Impact of phosphate fertiliser derived fluorine on soil microbiology and white clover (*Trifolium repens* L)

A thesis presented in partial fulfilment of the

requirements for the degree of

Doctor of Philosophy (PhD)

In

Environmental Sciences

at Massey University, Palmerston North,

New Zealand

Thangavelautham Geretharan

2019

ABSTRACT

Fluorine (F) is a significant contaminant in most phosphate fertilisers and fertiliser-derived F is accumulating in New Zealand agricultural soils as a consequent of phosphate fertiliser application. There is potential for soil fluoride (F^-) to detrimentally affect soil biological functions such as nitrogen fixation by *Rhizobium leguminosarum,* and to alter soil properties. Fluorine accumulation in soil may require changes to future land use and management practices. The aim of this thesis is to investigate whether phosphate fertiliser-derived soil F has a detrimental effect on soil microorganisms.

A novel analytical method for soil F analysis was developed to measure the total soil F concentration based on extraction with dilute NaOH. The relative error between a novel 4 mol L^{-1} NaOH extraction and the conventional fusion method was < 2 for organic-matter and volcanic parent material derived soils but was > 2 for recent and pallic soils. Precision of the 4 mol L^{-1} NaOH extraction method, measured through repeat analysis of three further soils (n = 270), was calculated as < 9% Relative Standard Deviation (RSD). To define a standard method to quantify the bioavailable F concentration in soil, samples were extracted with water, 1 mol L^{-1} HCl, 0.01 mol L^{-1} $CaCl_2$, 0.01 mol L^{-1} KCl, and 1 mol L^{-1} NH_4Cl. Compared to water, 0.01 mol L^{-1} $CaCl_2$ had high relative recovery (of bioavailable F) in soils which have elevated Fe and Al content. Therefore, 0.01 mol L^{-1} $CaCl_2$ is recommended to measure the bioavailable F concentration of New Zealand pastoral soils.

There is no data available which describes the toxic effect of bioavailable F on *R. leguminosarum* in New Zealand soils. A laboratory incubation experiment and

MicroResp 96-well format respiration-inhibition assay were conducted to investigate the effect of F on *R. leguminosarum* and white clover. *Rhizobium leguminosarum* growth was not significantly suppressed by F^- concentrations less than 100 mg L^{-1}. The normal rod-shaped bacterium cell of *R. leguminosarum* was morphologically altered when exposed to F^- concentrations above 100 mg L^{-1}. The IC_{10} values determined for F^- toxicity to *R. leguminosarum* were higher than 100 mg F^- L^{-1}. Pottle-based experiments showed that white clover growth was not significantly supressed at a F^- concentration < 70 mg L^{-1}, while healthy nodules were formed up to a F^- concentration of 100 mg L^{-1}. Light and TEM micrographs of nodules revealed that the *Rhizobium*-white clover interaction was not influenced by F^- concentrations up to 100 mg L^{-1}.

To assess the potential effects of lime and compost amendment on the bioavailability of F, laboratory F^- adsorption/desorption experiments were conducted. Results revealed that at pH < 6, F^- adsorption significantly ($p < 0.05$) increased as a function of compost application. At soil pH > 6, F^- adsorption was not significantly ($p > 0.05$) influenced by compost. Lime application increased the soil pH and maximum F^- adsorption was recorded at soil pH between 5.5 – 6.8. These results showed that soil pH significantly influences ($p < 0.05$) F^- desorption and this should be considered in the management of pastoral soil with elevated F.

A pot trial was conducted to quantify the effect of added F (equivalent to 0 - 50 years of F accumulation via the continuous application of phosphate fertiliser) on soil properties, soil microbial activity, white clover growth, and *R. leguminosarum* in an Allophanic soil. F addition (0 – 385 mg kg^{-1}) significantly ($p < 0.05$) increased soil pH and Dissolved Organic Carbon (DOC) from 5.18 to 5.53 and from 270.5 to 331.3 mg kg^{-1}, respectively.

The $CaCl_2$-extractable F concentration increased from 4.95 to 12.67 units as a function of added F. Microbial biomass carbon and soil enzyme activities, and white clover growth and interaction with *R. leguminosarum,* were not influenced by added F^- up to the highest concentration used in this study. White clover variety Merlyn and Tribute shoot F concentration was increased from 4.9 to 19.9 mg kg^{-1} DM and 5.12 to 16.68 mg kg^{-1} DM, respectively, however these concentrations are not expected to represent a risk to grazing livestock.

This study highlights that the 4 mol L^{-1} NaOH extraction method is a simple and accurate technique to measure the total F concentration for soils which have high Fe, Al and organic matter content. Water extractable and 0.01 mol L^{-1} $CaCl_2$-extractable F concentration are recommended to measure the bioavailable concentration of F in New Zealand soils. Current New Zealand bioavailable F concentrations are orders of magnitude lower than the F^- concentration assessed to be toxic to *R. leguminosarum* and white clover, and this suggests there is no imminent risk of soil F to *R. leguminosarum*. Compost is not recommended as an amendment for soils which have a pH above 6.0 to minimize the bioavailable soil F^- concentration. Lime application is suitable in such soils to minimize the bioavailable soil F^- concentration through altering soil pH. The major fraction of added F is immobilised by Allophanic soil and this effectively reduces the available F concentration to plants and soil microorganisms.

Future work is recommended to investigate the uptake mechanism of bioavailable F into white clover shoots and roots. However, there is no evidence to suggest that F concentrations in New Zealand soil are a risk to New Zealand's pasture-based farming systems.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank God Anjaneya for being with me throughout my life. I always felt his presence during my PhD research. Jai Sri Ram!

I am grateful to have been supervised by Professor Chris Anderson, Dr Paramsothy Jeyakumar and Dr Michael Bretherton.

I sincerely thank my chief supervisor Professor Chris Anderson to accept me to do the PhD under his supervision. Your guidance, encouragement, support and valuable recommendations were significant to this PhD research and for my academic career. Your guidance helped me in all time of research and writing of this thesis. My PhD has been an amazing experience and I thank Professor Chris wholeheartedly, not only for his tremendous academic support, but also for giving me so many wonderful opportunities for my career development.

I am highly indebted to my supervisor Dr Paramsothy Jeyakumar for his advice, encouragement and guidance. It is not possible to get this PhD at Massey without his effort. Thank you very much for all your valuable suggestions and advice, not only to my PhD research but also for my personal life. Your motivating discussions helped me at all times to stay focused and accomplish the task.

I am grateful to my supervisor Dr Michael Bretherton for his detailed review, constructive criticisms and extensive comments to enrich the ideas presented in this thesis. Thank you very much for your valuable comments and inputs.

I am also grateful to all my supervisors for their valuable comments and corrections on my manuscripts, their encouragement helps me to attain considerable amount of publications. These will be invaluable additions to my future academic career.

I am thankful to New Zealand Soil Science Society for selecting me for outstanding ‘Bert Quin PhD student award’ in 2018. I gratefully acknowledge the funding received towards my PhD from the Fertiliser Association of New Zealand which covered the tuition fee and research cost of the PhD research. I am also grateful to the Applied Academic Postgraduate Scholarships which I have received through my PhD research; the Helen E

Akers PhD Scholarship, the Ravensdown PhD Scholarship, and the George Mason Sustainable Land Use PhD Scholarship. Thank you very much Dr Kerry Harrington and the Scholarship Committee for selecting me as one of the recipients of these scholarships and your support to my PhD research.

I am also thankful to Dr T Ramilan for his valuable support during my PhD study.

Special mention of gratitude goes to the Eastern University, Sri Lanka for granting me paid study leave to pursue the PhD at Massey University, New Zealand.

I would like to thank Denise Stewart, Fiona Bardell and Sharon Wright for their administrative support. My deep appreciation goes out to the laboratory technicians Glenys Wallace, Ian Furkert and Bob Tose for their kind support throughout my PhD research.

I thank my fantastic postgraduate mates in Massey; Yang Li, Nilusha, Khadija, May, Aldrin, Ahamed, Hamed, Arkinson, Kamal and Stanislav for their stimulating discussions and all the fun we had during my life in Massey. My gratitude also extends to all the Palmy Sri Lankan community for their hospitality and lovely foods offered during my PhD study.

Last but not least, I would like to thank my mother, mother-in-law, and brothers for their love, encouragement, and supporting me spiritually throughout the PhD research and my life in general. My wife, we are so blessed to undertake our Bachelor, Masters and Doctoral Degrees together. I would like to say a heartfelt thank you to my wife who has been by my side through this PhD, even she engaged in her PhD research.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ACP	Acid phosphatase
ALP	Alkaline phosphatase
ATP	Adenosine triphosphate
CW	Cell wall
DOC	Dissolved Organic Carbon
FGD	Flue gas desulphurisation
IC	Ion chromatography
ISE	Ion-selective electrode
LM	Light
MSM	Mineral salt medium
MUB	Modified universal buffer
NAA	Neutron activation analysis
NES	National Environmental Standards
OD	Optical Density
PIGE	Particle induced gamma ray emission
PM	Peribacteroid membrane
RNA	Ribonucleic acid
RSD	Relative Standard Deviation
SEM	Scanning electron microscope
SOM	Soil organic matter
SSP	Single superphosphate
TEM	Transmission Electron Microscope
TES	Trace element solution
TISAB	Total Ionic Strength Adjustment Buffer’
TPF	Tri phenyl formazan
TXRF	Reflection X-ray fluorescence
XRF	X-ray fluorescence
YMB	Yeast extract Mannitol Broth

CHAPTER 1

Introduction

Primary production is the backbone of the New Zealand economy and the dairy sector is a dominant component in New Zealand's export earnings (MacLeod and Moller, 2006; Foote et al., 2015). In 2017, 26% of New Zealand's annual export revenue was earned from dairy products (Statistics New Zealand, 2017). The global competitiveness of New Zealand's dairy sector is governed by its low-cost pasture system developed on healthy and productive soil (Monaghan et al., 2008).

Legume-based pastoral farming is the predominant agricultural system in New Zealand, with white clover and ryegrass being the dominant herbage species (Loganathan et al., 2003). In this system, the symbiotic relationship between white clover and *R. leguminosarum* converts atmospheric nitrogen into plant-available nitrogen, thus increasing soil fertility (Delestre et al., 2015). In New Zealand, 1.57 million tonnes of nitrogen is fixed on an annual basis by *Rhizobium*-white clover symbiosis across 13.5 million ha of pasture land (Caradus et al., 1996). Biological nitrogen fixation supports ryegrass production by increasing soil nitrogen (Wakelin et al., 2018). In addition to nitrogen fixation, soil microorganisms help underpin the New Zealand pasture system by involving themselves in the Phosphorus (P) and Sulfur (S) cycles, plant disease control, and carbon mineralisation (Sarathchandra et al., 1984; Wakelin et al., 2013).

Effective white clover and ryegrass production requires regular phosphate fertiliser applications to overcome the high phosphorus retention capacity of New Zealand's productive dairying pasture soils (Loganathan et al., 2003). Phosphate fertiliser has been

used to assist pasture production in New Zealand since 1867 (Kim, 2008). Over the last two decades, dairy farm intensification has resulted in an increase in phosphate fertiliser usage in New Zealand (Foote et al., 2015). Annually, 500 kg ha^{-1} phosphate fertiliser is applied to maintain high plant phosphate availability in high P-retaining pasture soils (Loganathan et al., 2003).

Phosphate fertiliser used in New Zealand has dominantly been manufactured from the acidification of phosphate rock which often contains F as a natural impurity (Loganathan et al., 2007). The F is carried through to the final product in the manufacturing process, so that phosphate fertiliser contains 1.5 - 2% F (Loganathan et al., 2001). Studies conducted over 20 years in New Zealand have shown that continuous phosphate fertiliser application increases total soil F concentrations in New Zealand agricultural soils (Loganathan et al., 2001; Kim et al., 2016; Gray, 2018). Loganathan et al. (2001) reported that 20 years of phosphate fertiliser application has increased total soil F concentrations from 116 to 259 mg kg^{-1} in New Zealand pasture soils. In 2016, Kim et al. (2016) found that over 50 years of phosphate fertiliser application had increased total soil F concentrations from 220 to 650 mg kg^{-1} in dairy farms in the Bay of Plenty and in the Waikato regions of New Zealand. Recently, Gray (2018) reported that 63-years of phosphate fertiliser application at a rate of 376 kg ha^{-1} had elevated the total soil F concentration from 251 to 430 mg kg^{-1} in Canterbury, New Zealand.

Increased soil F concentrations have the potential to cause negative impacts on micro-biota living within the soil (Tscherko and Kandeler, 1997; Ropelewska et al., 2016). Of most concern are potential detrimental effects of F on *Rhizobium*-clover interactions which could have adverse consequences on pastoral dairy systems, and therefore on New

Zealand's economy. A comprehensive understanding of the interaction between added F and soil chemistry is therefore necessary to assess the effects of soil F on nitrogen-fixing bacteria.

Increasing soil F concentrations in New Zealand agricultural soils have initiated discussion on the need for soil F management guideline values to protect soil organisms and to measure the potential impacts of F on soil environment. The development of soil F guideline values requires quantification of F toxic concentration values for soil organisms. To date the toxic F concentration for *R. leguminosarum* has not been reported.

Accurate and useful soil F research must be underpinned by reliable and simple tests to determine total and bioavailable F concentrations in soil. Conventional techniques for total soil F analysis are complex, time-consuming and expensive (Yiping and Caiyun, 2010), and limited research has been done to standardise a method for the analysis of the bioavailable F concentration in soil. Jeyakumar and Anderson (2015) found that simple extraction of soil with dilute NaOH (4 mol L^{-1}) consistently reported 80% of the total soil F for Allophanic soils. However, the authors of this methodology provided the caveat that the test requires ongoing validation through multiple repeat assessments.

In addition to developing F guideline values to protect New Zealand pasture ecosystems, the impact of current soil management practices should be considered in terms of the current soil F concentration, and its toxicity in the environment. In New Zealand pasture systems, lime is applied to increase phosphorus availability and to alter the soil pH for optimum pasture production (Edmeades et al., 1984). The use of compost and lime as soil amendments to reduce cadmium uptake by plants has been a feature of recent soil research

(Cavanagh et al., 2018). Such soil management practices may have the potential to change the concentration of available F in soil as a result of changing soil properties; bioavailable F concentrations are highly controlled by soil properties (Quintáns-Fondo et al., 2016a). There is a lack of information on how soil amendment with lime and compost may influence the bioavailability of F in New Zealand soils. Furthermore, the F retention capacity of New Zealand soils, and how this is correlated with soil properties, has not been reported. Such information is vital to develop F guideline values and soil management practices to ensure any potential risk of soil F to New Zealand agriculture can be predicted and managed.

1.1 Research focus

The aim of this doctoral study is to find effects of soil F derived from phosphate fertilisers on soil microorganisms, specifically nitrogen fixing bacteria. Accordingly, this study was carried out with the following objectives:

1. To validate a NaOH extraction method to assess total soil F and propose a standard method to measure the bioavailable F concentration for New Zealand pasture soil.
2. To examine the effect of F^- ion on *Rhizobium*-white clover symbiosis.
3. To measure the effect of lime and compost application on F availability to soil organisms.
4. To find the effect of long-term F accumulation on soil microbial activity in a New Zealand pasture soil.

1.2 Thesis structure

This thesis is comprised of seven chapters which include this introduction. Chapter 2 is a literature review that provides detailed information regarding F chemistry, factors that influence F availability in soil, and the methodologies associated with total and extractable F analyses. Chapter 3 describes the development of methodology for total soil F analysis, and a standard method to measure the bioavailable F concentration in soil. Chapter 4 presents research outputs investigating the effects of F on *R. leguminosarum* and white clover – *Rhizobium* interactions. Chapter 5 describes how compost and lime application influence F^- adsorption. Chapter 6 presents research outputs of a pot experiment which investigated the consequences of F accumulation in Allophanic soil. Finally, Chapter 7 provides a summary of the research outcomes from this thesis and lists recommendations for further study. Each research chapter is presented as an abstract, introduction, material and methods, results and discussion and conclusion.

CHAPTER 2

Literature Review

2.1 Introduction

Fluorine is a soil contaminant, widely spread across the globe. Weathering of rocks and volcanic eruptions naturally increase the concentration of F in soil (Vithanage and Bhattacharya, 2015) with industrial activity the principle source of anthropogenic release (Notcutt and Davies, 2001).

Soil parent material and its components play a significant role in the levels of naturally available soil F. Naturally occurring F exists mostly as fluorspar (CaF_2), sellaite (MgF_2), fluorapatite [$3Ca_3(PO4)_2Ca(F,Cl_2)$] and cryolite (Na_3AlF_6). Sedimentary rocks mainly contain fluorspar, while cryolite is found in igneous rocks (Mohapatra et al., 2009). Topaz, lepidolite, zinnwaldite, and the mica group of minerals also contain a significant amount of F (Loganathan et al., 2003). These F-rich materials release F to soil duing the weathering processes. In New Zealand, the highest native F^- levels recorded in soil (a mean of 254 mg kg^{-1}) are derived from volcanic parent materials such as pumice, rhyolite and ignimbrite (Kim et al., 2016).

In addtion to the weathering of rocks and soil parent materials, volcanic activity increases the soil F concentration. During a volcanic erruption, HF (Hydrogen fluoride) gas is released and this can be absorbed by volcanic ash (Óskarsson, 1980; Symonds et al., 1988). For example, Mount Etna in Italy releases 70 000 metric tons of HF to the

amosphere annually with F deposition to soil ranging from 16 to 917 mg m^{-2} (Francis et al., 1998; Bellomo et al., 2007). In New Zealand, the volcano Mt Ruapehu errupted over 1995 – 1996 depositing tephra across 25 000 km^2 of agricultural land in the central North Island of New Zealand. The F concentration in this tephra ranged from 138 to 1797 mg kg^{-1} (Cronin et al., 2003).

Anthropogenic inputs of F to soil is mainly derived from emissions from the aluminium, brick, steel, glass and fertiliser manufacturing industries; the burning of fossil fuels; and the application of phosphorus (P) fertilisers to land (Arnesen, 1997; Cronin et al., 2000; Hedley et al., 2007; Mirlean and Roisenberg, 2007). A summary of the amounts of F emitted from different sources globally is presented in Table 2.1.

In aluminium smelters, molten cryolite (Na_3AlF_6) is used to dissolve alumina (Al_2O_3) for Al production. During production HF, CF_4, C_2F_6 and SiF_4 are created, with hydrogen fluoride gas HF the major component (Gago et al., 2014). Pomazkina et al. (2008) reported that the Irkutsk aluminium smelter in Siberia emitted 6 000 tons of NaF annually. Zouari et al. (2014) reported that 700 metric tons of F is emitted annually by the phosphate industry located in Sfax city (South Tunisia).

Non-industrial inputs of anthropogenic F to soils are mainly via the application of phosphate fertilisers. A summary of the range of total soil F concentrations that have been reported from around the world due to anthropogenic inputs of F is presented in Table 2.2.

Table 2. 1. Amount of F released from different sources globally (modified from Fuge (2019)).

F source	Added F (tons yr^{-1})
Brick manufacture	1 800 000
Coal combustion	200 000 – 300 000
Aluminium smelting	41 000
Vulcanicity	300 000 – 700 000
Phosphate fertiliser production	70 000 – 100 000
Ceramic tile production	290 000
Phosphate fertiliser application	2 300 000

Table 2. 2. Total F concentrations (mg F kg^{-1}) of top soils from various countries (modified from Loganathan et al., 2006).

Country	Soil F concentration (mg kg^{-1})	Main source of F	References
China (Paddy field)	409-1356	lead-zinc mining	Zhang et al. (2010)
China (Aerated field)	476-1553	lead-zinc mining	Zhang et al. (2010)
India	366-1178	-	Kumar et al. (2016)
Australia	44-156	P fertiliser	McLaughlin et al. (2001)
Austria	217-1074	Al smelter	Wenzel and Blum (1992)
Canada	16-909	P reaction plant	Thompson et al. (1979)
India	322-456	Brick industry	Jha et al. (2008)
New Zealand	117-900	P fertiliser	Kim et al. (2016)
Australia	< 40-2100	-	Mikkonen et al. (2018)
China	400 – 1612	P factory	Wang et al. (2018)

2.2 Soil F in New Zealand agricultural soils

New Zealand's economy is dependent (53% of merchandise exports) on the agricultural sector (MacLeod and Moller, 2006), with legume-based pastoral agriculture (composite ryegrass and clover) being the predominant farming system in New Zealand (Verkerk, 2003). *Rhizobium leguminosarum* is the fundamental component of New Zealand pasture systems as it supports legume pasture growth by fixing nitrogen from the atmosphere (Wakelin et al., 2016). Forty-eight percent of New Zealand's export earnings come from livestock production (Hedley et al., 2011), and pasture-based farming has intensified over the last 10 years (Basset-Mens et al., 2009). A consequence of this is inputs such as fertiliser, energy, irrigation, capital, and knowledge use has increased to produce more milk and meat from the same land area.

The F concentration in superphosphate (SP) fertiliser is 1.5-2% (Loganathan et al., 2001), and continuous phosphate fertiliser application to soil is the primary mechanism for the introduction of F to New Zealand agricultural ecosystems (Kim et al., 2016). The F concentration in phosphate fertiliser varies as a function of manufacture and formulation (Table 2.3). Hedley et al. (2007) estimated that phosphorus fertiliser application at a rate of 10–30 kg P ha^{-1} yr^{-1} will add 1–6 kg F^- ha^{-1} yr^{-1} to (New Zealand) agricultural soils leading to a doubling period for the F concentration for New Zealand pastoral soils of 51 years. Loganathan et al. (2006) reported that the total F concentration of New Zealand pastoral soils was 212-617 mg kg^{-1}.

Table 2. 3. Fluorine concentration of different phosphate fertiliser (modified from Fuge (2019)).

	Fertiliser	F (%)	Reference
New Zealand	Single superphosphate	1.08–1.84	Cronin et al. (2000)
	Triple superphosphate	1.30–2.40	Cronin et al. (2000)
	Monoammonium phosphate	1.60–2.20	Cronin et al. (2000)
	Diammonium phosphate	1.20–3.00	Cronin et al. (2000)
World	Phosphate fertiliser	0.85–3.80	Kabata-Pendias (2000)

Loganathan et al. (2006) proposed that soils with a total F concentration greater than 500 mg kg^{-1} requires management practices to avoid potential future F toxicity. Kim et al. (2016) measured the total soil F concentrations across different New Zealand agricultural land use systems (Table 2.4) and reported that the average F accumulation rate is 2.1% per year. These authors reported that over the last 50 years, the mean topsoil (10 cm) F concentration has increased from around 220 mg kg^{-1} to 440 mg kg^{-1}. In their study, the total soil F concentration for 44% of New Zealand's dairy farms had increased to above 500 mg F kg^{-1}, with 10% of the farms being above 650 mg F kg^{-1} (Figure 2.1). Recently, Gray (2018) demonstrated that in New Zealand, 63 years of continuous phosphorus fertiliser application increased total soil F concentration by 31 - 71%.

Table 2. 4. Total soil F concentrations (mg F kg^{-1}) for different agricultural land use systems in New Zealand (Kim et al., 2016).

Land use	Soil F concentration (mg kg^{-1})
Dairy	128-830
Sheep and beef	117-900
Arable	260-635
Orchard	119-680

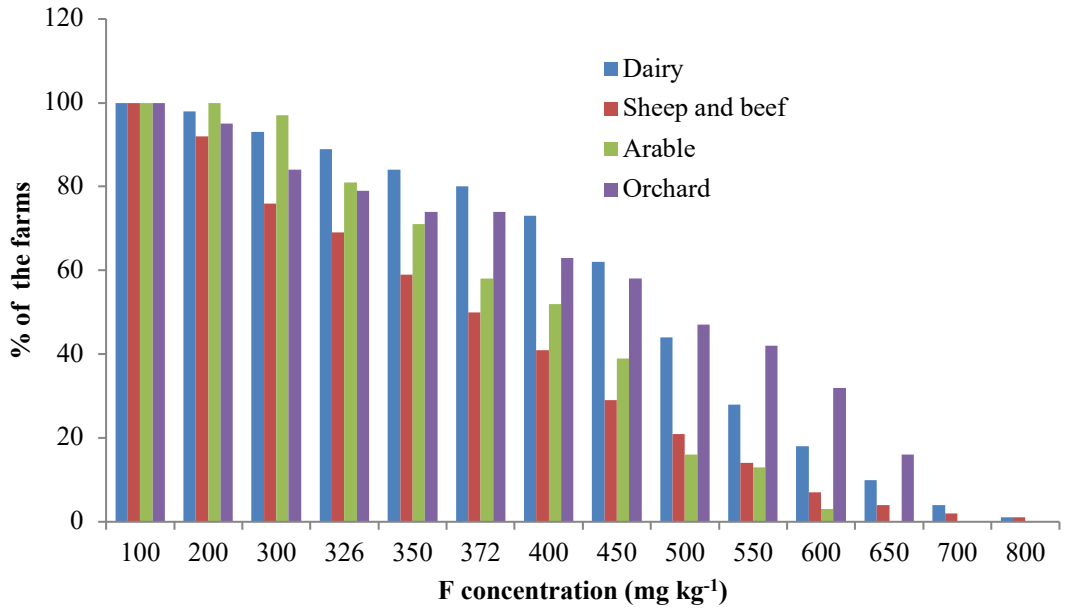


Figure 2. 1. Total F concentrations (mg F kg^{-1}) in varying farm lands (%) in New Zealand (Kim et al., 2016).

2.3 Chemical forms of F in soils

Total soil F can be divided into three different fractions; the pools of 1) Native F, 2) Immobile F, and 3) Labile F (Loganathan et al., 2007). Topaz [$Al_2(SiO_4)F_2$], apatite [$Ca_5(PO4)_3F$], fluorite (CaF_2), and cryolite (Na_3AlF_6) are all examples of naturally existing F-bearing soil minerals (Dehbandi et al., 2017), and constitute the native F pool. Fluorine weathered from soil minerals or derived from anthropogenic sources, and which is adsorbed by non-crystalline Al and Fe and organic matter particles in soil (Loganathan et al., 2006; Gray, 2018), is defined as the immobile F pool. According to literature, exchangeable and soluble soil F fractions are defined as the labile F pool (Álvarez et al., 2002). As shown in Figure 2.2, anthropogenic F has potential to directly reach the soil labile F pool.

Fluorine bearing minerals such as SiF_4 (hydrates) and cryolite (Na_3AlF_6) are water soluble in soil (Pickering, 1985) and thus add F^- from the native F pool to the labile F pool. The immobile F pool is dominated by F adsorbed onto non-crystalline Fe and Al through ligand exchange (Harrington et al., 2003), and organic matter through multivalent metallic cation bridges (Gago et al., 2012). At high soil pH (pH > 6.5), F bound with Al and Fe is exchanged by OH^- releasing F^- into the labile F pool (Larsen and Widdowson, 1971), whereas at low soil pH (pH < 5.5), Al–F complexes are released into the labile F pool (Loganathan et al., 2006). Fluorine adsorbed by organic matter is released into the labile F pool at higher soil pH as Al-F compounds are not stable at elevated soil pH (Gago et al., 2012). In addition, low molecular weight organic acids (oxalic acid, citric acid and malic acid) are secreted by plant roots and these have the ability to promote the transfer of F^- into the labile F pool from the immobile F pool by displacing adsorbed F^- from

adsorption sites, and by dissolving Al which is a key binding site for adsorbed F (Xu et al., 2006). Loganathan et al. (2008) reported that Al-F complexes are soluble and release F^- ions into the soil solution phase which indicates that Al-F complexes are essentially labile components which release F^- into soil solution. Available literature shows that F^- and Al-F complexes are released into the soil labile F pool from both native and immobile F pools. This F, which has potential to transfer to the labile pool, is defined as soluble and exchangeable F in the soil.

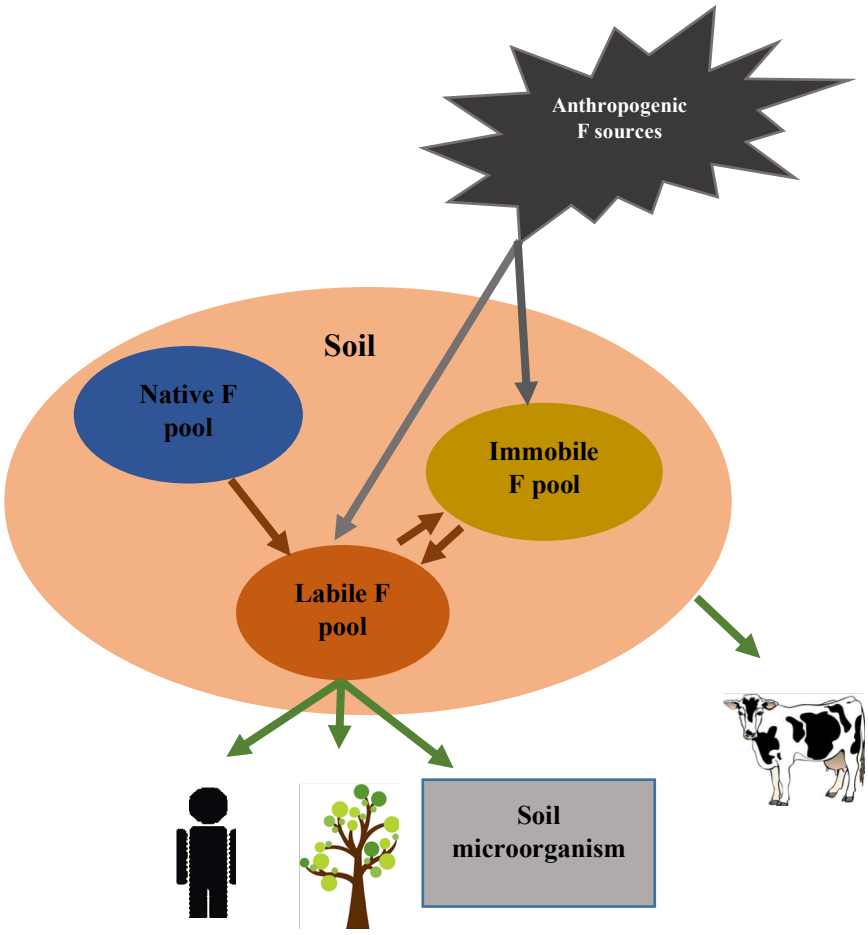


Figure 2. 2. Different forms of soil F and its link with living organisms

The concept of 'bioavailability' of soil elements has been defined by many authors (e.g. Stone and Marsalek, 1996; Chojnacka et al., 2005; Jeyakumar et al., 2010; Romić et al.,

2014; Varshney et al., 2016). Jeyakumar et al. (2010) reported that the bioavailability of soil elements can be defined as the potential soil fraction of an element that can be transferred from soil to living organisms. Romić et al. (2014) defined soil exchangeable and water-soluble copper as bioavailable copper fractions. Varshney et al. (2016) defined exchangeable and soluble forms of Pb, Mn, Al, Cu, Fe as bioavailable in their study. Chojnacka et al. (2005) and Stone and Marsalek (1996) defined the bioavailable fraction in terms of extractable concentrations.

Considering the literature reported in this section, this thesis defines labile F as bioavailable F. The term bioavailable F is used in preference to the term labile F throughout this work.

D'Alessandro et al. (2012) reported that the total soil F concentration is one to three orders of magnitude higher than the bioavailable soil F concentration. In soil solution, F can exist as either the free anion or as complexes with Al (Alvarez et al., 2002, 2005, 2011). Manoharan et al. (1996) found that in New Zealand pasture soils, the solution F concentration ranged from 0.05 to 0.45 mg L^{-1}. McLaughlin et al. (2001) reported that 95 - 98% of F in solution formed complexes with Al in Australian pastoral soil solutions at pH 4.7-4.8. The bioavailable soil F concentration is strongly associated with F toxicity in microorganisms, plants, and ground water F contamination, and with respect to environmental protection, the free F^- ion is the species of greatest concern.

2.4 Effects of soil F on living organisms

2.4.1 Human

Fluorine is an important element for humans and animals. At low concentration F is essential for dental enamel formation and bone development (Zhang et al., 2010). Fluorine enters the human body primarily via drinking water and food (Gago et al., 2014). Excessive F^- consumption can cause serious health problems such as skeletal malformation and dental fluorosis (Barbier et al., 2010). Shyam and Kalwania (2012) reported that drinking water F concentrations greater than 1.3 mg L^{-1} cause mottling of teeth and dental fluorosis, and that F concentrations above 3 mg L^{-1} may lead to skeletal fluorosis.

2.4.2 Plants

In plants, F uptake is via root and areal pathways. Gaseous form of F (HF) enters into the plants via leaf stomata and cuticles, causing toxicity (Louback et al., 2016).

The phytotoxicity effect caused by bioavailable fluoride (F) is considered a serious global environmental problem. Many authors have documented that plant growth and yield are significantly influenced by an elevated concentration of bioavailable F (Horner and Bell, 1995; Jha et al., 2009).

Bioavailable F enters plants through the root system and is concentrated in leaves by xylematic flow and can cause acute damage to a plant at high concentration (Klumpp et

al., 1998; Klumpp et al., 2000). Tip burn (margin necrosis), chlorosis, necrosis, leaf deformation, tissue desiccation, and changing colour symptoms are observed in monocotyledon and dicotyledon plant leaves due to excessive F uptake (Weinstein and Halscher-Herman, 1982).

Fluorine's effect on plants is dependent on plant species, the F concentration in the environment, and the duration of contact (Weinstein and Davison, 2004). Table 2.5 summarises studies that have been conducted to investigate the F effect on different plant species. Loganathan et al. (2001) measured the F concentration of mixed herbage (white clover and ryegrass) collected from New Zealand dairy farms which have a long-term P fertiliser application history and reported that the F concentration of mixed herbage was > 10 mg kg^{-1}. Analysis of this data suggests that mixed herbage in New Zealand dairy farms has average F concentrations which are lower than the dietary intake toxic threshold for cattle (40 - 50 mg F kg^{-1} DM) and ewes (60 mg F kg^{-1} DM).

2.4.3 Grazing animals

Fluorine accumulates in the topsoil (7.5 cm). At times of limited pasture growth or poor soil structure, for example during winter, an increased mass of soil is ingested by animals. It has been estimated that dairy cows each consume 908 mg F day^{-1} (52 mg F kg^{-1} DM), and sheep consume 120 mg F day^{-1} during winter (equivalent to consuming 275 g soil day^{-1}) (Lee, 1996). Animal welfare guidelines suggest that cattle which consume more than 40 mg F kg DM day^{-1} over months or years, are at risk of chronic fluorosis. Grace et al. (2005) documented that chronic fluorosis causes mottled teeth, hyperostotic chalky white bone lesions, anorexia, stiffness, and reduced milk production. Grace et al. (2003)

reported that a safe threshold daily dietary intake of F for breeding ewes and lambs is 60 mg kg^{-1} DM and 150 mg kg^{-1} DM, respectively.

2.4.4 Soil microfauna

Qiao et al. (2012) conducted a laboratory study to investigate the effect of NaF on nematodes (*Caenorhabditise legans*). Their study revealed that NaF increases oxidative stress and influences the number of nematodes in soil. Breimer and Vogel (1989) measured the total F concentration of earthworms collected from a soil in southern Germany affected by long-term industrial emission, and showed that total soil F concentration (10 – 670 mg F kg^{-1}) was significantly correlated with earthworm F concentration (5 – 130 mg F kg^{-1}). The study further revealed that accumulation of F in earthworms may lead to bioaccumulation of F in other living organisms which consume earthworms.

Table 2. 5. Studies that evaluated the effect of F on different plants.

Plant	Added F concentration range	Results	References
Onion (*Allium cepa*)	0–800 mg NaF kg^{-1} (0–361 mg F kg^{-1})	Tip burning and plant death were observed at > 400 mg NaF kg^{-1} (> 180 mg F kg^{-1}).	Jha et al. (2009)
Spinach (*Spinacea oleracea*)	0–800 mg NaF kg^{-1} (0–361 mg F kg^{-1})	Phyto-toxicity symptoms were not observed within the added F concentration range.	Jha et al. (2008)
Oats (*Avena sativa*)	0–128 mg F L^{-1}	Oats growth was not influenced by F within the F concentration range.	Stevens et al. (1998)
Tomatoes (*Lycopersicon esculentum*)	0–128 mg F L^{-1}	Dry weight was reduced at 62 mg F^- L^{-1}.	Stevens et al. (1998)
Willow tree (*Salix viminalis*)	0–400 mg F L^{-1}	Growth was not inhibited up to a F concentration of 100 mg L^{-1}.	Clausen et al. (2015)
Ryegrass (*Lolium multiflorium*)	0–800 mg NaF kg^{-1} (0–361 mg F kg^{-1})	Visible toxicity symptoms were not observed up to 200 mg F kg^{-1}.	Arnesen (1997)
White clover (*Trifolium repens*)	0–800 mg NaF kg^{-1} (0–361 mg F kg^{-1})	Visible toxicity symptoms observed at 200 mg F kg^{-1}.	Arnesen (1997)
Olive trees *(Olea europaea* L.)	20 and 1,770 mg F kg^{-1}	Toxicity symptoms observed at 100–1770 mg F kg^{-1}.	Zouri et al. (2014)
Alfalfa (*Medicago sativa* L.)	0.01 – 100 mg F L^{-1}	$N_2[C_2H_2]$ fixation, respiration rate, leaf Chl and net photosynthetic rate were not influenced by F up to 100 mg F L^{-1}.	Porter and Sheridan (1981)

2.4.5 Microorganisms

Soil microbial activity and its diversity are vital for sustainable agricultural food production and activity is adversely affected by organic and inorganic pollutants (Wakelin et al., 2013).

Marquis et al. (2003) reported that microbial cells are negatively affected by fluoride through different mechanisms; 1) F forms beryllium fluoride and alumino-fluoride compounds, both of which can act as phosphate analogues and therefore potentially influence the activity of phosphatase which is the phosphate translocating enzyme; 2) F^- or HF directly retards enzymes such as catalase, phosphatase, enolase and urease; and 3) HF can act as a transmembrane proton transporter which modifies the pH in cells. Marquis et al. (2003) reported that enolase activity is inhibited at a F^- concentration of 54 mg L^{-1}.

Enzymes, important to the Krebs cycle and the glycolytic pathway, are highly susceptible to F^- inhibition. In cells, the inhibition of Na+/K+ -ATPases (Adenosine triphosphate) by F^- reduces ATP production, leading to suppression of cellular respiration (Adamek et al., 2005). Laboratory studies have been conducted to quantify the effect of F on microorganisms under controlled conditions (Table 2.6).

Soil F concentrations elevated by anthropogenic activities therefore have potential to influence soil microbial activity. Tscherko and Kandeler (1997) conducted a study to determine the effect of environmental F accumulation on soil microorganisms. For this study soil was collected from close to an aluminium smelter at Ranshofen in Austria. Their study revealed that there was an 80% reduction of microbial biomass in the soil

when the water-extractable F concentration increased above 100 mg kg^{-1} soil. Fluorine accumulation inhibits microbial activity that decomposes organic material, resulting in an increase in organic matter. The resultant bulking of organic matter in soil changes humus dynamics, which then interrupts the soil carbon cycle which is likely to be detrimental to the soil ecosystem. In a case study example, Rao and Pal (1978) reported that near an aluminium factory at Renukoot, India, soil organic matter content increased with the accumulation of F in litter (> 73 mg F kg^{-1}) and soil (> 860 mg F kg^{-1}).

Agalakova and Gusev (2011) and Langer and Gunther (2001) reported that deposition of waste from the phosphate fertiliser industry to soil suppressed microbial biomass and microbial enzyme activities for a soil F concentration above 5300 mg F kg^{-1}.

García-Gil et al. (2013) conducted the field experiment to analyse the effect of F^- and heavy metal pollution on microbial enzyme activities, basal respiration and biomass C in soils close to an aluminium smelter in Slovakia. The water-extractable F concentration was high (96 mg kg^{-1} soil) for soil collected close to the aluminium smelter compared with a control soil collected from an area not impacted by industrial waste (8 mg kg^{-1}). In this study, contaminated soil had low microbial biomass carbon and dehydrogenase activity compared with the control.

An experiment was carried out by Ochoa-Herrera et al. (2009) to investigate the impact of F on microbial colonies used to remove nutrients and organic substances as part of a wastewater purifying process. This study demonstrated that anaerobic microorganisms are very susceptible to F^- (> 500 mg F L^{-1}).

Table 2. 6. Different studies conducted to investigate the effects of F on soil microbial activities.

Author	Objective	Methodology	Results
Chae et al. (2018)	to evaluate the F toxicity on soil microbial enzyme activity.	Different concentrations of NaF (0–905 mg F^- kg^{-1}) were added to soil.	After 20 days of F addition, for the added F concentration 905 mg F^- kg^{-1}, acid phosphatase activity reduced by 28% while urease activity increased by 73%.
Ropelewska et al. (2016)	to find the effect of NaF on soil thermokinetics of glucose decomposition and microbial activity.	Six NaF concentrations - 1105, 2210, 4420, 6631, 8841 and 11 051 mg kg^{-1} DM soil were used in the experiment.	Microbial biomass content and kinetics of glucose decomposition were reduced by the highest NaF doses (> 8841 mg kg^{-1}).
Szostek et al. (2015)	to find the effects of soil F on microbial activity.	Different concentrations of F (0, 100, 200, 300 mg F $kg^{-1}$of soil) were added tocharcoal and lime amended soil (loam).	Response of microorganisms to F varied between the microbial species. At added F concentration 300 mg kg^{-1}, alkaline and acid phosphatase activities were suppressed while urease activity was not constant.
Poulsen (2011)	to study the effect of F^- pollution on soil microorganisms in an Icelandic Brown Andosol.	Two F concentrations - 100 mg L^{-1} and 1000 mg L^{-1} - and 3 pH level (3,7,10) were arranged as a factorial experiment.	At F^- concentration 1000 mg L^{-1}, phosphatase activity decreased.

2.4.5.1 Al – F and Fe – F complexes and microbial activity

The fluoride ion is commonly bound with metal cations such as Al^{3+} and Fe^{3+} in the soil environment and forms metal complexes (Rodrigues et al., 2016). Studies show that these metal complexes are not toxic to microorganisms. The effect of F^- and aluminium on ferrous bio-oxidation by *Sulfobacillus thermosulfidooxidans* was investigated by Veloso et al. (2012), who revealed that at a F^- concentration of 10 mg L^{-1}, bacterial growth was completely inhibited by F^- in Norris growth medium. After addition of Al, at an Al:F molar ratio of 4 (2.0 mmol L^{-1} Al – 0.5 mmol L^{-1} F^-), bacterial growth was similar to the control (no F^- and Al^{3+}). Sicupira et al. (2011) assessed the effect of Al and F on *Sulfobacillus thermosulfidooxidan* growth in Norris medium. Their results revealed that at an Al:F molar ratio equal to or lower than 1, the bacteria count did not increase, while at an Al:F molar ratio of 4 (1:4), the bacteria cell count increased. Ahoranta et al. (2017) evaluated the effect of Al^{3+} on indigenous iron-oxidising microorganisms in trace element solution (TES) and in a mineral salt medium (MSM). Their study showed that Al^{3+} up to a concentration of 6 g L^{-1} increased microbial iron oxidising activity by overcoming F^- toxicity. Similarly, Rodrigues et al. (2016) reported that ferric iron produced by bacteria may overcome F^- toxicity. These studies suggest that Al^{3+} and Fe^{3+} can overcome F^- toxicity by forming Al-F and Fe-F complexes which cannot enter into microbial cells; these metal complexes are unable to cross microbial cell membranes (Brierley and Kuhn, 2010; Veloso et al., 2012).

2.5 Fate of F derived from phosphate fertiliser in soil

Fluorine added to soil through phosphate fertiliser application undergoes various reactions, depending on the chemical, physical and biological properties of the soil, as well as environmental factors (Loganathan et al., 2008). The fate of fertiliser-derived F is illustrated in Figure 2.3. When phosphate fertiliser is added to the soil, a fraction of the F content in the phosphate fertiliser precipitates as calcium fluoro-apatite, and the remainder of the F enters soil solution. Once there, a fraction of this F reacts with Al and Fe and forms Al-F and Fe-F complexes, with the remaining F existing as the F^- ion (F^-). Plants take up F as the F^- anion. Soil particles both adsorb F^- from solution and release F^- back into soil solution.

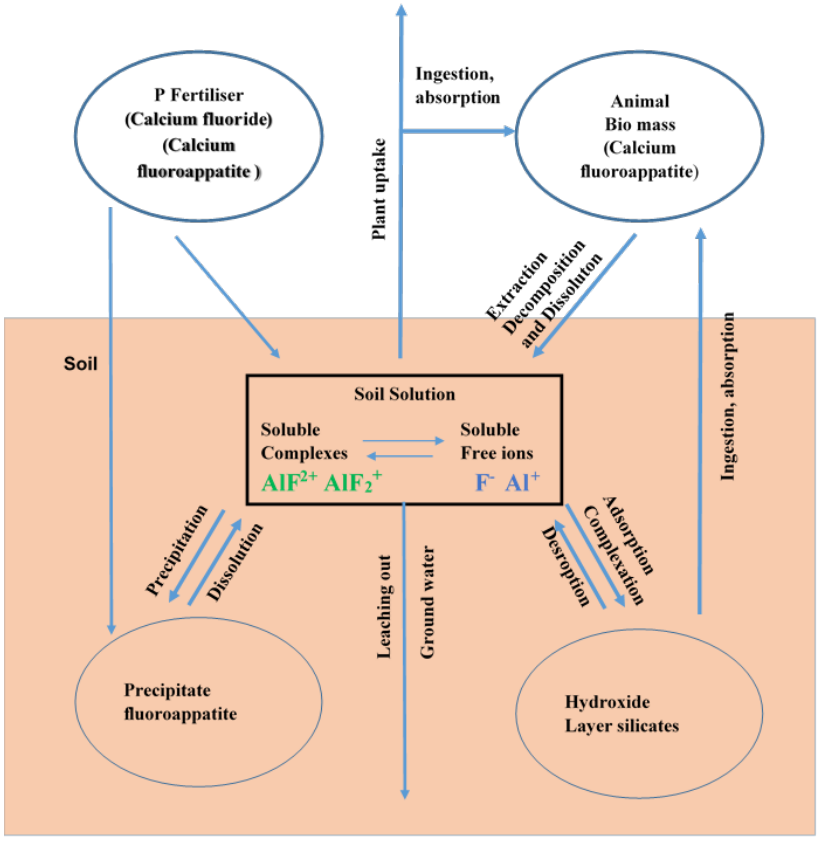


Figure 2. 3. Effects of physical, chemical and biological reactions on F dynamics in soil (Hedley et al., 2007)

2.6 Fluorine adsorption reactions in soil

The word ‘adsorption’ explains the process by which material is removed from a solution by surfaces (Barrow, 2008). In soil solution, the behaviour of contaminants and essential nutrients is highly dependent on their interactions with soil surfaces (Barrow, 2008). The fraction of total soil contaminant that is present in soil solution determines the amount of the contaminant that can be absorbed by plants through roots, or removed from the soil as leachate. These processes are important as they control the amount of F that can enter the food chain.

2.6.1 Fluorine adsorption mechanisms

Loganathan et al. (2013) reported five main F adsorption mechanisms; chemical modification of the adsorbent surface, hydrogen bonding (H-bonding) (inner-sphere surface complexation), van der Waals forces (outer-sphere surface complexation), ligand exchange (inner-sphere surface complexation) and ion exchange (outer-sphere surface complexation). These are described in this section.

Fluorine adsorption by van der Waals forces and ion exchange are not specific to F and are characterised by weak physical adsorption. In contrast, hydrogen bonding and ligand exchange mechanisms are strong adsorption processes that are unique to F and other strongly adsorbing anions. Chemical modification of the adsorbent surface can lead to both non-specific adsorption and specific adsorption.

Hydrogen or H bonding (Figure 2.4) occurs when a strongly electro-positive H atom bonds with highly electronegative F through a dipole–dipole attractive force (Loganathan et al., 2013).

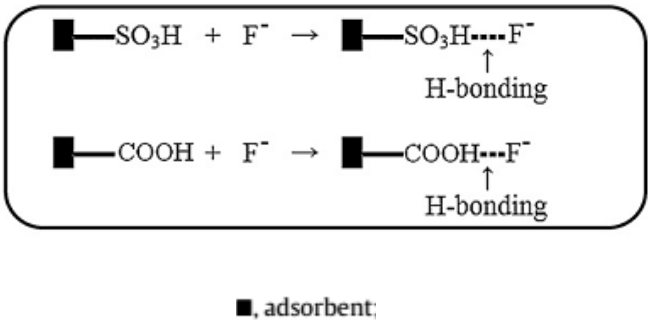


Figure 2. 4. H bonding F adsorption mechanism **(**Loganathan et al., 2013)

Van der Waals bonding (Figure 2.5) occurs when two atoms are bound by weak short-range forces which depend on the molecular weight of the adsorbates. Teng et al. (2009) reported that at high pH (pH > 6), manganese oxide-coated aluminium adsorbs F^- ions by van der Waals forces.

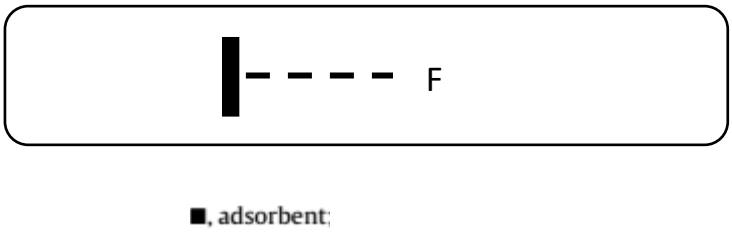


Figure 2. 5. Van der Waals bond F adsorption mechanism

During ligand exchange, strongly covalent chemical bonds are created between F^- and the metallic cation on the adsorbent surface (Figure 2.6). Loganathan et al. (2013) reported that inorganic adsorbents, which have high adsorption capacities, adsorb F mainly by ligand exchange mechanisms.

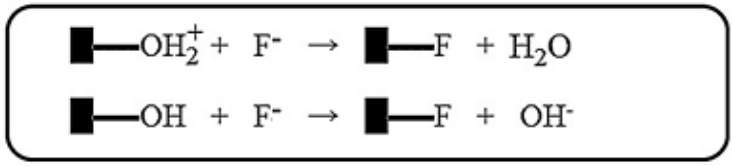


Figure 2. 6. Ligand exchange F adsorption mechanism **(**Loganathan et al., 2013)

During the process of ion exchange, the departure of an ion from an exchange surface requires the replacement by another ion of the same mole concentration and valency to maintain charge neutrality (Figure 2.7). This process is rapid and reversible. This mechanistic process can be applied to practical use. For example, ion exchange resins are used to remove F from solution by ion exchange mechanisms (Meenakshi et al., 2008).

