fonterra Waitoa dairy site.

(Source: Map Toaster Topo/NZ)

Table 5.2 EDTA analytical results relating to wastewater discharge into the Waitoa River from the Wallace Corporation Limited, sampled on 30 May 2008.

Surface water samples	EDTA concentration (µg/L)
Upstream Wallace boundary	1.0
Wallace wastewater pond	282.4
Downstream Wallace boundary	3.6
State Highway 26 Bridge	3.1
Upstream Waitoa (10m)	3.1

Waitoa effluent discharge	102.7
Downstream Waitoa (60 m)	3.1

5.2 ASSOCIATED METALS IN THE WAITOA RIVER

5.2.1 Metals in the Dairy Wastewater

The minor and trace element contents in milk and dairy products have been well documented and could differ from the diet, seasonal and geographic changes (Coni et al., 1994, 1995 and 1996; Lindmark-Månsson et al., 2003; García et al., 2006). Table 5.3 demonstrates metal differences of Na, K, Ca and Mg between dairy effluents from the Fonterra Waitoa dairy site and local (Morrisville) raw waters. It can be seen that concentrations of Na, K, Ca and Mg in dairy effluents have been increased significantly compared with the local raw water. The increase may originate from milk itself or chemicals used in the dairy processing, such as the addition of nutrient ingredients of calcium (Ca).

Table 5.3 Comparison of metal contents between dairy effluents from the Fonterra Waitoa dairy site and local raw waters (Morrisville).

	Dairy Effluent				Clean Water			
Date	**Total Ca (mg/L)**	**Total Mg (mg/L)**	**Total Na (mg/L)**	**Total K (mg/L)**	**Total Ca (mg/L)**	**Total Mg (mg/L)**	**Total Na (mg/L)**	**Total K (mg/L)**
Nov.06	36.7	4	686	167	-	-	-	-
Oct. 06	41.5	3.5	728	141	3.37	2.4	14.7	2.53
Oct. 06	-	-	-	-	3.87*	2.72*	16.3*	2.55*
Sep. 06	37.6	5.32	678	152	3.27	2.33	14.7	2.59
Aug.06	40.1	4.06	6.5	125	4.54	2.95	19.2	3.44
Aug. 06	34.2	4.86	802	88.8	5.84*	1.23*	5.89*	1.64*
July 06	24.7	6.84	477	37.1	-	-	-	-
June 06	25.3	8.31	541	58.6	3.17	1.79	11.2	1.96

* dissolved value

(Source: Fonterra Co-operative Group Limited, Waitoa)

5.2.2 Heavy Metals in the Waitoa River

One of the environmental concerns regarding EDTA is that high concentrations of EDTA may remobilize heavy metals from sediments, and transferred them into the aquatic phase where they could harm organisms in the aquatic system, or consequently pose a risk to groundwater and drinking waters (Sillanpää et al., 2001; Nowack and VanBriesen, 2005). Thus, it is important to identify heavy metals present in the Waitoa River. Tested metals included Na, K, Ca, Mg, Zn, Fe, Cd, Pb, Ni and Cr. A special heavy metal – Cr was selected as wastewater from a tannery factory, namely the Wallace Corporation Limited, is discharged upstream into the Waitoa River. All metals in the Waitoa River were analysed as dissolved metals by ICP-MS in R. J. Hill Laboratories of Hamilton (see appendix 3). The test results are shown and illustrated in Figure 5.13 - Figure 5.20.

Table 5.4 Analytical results for metal concentrations in surface water of the Waitoa River, August and October 2007.

Date		28 Aug.	2007	29 Aug.	2007	30 Aug.	2007	22 Oct.	2007	23 Oct.	2007	24 Oct.	2007	Mean	STDEV
River flow (m^3/s)		2.61		2.21		1.80		6.76		3.78		3.19		3.393	1794
River height (m)		13.94		13.83		13.72		14.32		13.82		13.70		13.89	0
Samples		**am**	**pm**	**am**	**pm**	**am**	**pm**	**am**	**pm**	**am**	**pm**	**am**	**pm**	-	-
Sodium ($mg\ L^{-1}$)	**US 2500 m**	17	17	16	15	18	16	12	12	15	12	16	12	15	2
	US 10 m	18	17	16	15	19	19	12	11	15	13	18	13	16	3
	DS 10 m	19	19	16	18	23	20	14	13	18	14	18	14	17	3
	DS 60 m	27	20	21	20	28	26	16	18	21	17	21	15	21	4
Potassium ($mg\ L^{-1}$)	**US 2500 m**	5.7	5.7	6.4	6.1	6.6	5.8	6.7	7.8	6.3	6.0	6.1	5.1	6.2	1
	US 10 m	5.8	5.9	6.5	6.2	6.7	6.7	6.9	6.7	6.9	6.4	7.9	6.0	6.6	1
	DS 10 m	6.0	6.0	6.0	6.2	6.9	6.2	6.7	6.2	7.0	5.7	6.3	5.3	6.2	0
	DS 60 m	7.2	5.8	6.4	6.6	7.8	7.1	6.4	7.0	7.4	6.1	6.5	6.6	6.7	1
Calcium ($mg\ L^{-1}$)	**US 2500 m**	9.6	9.6	8.7	8.3	9.1	8.7	8.0	8.1	8.2	8.1	8.8	7.9	8.6	1
	US 10 m	9.6	9.7	9.0	8.5	9.2	9.6	8.5	8.3	8.5	8.0	9.0	8.1	8.8	1
	DS 10 m	9.6	9.5	8.8	8.7	9.7	9.7	8.6	8.6	8.5	8.1	9.0	8.4	8.9	1
	DS 60 m	11.0	10.0	10.0	9.9	11.0	11.0	9.6	9.6	9.7	9.6	10.0	15.0	10.5	2
Magnesium ($mg\ L^{-1}$)	**US 2500 m**	4.2	4.3	3.9	3.7	3.9	3.9	3.5	3.6	3.7	3.6	3.9	3.6	3.8	0
	US 10 m	4.3	4.4	4	3.8	4	4.3	3.7	3.6	3.8	3.7	4.2	3.9	4.0	0
	DS 10 m	4.6	4.5	3.8	4.1	4.5	4.3	3.8	3.8	4.1	3.9	4.1	3.9	4.1	0
	DS 60 m	5	4.6	4.5	4.4	5	5	4.2	4.3	4.5	4.4	4.5	4	4.5	0
Zinc ($x10^{-3}$ mg/L)	**US 2500 m**	1.1	1.4	1.9	1.9	2.3	1.3	21	9.5	7.7	4.7	11	2.6	5.5	6
	US 10 m	2.8	<1.0	2	1.5	1.8	1.6	8.1	6.2	9.1	4.4	12	11	5.5	4
	DS 10 m	<1.0	2.1	1.7	1.5	1.4	1.3	7	7.5	5.8	6.3	6.6	3.4	4.1	3
	DS 60 m	1.7	<1.0	1.6	1.4	2.9	1.1	5.6	6.9	6.2	5.2	4.5	5.8	3.9	2

Cont.

	US 2500 m	55	51	69	43	55	59	230	190	240	180	240	310	144	0
Iron	**US 10 m**	58	58	55	59	66	68	270	250	230	280	160	300	155	104
($x10^{-3}$mg/L)	**DS 10 m**	57	61	68	48	59	80	280	260	240	290	180	290	159	106
	DS 60 m	82	60	53	57	79	55	260	210	220	280	230	240	152	94
	US 2500 m	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	-	-
Nickel	**US 10 m**	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	-	-
($x10^{-3}$ mg/L)	**DS 10 m**	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	-	-
	DS 60 m	0.63	<0.50	<0.50	0.55	0.72	0.63	0.56	0.51	<0.50	<0.50	<0.50	<0.50	0.6	0
	US 2500 m	0.58	0.71	0.94	0.88	0.83	0.77	0.98	0.93	0.73	0.71	0.72	0.73	0.8	0
Copper	**US 10 m**	0.66	0.55	0.77	0.77	0.77	0.74	0.99	0.83	0.67	0.7	0.66	0.7	0.7	0
($x10^{-3}$ mg/L)	**DS 10 m**	0.62	0.61	0.75	0.71	0.73	0.73	0.92	0.88	0.65	0.66	0.57	0.59	0.7	0
	DS 60 m	0.58	0.59	0.63	0.69	0.57	0.57	0.76	0.76	0.54	0.63	0.51	0.68	0.6	0
	US 2500 m	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	0.57	<0.50	0.6	-
Chromium	**US 10 m**	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	0.77	<0.50	0.8	-
($x10^{-3}$ mg/L)	**DS 10 m**	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	0.61	<0.50	0.6	-
	DS 60 m	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	0.52	<0.50	0.5	-
	US 2500 m	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	0.12	<0.050	<0.050	<0.050	<0.050	<0.050	-	-
Cadmium	**US 10 m**	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	-	-
($x10^{-3}$ mg/L)	**DS 10 m**	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	-	-
	DS 60 m	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	-	-
	US 2500 m	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	-	-
Lead	**US 10 m**	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	-	-
($x10^{-3}$ mg/L)	**DS 10 m**	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	-	-
	DS 60 m	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	-	-

Notes: (i) US (upstream) or DS (downstream) of the Waitoa dairy effluent discharge point
(ii) Analyses carried out by RJ Hill laboratories of Hamilton using ICP-MS

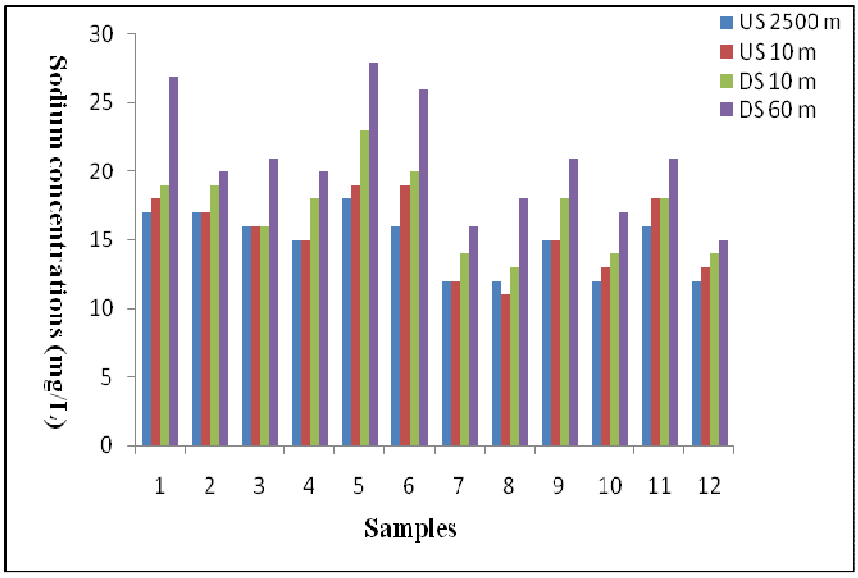

Figure 5.13 Test results of sodium (Na) concentrations for 12 samples collected upstream (US) and downstream (DS) from the Waitoa dairy effluent discharge point in the Waitoa River in August and October, 2007.

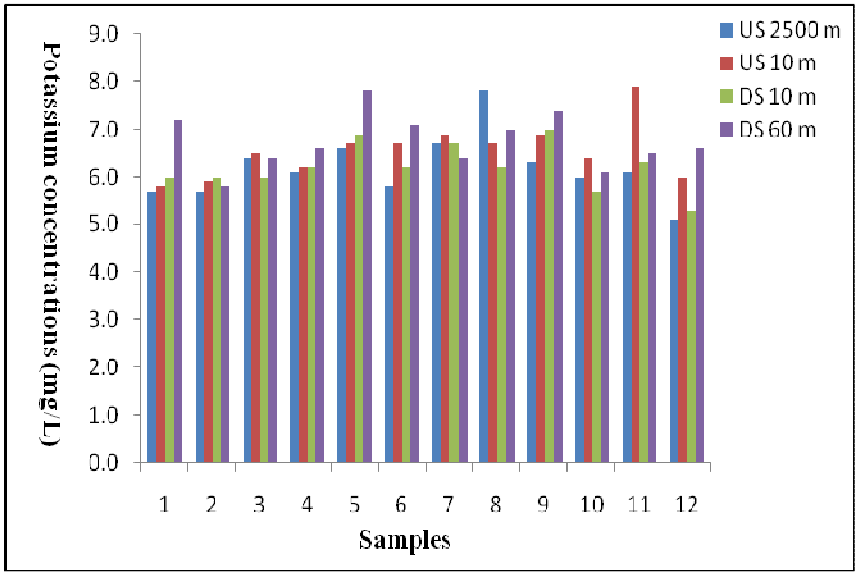

Figure 5.14 Test results of potassium (K) concentrations for 12 samples collected upstream (US) and downstream (DS) of the Waitoa dairy effluent discharge point in the Waitoa River in August and October, 2007.

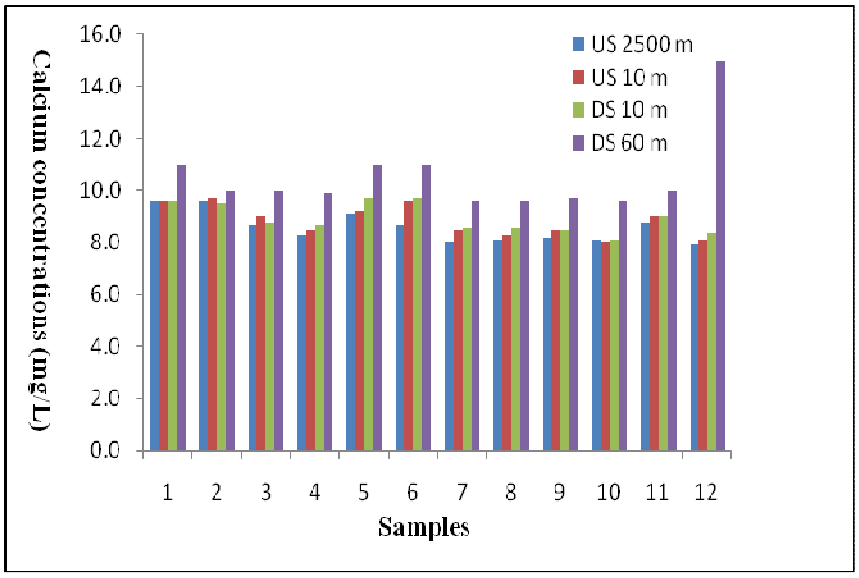

Figure 5.15 Test results of calcium (Ca) concentrations for 12 samples collected upstream (US) and downstream (DS) of the Waitoa dairy effluent discharge point in the Waitoa River in August and October, 2007.

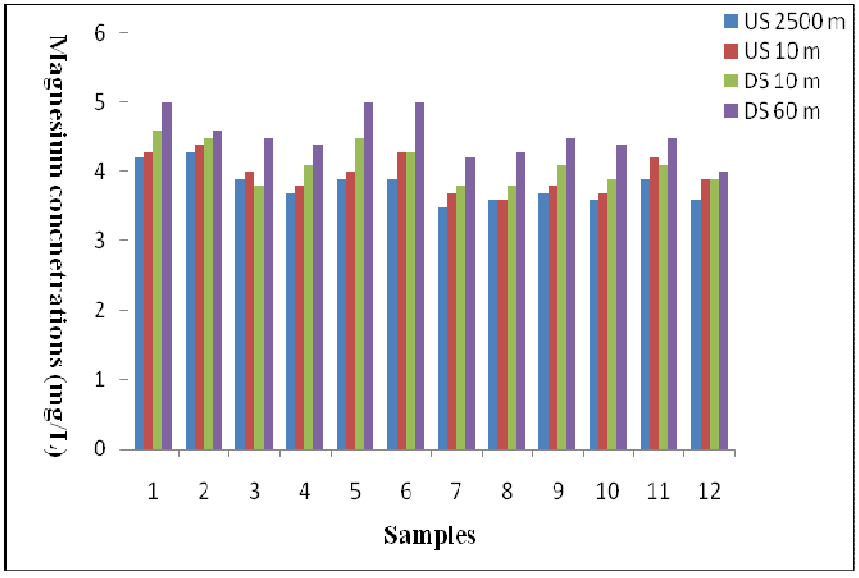

Figure 5.16 Test results of magnesium (Mg) concentrations for 12 samples collected upstream (US) and downstream (DS) of the Waitoa dairy effluent discharge point in the Waitoa River in August and October, 2007.

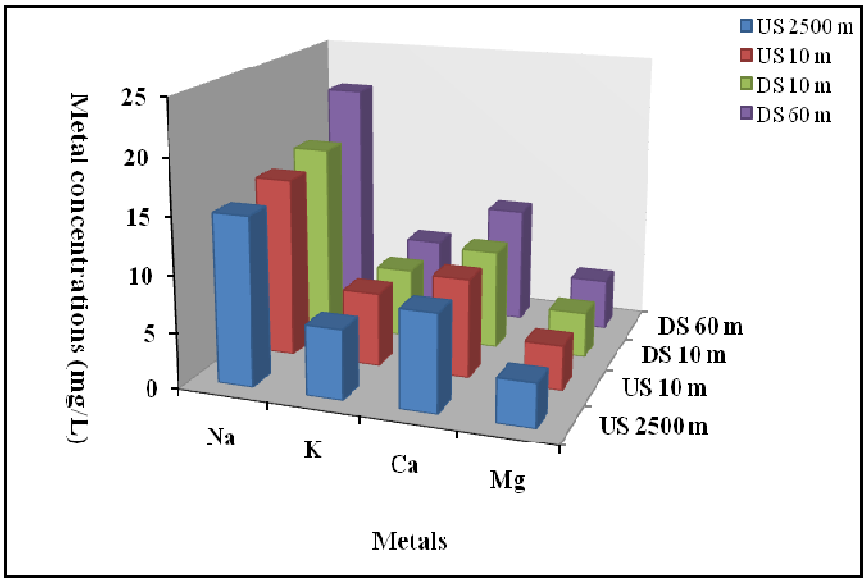

Figure 5.17 Mean concentrations of Na, K, Ca and Mg for 12 samples collected upstream (US) and downstream (DS) of the Waitoa dairy effluent discharge point in the Waitoa River in August and October, 2007.

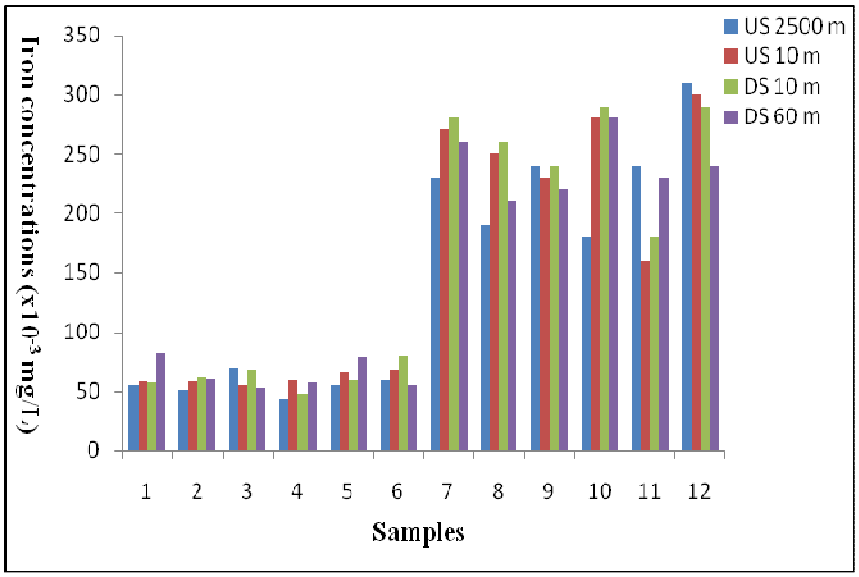

Figure 5.18 Test results of iron (Fe) concentrations for 12 samples collected upstream (US) and downstream (DS) of the Waitoa dairy effluent discharge point in the Waitoa River in August and October, 2007.

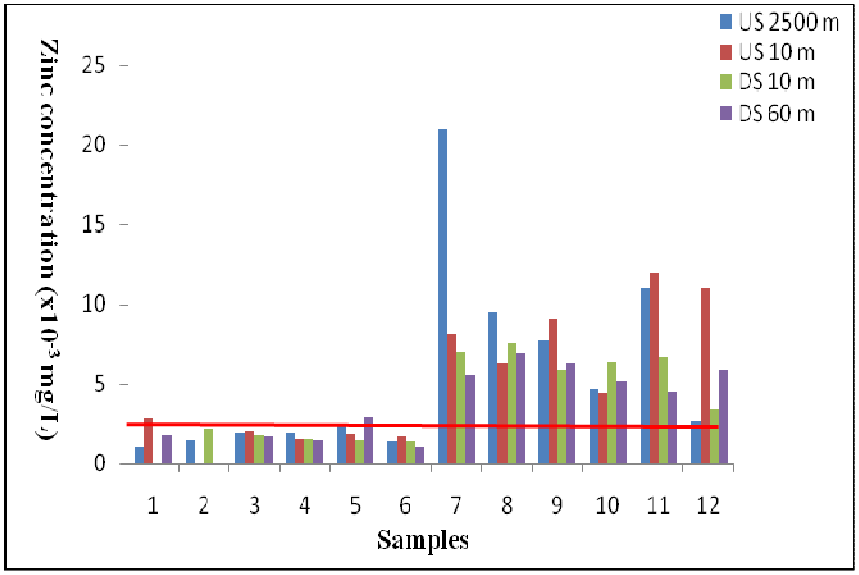

Figure 5.19 Test results of zinc (Zn) concentrations for 12 samples collected upstream (US) and downstream (DS) of the Waitoa dairy effluent discharge point in the Waitoa River in August and October, 2007

(New Zealand trigger value for toxicants of Zn shown by the red line)

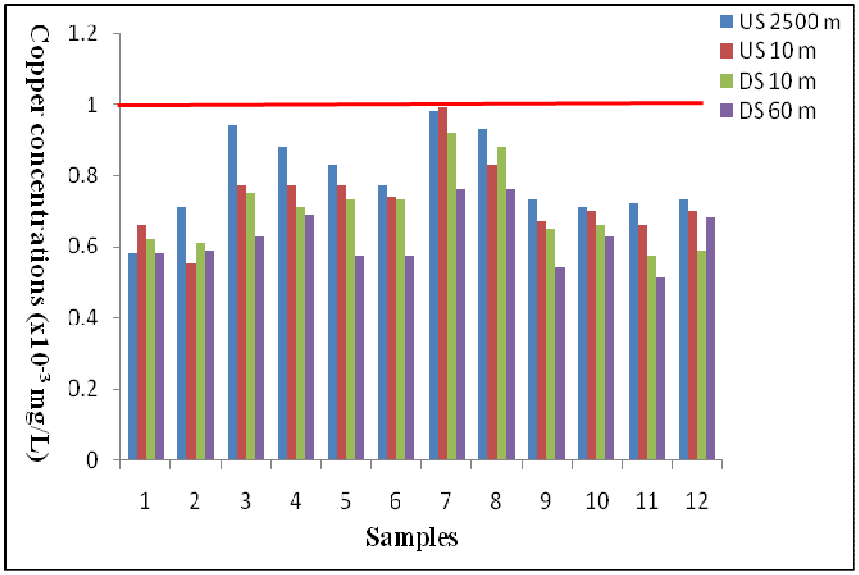

Figure 5.20 Test results of copper (Cu) concentrations for 12 samples collected upstream (US) and downstream (DS) of the Waitoa dairy effluent discharge point in the Waitoa River in August and October, 2007.

(New Zealand trigger value for toxicants of Cu shown by the red line)

The following findings may be drawn from Figure 5.13 - Figure 5.20:

i. Concentrations of Na, Ca and Mg at 60 m downstream of the dairy effluent diffuser were constantly higher than other sampling sites except K, which demonstrates the contribution of dairy effluents to the Waitoa River (Figure 5.13 - Figure 5.16). Mean concentrations of Na, K, Ca and Mg are also illustrated in Figure 5.17, showing increases of Na, K, Ca, and Mg 60 m downstream due to the Waitoa dairy effluent discharge.

ii. Significant differences of Fe and Zn concentrations occurred in the Waitoa River between the sampling period of August and October 2007 (Figure 5.18 and Figure 5.19). During the sampling period of 22-24, October 2007, the river flow was relatively high at 6.8, 3.8 and 3.2 m^3/s, separately. The Fe and Zn may come from runoff of pasture land because the best time to put fertilizers on pasture land is spring or autumn in New Zealand. In this case, the level of zinc was over the zinc trigger value of 2.4 μg/L for freshwater (Australian and New Zealand Environment and Conservation Council, 2000).

iii. In terms of Cu, it was detected both upstream and downstream. It can be seen from Figure 5.20 that concentrations of Cu downstream were lower than those at upstream sites due to the dilution of dairy effluent flux. The value of copper in the Waitoa River is however below the trigger value for toxicants of fresh water (Australian and New Zealand Environment and Conservation Council, 2000).

iv. Regarding Cr in the Waitoa River, it was only detectable on one day, and that might relate to the wastewater discharge from the Wallace Corporation Limited - upstream of the dairy effluent discharge.

v. Concentrations of Pb, Cd, and Ni in the Waitoa River were lower than their detection limits for most of samples.

5.3 DISCUSSION

5.3.1 Water Quality of the Waitoa River

To effectively manage water quality, the Waitoa River is one of the routinely monitored (monthly) rivers in the regional water quality monitoring programme (Beard, 2008). The quality of the Waitoa River for ecology at Mellon Road (approximate 7 km downstream of the Waitoa dairy site) and the comparison with other regional water quality monitored site are illustrated in Figure 5.21 and Figure 5.22, respectively.

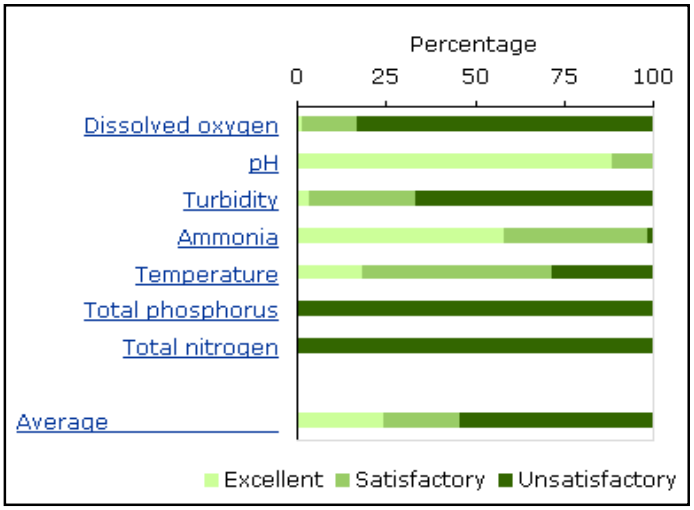

Figure 5.21 Water quality, as determined by the percentage of samples rated as excellent, satisfactory, or unsatisfactory against accepted ecological standards, of routinely monitored factors in the Waitoa River at Mellon Road..

(Source: Beard, 2008)

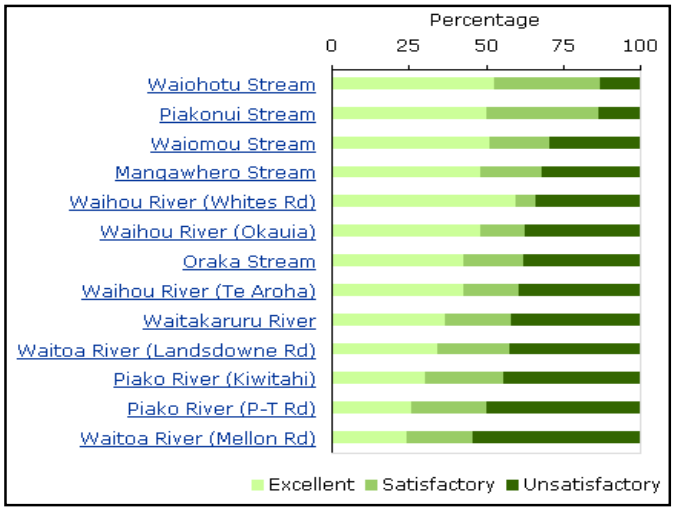

Figure 5.22 Averaged water quality score of the Waitoa River at Mellon Road compared with other sites in this region for ecology

(Source: Beard, 2008)

It can be seen from Figure 5.21 that 100% of total nitrogen samples from the Waitoa River exceeded their ecological standards and the general water quality was rated the worst of all of the sites. However, the results of this experiment indicate that, although EDTA is comprised of ~10% nitrogen, very little of the river nitrogen is from EDTA. This is because the EDTA concentration in the river is very low (1-2.7 μg/L) compared to the total nitrogen concentration (Beard, 2008).

5.3.2 Analysis of EDTA Speciation in the Waitoa River

EDTA concentrations of 1 ~ 2.7 μg/L were found 60 m downstream of the dairy effluent discharge. The species of EDTA complex in the Waitoa River depends not only on the dissolved concentrations of the associated cations and other natural ligands which determine the equilibrium speciation, but also on the released EDTA species from the dairy wastewater. Due to the low concentration of EDTA and dissolved metals detected in the Waitoa River, EDTA speciation was predicted only by its complex stability constants. Comparison of the EDTA concentration with concentrations of associated dissolved metals is shown in Table 5.5 for the site 60 meters downstream of the dairy discharge point in the Waitoa River.

Theoretically, EDTA is completely complexed with Fe(III) at a concentration of 0.01 μM Fe(III)EDTA in the Waitoa River. Nonetheless, further measurement is needed to verify its certainty.

Table 5.5 Comparison of concentrations of EDTA and dissolved metals with their complex stability constants 60 m downstream of the dairy outfall.