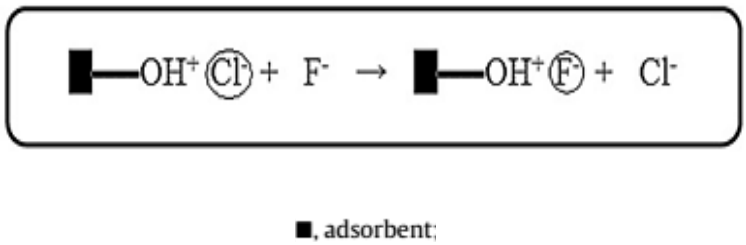
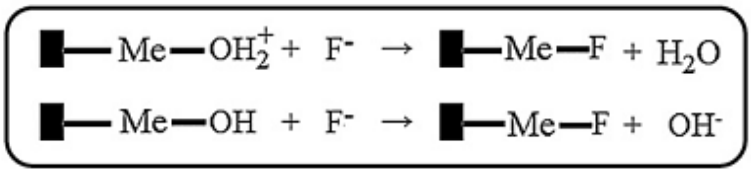


Figure 2. 7. Ion exchange F adsorption mechanism **(**Loganathan et al., 2013)

Chemical changes to an adsorbent surface can influence F adsorption. For example, F^- ions are repelled by adsorbents when a negative surface charge exists. However, when cations such as Al^{3+}, La^{4+}, Zr^{4+}, Fe^{3+}, and Ce^{3+} are adsorbed on the soil surface (Figure 2.8), they act as a bridge between F and the negatively charged adsorbent (Luo and Inoue, 2004; Samatya et al., 2007).



(■, adsorbent; Me, multivalent metallic cation)

Figure 2. 8. Surface chemical modification F adsorption mechanism **(**Loganathan et al., 2013)

2.7 Effect of soil properties on F bioavailability

Soluble ionic forms of an element can enter living cells and affect a living organism (Rensing and Maier, 2003). The bioavailability of F depends on soil type and the species of F which exists in the soil (Cronin et al., 2000). Fluorine solubility is controlled by the content of clay, amount of organic matter, pH, and quantity of Al oxides/hydroxides in the soil (Larsen and Widdowson, 1971; Omueti and Jones, 1977; Wenzel and Blum, 1992) (Table 2.7).

Table 2. 7. Effect of soil chemical and physical properties on F availability in soils.

Soil properties	F availability	References
pH	F availability is enhanced in soils with both low and high pH. With intermediate pH values, F availability is low.	Omueti and Jones (1977) Arnesen and Krogstad (1998) Farrah et al. (1987) D'Alessandro et al. (2012) Hedley et al. (2007)
Soil organic matter	Effect of organic matter on F availability depends on soil pH and the Al concentration of the soil.	Pickering (1985) Gago et al. (2014) Romar et al. (2009) Loganathan et al. (2006)
Soil texture	F availability is low in fine textured soil compared to soil with coarser textures.	Pickering (1985)
Al and Fe	Increasing Al and Fe content of soil reduces F bioavailability as F strongly binds with Al and Fe (ionic or particulate Fe and Al).	Harrington et al. (2003) Romar et al. (2009) Alvarez et al. (1992; 2002; 2005)
Cations in soils	An increase in Ca concentration reduces F availability.	Ruan et al. (2004) Zhang et al. (2010)

2.7.1 Soil pH

Soil pH has been identified as an important determinant of F solubility and its bioavailability in soil (Larsen and Widdowson, 1971). Gago et al. (2014) conducted a laboratory study to analyse F^- adsorption and desorption on soil collected at variable distance from an Al smelter located in Galicia Spain. Their results show that F adsorption to soil surfaces is influenced by soil pH (Figure 2.9).

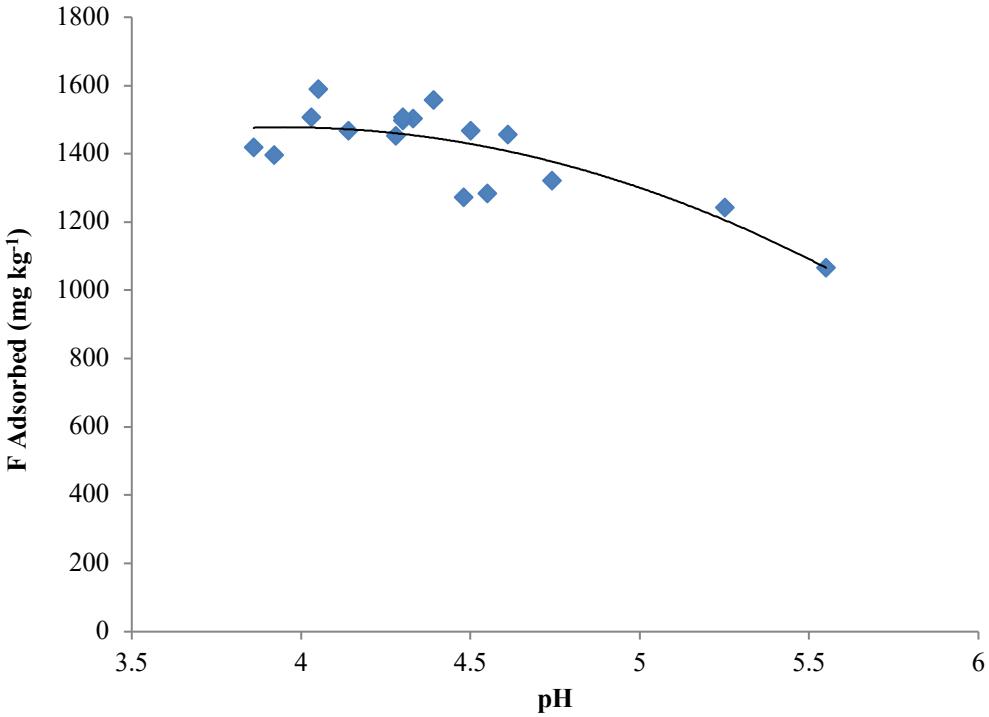


Figure 2. 9. Relationship between soil pH and F^- adsorption (Gago et al., 2014).

Omueti and Jones (1977) reported that F solubility in soil solution increases sharply at low and high pH but F solubility is low for pH between 5.5 and 6.5. At low pH (varies with soil type, Table 2.8), F solubility is the result of the release of small amounts of F^- ions into soil solution as Al-F complexes. The pH at which maximum F adsorption occurs varies among soils (Table 2.8).

Table 2. 8. Soil pH values for maximum F adsorption in various studies.

Soil and location	pH range tested	pH for F adsorption	Reference
Illinois soils (U.S.A)	4.4 – 7.7	5.5-6.5	Omueti and Jones (1977)
Luvisols and Regosols	4.01 – 7.87	6.0-6.5	Wenzel and Blum (1992)
Podzol and Arenosols	4.06 – 7.58	4.8-5.5	Arnesen and Krogstad
Volcanic soils (Italy)	5.30 – 6.52	5.3-6.0	D'Alessandro et al.
Umbrisols (Spain)	3.86 – 5.5	4.0-4.5	Gago et al. (2014)

F adsorption is influenced by pH because the surface charge of a number of soil components varies with pH. Hedley et al. (2007) reported that F retention onto soils decreases at higher pH (varies with soil type, Table 2.8) since soil particles at these pHs have a variable overall negative net charge which repels F^-. Farrah et al. (1987) reported that at a soil pH above 6.5, F^- adsorbed on soil surfaces is replaced by OH^- because higher concentrations of OH^- ions in solution at this pH are more likely to occupy exchange sites. Liu et al. (2014) reported that the F^- adsorption capacity of alkaline soils is 10 times lower than slightly acidic soils.

2.7.2 Soil organic matter

Soil organic matter content controls F availability in soils (Larsen and Widdowson, 1971; Omueti and Jones, 1977). Aluminium/Fe cations can act as a bridge between organic matter and F (Gago et al., 2014). Romar-Gasalla et al. (2018) reported that F can be adsorbed onto organic matter surfaces through protonated groups (NH_4^+), polyvalent cations (Al/Fe), and hydrogen bonds.

Gago et al. (2002) reported that the complex-forming capacity of organic matter with aluminium increases at higher pH due to the ionisation of acidic functional groups. Pickering (1985) reported that F adsorption by soils is influenced by the interactive effect of pH and organic matter. The author observed that complexation between organic matter and Al polymers increased with increasing organic matter content in soil. When the pH is low, Al polymers dissolve and release F^- into soil solution.

2.7.3 Soil clay minerals

Fluoride ions can be adsorbed from soil solution onto clay minerals (Wang et al., 2002) and Arnesen et al. (1995) reported that clay has a higher F^- adsorption capacity than soil organic matter. Yu et al. (2003) and Zhang et al. (2007) reported that, in clay minerals, OH^- is replaced by F^-, which increases the pH of the soil solution. Aluminium oxide, amorphous iron and aluminium, and free iron (Fe^{3+}) in soil solution, influence the electrochemical properties of the clay minerals which influences the adsorption capacity of clay minerals for F^- (Fung et al., 1999; Zhuang and Yu, 2002; Liu et al., 2014). Liu et al. (2014) reported the order of the various clay minerals' F adsorption capacity as: (vermiculite, goethite, and layered silicate minerals) > (various oxides, F containing $Al(OH)_3$, bentonite, and $Al(OH)_3$) > halloysite > (gibbsite, kaolinite) > soapalkaline soil.

2.7.4 Iron and aluminium

Fluorine is highly adsorbed by Al oxides and hydroxides. Bruce-Martin (1996) reported that, among the metal oxides hydroxides, F^- binds most strongly with Al oxides and hydroxides. In soils, F is highly adsorbed by low order amorphous Al and Fe oxides

(Omueti and Jones, 1977). Simard and Lafrance (1996) reported that F adsorption mainly occurs through exchange of -OH groups of the poorly ordered Fe and Al oxyhydroxides by F^-. Fluorine can also be adsorbed by crystalline Fe oxides, but its adsorption degree is low compared with Al oxides (Loganathan et al., 2006). Gago et al. (2014) conducted a study to find the F adsorption and desorption capacities of a soil located near an aluminium smelter in Galicia (NW Spain). The study revealed that F adsorption was positively correlated with non-crystalline Al ($r = 0.63$) and Fe ($r = 0.52$) content of the soil.

2.7. 5 Soil amendment

Soil amendments have potential to increase F adsorption to soil surfaces by altering soil properties (Quintáns-Fondo et al., 2016a). Many studies have shown the effect of soil amendments on F availability in soil (Table 2.9). The results of these studies suggest that soil amendments can be used to minimise F availability in soil by increasing F adsorption. Information on the effect of soil amendments on F^- availability will be useful to minimise the F effect on soil microorganisms.

Table 2. 9. Summary of studies that evaluate the effect of different amendments on F availability in soils.

Description	Findings	Source
Forest soil, vineyard soil, pyritic material and granitic material were amended with mussel shell at a rate of 48 t ha^{-1}. F^- adsorption and desorption in the amended soils was analysed by adding different concentrations of F^- (0 – 100 mg L^{-1}).	Mussel shell increased F^- adsorption up to > 90% in pyritic material while it did not influence desorption.	Quintáns-Fondo et al. (2016a)
Six soils were collected from close to an aluminium smelter in Norway and filled into 5 L pots. Soils were limed, and unlimed soils were considered as control. Common bent (*Agrostis capillaris*) was planted in each pot.	Fluorine availability in the soil was reduced by liming. Liming reduced Common bent F uptake by 10 mg F kg^{-1}.	Arnesen (1997)
Different rates of CaO (0, 1.05, 1.65, 2.15 and 2.70 g kg^{-1}) were mixed with air-dried soil and filled into pots. Tea seeds were planted.	At a lime application 2.70 g kg^{-1}, tea-leaf F concentration was reduced by 88.7%.	Ruan et al. (2004)
A pot experiment was conducted using soil with different rates of flue gas desulphurisation (FGD) gypsum (0, 1, 2, 5 and 10% (w/w)). Ryegrass was sown in each pot.	At a FGD gypsum application rate of 5%, the soluble F concentration reduced from 8.37 (control) to 5.31 mg kg^{-1}.	Álvarez et al. (2011)
Oak ash, pine bark, and fine shell were added to forest soil, vineyard soil and pyritic material individually at the rate of 48 t ha^{-1}.	Overall, Oak ash, pine bark, and fine shell increase F^- adsorption in the forest soil, vineyard soil and pyritic material. When Pine bark was added to forest soil, F^- adsorption ranged between 87.8 and 93.2%.	Romar-Gasalla et al. (2018)
Pot experiments were conducted where tea plants were cultivated in soil with different amendment rates (0.5, 1.0, 2.5, and 5.0% (w/w)) of bamboo charcoal.	The water-soluble F concentration of bamboo charcoal and charcoal amended soils was decreased from 2.87 to 2.04 mg kg^{-1} and from 2.87 to 2.21 mg kg^{-1}, respectively with an increasing application rate of amendments.	Gao et al. (2012)

2.8 Soil F guideline values

Various policies and regulations have been developed to manage soil quality. In New Zealand, a National Cadmium Management Strategy was released in February 2011 with an aim to minimise cadmium accumulation in agricultural soils. National Environmental Standards (NES) were introduced in 2012 to manage a range of soil contaminants to safeguard human health. Guideline values are currently being developed which are relevant to organic waste disposal (Cavanagh, 2015). Soil guideline values for contaminants are vital to protect soil organisms. They readily assess the potential environmental risk of soil contaminants. Soil F guideline values have been developed for several countries, and these are presented in Table 2.10. However, soil F guideline values have not yet been developed for New Zealand agricultural soils (Gray, 2018).

Table 2. 10. Soil F guideline values for different countries (modified from Mikkonen et al. (2018))

Country/Region	Guide value (mg kg^{-1})	Land use	Reference
Canada (Alberta)	200	Agricultural/Residential	Government of Alberta (2010)
Canada (Alberta)	2000	Commercial/Industrial	Government of Alberta (2010)
Switzerland	700	-	Wang et al. (2018)
Switzerland	20 (WE)	-	Wang et al. (2018)
Canada	200	Agricultural	CCME (2006)
Canada	400	Residential/Parkland	CCME (2006)
Australia	450	Industrial	EPA Victoria (2009)

WE :- Water extractable

2.9 Methods to analyse F in soil

2.9.1 Total soil F measurement methods

Total soil F concentration can be quantified using a number of analytical techniques including: ion-selective electrode (F-ISE), X-ray fluorescence (XRF), Ion chromatography (IC), neutron activation analysis (NAA), and particle induced gamma ray emission (PIGE) (Sucman and Bednar, 2012; Srivastava et al., 2014). The following sections discuss these methods in detail.

2.9.1.1 Fluoride-ion-selective electrode (F-ISE)

The fluoride ion selective electrode (F-ISE) technique to determine the F concentration in solution was developed by Frant and Ross in 1966 (Borjigin et al., 2009). The minimum F determination limit is 0.0057 mg kg^{-1} per sample (Van den Hoop et al., 1996). This is a destructive method as the sample undergoes a series of physicochemical treatments before analysis. This technique measures only the free F^- ions, not soluble F complexes. The determination of total F concentration using F-ISE becomes complicated when F^- in the sample is bound with ions such as aluminium and iron (Borjigin et al., 2010). The technique is also sensitive to hydroxide ion concentration. To overcome these issues, 'Total Ionic Strength Adjustment Buffer' (TISAB) is added with a sample during the measurement of F concentrations. The TISAB-solution maintains a constant ionic strength in solution to minimize the variation of ionic strength between a sample and standard. The TISAB solution contains citrate which acts as a decomplexing reagent to stabilise free F^-ions (Campbell, 1987).

Extraction of F from a sample is a critical step in using F-ISE to determine the F concentration in solution, and various techniques have been used to extract complexed as well as covalent- and ionic-bound F^- from soil media. Haldinmann and Zimmerli (1993) and Malde et al. (2001) reported that fusion, acid digestion, oxygen combustion and open ashing methods are the most commonly used techniques to decompose biological samples containing F.

The alkali fusion method is widely used to extract F from soil samples for quantification of total soil F. However, there are issue with this method. Campbell (1987) reported that F loss at temperatures above 550 °C is significant when fusion takes place while Yiping and Caiyun (2010) reported that fusion is a laborious and time-consuming method.

To overcome these issues, Jeyakumar and Anderson (2015) developed the 4 mol L^{-1} NaOH extraction method. In this method, a finely ground subsample (0.5 g) of oven dried soil is weighed into a polypropylene centrifuge tube and NaOH (10 mL of 4 mol L^{-1}) is added. The suspension is kept at 100 °C in a water bath for 24 hours with frequent agitation. Samples are then transferred into screw-top plastic containers. The sample pH is then adjusted to 8.5 by adding 6 mol L^{-1} HCl and made up to a final volume of 100 ml with deionised water before each suspension is filtered. This method is a low-cost analytical option as it requires lower amounts of acid, low concentration of NaOH and less time for the analysis. Furthermore, the chances of F losses are minimal in this method as fewer steps are involved compared to the Alkali fusion method. However, this methodology needs to be validated through multiple repeat assessments.

2.9.1.2 Instrumental Neutron activation analysis (INAA)

Instrumental Neutron Activation is used in many mining applications to quantify the concentration of trace elements in soil or rock. Havranek et al. (2004) reported that in INAA, the minimum F detection limit is 5 mg kg^{-1}. A major advantage of this method is that it is non-destructive and samples do not need to be subjected to physicochemical treatment before analysis (Zaim et al., 2016). INAA can measure multiple elements rapidly at the same time. However, the technique requires costly equipment. Furthermore, nuclear interference from sodium, aluminium, and oxygen means that INAA has limited sensitivity for F (Jankowski et al., 2007). INAA requires high sample homogeneity, as small quantities are used for the analysis.

2.9.1.3 Ion chromatography (IC)

Ion chromatography measures anions such as chloride, F^- and sulphate directly in solution. It is a destructive method (Yiping and Caiyun, 2010) and the minimum F^- detection limit is 0.2 μ mol L^{-1} (Van den Hoop et al., 1996). Interferences of calcium, aluminium and iron are the major limitations of ion chromatography in the measurement of total F concentrations. IC measures a specific fraction of the total F (free F^-) concentration (Van den Hoop et al., 1996).

2.91.4 Particle induced gamma ray emission (PIGE)

Particle induced gamma ray emission spectroscopy can be used to measure soil and food F concentrations (Havránek et al., 2004; Srivastava et al., 2014). The PIGE method is a

non-destructive and interference-free method (Havránek et al., 2004). Molla (2007) reported that the PIGE technique is suitable for F analysis as F has a low atomic number. However, the minimum detection limit of F in the PIGE technique is 22.13 mg kg^{-1} (Molla, 2007), which means that this method is not suitable to analyse the F concentration of samples with a low F concentration.

2.9.1.5 X-ray fluorescence (XRF)

X-ray fluorescence (XRF) is a non-destructive analytical technique to quantify elemental concentrations in soil samples (Marguí et al., 2005). Tarsoly et al. (2010) reported that determination of F by XRF is not common, as this element has a low atomic number (< 15) and therefore has low fluorescence. Only in recent years has this technique been able to accurately quantify the concentration of F in analytical samples using total reflection X-ray fluorescence (TXRF). However, the detection limit is not sufficient to measure the concentration of F in water samples (Tarsoly et al., 2010). Therefore, its application in F analyses is low compared with F-ISE and Ion chromatography. Jeyakumar and Anderson (2015) measured the soil F concentration of 12 soil samples by XRF and alkali fusion/ISE method at Massey University, New Zealand and found that the F concentration of seven soils determined by XRF were low compared with the F concentrations measured by alkali fusion/ISE method.

2.9.2 Soil bioavailable F measurement methods

Soil bioavailable F can be measured through extraction of a soil with water, calcium chloride or dilute acid. This bioavailable F can also be assessed through the extraction of F into a resin. These methods are described in detail here.

2.9.2.1 Water extraction

The water extraction method has been widely used to measure the bioavailable F concentration of soil (Table 2.11). Pickering (1985) reported that water can extract several soil F components including soluble fluoro-organic, water-exchangeable, and readily displaced F compounds. D'Alessandro et al. (2012) reported that the concentration of F in a plant does not correlate with total soil F concentration. Instead, these authors proposed that the water extractable F concentration is a more appropriate method to estimate potential plant uptake of F as the water extractable F concentration correlates with the plant F concentration. Loganathan et al. (2006) measured the water-soluble F concentration of 27 pasture sites and reported water soluble F concentrations ranging between 0.5 and 4.8 mg kg^{-1} (from 0.23 - 0.7% of total F). These values did not correlate with total soil F concentrations. Rodríguez et al. (2001) reported that water extractable F was a good parameter to measure F deposited to soil through atmospheric pollution. The water extractable F concentration of a soil is therefore an estimate of the bioavailable soil F available for plant uptake (Tscherko and Kandeler, 1997). In various studies, the water extraction method has been used to measure bioavailable F concentrations (Table 2.11). This method is widely used to measure the bioavailable F concentration of soils which are contaminated by point sources.

Table 2. 11. Examples of studies which have used the water extraction method to measure bioavailable F

Reference	Objective of the study	Results
Tscherko and Kandeler (1997)	to find the effect of F on soil microbial activities in soil surrounding an aluminium smelter	Bioavailable F concentration for soil microorganism was measured by water extraction. The correlations between microbial biomass ($r = 0.8$), dehydrogenase ($r = -0.86$) and arylsulphatase activity ($r = -0.84$) and water-extractable F concentrations were significant.
García-Gil et al. (2013)	to study the effect of F added from an aluminium smelter on the microbial activity of two soils	Bioavailable F concentration (for microorganisms) was evaluated using the water extractable F concentration. Microbial biomass and enzyme activities were low in soils which have higher water extractable F.
Arnesen (1997)	to investigate the uptake of F by *Trifolium repens* (white clover) and *Lolium multiflorium* (ryegrass)	Plant available F concentration (bioavailable F) was measured by water extraction and 0.01mol L^{-1} $CaCl_2$ extraction. A significant strong positive correlation ($r = 0.95–0.98$, $p < 0.001$) was recorded between water extractable F in soil and F accumulated in grass and clover.
Clausen et al. (2015)	to find the effect of F on willow tree (*Salix viminalis*)	Soil with a high water extractable F concentration significantly supressed transpiration ($p < 0.01$, one sided t-test).
Ruan et al. (2004)	to find the effect of pH and Ca on F uptake by tea plants (*Camellia sinensis* L)	The plant available F concentration (bioavailable F concentration) was measured using the water extraction method. The water extracted F concentration significantly correlated with tea shoot F concentration.
Ruan et al. (2003)	to investigate F uptake of tea plants (*Camellia sinensis* L)	The soil F fraction taken up by tea plants (bioavailable) was measured using the water extraction method. Correlation between the tea leaf F concentration and water extractable (water soluble) F was significant ($r^2 = 0.88$, $p < 0.001$).
Singh et al. (1979)	to study F uptake in wheat plants	In this study the water extractable F concentration was used to measure the bioavailable F concentration. A significant positive correlation ($r = 0.89$) was recorded between the F uptake by plants and the soil water-extractable F concentration

2.9.2.2 $CaCl_2$ extraction

Soil bioavailable F concentrations have been measured by 0.01 mol L^{-1} $CaCl_2$-extraction in several studies (McLaughlin et al., 2001; Jha et al., 2009). The 0.01 mol L^{-1} $CaCl_2$ solution extracts exchangeable F (F on colloidal surfaces) in the soil (McLaughlin et al., 2001). Keerthisinghe et al. (1991) reported that calcium-chloride extractable F has been used to estimate the concentration of plant-available F in soil. McLaughlin et al. (2001) conducted a study to measure the F distribution in permanent pasture soil profiles in Australia and reported that the 0.01 mol L^{-1} $CaCl_2$-extractable F concentration ranged from 0.5-4.5 mg kg^{-1} with no observable relationship between 0.01 mol L^{-1} $CaCl_2$-extractable F and total F. Arnesen (1997) reported that the 0.01 mol L^{-1} $CaCl_2$-extractable F concentration in soil near an Al smelter at Ardal, Norway, ranged from 4.88-81.4 mg kg^{-1}. In another study, the $CaCl_2$-extractable F concentrations of crop soils and forest soils collected close to an aluminium smelter in Galicia, Spain, ranged from 0.24 - 16.70 mg kg^{-1} and 0.52 - 39.77mg kg^{-1} respectively (Rodríguez et al., 2001). 0.01 mol L^{-1} $CaCl_2$ has the ability to displace exchangeable Al more effectively compared with water (Rodríguez et al., 2001). Generally, exchangeable Al is often bound with F in soil, and therefore, the 0.01 mol L-1 $CaCl_2$-extraction method is a suitable method to measure the bioavailability of F in soils which have high Al content.

2.9.2.3 KCl extraction

KCl solutions can also be used to measure the soil bioavailable F concentration (Loganathan et al., 2006). KCl extracts soluble fluoro-organic, water-exchangeable, and readily displaced F compounds from soil (Pickering, 1985). Loganathan et al. (2006)

conducted a study to identify the relationship between soil properties and different F extraction methods. Soils for this experiment were collected from both the North and South Islands of New Zealand which were highly contaminated by fertiliser–derived F. These authors reported that 0.01 mol L^{-1} KCl-extractable F concentrations were two orders of magnitude less than the total soil F concentration and ranged over 0.3–5.2 mg kg^{-1} soil. Recently, Wang et al. (2015) used 0.1 mol L^{-1} KCl to measure the bioavailable F concentration of several samples (coal, slag, filter ash and ash) from a coal-fired power plant and reported that bioavailable F concentrations ranged between 4.9-365.3 mg kg^{-1}.

2.9.2.4 HCl extraction

HCl has also been used to determine the extractable F concentration in soil (Arnesen, 1997; Breimer and Vogel, 1989). Pickering (1985) reported that dilute acid can extract organically combined and strongly adsorbed F from soil surfaces. Breimer and Vogel (1989) measured the extractable F concentrations of soils collected from industrial sites in Germany by using 1 mol L^{-1} HCl. The study reported that HCl-extractable F concentrations ranged from 65-276 mg kg^{-1}. Moreover, Eyde (1983) reported that the 1 mol L^{-1} HCl extraction method is a good predictor to determine the total F concentration in commercial fertiliser. However, Arnesen (1997) reported that 1 mol L^{-1} HCl extractable F concentrations were not correlated with the F concentrations of plants. The author concluded that using 1 mol L^{-1} HCl was not a good predictor of plant F because not all F compounds extracted by 1 mol L^{-1} HCl were absorbed by plants.

2.9.2.5 Resin extraction

This method was initially used to extract phosphorus (P) from soils (Saggar et al., 1990). The resin method can also be used to determine the bioavailable F concentration (Loganathan et al., 2006). When soil solution is shaken with an anion exchange resin, ions from the soil solution are adsorbed by the resin. The absorbed ions are then removed from the resin by shaking in suitable solutions (NaCl) (Sibbesen, 1977). Loganathan et al. (2006) measured the bioavailable F concentration of New Zealand pasture soil by the resin extraction method. In this experiment, cation (Na^{+}) and anion (HCO^{3-}) saturated ion-exchange resin strips were used and the adsorbed resin F was removed by shaking with 0.5 mol L^{-1} NaCl solution. Loganathan et al. (2006) reported that the resin-extractable F concentration of New Zealand pasture sites ranged from 0.7-55 mg kg^{-1}, this range being higher than the water extractable F of the same soil samples. Larsen and Widdowson (1971) measured the bioavailable F concentration of 100 agricultural soils from United Kingdom using 0.01 mol L^{-1} $CaCl_2$ extraction, and resin extraction methods. This study reported that $CaCl_2$ extractable F concentration and resin extractable F ranged from 0.02-1.30 and 4-24 mg kg^{-1}, respectively.

2.9.2.6 NH_4Cl extraction

The NH_4Cl extraction method to determine extractable F concentrations was developed by Rodríguez et al. (2001). However, this method was not used in further studies and there is limited detail of its use in literature. During the extraction of soil with NH_4Cl, F bound with Al is displaced in solution by NH_4Cl as NH_4^{+} has high exchange capacity (Rodríguez et al., 2001). The authors tested four different extraction methodologies: water,

1 mol L^{-1} NH_4Cl, 0.01 mol L^{-1} $CaCl_2$, and 0.02 mol L^{-1} NH_4Cl to determine the bioavailable F concentration in 47 cropped soil samples and 72 acid forest soil samples collected near aluminium smelters in Galicia, Spain. The study reported that 0.01 mol L^{-1} $CaCl_2$ extracted more F than water in the forest soil containing higher exchangeable aluminium, whereas in cropped soil, higher quantities of F were extracted by water due to the formation of CaF_2 precipitate which reduced the F concentration in solution, with the concentration of F extracted by NH_4Cl was lower than water in the cropped soil as exchangeable Ca was also replaced by ammonium this then led to the formation of a CaF_2 precipitate.

According to the available literature there is no standard method to measure the bioavailable F concentrations. In different studies, different extraction methods have been used. But, water and 0.01 mol L^{-1} $CaCl_2$ – extraction methods are widely used to measure the soil bioavailable F concentration. Soil F monitoring requires standard extraction method to measure the F toxicity for different soil organisms.

2.9.2.7 Relationship between extractable F concentration and solution F concentration

The extractable F concentration of soil is most commonly expressed on a dry weight soil basis (mg kg^{-1}) to eliminate the effect of soil moisture (Loganathan et al., 2006; Arnesen, 2007; Jha et al., 2008; Jha et al., 2009. Haidouti (1991) reported that rhizosphere soil solution F was 12% of the water extractable F concentration of soil collected from land surrounding an aluminium smelter in Greece. McLaughlin et al. (2001) measured the rhizosphere soil solution F concentration of fertilised pasture soil in Australia and found that soil solution F was 9% of the 0.01 mol L^{-1} $CaCl_2$ extractable F concentration. This

conversion factor to link the relationship between rhizosphere soil solution F and extractable F in soil was used by Loganathan et al. (2006). They conducted a comprehensive investigation of the rhizosphere soil solution F concentration in New Zealand pastoral soil (27 sites) and found that soil solution F concentration was less than 0.04 mg L^{-1}.

2.10 Summary and knowledge gap

Globally, the application of phosphate fertiliser continues to add large amounts of F to the soil relative to other anthropogenic sources. In New Zealand, the continuous application of F-containing phosphate fertiliser has significantly increased the total F concentration in New Zealand agriculture soils. Literature shows that F is toxic to human, plants, animals, micro fauna and microorganisms at high concentration. However, such studies are generally limited to areas of industrial soil contamination. Limited data is available on the consequences of phosphate fertiliser-derived F in agriculture soils.

The total soil F concentration can be measured by several methods, but these are complex, laborious and time consuming. Furthermore, literature shows that no standard method is available to measure the bioavailable F concentration in New Zealand agricultural soils. This lack of standardised method limits an assessment of the environmental impact of fertiliser-derived soil F.

Soil environmental studies report that organic matter decomposition and soil microbial activity are supressed by F addition. However, the effect of F on *R. leguminosarum* has not yet been recorded. *Rhizobium leguminosarum* is a nitrogen fixing bacterium which is

a fundamental component of New Zealand legume-based pastoral farming. Any impact of F on *R. leguminosarum* should be assessed.

In previous literatures, the effect of F on different plants in different countries has been reported. However, the effect of F on pastures grown in New Zealand soil conditions has not yet been reported (Gray, 2018). White clover contributes to the New Zealand economy in the form of nitrogen fixing and increased forage yield. Quantifying the effect of F on white clover and its N fixation is therefore necessary to ensure that the increasing F concentration of New Zealand soils does not threaten the value of this ecosystem service.

According to previous studies, soil solution F concentration is influenced by soil pH and soil organic matter content, and this suggests that lime and compost application may have potential to minimise the soil solution F concentration in New Zealand soils. However, the effect of lime and compost application on F^- adsorption in New Zealand agriculture soils has not been studied. Furthermore, the soil pH range for maximum F adsorption in New Zealand agricultural soils has not been revealed. These facts are vital to the development of comprehensive soil management guidelines.

Increasing soil F concentrations in New Zealand agricultural soils requires regulation, policy framework and soil F guideline values to measure and manage the risk of F accumulation in these soils. At present, policy framework, regulations and soil guideline values for soil F have not yet been developed. The development of soil F guideline values requires more data related to toxicity of F to soil organism and should proceed through standard experiments (Cavanagh and Munir, 2016). Ecotoxicological studies in literature

assess F toxicity by adding different concentrations F to soil. However, the relationship between added F concentration and the bioavailable F concentration, and their effect on soil properties, soil organisms, and white clover in New Zealand agricultural soils, have not yet been explored.

2.11 Research Objectives

Based on the knowledge gaps identified in the literature review, the following hypothesis and objectives for the current research have been designed.

The hypothesis of the study is that F added to soil through phosphate fertilisation has a detrimental impact on soil microbial activity, and specifically on nitrogen-fixing bacteria. This study was carried out with the following objectives:

1. To validate a 4 mol L^{-1} NaOH extraction method to assess total soil F by analysing soil samples representing a wide range of soil types, land uses, and locations, within New Zealand, and propose a standard method to measure the total and bioavailable F concentration in New Zealand pasture soils.
2. To evaluate the effect of F on white clover and *R. leguminosarum* (nitrogen-fixing bacteria), and to identify the F concentration at which N-fixation of white clover and the growth rates of *R. leguminosarum* begin to be impaired.
3. To determine the effect of lime and compost and varying rates of these amendments on soil properties and F availability to soil organisms.
4. To simulate the effect of long-term F accumulation (via phosphate fertiliser addition) on selected soil properties, soil microorganisms, and white clover, using a productive New Zealand soil.

CHAPTER 3

Defining a standard method to measure the total and bioavailable concentration of fluorine in New Zealand soils

---

This chapter has been published in *Microchemical Journal*. Citation:

Geretharan, T., Jeyakumar, P., Bretherton, M., Anderson, C.W.N., 2018. Defining a standard method to measure the total and bioavailable concentration of fluorine in New Zealand soils. *Microchemical Journal* 142, 94–101. doi:10.1016/j.microc.2018.06.018

3.1 Abstract

Ongoing soil F monitoring to underpin comprehensive soil F management practices requires an accurate and simple test to quantify both the total and bioavailable concentration of F in soil. In this study, soils were collected from various locations across New Zealand, representing different soil orders and land uses. The total soil F concentration was measured using an ion specific electrode following extraction with four different concentrations of NaOH (4 mol L^{-1}, 8 mol L^{-1}, 12 mol L^{-1}, and 16 mol L^{-1}), or fusion with NaOH (the conventional method used to analyse total soil F). We concluded that NaOH extraction gave an acceptable level of accuracy for organic-matter and volcanic parent material derived soils. Agreement was, however, less strong for recent and pallic soils. The extraction method was subsequently validated through repeat analysis of three further soils (n = 270).

To define a method for quantification of the bioavailable concentration of F in soil, samples were extracted with water, 1 mol L^{-1} HCl, 0.01 mol L^{-1} $CaCl_2$, 0.01 mol L^{-1} KCl, and 1 mol L^{-1} NH_4Cl. The correlation between 0.01 mol L^{-1} $CaCl_2$, 0.01 mol L^{-1} KCl, and water extracted F concentrations were significant ($p < 0.05$), and extracted the same soil F fractions. Results were normalised to the water-extractable concentration to compare recovery as a function of soil order. The recovery percentage of 0.01 mol L^{-1} $CaCl_2$ was high compared with water for soils which have high Al and Fe contents. The results of the study propose that 0.01 mol L^{-1} $CaCl_2$ extraction should be adopted as a standard method to assess the bioavailable F concentration of New Zealand pastoral soils.

Keywords

Fluorine; Fusion; Extraction; Bioavailability; Ion Selective Electrode; Soil

3.2 Introduction

3.2.1 Total soil F Analyses

The total F concentration in soil can be determined by a number of analytical methods. These include NaOH fusion followed by ion selective electrode (ISE); X-ray fluorescence (XRF); ion chromatography; instrumental neutron activation analysis (INAA); and atomic and molecular spectroscopy (Knight et al., 1977; Campbell, 1987; Saha and Kundu, 2003; Gao et al., 2012). The accuracy of these methods in quantifying the concentration of F in analytical preparations (soil extracts or solid-phase samples) is frequently compromised by interfering elements in the sample. Aluminium cations form very strong complexes with F^- ions under acidic conditions limiting sensitivity to ISE

(Thomas et al., 1977; D'Alessandro et al., 2008), and will also interfere with the analysis of F using XRF (An et al., 2012) and INAA (Knight et al., 1977). Environmental research in New Zealand related to soil F has been hampered by the lack of a standard, reliable, and simple test for total soil F, and this has limited large-scale screening of New Zealand soils to establish accurate baseline levels for soil F.

The standard method most commonly used for the analysis of total F in soil employs NaOH fusion followed by ISE. While ISE analysis is sensitive and rapid with a reported recovery rate greater than 90%, its trueness is greatly dependent on careful sample preparation (Campbell, 1987), particularly during the fusion of the soil sample with NaOH. This latter process is complex, time-consuming, and (consequently) expensive (Yiping and Caiyun, 2010). There is, therefore, considerable interest in the development of a simple and reliable alternative standard method for total soil F analysis that will help underpin more extensive environmental monitoring.

3.2.2 Bioavailable F Analyses

While analysis of the total F concentration of soil provides a useful basis for comparison of soils, this parameter generally shows poor correlation with the environmental risk that might be posed (Rodríguez et al., 2001). Instead, the term bioavailable is used to model the fraction of total soil F which is available to plants and microorganisms growing in the soil. Bioavailable F can be chemically defined as F that is water soluble or non-specifically adsorbed to exchange sites on soil surfaces (Loganathan et al., 2003). There is growing concern that increasing total soil F concentrations in New Zealand agricultural

systems may lead to an increase in the concentration of bioavailable F, and that this may negatively influence the function of soil microorganisms which underpin nutrient cycles.

Methods to estimate the bioavailable fraction of total soil F include water extractable, resin-extractable, low concentration acid-extractable (1 mol L^{-1} HCl) (Cronin et al., 2000), and $CaCl_2$-extractable (Arnesen, 1997) laboratory procedures. Of these, water extraction has been used to model the bioavailable F concentration in Al-contaminated industrial environments (Pomazkina et al., 2008; García-Gil et al., 2013). However, there is very little published literature regarding the bioavailable F concentration of soils subject to continuous applications of phosphate fertiliser.

The bioavailability of F in soil is dependent on a range of soil properties such as pH, clay mineralogy, organic matter content, and the presence of Fe and Al oxyhydroxides (Gago et al., 2014). Linking these properties with bioavailable F in soil will help to define critical toxic F concentration levels for soil microorganisms (Ropelewska et al., 2016), and also help guide management practices to minimise F bioavailability to microorganisms in New Zealand agricultural soils (Loganathan et al., 2006). However, according to literature, there are no standard methods to determine the bioavailable fraction of F in soil (Loganathan et al., 2006) and this limits the usefulness of bioavialable F as a measure of environmental risk in New Zealand pastoral soils.

The current study was designed to develop and test reliable and simple methodologies to measure both total soil F and the bioavailable concentration of F in soil to underpin the ongoing sustainability of New Zealand agricultural systems.

3.3 Material and Methods

3.3.1 Soil locations and sampling procedure

Soil samples were collected from operating farms which were selected to represent the dominant productive New Zealand soil orders (Table 3.1 and Appendix 1). Paddocks were randomly selected within each farm and samples were collected across the paddock along two linear transects to 150 mm depth using a stainless steel soil corer (2.5 cm diameter). Ten cores were collected from each transect and combined into a composite paddock sample. Soil cores were air-dried until constant weight, then passed through a 2 mm stainless steel sieve before storage at room temperature. Three of the locations (Canterbury, Pukekawa, and Kairanga) each had 30 pre-established research plots. For these locations, a composite core sample was collected from each plot to generate replicate samples for repeat analysis.

Table 3. 1. Location, land use, soil order (New Zealand and US soil classification scheme) of sampled soils in randomly selected paddocks.

Location	Land use	Soil Order		Analytical tests performed[#]
		US classification*	NZ classification	
Otorohanga	Dairy	Andosols	Allophanic	1,3,4
Reporoa	Sheep/Beef	Vitric andosols	Pumice	1,3,4
Newstead	Dairy	Andosols	Allophanic	1,3,4
Tokomaru	Dairy/Sheep	Luvisols	Pallic	1,3,4
Gordonton	Dairy	Histosols	Organic	1
Manawatu	Dairy	Fluvisols	Recent Sedimentary	1
Putaruru	Dairy	Andosols	Allophanic	1
Te Anau	Sheep/Beef/Deer	Cambisols	Brown	1
Tuapaka	Dairy/Beef	Luvisols	Pallic	1
Te Aroha	Dairy	Gleysols	Gley	1
Kairanga**	Horticulture	Gleysols	Gley	2,3,4
Pukekawa**	Horticulture	Ferralsols	Granular	2,4
Canterbury**	Horticulture	Luvisols	Pallic	2,4

*IUSS Working Group WRB, 2006; **pre-established research plots

1 – Method development for total soil F concentration using NaOH extractions (4 mol L^{-1}, 8 mol L^{-1}, 12 mol L^{-1} and 16 mol L^{-1})

2 – Method verification for total soil F concentration using NaOH extractions (4 mol L^{-1})

3 – Method development for bioavailable concentration of F in soil

4 – Analysis of relationship between total, proposed extractable F fraction (H_2O and 0.01 mol L^{-1} $CaCl_2$) and dominant soil properties

3.3.2 Total soil F analyses

3.3.2.1 NaOH extractions and ISE measurement of total soil F

Triplicate subsamples of finely ground (< 150 μm) soil (0.5 g) were accurately weighed into polypropylene centrifuge tubes. Sodium hydroxide solution (10 mL of 4 mol L^{-1}, 8 mol L^{-1}, 12 mol L^{-1} or 16 mol L^{-1} NaOH) was then added and the suspension was maintained at 100 ºC in a water bath for 24 hours with frequent end-over-end agitation. These suspensions were then quantitatively transferred to screw-top plastic containers (Jeyakumar and Anderson, 2016).

To minimise Al and Fe interferences in the ISE analysis, the pH of each suspension was adjusted to 8.5 by the slow addition of 3 to 6 mL of 6 mol L^{-1} HCl. Each sample suspension was then made up to 100 mL with deionised water, filtered through Whatman No.2 paper, and stored in a screw-top plastic container in a refrigerator for subsequent ISE analysis.

Prior to analysis of each filtrate, an aliquot was mixed with a total ionic strength adjusting buffer (TISAB IV) at a 1:1 ratio in order to maintain a constant ionic strength in solution and to stabilise free F^- ions (Campbell, 1987). The TISAB-filtrate sample was then analysed using Orion F^- Ion Selective Electrode (ThermoFisher Scientific, USA).

3.3.2.2 NaOH fusion and ISE measurement of total soil F

This is the conventional method employed to measure total F in soil samples (McQuaker and Gurney, 1977) and was used to compare the efficacy of the various NaOH extraction methods to quantify total soil F.

Triplicate subsamples of oven-dried (105 ºC overnight) soil (0.25 g) were accurately weighed into nickel crucibles. The samples were then moistened with a small amount of deionised water. A few drops of ethanol (70% v/v) were added to improve water absorption in those instances where the dried soil was hydrophobic. Six mL of 17 mol L^{-1} NaOH was then added to the moistened soil, mixed, and dried overnight in a 105 ºC oven to remove free water. The samples were then placed in a ventilated muffle furnace and the temperature was gradually increased to 600 °C, kept at that temperature for 1 h to fuse the NaOH with the soil sample, and then allowed to cool to room temperature. Approximately 10 mL of deionised water was then added to dissolve each fusion mass.

The resulting solution was quantitatively transferred to 100 mL-screw-top plastic containers. Subsequent F analysis followed the procedure for NaOH extraction and ISE measurement of total soil F. Briefly, the pH of each suspension was adjusted to 8.5 and then made to 100 mL with deionised water, filtered through Whatman No.2 paper. An aliquot was mixed with TISAB-buffer at a 1:1 ratio, and then analysed using ISE.

3.3.3. Bioavailable soil F

The purpose of a soil extractant is to mimic solubility processes that are occurring in the soil environment (Haney et al., 2006). These processes are generally chemistry dependent, and weak salt solutions are used to approximate chemical species that might be present in soil solution. In the current study, five extractants (Table 3.2) were compared for their ability to quantify bioavailable soil F concentrations. Rationale for selection of these methods is described in Appendix 6 under section A6.1.

3.3.4 Analysis of soil chemical properties

Soil pH was measured at 1: 2.5 (soil: water) ratio (m/v) using a Eutech Instruments CyberScan 310 pH meter.

Non-crystalline Fe and Al were extracted using the acid ammonium oxalate extraction method (Blakemore et al., 1987). The resulting elemental concentrations in solution were analysed using Microwave Plasma Atomic Emission Spectroscopy (4200 MP-AES, Agilent, USA).

Total N (%) and organic C (%) was determined using a Vario MACRO cube CHNS elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany). Soil Organic C was multiplied by two to convert to soil organic matter (SOM %) (Pribyl, 2010).

Table 3. 2**.** Extractants used to emulate soil solution.

	Extractant	Procedure	References
1	0.01 mol L^{-1} $CaCl_2$	5 g soil extracted with 37 mL 0.01 mol L^{-1} $CaCl_2$ in an end-over-end shaker for 16 h. The suspension was centrifuged at 7600 g for 30 min and filtered through a Schleicher and Shuell blue ribbon filter. The F concentration was determined with an F-ion selective electrode.	Arnesen (1997)
2	1 mol L^{-1} HCl	1 g soil extracted with 50 mL 1 mol L^{-1} HCl in an end-over-end shaker for 15 min. The suspension was centrifuged at 7600 g for 30 min and filtered through a Schleicher and Shuell blue ribbon filter. The F concentration was determined with an F-ion selective electrode.	Eyde (1983)
3	Water	5 g soil extracted with 30 mL deionised water for 2 hours in an end-over-end shaker. The suspension was centrifuged at 7600 g for 30 min, and filtered through a Schleicher and Shuell blue ribbon filter. The F concentration was determined with an F-ion selective electrode.	Loganathan et al. (2006)
4	0.01 mol L^{-1} KCl	5 g soil extracted with 30 mL 0.01 mol L^{-1} KCl, for 2 h in an end-over-end shaker. The suspension was centrifuged at 7600 g for 30 min, and filtered through a Schleicher and Shuell blue ribbon filter. The F concentration was determined with an F-ion selective electrode.	Loganathan et al. (2006)
5	1 mol L^{-1} NH_4Cl	16 g soil extracted with 50 mL 1 mol L^{-1} NH_4Cl for 16 h in an end-over-end shaker. The suspension was centrifuged and filtered through a Schleicher and Shuell blue ribbon filter. The F concentration was determined with an F-ion selective electrode.	Rodríguez et al. (2001)

3.3.5 Quality control

Analytical grade reagents were used in all analyses. All glassware were soaked in 0.01 mol L^{-1} HCl overnight and rinsed using deionised water between analyses. Quality control was performed using two internal soil standards (Soil A: S07052003A = 168 ± 29 µg g^{-1} F soil; Soil B: S07052003B = 542 ± 30 µg g^{-1} F soil) as standard reference materials for total soil F analysis (Loganathan et al., 2007). These had been analysed multiple times by the accepted conventional NaOH fusion and ISE method.

3.3.6 Statistical analysis

All data are presented as a mean ± standard deviation. ANOVA was performed using SAS@ 9.1 statistical software SAS 9.1.2 to determine significant differences between mean values, and to perform regression and correlation analyses to determine relationships between soil chemical properties and soil F content (both total soil F and bioavailable soil F).

3.4 Results and Discussion

3.4.1 Developing a method for total soil F analysis using NaOH extraction

A range of approaches can be used to validate the development of new analytical methodologies that will quantify soil components. Samples spiked with test elements, replicate analysis of a large data set, and the use of standard reference materials, are techniques that have been successfully used for method development by a large number

of authors (Mehlich, 1978; McGrath and Cunliffe, 1985; Yeomans and Bremner; 1988; Taylor, 2000; Ramos et al., 2005; Yiping and Caiyun, 2010; An et al., 2012; Boschetti et al., 2017). Chen and Ma (2001) reported that the precision and trueness of an analytical method is dependent on soil properties and the origin of the soil (natural deposits vs. anthropogenic).

In the present study, different soils were used to exhibit a wide range of soil properties, which containing variable amounts of soil F, in order to compare the total soil F analysis by NaOH extraction with the NaOH fusion method. Correlation of the total soil F concentration determined by 4 mol L^{-1}, 8 mol L^{-1}, 12 mol L^{-1}, and 16 mol L^{-1} NaOH extraction and fusion was significant for each NaOH extractant used, with correlation coefficients (r) of 0.95, 0.95, 0.96 and 0.95 respectively (Figure 3.1). These results provide a strong indication that both NaOH fusion and NaOH extraction removed the same form of F from the soil.

An et al. (2012) used relative error to compare the total soil F concentration determined by alkali fusion-ion selective electrode (ISE) and wavelength dispersive X-ray fluorescence (WD-XRF). Relative error was defined in this work as the difference between the F concentrations determined with ISE and WD-XRF divided by the F concentration determined with ISE (the accepted conventional method). In the current study, the measure of relative error was adopted and defined this parameter as the difference between the F concentration measured by NaOH extraction and the fusion method, divided by the F concentration measured by fusion. The relative error of the soils used in the current study can be grouped in those less than 0.2: Gordonton Organic (< 0.06), Otorohanga Allophanic (< 0.1), Putaruru Allophanic (< 0.14), Reporoa Pumice

(< 0.17), Newstead Allophanic (< 0.19) and Te Aroha Gley (< 0.12); and those greater than 0.2: Tokomaru Pallic (> 0.3), Tuapaka Pallic (>0.29), Te Anau Brown (> 0.31) and Manawatu Recent Sedimentary (> 0.55) (Figure 3.1).

Soils in each of these two groupings, differentiated by relative error, show similar properties. Soils which have low relative error contain high contents of Fe and Al hydroxides and/or organic matter, and therefore relatively high anion exchange capacities. The study proposes that the ISE methods (both fusion and extraction) are highly effective in quantifying the F concentration in soils that are high in Fe and Al, as the methodology has been designed to resolve issues with interferences (McQuaker and Gurney, 1977) from these elements. These soils represent New Zealand's most productive soil orders and have a history of regular and sometimes high rates of fertiliser application. Extraction with NaOH might remove only the F pool 'added' to the soil through superphosphate fertilisation. For these soils, this fraction of soil F is likely to be a high component of the total soil F. There were no significant differences in the total soil F concentration determined using NaOH extraction as a function of hydroxide concentration for any of the tested soils. Therefore, based on the study it is proposed that 4 mol L^{-1} NaOH extraction followed by ISE quantification of the total soil F concentration, is a reliable method to determine the total soil F concentration in volcanic and organic matter-rich soils.

In contrast, the grouping of Pallic, Brown, and Recent Sedimentary soils (Tokomaru Pallic, Te Anau Pallic, Te Anau Brown, and Manawatu Recent Sedimentary) had high relative error. These soils have lower organic matter and non-crystalline Fe and Al content (Gray et al., 1998; Gray et al., 1999). Fluorine is mainly adsorbed by non-crystalline

materials and organic matter (Gago et al., 2012), and the relative fraction of total F added to these soils from phosphate fertilisation is likely to be low. Therefore, the total soil F content of these soils is mainly affiliated with the geochemical soil matrix that may not be extracted by NaOH. Based on the study results, these soils are not suitable for NaOH extraction, and alkali fusion should be used to prepare them for total F analysis.

3.4.2 Validation of 4 mol L^{-1} NaOH method for total soil F analysis

In general, repeated validation against certified reference materials is used for new method development in soil elemental analysis (Frentiu et al., 2013; Srivastava et al., 2014; Rietig and Acker, 2017). For example, Boschetti et al. (2017) recently developed a total soil F analysis method and examined the trueness by reference to certified reference samples. They also tested the precision by analysis of 10 replicates of each sample, reported as relative standard deviation (RSD). In the present study, trueness of the 4 mol L^{-1} NaOH extraction method was measured by analysis of standard reference soils (Soil A (S07052003A) and Soil B (S07052003B), analysed multiple times by the standard Na fusion and ISE technique - Section 3.3.5) with a mean relative error of 0.09 and 0.11, respectively.

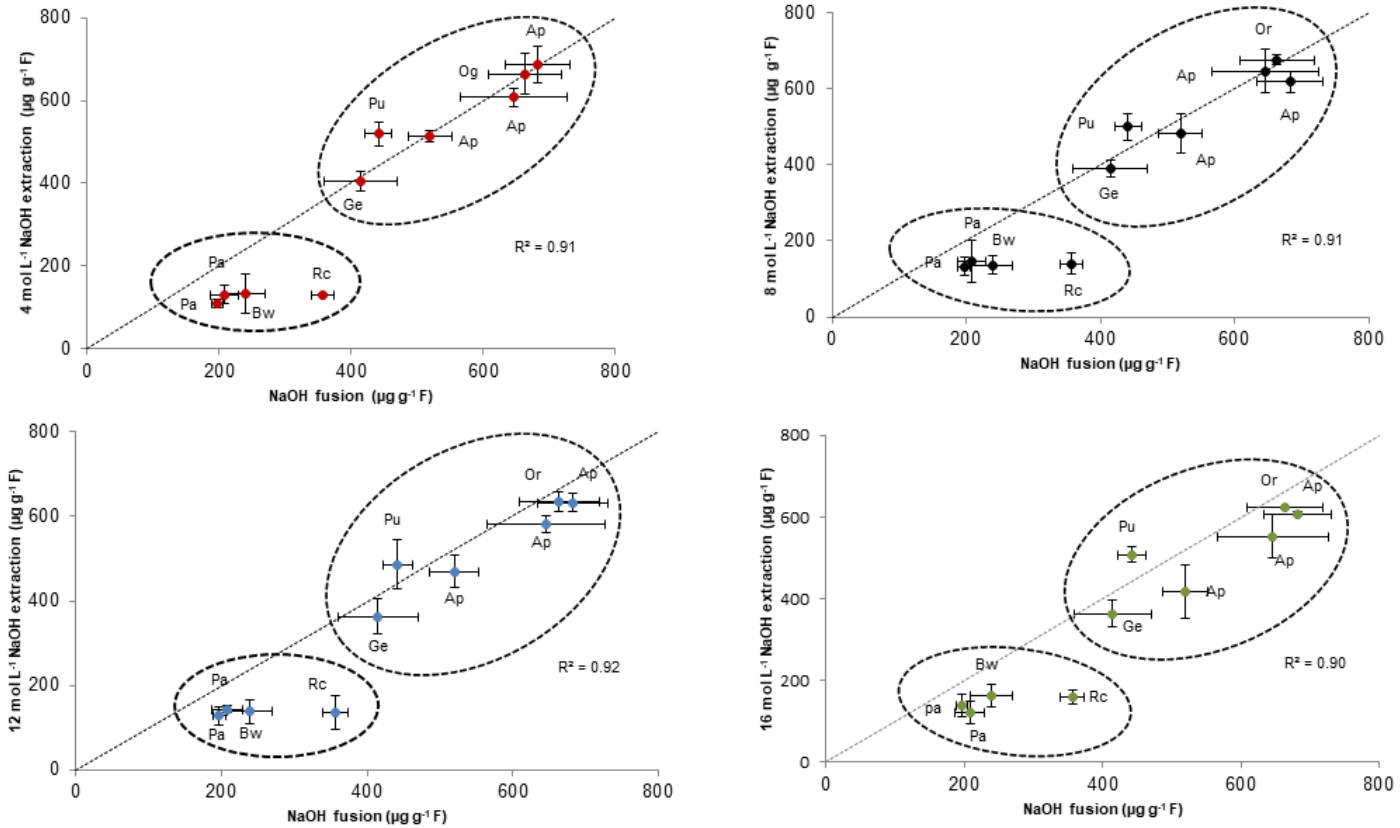


Figure 3. 1. Comparison of the total soil F concentration in twelve soil samples as determined by NaOH (4, 8, 12 and 16 mol L^{-1}) extraction and fusion. Ap – Allophanic, Pu – Pumice, Pa- Pallic, Or- Organic Rc- Recent Sedimentary Bw- Brown Ge- Gley.

The precision of the 4 mol L^{-1} NaOH extraction method for total soil F as an alternative to fusion was measured by replicate analysis of samples collected from three field stations that were used for soil Cd research in 2017 (Canterbury, Kairanga, Pukekawa). Triplicate sub-samples from each of 30 pre-established research plots were analysed using 4 mol L^{-1} NaOH and alkali fusion (270 samples in total). The RSD value of 4 mol L^{-1} NaOH extraction method for Canterbury, Kairanga, and Pukekawa soil was 8.8%, 7.6%, and 7.0%, respectively. The resulting low RSD values (< 9%) of the 4 mol L^{-1} NaOH extraction method indicate its high precision in measuring total soil F. The mean total soil F concentration determined using the 4 mol L^{-1} NaOH extraction method was 0.7% higher for Pukekawa soil, and 3.7% and 6.8% lower in the Kairanga and Canterbury soils respectively, compared to the total fusion method (Table 3.3).

For the volcanic Pukekawa soil, the difference in mean total soil F concentration determined by the two techniques was non-significant ($p > 0.05$) (Table 3.3). For the Canterbury Pallic soil, the difference was significant ($p < 0.0001$), and moderately significant for the Kairanga Gley soil ($p < 0.05$). These results confirm the results of the original method development tests (Section 3.4.1) and support the applicability of the 4 mol L^{-1} NaOH extraction technique as an analytical method to quantify the total F concentration in volcanic soils. Applicability of this test to Recent or Pallic soils is more limited. However, there is a strong indication that the technique could be very useful to determine the total F concentration in a range of organic-matter rich soils across New Zealand (in this case, Gley soils). Volcanic-derived soils generally report the greatest accumulation of soil F from superphosphate application, and such soils underpin much of New Zealand's most fertile land. These high-risk soils are those that are of regulatory concern regarding their increasing soil F concentration. Therefore, adoption of the 4 mol

L^{-1} NaOH extraction method would be a useful tool for both fertiliser management and environmental protection based on the ease of use, speed, and cost benefits associated with this method, relative to that of fusion.

Table 3. 3. Total soil F concentration of three horticultural soils analysed by fusion and 4 mol L^{-1} NaOH extraction methods (mean ± SD, n = 90).

Location	Soil order	Mean total soil F concentration (mg kg^{-1})	
		Fusion	4 mol L^{-1} NaOH
Canterbury	Pallic	257.4^{a} ± 19.9	241.0^{b} ± 21.4
Kairanga	Gley	167.4^{a} ± 18.3	161.3^{b} ± 12.4
Pukekawa	Granular	340.4^{a} ± 23.4	343.1^{a} ± 24.1

Means with the same letter in a row indicate differences are not significant from one another ($p < 0.05$)
SD = standard deviation

3.4.3 Comparison of different F extraction methods to determine bioavailable F

The bioavailability of elements is defined as a measure of the potential for an element to be transferred from a soil compartment to a living organism (Jeyakumar et al., 2010). Tscherko and Kandeler (1997) reported that the water-extractable F fraction defined the total amount of bioavailable F in soil, including water soluble F and that non-specifically adsorbed to exchange sites. Moreover, Pickering (1985) reported that soil F fractions such as soluble fluoro-organic, water-exchangeable, and readily displaced F compounds can be extracted by water. All these studies indicate that the water extractable F fraction mimics the bioavailable F component. In this study the water-extractable fraction was

considered as a baseline estimate of the soil bioavailable F fraction of the total soil F content.

Five methods reported in literature (Table 3.2) were used to model the bioavailable fraction of soil F. A subset (five soils) of the soils used to develop and verify the 4 mol L^{-1} NaOH extraction method for total F analysis was used for this experiment. The bioavailable soil F concentrations determined through extraction with 0.01 mol L^{-1} $CaCl_2$, 1 mol L^{-1} HCl, 0.01 mol L^{-1} KCl, 1 mol L^{-1} NH_4Cl, and water were significantly lower than the corresponding total soil F concentration (Table 3.4), and range from 0.06% to 3.17% of the total soil F. The findings of current study agree with the study of Loganathan et al. (2006) who reported that the water extractable F concentration and 0.01 mol L^{-1} KCl extractable F concentration of New Zealand pastoral soils were in the range of 0.01% to 0.7% of total soil F.

Table 3. 4. Bioavailable F concentrations (mg kg^{-1}) for five different soils extracted by different F extraction methods (mean ± SD).

Location	Soil Order	Bioavailable F concentration (mg kg^{-1})				
		0.01 mol L^{-1} $CaCl_2$	1 mol L^{-1} HCl	0.01 mol L^{-1} KCl	H_2O	1 mol L^{-1} NH_4Cl
Newstead	Allophanic	2.80 ± 0.2	2.57 ± 2.0	0.83 ± 0.1	1.79 ± 0.2	3.94 ± 0.3
Kairanga	Gley	3.12 ± 0.2	1.14 ± 0.4	0.87 ± 0.1	2.59 ± 0.3	1.56 ± 0.4
Otorohanga	Allophanic	2.49 ± 0.5	3.66 ± 2.4	0.60 ± 0.6	1.70 ± 0.1	2.48 ± 0.3
Reporoa	Pumice	2.65 ± 0.7	1.68 ± 1.2	1.27 ± 0.1	2.47 ± 0.2	2.50 ± 0.6
Tokomaru	Pallic	4.61 ± 0.5	5.18 ± 6.7	2.65 ± 0.1	6.45 ± 0.2	3.29 ± 0.5

SD = standard deviation

Variation in results generated by different extraction methods can be compared using correlation analysis. Wuenscher et al. (2015) used correlation analysis to compare 14 different soil phosphorus extraction methods, while Ivezić et al. (2013) used the same technique to compare concentrations of trace metal extraction from a range of soils. Correlation analysis was therefore used on the results from the extraction data in our study, with the correlation coefficients (r) for the five F extraction methods provided in Table 3.5. The correlation between the concentration of F extracted by water, and 0.01 mol L^{-1} $CaCl_2$ and 0.01 mol L^{-1} KCl was positive and significant ($p < 0.01$), whereas the F concentration extracted by 1 mol L^{-1} HCl and 1 mol L^{-1} NH_4Cl was not significantly ($p > 0.05$) correlated with that extracted by water. Rodríguez et al. (2001) reported that the use of different extraction methods will lead to differences in the fraction of soil F that is extracted. Pickering (1985) proposed that dilute acid extracts will desorb F that is strongly adsorbed to soil organic matter surfaces, while water will extract less strongly bound fractions of soil F. It is believed the poor correlation between the water-extractable concentration and 1 mol L^{-1} HCl and 1 mol L^{-1} NH_4Cl may be a function of these extractants desorbing different fractions of soil F due to the low pH and/or the relatively high salt concentration of these two extractants.

Table 3. 5. Pearson correlation coefficients (r) between the different soil F extraction methods.

	$F(CaCl_2)$	F(HCl)	F(KCl)	$F(H_2O)$
F(HCl)	0.651			
F(KCl)	0.929*	0.655		
$F(H_2O)$	0.977**	0.678	0.977**	
$F(NH_4Cl)$	0.229	0.522	0.299	0.195

*p < 0.05, **p < 0.01

The significantly different results for bioavailable F determined using 1 mol L^{-1} HCl and 1 mol L^{-1} NH_4Cl indicate that these extractants are not a good estimate for bioavailable F in New Zealand agriculture soils. Arnesen (1997) conducted a pot experiment and reported that water and 0.01 mol L^{-1} $CaCl_2$ extractable F concentration were highly correlated with the F concentration of ryegrass and white clover, but not with 1 mol L^{-1} HCl extractable F. Arnesan (1997) concluded that extraction with water or 0.01 mol L^{-1} $CaCl_2$ is a good indicator of the plant available F concentration in the soil whereas prediction of bioavailable F using 1 mol L^{-1} HCl is poor due to the extraction of F compounds which are not absorbed by plants.

To further quantify the relative potential of the $CaCl_2$ and KCl extraction methods to model bioavailable F, the extracted concentration of soil F was normalised to the water extractable F concentration and expressed as a percentage recovery of the soluble F concentration extracted using water (Equation 1).

$$Recovery\ \% = \left(\frac{Extractable\ F\ concentration - Water\ extractable\ F\ concentration}{Water\ extractable\ F\ concentration}\right) X\ 100 \quad \ldots (1)$$

The extractable F concentration quantified using 0.01 mol L^{-1} KCl was nearly 50% lower than that determined using water. The potential of 0.01 mol L^{-1} $CaCl_2$ to extract F was high (56.42%) when compared with water. These soils have a high Al content and the $CaCl_2$ results of the current study attribute to a reported ability of $CaCl_2$ to displace exchangeable Al from soil surfaces more effectively than water (Rodríguez et al., 2001). In general, F has an association with exchangeable Al in soil, and this F fraction is easily available to plant and microorganisms. Calcium chloride displaces exchangeable Al-F complexes which are bioavailable (Rodríguez et al., 2001). It is therefore, proposed that $CaCl_2$-extraction is a suitable method to measure the bioavailability of F in soils which have a high Al content, including the volcanic material-derived soils of New Zealand.

3.4.4 Effect of soil properties on soil F extractability

Fluorine solubility, availability and extractability are influenced by soil properties such as pH, clay content, organic matter, and the presence of Fe and Al hydroxides (Cronin et al., 2000). The effect of soil properties on the total soil F concentration determined by extraction with 4 mol L^{-1} NaOH was assessed for seven New Zealand agricultural soils (Table 3.6). The data were then further analysed to explore the relationship between soil properties and the bioavailable F concentration obtained using 0.01 mol L^{-1} $CaCl_2$ and water.

3.4.4.1 Variability in total soil F determined by 4 mol L^{-1} NaOH extraction

The total soil F concentration of seven soils, determined by 4 mol L^{-1} NaOH extraction, is presented in Table 3.6, and ranged between 153 and 1015 mg kg^{-1}. Soil from Otorohanga had the highest soil total F concentration and this was significantly different ($p < 0.05$) from the other locations. The total soil F concentration was not significantly different between the Reporoa and Newstead samples. The lowest total soil F concentrations were recorded for the Kairanga (153 mg kg^{-1}), Canterbury (205 mg kg^{-1}) and Tokomaru (203 mg kg^{-1}) soils which were not significantly different from each other ($p > 0.05$). Kim et al. (2016) measured the total soil F concentration of 264 New Zealand pastoral and horticultural soils using the NaOH fusion method and reported the range for F concentration to be 117-900 and 119-680 mg kg^{-1}, respectively. In another study, Loganathan et al. (2006) measured the total soil F concentration of 27 pasture sites by using the NaOH fusion method and reported that total F concentrations ranged between 212-617 mg kg^{-1}. In the current study, a similar range (153 - 1015 mg kg^{-1}) for total soil F concentration was obtained in both pastoral and horticultural soils using 4 mol L^{-1} NaOH. Furthermore, the total F concentration in soils collected from horticulture sites (Pukekawa, Kairanga and Canterbury) was lower than for pastoral sites. This is likely due to continuous annual ploughing which is a common practice for horticultural soils. The surface soil F concentration is diluted as ploughing mixes the top soil with subsurface soil (Kim et al., 2016).

Table 3. 6. Soil chemical characteristics of seven contrasting soils in New Zealand.

	Otorohanga	Reporoa	Newstead	Kairanga	Tokomaru	Pukekawa	Canterbury
pH	5.56^{b}	5.56^{b}	5.65^{b}	5.37^{c}	4.96^{e}	6.27^{a}	5.12^{d}
F ($CaCl_2$ /mg kg^{-1})	2.49^{cd}	2.65^{cd}	2.80^{c}	3.12^{c}	4.61^{b}	1.57^{d}	6.23^{a}
F (H_2O/mg kg^{-1})	1.70^{d}	2.47^{bc}	1.79^{cd}	2.59^{b}	6.45^{a}	1.91^{bcd}	2.00^{bcd}
F (Total /mg kg^{-1})	1014.76^{a}	673.15^{b}	754.89^{b}	153.00^{d}	203.28^{d}	342.33^{c}	204.67^{d}
Fe (%)	0.84^{a}	0.42^{c}	0.60^{b}	0.25^{d}	0.37^{c}	0.37^{c}	0.25^{d}
Al (%)	7.12^{a}	1.43^{c}	4.67^{b}	0.22^{e}	0.48^{de}	0.78^{d}	0.20^{e}
SOM (%)	20.12^{a}	13.80^{b}	12.62^{c}	4.69^{f}	7.28^{d}	5.36^{e}	4.58^{f}
N (%)	0.97^{a}	0.58^{c}	0.65^{b}	0.22^{f}	0.34^{d}	0.26^{e}	0.22^{f}
K (mg kg^{-1})	10.30^{a}	4.90^{c}	4.76^{c}	2.87^{d}	1.82^{e}	7.13^{b}	2.37^{de}
Ca (mg kg^{-1})	54.26^{a}	43.44^{b}	32.82^{cd}	21.23^{e}	37.27^{c}	30.17^{d}	20.04^{e}
Mg (mg kg^{-1})	5.33^{a}	2.28^{c}	2.22^{c}	1.94^{d}	4.64^{b}	1.83^{d}	0.68^{e}
Na (mg kg^{-1})	1.16^{a}	0.95^{ab}	0.73^{bc}	0.33^{d}	0.55^{ed}	0.35^{cd}	0.70^{bcd}
CEC	31.32^{a}	17.18^{b}	16.92^{b}	14.11^{bc}	12.20^{c}	14.61^{bc}	14.58^{bc}

Mean values with same letter among the soils are not significantly different. All mean values were calculated from 3 replicates ($p < 0.05$)

3.4.4.2 Effect of soil pH on soil F extractability

The pH of the seven soils ranged from 4.96 to 6.27 (Table 3.6). There was no clear relationship between soil pH and the total F concentration measured by 4 mol L^{-1} NaOH. This finding agrees with the study of Loganathan et al. (2006) who reported that total soil F measured by NaOH fusion was not correlated ($p > 0.05$) with soil pH. However, within this soil pH range, the relationship between soil pH and $CaCl_2$-extractable F was significant ($p < 0.05$) ($r = -0.83$) with the concentration of extractable F decreasing as a function of increasing pH (Figure. 3.2). The lowest pH was measured in the Tokomaru soil (pH = 4.96). This explains the high $CaCl_2$ extractable F concentration for the Tokomaru soil despite the low total F concentration. At lower soil pH, free F^- begins to bind with Al to form positively charged Al-F complexes (AlF^{2+}, AlF_2^+). These positively charged complexes are repelled by positively charged soil surfaces which begin to form at low soil pH (Gago et al., 2014). Wenzel and Blum (1992) studied F solubility in Austrian Luvisols and Regosols soils and reported that F solubility increased at both soil pH < 6.0 and > 6.5. In the present experiment, the reported soil pH range was not higher than the pH needed to desorb free F^- under alkaline soil condition (pH 6.5).

Both soil pH and $CaCl_2$ extractable F content were not significantly correlated ($p > 0.05$) with exchangeable calcium content. These results suggest that the calcium content of the soil does not influence soil F extracted by $CaCl_2$.

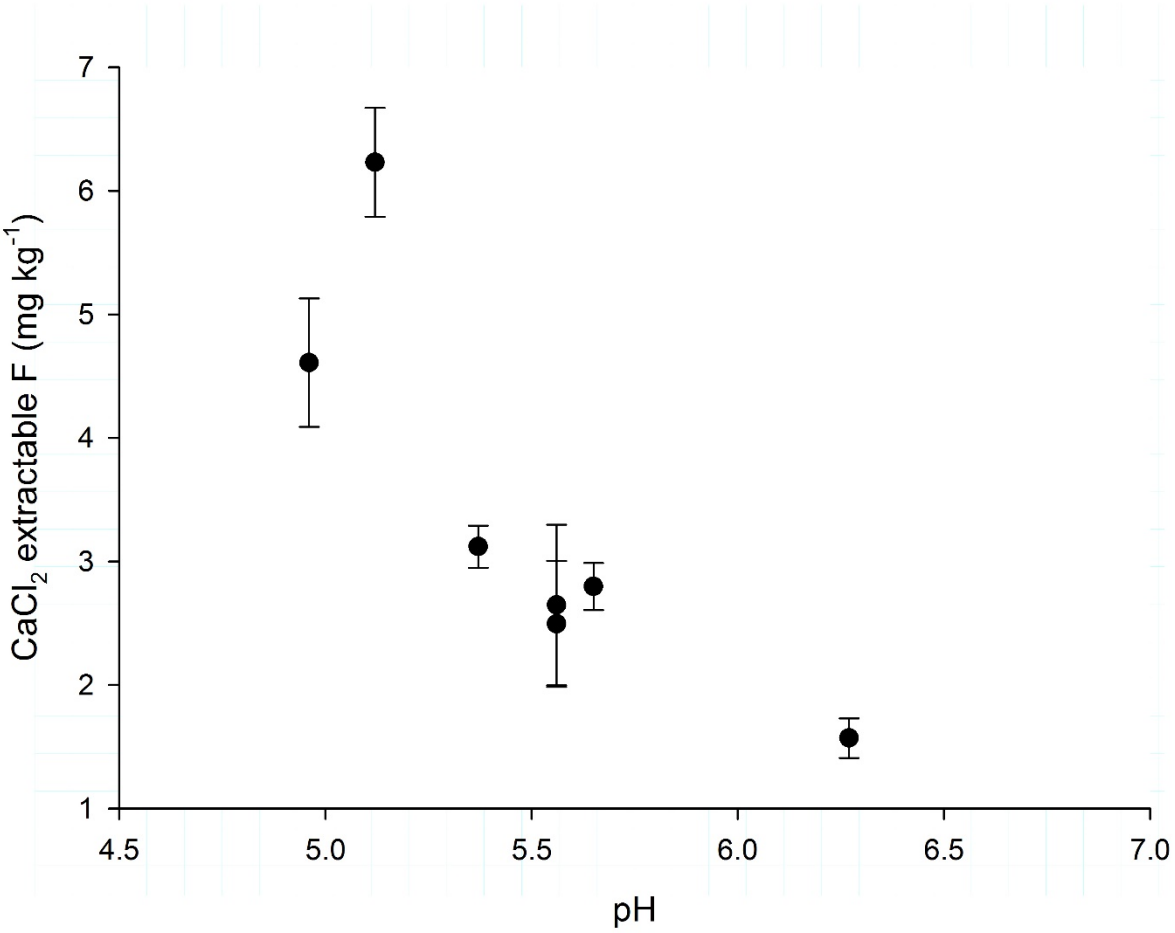


Figure 3. 2. Relationship between soil pH and $CaCl_2$ extractable F concentration in 7 New Zealand agriculture soils. Error bars indicate standard deviation (n =3).

3.4.4.3 Effect of soil extractable Fe and Al, and soil organic matter (SOM) on soil F extraction

The mean values for extractable Fe and Al concentration, CEC, and soil organic matter (SOM) for the seven soils are presented in Table 3.6. The soils generally showed low variation in Fe content (0.25–0.84%), in contrast with that of Al (0.20-7.12%). The SOM and CEC of the soils ranged from 4.58 to 20.12%, and from 12.20 to 31.32%, respectively. Total soil F concentration determined by extraction with 4 mol L^{-1} NaOH was significantly and positively ($p < 0.05$) correlated with acid ammonium oxalate

extractable Fe (r = 0.93) and Al (r = 0.92) (Figure. 3.3). Acid ammonium oxalate extracted Fe and Al represents the pool of non-crystalline forms of Fe and Al in soil. Gago et al. (2014) and Gago et al. (2012) reported that due to strong affinity between Al and F, F is adsorbed onto non-crystalline Al compounds. Kaufhold et al. (2010) reported that non-crystalline silicates such as allophane had a high F adsorption capacity compared with crystalline silicates. The strong affinity between non-crystalline Fe and Al with F, increases the total soil F concentration in soils that have received F from phosphate fertilisers.

The significant positive correlation between acid ammonium oxalate extractable Fe and Al, and total soil F measured by 4 mol L^{-1} NaOH, indicates that 4 mol L^{-1} NaOH extracts a significant amount of F from the pool of soil F adsorbed to non-crystalline Fe and Al. This analysis confirms the validity of 4 mol L^{-1} NaOH as an accurate measure of the total soil F concentration of New Zealand productive soils that have a high Al and Fe content. However, water-extractable F and $CaCl_2$-extractable F did not significantly correlate ($p > 0.05$) with the concentration of Fe and Al extracted by acid ammonium oxalate. This may be a function of the dependency of the formation of the Al-F complexes on both Al and Fe concentrations and soil pH.

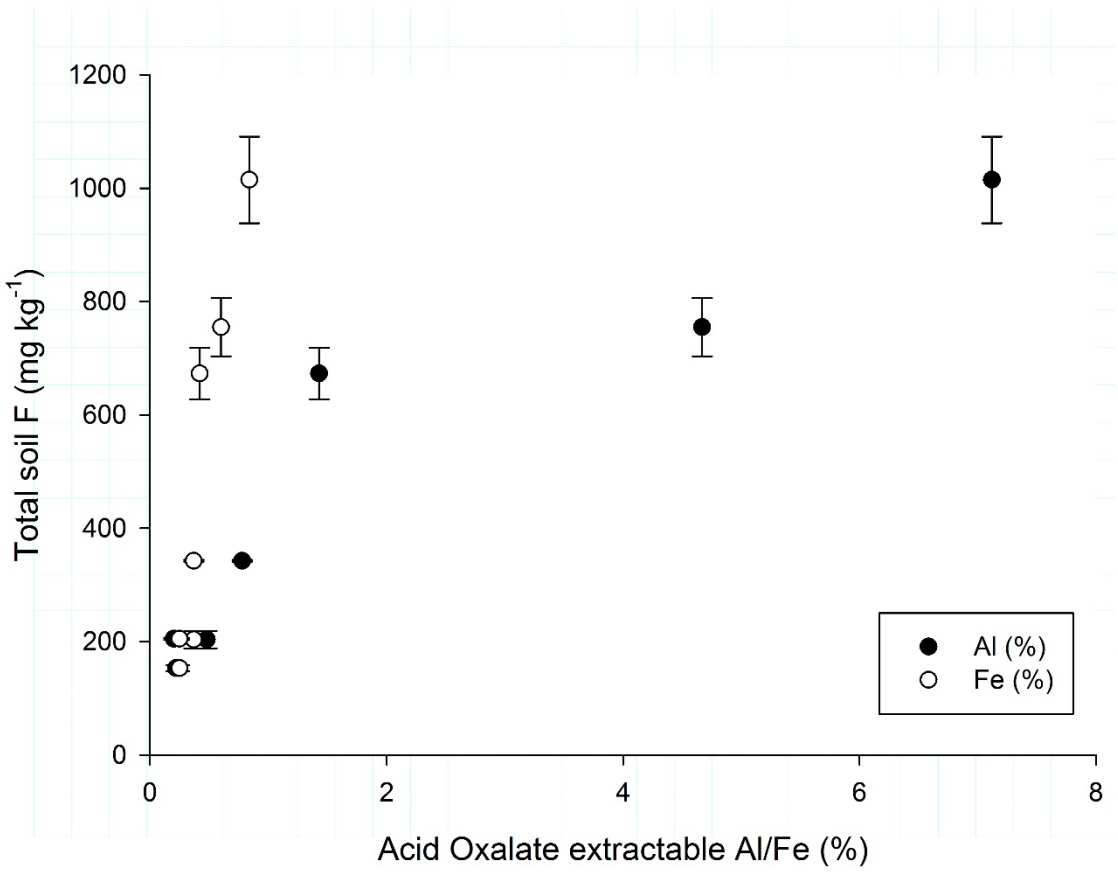


Figure 3. 3. Relationship between total soil F and acid ammonium oxalate extractable Al and Fe across the soils (n = 3). Error bars indicate standard deviation (n = 3)

A linear positive and significant relationship ($p < 0.05$; $r = 0.96$) was observed between SOM (%) and the total F concentration of the seven soils (Figure 3.4). However, neither water-extractable F nor $CaCl_2$-extractable F correlated with SOM. Quintáns-Fondo et al. (2016b) reported that anions are absorbed by organic matter through two different mechanisms. Multivalent cations (such as Al) bind to negative charges on organic matter surfaces and act as cationic bridges between F^- and organic matter, and F^- can also bind with organic matter through H bonds (–COOH functional groups form H bonds with F). The results of the current study confirm that 4 mol L^{-1} NaOH extraction has the ability to

extract F from the organic pool, and that extraction is appropriate for New Zealand productive soils which have a high organic matter content.

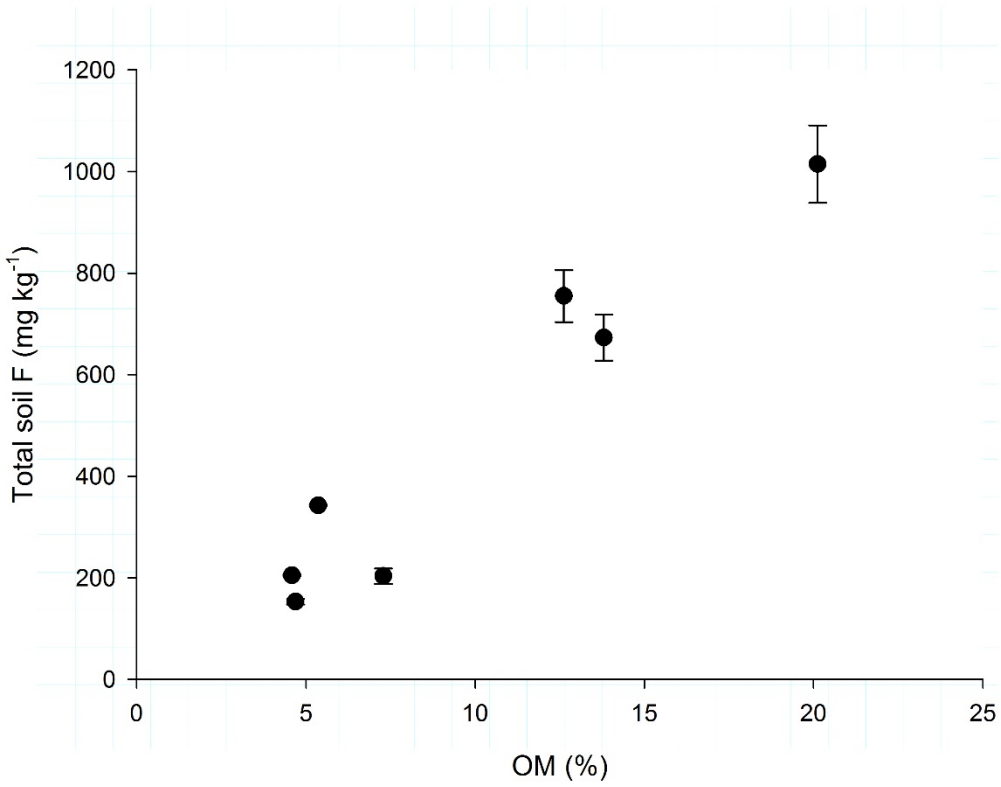


Figure 3. 4. Relationship between organic matter and total F concentrations across the soils. Error bars indicate standard deviation (n = 3)

3.5 Conclusions

Development of an accurate and cost-effective method for soil F analysis is vital for the ongoing management of an increasing F burden to New Zealand soils. In this study, a comprehensive suite of soil samples across different soil types indicated that the total soil F concentration determined using a 4 mol L^{-1} NaOH extraction method agreed with the concentration determined by alkali fusion. Precision of the 4 mol L^{-1} NaOH extraction method was presented as RSD which was less than 9% with the limit of detection (LOD)

of the electrode measured as 0.02 mg L^{-1} F. Relatively speaking, this alternative method is a lower cost analytical option as it requires lower amounts of acid, a lower concentration of NaOH, and less time for the analysis. Furthermore, the chances of F losses are less likely in this method as it requires fewer steps compared to the alkali fusion method. However, accuracy of the alternative method is dependent on specific soil characteristics. Extraction and alkali fusion/ISE showed the highest similarity for high-F Allophanic soils. These are soils where increasing F concentration is of greatest concern in NZ, and are therefore the soils where total soil F analysis using an acceptable low-cost alternative method is in greatest demand. Although the NaOH extraction method offers several advantages compared with the fusion method for total soil F analysis, it is not recommended for soils whose F content is dominated by geogenic sources.

The $CaCl_2$ extraction method demonstrated reliability in terms of evaluating the bioavailable F concentration for soils with high levels of Al, while the water extraction method can be recommended for all other soils. Adoption of environmental monitoring using the new techniques developed in this study will allow the New Zealand agricultural and horticultural sector to increase their environmental F monitoring capability and potentially broaden opportunities for the mitigation of F in New Zealand agricultural soils.

Acknowledgement

This paper is part of the PhD research project 'Determination of total soil fluorine by various standard methods of analysis' commissioned by the Fertiliser Association of New Zealand (FANZ). The authors thank the FANZ for funding this study.

CHAPTER 4

Fluorine and white clover: Assessing F's impact on *Rhizobium leguminosarum* morphology and respiration

---

Part of the results of this chapter was presented in the 2018 Fertilizer and Lime Research Centre Conference and NZSSS Conference. Citation:

Geretharan, T., Jeyakumar, P., Bretherton, M., Anderson, C. W. N. 2018. Effect of Fluorine on *Rhizobia*: A respiration-inhibition assay. In: *Farm environmental planning – Science, policy and practice*. (Eds L. D. Currie and C. L. Christensen). http://flrc.massey.ac.nz/publications.html. Occasional Report No. 31. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand. 4 pages.

Geretharan, T., Jeyakumar, P., Bretherton, M., Anderson, C. W. N. 2018. Effect of Fluorine (F) on *Rhizobia* growth and morphology NZSSS conference held in Napier. 3-6 December 2018.

4.1 Abstract

Rhizobium leguminosarum is a nitrogen-fixing soil bacterium that is a fundamental component in New Zealand legume-based pastoral farming. Any impact of soil F on *R. leguminosarum* will have an adverse effect on New Zealand pasture production. In this study, F toxicity to *R. leguminosarum* was examined as a first step to help develop F guideline values for New Zealand agricultural soils. Pottle-based experiments were conducted to examine the effect of the F^- ion on *Rhizobium*-white clover symbiosis by observing nodule morphology and growth. Results indicate that the IC_{10} (F^- concentration that causes 10% inhibition of *R. leguminosarum* respiration) values for F^- toxicity to *R. leguminosarum* were higher than 100 mg F^- L^{-1}. Significant morphological changes occurred when *R. leguminosarum* was exposed to F^- concentrations of 500 mg L^{-1} and 1000 mg L^{-1}. Both Light and TEM micrographs confirmed that *R. leguminosarum*-white clover

interaction was not influenced by F^- concentrations under 100 mg L^{-1}. The toxic F^- concentration for *R. leguminosarum* determined in this study is orders of magnitude higher than the F^- concentration in New Zealand agriculture soils under 'normal conditions'. There appears to be no indication of imminent risk of soil F to *R. leguminosarum.*

Keywords: Circularity, Fluoride, MicroResp, *Rhizobium leguminosarum,* White clover

4.2 Introduction

Rhizobium leguminosarum is a gram-negative soil bacterium which has a symbiotic relationship with legume plants and converts atmospheric nitrogen into plant available nitrogen to maintain soil fertility. Annually, legume-*Rhizobium* symbiosis provides 40 million tons of nitrogen to world food production (Herridge et al., 2008). Legume-based mixed pasture (clover and ryegrass) production systems are a key part of New Zealand primary production (Basset-Mens et al., 2009). Delestre et al. (2015) reported that legume pasture, predominantly white clover, is cultivated in more than 11,400 farms covering 7.88 million hectares in New Zealand. *R. leguminosarum* is therefore an important component of legume-based pasture farming. A range of studies have shown that *R. leguminosarum* growth, morphology, and interaction with legumes for N fixation are affected by many soil contaminants (Chaudri et al., 2000; Chen et al., 2003; Huang et al., 2014; Abd-Alla et al., 2016). Increasing F concentrations in New Zealand agricultural soils resulting from phosphate fertiliser applications may potentially threaten pasture production.

In soil solution, F can exist as the F^- ion and metal complexes (Al-F, Fe-F) (Elrashidi and Lindsay, 1986; Stevens et al., 1998). Al-F complex formation is highly dependent on pH and the F^- to Al ratio (Liu et al., 2013). A number of different studies have shown that Al-F and Fe-F complexes are not toxic to microorganisms, as these species are unable to cross the cellular membranes of microorganisms, since the membrane pore size is smaller than the size of these metal complexes (Brierley and Kuhn, 2010; Sicupira et al., 2011; Veloso et al., 2012; Ahoranta et al., 2017; Ma et al., 2017). Therefore, with respect to soil microorganisms, F^- is the potential toxic component.

In microorganisms, F^- alters cellular enzyme functions, thus reducing respiration and growth (Marquis et al., 2003; Barbier et al., 2010; Yasuda et al., 2017). Ma et al. (2014) reported that growth of *Escherichia coli* grown in Luria Bertani broth is reduced at an F^- concentration above 190 mg L^{-1}. In a bioleaching experiment, growth of the moderate thermophilic bacteria *Sulfobacillus thermosulfidooxidan* in Norris medium was shown to be inhibited at a F^- concentration of 270 mg L^{-1} (Sicupira et al., 2011). In another study, growth of the iron oxidizing bacteria *Acidithiobacillus ferrooxidans* in 9 potassium basal media was inhibited at F^- concentrations above 140 mg L^{-1} (Ma et al., 2017). With respect to soil, an F toxic effect on microorganisms has not previously been reported in terms of F^- concentration. However, Tscherko and Kandeler (1997) reported toxicity in terms of water-extractable F concentrations, and reported that F accumulation from an Austrian aluminium smelter inhibited arylsulphatase and dehydrogenase enzyme activities when water-extractable F concentrations increased above 20 and 100 mg kg^{-1} soil, respectively.

Bioavailable F in New Zealand pastoral soils, reported in the literature, ranges from 1.70 to 6.45 mg kg^{-1} (< 0.58 mg F^- L^{-1} soil solution); two orders of magnitude lower than that

of total F (Loganathan et al., 2006; Geretharan et al., 2018). However, there is no data available to determine if current New Zealand soil bioavailable F concentrations are harmful to *R. leguminosarum*.

The current study was therefore designed to investigate the effect of F^- on R. *leguminosarum* and its interaction with legumes. White clover was chosen as a model plant for this work as this species is an important and widely cultivated legume species in New Zealand. An assessment of F^- Inhibitory Concentration (IC) values for *R. leguminosarum* was undertaken to develop provisional F guideline values for New Zealand agricultural soils.

4.3 Materials and Methods

To examine the effect of F^- on *R. leguminosarum* and its interaction with white clover, a series of experiments were conducted in the microbiology laboratory of the School of Food and Advanced Technology, and using the growth chambers of the School of Agriculture and Environment, Massey University, New Zealand. White clover (*Trifolium repens* L) cultivar Tribute, and *R. leguminosarum* strain TA1, were used in the experiments as this cultivars and strains are widely used in New Zealand pastoral systems.

The toxic effect of F^- on *R. leguminosarum* was measured by the growth and shape of the *R. leguminosarum* bacteria, and the effect of F^- on the white clover-*Rhizobium* interaction was analysed using light and transmission electron micrographs of the white clover nodules.

4.3.1 Rhizobium leguminosarum growth

Rhizobium leguminosarum growth is a sensitive indicator of trace element contamination and toxicity (Pereira et al., 2006), and can be measured by Optical Density (OD) (Yessica et al., 2013; Aparicio et al., 2014). A *R. leguminosarum* starter culture (100 µL, OD_{600nm} = 0.1) was prepared by inoculating *R. leguminosarum* into 100 ml of Yeast extract Mannitol Broth (YMB) overnight at 27 °C on a shaking incubator (200 rev min^{-1}). To study the effect of F^- on *R. leguminosarum* growth, 5 mL of YMB was added to 25 mL glass bottles, along with 1 mL *R. leguminosarum* starter culture. A range of F^- concentrations (0, 0.5, 1, 5, 10, 20, 50, 70, 100, 500 and 1000 mg L^{-1}) was added using three different F salts (NaF, KF and NH_4F) to *R. leguminosarum*-treated bottles. The rationale for selecting this range of F^- concentrations is described in Appendix 6 section A6.2. The total volume was maintained at 10 mL per bottle. The three different F salts were used to test the effect of cations on the F^- toxicity. Each treatment was replicated three times and incubated at 27 °C for 48 h. After 24 and 48 h incubation, a 100 µL aliquot of each sample was added to a 96-well microplate and *R. leguminosarum* growth was measured by optical density at 600 nm using a microplate reader (SPECTROstar Nano; BMG Labtech, Ortenberg, Germany).

4.3.2 Rhizobium leguminosarum morphology

Morphological changes occur when bacteria are exposed to a toxic component (Ahmad et al., 2012). Scanning electron micrographs were used to quantify the toxic effect of F^- on *R. leguminosarum* morphology. The samples were prepared by incubating *R. leguminosarum* at 27 °C for 48 h with variable F^- concentrations (0, 100, 500 and 1000

mg L^{-1}) prepared from F salts (NaF, KF and NH_4F). The *R. leguminosarum* cells were then suspended in a modified Karnovsky's fixative (3% gluteraldehyde, 2% formaldehyde in 0.1 mol L^{-1} phosphate buffer, pH 7.2) for at least 8 h and then passed through a syringe filter holder (13 mm Swinney filter holder, Pall Corporation) containing a 0.4 μm membrane filter (Isopore, Merck Millipore LTD) to collect the cells. This membrane filter was then removed and washed three times in 0.1 mol L^{-1} phosphate buffer (15 min each). Once the phosphate buffer was removed, the membrane filters containing the samples were immersed in an increasing concentration of ethanol (15 min each at 25%, 50%, 75%, 95% and 100% before being replaced with a higher concentration) to dehydrate the samples.

The dehydrated samples were mounted onto aluminium stubs, sputter coated with approximately 100 nm of gold (BAL-TEC SCD 005 sputter coater), and then viewed under a scanning electron microscope (FEI Quanta 200, Netherlands) at an accelerating voltage of 25 kV.

The *R. leguminosarum* shape was described by circularity (ϕ). Circularity (ϕ) is given by the following equation (Huang et al., 2014).

$$\phi = \frac{4\pi A}{P^2}$$

Where A is the area and P is the perimeter of a single *R. leguminosarum* bacterium. From the SEM images of *R. leguminosarum*, area and perimeter were measured using ImageJ® software. Circularity (ϕ) provides a value range of 0-1, with 1 denoting a perfect circle.

4.3.3 Rhizobium – white clover interaction

To examine the effect of increasing F^- on white clover growth and its interaction with *R. leguminosarum,* a pottle-based experiment was conducted in a plant growth chamber. Sterile plastic pottles (50 ml) were filled with N-free sterile vermiculite.

Different F^- (NaF) concentrations (0, 1, 5, 10, 20, 50, 70, 100, 500, and 750 mg L^{-1}) of 38 mL N-free McKnights solutions were added to 10 g of vermiculite-filled pottles (Wakelin et al., 2016). Each treatment was replicated 6 times.

White clover seeds were sterilised using 70% ethanol for 15 s, then washed with sterile water. These seeds were then placed in a paper towel for 24 h in the dark for germination. A germinated seed was then placed in each pottle.

The *R. leguminosarum* pre-culture was prepared as explained in section 4.3.1. After incubation, 1 mL of pre-culture was introduced to a 250 mL flask containing 100 mL YM broth and incubated at 27 ºC for 48 h. The experimental design included a NaCl treatment to establish any Na ion effect on white clover–*Rhizobium* interaction (the Na concentration was equimolar with Na for the NaF treatment). To achieve this, the NaCl concentration was maintained at 5.2 mmol L^{-1} in each pottle for an equal amount of Na relative to the NaF treatment with 100 mg $F^- L^{-1}$. After incubation, the culture was diluted to 1.5×10^4 cfu mL^{-1} with 25 mL YMB and centrifuged at 4200 g for 15 min. The supernatant was discarded, and the remaining cell residue re-suspended with 25 mL of 0.85% NaCl.

One mL of this suspension was added to all pottles four days after the addition of germinated clover seeds. The inoculated pottles were kept in the growth chamber for six weeks, with l mL de-ionised sterile water being added every week.

The white clover plants were harvested after 6 weeks in the growth chamber. Plant roots were photographed using a digital camera (Olympus SC30, Germany), placed in a stereomicroscope (Leica MZ12, Germany), and the nodule length was measured using ImageJ® software. Total plant dry weight was determined after 72 h drying at 70 °C.

4.3.3.1 Micrograph analyses of clover nodules

Effective legume–*Rhizobium* symbiosis is indicated by *R. leguminosarum* density in infected cells and the presence of peribacteroid membranes in nodules, and by nodule formation in roots. The toxic effect on legume nodules was determined by Transmission Electron Microscopy (TEM) and Light Microscopy (LM) (Fan et al., 2014; Abd-Alla et al., 2016).

The six week-old nodules were cut into halves or thirds longitudinally and fixed in modified Karnovsky's Fixative (3% Gluteraldehyde (v/v) and 2% Formaldehyde (w/v) in 0.1 mol L^{-1} Phosphate Buffer (pH 7.2) for at least 12 h. These were then buffer-washed 3 times in 0.1 mol L^{-1} phosphate buffer (pH 7.2) for 10 min each before being post-fixed in 1% Osmium Tetroxide in 0.1 mol L^{-1} phosphate buffer for 1 h maximum. They were then again buffer washed 3 times (as above) for 10 min each and dehydrated using a graded acetone series (25%, 50%, 75%, 95%, and 100%) for 10-15 min each followed by 2x changes of 100% for 1 h each. The dehydrated samples were then placed into 50: 50

resin: acetone and then stirred overnight. The nodules slowly infiltrated with resin over a 3 d period before being placed into silicon moulds with fresh resin, and cured for 48 h to give rectangular blocks that could be cut with a microtome.

For the visible light microscope analysis, thin sections (1 micron) were cut using an ultramicrotome (Leica EM UC7, Germany) and heat-fixed onto glass slides. These were stained with 0.05% Toluidine Blue for approximately 12 s and viewed under a Zeiss Axiophot photomicroscope (Germany).

For the Transmission Electron Microscope analysis, the block of resin containing the nodules was then trimmed down to the selected area using a Diamond Knife (Diatome, Austria) set at 100 nm. These were stretched with chloroform to prevent wrinkle, and mounted on a grid using a Quick Coat G glue pen (Saiko, Japan).

The 100 nm sections mounted on grids were stained in Saturated Uranyl Acetate in 50% ethanol for 4 min, washed with 50% ethanol and MilliQ water and then stained in lead citrate (Venable and Coggeshall, 1965) for a further 4 min. This was followed by a wash in MilliQ water. Samples were viewed using a FEI Tecnai G^2 Spirit BioTWIN Transmission Electron Microscope (Czech Republic).

4.3.4 Rhizobium leguminosarum respiration-inhibition assay

Initial efforts were made in the current work to determine IC_{10} limits for F toxicity to *R. leguminosarum* as a preliminary step to develop F guideline values for New Zealand

agricultural soils. In this study, IC_{10} is defined as the inhibitory F^- concentration that causes 10% inhibition of *R. leguminosarum* respiration.

The F^- inhibitory concentration (IC) value for *R. leguminosarum* was determined using the MicroResp 96-well format respiration-inhibition assay developed by Campbell et al. (2003). MicroResp is a microplate system, containing two 96-well microplates; one being a deep-well microplate, the other being a detection microplate. Both plates are placed face-to-face and are separated by a rubber interface that facilitates the movement of carbon dioxide released by microbial respiration from the deep well plate to the detection plate containing a pH sensitive dye. The evolved carbon dioxide react with dye that leads colour changes is measured by colorimetry method.

In this experiment, each deep-well was charged with 0.25 mL of YMB. In addition, 0.2 mL of NaF, KF, and NH_4F (0, 0.5, 1, 5, 10, 20, 50, 70, 100, 500 and 1000 mg $F^- L^{-1}$) were added to eight-well columns across the deep-well micro plate. The total volume was maintained at 0.5 mL per well. *Rhizobium leguminosarum* activity in each plate was determined by respiration, evaluated as the amount of CO_2 released from the plate. The amount of CO_2 released by *R. leguminosarum* in each well was determined according to the method described by Campbell et al. (2003). Briefly, an initial absorbance value (A_{590}) of each well was recorded calorimetrically (t=0), then the microplate was sealed with a detection microplate and incubated for 24 h at 27 ºC. Detection microplate absorbance values were then measured (t=24) and absorbance differences (ΔA_{590}) were calculated. The absorbance difference (ΔA_{590}) is used to calculate the amount of CO_2 released (Wakelin et al., 2014) from the *R. leguminosarum*.

Respiration percentage (RP) of each well was calculated using the following equation.

$$RP(\%) = \left(\frac{\Delta A_{590}}{Mean\ \Delta A_{590}\ of\ control}\right) \times 100$$

Inhibition concentration values were determined by a 4-parameter logistic model (Sebaugh, 2011) and the equation is expressed as;

$$Y = D + \frac{A - D}{1 + \left(\frac{X}{C}\right)^{B}}$$

Where Y is respiration percentage (percentage of control), X is the F^- concentration (mg L^{-1}), A is the first asymptote, D is the second asymptote, B is the slope parameter, and C is the value at the inflection point.