Items	Molarity	LogKa	Ka*[M] (μM)
EDTA	0.01 μM	-	-
Ca	0.26 mM	10.61	1.0E+13
Mg	0.19 mM	8.83	1.3E+11
Zn	0.06 μM	16.44	1.7E+15
Fe	2.72 μM	25	2.7E+25
Ni	0.01 μM	18.52	3.3E+16

Cu	0.01 µM	18.7	5.0E+16
Cd	ND*	16.36	-
Cr	ND	-	-

*-the concentration was below the method detection limit

5.4 CONCLUSIONS

i. Surface water samples were collected 2,500 m and 10 m upstream, and 10 m and 60 m downstream of the effluent diffuser in the Waitoa River to investigate concentrations of EDTA in the aquatic environment receiving large volume of dairy wastewater from the Fonterra Waitoa dairy site.

ii. A necessary pre-concentration step was needed for the determination of EDTA in the surface waters as the level of EDTA was too low. This was achieved by heating the surface water sample (10 mL) to dryness in 90°C dry oven and adding 1.5 mL mobile and 0.5 mL Fe^{3+} (1.94 mg/L) solution for a 5-fold pre-concentration.

iii. The HPLC-UV method was applied to analyze EDTA concentrations in surface water samples. A daily calibration curve was established at the concentration range of 0 – 150 μg/L EDTA. A blank, a duplicate every 10^{th} sample and a spike recovery of 50 μg/L EDTA standard every 20^{th} sample were undertaken per run for quality control. The averaged duplicate variability was 8.1 % (n=5) and the spike recovery varied from 97 – 107 % (n=3).

iv. Analytical results show a slight increase of the EDTA concentration 60 m downstream from the dairy effluent discharge point. The highest EDTA concentration of 2.7 μg/L was observed 60 m downstream during the sampling period of August and October, 2007. This value is well under the Predicted Effect Concentration (PEC) of 2.2 mg/L for aquatic environments advised by the European Union, almost half of EDTA target value of 5 μg/L for surface waters in the Rhine catchment area, recommended by the International Association of Waterworks, and also well below the New Zealand Drinking Water Standards of 0.7 mg/L EDTA for health purposes. These data therefore suggest that the discharge of dairy effluent at the Fonterra Waitoa site appears not leading to a significant effect for the adjacent natural river flow. However, further study on dispersion of dairy wastewater in the Waitoa River is going to be undertaken in Chapter 6 for this potential effect.

v. Analytical results for associated heavy metals in the Waitoa River reveal (a) concentrations of Na, K, Ca and Mg were increased downstream of the dairy effluent diffuser in the Waitoa River, which demonstrates the contribution of effluents at the Fonterra Waitoa dairy site; (b) concentrations of Fe and Zn showed significant differences between the sampling periods of August and October, with the level of Zn collected in October obviously exceeded the trigger value of 2.4 mg/L for natural waters; (c) copper was detectable both upstream and downstream, but their concentrations were below the trigger value (1.0 mg/L) for toxicants in fresh water; (d) Cr was only detectable on one day out of 6 days during the sampling periods that may relate to the tannery factory effluent discharge (wallace) and (e) Pb, Cd and Ni seemed to be all under detection limits, and pose no particular concern.

vi. In addition to the wastewater discharge containing EDTA from the Fonterra Waitoa dairy site into the Waitoa River, another EDTA source originated from the Wallace Corporation Limited plant, where EDTA is applied in the tannery. The analytical results of random samples collected show not only the presence of EDTA in the wastewater pond at the Wallace plant, but also the contribution of EDTA to surface water samples from comparison of EDTA concentrations between upstream and downstream of the company boundaries along the Waitoa River.

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6.0 CHAPTER SIX: SIMULATION OF EDTA DISPERSION WITHIN THE WAITOA RIVER

6.1 INTRODUCTION

EDTA was detected from the limited surface water sample collection (see Chapter 5). Generally, EDTA is not included in routine monitoring parameters for the surface water quality so that there is insufficient data to reveal whether the dairy effluent discharge from the Fonterra Waitoa dairy factory has a significant effect on the EDTA concentration in the Waitoa River. However, numerical techniques with advanced computer performance such as modelling have been widely used to fill in this knowledge gap and provide an effective tool for simulating transport of dissolved pollutants in freshwater systems (Cox, 2003).

When EDTA contained in dairy effluent enters the Waitoa River, two things happen to its transport. Firstly, EDTA is carried away by the water flow, a process which is termed advection; and secondly, it spreads out due to the concentration gradient, a process which is termed dispersion or diffusion (Ruthford, 1981; Furukawa et al. 2007). Accordingly, in this chapter, two approaches namely (i) approximate calculations using quasi one-dimension vertical mixing model; and (ii) a numerical simulation of the hydrodynamic processes and effluent mixing in two-dimensions (depth-averaged) were undertaken to enhance the understanding of the fundamental aspects of the transport of EDTA within the Waitoa River. For a worst case scenario of high volumes of dairy effluent discharge into the Waitoa River at a low river flow, both dispersal path and concentration of EDTA are determined through model output analysis.

6.2 DISCHARGE AND MOVEMENT OF DAIRY EFFLUENT INTO THE WAITOA RIVER

6.2.1 Wastewater Outfall Diffuser

Wastewater from the Fonterra Waitoa dairy processing plants, treated by an extended aeration sludge biological treatment at the wastewater treatment plant, is discharged into the Waitoa River via a sub-fluvial pipe routed through an adjacent wetland. The discharge pipe consists of a 44 cm outside diameter steel pipe with an approximate wall thickness of 10-12 mm (Figure 6.1). The diffuser extends into the river 6.6 meters from the "0" point, which is submerged on the base of the river with multiple discharging points (Figure 6.2). The dairy effluent containing EDTA discharge into the Waitoa River occurs continually year round. On a daily basis, the discharge is relatively constant, about 7,000 m^3/day on average. The permit of the effluent discharge is up to 10,000 cubic meters per day, and the discharge rate should not exceed 175 litres per second (Waikato Regional Council, 1993).

Figure 6.1 End view of wastewater discharge pipe from the Fonterra Waitoa dairy site into the Waitoa River.

(Source: Fonterra Waitoa dairy factory**)**

Figure 6.2 Wastewater diffuser pipe from the Fonterra Waitoa dairy site into the Waitoa River.

(Source: Fonterra Waitoa dairy factory)

6.2.2 Waitoa River Flow

Flow rates of the Waitoa River vary during the year. However, between late October to mid-June, flow rates are generally less than 2000 litres (2 m^3) per second (Coffey, 2003). The Waikato Regional Council has authorised Fonterra's Waitoa dairy factory to utilise separate discharge loads when the river flow rates are <600 L/s, 600-1400 L/s, 1400-1700 L/s, 1700-2000 and >2000 L/s (Waikato Regional Council, 1993).

6.2.3 Movement of Dairy Effluent in the Waitoa River

In general, advection and dispersion occur in each of the three coordinate directions, and the governing equations will be comparatively complex, such as for the **Eulerian** advection/diffusion equation (Black, 2002). Under conditions of non-steady flow, the flux of a tracer can be taken as a sum of the advective motion of the fluid, driving by gravity and turbulent diffusion. The concentration (C) will be governed by the advection/diffusion equation 7.1:

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial x}\left[E_x \frac{\partial C}{\partial x} - UC\right] + \frac{\partial}{\partial y}\left[E_y \frac{\partial C}{\partial y} - VC\right] + \frac{\partial}{\partial z}\left[E_z \frac{\partial C}{\partial z} + w_s C - WC\right] - kC + S_s$$

(Equation 7.1)

where t is time; x, y, z are orthogonal spatial coordinates; E_x, E_y and E_z are coefficients of eddy diffusivity; w_s is the still water fall velocity under gravity of the tracer (position upwards); U, V, W are horizontal and vertical components of the fluid velocity; k is the decay coefficient for a non-conservative tracer; and S_s is the tracer/effluent source term.

The input parameters vary for each model ranging from one-dimensional models to the more complicated two- and three-dimensional models. However, the dispersion coefficient is more difficult to estimate, and varies in space, time, and for the model type. The dependence of the dispersion coefficient on the model complexity is illustrated in Figure 6.3. Apparently, the model coefficient decreases with model complexity.

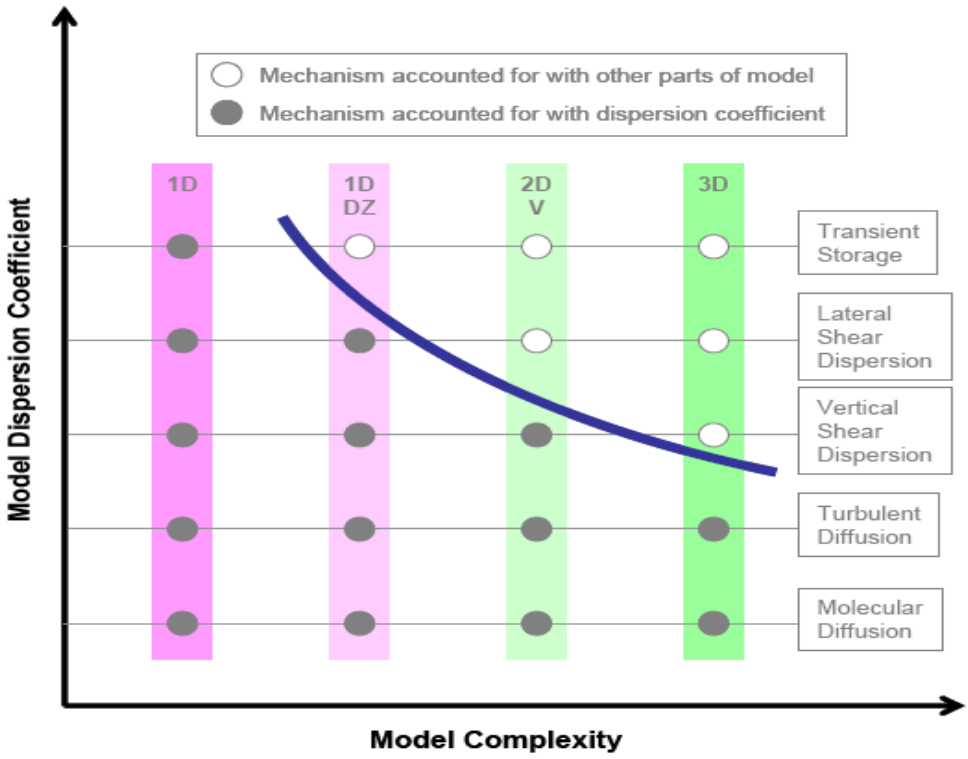

Figure 6.3 Model dispersion coefficient vs. model complexity. The model coefficient decreases with model complexity.

Model type: 1D = one-dimensional, 1D DZ = one-dimensional with dead zones, 2D V = two-dimensional vertically integrated, 3D = three-dimensional

(Source: Hellweger, 2005)

6.3 QUASI ONE DIMENSIONAL PRESENTATION OF EDTA DISPERSION

In many practical problems, the analysis can be simplified by neglecting terms which are small (Rutherford, 1981). For instance, in the case of Waitoa dairy effluent discharges with a steady uniform transverse line-source (Figure 6.4); transverse concentration gradients are negligible due to the uniform line-source. Longitudinal gradients are also negligible because the source is steady. Thus, the dispersion of EDTA can be simplified to become quasi one-dimensional, which is only vertical mixing by neglecting the horizontal and transversal dispersion.

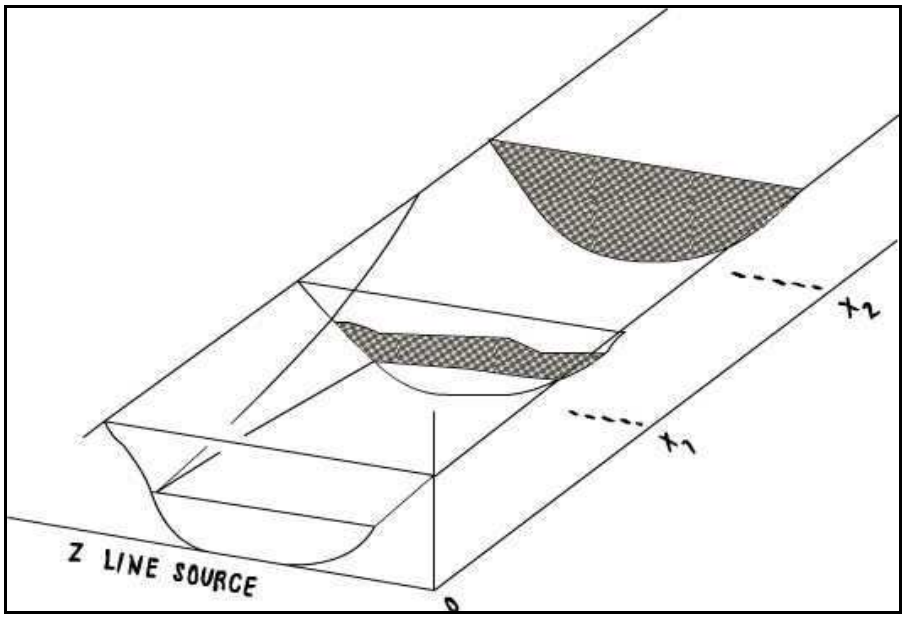

Figure 6.4 Diagram of quasi one-dimensional vertical mixing of EDTA from a steady uniform transverse line source, as for example from the Fonterra Waitoa dairy wastewater into the Waitoa River.

(Source: Rutherford, 1981)

6.3.1 Mixing Mechanism and Approximate Calculations

In channels with no secondary circulation, the principle mechanism causing vertical mixing is turbulence generated by velocity. The dispersion coefficient indicated by Elder (Rutherford, 1981) depends upon both depth and shear velocity as per the following equation

$$D_y(y) = y/d\ (1-y/d)\ K\ d\ u^*$$

(Equation 7.2)

where D_y is vertical dispersion coefficient; d is depth of flow; K is von Kármán's constant (= 0.4); and u^* is shear velocity (= $\sqrt{gds}$, where s is channel slope). For many practical problems the depth average is used

$$Dy = 0.067\ d\ u^*$$ (Rutherford, 1981)

(Equation 7.3)

For a natural channel, vertical secondary circulations can be expected to increase the rate of vertical mixing. It appears that

$$0.067 < Dy/d\ u^* < 0.33$$ (Rutherford, 1981)

(Equation 7.4)

Figure 6.4, Figure 6.5 and Figure 6.6 shows vertical mixing with equal concentration located downstream from a steady uniform transverse line-source of the Fonterra Waitoa dairy effluent at three different depths. It also indicates the length and width of the EDTA plume where the concentrations exceed a specified level. Variables are expressed in non-dimensional form so that many parameter combinations may appear on the same graph (Rutherford, 1981).

$$C^* = C/\bar{C} = CUbd/q$$

(Equation 7.5)

$$y^* = y/d$$

(Equation 7.6)

$$x^* = x\ D_y/Ud^2$$

(Equation 7.7)

where C^*, y^* and x^* are non-dimensional concentration, vertical displacement, and downstream displacement respectively; C is concentration; $\bar{C}$ is fully mixed concentration; U is mean velocity; D_y is depth averaged vertical dispersion

coefficient; *d* is river depth; *b* is river width; and *q* is tracer of EDTA mass inflow rate.

The vertical mixing is symmetrical in the direction *y* as the flow velocity is assumed to be uniform with the bed and water surface located at $y^* = 0$ and $y^* = 1$, Clearly $0 < y^* < 1$ and $C^* = 1$ appear at a long distance downstream from the outfall. The regions to the left of the C* = 0.001 contour do not contain any tracer of EDTA, while in the region to the right of the C* = 1.01 and 0.99 contours, the EDTA is fully mixed.

However, Figure 6.5 may overestimate the rate of dispersion downstream from an outfall on the bed of a rough natural channel due to the low velocity and low dispersion coefficient close to the boundary, and irregularities in the bed or dead zones causing locally high concentrations in these areas (Rutherford, 1981). The complete mixing can be attained within a distance (Figure 6.4)

$$x_m \approx 0.4\, Ud^2/D_y$$

(Equation 7.8)

downstream from an outfall on the bed or at the surface (Rutherford, 1981). It can be seen from Figure 6.6 that complete mixing appears to be attained with a distance

$$x_m \approx 0.1\, Ud^2/D_y$$

(Equation 7.9)

downstream from an outfall located at mid-depth (Rutherford, 1981).

a

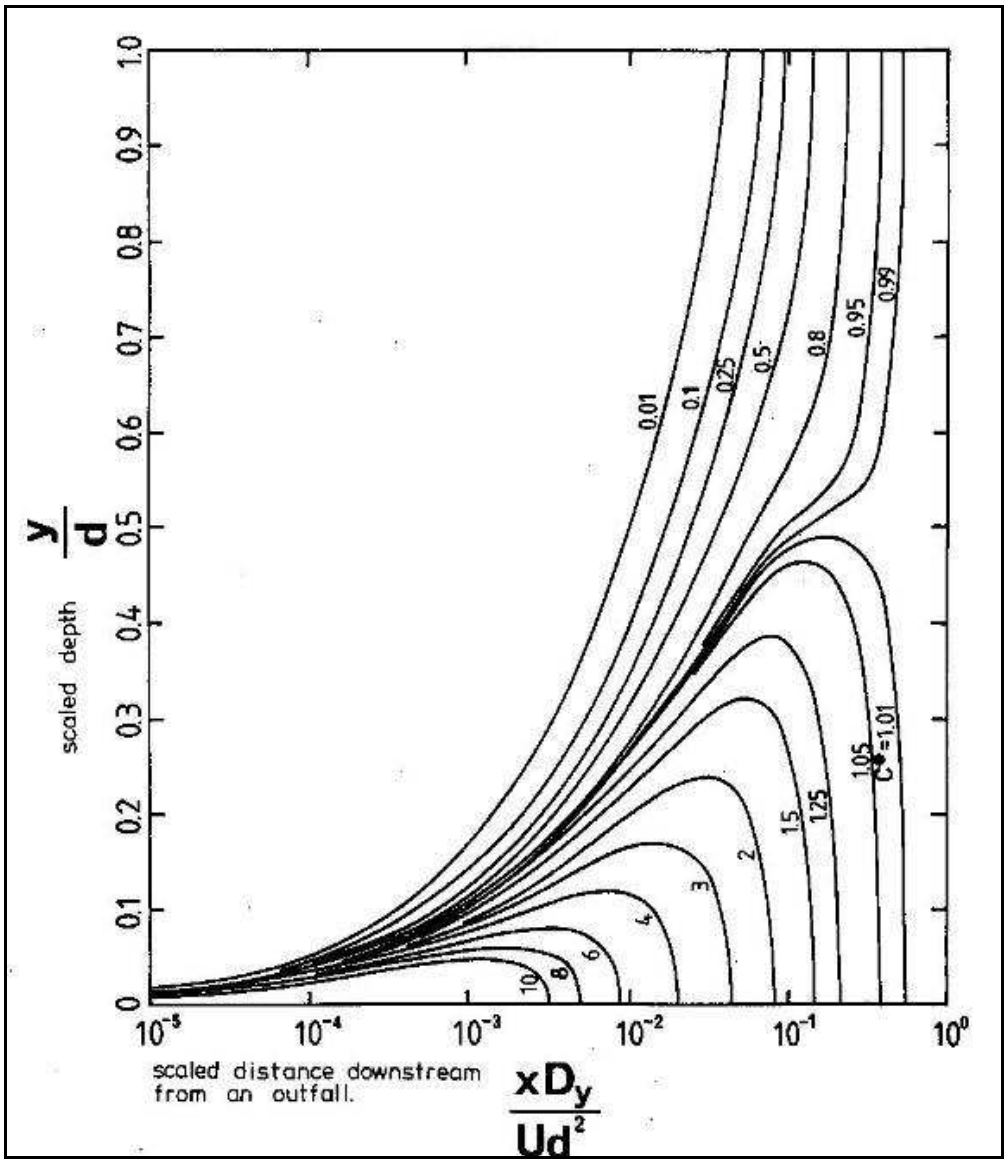

Figure 6.5 Concentration contours downstream from a steady transverse line source, applicable to the Waitoa dairy wastewater discharge, located on the channel bed of the Waitoa River.

(Source: Rutherford, 1981)

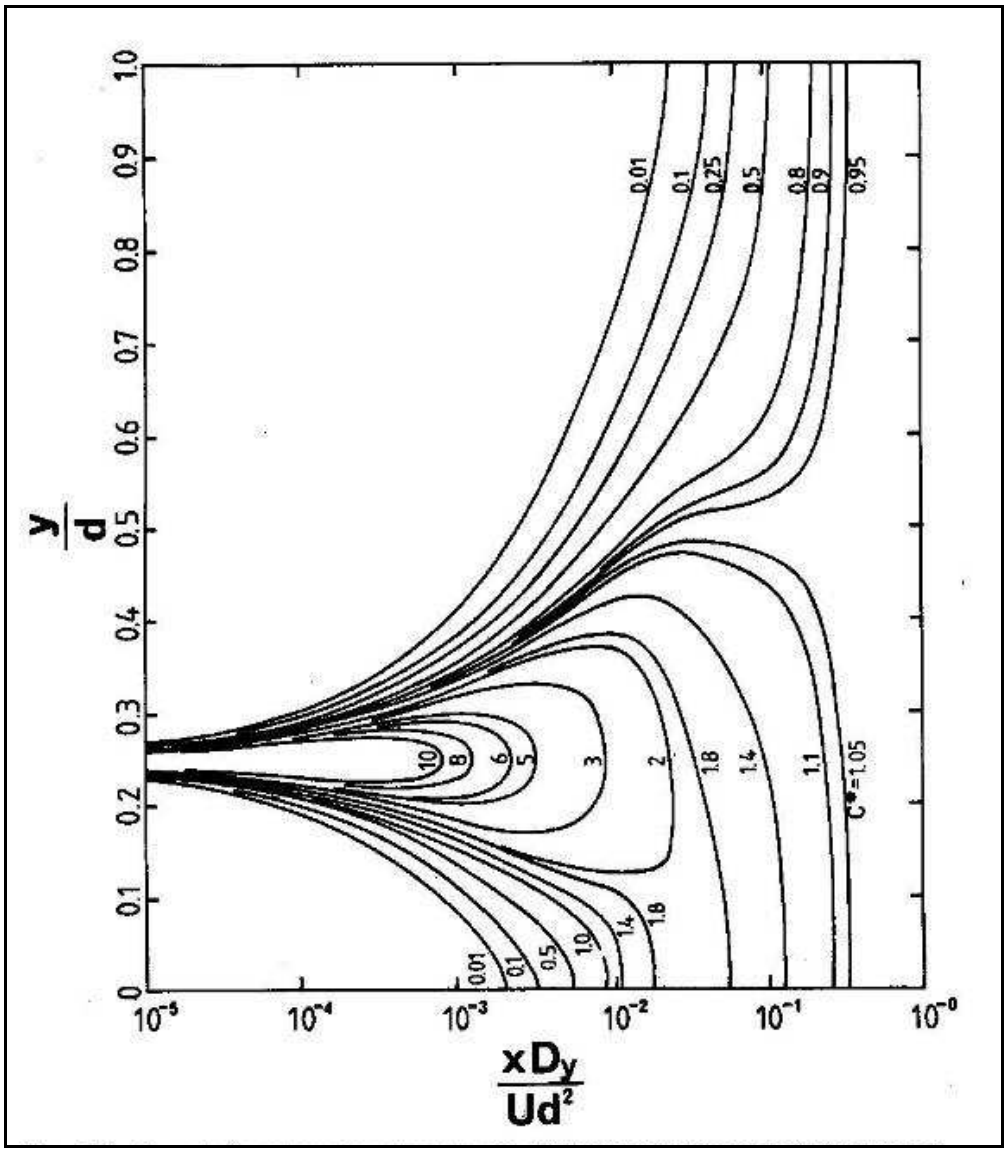

Figure 6.6 Concentration contours downstream from a steady transverse line source, applicable to the Waitoa dairy wastewater discharge, located at three-quarters depth of the river channel of the Waitoa River.

(Source: Rutherford, 1981)

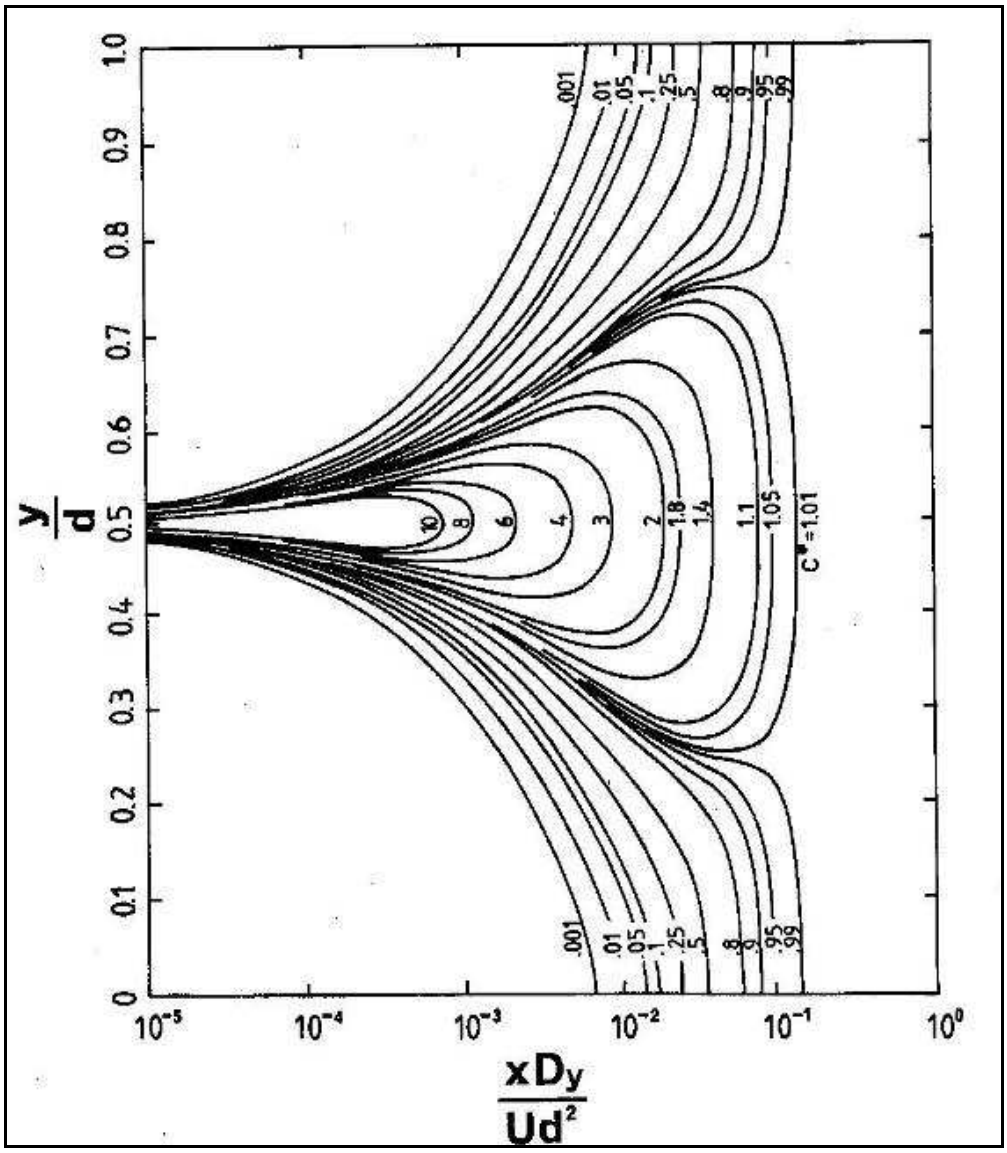

Figure 6.7 Concentration contours downstream from a steady transverse line source, applicable to the Waitoa dairy wastewater discharge, located at mid-depth of the river channel of the Waitoa River. The regions to the left of the C* = 0.001 contour do not contain any EDTA, while in the region to the right of the C* = 1.01 and 0.99 contours, the EDTA is fully mixed.

(Source: Rutherford, 1981)

6.3.2 Application of Quasi One-dimensional Mixing Mechanism

The above quasi one-dimensional mixing mechanism was applied for a specific monitored case of the Waitoa dairy wastewater discharge into the Waitoa River.

Field measurements such as water depth, currents, and EDTA concentration in dairy wastewater discharge were collected on 30 May, 2008. Mass flow of the EDTA was calculated based upon the effluent discharge volume (4,712,000 litres) for that particular day and measured EDTA concentration (107 μg/L) for the 24-hr composite dairy effluent sample.

An impact from EDTA may be evident in some cases at sites where there is an output source (European Chemicals Bureau, 2004; Grundler et al., 2005). The worst scenario for the Waitoa case is if significant volumes of dairy effluent are discharged into the Waitoa River to result in high EDTA concentrations in the waterway. Hence, the worst case is assumed as the maximum usage of EDTA in the processing plant combined with low river flow rate (<600 L/s) of the Waitoa River. In accordance with usage of Eliminator/Eliminator LF (34-35% EDTA contents) at the Fonterra Waitoa dairy factory for the year 2008, the maximum used amount was 4180 litres in total for January, 2008. The consumed EDTA on a daily basis was 4180 litres x 1.3 kg/L (density) x 35% (EDTA contents)/31days = 61.4 kg/day. The daily EDTA mass flow rate into the Waitoa River with an approximate 90% EDTA removal (see Chapter 4) by the bio-treatment is calculated as 61.4 x (1-0.9) x 1000 / (24 x 60 x 60) = 0.071 g/s.

When dairy effluent containing EDTA from the Fonterra Waitoa dairy site is discharged into the Waitoa River with a steady uniform transverse line source (Figure 6.4), the EDTA fully mixed concentration, and the distance downstream from the outfall at the surface and the mid-depth for both monitored case and the worst case are listed in Table 6.1. The results show that the distance of completely mixed conditions for both cases is 6 meters downstream from the outfall, and the complete mixed concentrations of EDTA are 3.18 μg/L for the monitored case and 40.3 μg/L for the worst case of the year 2008, respectively.

Table 6.1 EDTA dispersion results downstream from the dairy effluent outfall in the Waitoa River with a steady uniform transverse line-source, when quasi one-dimensional vertical mixing mechanism (Rutherford, 1981) is applied.

Items	Monitored case(30 May 08)	Worst case*	Comments
Waitoa River	Water depth: d =1.0m Width: b = 8 m Slope: $S = 2x10^{-4}$ Velocity: U = 0.22 m/s Shear velocity: u* = 0.0443 m/s	1.0 m 8 m 10^{-4} 0.20 m/s 0.0443 m/s	(source: Rutherford, 1981) $u^*= (gdS)^{1/2}$
Vertical dispersion coefficient (Dy)	$Dy = 0.33du^* = 146\ cm^2/s$	$146\ cm^2/s$	assuming as a natural and irregular river channel
Complete mixing distance at the surface	6 m	6 m	$Xm = 0.4\ Ud^2/Dy$
Complete mixing distance at the mid-depth	1.5 m	1.5 m	$Xm = 0.1\ Ud^2/Dy$
EDTA mass flow	0.0056 g/s	0.071 g/s	Field measurements
Fully mixed concentration	3.18 µg/L	40.3 µg/L	$\bar{C} = q/Udb$

*****Worst case - the maximum EDTA discharged into the Waitoa at a low river flow in the year 2008

6.4 3DD HYDRODYNAMIC MODEL SIMULATING EDTA DISPERSION IN THE WAITOA RIVER

6.4.1 Introduction

The 3-dimensional hydrodynamic model 3DD was developed by Professor Kerry Black and has been used successfully in numerous studies around the world and New Zealand for over 25 years (Black, 2002). The Model 3DD is based upon highly accurate mixed Eulerian/Lagrangian mathematical techniques and provides state-of-art –hydrodynamic and dispersal simulations. Rather than using an Eulerian finite difference scheme to solve equation 7.1, the dispersal model

POL3DD (POLlution dispersal coupled to 3DD) tracks dissolved materials as suspended "particles" to simulate water-borne dispersal and determines concentrations of pollutants from multiple sources in 3 dimensions (Black, 2002). In the shallow water environment, e.g. the Waitoa River, river currents only generated in a 2-dimensional (depth-averaged) using the 3DD hydrodynamic model, and the dispersal paths and concentrations of EDTA are then read by POL3DD to define the velocity fields. Therefore, the model will be described accordingly.

6.4.2 3DD Hydrodynamic Model Inputs and Outputs

The model 3DD requires an operation file (.DAT or .IN), a bathymetry file (.MD) and a boundary file (.BND). The operation file controls the input data and output file names, which contain the main characteristics of the site, including number of grid cells and sizes, friction coefficient, and time step.