4.3.5 Statistical analysis

The data were displayed as mean ± standard error. One-way analysis of variance was performed to measure the statistical difference among treatments using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). To compare the treatment means, the Tukey test was employed. Parameter estimates of the nonlinear regression equation were determined by using the PROC NLIN procedure of SAS (version 9.4). Residuals normality was tested by using the Ryan-Joiner test.

4.4 Results

4.4.1 Rhizobium leguminosarum growth

Incubation with F^- had a significant effect on the growth of *R. leguminosarum* as quantified by the parameter OD (Figure. 4.1).

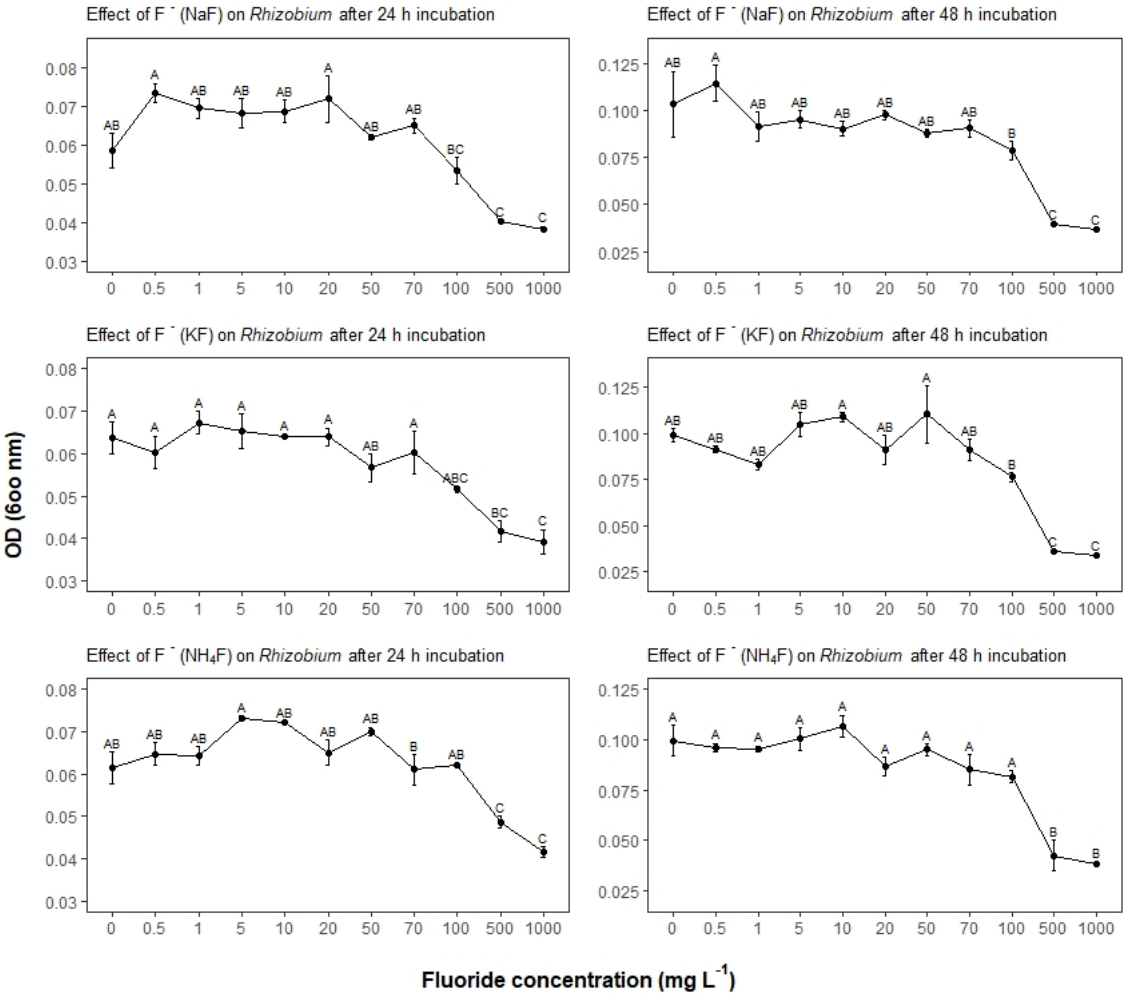


Figure 4. 1. Growth of *R. leguminosarum* culture when exposed to different F^- concentrations using three different F^- salts for 24 and 48 h incubation. Vertical bars indicate standard error of mean (n = 3). Means with the same letters are not significantly different at the $p > 0.05$ level (Tukey test).

R. leguminosarum growth after both 24 and 48 h was not significantly affected relative to the control ($F^- = 0$ mg L^{-1}) up to an F^- concentration of 100 mg L^{-1} for all salts of F used (NaF, KF, NH_4F), although there was a non-significant trend for decreased growth as a function of increasing F^- concentration. However, for both time points, and for all F salts, *R. leguminosarum* growth was significantly inhibited when exposed to F^- concentrations of 500 and 1000 mg L^{-1} (Figure 4.1).

4.4.2 Rhizobium leguminosarum morphological changes

In the absence of F^-, after 48 h of incubation, *R. leguminosarum* were rod-shaped (Figure 4.2a, e and i). However, as the concentration of F^- increased, the shape of *R. leguminosarum* progressively changed from rod to sphere. Circularity (ϕ) is an index that quantifies the 2D bacterial shape. In the present study, the effect of F^- on *R. leguminosarum* morphological changes was quantified by circularity (ϕ), and this was determined for individual *R. leguminosarum* bacterium through analysis of SEM micrographs (Table 4.1).

Table 4. 1. Effect of F^- on the morphological change of *R. leguminosarum* quantified using the parameter circularity (ϕ) for three different F salts (See Section 4.3.2)

F salts	F^- concentration (mg L^{-1})			
	0	100	500	1000
NaF	0.45 ± 0.019^{c}	0.49 ± 0.020^{c}	0.60 ± 0.028^{b}	0.87 ± 0.007^{a}
KF	0.49 ± 0.009^{c}	0.50 ± 0.018^{c}	0.65 ± 0.013^{b}	0.86 ± 0.011^{a}
NH_4F	0.49 ± 0.013^{c}	0.51 ± 0.021^{c}	0.68 ± 0.014^{b}	0.87 ± 0.005^{a}

Values denote means ± SEM (n=15). Means in the same table row with the same superscript letters are not significantly different at the $p > 0.05$ level (Tukey test).

Within a concentration value, circularity (ϕ) was not influenced by any of the F salts used (Table 4.1). Morphological changes can therefore be attributed to the effect of F^- rather than the cation used. At an F^- concentration of both 0 (control) and 100 mg L^{-1}, *R. leguminosarum* shapes (Figure 4.2) and mean circularity (Table 4.1) were the same for all three F salts. In the presence of an F^- concentration of 500 mg L^{-1}, circularity (ϕ) values for *R. leguminosarum* significantly ($p < 0.05$) increased to above 0.60 for all three salts and *R. leguminosarum* size had decreased (Figure 4.2c, g and k). When *R. leguminosarum* was exposed to an F^- concentration of 1000 mg L^{-1} for 48 h, *R. leguminosarum* morphology had changed from rods into spheres for all three salts (Figure 4.2d, h and l) and circularity (ϕ) values had significantly ($p < 0.05$) increased to above 0.85.

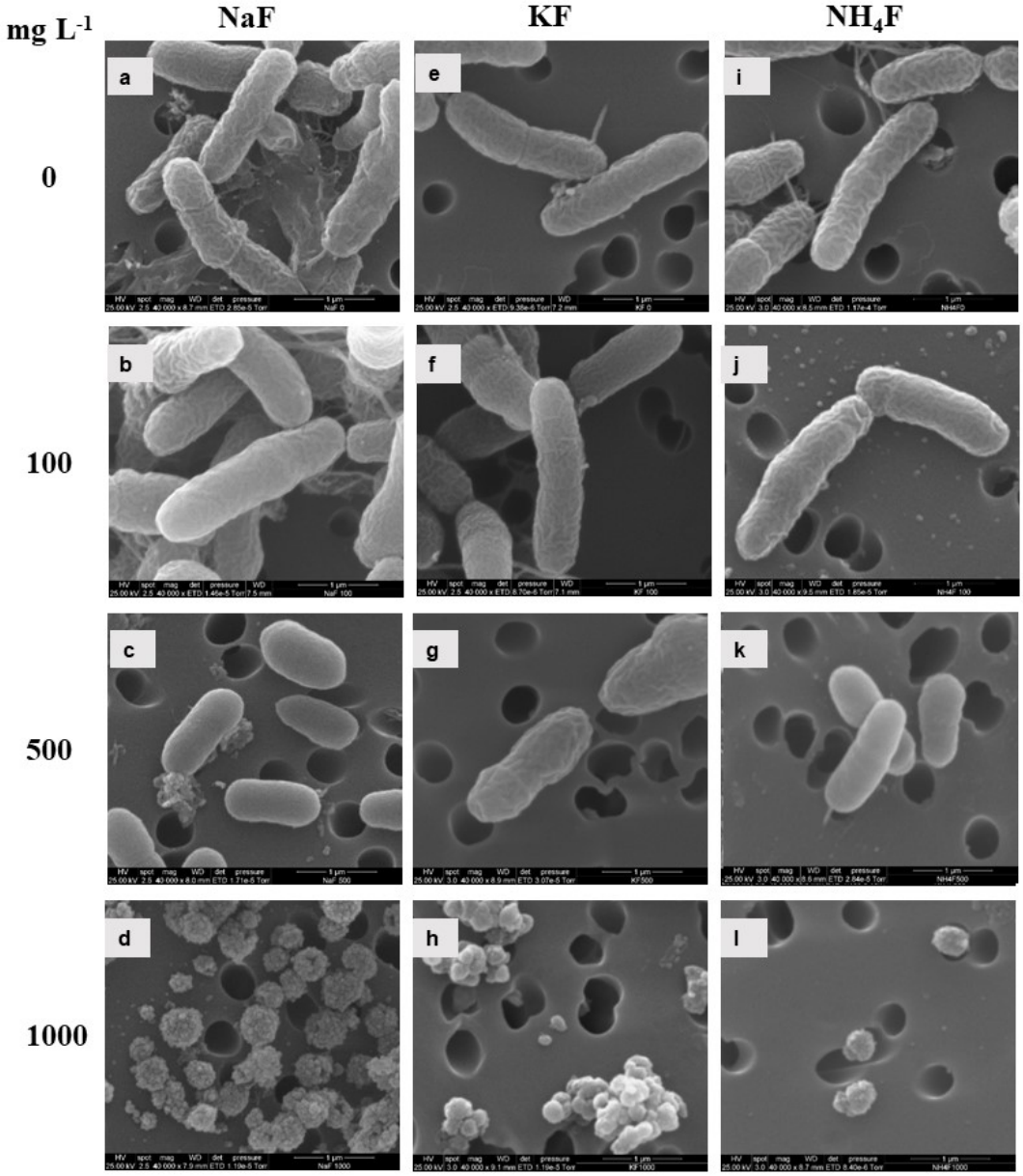


Figure 4. 2. SEM micrographs of *R. leguminosarum* exposed to NaF for 48 h at 27 °C, a) F^- concentration 0 mg L^{-1}, b) F^- concentration 100 mg L^{-1}, c) F^- concentration 500 mg L^{-1}, and d) F^- concentration 1000 mg L^{-1}. Exposed to KF for 48 h at 27 °C, e) F^- concentration 0 mg L^{-1}, f) F^- concentration 100 mg L^{-1}, g) F^- concentration 500 mg L^{-1}, and h) F^- concentration 1000 mg L^{-1}. Exposed to NH_4F for 48 h at 27 °C, i) F^- concentration 0 mg L^{-1}, j) F^- concentration 100 mg L^{-1}, k) F^- concentration 500 mg L^{-1}, and l) F^- concentration 1000 mg L^{-1}.

4.4.3 White clover-Rhizobium leguminosarum interaction

4.4.3.1 White clover dry weight

At concentrations of 0-70 mg F^- L^{-1}, F^- did not cause any significant effect on white clover dry weight (Figure 4.3). However, white clover dry weight significantly decreased when exposed to an F^- concentration of 100 mg L^{-1}.

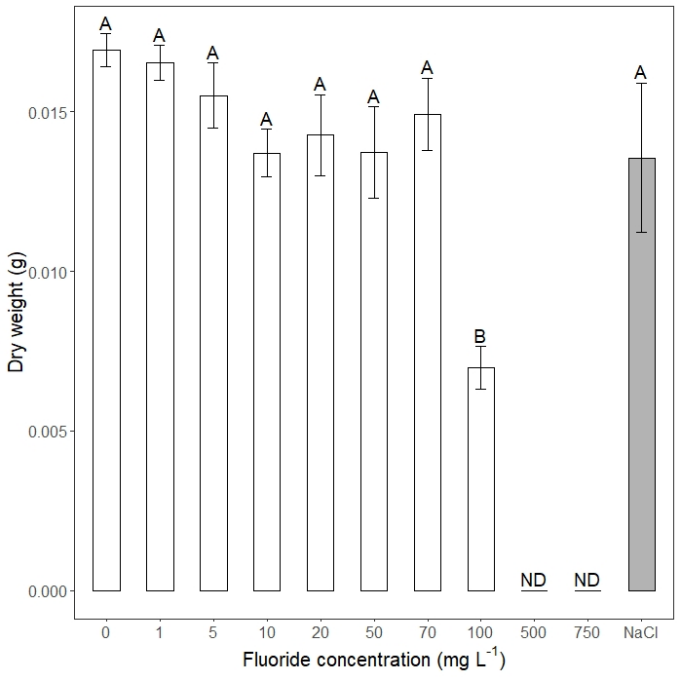


Figure 4. 3. Effect of different F^- concentrations on dry weight of white clover. Error bars indicate standard error of the mean (n = 5). Bars with same letters are not significantly different at the $p > 0.05$ level (Tukey test). ND – No data

Germinated white clover seeds did not mature into plants at F^- concentrations of 500 mg L^{-1} and 750 mg L^{-1}. Dry matter production for the white clover grown in the presence of NaCl was not significantly different for treatments exposed to F^- concentrations of up to

70 mg L^{-1}, but was significantly ($p < 0.05$) different to that for plants exposed to 100 mg L^{-1}.

4.4.3.2 White clover nodule length

The nodule lengths of 6-week old white clover at different F^- concentrations indicates that F^- had no significant effect on nodule length at a concentration range of 0-100 mg L^{-1} (Figure 4.4). Nodule lengths at F^- concentrations 500 mg L^{-1} and 750 mg L^{-1} were not recorded, as germinated seeds did not develop at these concentrations. The NaCl control shows that Na^+ ions also had no effect on nodule length.

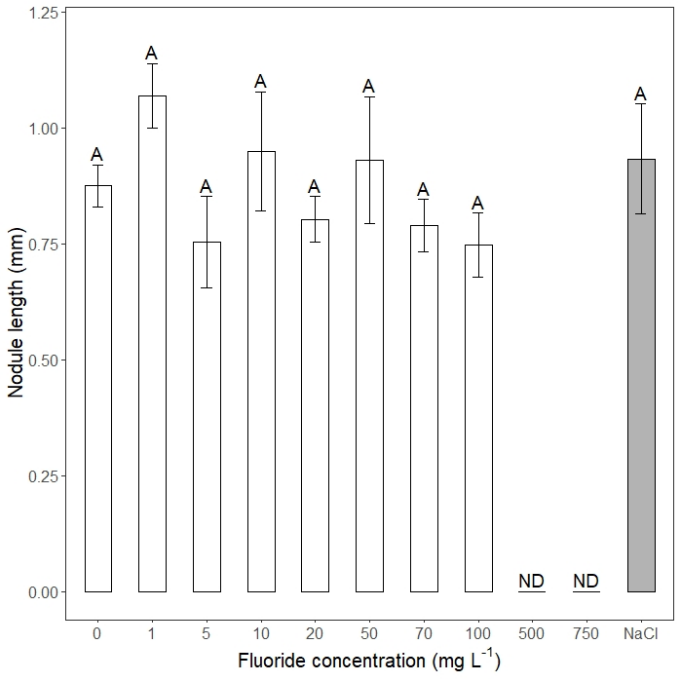


Figure 4. 4. Effect of different F^- concentrations on nodule length of white clover after 6 weeks growth. Error bars indicate standard error of the mean (n = 4). Bars with same letters are not significantly different at the $p > 0.05$ level (Tukey test). ND – No data.

4.4.3.3 Micrographs of white clover nodules

Light microscope images of nodules developed when exposed to 0, 50, 70, and 100 mg L^{-1} F^- concentrations are shown in Figure 4.5. The nodules of *R. leguminosarum* infected cells were densely packed with bacteroids (nitrogen-fixing form of *Rhizobia*) at F^- concentrations of 0, 50, 70, and 100 mg L^{-1}. Nodules were not apparent in the micrographs for F^- concentrations at 500 mg L^{-1} and 750 mg L^{-1} due to germinated seeds failing to develop into plants at these higher F^- concentrations.

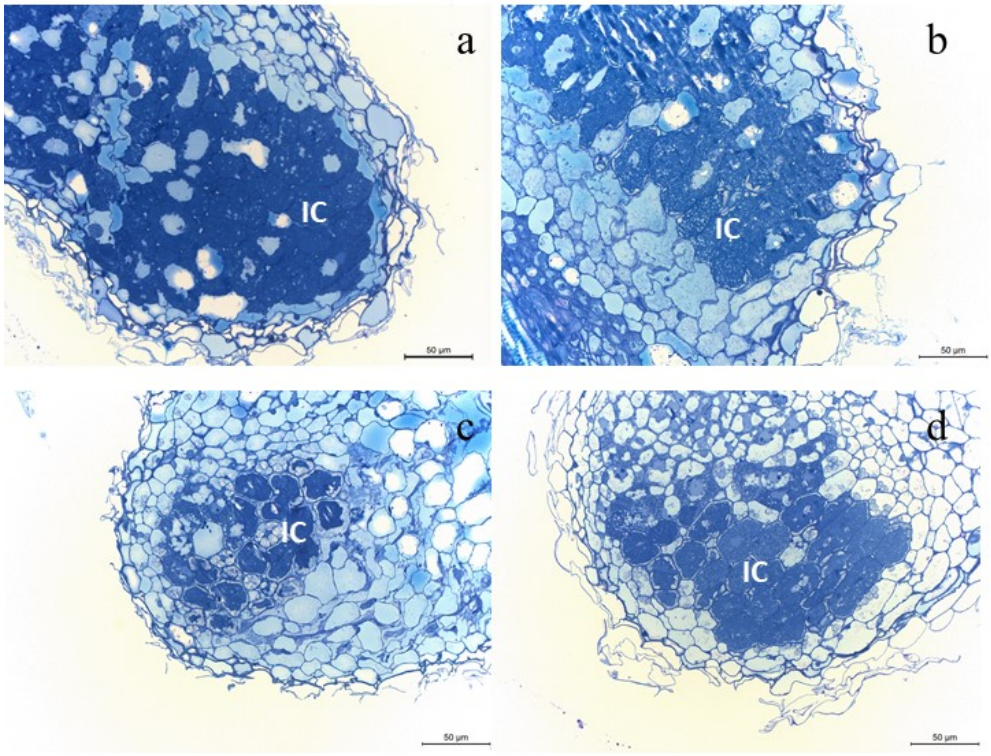


Figure 4. 5. Light Microscope (LM) images of six-week old white clover root nodule structures; (a) untreated (0 mg L^{-1} F^-) white clover root nodule, (b) 50 mg L^{-1} F^- treated white clover root nodule, (c) 70 mg L^{-1} F^- treated white clover root nodule and (d) 100 mg L^{-1} F^- treated white clover root nodule. Abbreviation: IC - infected cells

Transmission electron micrographs of white clover nodules exposed to 0, 50, 70, and 100 mg L^{-1} F^- concentrations are shown in Figure 4.6. Bacteroids were observed in the nodules of white clover treated with 0, 50, 70, and 100 mg L^{-1} F^- concentrations, each surrounded by a peribacteroid membrane to form a symbiosome at all F^- concentrations. A symbiosome is a unique structure that acts as an interface between the legume plant and *R. leguminosarum*. The symbiosome develops as a result of *R. leguminosarum* infection of legume roots (Emerich and Krishnan, 2014).

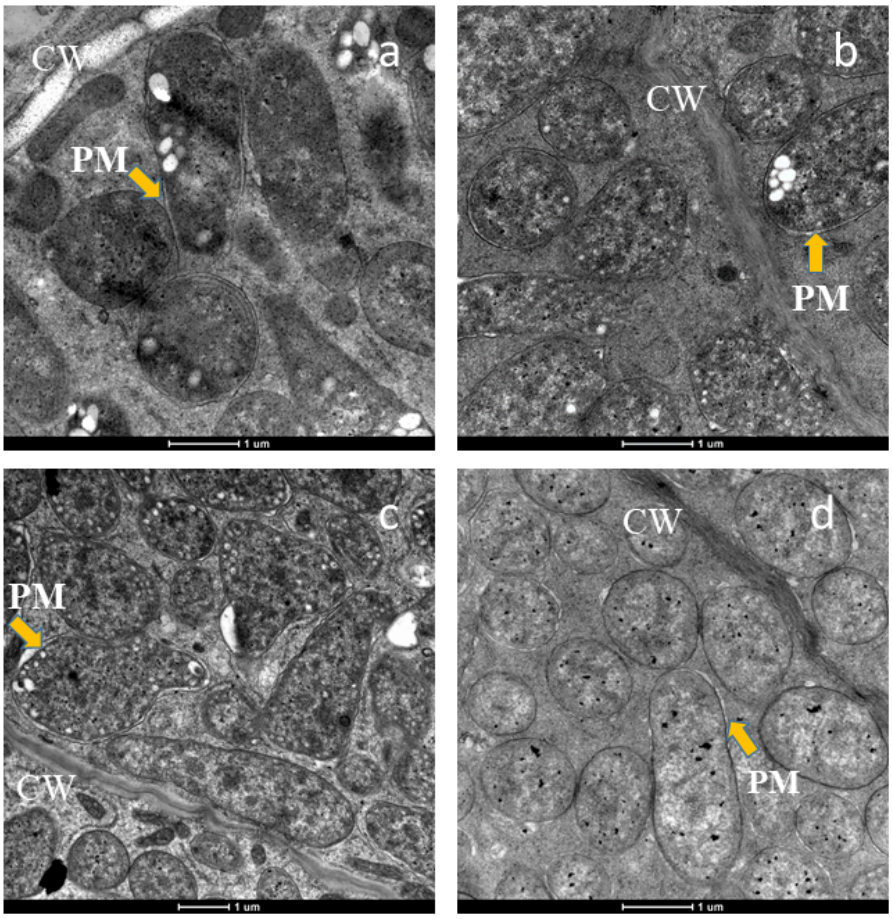


Figure 4. 6. Transmission Electron micrographs of six-week-old root nodules; (a) untreated (0 mg L^{-1}) white clover root nodule, (b) 50 mg F^- L^{-1} treated white clover root nodule, (c) 70 mg F^- L^{-1} treated white clover root nodule, and (d) 100 mg F^- L^{-1} treated white clover root nodule. Abbreviations: CW – cell wall, PM- peribacteroid membrane (symbiosome)

4.4.4 IC_{10} value for F^- toxicity to Rhizobium leguminosarum

Rhizobium leguminosarum respiration was significantly ($p < 0.05$) inhibited when exposed to a F^- concentration greater than 100 mg L^{-1} as indicated by the respiration assay (Figure 4.7).

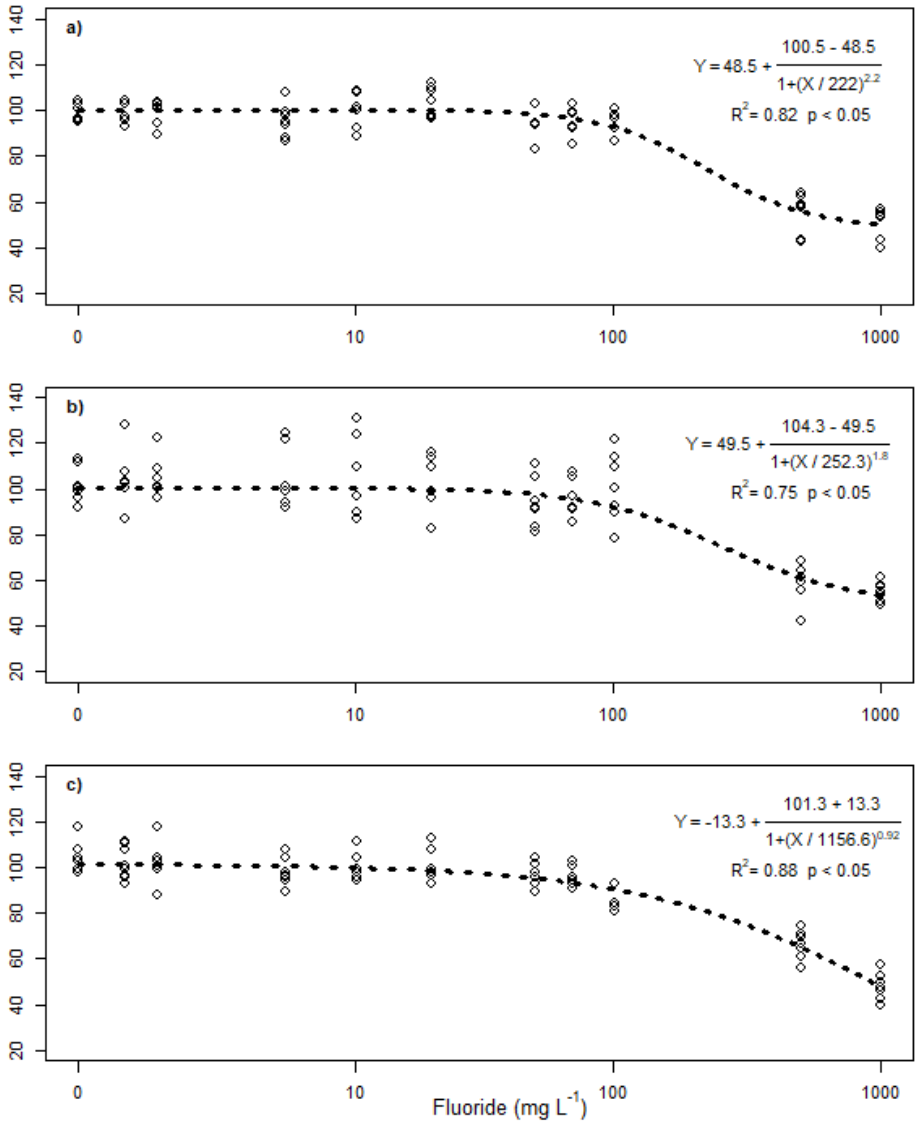


Figure 4. 7. Dose response relationships between F^- concentrations and *R. leguminosarum* respiration as a percentage of the control. Three different F salts were used; (a) NaF (b) KF, and (c) NH_4F

A four-parameter logistic model was used to determine the IC_{10} limits for F^- toxicity to *R. leguminosarum* to help set a provisional environmental quality guideline for soluble soil F^- concentrations. All four parameters were significant ($p < 0.05$). The IC_{10} values for F^- toxicity to *R. leguminosarum* are more than 100 mg F^- L^{-1} for each F salt used in the assay (Figure 4.7). In other words, F^- inhibits respiration by less than 10% when *R. leguminosarum* is exposed to F^- concentrations up to 100 mg F^- L^{-1}. There was a range of IC_{10} values between the three F salts, with the lowest value recorded for NH_4F. (IC_{10} value calculation is described in Appendix 2).

4.5 Discussion

4.5.1 Effect of F^- concentrations on Rhizobium leguminosarum growth

The results presented here suggest that F^- toxicity on *R. leguminosarum* growth depends on the F^- solution concentration. *Rhizobium leguminosarum* growth is not negatively influenced by F^- up to a concentration of 100 mg L^{-1} suggesting that *R. leguminosarum* is able to tolerate F^- below this value. Liu et al. (2017) reported that *Enterobacter cloacae* (strain FRM) is able to grow at an F^- concentration of 4000 mg L^{-1} in Burk broth, which suggests that some bacteria can grow at even higher F^- concentrations. Bacteria have known F^- toxicity mitigation mechanisms. When F^- concentration increases in the cell, F^- is identified by a F^- riboswitch (RNA molecules that bind with F^-) to activate an F^- exporter which ejects F^- from the bacteria cell (Baker et al., 2012; Breaker, 2012). *Rhizobium leguminosarum* growth suppression at an F^- concentration of 500 mg L^{-1} and 1000 mg L^{-1} could be explained by the antimicrobial activity of F^- (Marquis, 1995; Barbier et al., 2010) and inhibition of the F^- mitigation mechanism at this high F^-

concentration (Breaker, 2012). Ma et al. (2014) reported that *Escherichia coli* growth in Luria Bertani broth was reduced at F^- concentrations > 190 mg L^{-1} due to both of these mechanisms (antimicrobial activity and the concentration-induced absence of a F^- mitigation mechanism). Further research is needed to identify specific F^- mitigation mechanism in *R. leguminosarum*.

4.5.2 Effect of F^- on Rhizobium leguminosarum morphological changes

In the present study, a F^- concentration of 100 mg L^{-1} did not appear to induce a detrimental effect on *R. leguminosarum* morphology (Table 4.1). This is presumably due to F^- tolerance at this F^- concentration. Similar scanning electron micrograph studies showed that the soil bacteria *Bacillus marisflavi* and *Bacillus cereus* can grow in F^- concentrations as high as 1500 mg L^{-1} without any morphological change (Banerjee et al., 2016). Changes in *R. leguminosarum* cell morphology from a rod shape into an oval/round shape when exposed to higher F^- concentrations observed in this study may be due to cell wall deformation and changes in intercellular material induced by F^- (Barbier et al., 2010). While morphological changes in *R. leguminosarum* due to F^- toxicity have not previously been reported, similar morphological changes have been recorded for *R. leguminosarum* when exposed to other toxic substances. Huang et al. (2014) reported that *R. leguminosarum* morphed into oval/rounded shapes from rod shapes when exposed to 750 mg L^{-1} of nano-ZnO (nZnO) for 24 h. Deepika et al. (2016) reported that morphological changes occurred in *R. radioabacter* when exposed to 375 mg L^{-1} (5 mmol L^{-1}) arsenate.

4.5.3 Effect of F^- on white clover dry weight

Results from the *R. leguminosarum* pottle study indicate that white clover growth is not affected when the plant is exposed to F^- concentrations up to 70 mg L^{-1}. This is in agreement with the results from studies of other plants. Elloumi et al. (2005) showed that almond (*Amygdalis communis* L.) seedling dry weight was not reduced at an F^- concentration of 95 mg L^{-1}. Stevens et al. (1998), working with tomato (*Lycopersicon esculentum*), showed that shoot dry weight was not significantly ($p > 0.05$) affected by F^- concentrations up to 62 mg L^{-1} but significantly reduced by 18% relative to a control at an F^- concentration of 101 mg L^{-1}. In this study, F^- tolerance in white clover up to an F^- concentration of 70 mg L^{-1} could potentially be explained by F^- detoxification at the cellular level or the elimination of F^- at the root level. These mechanisms have been previously reported to explain the tolerance of Oats (*Avena sativa*) to F^- at 101 mg F^- L^{-1} (Stevens et al., 1998). At higher F^- concentrations (> 70 mg L^{-1}), detrimental effects on white clover may be due to F^- induced cellular injury, as well as physiological (photosynthesis) and biochemical (enzyme activities) changes in the plants. At an F^- concentration of 95 mg L^{-1}, cellular injury, chloroplast alteration, and detrimental effects on enzyme activity have been observed in wild shrub *Hypericum perforatum* (Fornasiero, 2001).

Non-significant differences ($p > 0.05$) in dry weight between the NaCl and F^- treatments (NaF) in this study suggest that Na did not affect white clover growth. Chartzoulakis and Klapaki (2000) reported that Na concentrations up to 10 mmol L^{-1} did not affect the dry matter weight of bell-pepper (*Capsicum annum*). The Na concentration in the NaCl

treatment (5.2 mmol L^{-1}) was equivalent to the Na concentration in the 100 mg F^- L^{-1} (as NaF) treatment. This value is lower than the reported plant tolerable Na concentration.

4.5.4 Effect of F^- on white clover nodule length

White clover continued to develop nodules when grown in a F^- concentration up to 100 mg L^{-1}. Although plant dry weight was significantly different between the control (0 mg F^- L^{-1}) and 100 mg L^{-1} F^- concentrations, nodule length was not significantly different between the two concentrations. This may be linked with *R. leguminosarum* tolerance to F^-. Similar results were reported by Manier et al. (2009) who stated that although the fresh biomass of white clover varied between Cd, Zn, and Pb contaminated and uncontaminated soils, nodule size was not significantly different.

In this study, Na concentration did not affect white clover nodulation. This was confirmed by non-significant differences ($p > 0.05$) in nodule lengths between the NaCl treatment and other F^- treatments. Borucki and Sujkowska (2008) reported that nodule volume per pea (*Pisum sativum* L.) plant was not influenced by Na up to 25 mmol L^{-1} Na, a concentration lower than the Na concentration (5.2 mmol L^{-1}) in the current study.

4.5.5 Effect of F^- on legume – Rhizobium leguminosarum symbiosis

The toxic effect of F^- on legume – *Rhizobium* symbiosis was analysed using TEM and LM micrographs of legume nodules. Bacteroid density in the nodule-infected cells was imaged by LM to show *Rhizobium* – legume symbiosis potential. Fan et al. (2014) studied the effect of nano-TiO_2 on *Rhizobium* – legume interaction and reported that the density

of bacteroids in infected cells was low in nano-TiO_2 treated nodules compared with untreated plant nodules. In the present study, LM micrographs revealed that the nodules exposed to 100 mg F^- L^{-1} were densely packed with bacteroids, indicating that F^- has no apparent effect on *R. leguminosarum's* potential to interact with white clover for N-fixation up to an F^- concentration of 100 mg L^{-1}.

TEM micrographs were used for further analysis of the *R. leguminosarum* structure in the infected cells. Abd-Alla et al. (2016) studied the impact of silver nanoparticles on faba bean- *R. leguminosarum* symbiosis and reported that deformed *R. leguminosarum* and peribacteroid membranes were observed in transmission electron micrographs of plant nodules treated with silver nanoparticles. In the current study, deformed *R. leguminosarum* were not observed in the TEM micrographs of nodules exposed to 100 mg F^- L^{-1}, and the presence of peribacteroid membranes at this concentration suggest that *R. leguminosarum* has a continued ability to fix nitrogen.

The microscopic studies conducted in the current work confirmed that *R. leguminosarum* is able to develop a symbiotic relationship with white clover at an F^- concentration of 100 mg L^{-1}. This is in agreement with Porter and Sheridan (1981) who reported that nitrogen fixation was not significantly influenced when alfalfa was exposed to an F^- concentration of 100 mg L^{-1}.

4.5.6 Respiration assay and derivation of an IC_{10} value for F^- toxicity to Rhizobium leguminosarum

Respiration assays indicated that *R. leguminosarum* activity is stable with increasing F^- concentrations up to 100 mg L^{-1}. Respiration inhibition at high F^- concentrations may be via the suppression of respiration enzyme activities and mitochondria functions. In microbial cells, F^- inhibits enolase and the ATPases enzymes required for the glycolytic pathway of the Krebs cycle, leading to suppression of cell respiration (Sutton et al., 1987; Marquis et al., 2003; Barbier et al., 2010; Yasuda et al., 2017). Cittanova et al. (1996) reported that F^- can reduce the activity of mitochondria at 95 mg L^{-1} which is an important cellular organ for respiration.

The IC_{10} value for F^- toxicity to *R. leguminosarum* determined in the current study is above 100 mg L^{-1} indicating that *R. leguminosarum* shows limited sensitivity to F^- concentrations up to this value. An inhibition concentration value for F^- toxicity to *R. leguminosarum* has not previously been reported, although some research has investigated the toxicity of F^- to nitrifying microorganisms in aerobic sewage sludge. Carrera et al. (2003) reported that nitrifying activity was inhibited by 50% when F^- concentrations were increased to 630 mg L^{-1}, while Ochoa-Herrera et al. (2009) reported that the denitrification process was not significantly influenced by F^- concentrations up to 500 mg L^{-1}.

The combined information from previous literature and from the current study suggest that present New Zealand soil F concentrations are not detrimental to *R. leguminosarum* and the *Rhizobium*–white clover symbiosis. Gao et al. (2012) reported that exchangeable

and water-soluble Fs are highly available to microorganisms and these are the forms of soil F that are toxic to microorganisms. Geretharan et al. (2018) measured the water-soluble F concentrations of soils representing different soil orders in New Zealand [Chapter 3] and reported that water-soluble F concentrations range between 1.70 to 6.45 mg kg^{-1}, with a free F^- concentration in soil solution less than 0.58 mg F^- L^{-1}. This range of 'field' soil solution F^- concentrations (< 0.58 mg L^{-1}) is at least 2 orders of magnitude lower than the IC_{10} value (100 mg L^{-1}) determined for 3 different F salts in this study.

4.6 Conclusions

The effect of F^- on *R. leguminosarum* was measured in the current study using three parameters: growth, morphological change, and microbial respiration. To quantify F^- toxicity to *R. leguminosarum*, the IC_{10} value was determined.

Rhizobium leguminosarum growth was not significantly supressed by F^- concentrations up to 100 mg L^{-1}. These results were confirmed by using 3 different F salts. Morphological changes were observed when *R. leguminosarum* was exposed to F^- concentrations at 500 and 1000 mg L^{-1}. In addition, pottle-based experiments indicated that *R. leguminosarum* was able to form healthy nodules with white clover at F^- concentrations up to 100 mg L^{-1}. Nodule size was not significantly affected by F^- concentrations up to this level, and nodule growth was not significantly supressed by F^- concentrations up to 70 mg L^{-1}. TEM and LM micrographs of white clover nodules indicate that *R. leguminosarum* and white clover symbiosis did not appear to be influenced by F^- concentrations up to 100 mg L^{-1}. Respiration studies indicated that *R. leguminosarum* respiration was inhibited less than 10% for F^- concentrations up to 100 mg L^{-1}.

Toxic effects of F^- on *R. leguminosarum* were recorded at solution concentrations two orders of magnitude higher than those measured for a range of New Zealand agricultural soils under field conditions. Accordingly, there appears to be no indication of imminent risk of soil solution F^- concentrations depressing *R. leguminosarum* growth under field conditions.

Acknowledgements

We thank The Fertiliser Association of New Zealand for financial support of this project; Steve Wakelin, AgResearch Ltd, Lincoln Science Centre, Christchurch, New Zealand for provision of the MicroResp procedure; and Sandra Young, AgResearch Ltd, Lincoln Science Centre, Christchurch, New Zealand for provision of the *R. leguminosarum* (strain TA1) culture. Thanks also to Niki and Jordan (Manawatu Microscopy and Imaging Centre, Massey University) for their scanning and transmission electron microscopy expertise.

CHAPTER 5

Effect of lime and compost amendments on fluoride adsorption in New Zealand soils

---

Part of the results of this chapter was presented in the SAE Symposium 2018. Citation:

Geretharan, T., Jeyakumar, P., Bretherton, M., Anderson, C. W. N. Sorption and desorption of fluoride in compost and lime amended soils. SAE Symposium 2018, 19 November 2018

5.1 Abstract

Understanding F^- adsorption behaviour to soil and soil amendments is a key part of developing F^- soil management practices that will minimise any future risk of F^- to pasture production. Field experiments that analysed the level of F in horticultural soils collected from field locations in Kairanga, Pukekawa and Canterbury were designed. Each field location has a long history of superphosphate application. At each location, different soil pH levels were achieved by the addition of fine elemental sulphur (100% S) or Aglime (80% $CaCO_3$). Amendment rates of sulphur and lime were determined based on a detailed preliminary laboratory incubation study. A commercial compost was applied at a range varying from 0 to 50 tons ha^{-1} to attain different levels of organic matter in the soil. Soil samples were then collected after one month of amendment. Laboratory batch-type experiments were conducted to study F^- adsorption/desorption on individual (un-amended) and amended soils.

F^- adsorption varied with soil type. Compost application increased F^- adsorption when soil pH levels were less than 6, and did not influence F^- adsorption above this value. Lime application significantly ($p < 0.05$) influenced F^- adsorption/desorption via alteration of soil pH, with soil pH for maximum F^- adsorption ranging from 5.5 to 6.8.This study suggests that a combination of lime and compost amendments can be used to reduce the soil F^- concentration in New Zealand agricultural soils.

Key words: Adsorption; Freundlich; Lime; Compost

5.2 Introduction

Fluorine toxicity depends on the chemical nature of the F compound that exists in soil (Loganathan et al., 2001). F^- and metal complexes of F^- (Al-F, Fe-F) are the main species of F which exist in soil solution (Elrashidi and Lindsay, 1987; Stevens et al., 1997). Several authors (Stevens et al., 1997; Ma, 2000) have suggested that Al-F complexes are less phytotoxic than the free ion. Reduced toxicity is particularly apparent for microorganisms, as Al-F and Fe-F complexes are unable to cross microorganism cell membranes since the membrane pore size is smaller than the size of these metal complexes (Brierley and Kuhn., 2010; Sicupira et al., 2011; Veloso et al., 2012; Ahoranta et al., 2017; Ma et al., 2017). Quintáns-Fondo et al. (2016a) reported that F^- is the primary toxic F compound that enters into living organisms via soil solution. In soil, F^- availability to the plants, human and microorganisms can be minimised by increasing F^- adsorption to soil surfaces.

Fluoride adsorption by soil is influenced by soil properties such as organic matter, soil pH, clay content, and Fe, Al oxide/hydroxide content (Larsen and Widdowson, 1971; Omueti and Jones, 1977; Gago et al., 2012; Gago et al., 2014). The soil pH required for maximum F^- adsorption varies as a function of soil chemistry (Omueti and Jones, 1977; Wenzel and Blum, 1992; Arnesen, 1998; D'Alessandro et al., 2012). D'Alessandro et al. (2012) reported that in Etnean volcanic soils, maximum F^- adsorption occurred at a pH range of 5.26 - 6.02, while western Norway Cambic Podzol and Cambic Arenosols had maximum F^- adsorption at pHs between 4.8 - 5.5 (Arnesen, 1998). However, F^- adsorption behaviour as a function of pH (as well as other soil chemical properties) has not yet been reported for New Zealand soils.

The addition of lime or lime-based products to soil can increase F^- adsorption by increasing the soil pH (Quintáns-Fondo et al., 2016a). Quintáns-Fondo et al. (2016a) reported that addition of 48 tons ha^{-1} of mussel shell to pyritic material (copper mine spoil) increased the pH of the waste material from 3.9 to 5.8, and increased F^- adsorption by 15%. In New Zealand agriculture, lime is applied to increase soil pH to maintain sustainable and economical pasture production (Edmeades et al., 1984). The effects of lime application on F^- adsorption via changes to soil pH have not yet been studied for New Zealand agricultural soils.

The use of by-product and bio-sorbents as soil amendments for F^- adsorption has drawn interest in recent years. Soil amendments such as mussel shell, charcoal, and bamboo charcoal, can reduce F^- availability in soil (Gao et al., 2012; Quintáns-Fondo et al., 2016a; Quintáns-Fondo., 2016b). Romar-Gasalla et al. (2018) reported that F^- adsorption increased by 19% in forest soil when amended with pine bark at 48 tons ha^{-1}. Commercial

compost is widely used as a soil amendment in New Zealand agriculture. Compost application may influence soil F^- adsorption. However, influences of compost application on soil F^- adsorption have not been documented for New Zealand agricultural soils.

On the basis of the above discussion, a study was conducted to propose soil management practices to minimise F^- availability. The objective of the study was to measure the effect of lime and compost application and their interaction on soil F^- adsorption and F^- availability in three New Zealand agricultural soils.

5.3 Material and Methods

A laboratory F^- adsorption study was conducted with soils collected from three New Zealand field experimental sites (Figure 5.1) which had been amended with compost and lime. Each experimental site had been divided into 30 plots, each of which was 3.4 m x 4.0 m (Figure 5.2). These plots comprised of ten treatments replicated three times. The treatment combinations were formulated using 5 different Ag lime levels (0 – 30 kg $plot^{-1}$), and 3 compost application rates (0 – 50 tons ha^{-1}) to achieve a target pH range from 5.6 to 7 (Table 5.1). The exception was the Pukekawa soil which had a natural soil pH above 6. For this soil, elemental sulphur (100% S) was applied to reduce soil pH to 5.6. The application rates of lime for all three sites, and elemental sulphur for Pukekawa, were determined based on the results of a pH incubation experiment conducted by Thompson (2017). After application of soil amendments, the soil was thoroughly mixed using a garden rake.

Table 5. 1. Application rates of lime, compost and anticipated pH of the three experimental sites.

Canterbury		Kairanga		Pukekawa		Anticipated pH
Lime (kg plot^{-1})	Com (ton ha^{-1})	Lime (kg plot^{-1})	Com (ton ha^{-1})	Lime/S (kg plot^{-1})	Com (ton ha^{-1})	
0	0 25 50	0	0 25 50	1.1(S)	0	5.6
7	0 25	5	0 25	0	0 25 50	6.0
12	0 25	10	0 25	5.7	0 25	6.3
20	0 25	18	0 25	12.2	0 25	6.7
30	0	25	0	18	0 25	7.0

S - Elemental sulphur
Com – Compost

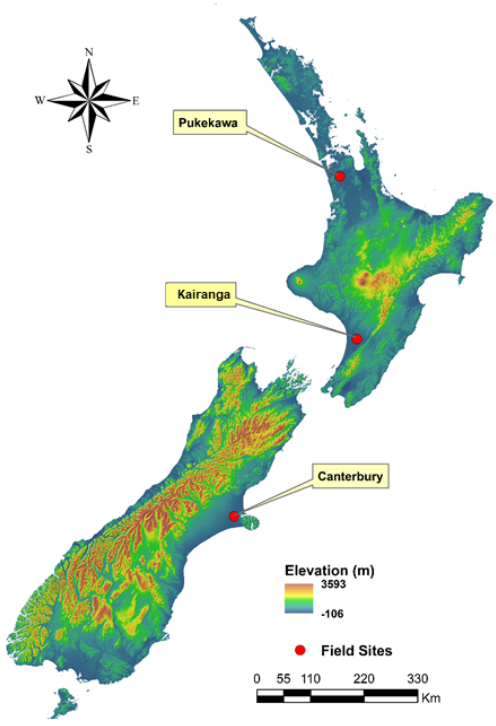


Figure 5. 1. Location of three field experimental sites.

Figure 5. 2. Field layout of Pukekawa site after lime and compost amendments.

5.3.1 Soil sampling

One month after the application of amendments, six soil cores (2.5 cm x 15 cm) were randomly collected across the plots for each treatment to form a composite soil sample. Collected samples were air dried at 20 °C until constant weight and passed through a 2 mm stainless steel sieve.

5.3.2 Soil chemical analysis

Soil pH was measured at a 1: 2.5 (soil: water) ratio using an Eutech Instrument Cyber Scan pH 310 meter. Non-crystalline Fe and Al were extracted using the acid ammonium oxalate extraction method (Blakemore et al., 1987), and the Fe and Al concentrations in solution were measured by MP-AES 4200 (Agilent, Germany). Total N (expressed as N%) and soil organic carbon (expressed as C%) were measured using a vario MACRO cube

CHNS elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany). To obtain soil organic matter (OM%), soil organic C content was multiplied by two (Pribyl, 2010). Total soil F was determined using the 4 mol L^{-1} NaOH extraction method (Jeyakumar and Anderson, 2015). $CaCl_2$ (0.01 mol L^{-1}) and water extractable F concentration was measured as per the method of Geretharan et al. (2018) [Chapter 3].

5.3.3 Fluoride adsorption experiment

In the adsorption experiment, soil samples were shaken with different concentrations of F^- to reach adsorption equilibrium. To achieve equilibrium, 30 mL of 0.03 mol L^{-1} NaCl (used to maintain a consistent ionic strength) containing 0, 5, 10, 20, 50, and 100 mg F^- L^{-1} was added to 3 g of each soil sample. The suspension was shaken for 24 hours, centrifuged at 6167 g for 15 minutes, and then filtered using Whatman 42 filer paper. Fluoride concentrations in the filtrate at equilibrium were then measured using an ion selective electrode (Orion USA), after adding an equal amount of TISAB (Total Ionic Strength Adjustment Buffer).

The mass of F^- adsorbed by the soil was calculated using the following equation (Gao et al., 2012).

$$q_e = \frac{(C_o - C_e)V}{M}$$

Where q_e is amount of F^- adsorbed by the soil (mg kg^{-1}), C_o is the initial concentration of F^- (mg L^{-1}), *Ce* is the equilibrium concentration of F^- (mg L^{-1}) and *V* is the volume of solution (L), and M is the mass of the soil (kg).

5.3.4 Fluoride desorption experiment

At the end of the adsorption experiment (section 5.3.3), F^- was desorbed from those soil samples which had been exposed to an F^- concentration of 100 mg L^{-1}. To achieve this, 30 mL of 0.03 mol L^{-1} NaCl solution was added to these samples. The resulting suspension was shaken for 24 hours, centrifuged, and then filtered with Whatman 42 filter paper. The desorbed F^- in the filtrate was measured using the same procedure employed for the adsorption experiment.

Data were fitted with a generalised Freundlich equation (Gago et al., 2014) described as:

$$q_e = K_F C_e^{1/n}$$

Where K_F and 1/n are fitted constants, Ce is the equilibrium concentration (mg L^{-1}), and the amount of F^- adsorbed at equilibrium is given by q_e (mg kg^{-1}).

5.3.5 Statistical analysis

One-way analysis of variance was performed to measure statistical differences between treatments using SAS 9.4. To compare the treatment means, the Tukey test was used. Parameter estimates of the Freundlich equation were determined using the PROC NLIN procedure of SAS (version 9.4).

5.4 Results and Discussion

5.4.1 General description of soils

Properties of the Canterbury, Kairanga, and Pukekawa soils are shown in Table 5.2. The selected soils had a wide range of important soil properties. Soil pH ranged between 5.31 – 6.34. The Pukekawa soil had higher acid extractable Al and Fe content (0.78% and 0.37 %, respectively) than the other two soils. The acid extractable Al concentration of the Canterbury soil was 0.37%, higher than that of the Kairanga soil (0.22%). The Pukekawa soil had the highest amount of organic matter content (5.36%) followed by the Canterbury (4.79 %) and Kairanga (4.69%) soils.

Table 5. 2. General chemical properties of the three soils used in this study.

	Kairanga	Pukekawa	Canterbury
pH	5.31	6.34	5.56
F ($CaCl_2$ /mg kg^{-1})	3.12	1.57	4.92
F (H_2O/mg kg^{-1})	2.59	1.91	2.06
F (Total /mg kg^{-1})	153.00	342.33	211.77
Fe (%)	0.25	0.37	0.22
Al (%)	0.22	0.78	0.37
SOM (%)	4.69	5.36	4.79
Soil Oder	Gley	Granular	Pallic

The highest total soil F concentration was recorded for the Pukekawa soil (342 mg kg^{-1}). The water-extractable F concentration ranged between 1.91-2.59 mg kg^{-1}, and decreased

in the order Kairanga > Canterbury > Pukekawa. In contrast, the Canterbury soil had a higher $CaCl_2$-extractable F concentration when compared with the Kairanga soil.

5.4.2 Effect of lime application on soil pH

The effect of different rates of lime application on soil pH of the three soils is displayed in Figure 5.3. After one month of soil amendment, lime application had increased the pH in each soil. Observed soil pH was higher than the targeted soil pH for all the lime application treatments, the differences being less than 0.5 pH units for all three soils. The difference may be due to environmental conditions in the field plots. Lime application rates were determined based on an incubation study (Thompson, 2017) where static environmental conditions were maintained. However, environmental conditions in the field were variable. Maier et al. (1997) reported that when soil is amended with lime, soil pH changes as a function of soil lime reaction rates, which are influenced by environmental conditions. In the Pukekawa soil, amendment with elemental sulphur (100% S) reduced the soil pH from 6.34 to 5.85 which was 0.28 unit higher than the targeted soil pH. Again, this differential in target vs actual pH may be due to the environmental conditions prevailing in the field.

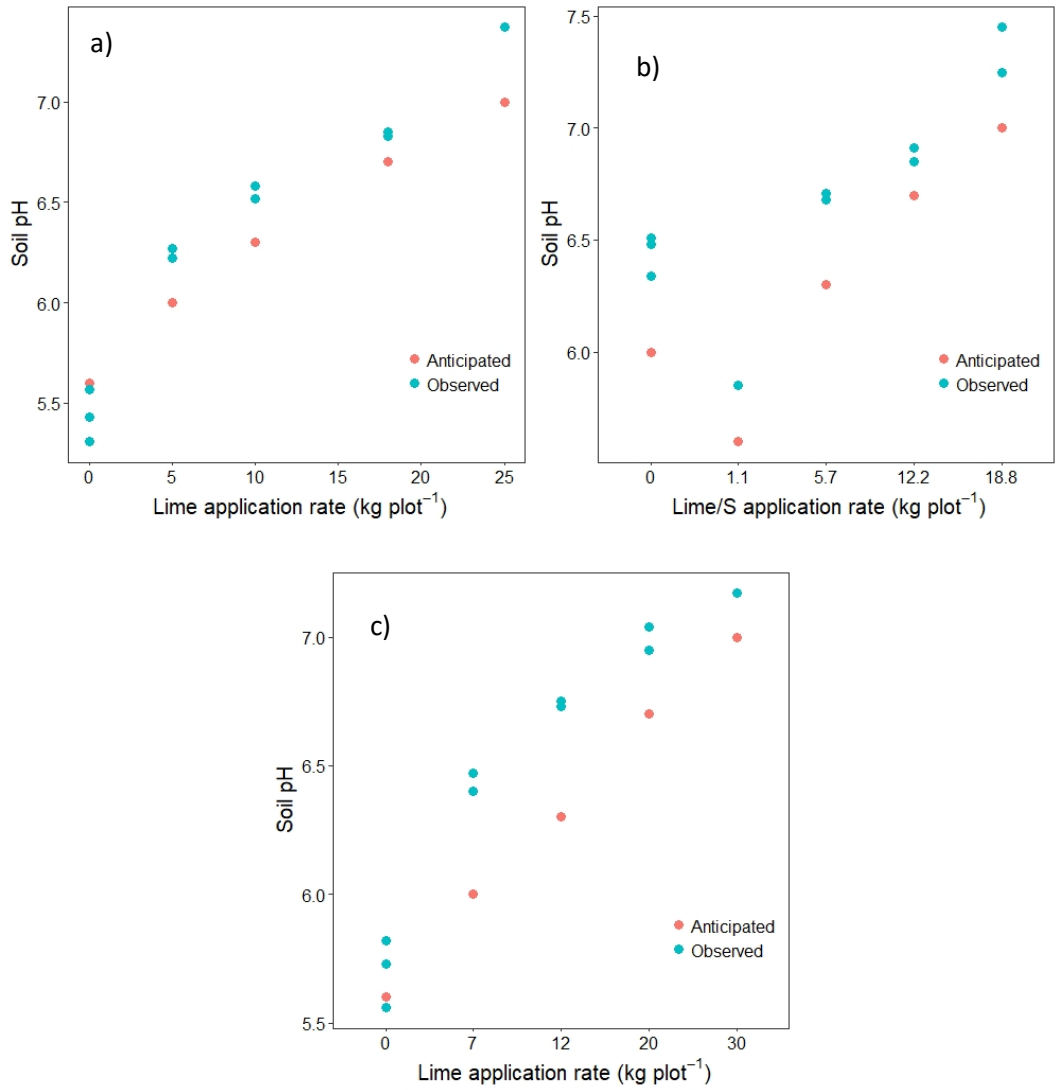


Figure 5. 3. Effect of lime application on soil pH under field conditions: a) Kairanga, b) Pukekawa, c) Canterbury (n=3).

5.4.3 Variation of F^- adsorption in different soils

Figure 5.4 shows the variation of F^- adsorption as a function of the different soils. The Pukekawa soil had greater F^- adsorption than the Kairanga and Canterbury soils.

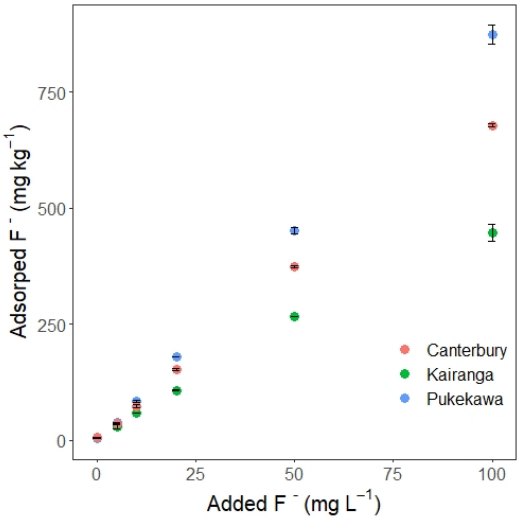


Figure 5. 4. Variation of F^- adsorption (mg kg^{-1}) for three different soils amended with different concentrations of F^- (mg L^{-1}). Error bars indicate standard error of the mean (n=3)

When 100 mg L^{-1} of F^- solution was added, the resulting adsorbed concentration for the Pukekawa soil was 873 mg F^- kg^{-1} of soil, while for the Kairanga and Canterbury soils the adsorbed F^- concentrations were 518 mg kg^{-1} and 677 mg kg^{-1}, respectively. This is equivalent to an adsorption of 87.3% of the added F^- to the Pukekawa soil, and 67.7% and 51.8% for the Canterbury and Kairanga soils respectively (Figure 5.8). Variation of F^- adsorption between the soils can be attributed to their differing soil properties. The Pukekawa soil had higher organic matter (5.36%), non-crystalline Al (0.78%), and Fe (0.37%) content than the Canterbury and Kairanga soils. Gago et al. (2012) reported that non-crystalline Al and Fe are mainly responsible for the F^- adsorption in soils. Similar results are reported by Quintáns-Fondo et al. (2016a), who found that concentration of adsorbed F^- was higher in a forest soil (761 mg kg^{-1}) relative to a vineyard soil (605 mg kg^{-1}) as it had a higher amount of organic matter and Al and Fe content.

5.4.4 Effect of compost application on F^- adsorption

The influence of compost on F^- adsorption as a function of increasing added F^- concentration is displayed in Figure 5.5.

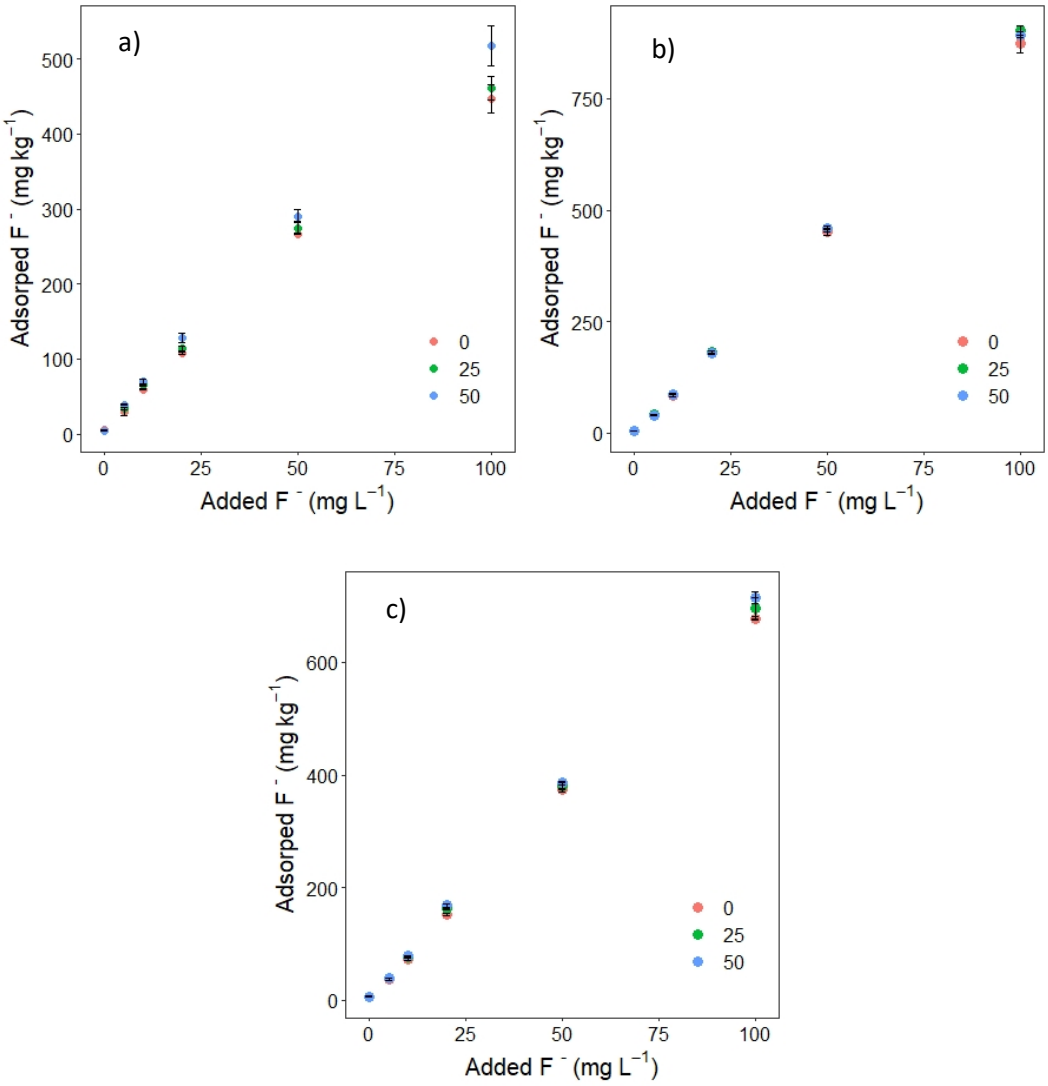


Figure 5. 5. Effect of compost application (tons ha^{-1}) on F^- adsorption as a function of increasing added F^- concentration: a) Kairanga b) Pukekawa and c) Canterbury. Error bars indicate standard error of the mean (n=3).

In the Canterbury and Kairanga soils, application of compost significantly increased F^- adsorption ($p < 0.05$) (Figure 5.5 a and c) after 1 month. In contrast, the application of compost did not significantly ($p > 0.05$) influence F^- adsorption in the Pukekawa soil (Figure 5.5 b).

The interactive effect of lime and compost application on the concentration of adsorbed F^- was investigated for the added F^- concentration of 100 mg L^{-1} (Figure 5.6). The effect of compost application on F^- adsorption was significantly ($p < 0.05$) influenced (positively) by lime in the Kairanga and Canterbury soils. However, this effect was not significant ($p > 0.05$) for the Pukekawa soil. At a soil pH < 6, when no lime was applied, compost addition increased F^- adsorption (Figure 5.6 a and c). However, the addition of compost did not influence F^- adsorption when lime was applied (Figure 5.6 b).

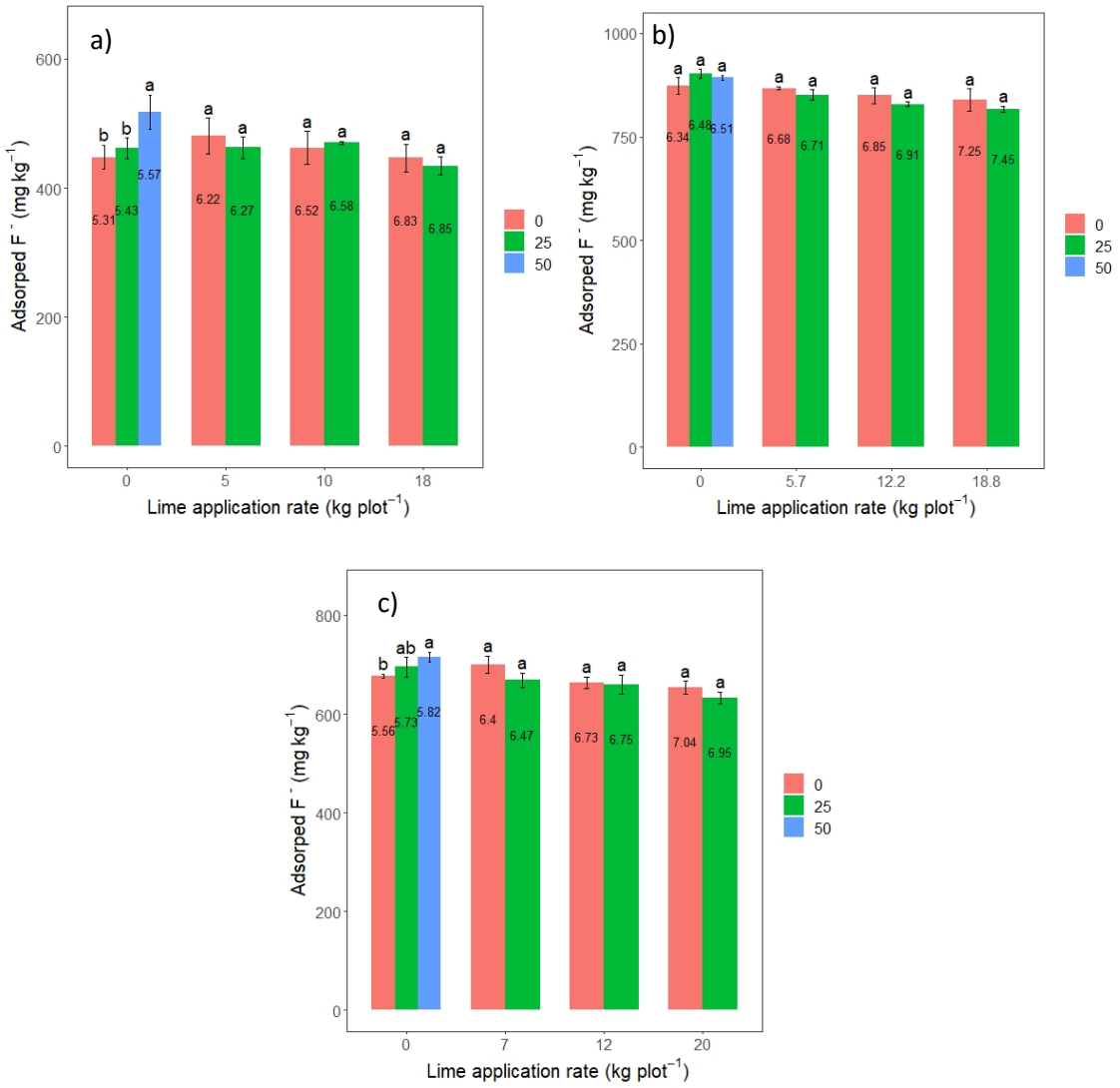


Figure 5. 6. Interaction effect of lime and compost application (0, 25, or 50 t ha^{-1}) on F^- adsorption (mg kg^{-1}) for a F^- concentration of 100 mg L^{-1}: a) Kairanga b) Pukekawa c) Canterbury. Numeric values presented inside the bars are soil pH. Error bars indicate standard error of the mean (n=3). Means with same letters are not significantly different at the $p > 0.05$ level (Tukey test) with in the lime application rates.

In the present study, the observation that F^- adsorption increased with compost addition when soil pH < 6 can be explained by F^- adsorption by organic matter via the cation bridge mechanisms (Quintáns-Fondo et al., 2016a). Romar-Gasalla et al. (2018) reported that organic matter can promote F^- adsorption by three different mechanisms: 1) free Fe and Al bind with the negatively charged sites of organic matter surfaces which then act as a

bridge between F^- and organic matter, 2) Organic matter can adsorp F^- via hydrogen bonds, 3) F^- can bind with protonated groups (- NH_4^+) of organic matter surfaces. The non-significant effect of compost addition on F^- adsorption at pH > 6 could be attributed to the instability of the cation bridge at higher soil pH. Al-F complexes are not stable at pH values above 6 (Gago et al., 2012) and, under alkaline conditions, OH^- competes with F^- to form Al-OH (Álvarez et al., 2009) reducing the rate of formation of Al-F complexes which bridge F with organic matter. Quintáns-Fondo et al. (2018) reported that F^- adsorption by oak wood ash was 100% at pH < 6, reducing to 30% at pH 10.

5.4.5 Effect of lime application on F^- adsorption

The effect of different soil pH levels on F^- adsorption, achieved through variable application rates of lime, is described in Figure 5.7. For the Kairanga soil, after the addition of 100 mg L^{-1} F^-, the highest F^- adsorption (494 mg kg^{-1}) occurred at a soil pH of 6.22, with adsorption being significantly ($p < 0.05$) reduced to 408 mg kg^{-1} at pH 7.37. A similar trend was observed for the Canterbury soil where the highest F^- adsorption concentration (700 mg kg^{-1}) was obtained at a soil pH of 6.40, with F^- adsorption significantly reducing to 624 mg kg^{-1} at a soil pH of 7.17. For the Pukekawa soil, the highest F^- adsorption concentration (907 mg kg^{-1}) was realised at a pH of 5.85, with F^- adsorption decreasing with increasing soil pH. Although soil pH varied between the different soils, maximum F^- adsorption was obtained across all soils at a pH range of 5.5 – 6.8. Lower F^- adsorption at lower soil pH levels can be explained by the formation of Al-F complexes (AlF^{2+}, AlF_2^+) which are repelled by positive charges on soil surfaces. At higher pH, lower adsorption rates occurred as a result of repulsion by the negatively charged soil surfaces (D'Alessandro et al., 2012).

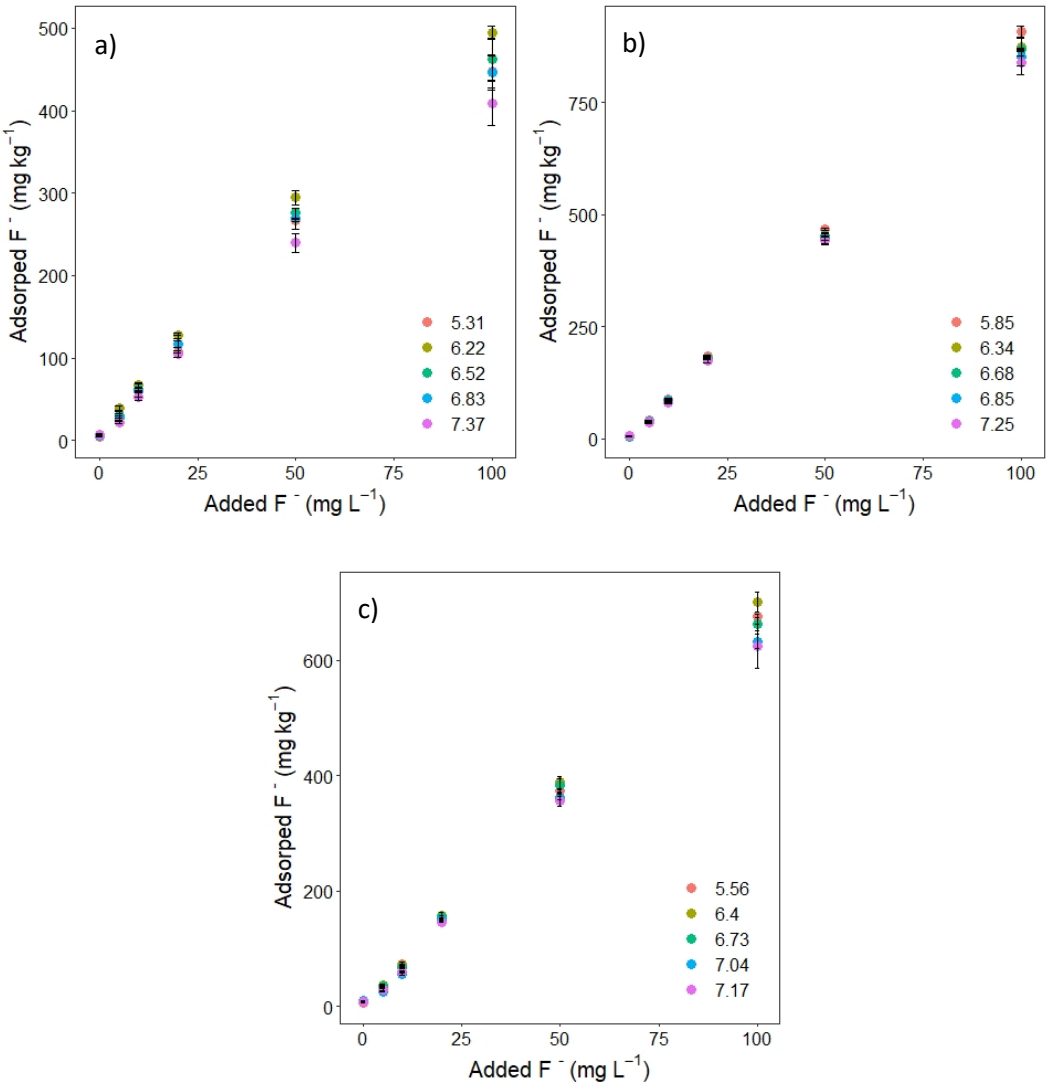


Figure 5. 7. The effect of soil pH on F^- adsorption as a function of the concentration of added F^-: a) Kairanga b) Pukekawa and c) Canterbury. Error bars indicate standard error of the mean (n=3).

5.4.6 Adsorption isotherm of F^-

The effect of soil amendments on F^- adsorption can be explained using an adsorption isotherm. An adsorption isotherm describes the relationship between the concentration of adsorbate on an adsorbent surface and in solution under equilibrium conditions (Wang et al., 2016). Langmuir and Freundlich isotherm models are widely used. The Langmuir model assumes adsorption takes place as a monolayer on a homogeneous surface, while the Freundlich isotherm assumes adsorption occurs on heterogeneous surfaces (Papageorgiou et al., 2006; Vargas et al., 2011; Wang et al., 2016). In the current study, where soil was amended with compost and lime, adsorption surfaces were heterogeneous, and therefore the Freundlich isotherm model is likely more suitable to explain observed results. Table 5.3 presents the Freundlich regression coefficients and R^2 values for lime and compost amended soils from the three locations. The Freundlich constant K_F is related to adsorption capacity (Ghorai and Pant, 2004; Lu et al., 2005; Bulut and Aydin, 2006; Quintáns-Fondo et al., 2016b). K_F is the ratio of adsorbate adsorbed to adsorbate remaining in soil solution, and can be used to compare adsorption among the treatments (Maiti et al., 2011; Shafqat and Pierzynski, 2014). In the present study, K_F (adsorption capacity) was used to describe the F^- adsorption effect of the compost and lime amended soils.

F^- adsorption capacity varied between the unamended (native) control soils. The Pukekawa soil had a higher F^- adsorption capacity (98.59) than the Canterbury (47.25) and Kairanga soil (32.82).

Table 5. 3. Freundlich constants and coefficients for different lime and compost application to three soils.

Location	Application amendments		Observed pH	Freundlich		
	Lime/S (kg plot^{-1})	Compost (kg ha^{-1})		K_F	*1/n*	R^2
Kairanga	0	0	5.31	23.52	0.7405	0.992
		25	5.43	28.12	0.7072	0.995
		50	5.57	32.82	0.7141	0.994
	5	0	6.22	34.71	0.6829	0.995
		25	6.27	36.02	0.6467	0.994
	10	0	6.52	26.99	0.7185	0.989
		25	6.58	28.60	0.7107	0.995
	18	0	6.83	26.49	0.7105	0.991
		25	6.85	30.61	0.6644	0.992
	25	0	7.37	18.89	0.7587	0.990
Pukekawa	1.1(S)	0	5.85	139.00	0.9354	0.971
	0	0	6.34	98.59	0.8946	0.973
		25	6.48	125.50	0.8705	0.964
		50	6.51	113.60	0.9028	0.965
	5.7	0	6.68	95.09	0.8934	0.971
		25	6.71	82.97	0.8955	0.963
	12.2	0	6.85	89.40	0.8524	0.973
		25	6.91	71.02	0.8934	0.981
	18.8	0	7.25	80.38	0.8586	0.962
		25	7.45	67.63	0.8848	0.978
Canterbury	0	0	5.56	47.25	0.7765	0.993
		25	5.73	55.49	0.7512	0.993
		50	5.82	61.07	0.7453	0.993
	7	0	6.40	51.10	0.7788	0.984
		25	6.47	48.60	0.7621	0.985
	12	0	6.73	50.18	0.744	0.981
		25	6.75	44.48	0.7745	0.983
	20	0	7.04	43.81	0.7508	0.985
		25	6.95	40.52	0.7815	0.980
	30	0	7.17	38.27	0.7792	0.976

S - Elemental sulphur

Unit for K_F - (mg/g) $(L/mg)^{1/n}$

Application of compost increased F^- adsorption capacity in the Kairanga soil (from 23.52 to 32.82) and Canterbury (from 47.25 to 61.07) soils (Table 5.3). Maximum F^- adsorption capacity (36.02) was obtained at a soil pH of 6.2 for the Kairanga soil, with F^- adsorption capacity decreasing with increasing soil pH from 6.2 to 7.3 (K_F 34.71 – 18.89). A similar

pattern was observed for the Canterbury soil where the soil at pH 5.82 had maximum F^- adsorption capacity (51.10) which decreased to 38.27 at soil pH 7.17. For the Pukekawa soil, maximum F^- adsorption was recorded at soil pH 5.85 (K_F = 139.00) and F^- adsorption decreased as a function of increased soil pH (K_F 139.00 – 67.63).

5.4.7 Effect of compost and lime applications on F^- desorption

The effect of compost and lime application on F^- desorption is presented in Figure 5.8 and Figure 5.9. Desorption percentages have been calculated in previous studies based on the amount of F^- adsorbed at the rate of maximum F^- concentration added to the soil (Gago et al., 2014; Quintáns-Fondo et al., 2016a). In the present study, F^- desorption percentages, as a function of different rates of lime and compost amendments, were determined based on the amount of F^- adsorbed for an added F^- concentration of 100 mg F^- L^{-1}. Without amendment, the Pukekawa soil desorbed 9.1% of adsorbed F^-, while Kairanga and Canterbury soils desorbed 35.1% and 24.8% of adsorbed F^-, respectively. Variable desorption between the soils can be explained by the strong adsorption capacity of non-crystalline Al and Fe oxyhydroxides. The Pukekawa soil had higher levels of non-crystalline Al and Fe, and it is well known that F^- is mainly adsorped by non-crystalline Fe and Al (Gago et al., 2012). Gago et al. (2014) reported that F^- adsorbed by Al and Fe shows little potential for desorption. For all three soils (without lime application), desorption percentages were not significantly different ($p > 0.05$) from each other as a function of compost application rate. In the current study, compost application significantly increased F^- adsorption for the Kairanga and Canterbury soils, but did not change the percentage of desorbed F^-. This result suggests that compost is a potential soil amendment to minimise F^- availability. In contrast, for the Pukekawa soil, F^- adsorption

and the percentage of desorbed F^- did not vary with increasing compost application. This indicates that the potential for F^- adsorption by compost is soil pH dependent.

Variation in soil pH as a result of the application of different rates of lime significantly influenced F^- desorption for all three soils (Figure 5.9). In the Kairanga soil, 57% of adsorbed F^- was desorbed at a soil pH of 7.37, but there was less F^- desorption at soil pH range 5.31 and 6.51 (35 and 44%, respectively). For the Pukekawa soil, F^- desorption at pH 7.25 (14%) was higher compared with F^- desorption in the pH range 5.31 and 6.22 (7 and 9%, respectively). This same trend was observed for the Canterbury soil where a higher fraction of F^- was desorbed (34%) at pH 7.17 compared to 25 and 26% at pH 5.56 and 6.4, respectively. Increased desorption at higher soil pH levels could be explained by repulsion of F^- by negative charges developed in soils at higher soil pH (Loganathan et al., 2001).

For all three soils, a higher percentage of F^- adsorption and lower percentage of F^- desorption recorded for a soil pH range from 5.5 to 6.8 suggests that soil solution F^- concentrations can be controlled by applying specific rates of lime to these New Zealand soils.

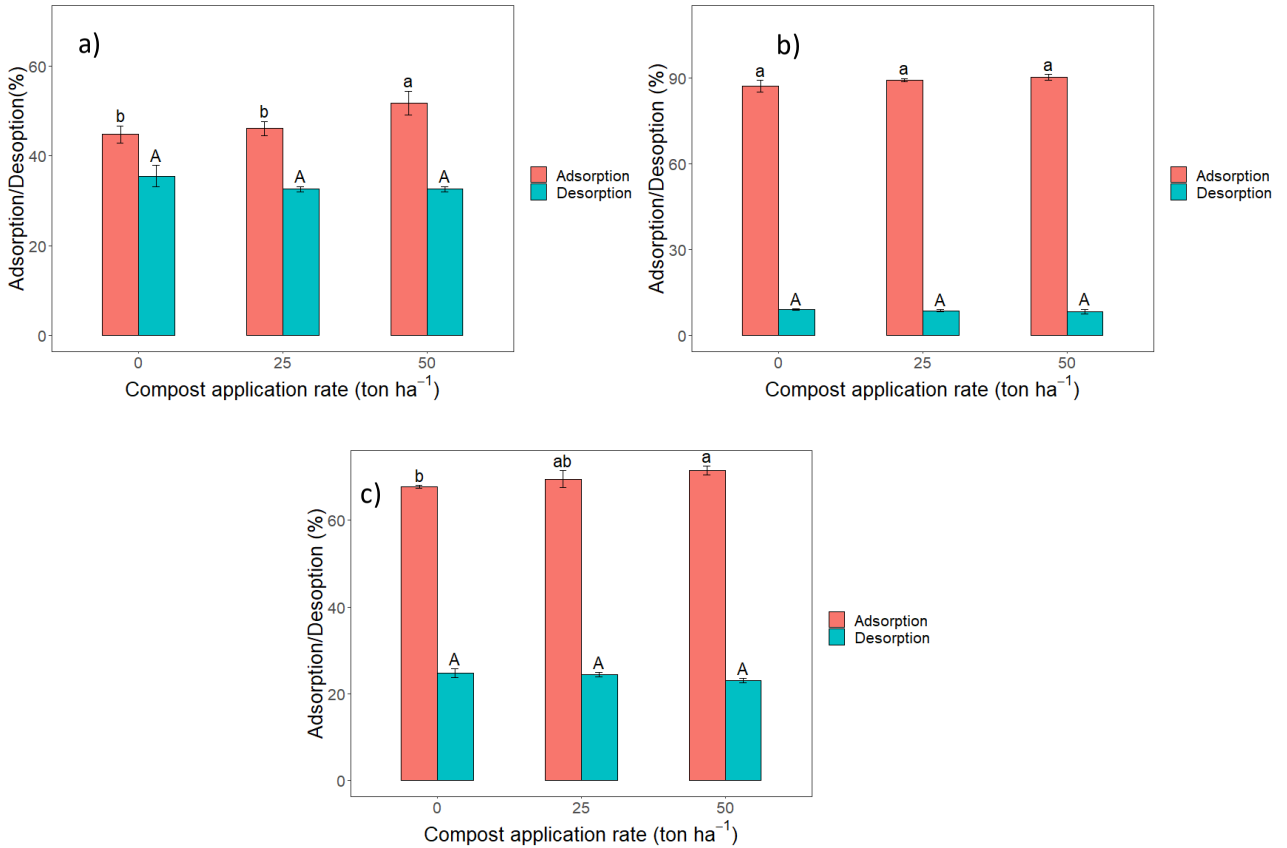


Figure 5. 8. Fluoride desorption (percentage of the amounts previously adsorbed) and F^- adsorption percentages for each compost application rate as a function of adding 100 mg L^{-1} F^-: a) Kairanga b) Pukekawa and c) Canterbury. Error bars indicate standard error of the mean (n=3). Means with same letters (upper case letters for desorption and lower case letters for adsorption) are not significantly different at the $p > 0.05$ level (Tukey test).

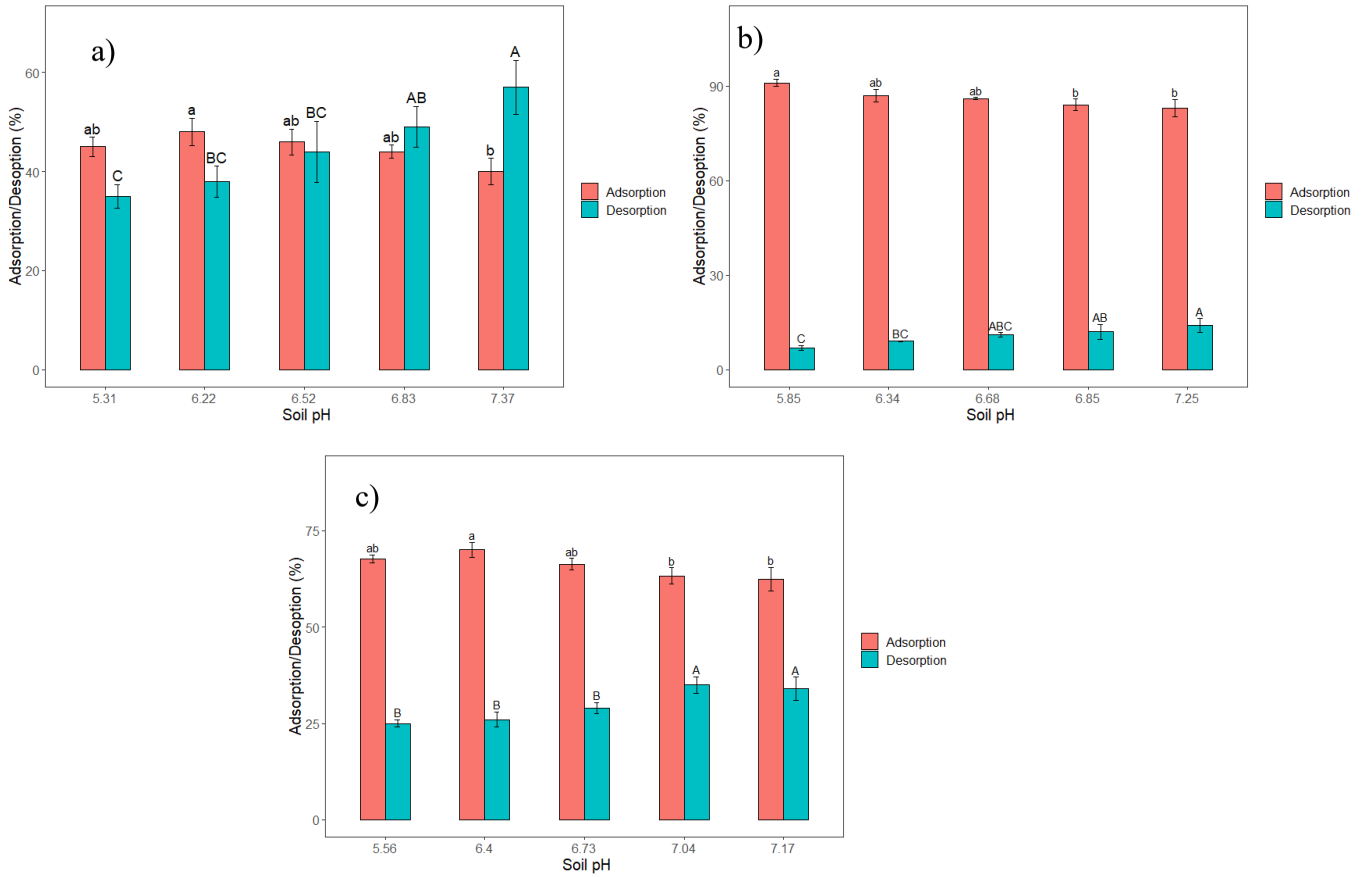


Figure 5. 9. Fluoride desorption (percentage of the amount previously adsorbed) and F^- adsorption percentages for different soil pH levels as a function of adding 100 mg L^{-1} F^- : a) Kairanga b) Pukekawa and c) Canterbury Error bars indicate standard error of the mean (n=3). Means with same letters (upper case letters for desorption and lower case letters for adsorption) are not significantly different at the $p > 0.05$ level (Tukey test).

5.5 Conclusions

Different rates of lime and compost were applied to three New Zealand soils in the field, and F^- adsorption behaviour was tested on these soils in the laboratory. A batch-type experiment confirmed that the soil pH required for maximum F^- adsorption varied with soil type and ranged between pH 5.5 – 6.8.

Fluoride adsorption in a compost-amended soil is pH dependent. Compost can be used to minimise the free soil F^- concentration at a soil pH less than 6.0 (by maximising adsorption of F^- to soil surfaces). However, compost is not suitable for minimising the soil solution F^- concentration when the pH is above 6.0 due to increased rates of desorption.

Soil solution F^- concentrations can be reduced through lime applications that increase soil pH. These application rates could be set based on a target soil pH for maximum F^- adsorption.

F^- adsorption and desorption is influenced by both soil pH and the Al and Fe content of the soil. The results from the current study should aid development of soil management practices to minimise the concentration of F^- in soil solution.

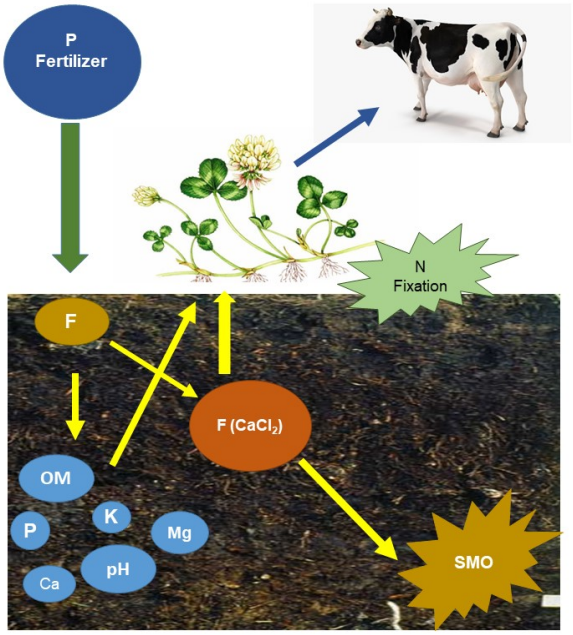
Acknowledgement

We thank The Fertiliser Association of New Zealand and the Ministry for Primary Industries for support of this project.

CHAPTER 6

Consequences of fluorine (F) accumulation in an Allophanic soil

6.1 Graphical Abstract



6.2 Abstract

Allophanic soil is one of the most productive soil orders in New Zealand. However, to support production, high rates of phosphate fertiliser have been used to overcome this soil order's high phosphorous (P) retention and this has resulted in high rates of F accumulation to these soils. To quantify the effect of added F on soil properties, soil microbial activity, and white clover growth, a bulk sample of Allophanic soil (478 mg kg^{-1} total F) was collected from a dairy farm near Hawera in Taranaki. Replicate greenhouse

pots were amended with increasing rates of F to model continuous long-term P application over 10-50 years, then planted with two varieties of white clover (Merlyn and Tribute).

Soil pH, dissolved organic carbon and $CaCl_2$-extractable F values increased as a function of treatment and were significantly ($p < 0.05$) correlated with the added F concentration. On average, only 1.49% of added F was extracted by 0.01 mol L^{-1} $CaCl_2$ suggesting that the major fraction of F added as NaF was immobilised in the soil. Soil microbial biomass carbon and soil enzyme activities were not significantly ($p > 0.05$) affected by the added F, and for both clover varieties there was no effect of F on shoot and root growth ($p > 0.05$). Shoot and root F concentration significantly ($p < 0.05$) increased as a function of increasing $CaCl_2$-extractable F concentration, and this was correlated with the concentration of added F. TEM and LM micrographs confirmed that the white clover – *Rhizobium* interaction was not influenced by added F concentrations for both varieties. Increasing soil pH and DOC as a result of F addition may impact on soil management, however this study shows that F added to Allophanic soil at rates equivalent to up to 50 years of continuous phosphate fertiliser application has no negative influence on soil microorganism activity, white clover growth and N fixation in Allophanic soil.

Key words: Microbial biomass carbon; Allophanic soil; White clover; Soil fluorine

6.3 Introduction

Soils derived from volcanic parental material represent a significant natural resource for New Zealand agriculture, and make up the land for more than 40% of the New Zealand animal herd (Hedley et al., 2011). Allophanic soil, a specific soil order derived from volcanic parent material, is characterized by high contents of non–crystalline Al, Fe and organic matter, and has a higher phosphorus fixing capacity (> 84%) than non – Allophanic soils (Jackman et al., 1964; Fraser and Vesely, 2011). However, this high phosphorus retention capacity requires high phosphorus fertiliser application rates to maintain pasture productivity. The consequence is increased F deposition rate (10 kg F ha^{-1} yr^{-1}) relative to other soil orders (Cronin et al., 2000; Loganathan et al., 2003; Loganathan et al., 2008; Kim et al., 2016). The non-crystalline Al and Fe content of Allophanic soil results in high F retention as non-crystalline Al and Fe hydrous oxides strongly adsorb F (Cronin et al., 2000).

The accumulation of F in soil has the potential to change soil properties. Pot experiments have shown that soil pH increased from 4.9 to 5.7 with increasing F^- addition from 0 to 200 mg kg^{-1} (Arnesen, 1997). Romar-Gasalla et al. (2018) reported that increased F^- concentration in a vineyard soil (1140 mg kg^{-1}) was associated with a significantly increased dissolved organic carbon concentration (increased by 30 mg L^{-1} relative to a control soil). Such changes in soil properties as a result of F accumulation may have implications on land use. An elevated soil F concentration can also directly and indirectly influence soil microbial activities (Tscherko and Kandeler, 1997; Ropelewska et al., 2016). Microorganisms are one of the important components of a soil ecosystem and are vital for environmental functions. Ropelewska et al. (2016) reported that microbial

biomass carbon significantly reduced from 664 to 616 mg C kg^{-1} at a F concentration of 4000 mg kg^{-1}. Tscherko and Kandeler (1997) reported that Arylsulphatase enzyme activity was suppressed at a water-extractable F concentration of 20 mg kg^{-1}. Despite the general understanding of the impact of F on soil properties, the impact of F accumulation on the chemical, physical and biological properties of New Zealand Allophanic soils is poorly studied.

Increasing F concentration also affects plant yield, dry matter production, photosynthesis activity, seed germination, seedling growth and transpiration (Fornasiero, 2001; Jha et al., 2009; Chakrabart et al., 2012; Clausen et al., 2015). Jha et al. (2008) reported that, at a F concentration of 362 mg kg^{-1}, Spinach (*Spinacea oleracea*) root and shoot biomasses were significantly decreased from 1 to 0.7 g $plant^{-1}$ and 4 to 3.3 g $plant^{-1}$, respectively. New Zealand's dairy farms are mainly pasture-based, and clover and ryegrass pastures are the major component in this system (Verkerk, 2003). Any negative impacts on pasture production have the potential to cause a consequential reduction in milk production. Despite the potential risk, the impact of increasing soil F concentration on pasture production for New Zealand soil conditions has not yet been studied (Gray, 2018).

In previous studies, the toxic effects of F on plants and microorganisms have been analysed by adding F salts (NaF). However, the bioavailable F concentration in soil amended with F salts has not been compared with that for fertiliser-derived F (Gray, 2018). Information about the relationship between added F concentration and the bioavailable F concentration in New Zealand agriculture soils and its effects on soil microorganisms and pasture is vital to assess the magnitude of any detrimental affect of phosphate fertiliser-derived F on soil microorganisms and pasture.

In response to this need for information, the work described in this chapter was conducted to investigate: 1) the effect of F accumulation on Allophanic soil properties; 2) the relationship between the added F concentration and the bioavailable F concentration in New Zealand soil; 3) the effect of F addition on white clover growth and nodulation; and 4) the effect of F accumulation on soil microbial activity.

6.4 Materials and Methods

6.4.1 Soil sampling

Allophanic soil used in this work was collected from a dairy farm near Hawera in Taranaki, with a history of long-term phosphate fertiliser application. Soil samples were collected from a paddock under white clover pasture to ensure the availability of *R. leguminosarum* in soil. Soil samples were collected from linear transects across the paddock at 0 – 15 cm depth. Samples were mixed to form a single composite sample. Following transport to the laboratory, pasture, root and other materials were eliminated from the sample by passing through a 4 mm sieve, with the sieved soil stored in a black polyethene bag at 4 ^{o}C in a cool room until use in a pot experiment.

6.4.2 Experimental design and treatments

The effect of F on white clover growth was assessed in a pot experiment where two white clover varieties (Tribute and Merlyn) and six different F concentrations were used. Each treatment was replicated three times and the experimental lay-out was arranged in a complete randomised block design. The pots were filled with 1 kg of soil and amended

with NaF to achieve different F concentrations of 77, 154, 231, 308 and 385 mg F kg^{-1} of soil. These F concentrations are equivalent to F accumulation as a result of long-term phosphate application from 10 to 50 years (assuming SSP consists 1.5% of F and soil bulk density = 780 kg m^{-3}). Soil with no F treatment was the control treatment. Three white clover seedlings (2 weeks old) were transplanted in each pot. Throughout the experiment soil moisture content was maintained at 80% 'pot field capacity' and the temperature was maintained at 16 ± 6 °C in a plant growth unit. The experiment was continued for 10 weeks after planting. At harvest, bulk and rhizosphere soil samples, root and above-ground biomass from each pot were collected.

6.4.3 Soil microbial analysis

Soil quality can be quantified through measuring microbial biomass carbon and the activity of soil enzymes (Vallejo et al., 2010; Velmourougane et al., 2014), and these indicators were used in the current work to investigate the effect of added F on soil microbial activity. Microbial biomass measures the amount of microbial cells in soils (Nogueira et al., 2006), and is influenced by F (Ropelewska et al., 2016).

6.4.3.1 Microbial biomass carbon

Soil microbial biomass C was measured using the chloroform fumigation and extraction method proposed by Vance et al. (1987). Briefly, moist soil subsamples equivalent to 50 g oven-dried soil were weighed in a 100 mL glass beaker and were fumigated in a desiccator with 25 mL of ethanol-free chloroform for 24 hours. Non-fumigated and fumigated soil samples (equivalent to 5 g of oven dry weight) were placed into 50 mL centrifuge tubes

and 20 mL of 0.5 mol L^{-1} K_2SO_4 was added. The centrifuge tubes were shaken for 30 minutes in an end-over-end shaker then centrifuged at 11 872 g for 5 minutes. The supernatant was then passed through Whatman No. 42 filter paper. Extracted C concentrations were analysed for TOC by using TOC - TN analyzer (TOC-V-CPN; Shimadzu Scientific Instruments Inc., Columbia, MD, USA).

6.4.3.2 Urease activity

The urease enzyme activity of soil samples was measured using the method of Shcherbakova (1983) where soil samples are incubated with urea solution for 4 hours at 37 ºC, before the amount of NH_4-N released is determined. Briefly, moist soil equivalent to 0.25 g of oven dried soil was placed in a 10 mL glass tube. Urea (2 mL of 0.3 mol L^{-1}) in phosphate buffer and 20 µL of toluene were added, mixed, and the preparation incubated for 4 hours at 37 ºC. At the end of the incubation, 100 µl of 50 % Trichloroacetic acid and 5 mL of 1 mol L^{-1} KCl were added and the reaction mixture passed through Whatman No. 1 filter paper. Supernatant (0.2 mL) was added to a 10 mL tube containing 0.2 mL of 50% Seignette reagent and 0.2 mL Nessler's reagent (Potassium tetraiodomercurate) in 4.4 mL of water. The NH_4-N concentration of the sample solution was measured using a spectrophotometer (DU-640; Beckman, Krefeld) at absorbance 400 nm.

6.4.3.3 Alkaline phosphatase (ALP) activity

Alkaline phosphatase (ALP) activity and Acid phosphatase (ACP) activity in soil were quantified using the procedure described by Tabatabai and Bremner (1969) where soil is

incubated with 4-Nitrophenylphosphate at 37 ^{o}C for 1 h, before the concentration of p–nitrophenol released by the soil is measured. Briefly, moist soil equivalent to 0.1 g of oven dry weight was added to a 10 mL tube. Successively, 2 mL of modified universal buffer (MUB, pH 11), 25 µL Toluene and 0.5 mL p-nitrophenyl phosphate were added, and the mixture incubated at 37 ^{o}C for 1 h. At the end of incubation, 0.5 mL of 0.5 mol L^{-1} $CaCl_2$ and 2 mL of 0.5 mol L^{-1} NaOH were added to stop the reaction and the sample solutions were passed through Whatman No. 1. The concentrations of p-nitrophenol in the filtered solutions was measured using a spectrophotometer (DU-640; Beckman, Krefeld) at absorbance 420 nm.

6.4.3.4 Acid phosphatase (ACP) activity

Acid phosphatase (ACP) activity in soil was measured following the same method as ALP, with the adjustment of the modified universal buffer pH to 6.5 using 0.1 mol L^{-1} HCl.