A precise bathymetry is essential for the accurate resolution of numerical modeling outputs (Black, 2002). The collection of bathymetric data was undertaken on 15 October, 2008 by two methods, (i) a standard GPS (TRIMBLE RTK GPS) station was set beside the wastewater treatment pond for a known point (Figure 6.8); and (ii) water depths were measured by an echosounder (KNUDSEN MP 329 Dual Frequency Echosounder) on a survey vessel (3.2 meter ZODIAC RIB) (Figure 6.9).

Figure 6.8 Setting up a standard GPS station for a known point before the collection of bathymetric data for the Waitoa River.

Figure 6.9 Collecting bathymetric data using an echosounder on a survey vessel for the purpose of hydrodynamic modelling within the Waitoa River.

A one meter by one meter bathymetric grid was created in ARC GIS and exported as a XYZ ASCII file. This file was then gridded using the software package

SURFER32 (Version 6.04, software by Golden Software Inc, 1997). The SURFER ASCII file was converted in-line with a 3DD standard format (I, J) as .md file (Figure 6.10). The cell (1, 1) is located at the bottom left corner of the grid and the maximum coordinate (Imax, Jmax) are at the top right corner. For simulation of EDTA in the Waitoa River, a grid size of 172 x 531(I, J) was created. The U velocity of x-direction is positive to the east, corresponding with an increasing I value, while the V velocity is positive north and corresponds with an increasing J. Each cell is referenced by its (I, J) coordinate as well as U and V velocities corresponding to that point. The Kriging method of interpolation was chosen with a search radius of 6 m to grid the bathymetry (Figure 6.11). A slight (1 cell radius) smoothing was performed for the bathymetry, shown in Figure 6.12.

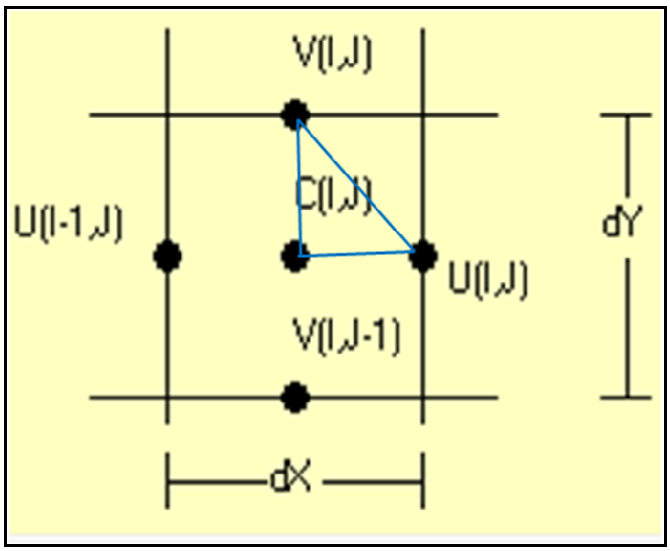

Figure 6.10 Structure of each model 'cell', where dX and dY are grid size (m), the x-direction (U velocity) is positive to the east and with corresponding an increasing I, and V velocity is positive to the north and corresponds with an increasing J. Each cell is referenced by its (I, J) coordinate and the U and V velocities in the triangle corresponding to that point.

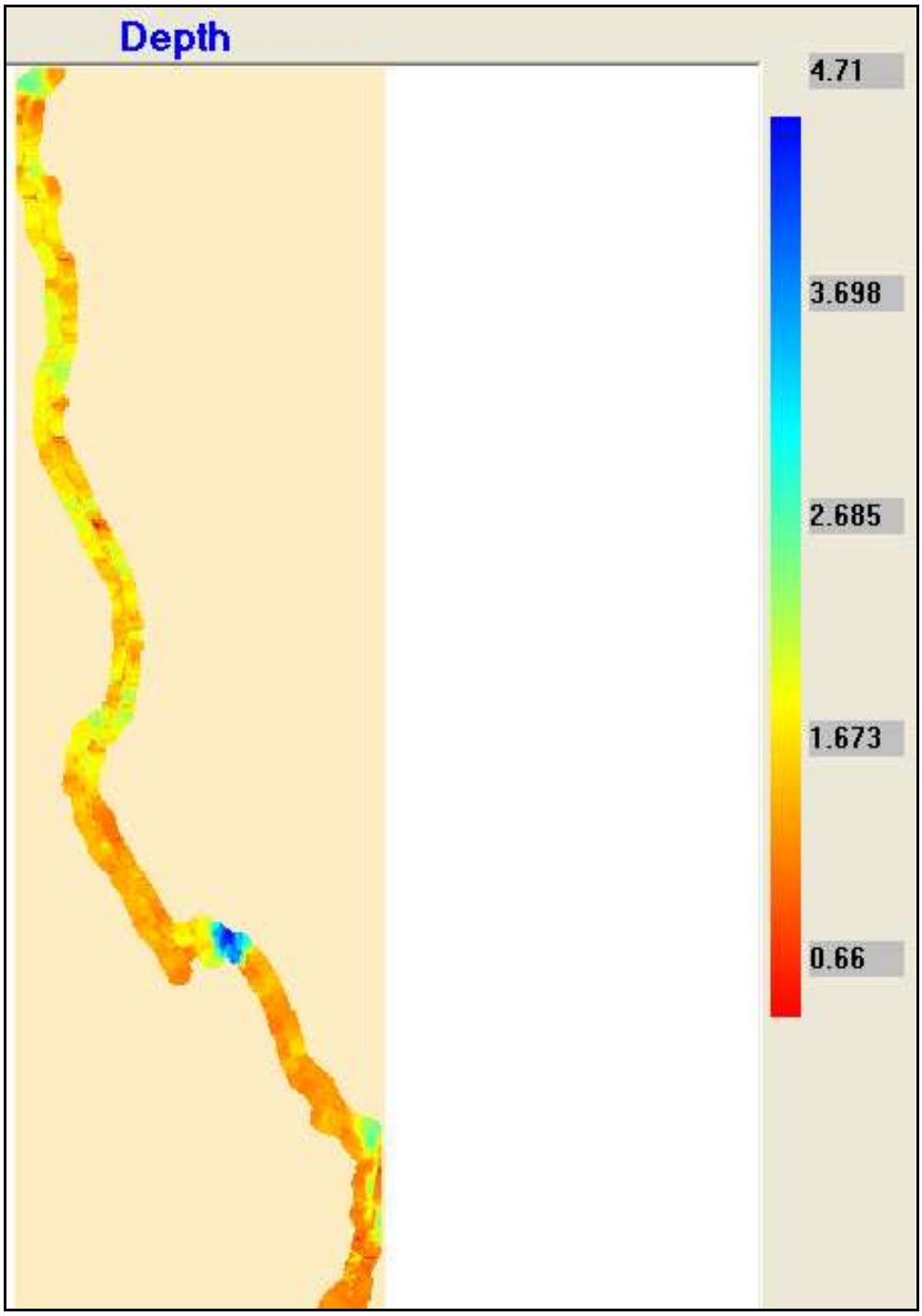

Figure 6.11 Coverage of KNUDSEN MP 329 Dual Frequency Echo-sounder in the Waitoa River. Survey was undertaken on 15 October, 2008, river flow and height were 3460 L/s and 13.6 m (sea level), respectively.

(River data: Fonterra Co-operative Group Limited, Waitoa dairy site)

Figure 6.12 A comparison of the created bathymetry for the 3DD hydrodynamic model with the aerial photo of the investigated region in the Waitoa River from the Google Earth.

(Source: Google Earth)

The boundary file contains boundary information, i.e. input data for the model at the boundaries such as the start and end I, J value.

The hydrodynamic model 3DD binary output file (.OUT) contains depth-averaged velocity data for each grid cell during the length of the time series. 3DD output files were examined and results presented in the MATLAB R2007a (Vision7.4.0) graphics using the 'plot3dd' command.

6.4.3 Hydrodynamic 3DD Model Processes and Calibration

1. Hydrodynamic processes

Model 3DD solves the momentum and continuity equations for the circulation explicitly on an Eulerian grid. In this case, only the two-dimensional capabilities of the 3DD model were applied to simulate the averaged-depth flow in the Waitoa River as a means of understanding how EDTA is dispersed in the receiving region. The model accounts for various parameters through bed roughness length and horizontal eddy viscosity. Within a shallow water environment of the Waitoa River, the dominant dispersion of EDTA is transverse with the main river flow, but not horizontal dispersal. Thus, the main effective parameter for the spatial variation is the bed roughness length.

2. Model calibration

Models can only produce meaningful results after proper calibration based upon comparisons of field measured data against model outputs. The model calibration was undertaken at a low river flow (425 L/s) by adjusting the roughness bed length in the model equation until the model-simulated river averaged velocities matched with the velocity measurements at the specific cross sections of the Waitoa River. A time series of the average velocity through the section over the full model simulation was extracted from the Model Support Manager of 3DD Suite (Figure 6.13). The actual field measurement was carried out using Pigmy current meter (P039) on 30 May 2008 assuming the river flow was consistent.

After calibrating the model parameters (shown in

Table **6.2**), another 48 hours simulation was undertaken. New depth-averaged velocities were then extracted to confirm that the model was performing consistently. For a comparison of depth-averaged velocities to the actual field measurements, the initial and calibrated 3DD model output is listed in Table 6.3.

These data reveal that

(i) the calibrated velocities confirmed that the 3DD hydrodynamic model was performing consistently by the same velocities; and

(ii) differences between the modelled and measured velocities were less than 4 %, and the averaged difference was -2.3%. This suggests that the river velocity was reflected well by the model.

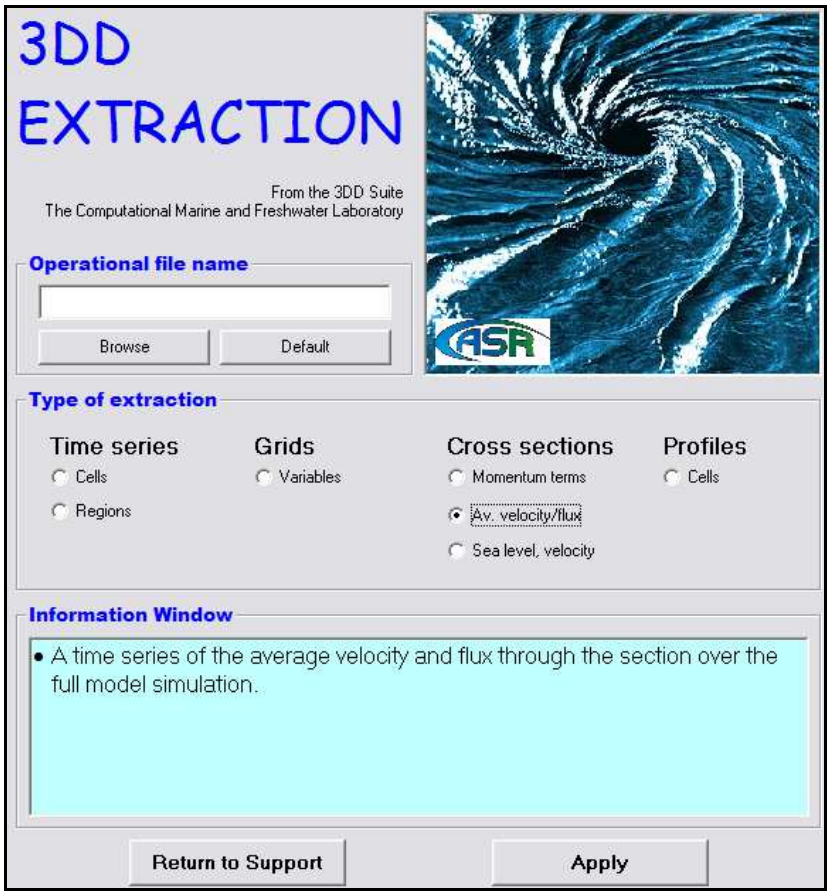

Figure 6.13 The 3DD model depth-averaged velocity data extraction window of cross sections from the 3DD Suite.

Table 6.2 Numerical parameters for the two-dimensional current hydrodynamic model of the Waitoa River receiving the Fonterra Waitoa dairy effluent containing EDTA.

Parameters	Value
Time steps	0.06 seconds
Model Duration	24 hours
Roughness length	0.01 m
Resistance length (uniform and constant)	0.01 m
Horizontal eddy viscosity (uniform and constant)	0.1 m^2/s
Grid resolutions	1 m x 1 m
Grid size	172 x 531
Rotation of relative to true north	0
Effective depth	0.3 m
Drying height	0.05 m
Boundaries	North and south boundary created

Table 6.3 Comparison of depth-averaged velocities from field measurements, the initial 3DD model outputs and the calibrated 3DD model outputs at a low river flow (425 L/s) for the Waitoa River.

Cross sections	Depth (cm)	Depth-averaged velocity (field) (m/s)	Depth-averaged velocity (3DD output) (m/S)	Calibrated depth-averaged velocity (m/s)	Differences* (%)
1	88	0.208	0.202	0.202	-2.9
2	150	0.228	0.220	0.220	-3.6
3	90	0.270	0.269	0.269	-0.4
				Mean	**-2.3**

* Differences (%) = (modeled velocity - measured velocity)/averaged velocity * 100

6.4.4 Simulation of River Flow by 3DD Model

The river flow patterns, velocity, and velocity vectors from the 3DD model output for the Waitoa River are shown in Figure 6.14 for a low river flow (425 L/s) and Figure 6.15 for a high river flow (>2000 L/s). Dead zones, caused by an eddy close to the river boundary and shown by the red dashed circle, occur during hydrodynamic current simulation at both low (0.2 m/s) and high (1.0 m/s) river currents. The velocity of dead zone (red dashed circle) is likely to be much lower

than the domain flows of the river. The peak velocities for both river flows can be seen to reach 0.3 m/s and 2.1 m/s in the Waitoa River.

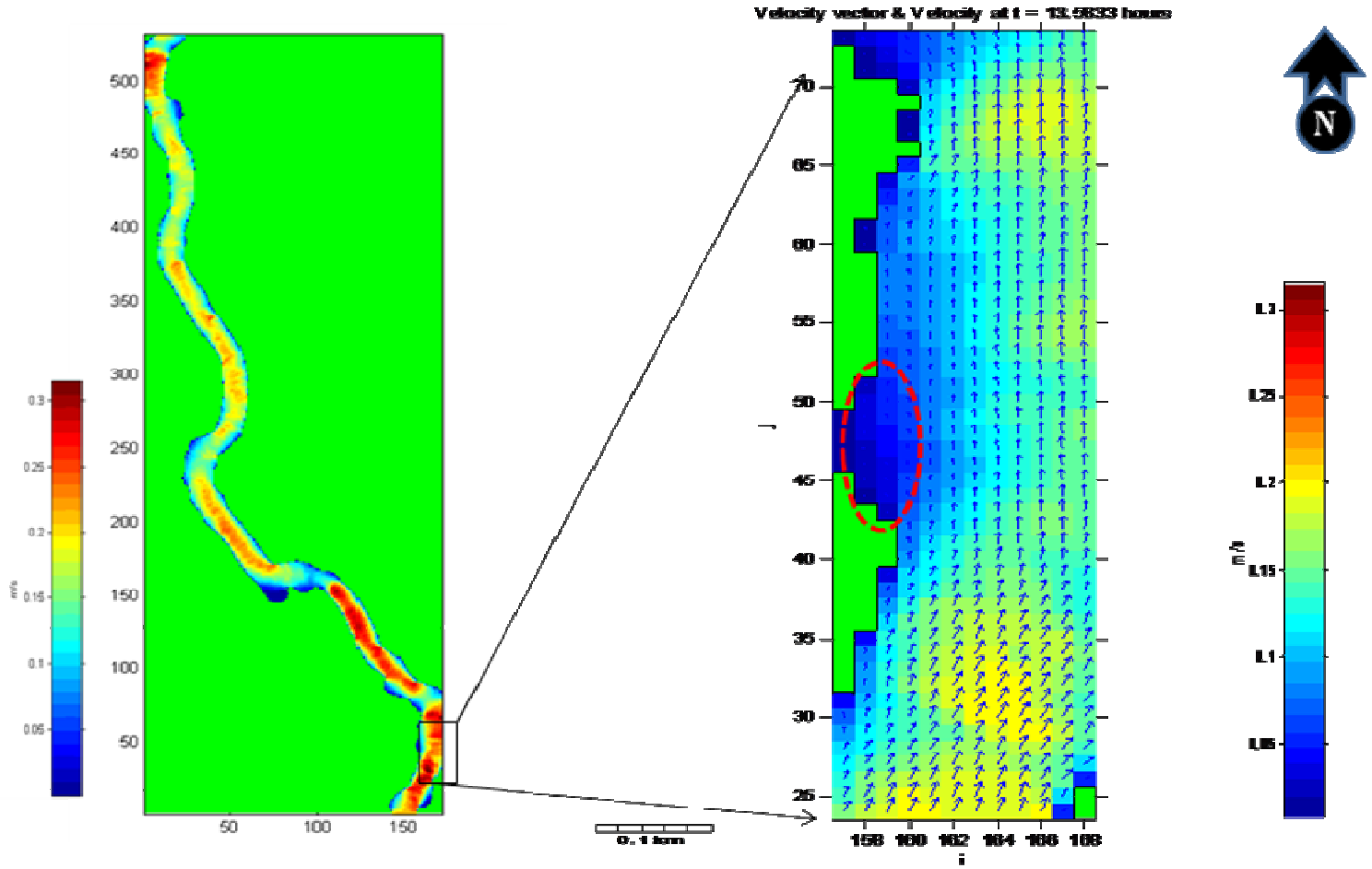

Figure 6.14 The simulated river flow, velocity vector pattern and velocity by the 3DD hydrodynamic model at a low river velocity of 0.2 m/s (river flow of 425 L/s) in the Waitoa River. The peak velocity can be seen to reach 0.31 m/s in the modelled region, and

the velocity of the dead zone caused by an eddy close to the boundary (red dash-circle) can be seen to be much smaller than the domain river velocities.

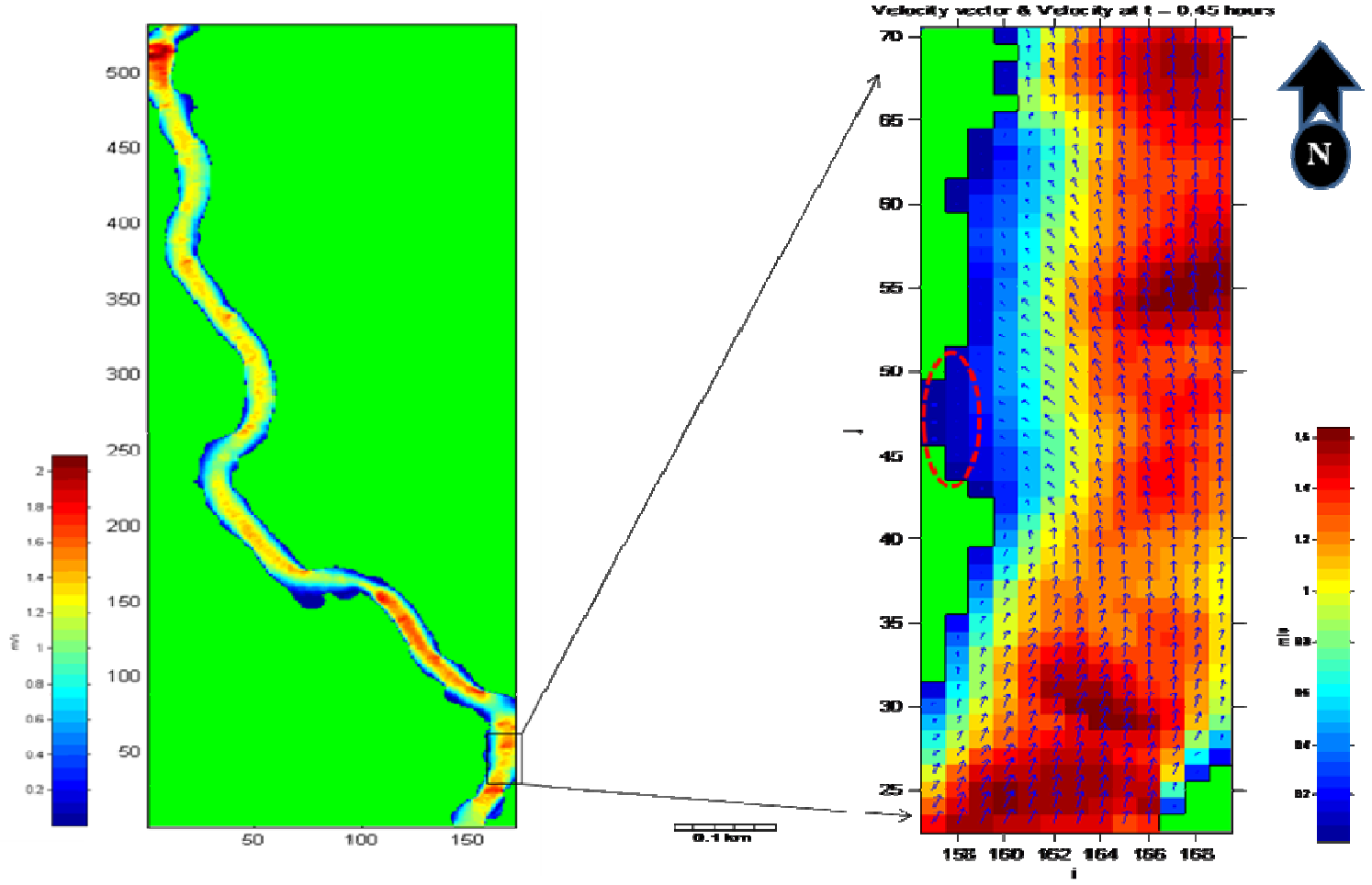

Figure 6.15 The simulated river flow, velocity vector pattern and velocity using the 3DD hydrodynamic model at a high river velocity of 1.0 m/s for the Waitoa River. The peak velocity can be seen to reach 2.1 m/s in the modelled region, and the velocity of the dead zone caused by an eddy close to the boundary (red dash-circle) can be seen to be much smaller than the domain river velocities.

6.4.5 Simulation of EDTA Dispersion in the Waitoa River Using POL3DD Model

POL3DD is a Lagrangian 3-dimensional numerical dispersal model for application to the transport of dissolved pollutants (EDTA in this case), and is linked to the 3DD hydrodynamic model (3DD) detailed flow patterns (Black, 2002). The model solves the dispersion equations using novel Lagrangian particle tracking techniques for the shallow water, which is the Waitoa River in the present study. The output from this model shows the EDTA dispersal paths and concentrations in the simulated region. The preparation of input files and extraction of results from the model output file are all supported by the 3DD Suite. The graphics programme, PLOT3DD - a MATLAB support tool, plots model outputs.

In essence, the POL3DD model works in four stages (Black, 2002) as follows:

(i) Boundary condition: EDTA particles are released each time step in accordance with a boundary condition, which, in this case, is volume input and EDTA concentration of dairy effluents;

(ii) Advection/Diffusion: EDTA is firstly advected by currents derived from a hydrodynamic model. Next, diffusion is modelled as a random walk, with position increments proportional to horizontal and vertical eddy diffusivity;

(iii) Accumulation: The EDTA concentration is determined in each model cell by accumulating the masses and volumes carried by the EDTA resident within the cells; and

(iv) Decay: EDTA is generally considered not to be biodegraded in the natural environment. Hence, EDTA is treated as conservative particles and no decay was applied.

1. POL3DD model parameters

The POL3DD model was undertaken to simulate the EDTA dispersal within the Waitoa River for the monitored case of 30 May 2008, and the worst case scenario for the year 2008. The chosen vertical velocity profiles around the averaged-depth current for the river flow pattern were generated by a two-dimensional

hydrodynamic model using the 3DD model described above. The parameters for POL3DD model are tabulated in Table 6.4.

Table 6.4 Numerical parameters for POL3DD to simulate the dispersal of EDTA within the Waitoa River receiving dairy effluent from the Fonterra Waitoa dairy site.

Parameters	Value
Center of release region (I, J)	(162, 27)
Constant release	0
Upper and lower Z from surface down	0, 1
Timing of release	0, 0
Uniform roughness length	0.01 m
Release EDTA concentration	
Monitored case	0.107 g/m^3
Worst case	0.88 g/m^3
Release volume	
Monitored case	0.055 m^3/s
Worst case	0.081 m^3/s
Horizontal diffusion option	1 (constant)
Vertical diffusion options	1 (constant)
Model time step	1 second
Model duration	72 hours

2. Modelling results

POL3DD of two-dimensional numerical dispersal model was applied to the transport of EDTA using novel Lagrangian particle tracking techniques within the Waitoa River subsequent to the Fonterra Waitoa dairy effluent discharge. The particle tracking results and distribution of EDTA concentrations within the Waitoa River for both monitored case (30 May 2008) and the worst case scenario for the year 2008 are shown in Figure 6.16 and Figure 6.17. EDTA concentrations are also plotted as 3-dimensional presence for the worst case scenario of dairy effluent discharge, shown in Figure 6.18.

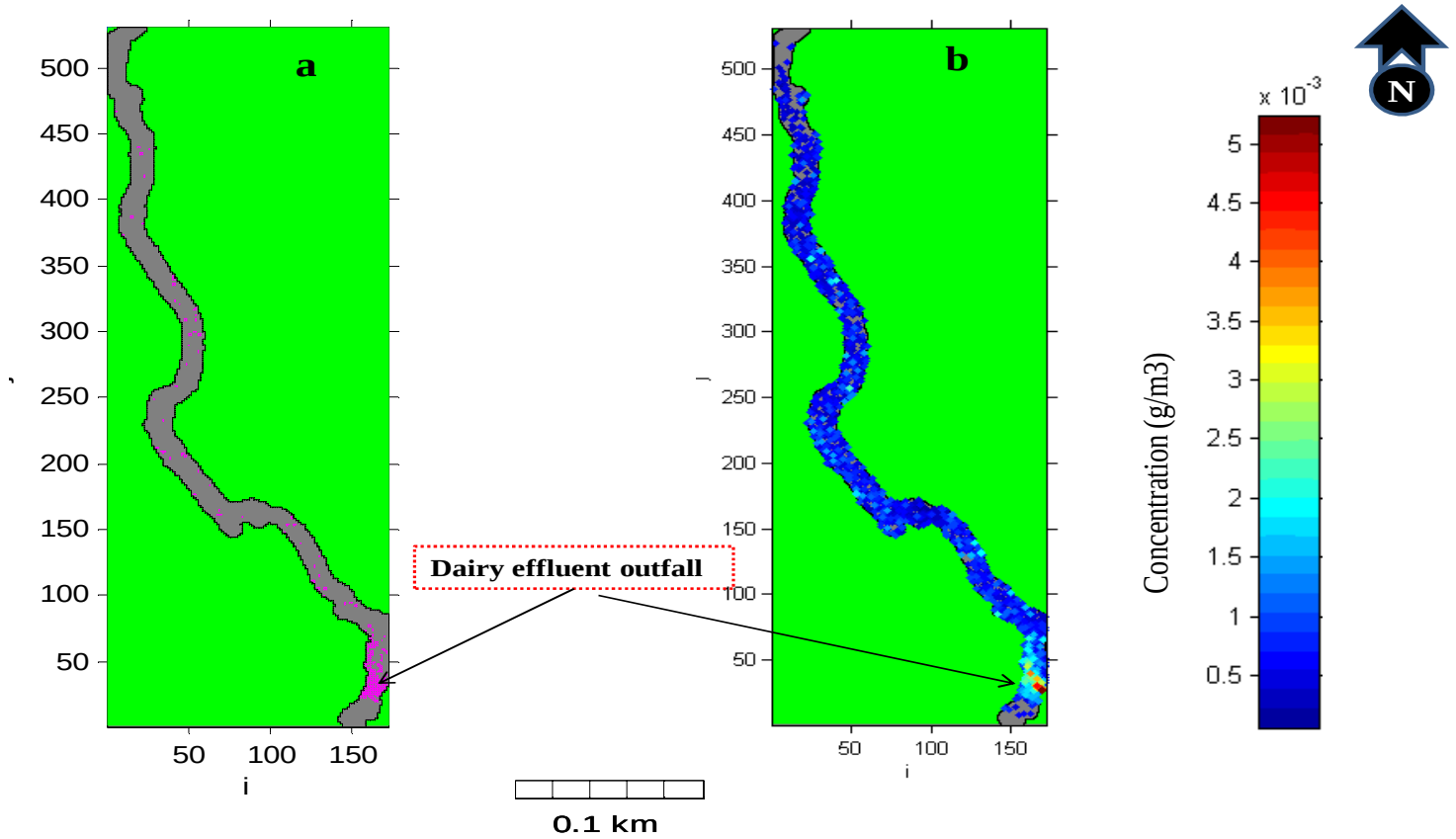

Figure 6.16 Particle tracking result (a) and simulated EDTA concentrations (g/m^3, e.g. 10^3 µg/L) (b) by the POL3DD model within the Waitoa River for the monitored case, as dairy effluent discharge volume of 0.055 m^3/s at the low river velocity of 0.2 m/s (or

river flow of 425 L/s). It can be seen that the maximum particle number and EDTA concentration occurred in the immediate vicinity of the dairy effluent outfall.