6.4.3.5 Peroxidase activity

Soil peroxidase activity was quantified following the procedure described by Garbuz et al. (2016) where hydroquinone is oxidized through incubation to 1–4-p-benzoquinone and measured. Briefly, moist soil equivalent to 0.25 g oven dry weight was placed in a 10 mL tube and 2.5 mL of 0.1 mol L^{-1} Hydroquinone and 0.25 mL of 5 % hydrogen peroxide were added. The substrate was incubated at 30 ºC for 30 minutes. At the end of the incubation, the enzyme reaction was stopped by addition of 2.5 mL of ethanol (96%)

before the mixture was filtered through Whatman No 1. The concentration of the released benzoquinone was determined using a Shimadzu UV-1800 spectrophotometer at 450 nm.

6.4.3.6 Polyphenol oxidase

The polyphenol oxidase activity in soil was measured following the same procedure used for peroxidase activity, but without the addition of hydrogen peroxide to the incubation.

6.4.3.7 Dehydrogenase activity

Dehydrogenase activity in soil was measured following the procedure described by Brookest and Chander (1991) where a soil sample is incubated with 2,3,5-triphenyl tetrazolium chloride (TTC) and total microbial activity is quantified as a function of the reduction of TTC to tri phenyl formazan (TPF). Briefly, moist soil equivalent to 4 g of oven dry soil was weighted into 35 mL plastic screw top containers, and 2 mL of TTC (3%) and 0.1 g $CaCO_3$ were added to the reaction. The sample mixtures were incubated at 28 °C for 24 h. After 24 hours, 15 mL of methanol was added to stop the reaction and the mixture was filtered through Watman No 1. The concentration of TPF released by soil was measured using a spectrophotometer (DU-640; Beckman, Krefeld) at 485 nm.

6.4.4 Soil analysis

A bulk sub-sample of the field-collected soil was air dried and passed through a 2 mm stainless steel sieve before analysis. Soil pH was determined (soil: water ratio 1: 2.5) using a Eutech Instruments Cyber Scan pH 310 meter.

The non-crystalline Fe and Al content of the soil was measured using the method described by Blakemore et al. (1987). Air-dried soil (0.4 g) and 40 mL of acid oxalate extractant (pH = 3) were added to a 50 mL centrifuged tube. The mixture was shaken on an end-over-end shaker for 4 hours in the dark then centrifuged at 11 872 g for 10 minutes. The resulting supernatant was filtered through Whatman No. 42 filter paper, and the concentration of extracted Fe and Al was measured by MP-AES (Agilent 4200, Germany).

The concentration of exchangeable cations in soil was quantified following soil leaching with 1 mol L^{-1} ammonium acetate (pH 7) (Blakemore et al., 1987). Briefly, 1 g of air-dried soil was mixed with 3 g of acid-washed sand and added into a leaching tube. Ammonium acetate (1 mol L^{-1} pH = 7) was passed through the leaching tube at the rate of 1 mL per minute and the leachate (50 mL) was collected in a plastic container. The concentration of Ca^{2+}, Mg^{2+}, K^{+} and Na^{+} in the leachate solution was measured by MP-AES (Agilent 4200, Germany).

Total N (expressed in N%) and soil organic carbon (expressed in C%) were measured using a vario MACRO cube CHNS elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany). The soil organic C was multiplied by two to obtain OM% (Pribyl, 2010).

The Olsen P concentration in soil was measured following the procedure described by Olsen et al. (1954). Briefly, 1g of soil was placed in a 50 mL centrifuge tube and extracted with 20 mL of 0.5 mol L^{-1} $NaHCO_3$ (pH 8.5) for 1 h using an end-over-end shaker. The extracted samples were centrifuged at 11 872 g for 5 min and the supernatant was filtered

through Whatman No 42. Subsequently, 4 mL of each sample extract was added to a 50 mL volumetric flask and 10 mL deionised water was added. Freshly prepared Murphy and Riley's reagent (10 mL) was added, and the volume made to 50 mL with deionised water. After mixing, the samples were allowed to stand for colour development, and the P concentration was measured using a spectrophotometer (DU-640; Beckman, Krefeld) at 712 nm.

The concentration of dissolved organic carbon in soil was quantified through end-over-end extraction (1 hour) of 5 g of soil with 25 mL of distilled water in a 50 mL centrifuge (Jones and Willett, 2006; Chantigny et al., 2014). The extraction was centrifuged at 7000 g for 10 min and the supernatant of each sample was filtered through Whatman No. 42 before the DOC concentration was measured using a TOC-TN analyzer (TOC-V-CPN; Shimadzu Scientific Instruments Inc., Columbia, MD, USA).

Total soil F was measured using the 4 mol L^{-1} NaOH extraction method described by Geretharan et al. (2018) [Chapter 3]. Briefly, 0.5 g soil was extracted with 4 mol L^{-1} NaOH at 100 °C. The residue was dissolved in water and the pH adjusted to 8.5. Following the addition of TISAB buffer, the F^- concentration was measured using an F^- specific ion electrode. The concentration of bioavailable F in soil was quantified through extraction with 0.01 mol L^{-1} $CaCl_2$ which was recommended by Geretharan et al. (2018) [Chapter 3] as suitable for soil with a high Fe/Al content. Soil (5 g) was extracted with 37 mL of 0.01 mol L^{-1} $CaCl_2$ in a reciprocating shaker for 16 h (Arnesen, 1997). The suspension was centrifuged (7600 for 30 min) and filtered through a Whatman No.2 paper, the supernatant F^- concentration was measured using a F^- specific ion electrode after addition of TISAB buffer.

6.4.5 Plant analysis

Freshly harvested white clover shoots were rinsed with distilled water to remove soil particles and blotter dried using filter paper. Shoot and root length, and fresh weight, were then measured. Roots were carefully washed with tap water to remove soil particles and the number of nodules per plant was counted. Plant dry weights were recorded following oven drying at 70 °C for 72 h.

The nodulation index has been proposed as a biological indicator to measure the impact of soil contaminants on *Rhizobium* – legume symbiosis (Manier et al., 2009), and has been suggested as an indicator to measure soil quality. In the present study, nodulation index was used as a parameter to quantify the effect of added F on the white clover – *Rhizobium* interaction. Nodulation index was determined as the total number of nodules divided by fresh biomass.

Nodule micrographs were recorded using Transmission Electron Microscopy (TEM) and Light Microscopy (LM) to visualise the legume – *Rhizobium* symbiosis (Procedures are described in Chapter 4 section 4.3.3.1).

The plant F concentration was measured using the procedure described by García-Ciudad et al. (1985). Briefly, dry plant samples were ground using a ball grinder to reduce the sample particle size below 1 mm. Approximately 0.2 g of ground plant sample was transferred to a 50 mL plastic screw top container and 10 mL of 0.1 mol L^{-1} perchloric acid was added. The mixture was shaken vigorously and kept on a desk overnight. The

sample F^- concentration was measured using a F^- specific ion electrode after addition of TISAB buffer. In this method, the recovery percentage of F^- was 95 – 103%.

6.4.6 Statistical procedure

All measured data were described as mean ± standard error (SE). To test significant among the treatments, data were subjected to an Analysis of Variance procedure (ANOVA) followed by Tukey test to compare the mean. Regression analysis was used to investigate the relationship between root and shoot F concentrations, and extractable F concentrations. Two-sample T test was performed to examine the difference between root and shoot F concentration. All statistical tests were performed at a significant level of 0.05. Minitab statistical software version 18 (Minitab Inc, USA) was used for the statistical analyses.

6.5. Results and Discussion

6.5.1 Effect of F addition on total and bioavailable F concentration

The F concentration of the soil used in this study was 478 mg kg^{-1} and the total soil F concentration significantly ($p < 0.05$) increased with F addition (Figure 6.1). At an added F concentration 385 mg kg^{-1}, the mean total soil F concentration was 823 mg kg^{-1}. As a linear relationship was obtained between the soil total F concentration and added F concentration, the results are discussed in this chapter with respect to the added F concentration.

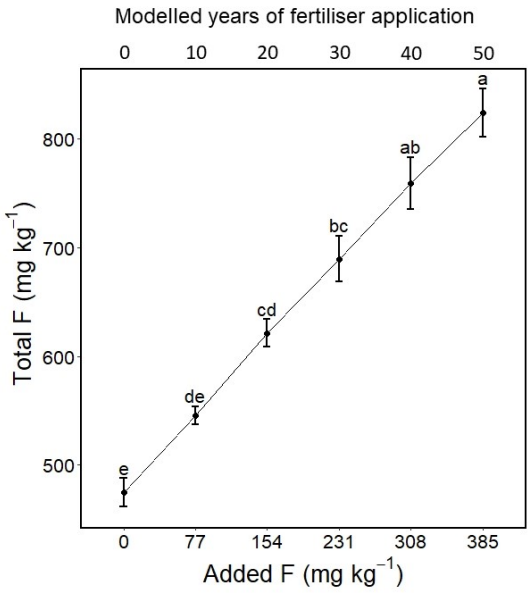


Figure 6. 1. Effect of F addition on the total soil F concentration. Vertical bars indicate standard error of mean (n = 6). Means with same letters are not significantly different at the $p > 0.05$ level (Tukey test).

The concentration of bioavailable F was quantified in the current study through extraction with 0.01 mol L^1 $CaCl_2$. The bioavailable F concentration was correlated with the added F concentration ($p < 0.05$), and ranged from 4.9 to 12.71 mg kg^{-1} (Figure 6.2).

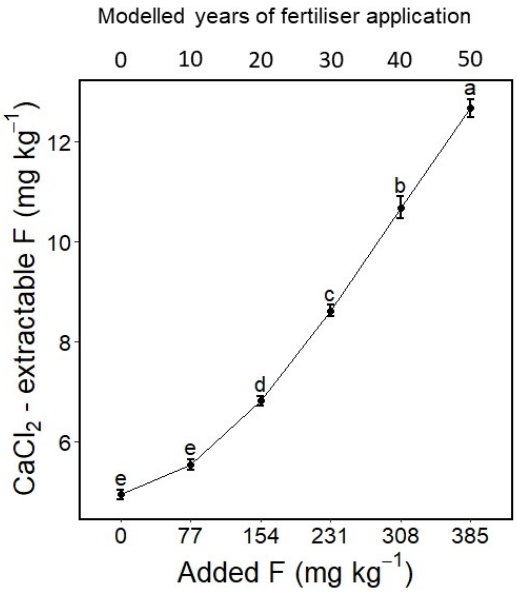


Figure 6. 2. Effect of F addition on the $CaCl_2$- extractable F concentration in soil. Vertical bars indicate standard error of mean (n = 6). Means with same letters are not significantly different at the $p > 0.05$ level (Tukey test).

The $CaCl_2$ - extractable F concentration of the amended soil was not significantly ($p > 0.05$) different to the control soil for the 77 mg kg^{-1} treatment, but significantly ($p < 0.05$) increased for the higher treatments. These results are in agreement with the study of Jha et al. (2008) who reported that the $CaCl_2$-extractable F concentration did not increase as a function of added F up to a treatment concentration of 50 mg NaF kg^{-1} but increased significantly ($p < 0.05$) at greater treatment. On average, F extracted by $CaCl_2$ was 1.49% of the added F, and suggests that the major fraction of F added as NaF was immobilised by the Allophanic soil. However, for all treatments, a constant % of added F remained bioavailable. In the present study, the ability of the soil to buffer changes in bioavailable F is likely due to retention of this F to binding sites which effectively immobilised added F. But the effect of this buffering continued to decrease as F rates increased (Figure. 6.2). This may be due to the binding (adsorption) becoming

increasingly saturated with the increasing load of F allowing more of the subsequently added F to remain in solution (bioavailable).

The maximum recorded bioavailable F concentration in the current study was 12.7 mg kg^{-1} for the 385 mg kg^{-1} total soil F treatment. This treatment models 50 years of continues phosphate fertiliser application at an application rate recommended for a soil. This bioavailable F concentration is in agreement with reported bioavailable F concentrations of New Zealand pasture soils which have long-term phosphate fertiliser application history. Geretharan et al. (2018) [Chapter 3] measured the bioavailable F concentration of soils representing different soil orders in New Zealand and reported that bioavailable F concentrations range between 1.70 to 6.45 mg kg^{-1}. However, the results of this 10 week study are not expected to be the same as that of 50 years of fertiliser application, even though the total F applied is the same. This is because most of the adsorbed F in field soils is likely to have diffused into the interior of the clay minerals/metal oxides (occluded) and formed new minerals over the 50 years. This would lead to a differential equilibrium between adsorbed F and the solution F. The observations here account for the difference between the maximum bioavailable F concentration in this study (12.7 mg kg^{-1}) and the field soils (6.45 mg kg^{-1}).

6.5.2 Effect of added F on Dissolved Organic Carbon (DOC)

In the current study, DOC ranged from 271 to 331 mg kg^{-1}, and significantly ($p < 0.05$) increased with increasing soil F concentration from 154 mg kg^{-1} to 385 mg kg^{-1} (Figure 6.3).

Changes in DOC may be due to the replacement of organic matter bound to Al or Fe or clay minerals by F^-. Romar et al. (2009) reported that F addition to soil increased soil solution organic matter via two possible reactions:

1) Clay-(Fe/Al)-OM + F^- → Clay-(Fe/Al)-F + OM; and

2) Clay-(Al/Fe)-OM + F^- → Clay + F-(Al/Fe)- OM.

DOC is the bioavailable soil carbon fraction for soil microorganisms (Marschner and Kalbitz, 2003) and increasing DOC concentrations due to F addition may have a potential positive effect on soil microbial activity. Tscherko and Kandeler (1997) reported that increasing DOC as a result of F addition to soil can overcome the negative effects of increasing F concentrations on soil microorganisms.

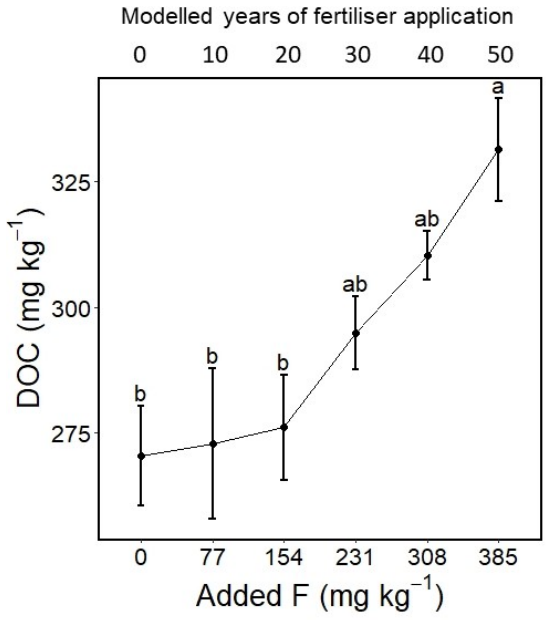


Figure 6. 3. Effect of F addition on DOC in soil. Vertical bars indicate standard error of mean (n = 6). Means with same letters are not significantly different at the $p > 0.05$ level (Tukey test).

6.5.3 Effect of added F on soil pH

Addition of F to soil significantly ($p < 0.05$) increased the soil pH with the magnitude of this effect greater at the highest rate of F addition. Soil pH for the 385 mg kg^{-1} F treatment was 0.4 pH units greater than the control soil (Figure 6.4). Changes in pH may be due to the substitution of OH^- bound to Fe or Al by F^-, increasing the OH^- ion concentration in soil solution (Harrington et al., 2003).

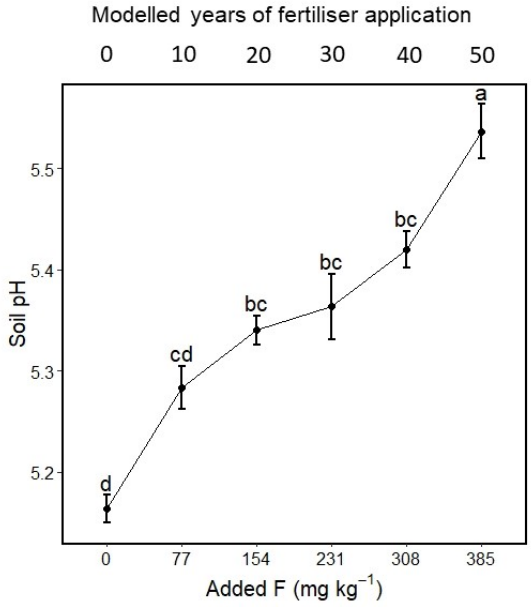


Figure 6. 4. Effect of F concentration on soil pH. Vertical bars indicate standard error of mean (n = 6). Means with same letters are not significantly different at the $p > 0.05$ level (Tukey test).

6.5.4 Effect of added F on exchangeable Ca, Mg, K and Olsen P

Exchangeable Ca, Mg and K concentrations ranged between 7.85 – 7.50, 2.15 – 2.23 and 0.57 – 0.63 meq 100 g^{-1}, respectively and were not significantly ($p > 0.05$) influenced by the added F concentration. Similarly, the Olsen P concentration ranged from 19.04 to 20.6

mg kg^{-1} and was not significantly ($p > 0.05$) influenced by the F concentration of the soil. Olsen P measures the plant-available P concentration in soils (Rivaie et al., 2008) and this can be linked toh the P retention capacity of the soil. Zhu et al. (2007) reported that F addition does not induce P desorption as P has a greater affinity for soil surfaces than F.

Table 6. 1. Effect of F concentration on exchangeable Ca, Mg, K and Olsen P in soil.

Added F mg kg^{-1}	Exchangeable Ca meq 100 g^{-1}	Exchangeable Mg meq 100 g^{-1}	Exchangeable K meq 100 g^{-1}	Olsen P mg kg^{-1}
0.00	7.06 ± 0.200[a]	2.15 ± 0.040[a]	0.60 ± 0.039[a]	20.69 ± .295[a]
77	7.25 ± 0.084[a]	2.23 ± 0.049[a]	0.61 ± 0.026[a]	19.98 ± .673[a]
154	7.20 ± 0.146[a]	2.16 ± 0.044[a]	0.59 ± 0.028[a]	20.06 ± .816[a]
231	7.50 ± 0.065[a]	2.22 ± 0.038[a]	0.57 ± 0.021[a]	19.41 ± .579[a]
308	6.85 ± 0.397[a]	2.16 ± 0.048[a]	0.61 ± 0.027[a]	19.04 ± .272[a]
385	6.95 ± 0.114[a]	2.21 ± 0.032[a]	0.63 ± 0.020[a]	19.85 ± .440[a]

Mean with same letters are within elements are not significant at the $p > 0.05$ level (Tukey test). Value described Mean ± SE (n=6).

Potassium, Ca, Mg and P are important elements for plant growth and are classified as major plant nutrients. The lack of any change in nutrient availability as a function of added F suggests that F addition to Allophanic soils will not directly influence major nutrient availability to plants.

6.5.5 Effect of added F concentration on soil microbial activity

Soil microbial biomass carbon ranged between 1519 – 1942 mg C kg^{-1} and was not significantly ($p > 0.05$) affected by the concentration of F added to the soil (Figure 6.5). This lack of any affect on microbial biomass carbon, for the treatment rates used in the

current study, agrees with Ropelewska et al. (2016) who reported that microbial biomass carbon was not negatively influenced by added F concentration up to 4000 mg kg^{-1}.

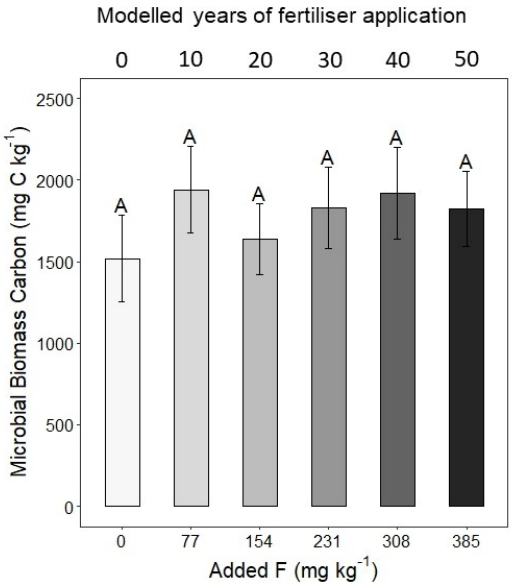


Figure 6. 5. Effect of F concentrations on microbial biomass carbon. Means with the same letters are not significantly different at the $p > 0.05$ level (Tukey test). Vertical bars indicate standard error of mean (n = 6).

The effect of added F on soil microorganisms was also measured by measuring the activity of soil enzyme activities (Figure 6.6). Urease activity, acid phosphatase and alkaline phosphatase ranged between 36.20 – 42.62 NH_4-N µg g^{-1} 4 h^{-1}, 133.4 – 152 NP g^{-1} h^{-1} and 109 – 157 NP g^{-1} h^{-1}, respectively, and were not significantly ($p > 0.05$) influenced by the concentration of added F. The same result was observed for dehydrogenase (61.3 – 71.38 TPF g $^{-1}$ h $^{-24}$), peroxidase (69 – 74 benzoquinone mmol L^{-1}) and ploy phenol oxidase (6.6 – 7.0 mmol L^{-1} benzoquinone) activity. Many studies have been conducted to measure the effect of F on soil enzymes, however, available results show no consistent trend. Chae et al. (2018) reported that acid phosphate activity was

suppressed by 28% at a F^- concentration of 905 mg kg^{-1}, while urease activity increased by 40% for the same F concentration.

In the current study there was no indication of toxicity at the concentrations of total and bioavailable F recorded for this work (Figure 6.6). However, toxicity effects have been reported at higher F concentration. Tscherko and Kandeler (1997) reported that dehydrogenase activity and microbial biomass carbon were significantly reduced from 190 to 26 µg TPF mg^{-1} and 315 to 63 mg C 100 g^{-1} soil, respectively, for a water-extractable F concentration greater than 100 mg kg^{-1}. García-Gil et al. (2013) reported that microbial biomass carbon and dehydrogenase activity were significantly reduced from 327 to 172 mg C kg^{-1} and 109.3 to 5.1 mg INTF g^{-1} soil for an increase in water soluble F concentration from 8 to 96 mg F kg^{-1}. In this same study, alkaline phosphatase significantly reduced from 94 to 63 mmol p-nitrophenol g^{-1} soil h^{-1} for a water-extractable F concentration of 47 mg F kg^{-1}, with urease activity significantly increasing from 0.21 to 0.27 mmol NH_4^+ g^{-1} soil h^{-1} at the same-water soluble F concentration.

In the present study, there was no significant effect of increasing F concentration on microbial biomass and soil enzyme activities. In the current study, the bioavailable F concentration, ranging from 4.9 to 12.7 mg kg^{-1}, was below the critical threshold for toxicity that has been established by previous studies. The total and bioavailable F concentrations in the soil of the current study present no toxicity risk to the enzymatic activity of soil microorganisms.

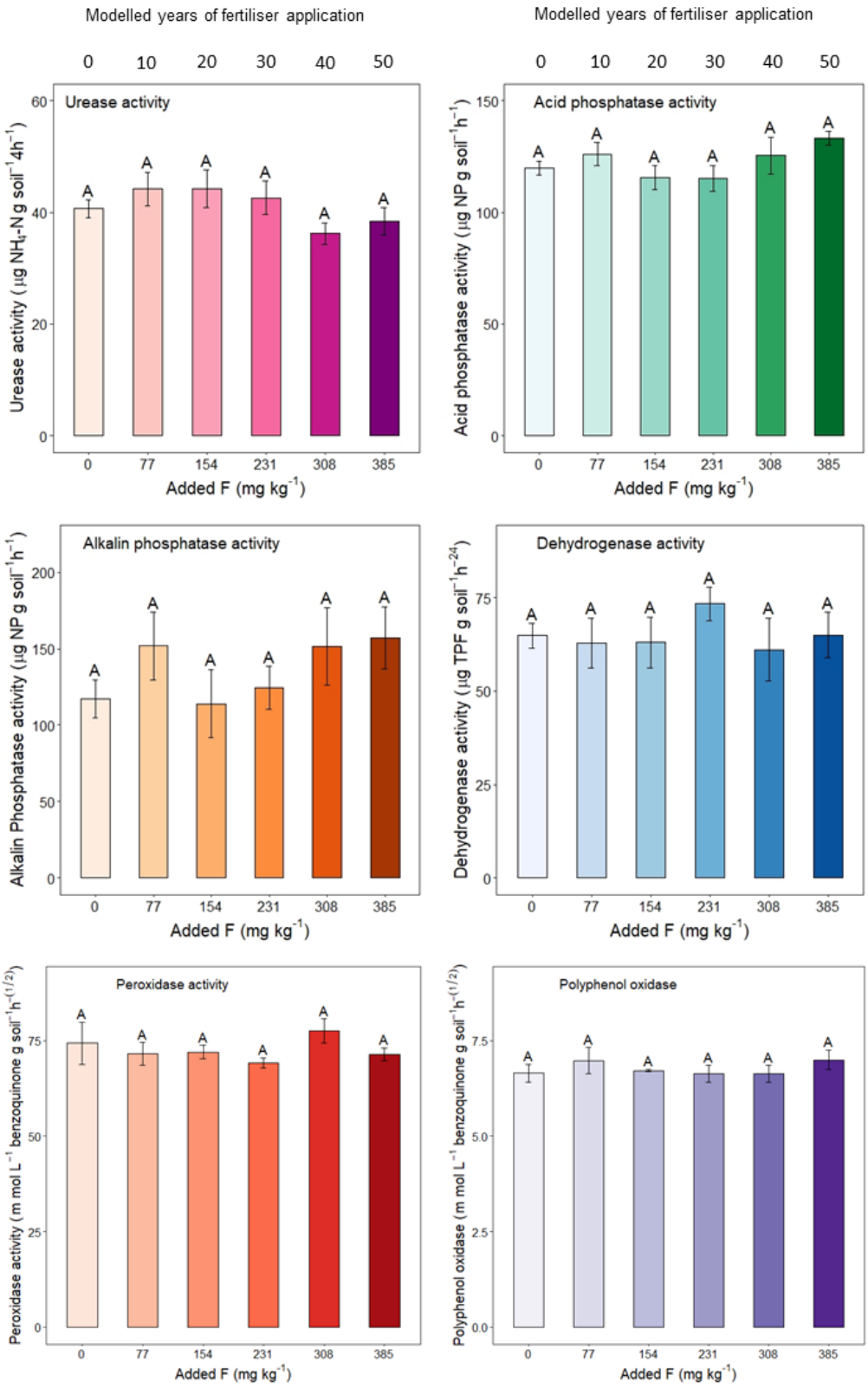


Figure 6. 6. Effect of F concentration on soil enzyme activity. Means with the same letters are not significantly different at the $p > 0.05$ level (Tukey test). Vertical bars indicate standard error of mean (n = 6).

6.5.6 Effect of added F on white clover root nodulation and growth

In addition to investigating the effects of increasing F concentration on soil microbial activity, the effect of added F on white clover root nodulation, growth and *Rhizobium* – legume symbiosis was also investigated.

Nodulation index was not significantly ($p > 0.05$) affected as a function of increasing F concentrations for the varieties Tribute (Appendix 3) and Merlyn (Appendix 4), and ranged between 19.2 – 24.9 and 21.2 – 25.6, respectively (Figure 6.7).

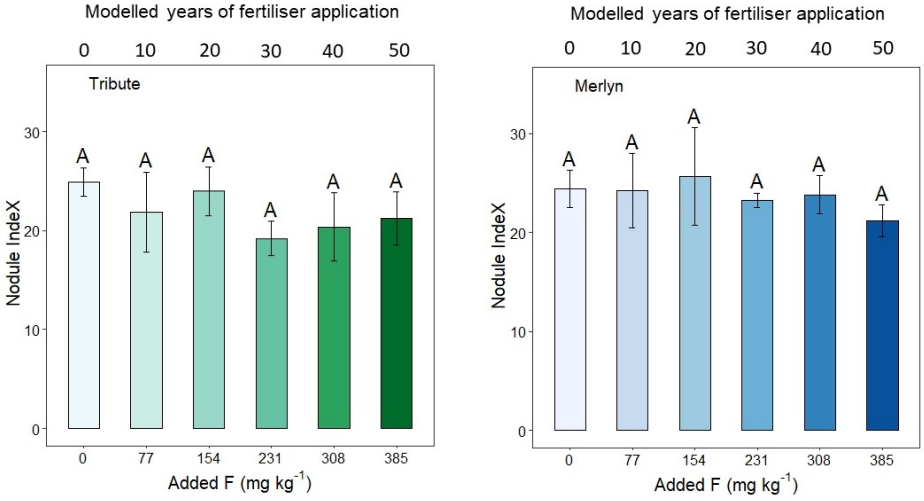


Figure 6. 7. Effect of F concentration on nodule index of white clover variety Tribute and Merlyn. Means with the same letters are not significantly different at the $p > 0.05$ level (Tukey test). Vertical bars indicate standard error of mean (n = 3).

Effects of F on legume nodulation have not been reported previously in the literature. However, Porter and Sheridan (1981) conducted a hydroponic experiment and reported that nitrogen fixation was not significantly influenced when alfalfa was exposed to a F^- concentration of 100 mg L^{-1}. In the present study, evidence suggests that the bioavailable F concentration of all treatments was lower than threshold for toxicity to nodulation.

6.5.7 Effect of added F concentration on root length, shoot length and biomass

The effect of F on root length, shoot length and biomass is presented in Figure 6.8. Root length was not significantly ($p > 0.05$) affected by the increasing F concentration for the two white clover varieties and ranged from 15.25 to 18.22 cm and from 19.53 to 21.93 cm for Merlyn and Tribute, respectively. A similar result was observed for shoot length, with a range from 10.3 to 11.2 cm and from 10.5 to 11.6 cm for the two varieties, respectively.

Clover biomass was not significantly ($p > 0.05$) affected by the increasing F concentration for both varieties. Jha et al. (2008) reported that spinach (*Spinacea oleracea*) biomass was not significantly affected up to a F concentration of 600 mg NaF kg^{-1} ($CaCl_2$ extractable F concentration was 13 mg kg^{-1}). Jha et al. (2009) reported that onion shoot, root and bulb biomass were not significantly affected by a F concentration up to 200 mg NaF kg^{-1} ($CaCl_2$ extractable F concentration 13 mg kg^{-1}).

6.5.8 Effect of spiked F concentration on shoot and root F accumulation

The shoot and root concentration of Tribute and Merlyn, as a function of the F concentration in the soil, is presented in Figure 6.9. For both varieties the root and shoot F concentrations increased as a function of the F concentration of the soil, with this increase was significant relative to the control for a soil treatment concentration greater than 77 mg kg^{-1}.

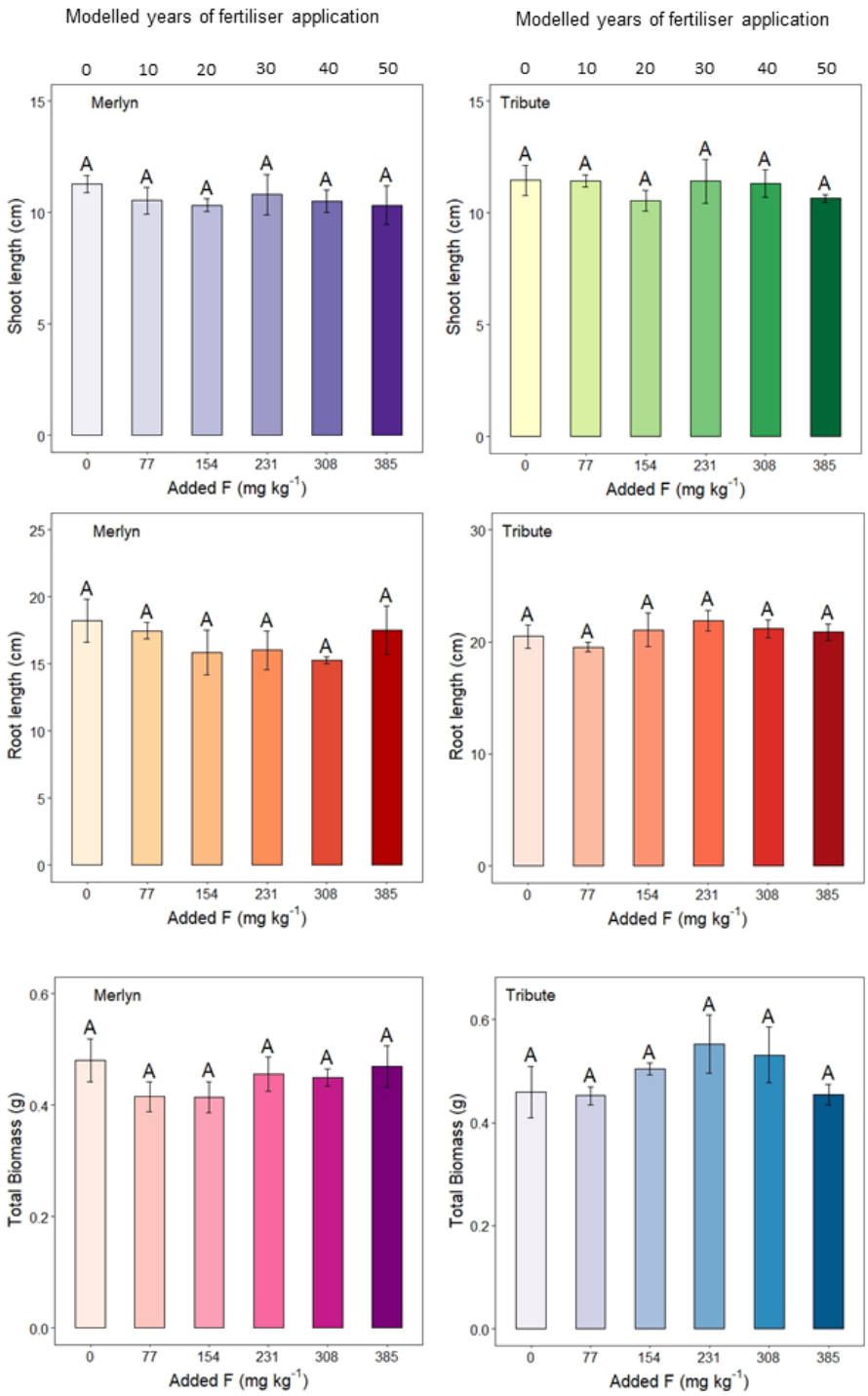


Figure 6. 8. Effect of F concentration on the root and shoot length and total biomass of white clover varieties Tribute and Merlyn. Means with the same letters are not significantly different at the $p > 0.05$ level (Tukey test). Vertical bars indicate standard error of mean (n = 3).

In the present study, the root and shoot F concentration in both varieties for the control treatment was less than 6 mg F kg^{-1}, and this result is in agreement with the previous study of Loganathan et al. (2001) who reported that the F concentration of herbage collected from soils with long-term fertiliser history was less than 10 mg F kg^{-1} DM. McLaughlin et al. (1997) reported a similar result for the F concentration of Australian herbage collected from phosphate fertiliser experimental sites (herbage concentration less than 10 mg F kg^{-1} DM). In the current study, the shoot and root F concentration for both varieties linearly increased with F addition, and this can be linked to the increasing $CaCl_2$ extractable F concentration, which is a function of the increasing treatment rate of F addition to the soil.

In the present study, the maximum shoot F concentration for the two clover varieties was recorded for the treatment concentration of 385 mg F kg^{-1} soil: the resulting herbage concentration was 16.6 mg F kg^{-1} DM and 19.9 mg F kg^{-1}DM for Tribute and Merlyn respectively. These shoot F concentrations are lower than the dietary intake toxic threshold for ewes (60 mg F kg^{-1} DM) and cattle (40 - 50 mg F kg^{-1} DM) (Loganathan et al., 2003) and suggest that a simulated F loading to soil through 50 years of phosphate fertiliser application will not cause a harmful effect to cattle and sheep through ingestion of F via white clover. However, white clover is only a component of New Zealand pasture systems. Ryegrass is a key species in New Zealand farm systems. Arneson (1997) reported that ryegrass has a lower F concentration in shoots than white clover. This gives confidence that the risk comment made here is appropriate, however, this provisional conclusion should be further analysed with respect to the F concentration of ryegrass.

The greater concentration of F in roots relative to shoots, for both varieties, for higher soil treatment levels, suggests a restriction of F translocation from roots to shoots. (Jha et al., 2008). Al-F complexes are accumulated in the root apoplast (*extracellular* space) and its movement from root to shoot is restricted as Al-F complexes are immobile within plants (MacLean et al., 1992; Yang et al., 2016).

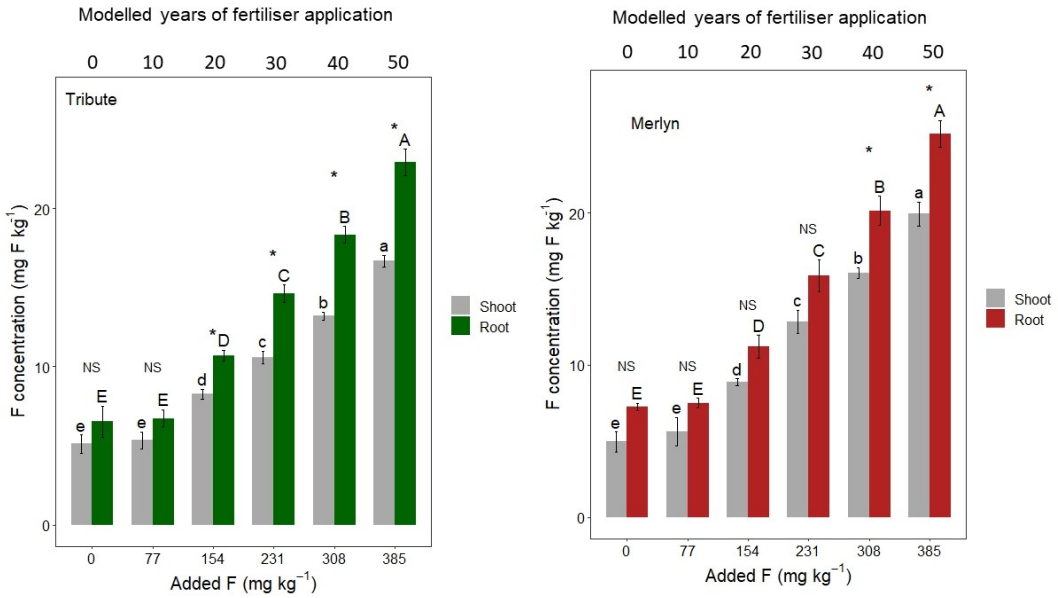


Figure 6. 9. Effect of F concentration on the shoot and root F concentration in the two white clover varieties Tribute and Merlyn. Means with the same letters (upper case letters for roots and lower case letters for shoots) are not significantly different at the $p > 0.05$ level in roots (Tukey test). Vertical bars indicate standard error of mean (n = 3). Significant difference between root and shoot F concentration is considered if $p < 0.05$, is denoted by "*" and $p > 0.05$ is denoted by NS (non-significant).

Visual inspection of the plants grown for this study showed no evidence of toxic symptoms that could be attributed to F for both varieties of white clover (Figure 6.10). Arnesen (1997) reported visible signs of F toxicity in clover above 200 mg F kg^{-1} in a

sandy loam soil (water extractable F concentration 23 mg kg^{-1})[1]. This threshold of toxicity reported by Arneson (1997) was not achieved in the present study.

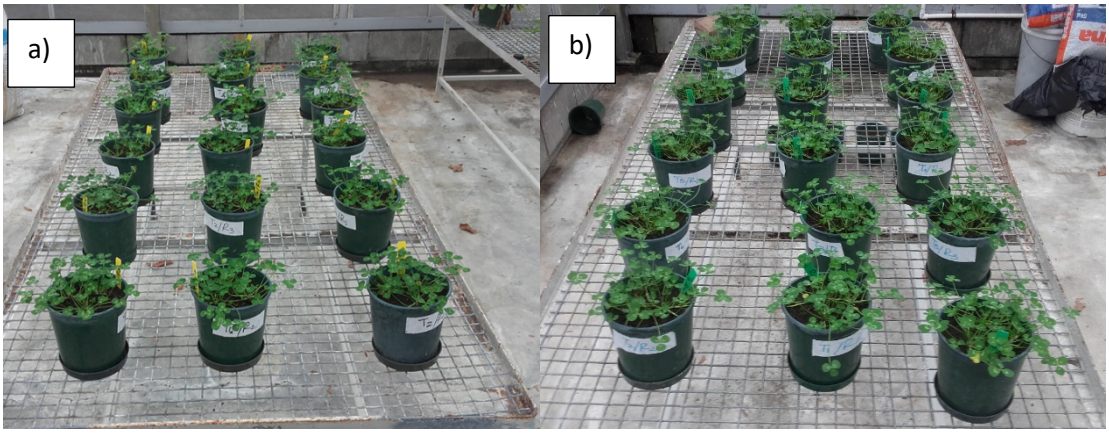


Figure 6. 10. White clover plants grown in soil with variable amended F concentration (0 – 385 mg F kg^{-1}); a) Merlyn white b) Tribute.

6.5.9 Relationship between root, shoot F concentrations and bioavailable F concentrations

A functional relationship between the shoot and root F concentrations and the bioavailable F concentration ($CaCl_2$-extractable F concentration) is presented in Table 6.2. Shoot and root F concentrations, for both varieties, was significantly and positively correlated ($p < 0.05$) with the bioavailable F concentration. Arnesen (1997) similarly reported that the relationship between the shoot F concentration of Common Bent (*Agrostis capillaris*) and the $CaCl_2$-extractable F concentration was positive and significant ($p < 0.05$).

[1] This value is higher than the bioavailable F concentration in Chapter 3, Table 3.4.

Table 6. 2. Relationship between shoot, root F concentrations and $CaCl_2$ extractable F concentrations for white clover varieties Tribute and Merlyn.

Variety	Equation	R^2	p value
Tribute	$F_{shoot} = -2.287 + 1.462\ F_{(CaCl_2)}$	97.6	$p < 0.05$
	$F_{root} = -4.190 + 2.105\ F_{(CaCl_2)}$	96.8	$p < 0.05$
Merlyn	$F_{shoot} = -4.744 + 1.987\ F_{(CaCl_2)}$	95.1	$p < 0.05$
	$F_{root} = -5.072 + 2.414\ F_{(CaCl_2)}$	96.1	$p < 0.05$

6.5.10 Effect of added F concentrations on nodule anatomy

The effect of added F on the *Rhizobium* – white clover interaction in the Allophanic soil of the current study was analysed using light and transmission electron micrographs (TEM). In the legume – *Rhizobium* symbiosis, bacteria are enclosed by a plant-derived membrane called the peri-bacteroid membrane (Emerich and Krishnan, 2014). Bacteria density in infected cells and the formation of the peri-bacteroid membrane are known to be sensitive to toxic components of the soil environment (Abd-Alla et al., 2016). Light micrographs show that infected root nodule cells for varieties Tribute (Figure 6.11) and Merlyn (Figure 6.12) had dense packing of *R. leguminosarum* for all F treatments: there was no difference in the density of *R. leguminosarum* between the control and greatest F soil treatment. TEM micrographs show that peri-bacteroid membranes were observed in Merlyn (Figure 6.13) and Tribute (Figure 6.14) root nodules for all F treatments, indicating that the symbiosis between *R. leguminosarum* and white clover was not affected by F for the soil concentrations used in the current study.

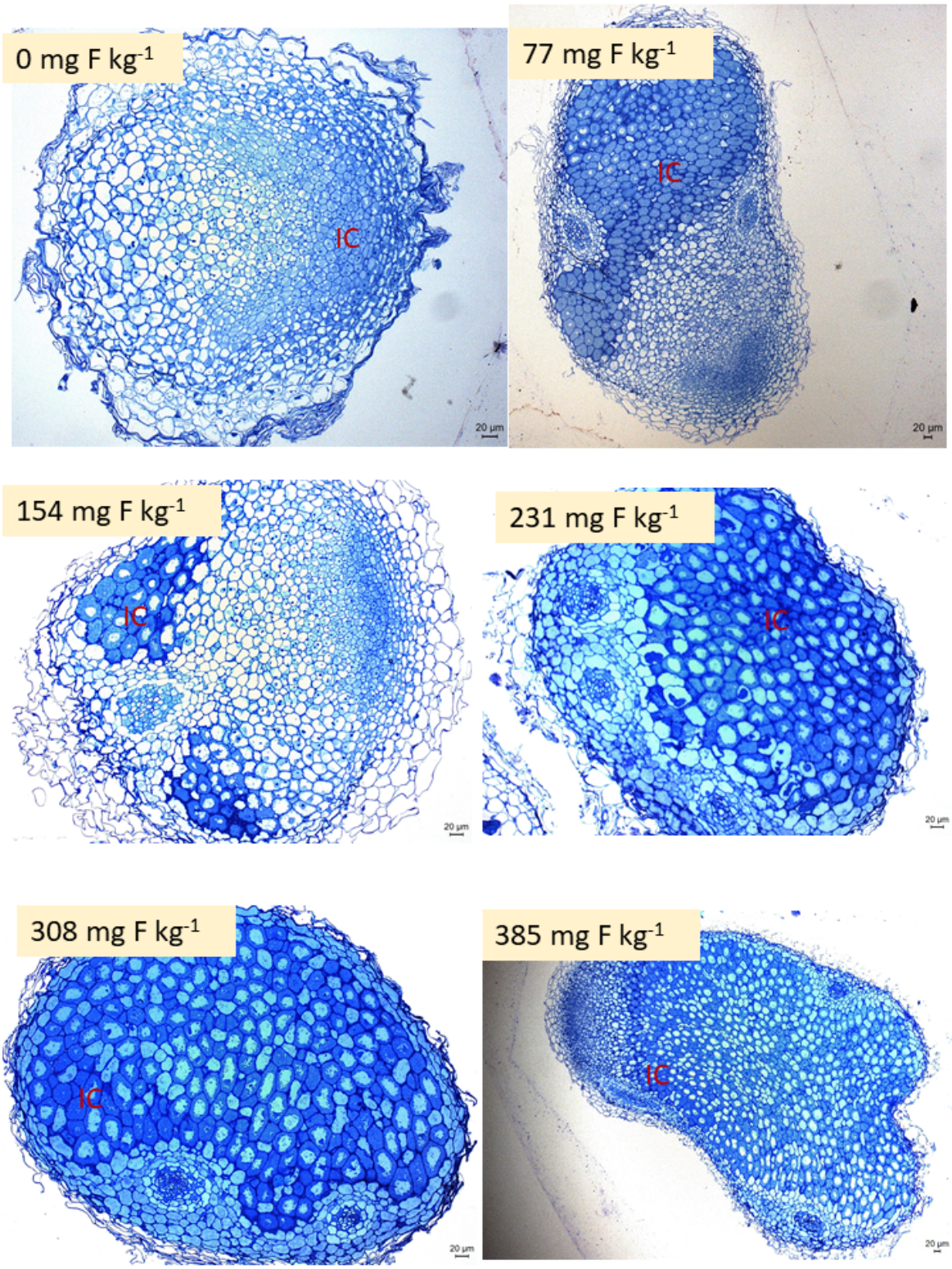


Figure 6. 11. Light Microscope (LM) images of root nodules structure of white clover variety Tribute grown in soil with added F concentration 0 – 385 mg kg^{-1}. Abbreviation: IC - infected cells.

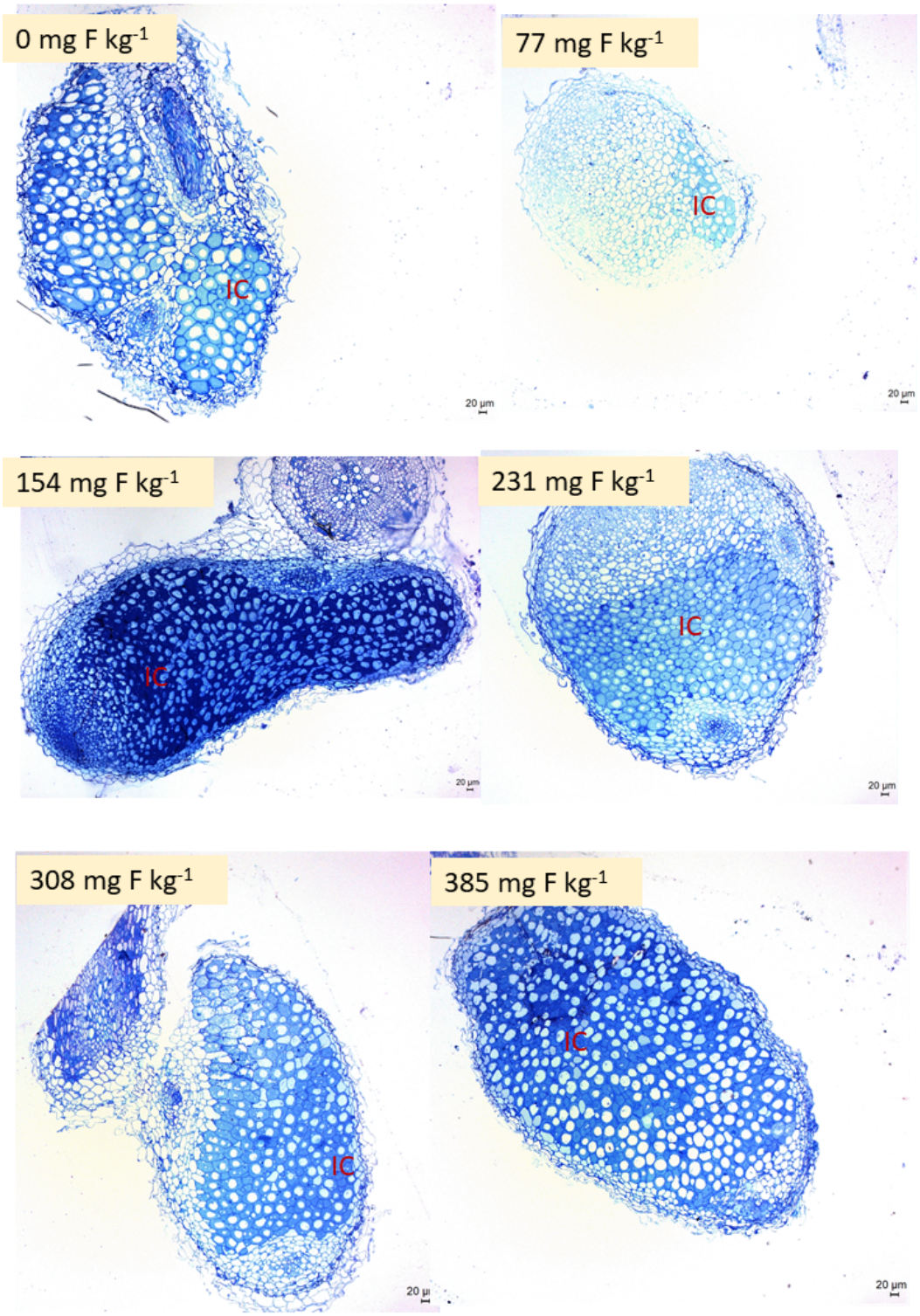


Figure 6. 12. Light Microscope (LM) images of root nodules structure of white clover variety Merlyn white grown in soil with added F concentration 0 –385 mg kg^{-1}. Abbreviation: IC - infected cells.

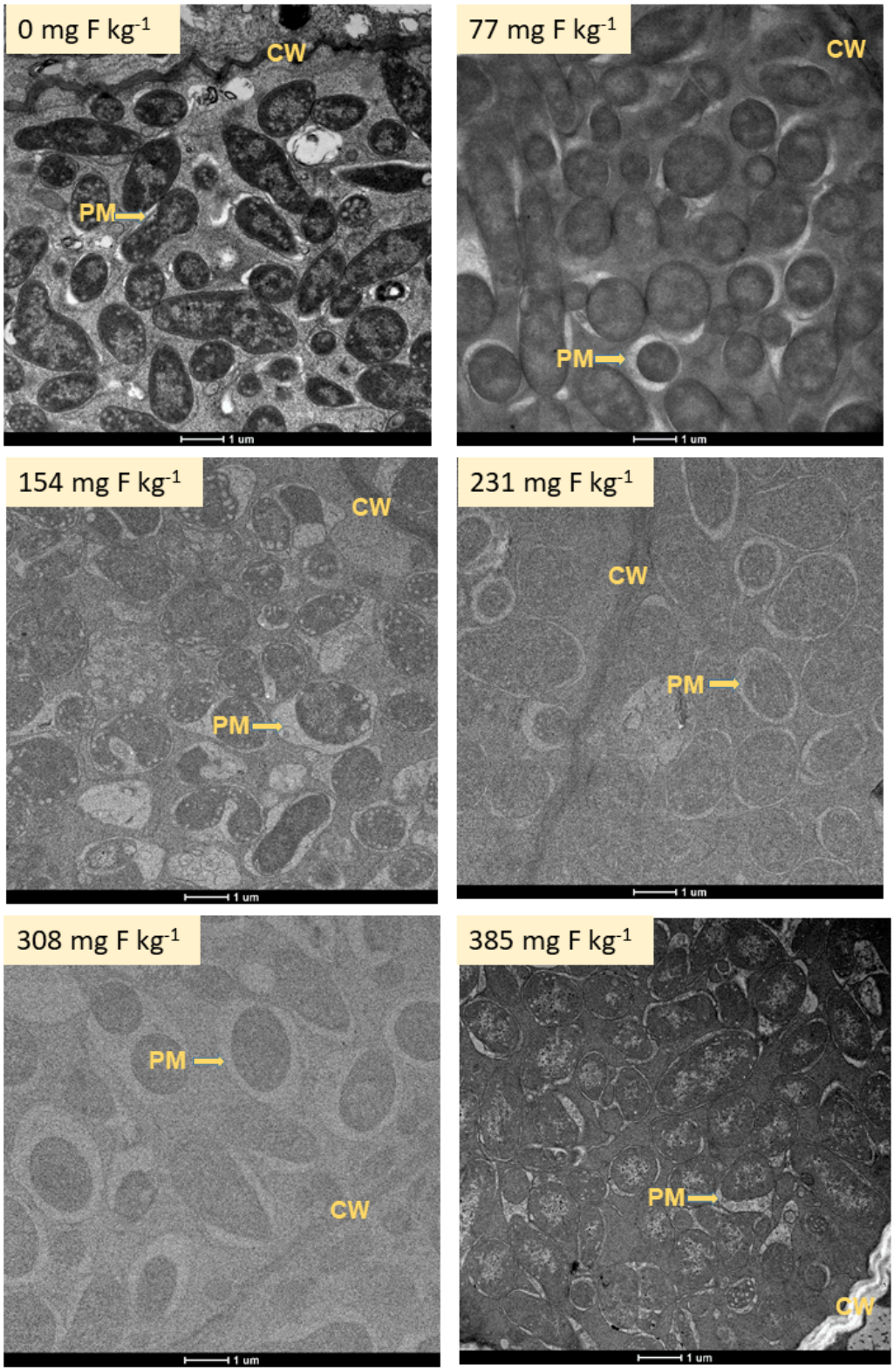


Figure 6. 13. Transmission Electron Micrographs of root nodules of white clover variety Merlyn white grown in soil with added F concentration 0 - 385 mg kg^{-1}. Abbreviations: CW – cell wall, PM- peribacteroid membrane (symbiosome).

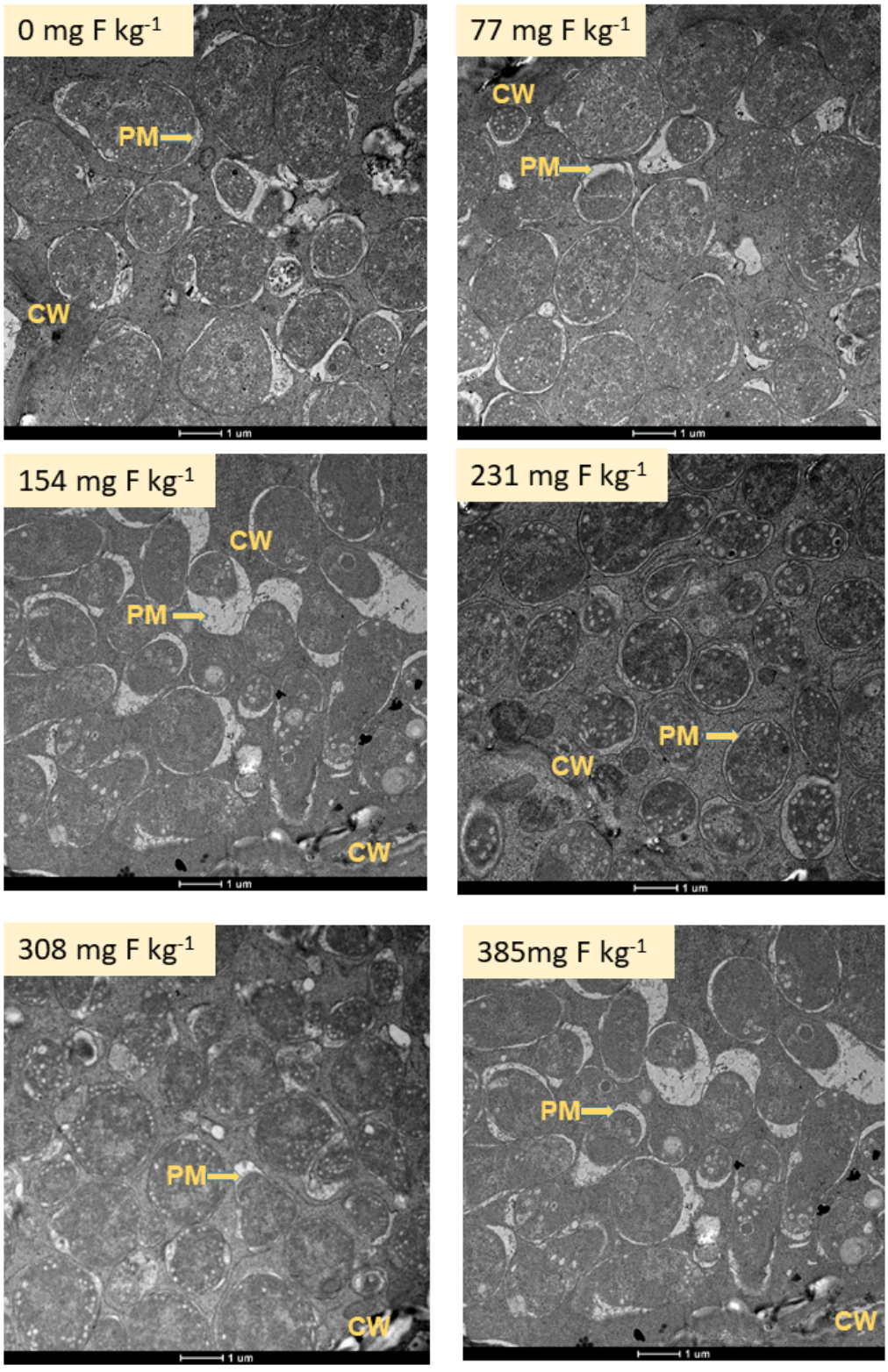


Figure 6. 14. Transmission Electron Micrographs of root nodules of white clover variety Tribute grown in soil with added F concentration 0 - 385 mg kg^{-1}. Abbreviations: CW – cell wall, PM- peribacteroid membrane (symbiosome).

6.6 Conclusions

The effect of increasing F addition on the chemical and biological properties of an Allophanic soil, was assessed using variable rates of F added to soil to model long-term P applications rates from 10-50 years. The major fraction of F added as NaF was adsorbed by the soil maintaining the bioavailable F concentration at orders of magnitude lower than the added F concentration. However, about 1.5% of added F remained bioavailable. The concentration of the plant essential nutrients Ca, Mg, K and P was not influenced by the increasing rate of added F. Soil pH was observed to increase as a function of F addition, and this may need to be considered in the context of lime application rates for Allophanic soils to maintain pasture productivity. The DOC concentration in soil solution was also shown to increase as a result of F addition and this may alter the C pool in Allophanic soils. The shoot and root F concentration of two varieties of white clover was shown to increase as a function of added F. However, the highest herbage concentration of F recorded in the current work (equivalent to 50 years of P fertiliser application) did not exceed the toxic threshold dietary intake value for cattle and sheep. There was a greater concentration of F in the roots than the shoots of white clover. However, light and transmission electron micrograph of root nodules showed that the *Rhizobium* - white clover symbiosis was not influenced by added F. This study showed that F added to soil at rates calculated to model up to 50 years of continuous P-fertiliser application to New Zealand's productive Allophianic soil order had no negative influence on soil biology, or the growth and performance of white clover. In New Zealand pasture system, white clover is mixed with ryegrass. Therefore, future research should also consider the F concentration of ryegrass to give more confidence to the recommendations of this work.

CHAPTER 7

Integrated discussion – Important research findings and recommendations for future research

7.1 Introduction

Increased F concentrations in agricultural soils have been linked to risks for soil health and, as a result, to potential impacts on primary production. In New Zealand, soil F concentrations have significantly increased in both pastoral and horticultural soil as the result of long-term phosphate fertiliser application. The development of soil F guideline values is therefore timely. However, the lack of standardised analytical methods for F in soil is a limiting factor that has been identified in this thesis.

Production from New Zealand pastoral farms is highly dependent on the white clover–*Rhizobium* symbiosis as a source of N to New Zealand farm systems and as a forage for grazing livestock. A review of the literature at the outset of this doctoral study highlighted that little was known about the potential effect of F on the performance of *R. leguminosarum* bacteria, and about the extent to which the F concentration in pasture may increase as a result of phosphate fertiliser, and represent a health risk to grazing livestock.

The toxicity of a soil contaminant to living organisms is a function of a contaminant's availability in soil to living organisms (bioavailability). Fluorine bioavailability is known to be influenced by soil properties. However, the extent to which the bioavailability of F can be regulated by New Zealand soils has not previously been reported. Soil amendments

have the potential to minimise F availability in soil through altering soil properties and through increasing adsorption onto soil surfaces. Lime application is a common practice in New Zealand to increase productivity and compost is becoming more widely used to increase soil carbon. The effects of lime and compost application to soil on the bioavailability of F, particularly to New Zealand soil orders, are poorly described in literature.

The primary goal of this doctoral study was, therefore, to determine whether phosphate fertiliser-derived F has a detrimental effect on soil microorganisms, particularly on nitrogen fixing bacteria, and to propose provisional management guidelines based on the F concentration in soil. This chapter presents and discusses the key findings of the research in the context of this high-level objective.

7.2 Important research findings and discussion

7.2.1 The new soil F analytical method substantiated in this thesis (4 mol L^{-1} NaOH extraction method) is a viable technique to measure the total soil F concentration

To underpin more widespread analysis of the F concentration of soil, a novel methodology for total F analysis, based on extraction with 4 mol L^{-1} NaOH, was validated [Chapter 3]. The correlation between the total soil F concentration measured by conventional fusion methodology and 4 mol L^{-1}, 8 mol L^{-1}, 12 mol L^{-1}, and 16 mol L^{-1} NaOH extraction was significant for each NaOH extractant used, with the correlation coefficients (r) analysed as 0.95, 0.95, 0.96 and 0.95, respectively. These results prove that both methods extract the same fraction of F from soil. The relative error for repeat

analysis of volcanic material (allophane) and organic matter-rich soils was less than 0.2, indicating that this method is highly suitable for soil orders developed from these parent materials.

The amount of F extracted from soil was not significantly influenced by the NaOH concentration. Therefore, further method development in the thesis used extraction with 4 mol L^{-1} NaOH to quantify the total F concentration in New Zealand soils.

The trueness of the 4 mol L^{-1} NaOH extraction method was measured through analysis of standard reference Soil A (S07052003A= 168 ± 29 µg F g^{-1}) and Soil B (S07052003B = 542 ± 30 µg F g^{-1}). The mean relative error from repeat analysis (n = 9) was 0.27 and 0.20, respectively. These results further validate 4 mol L^{-1} NaOH extraction is a reliable method to determine the total F concentration in New Zealand soils.

The precision of the 4 mol L^{-1} extraction method was measured by repeat analysis of soil samples collected from three field sites (Pukekawa, Kairanga and Canterbury) which were the subject of an MPI/FANZ study exploring the influence of soil properties on plant uptake of cadmium in New Zealand agricultural soils during the PhD period. Each of these sites consisted of 30 equal size (3.6 m x 4 m) treatment plots. Soil samples were collected from all plots prior to the application of soil amendments. These samples were analysed for total F by both the alkali fusion and NaOH (4 mol L^{-1}) extraction methods. Each analysis was replicated 3 times, and in total 270 samples were tested by each method. Relative standard deviation (RSD) was used to describe the precision of the method. The RSD for the fusion method and 4 mol L^{-1} NaOH method are given in Table

7.1. RSD values were low and less than 9% suggesting that 4 mol L^{-1} NaOH extraction method is a precise soil F analysis method for total soil F analysis.

Table 7. 1. RSD values (%) of fusion method and 4 mol L^{-1} NaOH method for the three soils

Location	Soil order	Fusion	4 mol L^{-1} NaOH
Kairanga	Gley	10.93	7.66
Pukekawa	Granular	6.88	7.0
Canterbury	Pallic	7.72	8.8

The measurable limit of the 4 mol L^{-1} NaOH extraction method was measured by analysing 7 New Zealand pasture and horticulture soils. The total soil F concentration ranged from 153 - 1015 mg kg^{-1} and was similar to the total soil F concentration measured by fusion method for New Zealand horticulture and pasture soils in previous studies (Loganathan et al., 2006; Kim et al., 2016).

In subsequent experimental work conducted for this thesis, extraction with 4 mol L^{-1} NaOH was adopted as the standard method for the quantification of the total F concentration in soil. A key outcome of the thesis is the proposal that this method be adopted as the standard method for total soil F analysis for soils across New Zealand.

7.2.2 This study proposes 0.01 mol $L^{-1}CaCl_2$ – extraction as a standard technique to measure the bioavailable F concentration of Allophanic and Organic soils

The extractable F concentration of New Zealand soils was quantified using a range of extractants to support the recommendation of a standard technique to measure the bioavailable F concentration of soil [Chapter 3]. Water-extractable F concentrations correlated well with 0.01 mol L^{-1} $CaCl_2$ and 0.01 mol L^{-1} KCl extractable F concentrations. However, 0.01 mol L^{-1} $CaCl_2$ showed a higher recovery percentage for soils with a high Al and Fe concentrations (specifically the Allophanic soil used in this study). The higher recovery percentage obtained using 0.01 mol L^{-1} $CaCl_2$ is due to $CaCl_2$ replacing Al bound F, which is bioavailable.

The strong positive and significant ($p < 0.05$) correlation obtained between 0.01 mol L^{-1} $CaCl_2$ extractable F concentration and the shoot and root F concentrations of white clover [Chapter 6], suggests that 0.01 mol L^{-1} $CaCl_2$ extractable F represents the bioavailable F concentration of soil. In combination with knowledge that $CaCl_2$ is an effective extractant for the important Allophanic soil order (Chapter 3: Allophanic soils have a long history of P fertilisation; have elevated F concentration; and are important soils for New Zealand agriculture), 0.01 mol L^{-1} $CaCl_2$ extraction method is recommended in this work as the standard method to measure the bioavailable F concentration of New Zealand pasture soils subject to high rates of P fertilisation and F retention.

7.2.3 There is no indication of imminent risk of soil F causing a toxic effect on Rhizobium – white clover interactions under normal soil conditions

A series of experiments were conducted to test the effect of F on *R. leguminosarum* and *Rhizobium*–white clover interactions [Chapter 4]. *Rhizobium leguminosarum* growth was not significantly ($p < 0.05$) supressed by a F^- concentration up to 100 mg L^{-1} and these results were confirmed by using 3 different F salts.

To confirm these results, *R. leguminosarum* morphology was analysed. *Rhizobium leguminosarum* was unaffected during exposure to 100 mg L^{-1} F relative to the control, maintaining a rod shape. At 500 mg L^{-1} F^-, *R. leguminosarum* size significantly decreased ($p < 0.05$) relative to the control and the morphology changed from rod to round shape at a F^- concentration of 1000 mg L^{-1}.

The MicroResp assay revealed that *R. leguminosarum* respiration was inhibited by less than 10% for a F^- concentration up to 100 mg L^{-1}.

In addition, pottle-based experiments were conducted to examine the effect of F^- on *Rhizobium*-white clover interactions by observing nodule morphology and growth. Visual results revealed that *R. leguminosarum* is able to form healthy nodules at a F^- concentration up to 100 mg L^{-1}. Both Light and TEM micrographs confirmed that the *Rhizobium* - white clover interaction was not influenced by F^- concentrations under 100 mg L^{-1}.

The toxic F^- concentration for *Rhizobium*-white clover recorded in this research (100 mg L^{-1}) is orders of magnitude higher than that recorded for New Zealand agriculture soils under 'normal conditions' where the F^- concentration is generally less than 0.58 mg L^{-1}.

To validate these results in the soil environment, a pot experiment was conducted [Chapter 6] where F was added at concentrations ranging from 0 – 385 mg F kg^{-1} chosen to model the F concentration in soil after 0 - 50 years of continuous phosphate fertiliser application. The bioavailable F concentration for all amendment scenarios was less than 12.67 mg kg^{-1} which is an order of magnitude lower than the reported toxic concentration in the literature (Tscherko and Kandeler, 1997).

The results from this experimental work highlights that soil is effective in immobilizing the majority of F that is added to soil. A key finding of this thesis is that F added through phosphate fertiliser will cause no detrimental effects on *R. leguminosarum* in the foreseeable future.

7.2.4 Fluorine accumulates in white clover shoots and roots proportional to the bioavailable F concentration in soil

The response of white clover growing in Allophanic soil to an increasing concentration of F in the soil was investigated in a pot experiment [Chapter 6]. The shoot F concentration for white clover variety Merlyn linearly increased from 4.9 to 19.9 mg kg^{-1} with increasing $CaCl_2$ ($p < 0.05$ and $r^2 = 95.1$) and water-extractable ($p < 0.05$ and $r^2 = 84.2$) F concentrations. A similar result was also observed for variety Tribute where shoot F concentration increased from 5.12 to 16.68 mg kg^{-1} with increasing $CaCl_2$ ($p < 0.05$ and

r^2 = 97.6) and water-extractable ($p < 0.05$ and r^2 = 88.9) F concentrations. However, even at the modelled 50-years of F application, the plant concentration was lower than the toxic dietary intake threshold for cattle (40 - 50 mg F kg^{-1} DM) and sheep (60 mg F kg^{-1} DM). Plant growth and yield were not visually affected by bioavailable F at any concentration used in this work.

The pottle experiment [Chapter 4] showed that there was no detrimental impact of F on white clover growth up to a F^- concentration of 70 mg L^{-1}. This is an order of magnitude higher than the modelled bioavailable F concentration in soil after 50 years of phosphate fertiliser application. The results from this thesis suggest that there is no immediate risk of soil F on white clover growth in New Zealand pastoral systems.

7.2.5 The major fraction of added F is adsorbed by Allophanic soils and is not available to soil microorganisms

A pot experiment [Chapter 6] revealed that, on average, F extracted by water (Appendix 5) and 0.01 M $CaCl_2$ was 0.37% and 1.49% of the added F (0 – 385 mg F kg^{-1}) respectively, and suggests that the major fraction of F added as NaF was immobilised by Allophanic soil.

These results suggest that Allophanic soils have strong capacity to adsorb F.

Pot experiment results [Chapter 6] reveal that soil microbial biomass carbon and soil enzyme activities were not affected by increasing added F concentrations due to the immobilisation of a major fraction (98.51- 99.63%) of the added F by the Allophanic soil.

Light microscopy and TEM micrographs confirmed that the increasing concentration of F did not influence N fixation by white clover, again due to the adsorption of added F by Allophanic soil.

7.2.6 Soil amendments such as lime and compost can minimize the bioavailable F^- concentration but efficacy is dependent on the rate of application of these amendments. The pH of maximum F adsorption occurred over a range of 5.5-6.8. Compost can be used to minimise free soil F^- concentrations in soils with a pH < 6.0 (by maximising adsorption of F^- to soil surfaces)

The affect of soil pH on the concentration of bioavailable F in soil was investigated in Chapter 3. $CaCl_2$-extractable F concentrations significantly ($p < 0.05$) decreased as soil pH increased from 4.96 to 6.27, suggesting that the bioavailable concentration of F in soil can be reduced by changing soil pH.

A significant and linear positive relationship ($p < 0.05$; $r = 0.96$) was recorded between the total F concentration and SOM (%) [Chapter 3] suggesting that F is retained by organic matter in soils and retention can be increased by manipulating SOM.

Parallel to this thesis, a field trial was conducted at locations in Kairanga, Pukekawa and Canterbury where different rates of lime and compost were applied to mitigate the risk of cadmium transfer to plants [Chapter 3]. The effect of amendments on F bioavailability was assessed for these trial locations during a laboratory trial as part of the thesis study.

The pH of maximum adsorption for an added F concentration of 100 mg L^{-1} was assessed for each soil. For the Kairanga soil, maximum adsorption occurred at a soil pH of 6.22 and reduced at soil pH 7.37. For the Canterbury soil, maximum adsorption occurred at pH 6.40 and reduced at pH 7.17. For the Pukekawa soil, maximum adsorption occurred at pH 5.85 and reduced with increasing pH. These results show that for soils representative of the major productive soil orders of New Zealand, maximum F^- adsorption occurs across a pH range of 5.5 – 6.8.

Manipulation of the field soils with compost also effected a change in F bioavailability. Compost application significantly ($p < 0.05$) increased F^- adsorption in the Canterbury and Kairanga soils (pH < 6), and reduced F bioavailability. However, there was no effect of compost amendment on the bioavailable F concentration in the relatively higher pH Pukekawa soil (pH > 6). This knowledge will underpin efforts to minimise the bioavailability of F in soil through adjusting soil pH and organic matter content.

7.2.7 An initial risk-based guideline value for New Zealand agricultural soils is 20 mg F kg^{-1} (Water / $CaCl_2$ – extractable F concentrations)

Fluorine guideline values for New Zealand agricultural soils have yet to be developed (Gray, 2018). As an effort to propose an initial F guideline value, the results of the current study, and F toxic values reported in literature, were considered. According to the findings of this thesis, the activity of soil microorganisms is not influenced by extractable F concentrations up to 12.67 mg kg^{-1}. Although white clover root and shoot F concentrations increased with F addition, plants were not negatively influenced by

bioavailable F concentrations up to 12.67 mg kg^{-1} and the interaction between *R. leguminosarum* and white clover is similarly not affected at this concentration.

Laboratory testing of the toxicity of F to *R. leguminosarum* reveals that the IC_{10} value for F^- toxicity to *R. leguminosarum* is greater than 100 mg F L^{-1}, independent of the salt of F used.

Pottle experiments conducted in Chapter 4 showed that for a soluble F^- concentration range of 0 – 70 mg L^{-1}, white clover growth was not reduced. In this work, white clover produced healthy nodules up to a soluble F^- concentration of 100 mg L^{-1}.

Very little available literature reports F toxicity in terms of bioavailable F concentrations (Gray, 2018). Most studies consider F toxicity in terms of the total F concentration of soil. Tscherko and Kandeler (1997) reported one of the few studies that does link toxicity to bioavailable F, and showed that arylsulphatase enzyme activity was suppressed at a water-extractable F concentration of 20 mg kg^{-1}. According to available literature, this is the minimum reported bioavailable F concentration value that negatively influences soil microorganism activity.

Fluorine soil guideline values have been developed in certain countries, including Canada, Australia and Switzerland (Mikkonen et al., 2018; Wang et al., 2018). Values are presented in terms of the total F concentration in soil with the exception of Switzerland where the guideline value is defined in terms of a water-soluble F concentration, which is 20 mg kg^{-1} (Wang et al., 2018). This Swiss guideline value correlates well with the findings of this thesis.

Considering the findings of the current work, a key outcome of this thesis is the proposal of 20 mg kg^{-1}, quantified using $CaCl_2$ extraction for Allophanic and organic soils, and water extraction for other soil orders, as a soil F guideline concentration for New Zealand agricultural soils to protect soil organisms.

7.3 Recommendations for future research

The research conducted for this thesis has identified that F will accumulate in white clover as a function of increasing bioavailable F concentrations. Glasshouse experiments should be conducted to quantify F accumulation / uptake in other pasture species which are widely cultivated across New Zealand. In addition, plant breeding trials should be conducted to explore how the selective use of cultivars may limit F uptake by pasture species. Further research should also investigate the mechanism of F uptake in different species.

This study found that F adsorption varied with soil type. Future studies should use scanning electron microscopy to investigate F adsorption onto soil surfaces as a function of interactive elements and the variable surfaces of New Zealand productive soils.

There is considerable scope for ongoing research into the ecotoxicology of F. Specifically, the accumulation of F in New Zealand earthworms should be investigated to establish the toxic F concentration for these important animals, and the relationship between earthworm F toxicity and the total and extractable F concentrations in soil should be explored.

REFERENCES

Abd-Alla, M.H., Nafady, N.A., Khalaf, D.M., 2016. Assessment of silver nanoparticles contamination on faba bean-*Rhizobium leguminosarum* bv. viciae-Glomus aggregatum symbiosis: Implications for induction of autophagy process in root nodule. Agriculture, Ecosystems and Environment 218, 163–177. doi:10.1016/j.agee.2015.11.022

Adamek, E., Pawłowska-Góral, K., Bober, K., 2005. In vitro and in vivo effects of fluoride ions on enzyme activity. Annales Academiae Medicae Stetinensis 51, 69–85.

Agalakova, N.I., Gusev, G.P., 2011. Effect of Inorganic Fluoride on Living Organisms of different phylogenetic level. Journal of Evolutionary Biochemistry and Physiology 47, 22–930. doi:10.1134/S002209301105001X

Ahmad, E., Zaidi, A., Khan, M.S., Oves, M., 2012. Heavy metal toxicity to symbiotic nitrogen-fixing microorganism and host legumes. In Toxicity of heavy metals to legumes and bioremediation (pp. 29-44). Springer, Vienna.

Ahoranta, S.H., Peltola, M.K., Lakaniemi, A.M., Puhakka, J.A., 2017. Enhancing the activity of iron-oxidising bacteria: A case study with process liquors from heap bioleaching of a complex sulphide ore. Hydrometallurgy 167, 163–172. doi:10.1016/j.hydromet.2016.11.010

Álvarez, E., Fernández-Marcos, M.L., Monterroso, C., Fernández-Sanjurjo, M.J., 2005. Application of aluminium toxicity indices to soils under various forest species. Forest Ecology and Management 211, 227-239. doi:10.1016/j.foreco.2005.02.044

Álvarez, E., Fernández-Sanjurjo, M., Otero, X.L., Macías, F., 2011. Aluminum speciation in the bulk and rhizospheric soil solution of the species colonizing an abandoned copper mine in Galicia (NW Spain). Journal of Soils and Sediments 11, 221–230. doi:10.1007/s11368-010-0295-2

Alvarez, E., Martinez, A., Calvo, R., 1992. Geochemical aspects of aluminium in forest soils in Galicia (NW Spain). Biogeochemistry 16, 167-180. doi:10.1007/BF00002817

Álvarez, E., Monterroso, C., Marcos, M.F., 2002. Aluminium fractionation in Galician (NW Spain) forest soils as related to vegetation and parent material. Forest Ecology and Management 166, 193-206. doi:10.1016/S0378-1127(01)00658-2

Álvarez, E., Viadé, A., Fernández-Marcos, M.L., 2009. Effect of liming with different sized limestone on the forms of aluminium in a Galician soil (NW Spain). Geoderma 152, 1–8. doi:10.1016/j.geoderma.2009.04.011

An, J., Kim, K.H., Yoon, H.O., Seo, J., 2012. Application of the wavelength dispersive X-ray fluorescence technique to determine soil fluorine with consideration of iron content in the matrix. Spectrochimica Acta Part B: Atomic Spectroscopy 69, 38–43. doi:10.1016/j.sab.2012.02.006

Aparicio, C., Urrestarazu, M., Cordovilla, M., del P., 2014. Comparative physiological analysis of salinity effects in six olive genotypes. HortScience 49, 901–904. doi:10.1080/00380768.1996.10414696

Arnesen, A.K.M., Abrahamsen, G., Sandvik, G., Krogstad, T., 1995. Aluminium-smelters and fluoride pollution of soil and soil solution in Norway. Science of the Total Environment 163, 39–53. doi:10.1016/0048-9697(95)04479-K

Arnesen, A.K.M., 1997. Availability of fluoride to plants grown in contaminated soils. Plant and Soil 191, 13–25. doi:10.1023/A:1004210713596

Arnesen, A.K.M., 1998. Effect of fluoride pollution on pH and solubility of Al, Fe, Ca, Mg, K and organic matter in soil from Ardal (Western Norway). Water, Air, and Soil Pollution 103, 375–388. doi:10.1023/A:1004921600022

Arnesen, A.K.M., Krogstad, T., 1998. Sorption and desorption of fluoride in soil polluted from the aluminium smelter at Ardal in Western Norway. Water, Air, and Soil Pollution 103, 357–373. doi:10.1023/A:1004900415952

Bailey, J.C., 1977. Fluorine in granitic rocks and melts: A review. Chemical Geology 19, 1–42. doi:10.1016/0009-2541(77)90002-X

Baker, J.L., Sudarsan, N., Weinberg, Z., Roth, A., Stockbridge, R.B., Breaker, R.R., 2012. Widespread genetic switches and toxicity resistance proteins for fluoride. Science 335, 233–235. doi:10.1126/science.1215063

Banerjee, G., Sengupta, A., Roy, T., Banerjee, P.P., Chattopadhyay, A., Ray, A.K., Bengal, W., 2016. Isolation and characterization of two fluoride resistant bacterial strains from fluoride endemic areas of west Bengal, India: Assessment of their fluoride absorption efficiency. Fluoride 49, 429–440.

Barbier, O., Arreola-Mendoza, L., Del-Razo, L.M., 2010. Molecular mechanisms of fluoride toxicity. Chemico-Biological Interactions 188, 319–333. doi:10.1016/j.cbi.2010.07.011

Barrow, N.J., 2008. The description of sorption curves. European Journal of Soil Science 59, 900–910. doi:10.1111/j.1365-2389.2008.01041.x

Basset-Mens, C., Ledgard, S., Boyes, M., 2009. Eco-efficiency of intensification scenarios for milk production in New Zealand. Ecological Economics 68, 1615–1625. doi:10.1016/j.ecolecon.2007.11.017

Bellomo, S., Aiuppa, A., D'Alessandro, W., Parello, F., 2007. Environmental impact of magmatic fluorine emission in the Mt. Etna area. Journal of Volcanology and Geothermal Research 165, 87–101. doi:10.1016/j.jvolgeores.2007.04.013

Blakemore, L.C., Searle, P.L., Day, B.K., 1987. Methods for chemical analysis of soils. New Zealand Soil Bureau, Scientific report 80, NZ Soil Bureau, Lower Hutt, New Zealand

Borjigin, S., Ashimura, Y., Yoshioka, T., Mizoguchi, T., 2009. Determination of fluoride using ion-selective electrodes in the presence of aluminum. Analytical Sciences : The International Journal of the Japan Society for Analytical Chemistry 25, 1437–43. doi:10.2116/analsci.25.1437

Borjigin, S., Yoshioka, T., Mizoguchi, T., 2010. Determination of total fluoride in boron-containing solutions. Analytical Sciences : The International Journal of the Japan Society for Analytical Chemistry 26, 603–606. doi:10.2116/analsci.26.603

Borucki, W., Sujkowska, M., 2008. The effects of sodium chloride-salinity upon growth, nodulation, and root nodule structure of pea (*Pisum sativum* L.) plants. Acta Physiologiae Plantarum 30, 293–301. doi:10.1007/s11738-007-0120-8

Boschetti, W., Dessuy, M.B., Pizzato, A.H., Vale, M.G.R., 2017. New analytical method for total fluorine determination in soil samples using high-resolution continuum source graphite furnace molecular absorption spectrometry. Microchemical Journal 130, 276–280. doi:10.1016/j.microc.2016.10.003

Breaker, R.R., 2012. New insight on the response of bacteria to fluoride. Caries Research 46, 78–81. doi:10.1159/000336397

Breimer, R.F., Vogel, J., 1989. Fluorine contamination of soils and earthworms (Lumbricus spp.) near a site of long-term industrial emission in Southern Germany. Biology and Fertility of Soils 7, 297-302. doi:10.1007/BF00257823

Brierley, J.A., Kuhn, M.C., 2010. Fluoride toxicity in a chalcocite bioleach heap process. Hydrometallurgy 104, 410–413. doi:10.1016/j.hydromet.2010.01.013

Brookest, P.C., Chander, K., 1991. Is the dehydrogenase assay invalid as a method to estimate microbial activity in copper-contaminated soil? Soil Biology and Biochemistry 23, 909–915.

Bruce-Martin, R., 1996. Ternary complexes of Al3+ and F^- with a third ligand. Coordination Chemistry Reviews 149, 23–32. doi:10.1016/S0010-8545(96)90008-9

Bulut, Y., Aydin, H., 2006. A kinetics and thermodynamics study of methylene blue adsorption on wheat shells. Desalination 194, 259–267. doi:10.1016/j.desal.2005.10.032

Campbell, A.D., 1987. Determination of fluoride in various matrices. Pure and Applied Chemistry 59, 695–702. doi:10.1351/pac198759050695

Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S., Potts, J.M., 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Applied and Environmental Microbiology 69, 3593–3599. doi:10.1128/AEM.69.6.3593

Caradus, J., Woodfield, D.R., Stewart, A.V., 1996. Overview and vision for white clover.

Agronomy Society of New Zealand Special Publication No. 11. Grassland Research and Practice Series No. 6, 1–6.

Carrera, J., Torrijos, M., Baeza, J.A., Lafuente, J., Vicent, T., 2003. Inhibition of nitrification by fluoride in high-strength ammonium wastewater in activated sludge. Process Biochemistry 39, 73–79. doi:10.1016/S0032-9592(02)00313-8

Cavanagh, J., 2015. Developing soil guideline values for the protection of soil biota in New Zealand. In: Moving farm systems to improved attenuation. (Eds L.D. Currie and L.L Burkitt). http://flrc.massey.ac.nz/publications.html. Occasional Report No. 28. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand. 13 pages.

Cavanagh, J.E., Munir, K., 2016. Development of soil guideline values for the protection of ecological receptors (Eco-SGVs): Technical document. Landcare Research Report for Envirolink Tools Grant C09X1402.

Cavanagh, J.E., Yi, Z., Gray, C., Young, S., Smith, S., Munir, K., Jeyakumar, P., Wakelin, S., Lehto, N.J., Robinson, B., Anderson, C., 2018. Assessing cadmium uptake in New Zealand. In: Farm environmental planning – Science, policy and practice. (Eds L.D. Currie and C.L. Christensen). http://flrc.massey.ac.nz/publications.html. Occasional Report No. 31. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand. 12 pages.

CCME, 2006. Soil quality guidelines for the protection of environmental and human health. In: Canadian Council of Ministers of the Environment. Retrieved 01. 06. 2019 from http://st-ts.ccme.ca/en/index.html?chems=97&chapters=all

Chae, Y., Kim, D., An, Y.J., 2018. Effects of fluorine on crops, soil exoenzyme activities, and earthworms in terrestrial ecosystems. Ecotoxicology and Environmental Safety 151, 21–27. doi:10.1016/j.ecoenv.2017.12.060

Chakrabarti, S., Patra, P.K., Mandal, B., Mahato, D., 2012. Effect of sodium fluoride on germination, seedling growth, and biochemistry of Bengal gram (*Cicer arieninum*). Fluoride 45, 257–262.

Chantigny, M.H., Harrison-Kirk, T., Curtin, D., Beare, M., 2014. Temperature and duration of extraction affect the biochemical composition of soil water-extractable organic matter. Soil Biology and Biochemistry 75, 161–166. doi:10.1016/j.soilbio.2014.04.011

Chartzoulakis, K., Klapaki, G., 2000. Response of two greenhouse pepper hybrids to NaCl salinity during different growth stages. Scientia Horticulturae 86, 247–260. doi:10.1016/S0304-4238(00)00151-5

Chaudri, A.M., Allain, C.M., Barbosa-Jefferson, V.L., Nicholson, F.A., Chambers, B.J., McGrath, S.P., 2000. A study of the impacts of Zn and Cu on two rhizobial species in soils of a long-term field experiment. Plant and Soil 221, 167–179. doi:10.1023/A:1004735705492

Chen, M., Ma, L.Q., 2001. Comparison of three Aqua Regia digestion methods for twenty Florida soils. Soil Science Society of America Journal 65, 491–499. doi:10.2136/sssaj2001.652491x

Chen, Y.X., He, Y.F., Yang, Y., Yu, Y.L., Zheng, S.J., Tian, G.M., Luo, Y.M., Wong, M.H., 2003. Effect of cadmium on nodulation and N2–fi xation of soybean in contaminated soils. Chemosphere 50, 781–787.

Chojnacka, K., Chojnacki, A., Gorecka, H., Górecki, H., 2005. Bioavailability of heavy metals from polluted soils to plants. Science of the Total Environment 337, 175–182.

Cittanova, M.L., Lelongt, B., Verpont, M.C., Geniteau-Legendre, M., Wahbe, F., Prie, D., Ronco, P.M., 1996. Fluoride ion toxicity in human kidney collecting duct cells. Anesthesiology: The Journal of the American Society of Anesthesiologists, 84, 428-435.

Clausen, L.P.W., Karlson, U.G., Trapp, S., 2015. Phytotoxicity of sodium fluoride and uptake of fluoride in willow trees. International Journal of Phytoremediation 174, 369-376. doi:10.1080/15226514.2014.910166

Cronin, S.J., Manoharan, V., Hedley, M.J., Loganathan, P., 2000. Fluoride: A review of its fate, bioavailability, and risks of fluorosis in grazed-pasture systems in New Zealand. New Zealand Journal of Agricultural Research 43, 295–321.

doi:10.1080/00288233.2000.9513430

Cronin, S.J., Neall, V.E., Lecointre, J.A., Hedley, M.J., Loganathan, P., 2003. Environmental hazards of fluoride in volcanic ash: A case study from Ruapehu volcano, New Zealand. Journal of Volcanology and Geothermal Research 121, 271–291. doi:10.1016/S0377-0273(02)00465-1

D'Alessandro, W., Bellomo, S., Parello, F., 2008. Fluorine speciation in topsoils of three active volcanoes of Sicily (Italy). Environmental Geology 56, 413–423. doi:10.1007/s00254-007-1179-7

D'Alessandro, W., Bellomo, S., Parello, F., 2012. Fluorine adsorption by volcanic soils at Mt. Etna, Italy. Applied Geochemistry 27, 1179–1188. doi:10.1016/j.apgeochem.2012.02.028

Deepika, K.V., Raghuram, M., Kariali, E., Bramhachari, P.V., 2016. Biological responses of symbiotic *Rhizobium radiobacter* strain VBCK1062 to the arsenic contaminated rhizosphere soils of mung bean. Ecotoxicology and Environmental Safety 134, 1–10. doi:10.1016/j.ecoenv.2016.08.008

Dehbandi, R., Moore, F., Keshavarzi, B., 2017. Provenance and geochemical behavior of fluorine in the soils of an endemic fluorosis belt, central Iran. Journal of African Earth Sciences 129, 56–71. doi:10.1016/j.jafrearsci.2016.12.016

Delestre, C., Laugraud, A., Ridgway, H., Ronson, C., O'Callaghan, M., Barrett, B.,

Ballard, R., Griffiths, A., Young, S., Blond, C., Gerard, E., Wakelin, S., 2015. Genome sequence of the clover symbiont *Rhizobium leguminosarum* bv. Trifolii strain CC275e. Standards in Genomic Sciences 10, 1–8. doi:10.1186/s40793-015-0110-1

Dissanayake, C.B., 1991. The fluoride problem in the ground water of Sri Lanka-environmental management and health. International Journal of Environmental Studies, 38, 137-155.

Edmeades, D.C., Feyter, C., O'Connor, M.B., 1984. Lime and phosphorus requirements for hill country yellow-brown earths. Proceedings of the New Zealand Grassland Association 45, 98–106.

Elloumi, N., Ben Abdallah, F., Mezghani, I., Rhouma, A., Boukhris, M., 2005. Effect of fluoride on almond seedlings in culture solution. Fluoride 38, 193–198.

Elrashidi, M.A., Lindsay, W.L., 1986. Chemical equilibria of fluorine in soils: a theoretical development. Soil Science, 141, 274-280. doi:10.1097/00010694-198604000-00004

Elrashidi, M.A., Lindsay, W.L., 1987. Effect of fluoride on pH, organic matter and solubility of elements in soils. Environmental Pollution 47, 123–133. doi:10.1016/0269-7491(87)90042-X

Emerich, D.W., Krishnan, H.B., 2014. Symbiosomes: temporary moonlighting organelles. Biochemical Journal 460, 1–11. doi:10.1042/BJ20130271

EPA Victoria, 2009. Industrial Waste Resource Guidelines. Retrieved 01. 06. 2019 from https://www.epa.vic.gov.au/~/media/Publications/IWRG621.pdf

Evdokimova, G.A., Korneykova, M.V., 2010. Microfungal communities in soil polluted with fluoride, Natural Science 2, 600-611. doi:10.4236/ns.2009.29125

Eyde, B., 1983. Determination of acid soluble fluoride in soils by means of an ion-selective electrode. Fresenius' Zeitschrift Für Analytische Chemie 316, 299–301. doi:10.1007/BF00468924

Fan, R., Huang, Y.C., Grusak, M.A., Huang, C.P., Sherrier, D.J., 2014. Effects of nano-TiO2 on the agronomically-relevant *Rhizobium*-legume symbiosis. Science of the Total Environment 466–467, 503–512. doi:10.1016/j.scitotenv.2013.07.032

Farrah, H., Slavek, J., Pickering, W.F., 1987. Fluoride interactions with hydrous aluminium oxides and alumina. Australian Journal of Soil Research 25, 55–69.

Foote, K.J., Joy, M.K., Death, R.G., 2015. New Zealand Dairy Farming: Milking our environment for all its worth. Environmental Management 56, 709–720. doi:10.1007/s00267-015-0517-x

Fornasiero, R.B., 2001. Phytotoxic effects of fluorides. Plant Science 161, 979–985. doi:10.1016/S0168-9452(01)00499-X

Francis, P., Burton, M.R., Oppenheimer, C., 1998. Remote measurements of volcanic gas compositions by solar occultation spectroscopy. Nature 396, 567.

Fraser, D.S., Vesely, E.T. 2011. Connecting North Island hill country farmers' nutrient requirements with soil mapping units. In: Adding to the knowledge base for the nutrient manager. (Eds L.D. Currie and C.L. Christensen). http://flrc.massey.ac.nz/publications.html. Occasional Report No. 24. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand. 8 pages.

Frentiu, T., Ponta, M., Hategan, R., 2013. Validation of an analytical method based on the high-resolution continuum source flame atomic absorption spectrometry for the fast-sequential determination of several hazardous/priority hazardous metals in soil. Chemistry Central Journal 7, 1–10. doi:10.1186/1752-153X-7-43

Fuge, R., 2019. Fluorine in the environment, a review of its sources and geochemistry. Applied Geochemistry 100, 393–406. doi:10.1016/j.apgeochem.2018.12.016

Fung, K.F., Zhang, Z.Q., Wong, J.W.C., Wong, M.H., 1999. Fluoride contents in tea and soil from tea plantations and the release of fluoride into tea liquor during infusion. Environmental Pollution 104, 197–205. doi:10.1016/S0269-7491(98)00187-0

Gago, C., Marcos, M.L.F., Lugo, E.Á., 2002. Aqueous Aluminium Species in Forest Soils affected by fluoride emissions from an aluminium smelter in NW Spain. Fluoride, 35, 110–121.

Gago, C., Romar, A., Fernández-Marcos, M.L., Álvarez, E., 2014. Fluoride sorption and desorption on soils located in the surroundings of an aluminium smelter in Galicia (NW Spain). Environmental Earth Sciences, 72, 4105-4114. doi.org/10.1007/s12665-014-3304-8

Gago, C., Romar, A., Fernández-Marcos, M.L., Álvarez, E., 2012. Fluorine sorption by soils developed from various parent materials in Galicia (NW Spain). Journal of Colloid and Interface Science 374, 232–236. doi:10.1016/j.jcis.2012.01.047

Gago, C., Romar, A., Fernández-Marcos, M.L., Álvarez, E., 2012. Fluorine sorption by soils developed from various parent materials in Galicia (NW Spain). Journal of Colloid and Interface Science 374, 232–236. doi:10.1016/j.jcis.2012.01.047

Gao, H., Zhang, Z., Wan, X., 2012. Influences of charcoal and bamboo charcoal amendment on soil-fluoride fractions and bioaccumulation of fluoride in tea plants. Environmental Geochemistry and Health 34, 551–562. doi:10.1007/s10653-012-9459-x

Garbuz, S.A., Yaroslavtseva, N.V., Kholodov, V.A., 2016. Enzymatic activity inside and outside of water-stable aggregates in soils under different land use. Eurasian Soil Science 49, 367–375. doi:10.1134/s1064229316030030

Garcia-Ciudad, A., Garcia-Criado, B., Emeterio, C.P.S., 1985. Determination of Fluoride in plant samples by a potentiometric method and Near-Infrared Reflectance

Spectroscopy. Communications in Soil Science and Plant Analysis 16, 1107–1122. doi:10.1080/00103628509367669

García-Gil, J.C., Kobza, J., Soler-Rovira, P., Javoreková, S., 2013. Soil microbial and enzyme activities response to pollution near an aluminium smelter. Clean - Soil, Air, Water 41, 485–492. doi:10.1002/clen.201200099

Geretharan, T., Jeyakumar, P., Bretherton, M., Anderson, C.W.N., 2018. Defining a standard method to measure the total and bioavailable concentration of fluorine in New Zealand soils. Microchemical Journal 142, 94–101. doi:10.1016/j.microc.2018.06.018

Ghorai, S., Pant, K.K., 2004. Investigations on the column performance of fluoride adsorption by activated alumina in a fixed-bed. Chemical Engineering Journal 98, 165–173. doi:10.1016/j.cej.2003.07.003

Government of Alberta, 2010. Alberta tier 1 soil and groundwater remediation guidelines. In: Air, L.a.W.P.B, Environmental Assurance Division. Edmonton, Alberta. Retrieved 02. 06. 2019 from https://open.alberta.ca/dataset/842becf6-dc0c-4cc7-8b29-e3f383133ddc/resource/1b851705-0622-485d-beee-752a627bdfc4/download/2016-albertatier1guidelines-feb02-2016a.pdf

Grace, N.D., Loganathan, P., Deighton, M.W., Molano, G., Hedley, M.J., 2005. Ingestion of soil fluorine: Its impact on the fluorine metabolism of dairy cows. New Zealand Journal of Agricultural Research 48, 23–27. doi:10.1080/00288233.2005.9513627

Grace, N.D., Loganathan, P., Hedley, M.J., Wallace, G.C., 2003. Ingestion of soil fluorine: Its impact on the fluorine metabolism and status of grazing young sheep. New Zealand Journal of Agricultural Research 46, 279–286. doi:10.1080/00288233.2003.9513555

Gray, C.W., 2018. Fluorine in soils under pasture following long-term application of phosphate fertiliser in New Zealand. Geoderma Regional 14, e00183. doi:10.1016/j.geodrs.2018.e00183

Gray, C.W., McLaren, R.G., Roberts, A.H.C., Condron, L.M., 1999. Solubility , sorption and desorption of native and added cadmium in relation to properties of soils in New Zealand. European Journal of Soil Science 50, 127-137. doi.org/10.1046/j.1365-2389.1999.00221.x

Gray, C.W., McLaren, R.G., Roberts, A.H.C., Condron, L.M., 1998. Sorption and desorption of cadmium from some New Zealand soils: Effect of pH and contact time, Australian Journal of Soil Research 36, 199–216. doi: 10.1071/S97085

Haidouti, C., 1991. Fluoride distribution in soils in the vicinity of a point emission source in Greece. Geoderma 49, 129–136.

Haldimann, M., Zimmerli, B., 1993. Evaluation of ashing procedures for the gas chromatographic determination of flouride in biological material.Analytica chimica acta 282, 589–601.

Haney, R., Haney, E., Hossner, L., Arnold, J., 2006. Development of a new soil extractant for simultaneous phosphorus, ammonium, and nitrate analysis. Communications in Soil Science and Plant Analysis 37, 1511–1523. doi:10.1080/00103620600709977

Harrington, L.F., Cooper, E.M., Vasudevan, D., 2003. Fluoride sorption and associated aluminum release in variable charge soils. Journal of Colloid and Interface Science 267, 302–313. doi:10.1016/S0021-9797(03)00609-X

Havránek, V., Kučera, J., Řanda, Z., Voseček, V., 2004. Comparison of fluorine determination in biological and environmental samples by NAA, PAA and PIXE. Journal of Radioanalytical and Nuclear Chemistry 259, 325–329. doi:10.1023/B:JRNC.0000017312.00776.e5

Hedley, M.J., Furness, H., Fick, J., 2011. Outlook for long-term P fertilizer demand and aspects of organic P re-cycling in New Zealand. In: Adding to the knowledge base for the nutrient manager. (Eds L.D. Currie and C.L. Christensen). http://flrc.massey.ac.nz/publications.html. Occasional Report No. 24. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand. 12 pages.

Hedley, M.J., Loganathan, P., Grace, N.D., 2007. Fertilizer-derived fluorine in grazed pasture systems. Australian Fertilizer Industry Conference, August, 2007.