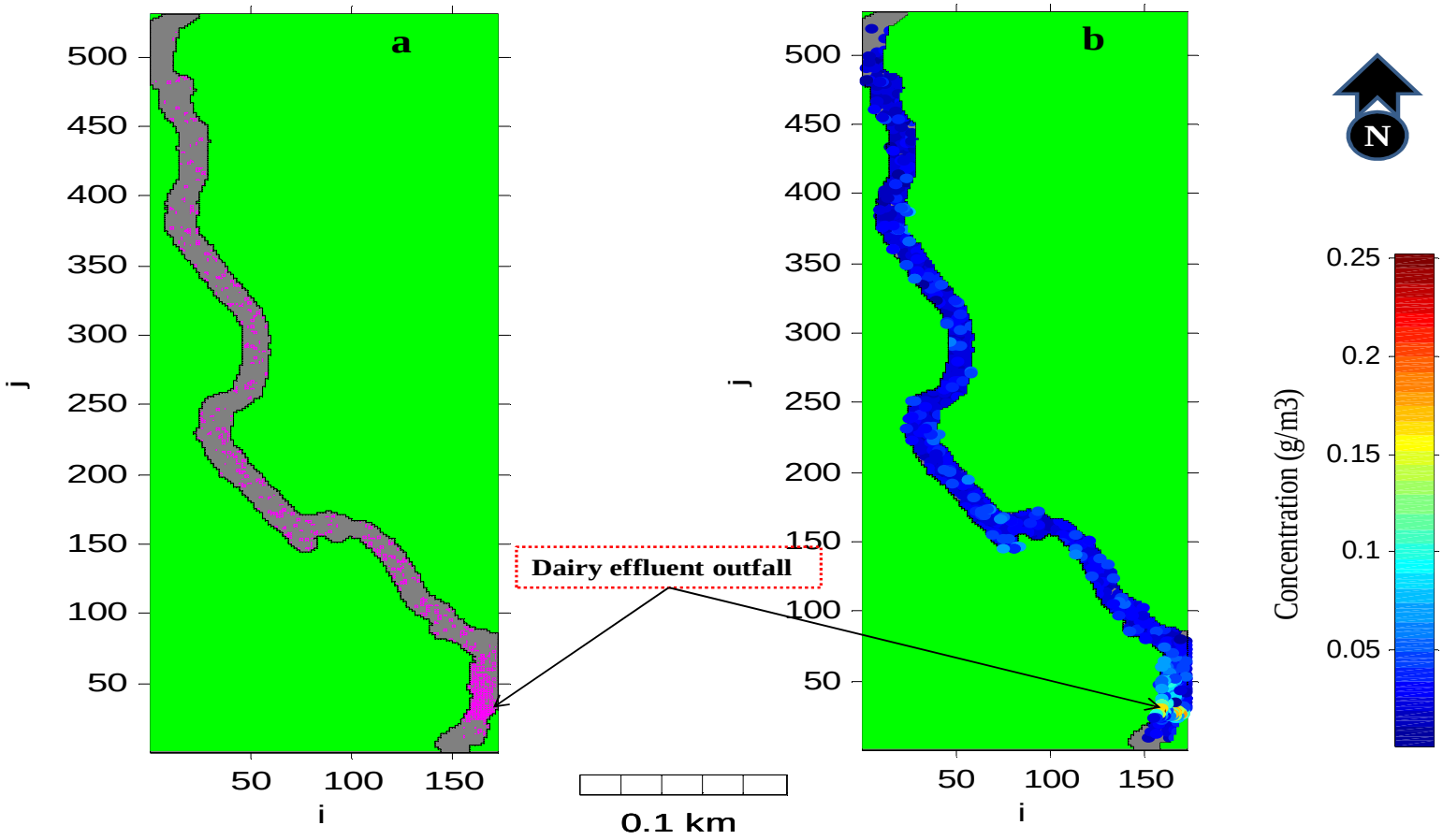

Figure 6.17 Particle tracking result (a) and simulated EDTA concentrations (g/m^3, e.g. 10^3 μg/L) (b) by the POL3DD model within the Waitoa River for the worst case, as dairy effluent discharge volume of 0.081 m^3/s at the low river velocity of 0.2 m/s (or river flow of 425 L/s). It can be seen that the maximum particle number and EDTA concentration occurred in the immediate vicinity of the dairy effluent outfall.

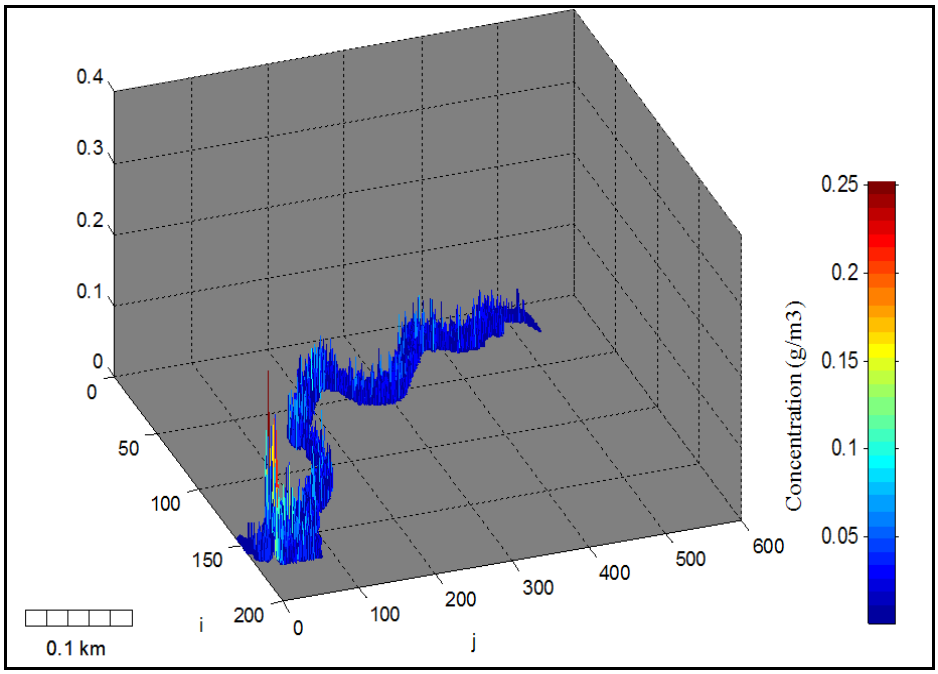

Figure 6.18 The simulated EDTA concentrations by the POL3DD model illustrated in three dimensions within the Waitoa River for the worst scenario of high volume discharge (0.081 m^3/s) and low river flow (425 L/s). It can be seen that the instantaneous EDTA concentration reached 0.25 g/m^3 at the immediate vicinity of the dairy effluent outfall.

It is evident that

(i) the maximum EDTA concentrations occurred at the immediate vicinity of dairy effluent outfalls for both cases; and

(ii) EDTA dispersal paths and concentrations were determined by the particle tracking results.

To explain how EDTA is dispersed within the Waitoa River due to the river water flux and dispersion, a series of EDTA averaged concentrations for each central cell of various transects were extracted, using the 3DD suite support manager, downstream from the dairy effluent outfall. Changes of EDTA concentrations for both cases within the Waitoa River are illustrated in Table 6.5 after the dairy effluent enter the stream. As expected, the concentration of EDTA had a significant decrease due to the dilution of water flux of the Waitoa River, but the important finding is that there was no obvious concentration change of EDTA beyond 50 meters downstream from the outfall.

Table 6.5 EDTA concentration extracted results of the central cell from each transect of varied distances downstream from the dairy effluent outfall for both monitored case and the worst case in the year 2008.

No.	Cell (I, J)	Approx. distance from outfalls (m)	EDTA concentration (μg/L)	
			Monitored case	Worst case
1	162, 27	0	1.80	16.81
2	164, 29	2	2.03	21.06
3	163, 35	10	1.23	15.63
4	166, 40	15	0.84	9.71
5	164, 45	20	0.80	9.78
6	165, 50	25	0.64	6.91
7	167, 66	40	0.28	3.58
8	168, 78	50	0.22	1.25
9	62, 175	200	0.14	1.66
10	22, 424	500	0.16	1.40

For the monitored case of 30 May 2008, the maximum simulated concentration (2.03 μg/L) of EDTA occurred around 2 m downstream from the dairy effluent discharge point, and then EDTA concentration was gradually reduced to 0.22 μg/L and 0.14 μg/L 50 m and 200 m downstream, respectively (Table 6.5 and Figure 6.19). The analytical results of EDTA concentrations in surface water, collected on 30 May 2008, show that there were no observable changes of EDTA concentrations upstream (10 m) and downstream (60 m) from the dairy effluent diffuser point (see Table 5.2 in Chapter 5).

For the worst Waitoa case, the maximum concentration of EDTA in the vicinity of the Fonterra Waitoa dairy effluent outfall was ~21 μg/L (Table 6.5). However, the instantaneous concentration of EDTA was likely to reach 0.25 g/m^3 (0.25 mg/L) in the vicinity of the outfall (Figure 6.18). The concentration of EDTA was then to be reduced to ~1.5 μg/L 50 m downstream of the dairy effluent outfall (Figure 6.20). These, therefore, suggest that the discharge of large volumes of effluents

from the Fonterra Waitoa dairy site appears to not result in a significant increase of EDTA concentrations in the Waitoa River.

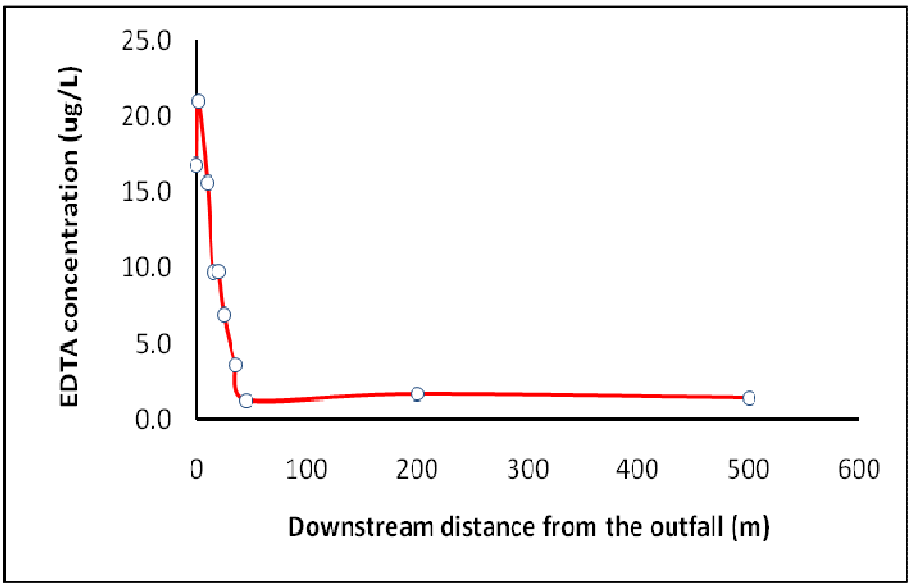

Figure 6.19 The trend of EDTA concentrations in the Waitoa River, where the Waitoa dairy effluent discharged for the monitored case of 30 May 2008.

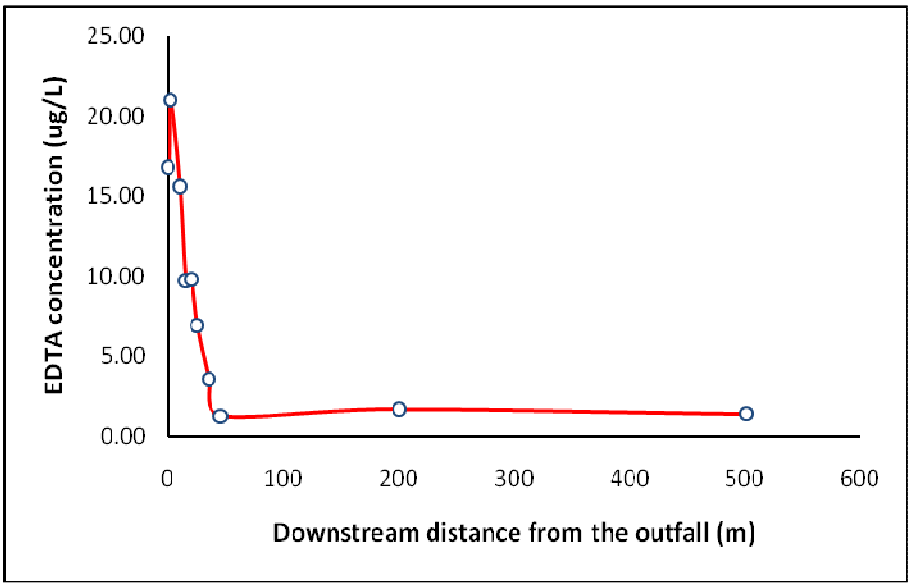

Figure 6.20 The trend of EDTA concentrations in the Waitoa River, where the Waitoa dairy effluent discharged for theworst case of the year of 2008.

6.5 DISCUSSION AND CONCLUSIONS

i. A large number of one- to three-dimensional models are used for describing water quality and simulating the transport of dissolved pollutants in freshwater systems or coastal seas (Rajar and Cetina, 1997; Periáñez, 2004; Rajar etal., 2004; Li et al., 2005). Models are usually based upon the environment, model purpose, the number of 'dimensions' considered etc. (Cox, 2003). However, three-dimensional (3D) models are highly sophisticated and usually reserved for large estuaries where the mixing patterns are complex (Rajar and Cetina, 1997; Rajar etal., 2004), but for water-quality in freshwater systems, such complex 3D solutions are not usually necessary. In this study, the environment is a mixing zone downstream of the dairy effluent input to the main water body of the Waitoa River, and the model purpose is to increase the understanding of the effects of dairy effluent discharge containing EDTA on the input stream. Both quasi one-dimensional empirical calculations (Rutherford, 1981) and the two-dimensional hydrodynamic (3DD model) and transport model (POL3DD) were undertaken to make a preliminary estimate of EDTA dispersal processes within the receiving stream of the Waitoa River.

ii. The one-dimensional model (Cox, 2003) generally presents the water flow and the advection and dispersion of solutes such as EDTA, in just one direction downstream in a river, where the river is assumed to be completely mixed across its width and depth. Based upon this, the National Institute of Water & Atmospheric Research (NIWA) (Hamilton) developed a dissolved oxygen (DO) model of STUDIO (Steady Uniform Flow Dissolved Oxygen) to investigate the transversal downstream of DO and to determine the effect of photosynthetic oxygen input from the Waitao dairy factory to the Waitoa River system (Oldman, 1996). However, when the Fonterra Waitoa dairy effluent discharges into the Waitoa River with a steady uniform transverse line-source, transverse concentration gradients are negligible due to the uniform line-source. Longitudinal gradients are also negligible because the source is steady. Thus, the dispersion of EDTA in the Waitoa River simplifies to become quasi one-dimensional of vertical mixing only (Rutherford, 1981). Rutherford (1981) used some empirical formula to calculate the vertically

complete mixing distance and concentration of EDTA occurring downstream of the outfalls in the Waitoa River, when the horizontal and transversal dispersion of EDTA are neglected.

iii. Based upon the quasi one-dimensional line source of the Waitoa dairy effluent discharge, the dispersion of EDTA in the Waitoa River was illustrated by vertical mixing dispersal contours along the river bed, at one-quarter depth and the mid-depth. It is evident that the vertical mixing is symmetrical at the mid-depth. However, it may be overestimated for the rate of EDTA dispersion downstream from the outfall on the bed of the rough natural channel of the Waitoa River due to the small velocity and dispersal coefficient close to the boundary. Estimated results of the empirical formula showed that the EDTA discharge from the Waitoa dairy effluent was completely mixed only 6 m downstream, assuming the river depth of 1 m, width of 8 m, slope of 2×10^{-4}, and mean velocity of 0.22 m/s. Fully mixed concentrations of EDTA were 3.18 µg/L for the monitored case (30 May 2008), and 40.3 µg/L for the worst case of the maximum EDTA used in the processing plant combined with a low river flow (425 L/s).

iv. A two-dimensional model either simulates dispersion across the width or depth of the stream (Cox, 2003). The Waitoa River is shallow, so that stratification is limited, but dispersion across the width of the river is slow. A two-dimensional, depth-averaged hydrodynamic model was, therefore, used to simulate EDTA dispersion in the Waitoa River. A similar principle of two-dimensional, vertically integrated hydrodynamic model is also employed in a multidam river system by Li et al. (2005).

v. The 3DD hydrodynamic model uses an explicit, finite difference scheme to solve momentum and continuity equation, and it is the ideal modelling tool for management, science and applied research (Black, 2002). Given a 1x1 m grid resolution with a precise bathymetry created by a single beam eco-sounder, a two-dimensional, depth-averaged hydrodynamic model (3DD) was set up for a low river velocity of 0.2 m/s. Outputs of the 3DD model concur well with the field measurements by an averaged difference of 2.3%. The 3DD hydrodynamic model was performing consistently by giving the exactly same velocities with different time series. A high river

velocity of 1.0 m/s was also simulated by the 3DD hydrodynamic model. Dead zones caused by an eddy close to the boundary were observed for both low and high river currents of 0.2 and 1.0 m/s, where vectors of the river velocity were much small corresponding to the domain river velocities.

vi. EDTA dispersion of the monitored case of 30 May 2008, and the worst case of high EDTA discharge combined with a low river flow were simulated in the Waitoa River. From the modelling, it can be seen that the maximum EDTA concentrations were present in the mediate vicinity of the dairy effluent outfall in the Waitoa River for both monitored and worst cases. The important finding was there was no significant increase of EDTA concentrations beyond 50 m downstream from the outfalls. The highest concentrations of EDTA seemed to be around 2 μg/L for the monitored case and 21 μg/L for the worst case. These values are nearly half of the quasi one-dimensional calculations. However, the quasi one-dimensional, vertical mixing of EDTA is only driven by turbulence generated by velocity, and the transversal mixing driven by the velocity of domain river flow was neglected. The estimated results are only indicative, less accurate for the EDTA dispersal in the river. The two-dimensional, depth-averaged 3DD hydrodynamic model considers the EDTA dispersal in both transversal and horizontal directions, which is believed to provide more practical simulation of EDTA dispersal in the Waitoa River.

vii. Another key point that should be noted is that the concentration of EDTA in the immediate vicinity of the receiving stream was as high as 21 μg/L (or 40.3 μg/L from approximate calculation) for the worst scenario of high volume dairy effluent discharged into the low flow rate of the Waitoa River, and then it was gradually diluted to ~1.5 μg/L due to the river flux and dispersion. These suggest that (i) the maximum EDTA concentration occurring in the immediate vicinity of the dairy outfall is well below the Predicted Effect Concentration (PEC) of 2.2 mg/L for aquatic environments advised by the European Union advised (European Chemicals Bureau, 2004); (ii) this value is also well under the New Zealand Drinking Water Standards of 0.7 mg/L EDTA for health purposes (Ministry of Health, 2005), and (iii) large volumes of dairy effluent

discharge seem to not result in a significant EDTA contribution to the Waitoa River.

In conclusion, the dairy effluent discharge from the Fonterra Waitoa dairy processing plants will not to lead to a significant increase of the EDTA concentration in the Waitoa River, based upon the estimated calculation and the simulation of EDTA dispersion within the Waitoa River by the 3DD hydrodynamic model.

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7.0 CHAPTER SEVEN: INVESTIGATION OF EDTA AND HEAVY METALS IN SOILS AND GROUNDWATER

The use of wastewater for irrigation on agricultural land is a world-wide practice, which offers an economic alternative to disposal into surface waters and it supplies nutrients to soils (Haruvy et al., 1999, Friedal et al., 2000, Angin et al., 2005). In New Zealand, dairy wastes are commonly applied to land as a means of fertilising or boosting the productivity of soils (Degens et al., 2000, Sparling et al., 2001). This is referred to as land application, and includes irrigation of dairy wastewater treatment sludge (also known as biomass or biosolid) and dairy effluent onto pasture land (Figure 7.1 and Figure 7.2). Long term land application can induce changes in the quality of soil, especially as trace element inputs are sustained over long periods and it may lead to groundwater contamination (Stuart and Milne, 2001, Silveira et al., 2003, Sinha et al., 2006, Xie et al., 2007). This chapter, therefore, investigates EDTA and heavy metals present in soils and groundwater in a pastoral area subjected to land application of wastes from dairy factories.

Figure 7.1 Dairy wastewater treatment sludge being spread onto nearby pastureland as an alternative disposal of dairy wastes in the New Zealand dairy industry.

(Photo provided by Fonterra Co-operative Group Limited)

Figure 7.2 Land spray irrigation system of dairy effluent in the New Zealand dairy industry.

(Photo provided by Fonterra Co-operative Group Limited)

7.1 LAND APPLICATION OF DAIRY WASTES IN NEW ZEALAND

Land application of wastes from dairy factories in this study includes dairy wastewater treatment sludge being spread onto pastureland and land spray irrigation of dairy wastewater. The Fonterra Waitoa dairy site was selected as a case study of biomass application onto pastureland (Figure 7.1), and the Fonterra Kauri dairy site as a case study of land spray irrigation of dairy wastewater (Figure 7.2).

7.1.1 Dairy Wastewater Treatment Sludge Disposal

At the Fonterra Waitoa dairy site, wastewater from dairy processing plants is treated by an extended aeration sludge biological treatment. About 300 m^3 of sludge from the wastewater treatment plants (WWTPs) with 2.5 - 3 % solid (biomass) is trucked away on a daily basis and spread onto nearby pasture (Figure 7.1).

7.1.2 Land Spray Irrigation of Dairy Wastewater

1. Wastewater system at the Fonterra Kauri dairy site

Wastewater from dairy processing plants at the Fonterra Kauri site is collected in a sump (Figure 7.3) and treated by the 'dissolved air flotation' (DAF) process (Figure 7.4). The treated wastewater is then pumped through pipelines to the irrigation station and spray irrigated onto pasture land, as illustrated in Figure 7.1.

Figure 7.3 A site wastewater sump at the Fonterra Kauri dairy factory, Northland in New Zealand.

Figure 7.4 Wastewater pre-treatment by the dissolved air flotation (DAF) process prior to the land spray irrigation at the Fonterra Kauri dairy site.

2. Pasture areas irrigated by wastewater from the Fonterra Kauri dairy site

Farms irrigated by dairy wastewater from the Fonterra Kauri site include the Jordan Valley, Hikurangi and Kauri farms. The Jordan and Hikurangi farms possess clay soils, and Kauri farm has volcanic soils (Figure 7.5).

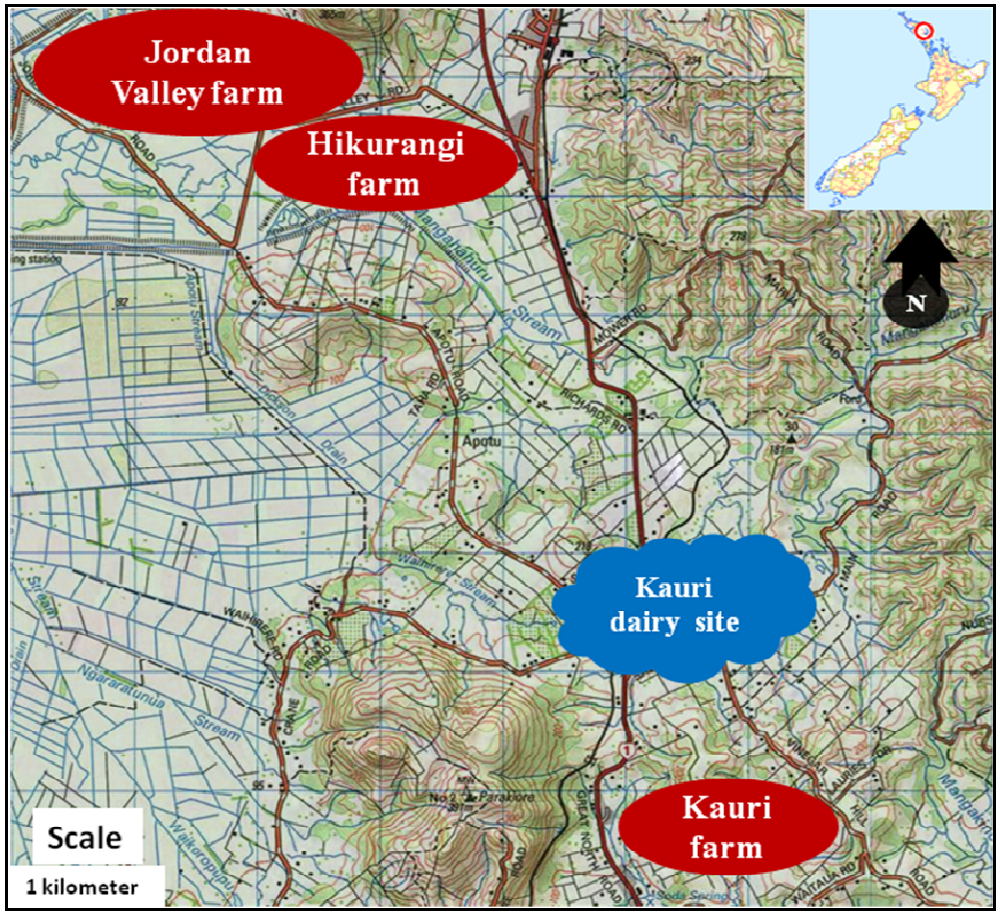

Figure 7.5 Layout of pasture areas irrigated by dairy wastewater, including the Jordan Valley, Hikurangi and Kauri farms, nearby the Fonterra Kauri dairy site.

(Map source: Map Toaster Topo/NZ)

7.2 METHODS

7.2.1 Dairy Wastewater Treatment Sludge

1. Dairy sludge sample collection

Dairy sludge samples were collected from the gravity belts when sludge was being pre-concentrated before trucking away (Figure 7.6)

Figure 7.6 Dairy sludge samples were collected from sludge pre-concentrated gravity belts at the Waitoa wastewater treatment plants (WWTPs).

2. Sludge sample pre–treatment

Pre-treatment of the sludge samples for analysis involved:

a. Spinned the sludge samples in a centrifuge and oven drying at 90^0C;
b. Weighed a 0.5 g dried sludge sample in a 20 mL vial;
c. Extracted EDTA with 15 mL of 0.002 M NaH_2PO_4 desorption solution in an ultrasonic bath (Bransonic 220) for 15 minutes (Nowack et al., 1996);
d. Took 5 mL supernatant and adding 0.1 mL 0.194 g/L Fe^{3+};
e. Allowed the complexing of Fe(III)EDTA overnight in the dark; and

f. Filtered the solution through a 0.45 µm cellulose nitrate filter (Phenomenex) using a syringe unit prior to HPLC-UV analysis.

7.2.2 Analyses of Soil Samples

1. Sample collection

Composite soil samples were taken from 0-75 mm within the pastoral topsoil, onto which dairy biomass or wastewater had been applied, by taking approximately 10 cores (1.5 meters between cores) per arm of a 'z' pattern across to give a total of 30 cores (Figure 7.7). A 75 mm deep auger was used and samples were pooled for one composite sample (Figure 7.8). Soil sampling sites of pastureland near the Fonterra Waitoa dairy site are presented in Figure 7.9, and two of each irrigated and un-irrigated paddocks were selected for the Hikurangi and Kauri farms, which possess clay and volcanic soils irrigated by dairy wastewater from the Fonterra Kauri dairy site. Soil samples from un-irrigated areas were collected for a comparison.

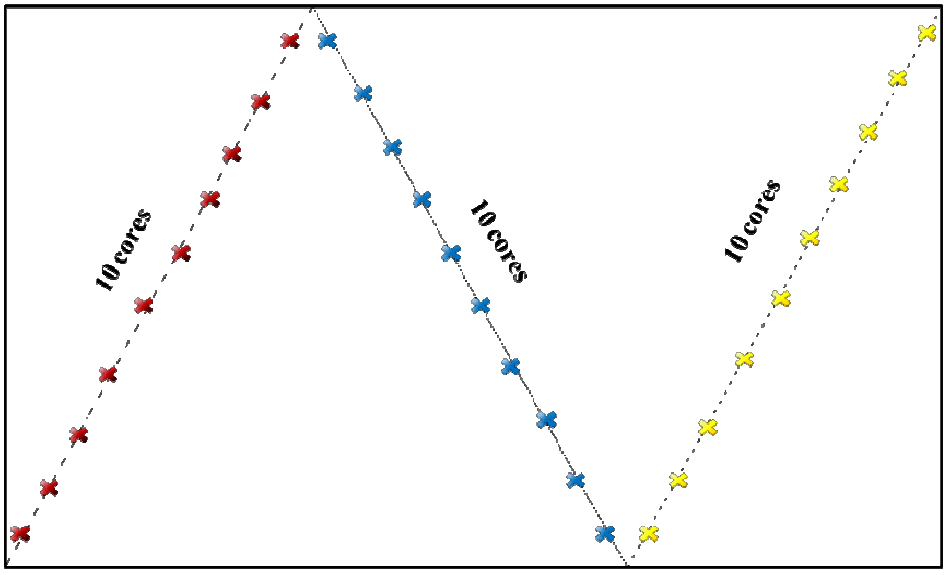

Figure 7.7 A pattern of approximately 10 cores (1.5 m between each core) apart per arm of a 0-75 mm deep 'z' shape of across to give a total of 30 cores were taken and pooled to produce one composite pastoral topsoil sample.

Figure 7.8 Sample was collected with a 75 mm deep auger and pooled into a plastic bag for a composite pastoral topsoil sample.

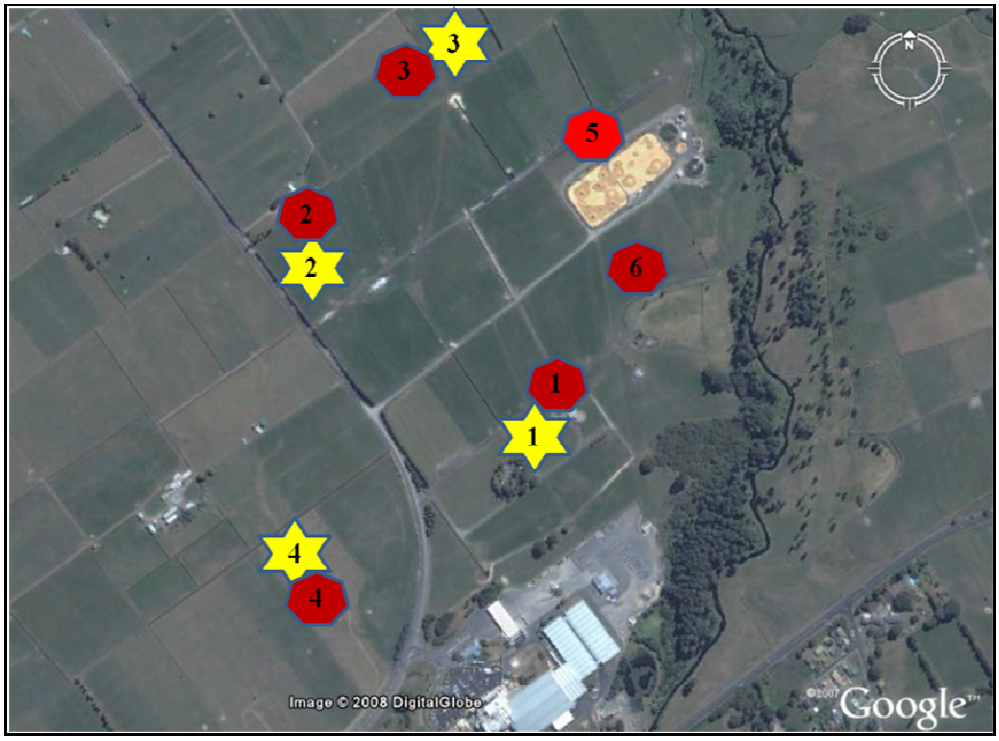

Figure 7.9 Layout of paddocks for soil sample collection and groundwater wells near the Fonterra Waitoa dairy site, where paddock 4 was a reference site as no dairy biomass had been applied, and paddock 5 was the heaviest dairy biomass spread paddock.

(- groundwater bores and - soil sampling sites)

(Map Source: Google Earth)

2. Soil sample pre-treatment

Soil samples were dried in a forced air convection drier at 35-40^{0}C and crushed to pass through a 2 mm sieve. This was conducted by Hill Laboratories Limited, Hamilton. A 0.5 gram soil sample was weighed in a 20 mL vial. EDTA was extracted by the method of Nowack et al. (1996) with 15 mL of 0.002 M NaH_2PO_4 desorption solution in an ultrasonic bath for 15 minutes. The extracted solution was kept in the dark and allowed complexing of Fe(III)EDTA overnight, and then filtered through a 0.45 µm cellulose nitrate filter (Phenomenex) prior to HPLC-UV analysis.

3. HPLC separation

Modification of the HPLC method was required there were many peaks from other compounds that eluted in the region of the Fe(III)EDTA peak. For example, Figure 7.10 illustrates the separation of analyte Fe(III)EDTA using a mobile phase containing 2 % MeOH and 15 mM TBABr within a pH 3.3 formic acid/formate buffer solution, at a flow rate of 0.9 mL/min.

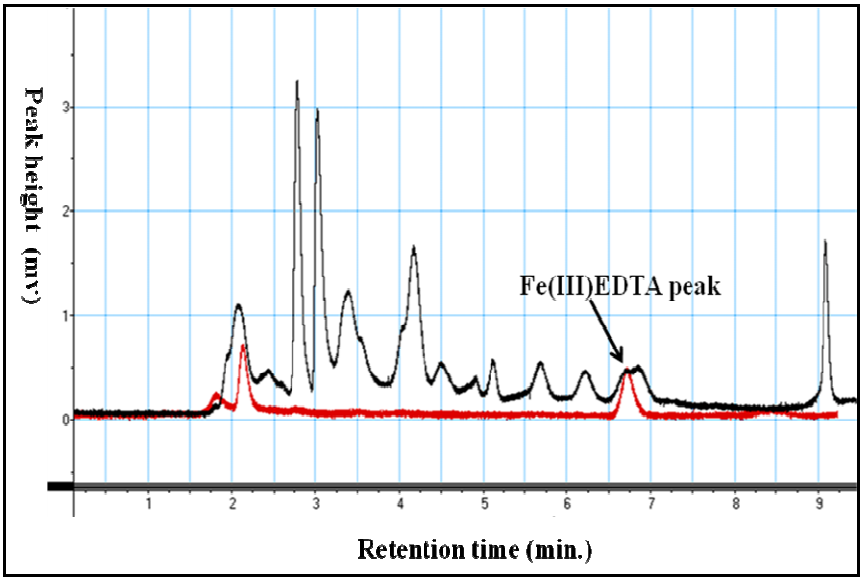

Figure 7.10 Overlay of chromatograms of a 50 µg/L EDTA standard (red line) and a pastoral topsoil sample (black line) from the Hikurangi irrigated farm with dairy wastewater from the Fonterra Kauri site.

(HPLC separation conditions: mobile phase of 2 % MeOH and 15 mM TBABr in pH 3.3 buffer solution at a flow rate of 0.9 mL/min.)

Based on experimental trials, final separation conditions were mobile phase with 1% MeOH and 10 mM TBABr in a pH 3.3 formic acid/formate buffer solution, at a flow rate of 1.0 mL/min. The chromatogram with an appropriately separated peak of Fe(III)EDTA for the determination of EDTA in the same soil sample is illustrated in Figure 7.11.

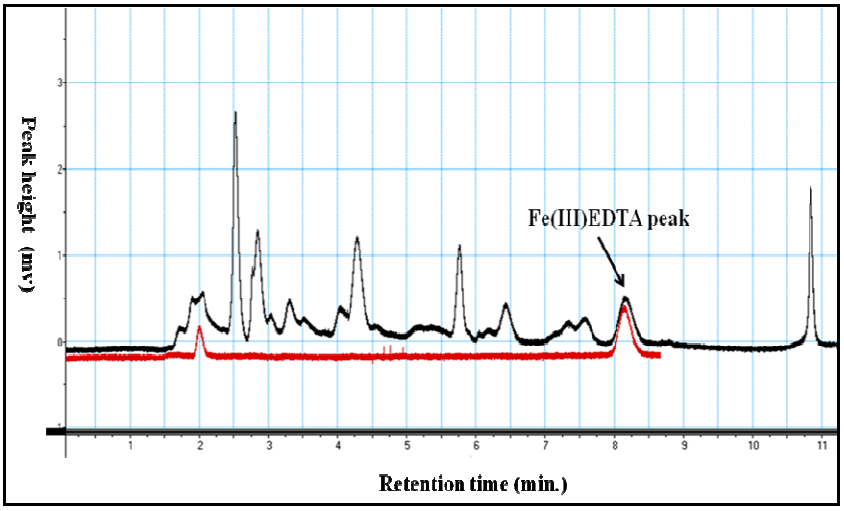

Figure 7.11 Overlay of chromatograms of an 80 µg/L EDTA standard (red line) and a pastoral topsoil sample (black line) from the Hikurangi irrigated farm with dairy wastewater from the Fonterra Kauri site.

(HPLC separation conditions: mobile phase of 1 % MeOH and 10 mM TBABr in pH 3.3 buffer solution at a flow rate of 1.0 mL/min)

4. Quality control and detection limit

The method was verified using a spiked recovery (≥80%, n=4) with a standard EDTA solution as there is no certified material reference available (Figure 7.12). Quality control of analyses was undertaken by running a blank, a duplicate sample every 10^{th} sample and a spiked recovery every 20^{th} sample. Duplicated results were within 8.7% (n=4) and spiked recoveries were 95 – 98% (n=2).

The method detection limit of 0.15 mg/kg (dry weight) for soils was calculated based on the HPLC-UV method of 5 µg/L of EDTA.

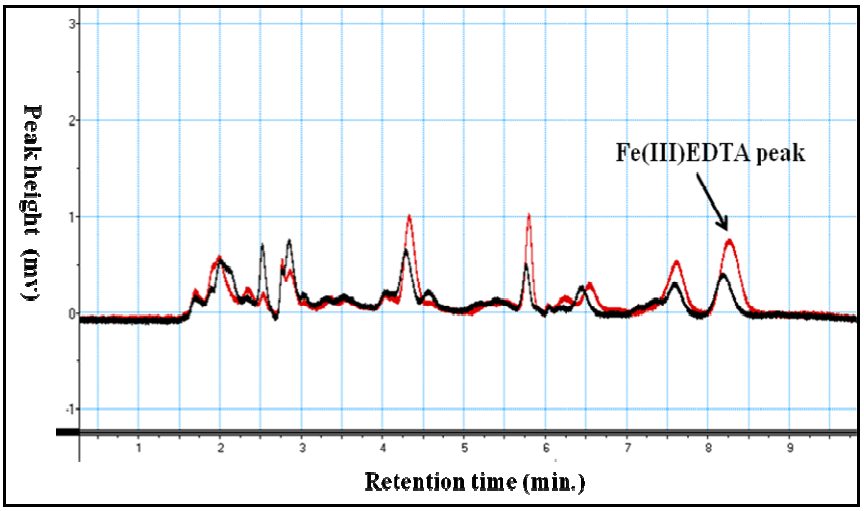

Figure 7.12 Overlay of HPLC-UV chromatograms of a 100 µg/L EDTA spiked soil sample (red line) and a pastoral topsoil sample only (black line) from the Hikurangi irrigated farm with dairy wastewater from the Fonterra Kauri site.

5. Other analyses for soil samples

Metals and other measurement for soils were analyzed by Hill Laboratories Limited, Hamilton. Method and details are shown in Appendix 4.

7.2.3 Analyses of Groundwater

1. Sample collection

Groundwater samples were collected in opaque PE bottles to avoid photolysis of the Fe(III)EDTA and stored at 4ºC until analysis. Sample collection sites for the Fonterra Waitoa dairy site are shown in Figure 7.9, where groundwater bores are located. Groundwater samples were collected on 4 November, 2007, 8 February and 15 July, 2008.

For the Fonterra Kauri dairy site, groundwater samples were obtained by digging a hole to reach the groundwater and pumping it through a plastic tube to a container (as illustrated in Figure 7.13) at both the Hikirangi and Kauri farms. Groundwater samples were collected on the same day as soils.

Figure 7.13 Groundwater sample was collected at both the Hikurangi and Kauri farms near the Fonterra Kauri dairy site by digging a hole to reach the groundwater and drawing it through a plastic tube to a container.

2. EDTA Analysis for groundwater using HPLC-UV

A pre-concentration step was needed for the determination of EDTA in groundwater samples because of their low concentrations. Groundwater samples (10 mL) were heated to dryness at 90^0C, reconstituted into a mobile phase, followed by complexing of Fe(III)EDTA and analysed using HPLC-UV. The pre-

concentrated factor of 2.5 was appropriate to attain a clear EDTA peak as illustrated in Figure 7.14 and Figure 7.15.

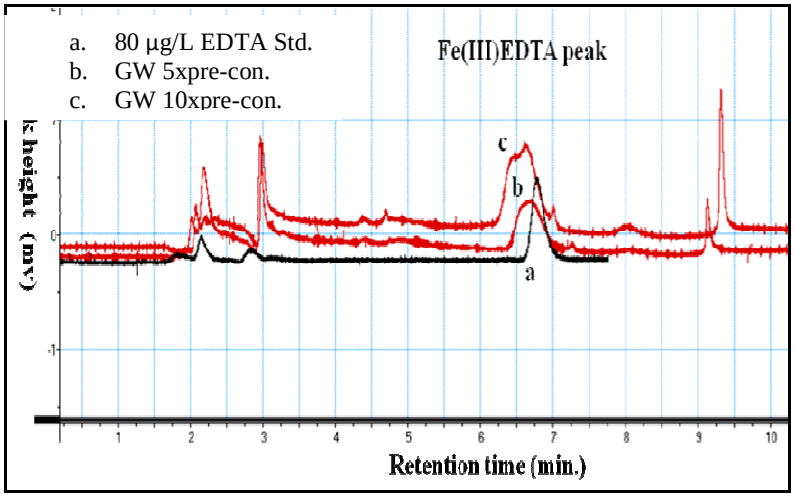

Figure 7.14 Overlay of HPLC-UV chromatograms of an 80 µg/L EDTA standard (black line) and a groundwater sample with a pre-concentrated factor of 5 and 10 (red lines of b and c respectively).

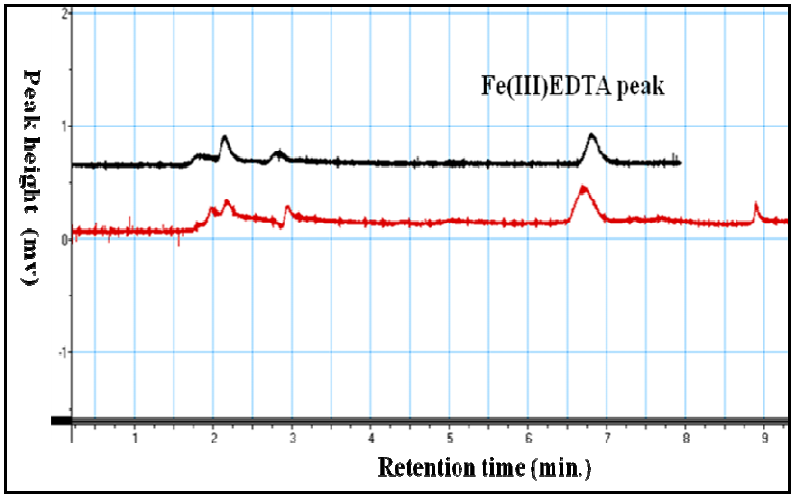

Figure 7.15 Overlay of HPL–UV chromatograms of a 25 µg/L of EDTA standard (black line) and a groundwater sample with a pre-concentrated factor of 2.5 (red line).

3. Quality of control and detection limit

Quality control of the analysis was the same as for previous sector. The duplicate limit and EDTA standard spiking recovery of 80 µg/L was within 7% (n=8) and

91-107% (n=5), respectively. The detection limit was 2 µg/L of EDTA due to the pre-concentrated factor.

4. Other analyses of groundwater

Associated heavy metals and other parameters of groundwater were obtained by Hill Laboratories Limited, Hamilton. Method and details are shown in Appendix

7.3 RESULTS

7.3.1 Presence of EDTA in dairy sludge

Chromatograms of dairy sludge samples were complicated due to a wide range of chemical and biological constitutions in the sludge. EDTA is generally identified by the retention time (minutes), and thus EDTA was likely to occur in sludge samples by comparing overlay of chromatograms of a standard EDTA and sludge sample (Figure 7.16). No further study was undertaken on its analytical conditions for this particular case.

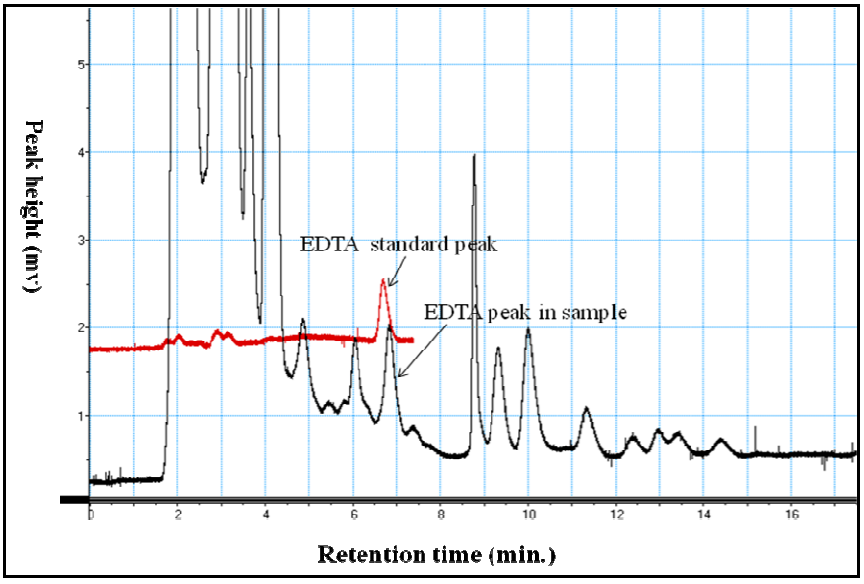

Figure 7.16 Overlay of chromatograms of a 100 µg/L EDTA standard solution (red line) and a dairy sludge sample (black line) from the Waitoa dairy wastewater treatment plant.

7.3.2 EDTA Occurrence in Pastoral Topsoil and Groundwater Following Land Application of Dairy Sludge

Analytical results of EDTA in pastoral topsoil and groundwater are tabulated in Table 7.1 and Table 7.2, where the dairy sludge from the Waitoa dairy wastewater treatment plant was spread onto pasture.

Table 7.1 EDTA analytical results for pastoral topsoil samples from the Fonterra Waitoa dairy site using HPLC-UV, where paddock 4 was a reference site as no sludge had been applied, and paddock 5 was the heavieist sludge pread paddock.

Sampling date	Sampling site	EDTA concentration (mg/kg)	Detection limit (mg/kg)
14-Feb-08	Paddock 1	0.41	0.15*
	Paddock 2	<0.15	0.15
	Paddock 3	< 0.15	0.15
	Paddock 4	< 0.15	0.15
	Paddock 5	< 0.15	0.15
	Paddock 6	< 0.15	0.15
15-Jul-08	Paddock 1	< 0.15	0.15
	Paddock 2	< 0.15	0.15
	Paddock 3	< 0.15	0.15
	Paddock 4	< 0.15	0.15
	Paddock 5	< 0.15	0.15
	Paddock 6	< 0.15	0.15

*0.15 mg/kg (dry weight) was calculated based on the method detection limit of 5µg/L EDTA for HPLC-UV

EDTA was only detected at paddock for both sample collecting dates at the Fonterra Waitoa dairy site. Otherwise EDTA appeared to be below the detection limit, even for the paddock 5 in which the heaviest biomass had been applied. There was no dairy biomass (sludge) actually having been spread on paddock 1 except a dairy shed from which some washing detergents used may contain EDTA for cleaning purposes. This suggests that the land application of dairy biomass seems not to significantly increase EDTA levels in pastoral top soils.

Table 7.2 EDTA analytical results in groundwater near the Fonterra Waitoa dairy site, where bore 4 was a reference site and there was no bore on the heaviest sludge spread paddock (paddock 5).

Sampling date	Sample	Groundwater depth (m)	Pre-concentrated factor	EDTA concentration (µg/L)
4-Nov-07	Bore 1	2.4	2.5	26.4
	Bore 2	1.2	2.5	<2*
	Bore 3	4.1	2.5	<2
8-Feb-08	Bore 1	3.1	2.5	20.1
	Bore 2	1.9	2.5	<2
	Bore 3	2.6	2.5	<2
	Bore 4	3.3	2.5	ND**
15-Jul-08	Bore 1	2.8	2.5	14.5
	Bore 2	1.0	2.5	<2
	Bore 3	2.1	2.5	<2
	Bore 4	2.8	2.5	ND**

* - less than detection limit
** - No EDTA peak observed

It can be concluded from Table 7.2 that

i. EDTA was only detected in bore 1, which also had the highest concentration in soil;
ii. the concentration of EDTA in the groundwater collected in July 2008 was less than that in November 2007 and February 2008; and
iii. the depth of groundwater was shallower in July than in November and February, except for bore 1.

7.3.3 EDTA Presence in Pastoral Topsoil Subjected to Land Irrigation of Dairy Effluent

1. EDTA concentration of wastewater from the Fonterra Kauri dairy site

Monthly treated composite wastewater samples from February to April 2008 were collected at the Fonterra Kauri dairy site. There were no wastewater samples from

May to August 2008 as dairy processing plants were under maintenance during low milk production season. Some water samples from a drainage ditch (testing for runoff) along the Hikurangi farm (Figure 7.17) were also collected in April and July 2008. Analytical results for both samples are presented in Table 7.3.

Figure 7.17 The drainage ditch where samples were collected for assessment of EDTA runoff at the Hikurangi farm irrigated by dairy wastewater from the Fonterra Kauri dairy factory.

Table 7.3 EDTA concentrations in dairy wastewater and water samples from the drainage ditch along the Hikurangi farm near the Fonterra Kauri dairy site.

Sampling date	Sample	EDTA concentration (µg/L)
Feb-08	Kauri monthly ww	197.1
Mar-08	Kauri monthly ww	11.4
Apr-08	Kauri monthly ww	172.6
11-April-08	Hika farm ditch #1	58.4
	Hika farm ditch #2	59.8
	Hika farm ditch #3	69.0
	Hika farm ditch #4	71.6
17-July-08	Hika farm ditch #1	99.7
	Hika farm ditch #2	79.7
	Hika farm ditch #3	81.6
	Hika farm ditch #4	101.8

Test results of EDTA show clearly that EDTA was detected in both dairy wastewater from the Fonterra Kauri dairy site and surface water samples (runoff) of the drainage ditch at concentrations of 11 – 200 µg/L.

2. Analytical results of pastoral topsoil samples

Analytical results of pastoral topsoil for both Hikurangi farm (clay soil) and Kauri farm (volcanic soil) are presented in Table 7.4.

Table 7.4 EDTA analytical results for pastoral topsoil samples from the Hikurangi and Kauri farms irrigated (I) and un-irrigated (U) with wastewater from the Fonterra Kauri dairy site using HPLC-UV.

Sampling date	Soil type	Site	Sample	EDTA concentration (mg/kg)	Detection limit (mg/kg)
11-April-08	Clay	Hika farm		< 0.15	0.15*
	Clay		U (B)	< 0.15	0.15
	Clay		I (A)	0.93	0.15
	Clay		I (B)	0.75	0.15
	Volcanic	Kauri farm	U (A)	<0.15	0.15
	Volcanic		U (B)	<0.15	0.15
	Volcanic		I (A)	<0.15	0.15
	Volcanic		I (B)	0.17	0.15
17-July-08	Clay	Hika farm	U (A)	<0.15	0.15
	Clay		U (B)	<0.15	0.15
	Clay		I (A)	<0.15	0.15
	Clay		I (B)	<0.15	0.15
	Volcanic	Kauri farm	U (A)	<0.15	0.15
	Volcanic		U (B)	<0.15	0.15
	Volcanic		I (A)	<0.15	0.15
	Volcanic		I (B)	<0.15	0.15

*HPLC-UV detection limit for soil (dry weight)

For land application of dairy wastewater from the Fonterra Kauri dairy site, EDTA was detectable for pastoral top soils at both farms irrigated with clay and volcanic soils for the relatively dry season (11 April, 2008). EDTA was not detected at both farms for the wet season (15 July, 2008), possibly because there was enough moisture within the soils and less dairy wastewater irrigation was required.

3. EDTA in groundwater

EDTA behaves as a persistent substance in its passage through groundwater (Nowack and Sigg, 1997). Various literature reports show that EDTA is widely observed in groundwater with a low concentration of 0.1 - 72μg/L range (Bucheli-Witschel and Egli, 2001, Schmidt et al., 2004, Nowack and VanBriesen, 2005).

In the present study, EDTA concentrations in groundwater were investigated in association with land application of dairy effluent at both the Fonterra Kauri sites. Analytical results of EDTA concentrations are shown in Table 7.5.

Table 7.5 EDTA analytical results in groundwater at both the Hikurangi and Kauri farms irrigated and un-irrigated with dairy wastewater from the Fonterra Kauri dairy site.

Sample Date	Sample	Groundwater depth (m)	Pre-concentrated factor	EDTA concentration (μg/L)
11-Apr-08	Hika un-irrigated farm	3.5	2.5	4.1
	Hika irrigated farm A	2.0	1	331.4
	Hika irrigated farm B	2.0	1	457.3
	Kauri un-irrigated farm A	1.2	1.25	6.4
	Kauri un-irrigated farm B	0.5	1.25	38.0
	Kauri irrigated farm A	1.0	1.25	10.1
	Kauri irrigated farm B	0.8	1.25	143.1
17-Jul-08	Hika un-irrigated farm	2.4	2.5	ND*
	Hika irrigated farm A	1.6	1	51.1
	Hika irrigated farm B	1.6	1	74.7
	Kauri un-irrigated farm A	0.4	2.5	<2**
	Kauri un-irrigated farm B	0.8	2.5	<2
	Kauri irrigated farm A	0.8	1	626.8
	Kauri irrigated farm B	0.4	1	110.3

* - no EDTA peak observed
** - EDTA peak observed but less than the detection limit of 2 μg/L

In Table 7.5, it is demonstrated that:

(i) EDTA was detectable for all groundwater samples collected on 11 April, 2008 and even quite high for groundwater of the irrigated areas; and

(ii) concentrations of EDTA are lower than the maximum acceptable value of 700 μg/L EDTA for New Zealand drinking water (2005).

7.3.4 Soil Characteristics Subsequent to Land Application of Dairy Effluent

At the present study, physical and chemical properties of clay soils at the Hikurangi farm irrigated with dairy wastewater from the Fonterra Kauri site were investigated and compared with the un-irrigated soils by the dairy wastewater. Samples were collected on 11 April 2008 for two of each irrigated and un-irrigated paddocks with dairy wastewater (Table 7.4). Soil samples were air-dried at 35–40^{0}C overnight (residual moisture typically 4%) and crushed to pass through a 2 mm screen. Analyses for soils, including basic soil test data, Mehlich 3 test data and associated heavy metals, were obtained by Hill Laboratories Limited, Hamilton. The original test results are attached in Appendix 4, and analytical results are summarised in Table 7.6.

Table 7.6 Analytical results of clay soil properties, collected on 11 April 2008, at the Hikurangi farm irrigated (I) and un-irrigated (U) with dairy wastewater.

Items	U (A)	U (B)	Mean (U)	I (A)	I (B)	Mean (I)	U/I
pH	6.1	5.9	6.0	6.3	6.2	6.3	**<1**
EDTA concentration (mg/kg dry wt)	0	0	0.00	0.9	0.7	0.8	**<1**
Total Recoverable Cd (mg/kg dry wt)	0.63	0.61	0.62	0.36	0.37	0.37	**>1**
Total Recoverable Fe(mg/kg dry wt)	33000	19000	26000	19000	18000	18500	**>1**
Total Recoverable Hg (mg/kg dry wt)	0.098	0.1	0.1	0.14	0.18	0.16	**<1**
Olsen P (mg/L)	26	42	34	121	125	123	**<1**
K (me/100g)	0.63	0.49	0.56	0.84	1.47	1.16	**<1**
Ca (me/100g)	19.2	21.4	20.3	24.5	22.22	23.4	**<1**
Mg (me/100g)	1.20	1.40	1.30	1.46	1.60	1.53	**<1**
Na (me/100g)	0.17	0.32	0.25	2.72	2.24	2.48	**<1**
CEC (me/100g)	26	29	27.5	34	32	33	**<1**
Base Saturation (%)	81	81	81	87	85	86	**<1**
Volume Weight (g/mL)	0.79	0.70	0.75	0.78	0.71	0.75	**1**
Total Nitrogen (%)	0.60	0.67	0.64	0.87	0.62	0.75	**<1**
Phosphorus (Mehlich 3) (mg/L)	31	52	42	197	156	177	**<1**
Iron (Mehlich 3) (mg/L)	193	384	289	409	399	404	**<1**
Manganese (Mehlich 3) (mg/L)	61.2	11.0	36.1	8.6	13.2	10.9	**>1**
Zinc (Mehlich 3) (mg/L)	2.04	1.90	1.97	2.46	3.15	2.81	**<1**
Copper (Mehlich 3) (mg/L)	1.9	1.5	1.7	1.0	1.3	1.2	**>1**
Boron (Mehlich 3) (mg/L)	<0.5	<0.5	No data	0.6	<0.5	No data	**<1**
Cobalt (Mehlich 3) (mg/L)	0.3	<0.1	No data	<0.1	<0.1	No data	**>1**
Aluminium (Mehlich 3) (mg/L)	532	481	507	546	410	478	**>1**

(Source: Hill Laboratories limited – appendix 4)

Comparing basic soil test data of irrigated and un-irrigated soil samples (Appendix 4), it can be concluded that:

- nutrient levels of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na) were boosted in irrigated soils,
- cation exchange capacity (CEC) (me/100) and base saturation (%) were increased slightly for irrigated soils, and
- volume weight (g/mL) stayed similar in both irrigated and un-irrigated soils.

For Mehlich 3 soil test, concentration of P was evidently boosted. Concentrations of heavy metals - Fe, Mn and Zn seemed to be enhanced as well with the dairy wastewater irrigation areas. Lower concentration of Cu in irrigated soils was found in contrast to the un-irrigated soils, and presence of Al was variable for both pastoral top soils.

7.3.5 Heavy Metals in Groundwater

To investigate the potential transportation of heavy metals by EDTA, further analysis was carried out for groundwater samples in irrigated areas with dairy wastewater, and the groundwater of non-irrigated area was sampled for a comparison. Tests were undertaken by Hill Laboratories at Hamilton, New Zealand. Original data is shown in Appendix 5 and analytical results are presented in Table 7.7.

Based on Table 7.7 it can be concluded that:

i. high concentrations of EDTA (highest ever reported), were detected in the groundwater, where dairy effluent containing EDTA directly irrigated onto pasture; and
ii. there is an indication that trace elements of Cd, Cu, Fe and Zn were transported to the groundwater, identified by the loss of metals in soils and the gain of metals in groundwater. However, further systematic research is needed to confirm this.

Table 7.7 Analytical results for groundwater collected on 11 April 2008 from the irrigated (I) and un-irrigated (I) areas with dairy effluent at the Hikurangi farm.

Items	U (A)	I (A)	I (B)	Mean (I)	U/I
Groundwater depth (m)	3.5	2.0	2.0	2.0	>1
EDTA concentration (µg/L)	4.1	331.4	447	389.2	<1
pH	6.34	5.28	6.42	5.9	>1
EC (mS/m)	17.6	62.5	93.1	77.8	<1
Dissolved Cd (g/m^3)	<0.000050	0.00028	0.00013	0.00021	<1
Dissolved Ca (g/m^3)	2.5	2.5	5.4	4.0	<1
Dissolved Cu (g/m^3)	0.0028	0.0063	0.013	0.0097	<1
Dissolved Fe (g/m^3)	<0.020	0.11	0.14	0.13	<1
Dissolved Mg (g/m^3)	3.9	1.1	3.5	2.3	>1
Dissolved Hg (g/m^3)	<0.000080	0.00008	<0.000080	No data	-
Dissolved K (g/m^3)	2.2	1.1	4.8	3.0	<1
Dissolved Na (g/m^3)	24	110	210	160.0	<1
Dissolved Zn (g/m^3)	0.0041	0.072	0.024	0.048	<1
Dissolved Reactive Phosphorus (g/m3)	0.14	0.0054	0.0046	0.005	>1
Total Phosphorus (g/m3)	1.9	0.12	0.89	0.5	>1
Nitrate-N+ nitrite-N (g/m^3)	0.055	2	0.36	1.2	<1
Total Kjeldahl Nitrogen (TKN) (g/m^3)	0.44	0.71	6.9	3.8	<1

(Source: Hill Laboratories limited – appendix 5)

7.4 DISCUSSION

Land application of dairy wastes, including dairy biomass and wastewater, is an economic and practical option due to their fertiliser benefits (Longhurst et al., 2000, MfE and NZWWA, 2003, Russell, 2007). However, long term or overdose of land application has been reported to induce changes in the characteristics of soils, such as trace element inputs, when sustained over long periods (Degens, et al. 2000, Longhurst et al. 2000, Sparling et al. 2001). López-Mosquera et al. (2000) suggested that the dairy sludge was a source of heavy metals for soils, but that even the short- or medium-term (4 y) did not lead to harmful accumulation of heavy metals in soils. They however indicated that long-term sludge application would eventually lead to a build-up of heavy metals in soils. The survey of Fonterra dairy wastewater and biomass by Russell (2007) from Fonterra Research

Center Limited also recommended that total heavy metal analyses should be determined on irrigation farms at 10-yearly intervals to ensure heavy metals levels are stable.

Furthermore, heavy metals may leach to groundwater and lead to a risk of groundwater contamination, in particular with EDTA chelates (Cooper et al., 1999, Tandy et al., 2006). Contaminants can be involved in many different reactions and processes in soils, but their ultimate fate can be summarized as shown in Figure 7.18.

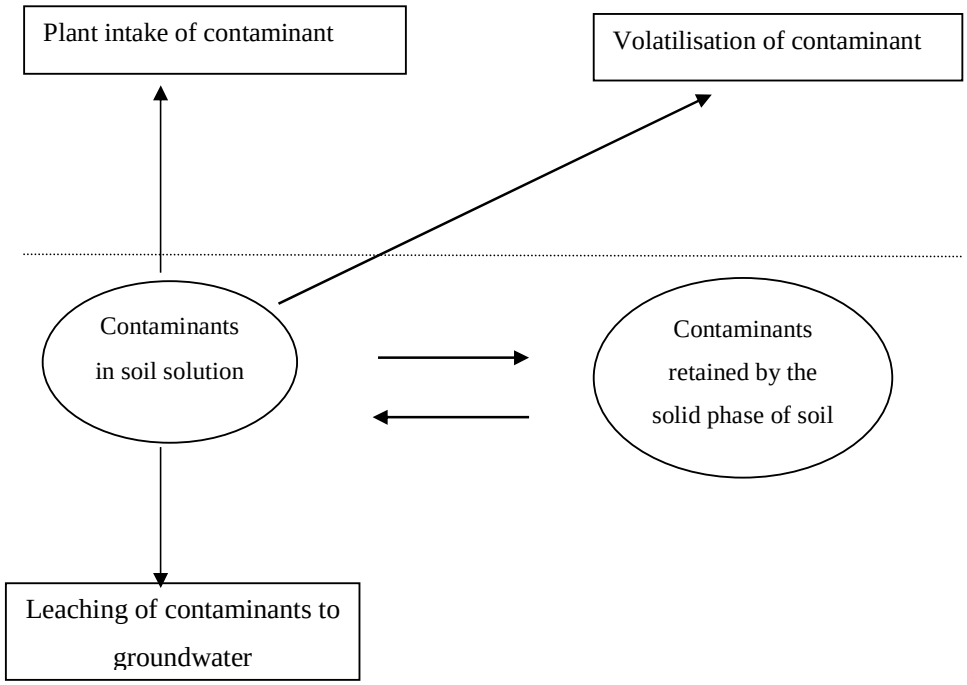

Figure 7.18 Simplified depiction of the fate of contaminants in soils.

(Source: MfE and NZWWA, 2003)

EDTA has the potential to remobilize metals adsorbed onto a solid (Nowack et al., 1996, Sillanpää et al., 1997, Sillanpää and Romo, 2001, Ceremigna et al., 2005), which suggests that metal ions are likely to be transported together with EDTA under environmental conditions (Xue et al., 1995). The process depends upon the competition between EDTA in solution and binding of metals to particular compounds, mostly by complexing with surface ligands (Knepper, 2003, Di Palma and Mecozzi, 2007).

7.4.1 Effects of Soil Properties and Management of Land Application of Dairy Wastewater

There are various reports describing effects on the physical and chemical properties of soils irrigated with dairy wastewater (Shahalam et al., 1998, Friedal et al., 2000, Zhou et al., 2003, Angin et al., 2005, Dawes and Goonetilleke, 2006). The current study has also shown the changes of soil properties, such as P, CEC, and minerals, induced by the irrigation of dairy wastewater. The most important point from this study is that heavy metals may be retained in soils because of the dairy wastewater irrigation. This disadvantage of the wastewater irrigation was also suggested by Angin et al. (2005).

In Table 7.6, if the value of U/I is greater than 1 (as for Fe, Cu, Co, Mn, Al and Cd), metals may either have been taken up into plants (stimulated growth by irrigation) by enhancing plant availability of EDTA; or lost to groundwater by leaching of the complexion with EDTA or free ions). If the value of U/I is less than 1 (as for P, N, K, Na, Ca and Mg), it can be inferred that the compounds have been retained in soils after irrigation due to metal exchanging, especially via inorganic soil colloids (MfE and NZWWA, 2003).

In terms of the EDTA concentration, it was under the method detection limit for pastoral topsoil where the dairy sludge was applied. This suggests that the land application of dairy biomass seems not to significantly increase EDTA levels in the pastoral topsoil. However, EDTA was detectable in both randomly picked paddocks irrigated by the Kauri dairy wastewater, as EDTA complexes themselves may be adsorbed onto the surface of soil (Nowack, 2002). Conversely, EDTA was not detectable in both un-irrigated paddocks.

Concentrations of nitrogen (N) of Nitrate-N (NO_3^--N) + Nitrite-N (NO_2^--N) and total Kjeidahl nitrogen (TKN) was higher in the irrigated groundwater than in the un-irrigated groundwater, which is possibly caused by nitrogen leaching from soils to groundwater due to nutrients in dairy wastewater (Longhurst et al. 2000, MfE and NZWWA, 2003). Moreover, the irrigated groundwater A seemed to have higher content of NO_3-N + NO_2-N than the irrigated groundwater B. On the

contrary, the irrigated groundwater A appeared to have lower content of TKN than the irrigated groundwater B, presenting nitrogen as ammonia (NH_3-N) or bound in organic compounds.

Nitrogen is also an essential nutrient element for pasture growing. However, it may pose a risk to the ground water if the management of land application loses its control. The existing study has demonstrated that higher levels of nitrogen were observed in the groundwater of irrigated areas versus the un-irrigated area, which were even far below the drinking water limit of 11.3 mg/L nitrate-N that is equal to a concentration of 50 mg/L (maximum acceptable value) nitrate ion (Ministry of Health, 2005). Nonetheless, this agrees with Longhurst et al. (2000) that the application of nitrogen from dairy wastewaters should be limited.

7.4.2 EDTA in Groundwater

It has been recorded in the literature that EDTA is widely observed in groundwater at a low μg/L concentration (0.1 – 72 μg/L) (Bucheli-Witschel and Egli, 2001, Schmidt et al., 2004, Nowack and VanBriesen, 2005). The present research has indicated that EDTA is likely to be found at a low concentration from the groundwater collected. Nonetheless, high concentrations of EDTA (highest ever reported) were also observed in the groundwater from the irrigated areas with dairy wastewater containing EDTA. On the other hand, this is also confirmed by the higher concentrations of total Kjeldahl nitrogen (TKN) in the irrigated groundwater as EDTA contains about 10% nitrogen.

7.4.3 Mobilisation of Heavy Metals from Soil to Groundwater

Heavy metals, such as cadmium, zinc, copper and mercury, can cause significant damage to the environment and human health due to the mobility and solubility. Some concern has been raised about the enhanced mobility of heavy metals in soils and their potential risk for leaching to groundwater with chelates (Cooper et al., 1999, Tandy et al., 2006).

There seems no conclusive evidence of heavy metals' remobilization potential by EDTA at environmentally realistic concentrations (Knepper, 2003). Some reports suggest that remobilization of metals from sediments by EDTA, is likely to happen under environmental conditions (Nowack and Sigg, 1997, Stumm, 1995, Sillanpää and Romo, 2001, Ceremigna et al., 2005). Consequently, EDTA has been used for many years as an extractant for metals from soils and sediments to characterize the plant-available fraction. EDTA chelates have also been proposed as enhancers for the phytoremediation of heavy metals by plants and soil washing (Hong and Jiang, 2005, Juang and Wang, 2000). Furthermore, some authors (Cooper et al., 1999, Tandy et al., 2006) have mentioned that heavy metals may leach to groundwater and lead to a risk of groundwater contamination, particularly with chelates.

The study undertaken for the Fonterra Kauri dairy site indicates that concentration changes of heavy metals were observed. For instance, Fe and Zn of Mehlich 3, and total recoverable Hg were consistently increased in irrigated soils versus un-irrigated soils with dairy wastewater from the Fonterra Kauri site. Conversely, total recoverable Cd and Fe were decreased in irrigated soils against un-irrigated soils. Table 7.8 and Figure 7.19 present the comparison of the metal mobility with its EDTA complex constant in the soil-groundwater system from changes of metal concentrations in the unirrigated and irrigated soil and groundwater.

Table 7.8 Comparison of the metal mobility with its EDTA complex constant in the soil-groundwater system from changes of the metal concentration in the unirrigated and irrigated soil and groundwater.

Heavy metals	Complex constant log K_{MeEDTA}	Unirrigated soil/water	Irrigated soil/water	Ratios (U/I) soil/water
Mg	8.8	0.3	0.7	0.5
Ca	10.6	8.1	5.9	1.4
Cd	16.4	12400	1780	7.1
Zn	16.4	480	58	7.8
Cu	18.7	607	119	5.1
Fe	25.0	1300000	148000	8.8

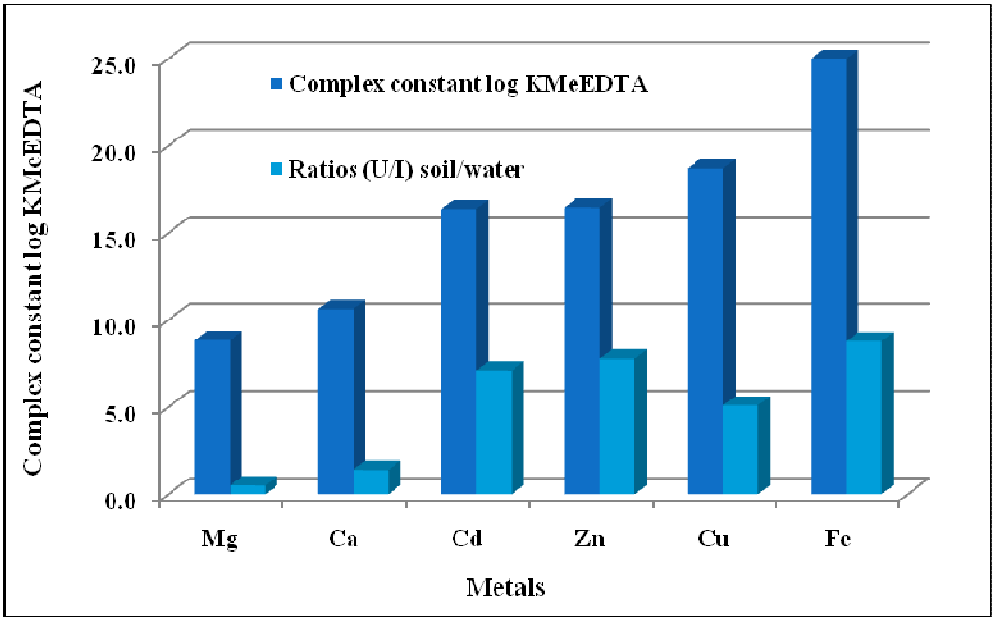

Figure 7.19 Comparison of the metal mobility with its EDTA complex constant in the soil-groundwater system from ratios of the metal concentration in the unirrigated (U) and irrigated (I) soil and groundwater.

To compare heavy metal contents in soils and groundwater with EDTA complexing constants (Table 7.8 and Figure 7.19), there is some correlation between the mobility of metals and the stability of EDTA complexes, indicating that the transportation of metals influenced by EDTA was increased with the increase of EDTA complex constants. Further research is however recommended to determine this with certainty.

7.4.4 Analysis of Metal-EDTA Present in Groundwater

To compare dissolved metals and EDTA in the groundwater (Table 7.9), the majority of the EDTA will be in the form of an iron complex in the groundwater as the K_{FeEDTA}*[Fe] is so much higher than other metals. The averaged EDTA concentration was observed at 389.2µg/L (1.35E-3 mM), which indicates that EDTA could all be complexed with Fe and form a Fe(III)EDTA (1:1) complex. Furthermore, the EDTA concentration is supposed to be 671µg/L if Fe(III) is fully complexed and formed as the form of Fe(III)EDTA, which means that the speciation of EDTA theoretically present in the groundwater was most likely to be

only Fe(III)EDTA for this case. Nonetheless, true speciation of metal-EDTA may differ from the theoretical calculations due to the complicated environmental conditions, such as organic matters from the dairy effluent. Further research is thus needed to confirm this.

Table 7.9 Analysis of metals theoretically present as metal-EDTA in the groundwater where the dairy effluent containing EDTA was spray-irrigated based upon EDTA-complex stability.

Metals	Concentration mg/L	M. Weight g/molar	Concentration (mM)	Complex constant log K_{MeEDTA}	K_{MeEDTA}*[M]
Mg	2.3	24.3	9.47E-2	8.8	5.97E+07
Ca	5.4	40.1	1.35E-1	10.6	5.36E+09
Cd	2.1E-4	112.4	1.87E-06	16.4	4.69E+10
Zn	4.8E-2	65.4	7.34E-4	16.4	1.84E+13
Cu	9.7E-3	63.6	1.53E-4	18.7	7.64E+14
Fe	1.3E-1	55.9	2.33E-3	25.0	2.33E+22

7.5 CONCLUSIONS

Application of dairy wastes onto pastureland is commonly undertaken in the New Zealand dairy industry. This approach offers the advantage of utilizing nutrients contained in dairy wastes for soils and plants, but has the disadvantage of retaining heavy metals in soils long term and likely posing a risk to groundwater. This chapter investigated the presence of EDTA and related heavy metals in soils and groundwater in association with land application of dairy wastes. The purpose was to identify the potential risk to groundwater of EDTA chelates.

The HPLC-UV analytical method was applied for identification of EDTA in both soils and groundwater. EDTA in soils was released by 0.002 M NaH_2PO_4 desorption solution in an ultrasonic bath, and EDTA in groundwater were appropriately pre-concentrated due to its low concentration. Quality control of the analysis was undertaken by a daily calibration curve with freshly made standard EDTA solutions, running a blank, a duplicate every 10^{th} sample and a standard spiked recovery every 20^{th} sample. Ranges of duplicated limits and recoveries

were within 8.7% (n=4) and 95–98% (n=2) for soils, and 7% (n=8) and 91-107% (n=5) for groundwater, respectively.

EDTA is likely to be under the method detection limit of 0.15 mg/kg (dry weight) in soils where dairy waste treatment sludge, also known as biomass or bio-solid from the Fonterra Waitoa dairy site, had been applied onto pastures. Analytical results of EDTA for pastoral top soils indicated that EDTA was, however, detected in relatively dry conditions for clay and volcanic soils from the irrigated areas with dairy wastewater containing EDTA from the Fonterra Kauri site. EDTA, otherwise, was under the method detection limit of 0.15 mg/kg (dry weight) for pastoral top soils in wet conditions.

Concentrations of EDTA appeared to be under the detection limit of 2 μg/L in groundwater in which dairy biomass had been spread on the pastures near the Fonterra Waitoa dairy site. In contrast, EDTA was detectable in groundwater whether the paddocks were irrigated or un-irrigated with dairy wastewater from the Fonterra Kauri dairy site under relatively dry conditions (April 2008). Furthermore, EDTA was detected, even likely to be at higher concentrations for irrigated areas under wet conditions (July 2008) while EDTA was below the detection limit for groundwater from the un-irrigated areas. Nonetheless, the detected concentrations of EDTA for the groundwater were all below the maximum acceptable value of 700 μg/L of EDTA for the New Zealand drinking water (2005).

It has showed that soil characteristics, for instance, the nutrient levels of N, P and K, was changed by comparing the basic soil test data of irrigated and un-irrigated pastoral top soils with the dairy wastewater from the Fonterra Kauri site.

Analytical results for soils and groundwater near the Fonterra Kauri dairy site, even with this limited data, appear to suggest that heavy metals may be built up over long periods of irrigation with dairy wastewater, and they are likely to be transported to the groundwater with the existence of EDTA. This finding is significant enough to suggest more research is required in the future.

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8.0 CHAPTER EIGHT: SUMMARY AND CONCLUSIONS

8.1 INTRODUCTION

Ethylene**D**iamine**T**etraacetatic **A**cid (EDTA) is a well-known chelating agent, used to control the effect of metals in many industrial processes for more than 50 years, such as in the dairy industry to remove calcium and magnesium in the cleaning process.

As EDTA is water-soluble and not volatile, it is mainly released with wastewater effluent. It is generally believed to be of low risk to human health and the environment. However, it came under scrutiny in Europe in the late of 1980's because of its widespread presence in the aqueous environment and its sequester ability to heavy metals.

This research was sponsored by Fonterra Co-operative Group Limited (Fonterra) and obtained support also from the Technology New Zealand. The rationale for this project arose from the use of EDTA as an additive in caustic agents to improve cleaning efficiency, and minimise dairy wastewater in the clean-in-place (CIP) system within the processing plants of the New Zealand dairy industry.

There are two major disposal methods for dairy effluent in New Zealand. They are:

(i) discharge into local waterways after treated by an extended aeration system (biological treatment) such as the Fonterra Waitoa dairy site (Waitoa), and

(ii) spray irrigation onto pasture land (land treatment system), like the Fonterra Kauri dairy site (Kauri).

A particular case of the Fonterra Waitoa dairy site was intensively studied as significant amounts of EDTA had been used in the CIP system, the reduction of EDTA from the existing wastewater treatment plants was unknown, and large

volumes of dairy effluent were discharged into a relatively small stream of the Waitoa River.

EDTA and associated heavy metals present in both pastoral top soils subjected to long term application of dairy effluent (Kauri) and dairy biomass (Waitoa) containing EDTA, and groundwater were investigated,

This project was undertaken with the following identified aims:

(i) Establish an HPLC-UV analytical method (not previously established in New Zealand) for determination of the presence of EDTA in environments;
(ii) Investigate concentrations of EDTA in dairy wastewater;
(iii) reveal EDTA removal efficiency by the existing wastewater treatment plants;
(iv) examine EDTA and associated heavy metals present in the local adjacent waterway, in this case the Waitoa River;
(v) conduct a dispersal simulation of EDTA in the Waitoa River using applicable models; and
(vi) undertake the investigation of EDTA in soils and ground waters following the application of dairy wastes (dairy biomass and effluent) via a land treatment system onto pasture land.

8.2 SUMMARY OF RESEARCH FINDINGS

8.2.1 Method Development for Analyzing EDTA Using HPLC-UV

A standard method for analysing EDTA has not been previously established in New Zealand (e.g. a leading analytical laboratory, R J Hill laboratories, has no such method). A method for measuring EDTA in dairy wastewater using reversed–phase ion-pair liquid chromatography was therefore established. This was achieved by optimizing the chromatographic separation including organic compositions and concentrations of the ion-pair reagent in mobile phase, studying

the effects of interfering compounds such as nitrate, Ca^{2+} and Mg^{2+}, and converting losses of EDTA between metal complexes. Validation procedures showed good linearity (r^2 0.9988 - 0.9998) and spiking recoveries (98 - 102%) (no certificated reference material available). The method standard deviation and detection limit (3*RSD) were 1.5% and 5 µg L^{-1} EDTA, respectively. This method was applied to the later research with pre-concentration steps for surface and ground waters, and an EDTA extraction step and modified separation conditions for soils.

8.2.2 EDTA Occurrences in Dairy Processing Wastewater

Thirteen 24–hour composite flow-proportional wastewater samples (August, October/November and December, 2007) were collected from drainage systems from cheese and wet process plants. These streams of wastewater were suggested as containing potentially high concentrations of EDTA at the Fonterra Waitoa dairy site.

Significant concentration of EDTA was observed in wastewater samples of the processing plants, where the commercial product of Eliminator, or Eliminator II, containing 34 – 36 % of EDTA was applied in the clean-in-place system. The highest concentration of EDTA detected for the cheese drain was approximately 77000 µg/L (77 mg/L) and for the wet process, was 83000 µg/L (83 mg/L). Nevertheless, those levels of EDTA were below the controlled value of EDTA (0.1%) by the process plants. Furthermore, analysis of EDTA concentrations in the processing wastewater showed marked variations in daily and seasonal composition.

8.2.3 EDTA Removal Efficiency by the Existing Wastewater Treatment Plants

The wastewater from 12 – 14 streams, generated from processing plants at the Fonterra Waitoa site, is collected in a sump on site and subsequently pumped to

the wastewater treatment plant (WWTP) for a treatment. The Waitoa WWTP, utilizing an extended aeration activated sludge treatment, includes two major ponds operated in series and two clarifiers (settling tanks) operated in parallel. The treatment mechanism is that:

(i) bacteria and other micro-organisms contained in the activated sludge mass use the dairy wastewater as a food source, consuming oxygen added continuously through aerators in the process ponds, and

(ii) the activated sludge mass flows from the ponds into either of two clarifiers, in which the floc is separated from the water. Oxygen is added to satisfy the micro-organisms.

It takes 6-7 days for the influent to go through the treatment process, operated at a pH value of 8.0 – 8.2 with a 3-week sludge retention time.

Reduction of EDTA during the treatment processes cannot be assessed on a daily basis due to large capacity of the ponds and clarifiers. Thus, EDTA removal efficiency was calculated as 93 %, based upon a mass difference of the overall averaged EDTA amounts between the influents and wastes discharged (including effluent and sludge) during the sampling period of August, October and December 2007.

The concentration of EDTA in dairy effluent discharged into the adjacent Waitoa River was found to be in the range of 72 – 261 μg/L based upon analytical results of 13 effluent samples collected in August, October and December 2007.

8.2.4 Presence of EDTA and Related Heavy Metals in the Waitoa River

Surface water samples were collected 2,500 m and 10 m upstream, and 10 m and 60 m downstream of the dairy effluent outfall in the Waitoa River in August and October 2007, for the purpose of investigating concentrations of EDTA and associated heavy metals in the aquatic environment receiving dairy wastewater from the Fonterra Waitoa site.

As the level of EDTA was too low, a 5-fold pre-concentration step was needed and achieved by heating the sample to dryness and reconstituting before the analysis of EDTA. A daily calibration curve was established at the concentration range of 0 – 150 μg/L EDTA. A blank, a duplicate every 10^{th} sample and a spiking recovery of an EDTA standard every 20^{th} sample were undertaken per run for quality control. The averaged duplicate variability was 8.1 % (n=5) and the spiking recovery varied from 97 – 107 % (n=3).

Analytical results showed a slight increase of the EDTA concentration 60 m downstream from the dairy effluent outfall based on analyses of 12 samples collected. The highest EDTA concentration was 2.7 μg/L. This value is almost half of the EDTA target value of 5 μg/L for surface waters in the Rhine catchment area, recommended by the International Association of Waterworks; is two orders of magnitude below the New Zealand Drinking Water Standards of 0.7 mg/L EDTA for health purposes.

In addition to the wastewater discharge from the Fonterra Waitoa dairy site into the Waitoa River, another EDTA source was identified from the Wallace Corporation Limited (Wallace) plant, where EDTA was applied in the tannery plant. Analytical results for the randomly collected samples on 30 May 2008, showed EDTA not only present in the wastewater pond at the Wallace site , but also in the Waitoa River based upon surface water samples upstream and downstream adjacent to the Wallace site, which is about 3km upstream of the Fonterra Waitoa discharge point.

Analytical results for associated heavy metals in the Waitoa River revealed that:

(i) concentrations of Na, K, Ca and Mg were increased downstream of the dairy effluent diffuser in the Waitoa River, which demonstrates the contribution of dairy effluent from the Fonterra Waitoa site;

(ii) concentrations of Fe and Zn showed significant differences between the sampling periods of August and October, 2007. The concentration of Zn for samples collected in October obviously exceeded the trigger value of 2.4 mg/L for New Zealand natural waters, which may relate to the fertilizers on the pasture;

(iii) copper (Cu)was detectable both upstream and downstream, but their concentrations were below the trigger value (1.0 mg/L) for toxicants in fresh water (New Zealand water quality);

(iv) chromium (Cr) was only detectable on one day out of 6 during the sampling periods that may relate to the tannery factory effluent discharge (Wallace); and

(v) Lead (Pb), cadmium (Cd) and nickel (Ni) were all under detection limits, and pose no particular concerns.

8.2.5 Simulation of EDTA Dispersion in the Waitoa River

Two approaches were undertaken to help the understanding of the fundamental aspects of the transport of EDTA in the Waitoa River. These are approximate calculations using quasi one-dimension vertical mixing model; and a numerical simulation of the hydrodynamic processes (3DD model) and effluent mixing (POL3DD) in two-dimensions (depth-averaged). Both specific monitored case (30 May 2008) and a worst case scenario of high EDTA discharge (the maximum EDTA usage at the Waitoa site for year 2008) combined with a low river flow (< 600 L/s) were simulated for the dispersal path and the concentration of EDTA through model output analyses.

The quasi one-dimension vertical mixing model (Rutherford model) is based upon the assumption that both transverse and longitudinal concentration gradients are negligible due to the uniform line-source, as for the example of the Waitoa dairy effluent released into the Waitoa River. Thus, the dispersion of EDTA in the river channel simplifies to become quasi one-dimensional from vertical mixing only. Estimated results showed the EDTA discharged from the Waitoa dairy effluent was only completely mixed 6 m downstream, assuming the river depth of 1 m, width of 8 m, slope of 2×10^{-4}, and mean velocity of 0.22 m/s. Fully mixed concentrations of EDTA were 3.18 μg/L for the monitored case (30 May 2008), and 40.3 μg/L for the worst case.

The Model 3DD is based upon highly accurate mixed Eulerian/Lagrangian mathematical techniques, and the dispersal model POL3DD (POLlution dispersal coupled to 3DD) tracks dissolved materials as suspended "particles" to simulate water-borne dispersal and determines concentrations of pollutants from multiple sources in 3 dimensions. In the Waitoa case of shallow water, river currents were only generated in 2-dimensions (depth-averaged) using the 3DD hydrodynamic model, and the dispersal paths and concentrations of EDTA were then read by POL3D.

Given a 1x1 m grid resolution with a precise bathymetry created by single beam echo-sounder, a two-dimensional, depth-averaged hydrodynamic model (3DD) was set up for a low river velocity of 0.2 m/s. Outputs of the 3DD model concur well with the field measurements by an averaged difference of 2.3%.

EDTA dispersion of the monitored case and the worst case were simulated in the Waitoa River. The modelling results suggest that the maximum EDTA concentrations were present in the mediate vicinity of the dairy effluent outfall in the Waitoa River for both cases. The instantaneous concentration of EDTA was likely to reach 0.25 g/m^3 (mg/L) for the worst case. But the important finding was there was no significant increase of EDTA concentrations beyond 50 m downstream from the outfalls. The highest concentrations of EDTA were around 2 μg/L for the monitored case and 21 μg/L for the worst case based on the data extraction of the central cell of transects. These values are nearly half of the quasi one-dimensional calculations. The estimated results from the quasi one-dimension vertical mixing are only indicative due to its assumption and limitation. The two-dimensional 3DD hydrodynamic model considers the EDTA dispersal in both transversal and horizontal directions, and is believed to provide a more practical and realistic simulation of EDTA dispersal in the Waitoa River.

From the quasi one-dimensional or the 2-dimensional simulation results for the worst case scenario, a key point is that the concentration of EDTA in the immediate vicinity of the receiving stream was as high as 21 μg/L for an average (40.3 μg/L from the approximate calculation) or 0.25 mg/L for an instantaneous

concentration, and then gradually reduced to ~1.5 μg/L beyond 50 m downstream due to the river flux and dispersion. This suggests that:

i. the EDTA concentration occurring in the immediate vicinity of the dairy outfall is well below the Predicted Effect Concentration (PEC) of 2.2 mg/L for aquatic environments advised by the European Union (European Chemicals Bureau, 2004);
ii. this value is also well under the New Zealand Drinking Water Standards of 0.7 mg/L EDTA for health purposes (Ministry of Health, 2005); and
iii. large volumes of dairy effluent discharge at this site appears not to result in a significant EDTA contribution to the Waitoa River.

In summary, the dairy effluent discharge from the Fonterra Waitoa dairy site will not to lead to a significant effect on the Waitoa River in terms of EDTA concentration, based upon the estimated results of the quasi one-dimension vertical mixing and the simulation of EDTA dispersion within the Waitoa River by the 3DD hydrodynamic model.

8.2.6 Investigation of EDTA and Related Heavy Metals in Soils and Groundwater Subjected to Land Treatment System of Dairy Wastes

In the New Zealand dairy industry, land treatment is an alternative for disposal of dairy wastes, including irrigation of dairy wastewater treatment sludge (also known as biomass or biosolid) and dairy wastewater onto pasture land. Long term land application could potentially induce changes in the quality of soil, especially as trace element inputs are sustained over long periods, and it may lead to groundwater contamination with the presence of EDTA. For this reason, EDTA and related heavy metals present in soils and groundwater were investigated for pastoral areas subjected to land application of dairy wastes.

The modified HPLC-UV analytical method was applied for identification of EDTA in both soils and groundwater. EDTA in soils were released by 0.002 M NaH_2PO_4 desorption solution in an ultrasonic bath, and an appropriate pre-

concentration was needed for measuring EDTA in groundwater due to its low concentration. The same quality control was undertaken for the analysis. Ranges of duplicated limits and recoveries were within 8.7% (n=4) and 95–98% (n=2) for soils, and 7% (n=8) and 91-107% (n=5) for groundwater, respectively. The detection limits for EDTA were 0.15 mg/kg (dry weight) in soils and 2 μg/L inr groundwater.

EDTA was under the method detection limit of 0.15 mg/kg in soils where dairy waste treatment sludge had been applied onto pastures at the Fonterra Waitoa dairy site. Analytical results of EDTA in pastoral top soils indicate that EDTA was, however, detectable for relatively dry conditions in both clay and volcanic soils, where dairy wastewater containing EDTA were spray-irrigated onto the pasture for a period of time at the Fonterra Kauri site. EDTA, otherwise, was under the method detection limit for wet conditions.

Concentrations of EDTA were under the detection limit of 2 μg/L in groundwater in which dairy biomass had been spread on the pastures near the Fonterra Waitoa dairy site. In contrast, EDTA was detectable in groundwater whether the paddocks were irrigated or un-irrigated with dairy wastewater under relatively dry conditions (April 2008) at the Fonterra Kauri site. Furthermore, EDTA was detectable, even likely to be at higher concentrations for irrigated areas under wet conditions (July 2008) while EDTA was below the detection limit in the un-irrigated areas. Nonetheless, the detected concentrations of EDTA in the groundwater were all below the maximum acceptable value of 0.7 mg/L (700 μg/L) of EDTA for New Zealand drinking water (2005).

It was shown that soil characteristics, for instance, the nutrient levels of N, P and K, were changed by comparing the basic soil test data between the irrigated and un-irrigated pastoral top soils with the dairy wastewater from the Fonterra Kauri site. Analytical results for soils and groundwater near the Fonterra Kauri dairy site appeared to suggest that heavy metals may be built up over long periods of irrigation with dairy wastewater, and they were likely to be transported to the groundwater with the existence of EDTA.

8.3 IMPLICATIONS OF THIS RESEARCH

This research has validated the current disposal methods of dairy effluent containing EDTA used by the New Zealand dairy industry in protecting the environment. Findings of the study imply that:

- The current environmental practices of discharge dairy effluent (treated) into the local stream do not appear to threaten to the water quality of the local aquatic environment. Nevertheless, the volume of effluent discharge and EDTA usage in the processing plants should be monitored, particularly when river flows are low (<600 L/s);
- Based upon analyses in this study, the present practice of dairy biomass spread onto pasture land does not appear to lead to potential EDTA contamination in the environment; however
- The general practice of dairy effluent spray-irrigation onto pasture land may retain and build up heavy metals, and then be transported to groundwater due to EDTA chelates. This may result in further groundwater contamination.

8.4 RECOMMENDATIONS FOR FUTURE RESEARCH

Accordingly, there are some concerns about that:

(i) long-term land application of dairy wastes would eventually lead to a build-up of heavy metals in soils; and

(ii) the enhanced mobility of heavy metals in soils poses a potential risk for leaching to groundwater with chelates.

Based on findings of this research, the same concern was also proposed for spray land irrigation of dairy effluent. This suggests more research is required in future for the New Zealand dairy industry.

The following lines of future research are recommended:

- Collect database of EDTA in soils relating to land application of dairy effluent;
- Metal spices in soils;
- Speciate metal–EDTA complexes in water and soils;
- Investigate the transportation of heavy metals by EDTA chelates in soils, e.g. by using column leaching studies; and
- Determine the potential risk to groundwater with the existence of EDTA chelates.

APPENDICES

A.1 EDTA occurrences in dairy wastewater of the Fonterra Waitoa site, analysed on 9 November 2007

Sample table on 9 November 2007.

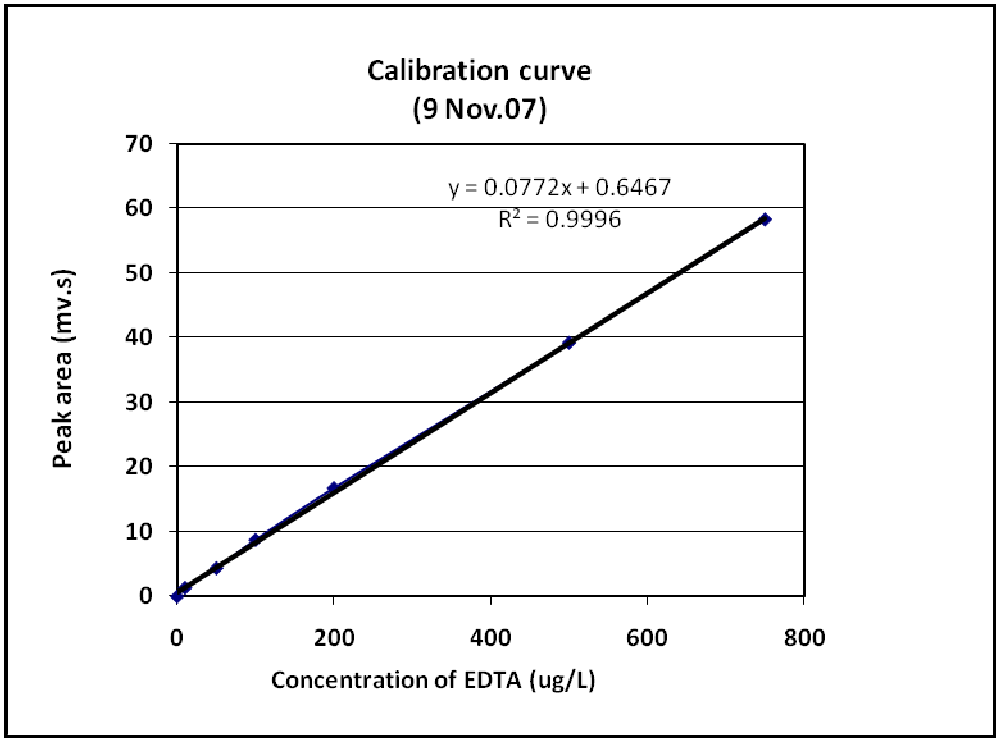
Sample Table for 9.Nov.07.pfwdat

Sample loop: 50 uL
Mobile phase: 2% MeOH, 15 mM TBABr in pH value of 3.3 sodium formate/formic acid buffer solution
Column: Hypersil C18 RP, 5 um, 200 x 4.6 mm
Temperature: Ambient Tem.

	Sample Control			
Runs	Sample Name	Vial	Volume	Comment
1	Mobile phase only		50.0	
2	mobile phase		50.0	
3	10 ug/L EDTA Std.		50.0	Cal curve - point 1
4	10 ug/L EDTA Std.		50.0	
5	10 ug/L EDTA Std.		50.0	
6	10 ug/L EDTA Std.		50.0	
7	50 ug/L EDTA Std.		50.0	point 2
8	50 ug/L EDTA Std.		50.0	
9	50 ug/L EDTA Std.		50.0	
10	100 ug/L EDTA Std.		50.0	point 3
11	100 ug/L EDTA Std.		50.0	
12	100 ug/L EDTA Std.		50.0	
13	200 ug/L EDTA Std.		50.0	point 4
14	200 ug/L EDTA Std.		50.0	
15	200 ug/L EDTA Std.		50.0	
16	500 ug/L EDTA Std.		50.0	point 5
17	500 ug/L EDTA Std.		50.0	
18	500 ug/L EDTA Std.		50.0	
19	750 ug/L EDTA Std.		50.0	point 6
20	750 ug/L EDTA Std.		50.0	
21	750 ug/L EDTA Std.		50.0	
22	10 ug/L EDTA Std.		50.0	Repeat
23	10 ug/L EDTA Std.		50.0	
24	Inf. 28/8/07	1	50.0	diluted factor: 2 - 5 ml sample + 5 ml Fe3+(1.94 mg/L) in the mobile phase
25	Inf. 28/8/07 dup	2	50.0	diluted factor: 2 - 5 ml sample + 5 ml Fe3+(1.94 mg/L) in the mobile phase
26	Eff. 28/8/07	3	50.0	diluted factor: 2 - 5 ml sample + 5 ml Fe3+(1.94 mg/L) in the mobile phase

	Sample Control			
Runs	Sample Name	Vial	Volume	Comment
27	Wet pro. 28/8/07	4	50.0	Dilution factor: 2
28	Pond 1 (1) 28/8/07	5	50.0	Dilution factor: 2
29	Pond 1 (2) 28/8/07	6	50.0	Dilution factor: 2; adding 0.1 ml MeOH to the sample, any difference? yes - no shoulder
30	Pond 1 (1) 28/8/07	5	50.0	Dilution factor: 2; adding 0.1 ml MeOH to the sample, any difference? yes
31	Pond 2 (1) 28/8/07	7	50.0	Dilution factor: 2; adding 0.1 ml MeOH to the sample, any difference? yes
32	Pond 2 (2) 28/8/07	8	50.0	Dilution factor: 2; adding 0.1 ml MeOH to the sample, any difference? yes
33	Inf. 29/8/07 + EDTA	10	50.0	Dilution factor: 2; Recovery test for the run.
34	Inf. 29/8/07	9	50.0	Dilution factor: 2
35	Eff. 29/8/07	11	50.0	Dilution factor: 2
36	Wet Pro. 29/08/07	12	50.0	Dilution factor: 2
37	Pond 1 (1) 29/08/07	13	50.0	Dilution factor: 2
38	Pond 1 (2) 29/08/07	14	50.0	Dilution factor: 2
39	Pond 2 (1) 29/08/07	15	50.0	Dilution factor: 2
40	Pond 2 (2) 29/08/07	16	50.0	Dilution factor: 2
41	200 ug/L EDTA Std.		50.0	Dilution factor: 2
42	Inf. 30/08/07	17	50.0	Dilution factor: 2
43	Eff. 30/08/07	19	50.0	Dilution factor: 2
44	Wet pro. 30/08/07	21	50.0	Dilution factor: 2
45	Pond 1 (1) 30/08/07	22	50.0	Dilution factor: 2
46	Pond 1(2) 30/08/07	23	50.0	Dilution factor: 2
47	Pond 2 (1) 30/08/07	24	50.0	Dilution factor: 2
48	Pond 2 (2) 30/08/07	25	50.0	Dilution factor: 2
49	Pond 1 (2) 29/08/07	14	50.0	Rpt.
50	Pond 1 (1) 22/10/07		50.0	Dilution factor: 2
51	Pond 1 (2) 22/10/07		50.0	Dilution factor: 2
52	Pond 2 (1) 22/10/07		50.0	Dilution factor: 2
53	Pond 2 (2) 22/10/07		50.0	Dilution factor: 2
54	Pond 1 (1) 24/10/07		50.0	Dilution factor: 2
55	Pond 1 (2) 24/10/07		50.0	Dilution factor: 2
56	Pond 2 (1) 42/10/07		50.0	Dilution factor: 2
57	Pond 2 (2) 24/10/07		50.0	Dilution factor: 2
58	200 ug/L EDTA Std.		50.0	
59	Pond 1 (2) 29/08/07 Rpt.		50.0	Dilution factor: 2 x 4 (2.5 mlLdiluted to 10 mL)
60	Pond 1 (2) 22/10/07 Rpt.		50.0	Dilution factor: 2 x 2 (1mL diluted to 2 mL)

Daily calibration curve of EDTA ranging from 0 – 750 µg/L on 9 November 2007.



Analytical results of EDTA in dairy wastewater from the Fonterra Waitoa dairy site, sample collected in August and October 2007and analysed on 9 November 2007.

Sample date	Sample name	Peak area (mv.s)	Cal. EDTA con. (ug/L)	Diluted factor	Sample EDTA con. (ug/L)
28 Aug. 07	Influent	12.15	149.01	2	298
	Influent (dup)	12.34	151.47	2	303
	Effluent	6.5	75.82	2	152
	Wet pro.	0.71	0.82	2	2
	Pond 1 (1)	22.11	278.02	2	556
	Pond 1 (2)	12.2	149.65	2	299
	Pond 2 (1)	10.06	121.93	2	244
	Pond 2 (2)	8.57	102.63	2	205
29 Aug. 07	Influent	4.22	46.29	2	93
	Influent recovery	20.92	Recovery: 99.6%		
	Effluent	5.42	61.83	2	124
	Wet pro.	1.05	5.22	2	10
	Pond 1 (1)	17.38	216.75	2	434
	Pond 1 (2)	19.63	245.90	8	1967
	Pond 2 (1)	11.75	143.83	2	288
	Pond 2 (2)	7.65	90.72	2	181
30 Aug. 07	Influent	9.36	112.87	2	226
	Effluent	6.86	80.48	2	161
	Wet pro.	ND			
	Pond 1 (1)	10.97	133.72	2	267
	Pond 1 (2)	12.93	159.11	2	318
	Pond 2 (1)	8.25	98.49	2	197
	Pond 2 (2)	6.84	80.22	2	160
22 Oct. 07	Pond 1 (1)	16.31	202.89	2	406
	Pond 1 (2)	11.12	135.66	4	543
	Pond 2 (1)	24.92	314.42	2	629
	Pond 2 (2)	14.79	183.20	2	366
24. Oct. 07	Pond 1 (1)	11.25	137.35	2	275
	Pond 1 (2)	9.24	111.31	2	223
	Pond 2 (1)	11.21	136.83	2	274
	Pond 2 (2)	9.79	118.44	2	237

A.2 EDTA analyses in surface water of the Waitoa River, sample collected in August 2007 and analysed on 13 March 2008

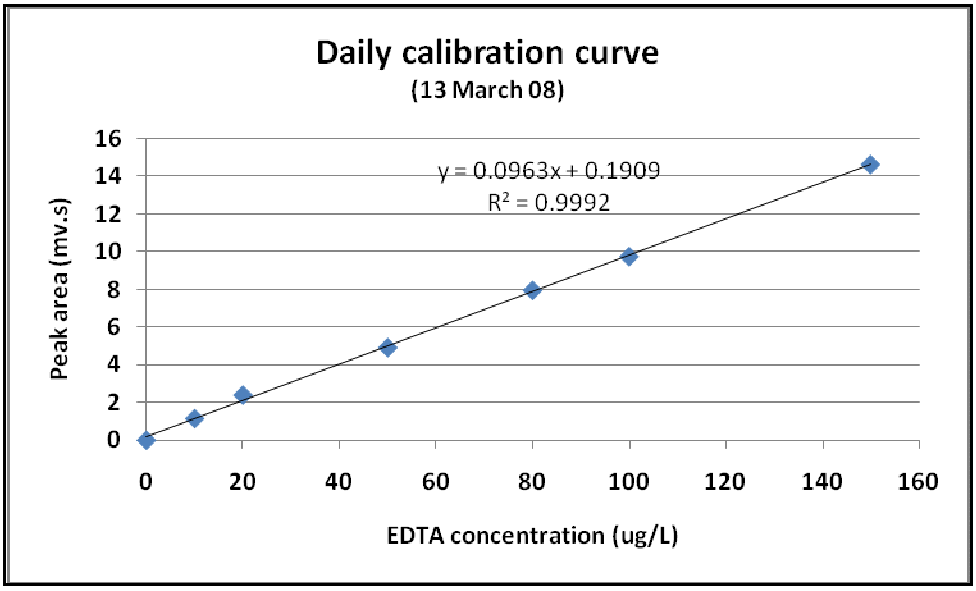
Sample table on 13 March 08.

Sample Table for 13 Mar 08.pfwdat

Mobile phase: 2%MeOH 15 mM TBABr with pH 3.3 formic acid/sodium formate buffer solution
Column: HyperClone ODS C18 5 um 200x4.6 mm
Flow rate: 0.9 mL/min
Sample loop: 50uL

	Sample Control			
Runs	Sample Name	Vial	Rpts	Comment
1	10 ug/L EDTA Std.		1	Daily Cal. Curve, point 1
2	10 ug/L EDTA Std.		1	
3	20 ug/L EDTA Std.		1	point 2
4	20 ug/L EDTA Std.		1	
5	50 ug/L EDTA Std.		1	point 3
6	50 ug/L EDTA Std.		1	
7	80 ug/L EDTA Std.		1	point 4
8	80 ug/L EDTA Std.		1	
9	100 ug/L EDTA Std.		1	point 5
10	100 ug/L EDTA Std.		1	
11	150 ug/L EDTA Std.		1	point 6
12	150 ug/L EDTA Std.		1	
13	mobile phase only		1	
14	SH 26 1 28.08.07	1	1	10 ml sample to dryness, reconstituted with 1.5 mL mp + 0.5 mL Fe3+ (1.94 mg/L)
15	SH 26 2	2	1	
16	US 20m 1	3	1	
17	US 20m 2	4	1	
18	DS 20m 1	5	1	
19	DS 20m 2	6	1	
20	DS 100m 1	7	1	
21	DS 100m 2	8	1	
22	SH 26 1 29.08.07	9	1	
23	SH 26 2	10	1	
24	SH 26 1 29.08.07	9	1	Rpt. to confirm
25	SH 26 2 Dup	11	1	
26	US 20m 1	12	1	
27	US 20m 2	13	1	
28	DS 20m 1	14	1	
29	DS 20m 2	15	1	
30	DS 100m 1	16	1	
31	DS 100m 2	17	1	
32	SH 26 1 30.08.07	18	1	
33	SH 26 2	19	1	
34	US 20m 1	20	1	
35	US 20m 2	21	1	
36	US 20m 2 up	22	1	Duplicate
37	US 20m 2 recovery	23	1	
38	50 ug/L EDTA Std.		1	
39	DS 20m 1	25	1	
40	DS 20m 2	26	1	
41	DS 100m 1	27	1	
42	DS 100m 2	28	1	
43	DS 100m 2 Dup	29	1	
44	Recovery	30	1	
45	50 ug/L EDTA Std.		1	
46	Recovery	30	1	Rpt.
47	US 20 m 1 28.8.07	3	1	Rpt.
48	SH 26 1 29.08.07	9	1	Rpt.

Daily calibration curve of EDTA ranging from 0 – 150 µg/L on 13 March 2008.



Analytical results of EDTA in surface water of the Waitoa River , sample collected in August 2007 and analysed on 13 March 2008.

Sample	Peak area (mv·s)	EDTA concentration (µg/L)	Precon factor	EDTA con in sample (µg/L)
SH 26 1 28.8.07	0.8	6.33	5	1.27
SH 26 2	1.08	9.23	5	1.85
US 10 m 1	0.62	4.46	5	0.89
US 10 m 2	0.54	3.63	5	0.73
DS 10 m 1	0.69	5.18	5	1.04
DS 10 m 2	0.72	5.49	5	1.1
DS 60 m 1	0.61	4.35	5	0.87
DS 60 m 2	1.47	13.28	5	2.66
SH 26 1 29.8.07	4.02	39.76	5	7.95
SH 26 2	0.56	3.83	5	0.77
Dup	0.61	4.35	5	0.87
US 10 m 1	0.69	5.18	5	1.04
US 10 m 2	0.5	3.21	5	0.64
DS 10 m 1	0.66	4.87	5	0.97
DS 10 m 2	0.55	3.73	5	0.75
DS 60 m 1	1.41	12.66	5	2.53
DS 60 m 2	0.9	7.36	5	1.47
SH 26 1 30.8.07	0.73	5.6	5	1.12
SH 26 2	0.87	7.05	5	1.41
US 10 m 1	1.41	12.66	5	2.53
US 10 m 2	0.65	4.77	5	0.95
Dup	0.66	4.87	5	0.97
Recovery	5.55		5	
50 ug/L EDTA	4.92			97%
DS 10 m 1	0.74	5.7	5	1.14
DS 10 m 2	1.33	11.83	5	2.37
DS 60 m 1	0.67	4.98	5	1
DS 10 m 2	0.9	7.36	5	1.47
DS 10 m 2 Dup	0.96	7.99	5	1.6
50ug/ EDTA Std.	4.92			

A.3 Analytical results of dissolved metals in the Waitoa River by ICP-MS

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ANALYSIS REPORT

Client:	University of Waikato	Lab No:	637754 SPv2
Contact:	Healy, Terry (Dr)	Date Registered:	09-Apr-2008
	Dept Earth & Ocean Sciences	Date Reported:	10-Oct-2008
	Ruakura Satellite Campus	Quote No:	32628
	Hamilton	Order No:	165158
		Client Reference:	Zoe Xie - Surface waters
		Submitted By:	Healy, Terry (Dr)

Sample Type: Clean waters

Sample Name:		SH26 Bridge 28/08/07 28-Aug-2007	SH26 Site 1 Bridge 28/08/07 28-Aug-2007	SH26 Bridge 1st-2nd 29/08/07 29-Aug-2007	SH26 Bridge 3rd-4th 29/08/07 29-Aug-2007	SH26 Bridge 1st-2nd 30/08/07 30-Aug-2007
Lab Number:		637754.1	637754.2	637754.3	637754.4	637754.5
Electrical Conductivity (EC)	mS/m	20	21	20	19	22
Dissolved Cadmium	g/m³	< 0.000050	< 0.000050	< 0.000050	< 0.000050	< 0.000050
Dissolved Calcium	g/m³	9.6	9.6	8.7	8.3	9.1
Dissolved Chromium	g/m³	< 0.00050	< 0.00050	< 0.00050	< 0.00050	< 0.00050
Dissolved Copper	g/m³	0.00058	0.00071	0.00094	0.00088	0.00083
Dissolved Iron	g/m³	0.055	0.051	0.069	0.043	0.055
Dissolved Lead	g/m³	< 0.00010	< 0.00010	< 0.00010	< 0.00010	< 0.00010
Dissolved Magnesium	g/m³	4.2	4.3	3.9	3.7	3.9
Dissolved Nickel	g/m³	< 0.00050	< 0.00050	< 0.00050	< 0.00050	< 0.00050
Dissolved Potassium	g/m³	5.7	5.7	6.4	6.1	6.6
Dissolved Sodium	g/m³	17	17	16	15	18
Dissolved Zinc	g/m³	0.0011	0.0014	0.0019	0.0019	0.0023

Sample Name:		SH26 Bridge 3rd-4th 30/08/07 30-Aug-2007	SH26 Bridge No.1 22/10/07 22-Oct-2007	SH26 Bridge No.2 22/10/07 22-Oct-2007	SH26 No.1 23/10/07 23-Oct-2007	SH26 No.2 23/10/07 23-Oct-2007
Lab Number:		637754.6	637754.7	637754.8	637754.9	637754.10
Electrical Conductivity (EC)	mS/m	21	17	17	19	17
Dissolved Cadmium	g/m³	< 0.000050	0.00012	< 0.000050	< 0.000050	< 0.000050
Dissolved Calcium	g/m³	8.7	8.0	8.1	8.2	8.1
Dissolved Chromium	g/m³	< 0.00050	< 0.00050	< 0.00050	< 0.00050	< 0.00050
Dissolved Copper	g/m³	0.00077	0.00098	0.00093	0.00073	0.00071
Dissolved Iron	g/m³	0.059	0.23	0.19	0.24	0.18
Dissolved Lead	g/m³	< 0.00010	< 0.00010	< 0.00010	< 0.00010	< 0.00010
Dissolved Magnesium	g/m³	3.9	3.5	3.6	3.7	3.6
Dissolved Nickel	g/m³	< 0.00050	< 0.00050	< 0.00050	< 0.00050	< 0.00050
Dissolved Potassium	g/m³	5.8	6.7	7.8	6.3	6.0
Dissolved Sodium	g/m³	16	12	12	15	12
Dissolved Zinc	g/m³	0.0013	0.021	0.0095	0.0077	0.0047

Sample Name:		SH26 No.1 24/10/07 24-Oct-2007	SH26 No.2 24/10/07 24-Oct-2007	Upstream 10m 12:00 28/08/07 28-Aug-2007	Upstream 10m 02:00 28/08/07 28-Aug-2007	Upstream 10m 1st-2nd 29/08/07 29-Aug-2007
Lab Number:		637754.11	637754.12	637754.13	637754.14	637754.15
Electrical Conductivity (EC)	mS/m	19	16	21	21	20
Dissolved Cadmium	g/m³	< 0.000050	< 0.000050	< 0.000050	< 0.000050	< 0.000050
Dissolved Calcium	g/m³	8.8	7.9	9.6	9.7	9.0
Dissolved Chromium	g/m³	0.00057	< 0.00050	< 0.00050	< 0.00050	< 0.00050
Dissolved Copper	g/m³	0.00072	0.00073	0.00066	0.00055	0.00077
Dissolved Iron	g/m³	0.24	0.31	0.058	0.058	0.055
Dissolved Lead	g/m³	< 0.00010	< 0.00010	< 0.00010	< 0.00010	< 0.00010

This Laboratory is accredited by International Accreditation New Zealand (IANZ), which represents New Zealand in the International Laboratory Accreditation Cooperation (ILAC). Through the ILAC Mutual Recognition Arrangement (ILAC-MRA) this accreditation is internationally recognised.
The tests reported herein have been performed in accordance with the terms of accreditation, with the exception of tests marked *, which are not accredited.

Sample Type: Clean waters						
	Sample Name:	SH26 No.1 24/10/07 24-Oct-2007	SH26 No.2 24/10/07 24-Oct-2007	Upstream 10m 12:00 28/08/07 28-Aug-2007	Upstream 10m 02:00 28/08/07 28-Aug-2007	Upstream 10m 1st-2nd 29/08/07 29-Aug-2007
	Lab Number:	637754.11	637754.12	637754.13	637754.14	637754.15
Dissolved Magnesium	g/m³	3.9	3.6	4.3	4.4	4.0
Dissolved Nickel	g/m³	< 0.00050	< 0.00050	< 0.00050	< 0.00050	< 0.00050
Dissolved Potassium	g/m³	6.1	5.1	5.8	5.9	6.5
Dissolved Sodium	g/m³	16	12	18	17	16
Dissolved Zinc	g/m³	0.011	0.0026	0.0028	< 0.0010	0.0020

	Sample Name:	Upstream 10m 3rd-4th 29/08/07 29-Aug-2007	Upstream 10m 1st-2nd 30/08/07 30-Aug-2007	Upstream 10m 3rd-4th 30/08/07 30-Aug-2007	Upstream 10m No.1 22/10/07 22-Oct-2007	Upstream 10m No.2 22/10/07 22-Oct-2007
	Lab Number:	637754.16	637754.17	637754.18	637754.19	637754.20
Electrical Conductivity (EC)	mS/m	19	22	22	17	17
Dissolved Cadmium	g/m³	< 0.000050	< 0.000050	< 0.000050	< 0.000050	< 0.000050
Dissolved Calcium	g/m³	8.5	9.2	9.6	8.5	8.3
Dissolved Chromium	g/m³	< 0.00050	< 0.00050	< 0.00050	< 0.00050	< 0.00050
Dissolved Copper	g/m³	0.00077	0.00077	0.00074	0.00099	0.00083
Dissolved Iron	g/m³	0.059	0.066	0.068	0.27	0.25
Dissolved Lead	g/m³	< 0.00010	< 0.00010	< 0.00010	< 0.00010	< 0.00010
Dissolved Magnesium	g/m³	3.8	4.0	4.3	3.7	3.6
Dissolved Nickel	g/m³	< 0.00050	< 0.00050	< 0.00050	< 0.00050	< 0.00050
Dissolved Potassium	g/m³	6.2	6.7	6.7	6.9	6.7
Dissolved Sodium	g/m³	15	19	19	12	11
Dissolved Zinc	g/m³	0.0015	0.0018	0.0016	0.0081	0.0062

	Sample Name:	Upstream 10m No.1 23/10/07 23-Oct-2007	Upstream 10m No.2 23/10/07 23-Oct-2007	Upstream 10m No.1 24/10/07 24-Oct-2007	Upstream 10m No.2 24/10/07 24-Oct-2007	Downstream 10m 10am-12pm 28/08/07 28-Aug-2007
	Lab Number:	637754.21	637754.22	637754.23	637754.24	637754.25
Electrical Conductivity (EC)	mS/m	19	17	20	17	22
Dissolved Cadmium	g/m³	< 0.000050	< 0.000050	< 0.000050	< 0.000050	< 0.000050
Dissolved Calcium	g/m³	8.5	8.0	9.0	8.1	9.6
Dissolved Chromium	g/m³	< 0.00050	< 0.00050	0.00077	< 0.00050	< 0.00050
Dissolved Copper	g/m³	0.00067	0.00070	0.00066	0.00070	0.00062
Dissolved Iron	g/m³	0.23	0.28	0.16	0.30	0.057
Dissolved Lead	g/m³	< 0.00010	< 0.00010	< 0.00010	< 0.00010	< 0.00010
Dissolved Magnesium	g/m³	3.8	3.7	4.2	3.9	4.6
Dissolved Nickel	g/m³	< 0.00050	< 0.00050	< 0.00050	< 0.00050	< 0.00050
Dissolved Potassium	g/m³	6.9	6.4	7.9	6.0	6.0
Dissolved Sodium	g/m³	15	13	18	13	19
Dissolved Zinc	g/m³	0.0091	0.0044	0.012	0.011	< 0.0010

	Sample Name:	Downstream 10m 2pm-4pm 28/08/07 28-Aug-2007	Downstream 10m 1st-2nd 29/08/07 29-Aug-2007	Downstream 10m 3rd-4th 29/08/07 29-Aug-2007	Downstream 10m 1st-2nd 30/08/07 30-Aug-2007	Downstream 10m 3rd-4th 30/08/07 30-Aug-2007
	Lab Number:	637754.26	637754.27	637754.28	637754.29	637754.30
Electrical Conductivity (EC)	mS/m	21	19	19	23	22
Dissolved Cadmium	g/m³	< 0.000050	< 0.000050	< 0.000050	< 0.000050	< 0.000050
Dissolved Calcium	g/m³	9.5	8.8	8.7	9.7	9.7
Dissolved Chromium	g/m³	< 0.00050	< 0.00050	< 0.00050	< 0.00050	< 0.00050
Dissolved Copper	g/m³	0.00061	0.00075	0.00071	0.00073	0.00073
Dissolved Iron	g/m³	0.061	0.068	0.048	0.059	0.080
Dissolved Lead	g/m³	< 0.00010	< 0.00010	< 0.00010	< 0.00010	< 0.00010
Dissolved Magnesium	g/m³	4.5	3.8	4.1	4.5	4.3
Dissolved Nickel	g/m³	< 0.00050	< 0.00050	< 0.00050	< 0.00050	< 0.00050
Dissolved Potassium	g/m³	6.0	6.0	6.2	6.9	6.2
Dissolved Sodium	g/m³	19	16	18	23	20
Dissolved Zinc	g/m³	0.0021	0.0017	0.0015	0.0014	0.0013

Sample Type: Clean waters						
	Sample Name:	Downstream 10m No.1 22/10/07 22-Oct-2007	Downstream 10m No.2 22/10/07 22-Oct-2007	Downstream 10m No.1 23/10/07 23-Oct-2007	Downstream 10m No.2 23/10/07 23-Oct-2007	Downstream 10m No.1 24/10/07 24-Oct-2007
	Lab Number:	637754.31	637754.32	637754.33	637754.34	637754.35
Electrical Conductivity (EC)	mS/m	17	16	19	17	20
Dissolved Cadmium	g/m³	< 0.000050	< 0.000050	< 0.000050	< 0.000050	< 0.000050
Dissolved Calcium	g/m³	8.6	8.6	8.5	8.1	9.0
Dissolved Chromium	g/m³	< 0.00050	< 0.00050	< 0.00050	< 0.00050	0.00061
Dissolved Copper	g/m³	0.00092	0.00088	0.00065	0.00066	0.00057
Dissolved Iron	g/m³	0.28	0.26	0.24	0.29	0.18
Dissolved Lead	g/m³	< 0.00010	< 0.00010	< 0.00010	< 0.00010	< 0.00010
Dissolved Magnesium	g/m³	3.8	3.8	4.1	3.9	4.1
Dissolved Nickel	g/m³	< 0.00050	< 0.00050	< 0.00050	< 0.00050	< 0.00050
Dissolved Potassium	g/m³	6.7	6.2	7.0	5.7	6.3
Dissolved Sodium	g/m³	14	13	18	14	18
Dissolved Zinc	g/m³	0.0070	0.0075	0.0058	0.0063	0.0066

	Sample Name:	Downstream 10m No.2 24/10/07 24-Oct-2007	Downstream 60m 2pm 28/08/07 28-Aug-2007	Downstream 60m 3pm 28/08/07 28-Aug-2007	Downstream 60m 1st-2nd 29/08/07 29-Aug-2007	Downstream 60m 3rd-4th 29/08/07 29-Aug-2007
	Lab Number:	637754.36	637754.37	637754.38	637754.39	637754.40
Electrical Conductivity (EC)	mS/m	17	26	21	21	21
Dissolved Cadmium	g/m³	< 0.000050	< 0.000050	< 0.000050	< 0.000050	< 0.000050
Dissolved Calcium	g/m³	8.4	11	10	10	9.9
Dissolved Chromium	g/m³	< 0.00050	< 0.00050	< 0.00050	< 0.00050	< 0.00050
Dissolved Copper	g/m³	0.00059	0.00058	0.00059	0.00063	0.00069
Dissolved Iron	g/m³	0.29	0.082	0.060	0.053	0.057
Dissolved Lead	g/m³	< 0.00010	< 0.00010	< 0.00010	< 0.00010	< 0.00010
Dissolved Magnesium	g/m³	3.9	5.0	4.6	4.5	4.4
Dissolved Nickel	g/m³	< 0.00050	0.00063	< 0.00050	< 0.00050	0.00055
Dissolved Potassium	g/m³	5.3	7.2	5.8	6.4	6.6
Dissolved Sodium	g/m³	14	27	20	21	20
Dissolved Zinc	g/m³	0.0034	0.0017	< 0.0010	0.0016	0.0014

	Sample Name:	Downstream 60m 1st-2nd 30/08/07 30-Aug-2007	Downstream 60m 3rd-4th 30/08/07 30-Aug-2007	Downstream 60m No.1 22/10/07 22-Oct-2007	Downstream 60m No.2 22/10/07 22-Oct-2007	Downstream 60m No.1 23/10/07 23-Oct-2007
	Lab Number:	637754.41	637754.42	637754.43	637754.44	637754.45
Electrical Conductivity (EC)	mS/m	27	25	20	21	22
Dissolved Cadmium	g/m³	< 0.000050	< 0.000050	< 0.000050	< 0.000050	< 0.000050
Dissolved Calcium	g/m³	11	11	9.6	9.6	9.7
Dissolved Chromium	g/m³	< 0.00050	< 0.00050	< 0.00050	< 0.00050	< 0.00050
Dissolved Copper	g/m³	0.00057	0.00057	0.00076	0.00076	0.00054
Dissolved Iron	g/m³	0.079	0.055	0.26	0.21	0.22
Dissolved Lead	g/m³	< 0.00010	< 0.00010	< 0.00010	< 0.00010	< 0.00010
Dissolved Magnesium	g/m³	5.0	5.0	4.2	4.3	4.5
Dissolved Nickel	g/m³	0.00072	0.00063	0.00056	0.00051	< 0.00050
Dissolved Potassium	g/m³	7.8	7.1	6.4	7.0	7.4
Dissolved Sodium	g/m³	28	26	16	18	21
Dissolved Zinc	g/m³	0.0029	0.0011	0.0056	0.0069	0.0062

	Sample Name:	Downstream 60m No.2 23/10/07 23-Oct-2007	Downstream 60m No.1 24/10/07 24-Oct-2007	Downstream 60m No.2 24/10/07 24-Oct-2007		
	Lab Number:	637754.46	637754.47	637754.48		
Electrical Conductivity (EC)	mS/m	20	22	20	-	-
Dissolved Cadmium	g/m³	< 0.000050	< 0.000050	< 0.000050	-	-
Dissolved Calcium	g/m³	9.6	10	9.1	-	-
Dissolved Chromium	g/m³	< 0.00050	0.00052	< 0.00050	-	-
Dissolved Copper	g/m³	0.00063	0.00051	0.00068	-	-
Dissolved Iron	g/m³	0.28	0.23	0.24	-	-
Dissolved Lead	g/m³	< 0.00010	< 0.00010	< 0.00010	-	-
Dissolved Magnesium	g/m³	4.4	4.5	4.0	-	-

Sample Type: Clean waters						
Sample Name:		Downstream 60m No.2 23/10/07 23-Oct-2007	Downstream 60m No.1 24/10/07 24-Oct-2007	Downstream 60m No.2 24/10/07 24-Oct-2007		
Lab Number:		637754.46	637754.47	637754.48		
Dissolved Nickel	g/m³	< 0.00050	< 0.00050	< 0.00050	-	-
Dissolved Potassium	g/m³	6.1	6.5	6.6	-	-
Dissolved Sodium	g/m³	17	21	15	-	-
Dissolved Zinc	g/m³	0.0052	0.0045	0.0058	-	-

SUMMARY OF METHODS

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively clean matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis.

Sample Type: Clean waters			
Test	Method Description	Default Detection Limit	Samples
Electrical Conductivity (EC)	Conductivity meter, 25°C. APHA 2510 B 21st ed. 2005.	0.1 mS/m	1-48
Filtration for dissolved metals analysis	Sample filtration through 0.45µm membrane filter and preservation with nitric acid. APHA 3030 B 21st ed. 2005.	-	1-48
Dissolved Cadmium	Filtered sample, ICP-MS, trace level. APHA 3125 B 21st ed. 2005.	0.000050 g/m³	1-48
Dissolved Calcium	Filtered sample, ICP-MS, trace level. APHA 3125 B 21st ed. 2005.	0.050 g/m³	1-48
Dissolved Chromium	Filtered sample, ICP-MS, trace level. APHA 3125 B 21st ed. 2005.	0.00050 g/m³	1-48
Dissolved Copper	Filtered sample, ICP-MS, trace level. APHA 3125 B 21st ed. 2005.	0.00050 g/m³	1-48
Dissolved Iron	Filtered sample, ICP-MS, trace level. APHA 3125 B 21st ed. 2005.	0.020 g/m³	1-48
Dissolved Lead	Filtered sample, ICP-MS, trace level. APHA 3125 B 21st ed. 2005.	0.00010 g/m³	1-48
Dissolved Magnesium	Filtered sample, ICP-MS, trace level. APHA 3125 B 21st ed. 2005.	0.020 g/m³	1-48
Dissolved Nickel	Filtered sample, ICP-MS, trace level. APHA 3125 B 21st ed. 2005.	0.00050 g/m³	1-48
Dissolved Potassium	Filtered sample, ICP-MS, trace level. APHA 3125 B 21st ed. 2005.	0.050 g/m³	1-48
Dissolved Sodium	Filtered sample, ICP-MS, trace level. APHA 3125 B 21st ed. 2005.	0.020 g/m³	1-48
Dissolved Zinc	Filtered sample, ICP-MS, trace level. APHA 3125 B 21st ed. 2005.	0.0010 g/m³	1-48

These samples were collected by yourselves (or your agent) and analysed as received at the laboratory.

Samples are held at the laboratory after reporting for a length of time depending on the preservation used and the stability of the analytes being tested. Once the storage period is completed the samples are discarded unless otherwise advised by the client.

This report must not be reproduced, except in full, without the written consent of the signatory.

Peter Robinson MSc (Hons), PhD, FNZIC
Client Services Manager - Environmental Division

A.4 Analytical results of soils subjected to land application of dairy effluent from the Fonterra Kauri site

Hill Laboratories

R J Hill Laboratories Limited

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Internet: www.hill-labs.co.nz

ANALYSIS RESULTS

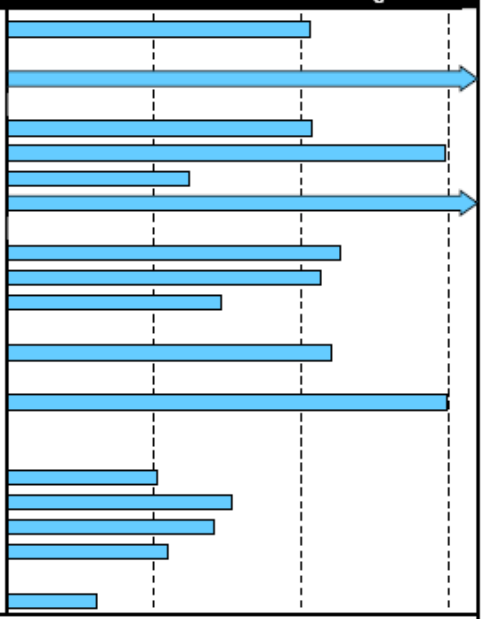
Client: University of Waikato
Address: Private Bag 3105, HAMILTON, New Zealand

Client Phone: 856 2889

Laboratory No.: 573968/1
Registered: 17-Sep-2008
Reported: 25-Sep-2008
Order No.: 174495
Submitted By: Dr T Healy
Client Ref:

Sample Name: Kauri Hika Irrigated A
Sample Type: SOIL Mixed Pasture (S1)

Analysis		Level Found	Medium Range
pH		6.3	5.8 - 6.3
Olsen P	(mg/L)	121	20 - 30
Potassium	(me/100g)	0.84	0.50 - 0.80
Calcium	(me/100g)	24.5	6.0 - 12.0
Magnesium	(me/100g)	1.46	1.00 - 3.00
Sodium	(me/100g)	2.72	0.20 - 0.50
CEC	(me/100g)	34	12 - 25
Base Saturation	(%)	87	50 - 85
Volume Weight	(g/mL)	0.78	0.60 - 1.00
Total Nitrogen	(%)	0.87	0.30 - 0.60
Phosphorus (Mehlich 3)	(mg/L)	197	40 - 70
Iron (Mehlich 3)	(mg/L)	409	
Manganese (Mehlich 3)	(mg/L)	8.6	8.0 - 65.0
Zinc (Mehlich 3)	(mg/L)	2.46	0.80 - 4.00
Copper (Mehlich 3)	(mg/L)	1.0	0.4 - 2.0
Boron (Mehlich 3)	(mg/L)	0.6	0.6 - 1.2
Cobalt (Mehlich 3)	(mg/L)	< 0.1	0.2 - 0.5
Aluminium (Mehlich 3)	(mg/L)	546	900 - 1300
Base Saturation		K 2.5 Ca 72	Mg 4.3 Na 8.0
MAF Units		K 13 Ca 24	Mg 26 Na 97



The above nutrient graph compares the levels found with reference interpretation levels. NOTE: It is important that the correct sample type be assigned, and that the recommended sampling procedure has been followed. R J Hill Laboratories Limited does not accept any responsibility for the resulting use of this information.

Laboratory Comments

Analysis Comments

Results for the Mehlich 3 soil test are shown above. Details of this test are available from our website and in a Technical Note, available on request.

The Mehlich 3 B test is considered to be a reliable measure for soils with moderate or high B status. For soils with low B levels (<1.5 mg/L), the test is much less reliable, and must be interpreted with appropriate caution. Plant herbage (leaf) B levels should be considered before recommending boron application.

End of Laboratory Comments

Submitter: Dr T Healy, University of Waikato, Dept Earth & Ocean Sciences, Ruakura Satellite Campus, Hamilton, New Zealand (Ph: 07 858 5200,

Hill Laboratories

R J Hill Laboratories Limited

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Facsimile: +64 (7) 858-2001
Email: mail@hill-labs.co.nz
Internet: www.hill-labs.co.nz

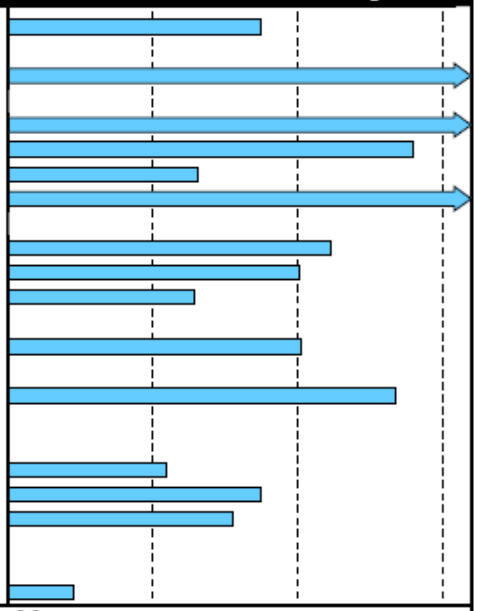
ANALYSIS RESULTS

Client: University of Waikato
Address: Private Bag 3105, HAMILTON, New Zealand
Client Phone: 856 2889

Laboratory No.: 573968/2
Registered: 17-Sep-2008
Reported: 25-Sep-2008
Order No.: 174495
Submitted By: Dr T Healy
Client Ref:

Sample Name: Kauri Hika Irrigated B
Sample Type: SOIL Mixed Pasture (S1)

Analysis		Level Found	Medium Range
pH		6.2	5.8 - 6.3
Olsen P	(mg/L)	125	20 - 30
Potassium	(me/100g)	1.47	0.50 - 0.80
Calcium	(me/100g)	22.2	6.0 - 12.0
Magnesium	(me/100g)	1.60	1.00 - 3.00
Sodium	(me/100g)	2.24	0.20 - 0.50
CEC	(me/100g)	32	12 - 25
Base Saturation	(%)	85	50 - 85
Volume Weight	(g/mL)	0.71	0.60 - 1.00
Total Nitrogen	(%)	0.62	0.30 - 0.60
Phosphorus (Mehlich 3)	(mg/L)	156	40 - 70
Iron (Mehlich 3)	(mg/L)	399	
Manganese (Mehlich 3)	(mg/L)	13.2	8.0 - 65.0
Zinc (Mehlich 3)	(mg/L)	3.15	0.80 - 4.00
Copper (Mehlich 3)	(mg/L)	1.3	0.4 - 2.0
Boron (Mehlich 3)	(mg/L)	< 0.5	0.6 - 1.2
Cobalt (Mehlich 3)	(mg/L)	< 0.1	0.2 - 0.5
Aluminium (Mehlich 3)	(mg/L)	410	900 - 1300
Base Saturation		K 4.6 Ca 69	Mg 4.9 Na 6.9
MAF Units		K 22 Ca 20	Mg 26 Na 74



The above nutrient graph compares the levels found with reference interpretation levels. NOTE: It is important that the correct sample type be assigned, and that the recommended sampling procedure has been followed. R J Hill Laboratories Limited does not accept any responsibility for the resulting use of this information.

Laboratory Comments

Analysis Comments
Results for the Mehlich 3 soil test are shown above. Details of this test are available from our website and in a Technical Note, available on request.

The Mehlich 3 B test is considered to be a reliable measure for soils with moderate or high B status. For soils with low B levels (<1.5 mg/L), the test is much less reliable, and must be interpreted with appropriate caution. Plant herbage (leaf) B levels should be considered before recommending boron application.

End of Laboratory Comments

Submitter: Dr T Healy, University of Waikato, Dept Earth & Ocean Sciences, Ruakura Satellite Campus, Hamilton, New Zealand (Ph: 07 858 5200,

Hill Laboratories

R J Hill Laboratories Limited

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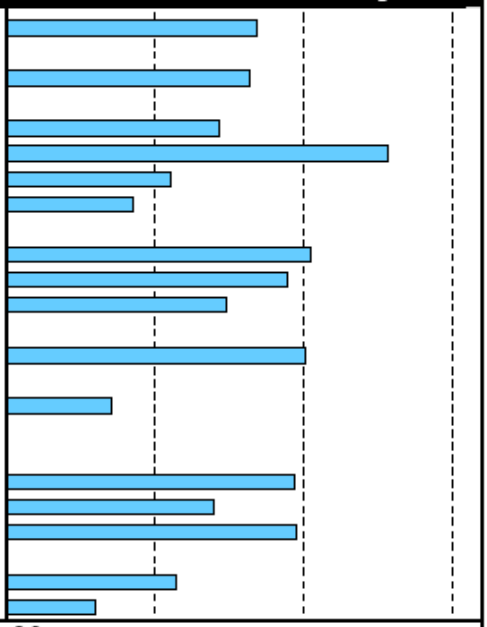
ANALYSIS RESULTS

Client: University of Waikato
Address: Private Bag 3105, HAMILTON, New Zealand
Client Phone: 856 2889

Laboratory No.: 573968/3
Registered: 17-Sep-2008
Reported: 25-Sep-2008
Order No.: 174495
Submitted By: Dr T Healy
Client Ref:

Sample Name: Kauri Hika Unirrigated A
Sample Type: SOIL Mixed Pasture (S1)

Analysis		Level Found	Medium Range
pH		6.1	5.8 - 6.3
Olsen P	(mg/L)	26	20 - 30
Potassium	(me/100g)	0.63	0.50 - 0.80
Calcium	(me/100g)	19.2	6.0 - 12.0
Magnesium	(me/100g)	1.20	1.00 - 3.00
Sodium	(me/100g)	0.17	0.20 - 0.50
CEC	(me/100g)	26	12 - 25
Base Saturation	(%)	81	50 - 85
Volume Weight	(g/mL)	0.79	0.60 - 1.00
Total Nitrogen	(%)	0.60	0.30 - 0.60
Phosphorus (Mehlich 3)	(mg/L)	31	40 - 70
Iron (Mehlich 3)	(mg/L)	193	
Manganese (Mehlich 3)	(mg/L)	61.2	8.0 - 65.0
Zinc (Mehlich 3)	(mg/L)	2.04	0.80 - 4.00
Copper (Mehlich 3)	(mg/L)	1.9	0.4 - 2.0
Boron (Mehlich 3)	(mg/L)	< 0.5	0.6 - 1.2
Cobalt (Mehlich 3)	(mg/L)	0.3	0.2 - 0.5
Aluminium (Mehlich 3)	(mg/L)	532	900 - 1300
Base Saturation		K 2.4 Ca 73	Mg 4.6 Na 0.6
MAF Units		K 10 Ca 19	Mg 21 Na 6



The above nutrient graph compares the levels found with reference interpretation levels. NOTE: It is important that the correct sample type be assigned, and that the recommended sampling procedure has been followed. R J Hill Laboratories Limited does not accept any responsibility for the resulting use of this information.

Laboratory Comments

Analysis Comments
Results for the Mehlich 3 soil test are shown above. Details of this test are available from our website and in a Technical Note, available on request.

The Mehlich 3 B test is considered to be a reliable measure for soils with moderate or high B status. For soils with low B levels (<1.5 mg/L), the test is much less reliable, and must be interpreted with appropriate caution. Plant herbage (leaf) B levels should be considered before recommending boron application.

End of Laboratory Comments

Submitter: Dr T Healy, University of Waikato, Dept Earth & Ocean Sciences, Ruakura Satellite Campus, Hamilton, New Zealand (Ph: 07 858 5200,

Hill Laboratories

R J Hill Laboratories Limited

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Email: mail@hill-labs.co.nz
Internet: www.hill-labs.co.nz

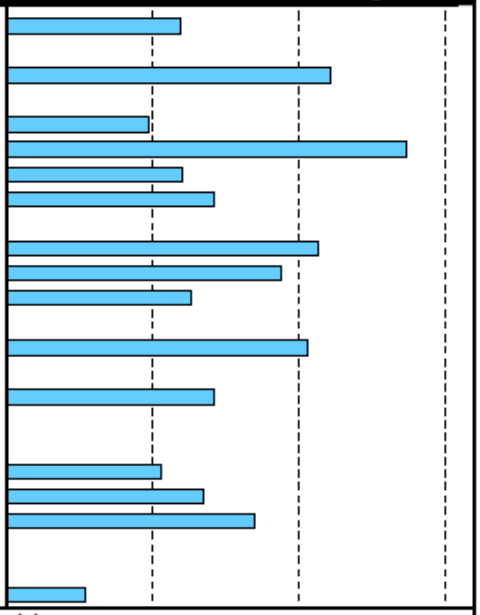
ANALYSIS RESULTS

Client: University of Waikato
Address: Private Bag 3105, HAMILTON, New Zealand
Client Phone: 856 2889

Laboratory No.: 573968/4
Registered: 17-Sep-2008
Reported: 25-Sep-2008
Order No.: 174495
Submitted By: Dr T Healy
Client Ref:

Sample Name: Kauri Hika Unirrigated B
Sample Type: SOIL Mixed Pasture (S1)

Analysis		Level Found	Medium Range
pH		5.9	5.8 - 6.3
Olsen P	(mg/L)	42	20 - 30
Potassium	(me/100g)	0.49	0.50 - 0.80
Calcium	(me/100g)	21.4	6.0 - 12.0
Magnesium	(me/100g)	1.40	1.00 - 3.00
Sodium	(me/100g)	0.32	0.20 - 0.50
CEC	(me/100g)	29	12 - 25
Base Saturation	(%)	81	50 - 85
Volume Weight	(g/mL)	0.70	0.60 - 1.00
Total Nitrogen	(%)	0.67	0.30 - 0.60
Phosphorus (Mehlich 3)	(mg/L)	52	40 - 70
Iron (Mehlich 3)	(mg/L)	384	
Manganese (Mehlich 3)	(mg/L)	11.0	8.0 - 65.0
Zinc (Mehlich 3)	(mg/L)	1.90	0.80 - 4.00
Copper (Mehlich 3)	(mg/L)	1.5	0.4 - 2.0
Boron (Mehlich 3)	(mg/L)	< 0.5	0.6 - 1.2
Cobalt (Mehlich 3)	(mg/L)	< 0.1	0.2 - 0.5
Aluminium (Mehlich 3)	(mg/L)	481	900 - 1300



Base Saturation	K 1.7	Ca 73	Mg 4.8	Na 1.1
MAF Units	K 7	Ca 19	Mg 22	Na 11

The above nutrient graph compares the levels found with reference interpretation levels. NOTE: It is important that the correct sample type be assigned, and that the recommended sampling procedure has been followed. R J Hill Laboratories Limited does not accept any responsibility for the resulting use of this information.

Laboratory Comments

Analysis Comments
Results for the Mehlich 3 soil test are shown above. Details of this test are available from our website and in a Technical Note, available on request.

The Mehlich 3 B test is considered to be a reliable measure for soils with moderate or high B status. For soils with low B levels (<1.5 mg/L), the test is much less reliable, and must be interpreted with appropriate caution. Plant herbage (leaf) B levels should be considered before recommending boron application.

End of Laboratory Comments

Submitter: Dr T Healy, University of Waikato, Dept Earth & Ocean Sciences, Ruakura Satellite Campus, Hamilton, New Zealand (Ph: 07 858 5200,

Hill Laboratories

R J Hill Laboratories Limited

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Email: mail@hill-labs.co.nz
Internet: www.hill-labs.co.nz

ANALYSIS RESULTS

Client: University of Waikato
Address: Private Bag 3105, HAMILTON, New Zealand

Client Phone: 856 2889

Laboratory No.: 573968
Registered: 17-Sep-2008
Reported: 25-Sep-2008
Order No.: 174495
Submitted By: Dr T Healy
Client Ref:

The following table gives a brief description of the analysis methods for this job. The COV (coeffient of variation) gives a measure of precision and is sometimes referred to as the Relative Standard Deviation, ie the standard deviation expressed as a percentage of the absolute value.

For further details and explanations, please contact the laboratory.
These samples were collected by yourselves (or your agent) and analysed as received at this laboratory.

Analyte	Method	COV(%)
Soil		
Volume Weight	The weight/volume ratio of dried, ground soil.	2
Base Saturation	Calculated from Extractable Cations and Cation Exchange Capacity.	4
M3-Phosphorus*, M3-Iron*, M3-Manganese*, M3-Zinc*, M3-Copper*, M3-Boron*, M3-Cobalt*, M3-Aluminium*	Mehlich 3 Extraction followed by ICP-OES.	-
Total Nitrogen*	Determined by NIR, calibration based on Total N by Dumas combustion.	-
Total Nitrogen	Dumas combustion.	-
CEC	Summation of extractable cations (K, Ca, Mg, Na) and extractable acidity.	4
Sample Registration*	Samples were collected by yourselves and analysed as received in the laboratory.	-
Soil Preparation (Dry and Grind)*	Air dried at 35 - 40°C overnight (residual moisture typically 4%) and crushed to pass through a 2 mm screen.	-
pH	1:2 (v/v) soil:water slurry followed by potentiometric determination of pH.	1
Potassium, Calcium, Magnesium, Sodium	1M Neutral ammonium acetate extraction followed by ICP-OES.	4
Phosphorus	Olsen extraction followed by Molybdenum Blue colorimetry.	6

* Indicates a non-accredited test.

Signatory:

Fiona Calvert
Client Services Manager

Submitter: Dr T Healy, University of Waikato, Dept Earth & Ocean Sciences, Ruakura Satellite Campus, Hamilton, New Zealand (Ph: 07 858 5200,

Hill Laboratories
BETTER TESTING BETTER RESULTS

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Private Bag 3205
Hamilton 3240, New Zealand

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Fax +64 7 858 2001
Email mail@hill-labs.co.nz
Web www.hill-labs.co.nz

ANALYSIS REPORT

Client:	University of Waikato	Lab No:	646820	SPv1
Contact:	Healy, Terry (Dr)	Date Registered:	20-Jun-2008	
	Dept Earth & Ocean Sciences	Date Reported:	19-Sep-2008	
	Ruakura Satellite Campus	Quote No:	32104	
	Hamilton	Order No:	174495	
		Client Reference:		
		Submitted By:	Xie, Zoe	

Sample Type: Soil

Sample Name:		Kauri. Hika Irrigated A	Kauri. Hika Irrigated B	Kauri. Hika Unirrigated A	Kauri. Hika Unirrigated B	
Lab Number:		646820.8	646820.9	646820.10	646820.11	
Total Recoverable Cadmium	mg/kg dry wt	0.36	0.37	0.63	0.61	-
Total Recoverable Iron	mg/kg dry wt	19000	18000	33000	19000	-
Total Recoverable Mercury	mg/kg dry wt	0.14	0.18	0.098	0.10	-

SUMMARY OF METHODS

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively clean matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis.

Sample Type: Soil

Test	Method Description	Default Detection Limit	Samples
Environmental Solids Sample Preparation*	Air dried at 35°C and sieved, <2mm fraction.	-	8-11
Total Recoverable digestion	Nitric / hydrochloric acid digestion. US EPA 200.2.	-	8-11
Total Recoverable Cadmium	Dried sample, sieved as specified (if required). Nitric/Hydrochloric acid digestion, ICP-MS, trace level. US EPA 200.2.	0.010 mg/kg dry wt	8-11
Total Recoverable Iron	Dried sample, sieved as specified (if required). Nitric/Hydrochloric acid digestion, ICP-MS, screen level. US EPA 200.2.	40 mg/kg dry wt	8-11
Total Recoverable Mercury	Dried sample, sieved as specified (if required). Nitric/Hydrochloric acid digestion, ICP-MS, trace level. US EPA 200.2.	0.010 mg/kg dry wt	8-11

These samples were collected by yourselves (or your agent) and analysed as received at the laboratory.

Samples are held at the laboratory after reporting for a length of time depending on the preservation used and the stability of the analytes being tested. Once the storage period is completed the samples are discarded unless otherwise advised by the client.

Peter Robinson MSc (Hons), PhD, FNZIC
Client Services Manager - Environmental Division

A.5 Analytical results of ground water subjected to land application of dairy effluent from the Fonterra Kauri site

Hill Laboratories
BETTER TESTING BETTER RESULTS

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Hamilton 3240, New Zealand

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Web www.hill-labs.co.nz

ANALYSIS REPORT

Client:	University of Waikato	Lab No:	646820 SPv1
Contact:	Healy, Terry (Dr)	Date Registered:	20-Jun-2008
	Dept Earth & Ocean Sciences	Date Reported:	19-Sep-2008
	Ruakura Satellite Campus	Quote No:	32104
	Hamilton	Order No:	174495
		Client Reference:	
		Submitted By:	Xie, Zoe

Sample Type: Soil

Sample Name:		Kauri. Hika Irrigated A	Kauri. Hika Irrigated B	Kauri. Hika Unirrigated A	Kauri. Hika Unirrigated B	
Lab Number:		646820.8	646820.9	646820.10	646820.11	
Total Recoverable Cadmium	mg/kg dry wt	0.36	0.37	0.63	0.61	-
Total Recoverable Iron	mg/kg dry wt	19000	18000	33000	19000	-
Total Recoverable Mercury	mg/kg dry wt	0.14	0.18	0.098	0.10	-

SUMMARY OF METHODS

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively clean matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis.

Sample Type: Soil

Test	Method Description	Default Detection Limit	Samples
Environmental Solids Sample Preparation*	Air dried at 35°C and sieved, <2mm fraction.	-	8-11
Total Recoverable digestion	Nitric / hydrochloric acid digestion. US EPA 200.2.	-	8-11
Total Recoverable Cadmium	Dried sample, sieved as specified (if required). Nitric/Hydrochloric acid digestion, ICP-MS, trace level. US EPA 200.2.	0.010 mg/kg dry wt	8-11
Total Recoverable Iron	Dried sample, sieved as specified (if required). Nitric/Hydrochloric acid digestion, ICP-MS, screen level. US EPA 200.2.	40 mg/kg dry wt	8-11
Total Recoverable Mercury	Dried sample, sieved as specified (if required). Nitric/Hydrochloric acid digestion, ICP-MS, trace level. US EPA 200.2.	0.010 mg/kg dry wt	8-11

These samples were collected by yourselves (or your agent) and analysed as received at the laboratory.

Samples are held at the laboratory after reporting for a length of time depending on the preservation used and the stability of the analytes being tested. Once the storage period is completed the samples are discarded unless otherwise advised by the client.

Peter Robinson MSc (Hons), PhD, FNZIC
Client Services Manager - Environmental Division

Hill Laboratories
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Hamilton 3240, New Zealand

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Web www.hill-labs.co.nz

ANALYSIS REPORT

Client:	University of Waikato	Lab No:	658285 SPv2
Contact:	Healy, Terry (Dr)	Date Registered:	17-Sep-2008
	Dept Earth & Ocean Sciences	Date Reported:	16-Oct-2008
	Ruakura Satellite Campus	Quote No:	33971
	Hamilton	Order No:	174495
		Client Reference:	Zoe Xie - Groundwaters
		Submitted By:	Xie, Zoe

Amended Report This report replaces an earlier report issued on the 30 Sep 2008 at 3:24 pm
Phosphorus tests have been added after discussion with the client.

Sample Type: Clean waters

Sample Name:		Kauri Hika Irrigated 1 pH 5.28 11-Apr-2008	Kauri Hika Irrigated 2 pH 6.42 11-Apr-2008	Kauri Hika Un-irrigated pH 6.34 11-Apr-2008		
Lab Number:		658285.1	658285.2	658285.3		
Electrical Conductivity (EC)	mS/m	62.5	93.1	17.6	-	-
Dissolved Cadmium	g/m³	0.00028	0.00013	< 0.000050	-	-
Dissolved Calcium	g/m³	2.5	5.4	2.5	-	-
Dissolved Copper	g/m³	0.0063	0.013	0.0028	-	-
Dissolved Iron	g/m³	0.11	0.14	< 0.020	-	-
Dissolved Magnesium	g/m³	1.1	3.5	3.9	-	-
Dissolved Mercury	g/m³	< 0.000080	< 0.000080	< 0.000080	-	-
Dissolved Potassium	g/m³	1.1	4.8	2.2	-	-
Dissolved Sodium	g/m³	110	210	24	-	-
Dissolved Zinc	g/m³	0.072	0.024	0.0041	-	-
Nitrate-N + Nitrite-N	g/m³	2.0	0.36	0.055	-	-
Total Kjeldahl Nitrogen (TKN)	g/m³	0.71	6.9	0.44	-	-
Dissolved Reactive Phosphorus	g/m³	0.0054	0.0046	0.014	-	-
Total Phosphorus	g/m³	0.12	0.89	1.9	-	-

SUMMARY OF METHODS

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively clean matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis.

Sample Type: Clean waters

Test	Method Description	Default Detection Limit	Samples
Filtration, Unpreserved	Sample filtration through 0.45µm membrane filter.	-	1-3
Total Kjeldahl Digestion	Sulphuric acid digestion with copper sulphate catalyst.	-	1-3
Total Phosphorus Digestion	Acid persulphate digestion.	-	1-3
Electrical Conductivity (EC)	Conductivity meter, 25°C. APHA 2510 B 21st ed. 2005.	0.1 mS/m	1-3
Filtration for dissolved metals analysis	Sample filtration through 0.45µm membrane filter and preservation with nitric acid. APHA 3030 B 21st ed. 2005.	-	1-3
Dissolved Cadmium	Filtered sample, ICP-MS, trace level. APHA 3125 B 21st ed. 2005.	0.000050 g/m³	1-3
Dissolved Calcium	Filtered sample, ICP-MS, trace level. APHA 3125 B 21st ed. 2005.	0.050 g/m³	1-3

Sample Type: Clean waters			
Test	**Method Description**	**Default Detection Limit**	**Samples**
Dissolved Copper	Filtered sample, ICP-MS, trace level. APHA 3125 B 21st ed. 2005.	0.00050 g/m³	1-3
Dissolved Iron	Filtered sample, ICP-MS, trace level. APHA 3125 B 21st ed. 2005.	0.020 g/m³	1-3
Dissolved Magnesium	Filtered sample, ICP-MS, trace level. APHA 3125 B 21st ed. 2005.	0.020 g/m³	1-3
Dissolved Mercury	Filtered sample. Permanganate / Persulphate digestion. Analysis by FIMS.	0.000080 g/m³	1-3
Dissolved Potassium	Filtered sample, ICP-MS, trace level. APHA 3125 B 21st ed. 2005.	0.050 g/m³	1-3
Dissolved Sodium	Filtered sample, ICP-MS, trace level. APHA 3125 B 21st ed. 2005.	0.020 g/m³	1-3
Dissolved Zinc	Filtered sample, ICP-MS, trace level. APHA 3125 B 21st ed. 2005.	0.0010 g/m³	1-3
Nitrate-N + Nitrite-N	Total oxidised nitrogen. Automated cadmium reduction, flow injection analyser. APHA 4500-NO_3- I (Proposed) 21st ed. 2005.	0.0020 g/m³	1-3
Total Kjeldahl Nitrogen (TKN)	Total Kjeldahl digestion, phenol/hypochlorite colorimetry. Discrete Analyser. APHA 4500-N_{org} C. (modified) 4500 NH_3 F (modified) 21st ed. 2005.	0.10 g/m³	1-3
Dissolved Reactive Phosphorus	Filtered sample. Molybdenum blue colorimetry. Discrete Analyser. APHA 4500-P E (modified from manual analysis) 21st ed. 2005.	0.0040 g/m³	1-3
Total Phosphorus	Total phosphorus digestion, ascorbic acid colorimetry. Discrete Analyser. APHA 4500-P E (modified from manual analysis) 21st ed. 2005.	0.0040 g/m³	1-3

These samples were collected by yourselves (or your agent) and analysed as received at the laboratory.

Samples are held at the laboratory after reporting for a length of time depending on the preservation used and the stability of the analytes being tested. Once the storage period is completed the samples are discarded unless otherwise advised by the client.

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