Herridge, D.F., Peoples, M.B., Boddey, R.M., 2008. Global inputs of biological nitrogen fixation in agricultural systems. Plant and Soil 311, 1–18. doi:10.1007/s11104-008-9668-3

Horner, J.M., Bell, J.N.B., 1995. Effects of fluoride and acidity on early plant growth. Agriculture, Ecosystems and Environment 52, 205–211. doi:10.1016/0167-8809(94)00533-K

Huang, Y.C., Fan, R., Grusak, M.A., Sherrier, J.D., Huang, C.P., 2014. Effects of nano-ZnO on the agronomically relevant *Rhizobium*-legume symbiosis. Science of the Total Environment 497–498, 78–90. doi:10.1016/j.scitotenv.2014.07.100

Ivezić, V., Lončarić, Z., Engler, M., Kerovec, D., Singh, B.R., 2013. Comparison of different extraction methods representing available and total concentrations of Cd, Cu, Fe, Mn and Zn in soil. Poljoprivreda 19, 53–58.

Jackman, R.H., 1964. Accumulation of organic matter in some New Zealand soils under permanent pasture: II. Rates of mineralisation of organic matter and the supply of available nutrients. New Zealand Journal of Agricultural Research, 7, 472–479.

Jankowski, K., Jackowska, A., Mrugalska, M., 2007. Direct spectrometric determination of total fluorine in geological materials by continuous powder introduction into helium microwave induced plasma. Journal of Analytical Atomic Spectrometry 22, 386–391. doi:10.1039/b616170g

Jeyakumar, P., Anderson, C.W.N., 2015. Determination of total soil fluorine by various standard methods of analysis. Confidential report prepared by the Fertilizer and Lime Research Centre, Massey University

Jeyakumar, P., Anderson, C.W.N., 2016. Recent methodology developments in soil Fluorine analysis. In: Integrated nutrient and water management for sustainable farming. (Eds L.D. Currie and R. Singh). http://flrc.massey.ac.nz/publications.html. OccasionalReport No. 29. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand, 6 pages.

Jeyakumar, P., Loganathan, P., Sivakumaran, S., Anderson, C.W.N., McLaren, R.G., 2010. Bioavailability of copper and zinc to poplar and microorganisms in a biosolids-amended soil. Australian Journal of Soil Research 48, 459–469. doi:10.1071/SR09169

Jha, S.K., Nayak, A.K., Sharma, Y.K., 2008. Response of spinach (*Spinacea oleracea*) to the added fluoride in an alkaline soil. Food and Chemical Toxicology 46, 2968–2971. doi:10.1016/j.fct.2008.05.024

Jha, S.K., Nayak, A.K., Sharma, Y.K., 2009. Fluoride toxicity effects in onion (*Allium cepa* L.) grown in contaminated soils. Chemosphere 76, 353–356. doi:10.1016/j.chemosphere.2009.03.044

Jones, D.L., Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biology and Biochemistry 38, 991–999. doi:10.1016/j.soilbio.2005.08.012

Kabata-Pendias, A., 2010. Trace elements in soils and plants. CRC press.

Kaufhold, S., Dohrmann, R., Abidin, Z., Henmi, T., Matsue, N., Eichinger, L., Kaufhold, A., Jahn, R., 2010. Allophane compared with other sorbent minerals for the removal of fluoride from water with particular focus on a mineable Ecuadorian allophane. Applied Clay Science 50, 25–33. doi:10.1016/j.clay.2010.06.018

Keerthisinghe, G., McLaughlin, M.J., Freney, J.R., 1991. Use of gypsum, phosphogypsum and fluoride to ameliorate subsurface acidity in a pasture soil. In Plant-Soil Interactions at Low pH (pp. 509-517). Springer Netherlands. doi:10.1007/978-94-011-3438-5_57

Kim, N., 2008. Cadmium accumulation in Waikato soils. WRC Technical Report TR2005/51 (ISSN 1172-4005), Waikato Regional Council, Hamilton, New Zealand. Retrieved 06. 01. 2019 from http://www.waikatoregion.govt.nz/Services/Publications/Technical-Reports/TR-200551/

Kim, N.D., Taylor, M.D., Drewry, J.J., 2016. Anthropogenic fluorine accumulation in the Waikato and Bay of Plenty regions of New Zealand: comparison of field data with projections. Environmental Earth Sciences 75, 147. doi:10.1007/s12665-015-4897-2

Klumpp, A., Domingos, M., de Moraes, R.M., Klumpp, G., 1998. Effects of complex air pollution on tree species of the atlantic rain forest near Cubatão, Brazil. Chemosphere 36, 989–994.

Klumpp, A., Domingos, M., Pignata, M.L., 2000. Air pollution and vegetation damage in South America–state of knowledge and perspectives (pp. 111-136). CRC Press LLC, United States of America.

Knight, H.G., Furr, A.K., Parkinson, T.F., 1977. Determination of Fluorine by Neutron Activation Analysis. Analytical Chemistry 49, 1507–1510. doi:10.1021/ac50019a013

Kumar, B., Naaz, A., Shukla, K., Narayan, C., Singh, G., Kumar, A., Ramanathan, A.L., 2016. Spatial variability of fluorine in agricultural soils around Sidhi District, Central India. Journal of the Geological Society of India 87, 227–235. doi:10.1007/s12594-016-0391-z

Langer, U., Günther, T., 2001. Effects of alkaline dust deposits from phosphate fertilizer production on microbial biomass and enzyme activities in grassland soils. Environmental Pollution 112, 321–327. doi:10.1016/S0269-7491(00)00148-2

Larsen, S., Widdowson, A.E., 1971. Soil Fluorine. European Journal of Soil Science 22, 210–221. doi:10.1111/j.1365-2389.1971.tb01608.x

Lee, J., 1996. Accumulation of cadmium with time in Romney sheep grazing ryegrass-white clover pasture : effect of cadmium from pasture and soil intake. Australian Journal of Agricultural Research 47, 877-94.

Liu, R., Zhu, L., Gong, W., Lan, H., Liu, H., Qu, J., 2013. Effects of fluoride on coagulation performance of aluminium chloride towards Kaolin suspension. Colloids and Surfaces A: Physicochemical and Engineering Aspects 421, 84–90. doi:10.1016/j.colsurfa.2012.12.047

Liu, X., Tian, J., Liu, L., Zhu, T., Yu, X., Chu, X., Yao, B., Wu, N., Fan, Y., 2017. Identification of an operon involved in fluoride resistance in Enterobacter cloacae FRM. Scientific Reports 7, 1–9. doi:10.1038/s41598-017-06988-1

Liu, X., Wang, B., Zheng, B., 2014. Geochemical process of fluorine in soil. Chinese Journal of Geochemistry 33, 277–279. doi:10.1007/s11631-014-0688-9

Loganathan, P., Bretherton, M.R., Hedley, M.J., 2007. Effect of soil cultivation and winter pugging on fluorine distribution in soil profiles under pasture following long-term applications of phosphate fertilisers. Australian Journal of Soil Research 45, 41–47. doi:10.1071/SR06094

Loganathan, P., Gray, C.W., Hedley, M.J., Roberts, A.H.C., 2006. Total and soluble fluorine concentrations in relation to properties of soils in New Zealand. European Journal of Soil Science 57, 411–421. doi:10.1111/j.1365-2389.2005.00751.x

Loganathan, P., Hedley, M.J., Wallace, G.C., Roberts, A.H.C., 2001. Fluoride accumulation in pasture forages and soils following long-term applications of phosphorus fertilisers. Environmental Pollution 115, 275–282. doi:10.1016/S0269-7491(01)00102-6

Loganathan, P., Hedley, M.J., Grace, N.D., 2008. Pasture soils contaminated with fertilizer derived cadmium and fluoride: livestock effects. Reviews of Environmental Contamination and Toxicology 192, 29–66. doi:10.1007/978-0-387-71724-1_2

Loganathan, P., Hedley, M.J., Grace, N.D., Lee, J., Cronin, S.J., Bolan, N.S., Zanders, J.M., 2003. Fertiliser contaminants in New Zealand grazed pasture with special reference to cadmium and fluorine: A review. Australian Journal of Soil Research 41, 501–532. doi:10.1071/SR02126

Loganathan, P., Vigneswaran, S., Kandasamy, J., Naidu, R., 2013. Defluoridation of drinking water using adsorption processes. Journal of Hazardous Materials 248–249, 1–19. doi:10.1016/j.jhazmat.2012.12.043

Louback, E., Pereira, T.A.R., de Souza, S.R., de Oliveira, J.A., da Silva, L.C., 2016. Vegetation damage in the vicinity of an aluminum smelter in Brazil. Ecological Indicators 67, 193–203. doi:10.1016/j.ecolind.2016.02.044

Lu, C., Chung, Y.L., Chang, K.F., 2005. Adsorption of trihalomethanes from water with carbon nanotubes. Water Research 39, 1183–1189. doi:10.1016/j.watres.2004.12.033

Luo, F., Inoue, K., 2004. The removal of Fluoride ion by using Metal(III)-Loaded Amberlite Resins. Solvent Extraction and Ion Exchange 22, 305–322.

doi:10.1081/SEI-120028007

Ma, H., Wu, X., Yang, M., Wang, J., Wang, J., Wang, J., 2014. Chemosphere Effects of fluoride on bacterial growth and its gene / protein expression. Chemosphere 100, 190–193. doi:10.1016/j.chemosphere.2013.11.032

Ma, J.F., 2000. Role of organic acids in detoxification of aluminum in higher plants. Plant and Cell Physiology 41, 383–390. doi:10.1093/pcp/41.4.383

Ma, L., Wang, X., Tao, J., Feng, X., Liu, X., Qin, W., 2017. Differential fluoride tolerance between sulfur and ferrous iron-grown *Acidithiobacillus ferrooxidans* and its mechanism analysis. Biochemical Engineering Journal 119, 59–66. doi:10.1016/j.bej.2016.12.013

MacLean, D.C., Hansen, K.S., Schneider, R.E., 1992. Amelioration of aluminium toxicity in wheat by fluoride. New Phytologists 121, 81– 88.

MacLeod, C.J., Moller, H., 2006. Intensification and diversification of New Zealand agriculture since 1960: An evaluation of current indicators of land use change. Agriculture, Ecosystems and Environment 115, 201–218. doi:10.1016/j.agee.2006.01.003

Maier, N.A., McLaughlin, M.J., Heap, M., Butt, M., Smart, M.K., Williams, C.M.J., 1997. Effect of current season applications of calcitic lime on pH, yield and cadmium concentration of potato (*Solanum tuberosum* L.) tubers. Nutrient Cycling in

Agroecosystems. 47, 1–12.

Maiti, A., Basu, J.K., De, S., 2011. Chemical treated laterite as promising fluoride adsorbent for aqueous system and kinetic modeling. Desalination 265, 28–36. doi:10.1016/j.desal.2010.07.026

Malde, M.K., Bjorvatn, K., Julshamn, K., 2001. Determination of fluoride in food by the use of alkali fusion and fluoride ion-selective electrode. Food Chemistry 73, 373–379.

Manier, N., Deram, A., Broos, K., Denayer, F.O., Van Haluwyn, C., 2009. White clover Nodulation Index in heavy metal contaminated soils–A Potential Bio indicator. Journal of Environmental Quality 38, 685–692. doi:10.2134/jeq2008.0013

Manoharan, V., Loganathan, P., Parfitt, R.L., Tillman, R.W., 1996. Changes in soil solution composition and aluminium speciation under legume-based pastures in response to long-term phosphate fertiliser applications. Australian Journal of Soil Research 34, 985–998.

Marguí, E., Hidalgo, M., Queralt, I., 2005. Multielemental fast analysis of vegetation samples by wavelength dispersive X-ray fluorescence spectrometry: Possibilities and drawbacks. Spectrochimica Acta – Part B Atomic Spectroscopy 60, 1363–1372. doi:10.1016/j.sab.2005.08.004

Marquis, R.E., 1995. Antimicrobial actions of fluoride for oral bacteria. Canadian Journal of Microbiology 41, 955–64. doi:10.1139/m95-133

Marquis, R.E., Clock, S.A., Mota-Meira, M., 2003. Fluoride and organic weak acids as modulators of microbial physiology. FEMS Microbiology Reviews 26, 493–510. doi:10.1016/S0168-6445(02)00143-2

Marschner, B., Kalbitz, K., 2003. Controls of bioavailability and biodegradability of dissolved organic matter in soils. Geoderma 113, 211–235. doi:10.1016/S0016-7061(02)00362-2

McGrath, S.P., Cunliffe, C.H., 1985. A simplified method for the extraction of the metals Fe, Zn, Cu, Ni, Cd, Pb, Cr, Co and Mn from soils and sewage sludges. Journal of the Science of Food and Agriculture 36, 794–798. doi:10.1002/jsfa.2740360906

McLaughlin, M.J, Simpson, P.G., Fleming, N., Stevens, D.P., Cozens, G., Smart, M.K., 1997. Effect of fertiliser type on cadmium and fluorine concentrations in clover herbage. Australian Journal of Experimental Agriculture 37, 1019–1026. doi:10.1071/EA98132

McLaughlin, M.J., Stevens, D.P., Keerthisinghe, D.G., Cayley, J.W.D., Ridley. A.M., 2001. Contamination of soil with fluoride by long-term application of superphosphates to pastures and risk to grazing animals. Australian Journal of Soil Research 40, 431–459. doi:10.1071/SR01114

McQuaker, N.R., Gurney, M., 1977. Determination of total Fluoride in soil and vegetation

using an Alkali Fusion-Selective Ion Electrode Technique. Analytical Chemistry 49, 53–56. doi:10.1021/ac50009a022

Meenakshi, S., Sundaram, C.S., Sukumar, R., 2008. Enhanced fluoride sorption by mechanochemically activated kaolinites. Journal of Hazardous Materials 153, 164–172. doi:10.1016/j.jhazmat.2007.08.031

Mehlich, A., 1978. New extractant for soil test evaluation of phosphorus, potassium, magnesium, calcium, sodium, manganese and zinc. Communications in Soil Science and Plant Analysis 9, 477–492. doi:10.1080/00103627809366824

Mikkonen, H.G., van de Graaff, R., Mikkonen, A.T., Clarke, B.O., Dasika, R., Wallis, C.J., Reichman, S.M., 2018. Environmental and anthropogenic influences on ambient background concentrations of fluoride in soil. Environmental Pollution 242, 1838–1849. doi:10.1016/j.envpol.2018.07.083

Mirlean, N., Roisenberg, A., 2007. Fluoride distribution in the environment along the gradient of a phosphate-fertilizer production emission (southern Brazil). Environmental Geochemistry and Health 29, 179–187. doi:10.1007/s10653-006-9061-1

Mohapatra, M., Anand, S., Mishra, B.K., Giles, D.E., Singh, P., 2009. Review of fluoride removal from drinking water. Journal of Environmental Management 91, 67–77. doi:10.1016/j.jenvman.2009.08.015

Molla, 2007. Measurement of the concentrations of fluorine in the soil of different areas of Savar and its effects on environment. BRAC University Journal 4, 13–17.

Monaghan, R.M., de Klein, C.A.M., Muirhead, R.W., 2008. Prioritisation of farm scale remediation efforts for reducing losses of nutrients and faecal indicator organisms to waterways: A case study of New Zealand dairy farming. Journal of Environmental Management 87, 609–622. doi:10.1016/j.jenvman.2006.07.017

Nogueira, M.A., Albino, U.B., Brandão-Junior, O., Braun, G., Cruz, M.F., Dias, B.A., Duarte, R.T.D., Gioppo, N.M.R., Menna, P., Orlandi, J.M., Raimam, M.P., Rampazo, L.G.L., Santos, M.A., Silva, M.E.Z., Vieira, F.P., Torezan, J.M.D., Hungria, M., Andrade, G., 2006. Promising indicators for assessment of agroecosystems alteration among natural, reforested and agricultural land use in southern Brazil. Agriculture, Ecosystems and Environment 115, 237–247. doi:10.1016/j.agee.2006.01.008

Notcutt, G., Davies, F., 2001. Environmental accumulation of airborne Fluorides in Romania. Environmental Geochemistry and Health 23, 43–51. doi:10.1023/A:1011062115049

Ochoa-Herrera, V., Banihani, Q., Len, G., Khatri, C., Field, J.A., Sierra-Alvarez, R., 2009. Toxicity of fluoride to microorganisms in biological wastewater treatment systems. Water Research 43, 3177–3186. doi:10.1016/j.watres.2009.04.032

Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of available

phosphorus in soils by extraction with sodium carbonate. USDA Department circular 939. Government Printing Office, Washington DC

Omueti, J.A.I., Jones, R.L., 1977. Fluoride adsorption by illinois soils. Journal of Soil Science 28, 564–572. doi:10.1111/j.1365-2389.1977.tb02264.x

Óskarsson, N., 1980. The interaction between volcanic gases and tephra: Fluorine adhering to tephra of the 1970 hekla eruption. Journal of Volcanology and Geothermal Research 8, 251–266. doi:10.1016/0377-0273(80)90107-9

Papageorgiou, S.K., Katsaros, F.K., Kouvelos, E.P., Nolan, J.W., Le Deit, H., Kanellopoulos, N.K., 2006. Heavy metal sorption by calcium alginate beads from Laminaria digitata. Journal of Hazardous Materials 137, 1765–1772. doi:10.1016/j.jhazmat.2006.05.017

Pereira, S.I.A., Lima, A.I.G., Figueira, E.M. de A.P., 2006. Heavy metal toxicity in *Rhizobium leguminosarum* biovar viciae isolated from soils subjected to different sources of heavy-metal contamination: Effects on protein expression. Applied Soil Ecology 33, 286–293. doi:10.1016/j.apsoil.2005.10.002

Pickering, W.F., 1985. The mobility of soluble fluoride in soils. Environmental Pollution. Series B, Chemical and Physical 9, 281–308. doi:10.1016/0143-148X(85)90004-7

Pomazkina, L.V., Kotova, L.G., Zorina, S.Y., Rybakova, A.V., 2008. Comparative assessment of agroecosystems developed on different types of soils contaminated by

fluorides from aluminum smelters in the cis-Baikal region. Eurasian Soil Science 41, 629–637. doi:10.1134/s1064229308060082

Porter, J.R., Sheridan, R.P., 1981. Inhibition of nitrogen fixation in alfalfa by arsenate, heavy metals, fluoride, and simulated acid rain. Plant Physiology 68, 143–148. doi:10.1104/pp.68.1.143

Poulsen, R., 2011. The effect of fluoride pollution on soil microorganisms. Dissertation, University of Iceland. Identifier: hdl.han- dle.net/1946/1039

Pribyl, D.W., 2010. A critical review of the conventional SOC to SOM conversion factor. Geoderma 156, 75–83. doi:10.1016/j.geoderma.2010.02.003

Qiao, L.I., Zhang, S.H., Yu, Y.H., Wang, L.P., Guan, S.W., Li, P.F., 2012. Toxicity of sodium fluoride to Caenorhabditis elegans. Biomedical and Environmental Sciences 25, 216–223.

Quintáns-Fondo, A., Ferreira-Coelho, G., Paradelo-Núñez, R., Nóvoa-Muñoz, J.C., Arias-Estévez, M., Fernández-Sanjurjo, M.J., Álvarez-Rodríguez, E., Núñez-Delgado, A., 2016a. Promoting sustainability in the mussel industry: mussel shell recycling to fight fluoride pollution. Journal of Cleaner Production 131, 485–490. doi:10.1016/j.jclepro.2016.04.154

Quintáns-Fondo, A., Ferreira-Coelho, G., Paradelo-Núñez, R., Nóvoa-Muñoz, J.C., Arias-Estévez, M., Fernández-Sanjurjo, M.J., Álvarez-Rodríguez, E., Núñez-

Delgado, A., 2016b. F sorption/desorption on two soils and on different by-products and waste materials. Environmental Science and Pollution Research 23, 14676–14685. doi:10.1007/s11356-016-6959-8

Quintáns-fondo, A., Santás-miguel, V., Nóvoa-muñoz, J.C., 2018. Effects of changing pH , incubation time, and As (V) competition, on F − retention on soils, natural adsorbents, by-products, and waste materials 6, 1–9. doi:10.3389/fchem.2018.00051

Ramos, A.A., Ohde, S., Hossain, M.M.M., Ozaki, H., Sirirattanachai, S., Apurado, J.L., 2005. Determination of fluorine in coral skeletons by instrumental neutron activation analysis. Journal of Radioanalytical and Nuclear Chemistry 266, 19–29. doi:10.1007/s10967-005-0863-x

Rao, D.N., Pal, D., 1978. Effect of fluoride pollution on the organic matter content of soil. Plant and Soil 49, 653-656. doi:10.1007/BF02183290

Rensing, C., Maier, R.M., 2003. Issues underlying use of biosensors to measure metal bioavailability. Ecotoxicology and Environmental Safety 56, 140–147. doi:10.1016/S0147-6513(03)00057-5

Rietig, A., Acker, J., 2017. Development and validation of a new method for the precise and accurate determination of trace elements in silicon by ICP-OES in high silicon matrices. Journal of Analytical Atomic Spectrometry. 32, 322–333. doi:10.1039/C6JA00241B

Rivaie, A.A., Loganathan, P., Graham, J.D., Tillman, R.W., Payn, T.W., 2008. Effect of phosphate rock and triple superphosphate on soil phosphorus fractions and their plant-availability and downward movement in two volcanic ash soils under *Pinus radiata* plantations in New Zealand. Nutrient Cycling in Agroecosystems 82, 75–88. doi:10.1007/s10705-008-9170-6

Rodrigues, M.L.M., Lopes, K.C.S., Leôncio, H.C., Silva, L.A.M., Leão, V.A., 2016. Bioleaching of fluoride-bearing secondary copper sulphides: Column experiments with *Acidithiobacillus ferrooxidans*. Chemical Engineering Journal 284, 1279–1286. doi:10.1016/j.cej.2015.09.020

Rodríguez, C.G., Rodríguez, E.Á., Marcos, M.L.F., 2001. Comparison of methods for fluoride extraction from forest and cropped soils in vicinity of an aluminum smelter in galicia (NW Spain). Communications in Soil Science and Plant Analysis 32, 2503–2517. doi:10.1081/CSS-120000387

Romar, A., Gago, C., Fernndez-Marcos, M.L., Lvarez, E., 2009. Influence of Fluoride addition on the composition of solutions in equilibrium with acid soils. Pedosphere 19, 60–70. doi:10.1016/S1002-0160(08)60084-3

Romar-Gasalla, A., Santás-miguel, V., Nóvoa-muñoz, J.C., Arias-estévez, M., Álvarez-rodríguez, E., Núñez-delgado, A., 2018. Chromium and fluoride sorption / desorption on un-amended and waste-amended forest and vineyard soils and pyritic material. Journal of Environmental Management 222, 3–11. doi:10.1016/j.jenvman.2018.05.050

Romić, M., Matijević, L., Bakić, H., Romić, D., 2014. Copper accumulation in vineyard soils: distribution, fractionation and bioavailability assessment. In Environmental risk assessment of soil contamination, Rijeka: InTechOpen

Ropelewska, E., Dziejowski, J., Zapotoczny, P., 2016. Changes in the microbial activity and thermal properties of soil treated with sodium fluoride. Applied Soil Ecology 98, 159–165. doi:10.1016/j.apsoil.2015.10.013

Ruan, J., Ma, L., Shi, Y., Han, W., 2003. Uptake of fluoride by tea plant (*Camellia sinensis* L) and the impact of aluminium. Journal of the Science of Food and Agriculture 83, 1342–1348. doi:10.1002/jsfa.1546

Ruan, J., Ma, L., Shi, Y., Han, W., 2004. The impact of pH and calcium on the uptake of fluoride by tea plants (*Camellia sinensis* L.). Annals of Botany 93, 97–105. doi:10.1093/aob/mch010

Saggar, S., Hedley, M.J., White, R.E., 1990. A simplified resin membrane technique for extracting phosphorus from soils. Fertilizer Research 24, 173–180. doi:10.1007/BF01073586

Saha, J.K., Kundu, S., 2003. Determination of Fluoride in soil water extract through Ion Chromatography. Communications in Soil Science and Plant Analysis 34, 181–188. doi:10.1081/CSS-120017424

Samatya, S., Yüksel, Ü., Yüksel, M., Kabay, N., 2007. Removal of Fluoride from water

by metal ions (Al^{3+}, La^{3+} and ZrO^{2+}) loaded natural zeolite. Separation Science and Technology 42, 2033–2047. doi:10.1080/01496390701310421

Sarathchandra, S.U., Perrott, K.W., Upsdell, M.P., 1984. Microbiological and biochemical characteristics of a range of New Zealand soils under established pasture. Soil Biology and Biochemistry 16, 177–183. doi:10.1016/0038-0717(84)90109-3

Sebaugh, J.L., 2011. Guidelines for accurate EC50/IC50 estimation. Pharmaceutical Statistics 10, 128–134. doi:10.1002/pst.426

Shafqat, M.N., Pierzynski, G.M., 2014. The Freundlich adsorption isotherm constants and prediction of phosphorus bioavailability as affected by different phosphorus sources in two Kansas soils. Chemosphere 99, 72–80. doi:10.1016/j.chemosphere.2013.10.009

Shcherbakova T.A., 1983. Enzymatic activity of soil and transformation of organic matter in natural and artificial phytocenoses. Nauka i Takhnika, Minsk. 222p

Shyam, R., Kalwania, G.S., 2012. Health risk assessment of fluoride with other parameters in ground water of Sikar city (India). Environmental Earth Sciences 65, 1275–1282. doi:10.1007/s12665-011-1375-3

Sibbesen, E., 1977. A simple ion-exchange resin procedure for extracting plant-available elements from soil. Plant and Soil 46, 665–669. doi:10.1007/BF00015928

Sicupira, L., Veloso, T., Reis, F., Leão, V., 2011. Assessing metal recovery from low-grade copper ores containing fluoride. Hydrometallurgy 109, 202–210. doi:10.1016/j.hydromet.2011.07.003

Simard, R.R., Lafrance, P., 1996. Fluoride sorption and desorption indices in Quebec soils. Communications in Soil Science and Plant Analysis 27, 853–866. doi:10.1080/00103629609369602

Singh, A., Chhabra, R., Abrol, I.P., 1979. Effect of fluorine and phosphorus applied to a sodic soil on their availability and on yield and chemical composition of wheat. Soil Science 128, 90–97

Srivastava, A., Chhillar, S., Singh, D., Acharya, R., Pujari, P.K., 2014. Determination of fluorine concentrations in soil samples using proton induced gamma-ray emission. Journal of Radioanalytical and Nuclear Chemistry 302, 1461–1464. doi:10.1007/s10967-014-3661-5

Statistics New Zealand (2017). Exports and imports hit new highs in 2017. Retrieved 15. 01. 2019 from https://www.stats.govt.nz/tereo/news/exports-and-imports-hit-new-highs-in-2017

Stevens, D.P., McLaughlin, M.J., Alston, A.M. 1997. Phytotoxicity of aluminium-fluoride complexes and their uptake from solution culture by *Avena sativa* and *Lycopersicon esculentum*. Plant and Soil 192, 81-93. doi:10.1023/A:100422452

Stevens, D.P., McLaughlin, M.J., Alston, A.M. 1998. Phytotoxicity of the fluoride ion and its uptake from solution culture by *Avena sativa* and *Lycopersicon esculentum*. Plant and Soil 200, 119-129. doi.org/10.1023/A:1004392801938

Stone, M., Marsalek, J., 1996. Trace metal composition and speciation in street sediment: Sault Ste. Marie, Canada. Water, Air, and Soil Pollution 87, 149-169.

Šucman, E., Bednár, J., 2012. Determination of fluoride in plant material using microwave induced oxygen combustion. Czech Journal of Food Sciences 30, 438–441. doi:10.2754/avb201281030319

Sutton, S.V.W., Bender, G.R., Marquis, R.E., 1987. Fluoride inhibition of proton-translocating ATPases of oral bacteria. Infection and Immunity 55, 2597–2603. doi:0019-9567/87/112597-07$02.00/0

Symonds, R.B., Rose, W.I., Reed, M.H., 1988. Contribution of C1-and F-bearing gases to the atmosphere by volcanoes. Nature 334, 415.

Szostek, R., Ciećko, Z., Walczak, M., Swiontek-brzezinska, M., 2015. Microbiological and enzymatic activity of soil after pollution with Fluorine. Polish Journal of Environmental Studies 24, 2641–2646. doi:10.15244/pjoes/59491

Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biology and Biochemistry 1, 301–307. doi:10.1016/0038-

0717(69)90012-1

Tarsoly, G., Óvári, M., Záray, G., 2010. Determination of fluorine by total reflection X-ray fluorescence spectrometry. Spectrochimica Acta - Part B Atomic Spectroscopy 65, 287–290. doi:10.1016/j.sab.2010.02.019

Taylor, M.D., 2000. Determination of total phosphorus in soil using simple Kjeldahl digestion. Communications in Soil Science and Plant Analysis 31, 2665–2670. doi:10.1080/00103620009370616

Teng, S.X., Wang, S.G., Gong, W.X., Liu, X.W., Gao, B.Y., 2009. Removal of fluoride by hydrous manganese oxide-coated alumina: Performance and mechanism. Journal of Hazardous Materials 168, 1004–1011. doi:10.1016/j.jhazmat.2009.02.133

Thomas, J., Glass, H.D., White, W.A., Trandel, R.M., 1977. Fluoride content of clay minerals and argillaceous earth materials. Clays and Clay Minerals 25, 278–284. doi:10.1346/CCMN.1977.0250405

Thompson, L.K., Sidhu, S.S., Roberts, B.A., 1979. Fluoride accumulations in soil and vegetation in the vicinity of a phosphorus plant. Environmental Pollution 18, 221-234. doi:10.1016/0013-9327(79)90104-6

Thompson, M.H., 2017. *Cadmium management in New Zealand's horticultural soils* (Masters thesis, Massey University, Palmerston North, New Zealand). Retrieved 24. 01. 2019 from https://mro.massey.ac.nz/handle/10179/12548

Tscherko, D., Kandeler, E., 1997. Ecotoxicological effects of fluorine deposits on microbial biomass and enzyme activity in grassland. European Journal of Soil Science 48, 329–335. doi:10.1111/j.1365-2389.1997.tb00553.x

Vallejo, V.E., Roldan, F., Dick, R.P., 2010. Soil enzymatic activities and microbial biomass in an integrated agroforestry chronosequence compared to monoculture and a native forest of Colombia. Biology and Fertility of Soils 46, 577–587. doi:10.1007/s00374-010-0466-8

Van den Hoop, M.A.G.T., Cleven, R.F.M.J., Van Staden, J.J., Neele, J., 1996. Analysis of fluoride in rain water. Comparison of capillary electrophoresis with ion chromatography and ion-selective electrode potentiometry. Journal of Chromatography A 739, 241–248. doi:10.1016/0021-9673(96)00029-5

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19, 703–707. doi:10.1016/0038-0717(87)90052-6

Vargas, A.M.M., Cazetta, A.L., Kunita, M.H., Silva, T.L., Almeida, V.C., 2011. Adsorption of methylene blue on activated carbon produced from flamboyant pods (*Delonix regia*): Study of adsorption isotherms and kinetic models. Chemical Engineering Journal 168, 722–730. doi:10.1016/j.cej.2011.01.067

Varshney, P., Saini, R., Taneja, A., 2016. Trace element concentration in fine particulate

matter (PM 2.5) and their bioavailability in different microenvironments in Agra, India: a case study. Environmental Geochemistry and Health 38, 593-605.

Velmourougane, K., Venugopalan, M.V., Bhattacharyya, T., Sarkar, D., Pal, D.K., Sahu, A., Chandran, P., Ray, S.K., Mandal, C., Nair, K.M., Prasad, J., Singh, R.S., Tiwary, P., 2014. Microbial biomass carbon status in agro-ecological sub regions of black soils in India. Proceedings of the National Academy of Sciences India Section B - Biological Sciences 84, 519–529. doi:10.1007/s40011-013-0238-y

Veloso, T.C., Sicupira, L.C., Rodrigues, I.C.B., Silva, L.A.M., Leo, V.A., 2012. The effects of fluoride and aluminum ions on ferrous-iron oxidation and copper sulfide bioleaching with *Sulfobacillus thermosulfidooxidans*. Biochemical Engineering Journal 62, 48–55. doi:10.1016/j.bej.2012.01.003

Venable, J.H., Coggeshall, R., 1965. A simplified lead citrate stain for use in electron microscopy. The Journal of Cell Biology, 25, 407. doi: 10.1083/jcb.25.2.407

Verkerk, G., 2003. Pasture-based dairying: challenges and rewards for New Zealand producers. Theriogenology 59, 553-561.

Vithanage, M., Bhattacharya, P., 2015. Fluoride in the environment: sources, distribution and defluoridation. Environmental Chemistry Letters 13, 131–147. doi:10.1007/s10311-015-0496-4

Wakelin, S., Cavanagh, J., Young, S., Gray, C., van Ham, R., 2016. Cadmium in New Zealand pasture soils: toxicity to *Rhizobia* and white clover. New Zealand Journal of Agricultural Research 59, 65–78. doi:10.1080/00288233.2015.1130725

Wakelin, S., Gerard, E., Black, A., Hamonts, K., Condron, L., Yuan, T., Nostrand, J. Van, Zhou, J., Callaghan, M.O., 2014. Mechanisms of pollution induced community tolerance in a soil microbial community exposed to Cu. Environmental Pollution 190, 1–9. doi:10.1016/j.envpol.2014.03.008

Wakelin, S., Lombi, E., Donner, E., Macdonald, L., Black, A., Callaghan, M.O., 2013. Application of MicroResp™ for soil ecotoxicology. Environmental Pollution 179, 177–184. doi:10.1016/j.envpol.2013.04.010

Wakelin, S., Tillard, G., van Ham, R., Ballard, R., Farquharson, E., Gerard, E., Geurts, R., Brown, M., Ridgway, H., O'Callaghan, M., 2018. High spatial variation in population size and symbiotic performance of *Rhizobium leguminosarum* bv. trifolii with white clover in New Zealand pasture soils. PLoS ONE 13, 1–16. doi:10.1371/journal.pone.0192607

Wakelin, S.A., Van Koten, C., O'Callaghan, M., Brown, M., 2013. Physicochemical properties of 50 New Zealand pasture soils: A starting point for assessing and managing soil microbial resources. New Zealand Journal of Agricultural Research 56, 248–260. doi:10.1080/00288233.2013.822003

Wang, G.M., Luo, Z.X., Zhang, J.Y., Zhao, Y.C., 2015. Modes of occurrence of Fluorine

by extraction and SEM method in a coal-fired power plant from Inner Mongolia, China. Minerals 5, 863–869. doi:10.3390/min5040530

Wang, M., Tang, Y., Anderson, C.W.N., Jeyakumar, P., Yang, J., 2018. Effect of simulated acid rain on fluorine mobility and the bacterial community of phosphogypsum. Environmental Science and Pollution Research 25, 15336-15348. doi: 10.1007/s11356-018-1408-5

Wang, W., Li, R., Tan, J., Luo, K., Yang, L., Li, H., Li, Y., 2002. Adsorption and leaching of fluoride in soils of China. Fluoride 35, 122–129.

Wang, Z., Shen, D., Shen, F., Li, T., 2016. Phosphate adsorption on lanthanum loaded biochar. Chemosphere 150, 1–7. doi:10.1016/j.chemosphere.2016.02.004

Weinstein, L.N., Halscher-Herman, R., 1982. Physiological responses of plants to fluorine. In Effects of gaseous air pollutants in agriculture and horticulture (pp. 139–167). Butterworths, London.

Weinstein, L.H., Davison, A.W., 2004. Fluorides in the Environment. Cambridge (UK): CABI Publishing.

Wenzel, W.W., Blum, W.E.H., 1992. Fluorine speciation and mobility in F-contaminated soils. Soil Science 153, 357-364. doi: 10.1097/00010694-199205000-00003

Wuenscher, R., Unterfrauner, H., Peticzka, R., Zehetner, F., 2015. A comparison of 14

soil phosphorus extraction methods applied to 50 agricultural soils from Central Europe. Plant, Soil and Environment 61, 86–96. doi:10.17221/932/2014-PSE

Xu, R., Wang, Y., Zhao, A., Ji, G., Zhang, H., 2006. Effect of low molecular weight organic acids on adsorption and desorption of fluoride on variable charge soils. Environmental Geochemistry and Health 28, 141-146.

Yang, Y., Liu, Y., Huang, C.F., de Silva, J., Zhao, F.J., 2016. Aluminium alleviates fluoride toxicity in tea (Camellia sinensis). Plant and Soil 402, 179-190.

Yasuda, K., Hsu, T., Gallini, C.A., Mclver, L.J., Schwager, E., Shi, A., Garrett, W.S., 2017. Fluoride depletes acidogenic taxa in oral but not gut microbial communities in mice. mSystems 2, e00047-17. doi:10.1128/mSystems.00047-17

Yeomans, J.C., Bremner, J.M., 1988. A rapid and precise method for routine determination of organic carbon in soil1. Communications in Soil Science and Plant Analysis 19, 1467–1476. doi:10.1080/00103628809368027

Yessica, G.P., Alejandro, A., Ronald, F.C., José, A.J., Esperanza, M.R., Samuel, C.S.J., Remedios, M.L.M.A., Ormeño-Orrillo, E., 2013. Tolerance, growth and degradation of phenanthrene and benzo[a]pyrene by *Rhizobium tropici* CIAT 899 in liquid culture medium. Applied Soil Ecology 63, 105–111. doi:10.1016/j.apsoil.2012.09.010

Yiping, H., Caiyun, W., 2010. Ion chromatography for rapid and sensitive determination

of fluoride in milk after headspace single-drop microextraction with in situ generation of volatile hydrogen fluoride. Analytica Chimica Acta 661, 161–166. doi:10.1016/j.aca.2009.12.018

Yu, P., Lee, A.P., Phillips, B.L., Casey, W.H., 2003. Potentiometric and 19F nuclear magnetic resonance spectroscopic study of fluoride substitution in the GaAl12 polyoxocation: Implications for aluminum (hydr)oxide mineral surfaces. Geochimica et Cosmochimica Acta 67, 1065–1080. doi:10.1016/S0016-7037(02)00919-5

Zaim, N., Dogan, C., Camtakan, Z., 2016. Neutron activation analysis of soil samples from different parts of Edirne in Turkey. Journal of Applied Spectroscopy 83, 271–276. doi:10.1007/s10812-016-0280-7

Zhang, C., Li, Z., Gu, M., Deng, C., Liu, M., Li, L., 2010. Spatial and vertical distribution and pollution assessment of soil fluorine in a lead-zinc mining area in the Karst region of Guangxi, China 2010, 282–287.

Zhang, H.M., Su, B.Y., Liu, P.H., Zhang, W., 2007. Experimental tudy of Fluorine transport rules in unsaturated stratified soil. Journal of China University of Mining and Technology 17, 382–386. doi:10.1016/S1006-1266(07)60110-2

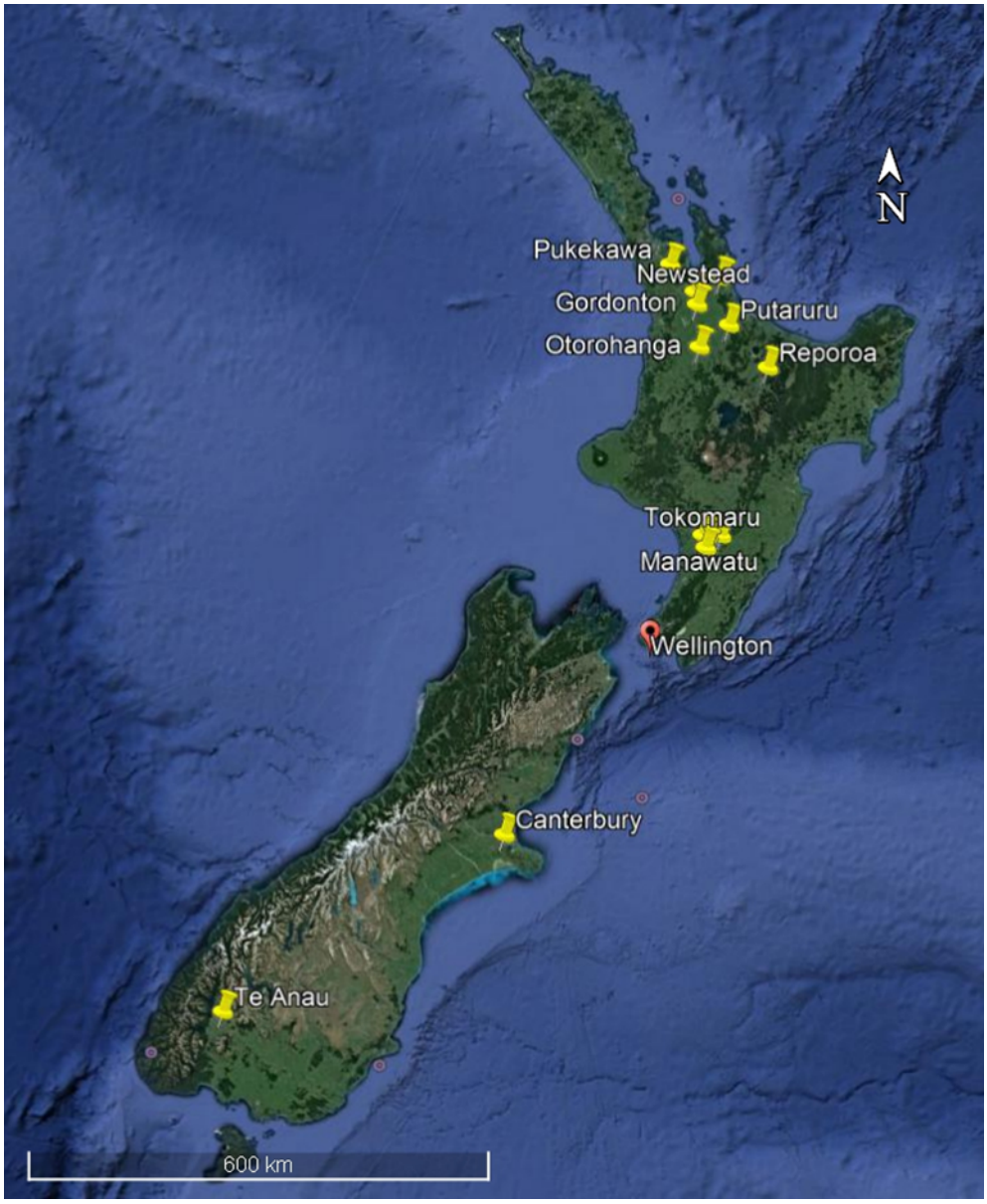
Zhu, M.X., Ding, K.Y., Jiang, X., Wang, H.H., 2007. Investigation on co-sorption and desorption of fluoride and phosphate in a red soil of China. Water, Air, and Soil Pollution 183, 455–465. doi:10.1007/s11270-007-9394-0

Zhuang, J., Yu, G.R., 2002. Effects of surface coatings on electrochemical properties and contaminant sorption of clay minerals. Chemosphere 49, 619–628. doi:10.1016/S0045-6535(02)00332-6

Zouari, M., Ben-Ahmed, C., Fourati, R., Delmail, D., Ben-Rouina, B., Labrousse, P., Ben-Abdallah, F., 2014. Soil fluoride spiking effects on olive trees (*Olea europaea* L. cv. Chemlali). Ecotoxicology and Environmental Safety 108, 78–83. doi:10.1016/j.ecoenv.2014.06.022

APPENDIX

APPENDIX 1 Locations of soil sample collected for total soil F analysis



f

APPENDIX 2 Calculation of IC_{10}

Table A1.1 Parameter and estimate of the four-parameter logistic model for NaF salt

Parameter	Estimate
D	48.5
A	100.5
B	2.2
C	222.0

$$90 = 48.5 + \frac{100.5 - 48.5}{1 + \left(\frac{X}{222.0}\right)^{2.2}}$$

$X = 118.86$

$IC_{10\ (NaF)} = 118.86$

Table A1.2 Parameter and estimate of the four-parameter logistic model for KF salt

Parameter	Estimate
D	49.5
A	104.3
B	1.8
C	252.3

$$90 = 49.5 + \frac{104.3 - 49.5}{1 + \left(\frac{X}{252.3}\right)^{1.8}}$$

$X = 141.49$

$IC_{10\ (KF)} = 141.49$

Table A1.3 Parameter and estimate of the four-parameter logistic model for NH_4F salt

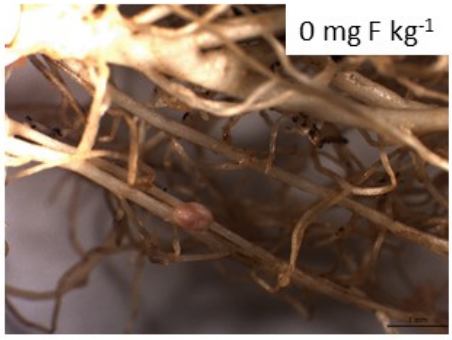
Parameter	Estimate
D	-13.29
A	101.3
B	0.92
C	1156.6

$$90 = -13.3 + \frac{101.3 + 13.3}{1 + \left(\frac{X}{1156.6}\right)^{0.92}}$$

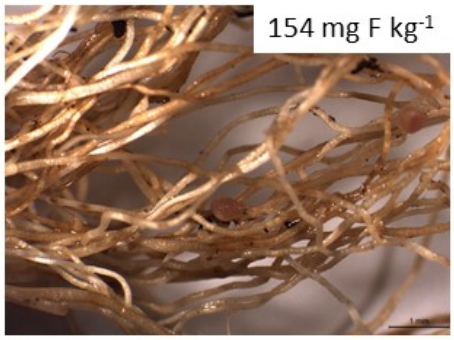
$X = 104.37$

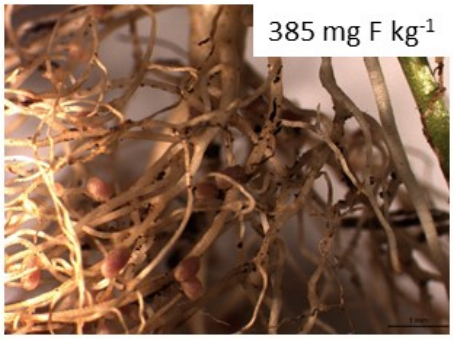
$IC_{10}(NH_4F) = 104.37$

APPENDIX 3 Root images of Tribute grown in different F concentrations added to Allophanic soil in the pot experiment



APPENDIX 4 Root images of Merlyn grown in different F concentrations added to Allophanic soil in the pot experiment





APPENDIX 5 Effect of F addition on water-extractable F in the Allophanic soil used for a pot experiment

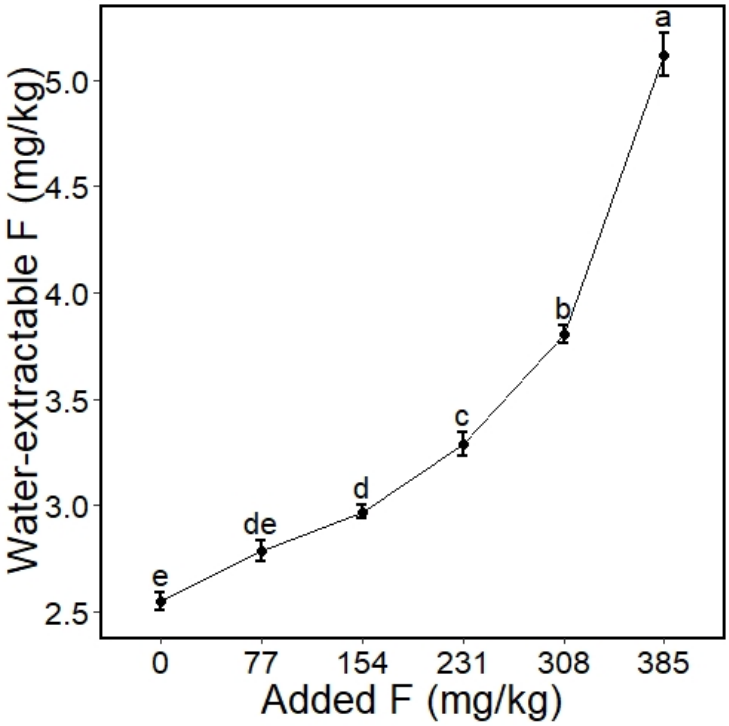


Figure A5.1. Effect of F addition on the water-extractable F concentration in an Allophanic soil. Vertical bars indicate standard error of mean (n = 6). Means with the same letters are not significantly different at the P > 0.05 level (Tukey test).

APPENDIX 6 Rationalise the experimental procedures

A6.1 Selection of 5 different extraction methods

According to literature (section 2.9.2), five extraction methods have been extensively used to extract F from the soluble and exchangeable (bioavailable F) soil F pool. However, the amount of F extracted by these extractants is influenced by soil properties (Rodríguez et al., 2001). The present study hypothesized that these five extraction methods will not extract the same amount of bioavailable F for any given New Zealand soil.

The aim of this work was to define an appropriate standard extractant (from a list of known and used extractants) that can be used to estimate the bioavailable F concentration in New Zealand soils.

A6.2 Selection of different F^- concentrations

Wakelin et al. (2016) conducted laboratory experiments to test the effect of Cd on *R. leguminosarum* and white clover in New Zealand. In these experiments a range of Cd concentrations (0, 0.5, 1, 5, 10, 20, 50, 70, 100, 500 and 1000 mg L^{-1}).were used to find the Cd toxicity on *R.leguminosarum* and white clover. In New Zealand soils Cd is also derived from phosphate fertiliser. Therefore, for this thesis work, a similar F^- concentration range was used to investigate the F^- toxicity concentration for *R. leguminosarum*.

APPENDIX 7: Statement of contribution Doctorate with publications/Manuscripts.

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors ha consented to their work being included in the thesis and they have accepted the candidat contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Thangavelautham Geretharan
Name/title of Primary Supervisor:	Professor Chris Aderson
Name of Research Output and full reference:	
Geretharan, T., Jeyakumar, P., Bretherton, M., Anderson, C.W.N., 2018.Defining a standard method to measure the total and bioavailable concentration of fluorine in New Zealand soils. Microchemical Journal 142, 94–101.	
In which Chapter is the Manuscript /Published work:	Chapter 03
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	~ 70%
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
I have conducted laboratory experiments to generate data for this manuscript to validate 4M NaOH method and standardized bioavailable F concentration, carried out data analysis and manuscript preparation.	
For manuscripts intended for publication please indicate target journal:	
Candidate's Signature:	T. G
Date:	15.07.2019
Primary Supervisor's Signature:	
Date:	15 July 2019

(This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis)