Amelioration of Aluminium Toxicity in Atlantic Salmon, *Salmo salar* L., with Particular Reference to Aluminium/ Silicon Interactions.

By

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For Michelle, whenever I may find her

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ABSTRACT

The aim of the research was to examine amelioration strategies for aluminium and in particular the potential of silicon to ameliorate the acute toxicity of Al in Atlantic salmon, Salmo salar, fry in a dilute acid water.

Methods were developed for the analysis of Al and Si using graphite furnace atomic absorption spectrometry. This technique was then combined with carefully defined pre-treatment procedures to investigate simple Al speciation and solubility in dilute aqueous milieu.

Research then examined the potential of $Ca(OH)_{2(s)}$, $CaCO_{3(s)}$ and $CaO/SiO_{2(s)}$ to ameliorate acute Al toxicity. A field study highlighted the inadequacies of $Ca(OH)_{(2)(s)}$ as an effective agent to ameliorate Al toxicity and laboratory studies, whilst substantiating these inadequacies for both $Ca(OH)_{2(s)}$ and $CaCO_{3(s)}$, highlighted the potential of $CaO/SiO_{2(s)}$, and in particular silicic acid, as a potential detoxification agent for Al in acid waters.

Detailed laboratory studies of the interactions between Al and silicic acid in dilute aqueous environments

demonstrated the formation of aluminosilicates, the stabilities of which were dependent on the pH and the silicic acid concentration. Stable, occasionally charged aluminosilicate species were formed at silicic acid concentrations above 1000 $\mu g l^{-1}$, probably through the interaction of silicic acid with the aluminate anion, $Al(OH)_4^-$ (aq). These species were slow to come out of solution and increased Al solubility with respect to amorphous $Al(OH)_{3(s)}$ in the short-term, (168h).

A flow-through toxicity system was developed to assess the results of these Al/Si interactions on the acute toxicity of Al to Atlantic salmon fry at pH 5.0. Acute Al toxicity was found to be abolished by a critical concentration of silicic acid, ($\simeq 750 < X < \simeq 2400\ \mu g l^{-1}$). The silicic acid rendered the Al biologically unavailable, Al neither being taken up by the fish nor associating with the gill surface, and this observation has outlined a potential and not previously considered role of silicon in biology.

CHAPTER ONE: GENERAL INTRODUCTION

1.1 Aluminium Toxicity to Fish

The acute toxicity of aluminium to fish has been known for many years, (Thomas 1915, Ellis 1937, Jones 1939), however, the environmental significance of aluminium toxicity has only recently been compounded through evidence of the close association of aluminium with environmental pollution and in particular, "Acid Rain", (e.g. Dickson 1978, Baker and Schofield 1980).

Aluminium has been shown to be both acutely and chronically toxic to fish (Table 1.1), and the nature of the toxicity is dependent on the solution chemistry, (Exley and Phillips 1988). Suggested causes of aluminium-induced mortalities in fish include: mucous clogging of the gills and resulting anoxia (Muniz and Leivestad 1980), impaired ion exchange across the gill membrane (McWilliams 1982), leading to a rapid loss of body sodium (Potts 1986) and death resulting from the concomitant effects of plasma ionodilution (Wood 1986). Sublethal effects are often manifested as gill damage (Schofield and Trojnar 1980, Muniz and Leivestad 1980, Exley 1985, Chevalier et al 1985, Karlsson-Norrgren et al 1986) and aluminium accumulation in the tissues (Hunter et al 1980, Stoner et al 1984, Exley 1985, Karlsson-Norrgren et al 1986). Effects on locomotion, appetite, ventilation and colouration have

also been recorded, (Ogilvie and Stechey 1983, Exley 1985, Kramer et al 1986, Malte 1986).

The present study is concerned with the acute toxicity of aluminium to fish. Recent field and/or laboratory studies on the acute toxicity of aluminium have been carried out on a wide range of different fish species including: Tilapia mosambica, (Murungi and Robinson 1987), Oreochromis aureus (Phillips and Saleh 1987), blueback herring, Alosa aestivalis, (Klauda et al 1987), striped bass, Morone saxatilis, (Buckler et al 1987), bluegill, Lepomis macrochirus, channel catfish, Ictalurus punctatus, fathead minnow, Pimephales promelas, (Palmer et al 1988), smallmouth bass, Micropterus dolomieui, (Kane and Rabeni 1987), sunfish, Lepomis gibbosus, lake trout, Salvelinus namaycush, (Hutchinson et al 1987), flagfish, Jordanella floridae, (Hutchinson and Sprague 1987), brook trout, Salvelinus fontinalis, (Hunn et al 1987, Wood et al 1988a, 1988b, 1988c, Booth et al 1988, Walker et al 1988), brown trout Salmo trutta, (Wood and McDonald 1987, Ormerod et al 1987, Muniz et al 1987, Segner et al 1988), rainbow trout Salmo gairdneri, (Evans et al 1988, Ramamoorthy 1988, Thomsen et al 1988, Heming and Blumhagen 1988, Witters et al 1987, Witters et al 1988, Goss and Wood 1988), and Atlantic salmon Salmo salar, (Ormerod et al 1987, Leivestad et al 1987, Jagoe et al 1987).

The sensitivity of different fish species to aluminium

varies widely, for example from mgl^{-1} for tilapia spp, (Phillips and Saleh 1987, Murungi and Robinson 1987), to μgl^{-1} for salmonids, (see Goss and Wood 1988), however the symptoms of acute toxicity fall into two distinct categories, osmo-/ionoregulatory stress, (e.g. Witters et al 1987, Dalziel et al 1987, Witters et al 1988, Heming and Blumhagen 1988), and respiratory/ventilatory stress, (e.g. Ormerod et al 1987, Walker et al 1988, Wood et al 1988a), the former being most pronounced in very low Ca waters, (see Booth et al 1988). Concomitant with these symptoms are often gross abnormalities of the gills, (Jagoe et al 1987, Youson and Neville 1987, Evans et al 1988, Tietge et al 1988), although gill cellular damage is not always present, particularly in younger fish, (Klauda et al 1987), where undefined physiological toxic mechanisms were postulated by these authors. The toxic mode of action associated with acute aluminium toxicity is still unknown and will vary according to the water quality and to some extent the fish species. Two general, but not mutually distinct, theories prevail at present, the solubility and the speciation theories.

The solubility theory, (Muniz and Leivestad 1980, Schofield and Trojnar 1980), relies upon aluminium hydroxide precipitation at the gill surface, acting as an irritant and inducing an inflammatory response in the gill tissue. Rapid aluminium precipitation at the gill surface would either require the interlamellar water to be heavily over-

saturated with respect to amorphous $Al(OH)_3$, or aluminium, at the gill surface, perhaps bound to anionic sites, to act as nucleation sites for the induced precipitation of aluminium.

The speciation theory, (see Booth *et al* 1988), relates aluminium toxicity to competition between aluminium species, hydrogen ions and other cations for binding/chelation/ adsorption at the gill surface. The positive charge on the aluminium species found in acid solution could induce the association of aluminium with anionic sites on the gill surface, anionic sites that may be integral to the gill physiology and biochemistry. The disruption of these processes by specific aluminium species may be acutely toxic to the fish.

It is likely that both of the above mechanisms are involved in aluminium toxicity and that their relative importance will be attributable to the prevailing water quality. One of the main aims of this study was to elucidate further the acute toxic mode of action of aluminium on salmon, *Salmo salar*, fry in acid water and to relate this to the relative contributions of the aforementioned theories.

1.2 Aluminium Chemistry

The acute toxicity of aluminium to fish, whether by $Al(OH)_{3(s)}$ precipitation or aluminium cation binding at the gill surface, is inextricably linked to the water

Table 1.1 Observations of Aluminium Toxicity to Fish in Acid Waters

Aluminium level ($\mu g l^{-1}$)	pH	Calcium ($mg l^{-1}$)	Fish and developmental stage or size	Observation	Reference
(1) 3,600	6.5	-	Salvelinus fontinalis	50% mortality (LD_{50}) observed at recorded total aluminium levels and pH	Decker and Menendez (1974)
4,450	6.0	-	130g		
4,000	5.5				
(2) 190	5.0	-	Salmo trutta	Potentiation of aluminium toxicity observed at this pH,(pH5.0).	Muniz and Leivestad (1980)
380	4.0	-	Salmo trutta	Aluminium ameliorated acid toxicity,(pH4.0).	
	6.0	-	Salmo trutta	No effect, alone or synergistically of aluminium or acidity,(pH6.0).	
(3) 0 → 500	4.2 → 5.6	2.2	Salvelinus fontinalis Catostomus commersoni Eggs, larvae and post-larvae	In the pH range, 4.2-4.8 and Al up to $200 \mu g l^{-1}$ for C. commersoni, and $500 \mu g l^{-1}$ for S. fontinalis, egg survival was enhanced through to the eyed stage in the presence of the aluminium. In the pH range, 4.2-5.6, $100 \mu g l^{-1}$ Al for C. commersoni and $200 \mu g l^{-1}$ Al for S. fontinalis, mortality of larvae and post-larvae was enhanced in the presence of Al. Potentiation of Al toxicity was at pH 5.2-5.4.	Baker and Schofield (1982)

cont'd...

Aluminium level ($\mu g l^{-1}$)		pH	Calcium ($mg l^{-1}$)	Fish and developmental stage or size	Observation	Reference
(4)	50 100 200 500	6.0	76	Salmo gairdneri 5-8cm long	After 26 h exposure, the cough rate had increased significantly and locomotor activity was reduced. Changes in the cough rate were proportional to the aluminium level, a coughing threshold of $100 \mu g l^{-1}$ was recorded.	Ogilvie and Stechey (1983)
(5)	200	5.0	-	Salmo salar 28g Salmo gairdneri 57g	Exposure to the said conditions reduced the activity of carbonic anhydrase and Na^+K^+-ATPase in the gills by 25-40% of control levels.	Staurnes et al (1984)
(6)	216	4.5 4.0	0.4	Salmo trutta 35g	The presence of aluminium significantly reduced $Na^+_{(aq)}$ influx across the gills at low pH. Aluminium showed no effect on $Na^+_{(aq)}$ efflux.	Dalziel et al (1985a)
(7)	54	4.5 4.0 5.4	-	Salmo trutta 45g	Aluminium induced a significant decrease in $Na^+_{(aq)}$ influx across the gill at pH 4.0 and 4.5, but had no additional effect on acidity at pH 5.4.	Dalziel et al (1985b)

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Aluminium level ($\mu g l^{-1}$)		pH	Calcium ($mg l^{-1}$)	Fish and developmental stage or size	Observation	Reference
(8)	374	5.2	2.0	Salmo salar parr 14g	50% mortality LC_{50}, was recorded for both fish at the recorded total Al levels and pH.	Exley (1985)
	183	5.2	2.0	Salmo gairdneri juveniles 57g		
(9)	76	4.0 - 6.5	2 - 6	Salmo gairdneri juveniles 197-469g	After 6-11 days exposure, physiological responses to toxicity were most severe at pH 6.1, 4.5 and 4.0, 40-90% of fish dying within a few days. Effects were moderate to slight at pH 5.5 and 5.0 and no effect was observed at pH 6.5 Mortality at pH 6.1 was attributed to hypoxia whereas electrolyte loss was the suggested cause at pH 4.0	Neville (1985)
(10)	100,000	5.0	-	Notemigonous crysoleucas (Golden Shiners)	All fish died within a few hours on the addition of Al. Mortality was due to aluminium gill interactions. Suffocation was ruled out since fish survived happily in suspended $Al(OH)_{3(s)}$ at pH 7.0.	Robinson and Deano (1985)

cont'd...

Aluminium level ($\mu g l^{-1}$)	pH	Calcium ($mg l^{-1}$)	Fish and developmental stage or size	Observation	Reference
(11) 300	4.5 5.2 7.2	3	Salvelinus fontinalis fry	Increased mortality of fry in the presence of aluminium at pH 4.5 and 5.2. Potentiation of effect at pH 5.2.	Cleveland et al (1986)
(12) 130	5.0	4	Notropis cornutus (Common shiners) fork length 4.9 - 11.8 cm.	Mortality was over 90% within 7 hours of exposure, no mortality in the absence of Al. Larger fish were more susceptible to aluminium toxicity.	Kramer et al (1986)
(13) 2,000	5.0	132	Salmo gairdneri 300-400g	No observed changes in the major plasma ions. Fish became hypoxic due to a fall in the dorsal pO_2 from 10[illegible]mmHg-40mmHg and an increase in the dorsal pCO_2. A 15% increase in the standard O_2 uptake was recorded as well as doubling of the ventilation frequency and swelling of erythrocytes.	Malte (1986)

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Aluminium level ($\mu g l^{-1}$)		pH	Calcium ($mg l^{-1}$)	Fish and developmental stage or size	Observation	Reference
(14)	87	5.1 - 5.3	2.9	Salmo gairdneri 3.5g	Improved tolerance to subsequent Al exposure was observed. A 1-2 week 'acclimation' increased their LC_{50} to 2 X their control LC_{50} of $175 ug l^{-1}$ total aluminium at pH 5.0.	Orr et al (1986)
(15)	75	5.06	1.29	Salmo salar smolts 38g	50% mortality of smolts occurred within 108 h. There was no mortality in the absence of labile aluminium.	Skogheim and Rosseland (1986)
(16)	200-1200	4.4 - 4.9	2	Salvelinus fontinalis juveniles	Repetitive intermittent exposure to Al/acid stress produced greater cumulative mortality and lower growth rates than continuous exposures to the same water quality. The effect was exacerbated at pH 4.9 for intermittent exposure and pH 4.4 for continuous exposure.	Siddens et al (1986)

cont'd...

Aluminium level (μgl^{-1})	pH	Calcium (mgl^{-1})	Fish and developmental stage or size	Observation	Reference
(17) 350	4.1	1.5	Salmo gairdneri 1 yr. old 180-350g	Aluminium promoted a massive whole body loss of Na, Cl, and K, twice the effect due to acid alone. The loss was via the branchial rather than urinary route. Increasing the ambient $Ca^{2+}_{(aq)}$ level to $7.5mgl^{-1}$ had no moderating effect on ion loss.	Witters (1986)
(18) 37 - 111	5.2	2	Salvelinus fontinalis yolk sac fry	Aluminium exhibited a clear protective effect over acid effects alone. Whole body Na, Cl, K and Ca were raised by 80% (Na and Cl) and 30% (K and Ca). Potentiation of effect was at pH 5.2.	Wood et al (1986)

quality of the milieu and the interlamellar water of the gill in particular. The water quality will largely dictate both aluminium speciation and aluminium solubility control and is, in turn, a direct consequence of both geo- and physico-chemical environmental constraints (Bache 1986, Nordstrom and Ball 1986, Lawrence et al 1986, Miller and Andelman 1987, Tipping et al 1988a, Exley and Phillips 1988). For example, storm flow due to heavy rain and/or snowmelt, may rapidly raise aluminium solubility, through a change in aluminium solubility control from gibbsite to amorphous $Al(OH)_{3(s)}$, (Goenaga and Williams 1988). Similarly, low water temperatures, particularly characteristic of snowmelt events (Elgmork et al 1973, Johanessen et al 1980, Hendershot et al 1986) will move complexation equilibria of soluble aluminium species from predominantly Al-fluoride to predominantly Al-hydroxy species, even in the presence of an excess of fluoride, (Plankey et al 1986, Plankey and Patterson 1987). Both of the aforementioned events are often associated with a low water pH and are acutely toxic to salmonids, (Driscoll et al 1980, Johnson et al 1981). Oversaturation of aluminium with respect to both amorphous and crystalline phases of $Al(OH)_{3(s)}$ may occur in natural waters rich in organics, in particular humic and fulvic acids, (Lovgren et al 1987, Backes and Tipping 1987, Tipping and Backes 1988, Tipping et al 1988b, Tipping et al 1988c), however the organo-aluminium complexes that predominate in such waters are generally accepted as not acutely toxic,

(Neville 1985, Gunn et al 1986, Kramer et al 1986, Robinson and Deano 1986, Hutchinson and Sprague 1987).

The undersaturation of aluminium with respect to known solubility controls is also noted in natural waters and has been attributed to aluminium adsorption by particulates, (Goenaga et al 1987, Bruggenwert et al 1987, Walker et al 1988, Goenaga and Williams 1988), adsorption to a solid humic phase (Cronan et al 1986)and aluminosilicate formation (Paces 1978, Farmer et al 1983, Farmer 1986).

The nature of the soluble fraction of aluminium will be governed by the different organic and inorganic ligands available for complexation. The nature of aquatic organics remains somewhat vague and generally all fall under the distinction of humic substances. Binding to aluminium is via carboxylate, phenolic and salicyclic acid-like groups, (see Plankey and Patterson 1987, Tipping et al 1988b), and is dependent on pH, temperature, and fluoride levels, though is largely independent of the calcium level.

Important inorganic complexing ligands include fluoride, sulphate, hydroxide and silicic acid, (Exley and Phillips 1988). In the absence of other complexing ligands soluble aluminium will be found as $Al^{3+}_{(aq)}$ and its hydrolysis products, $AlOH^{2+}_{(aq)}$, $Al(OH)_2^{+}{}_{(aq)}$, $Al(OH)_{3(aq)}$ and $Al(OH)^{-}_{4(aq)}$, and the relative proportions of each of

these species will be dependent on the solution pH and ionic strength, (Chapter Five). Both of the anions, fluoride ($F^-_{(aq)}$)and sulphate ($SO_4^{2-}{}_{(aq)}$) will complex aluminium and will affect both the absolute and relative proportions of the Al-hydroxy species at any one pH. In acidified natural waters of low aluminium, ($\lesssim 270\ \mu g l^{-1}$) and in the absence of organics, the concentrations of sulphate and fluoride are typically high enough, ($\sim 200 \mu g l^{-1}$ F^- and $\sim 9000 \mu g l^{-1}$ SO_4^{2-}) to complex most of the available aluminium, (Courtijn et al 1987).

Silicic acid, $Si(OH)_{4(aq)}$, is the predominant form of aqueous silica in natural waters and is generally low in acidified waters, (Farmer 1986). To date, the interactions between silicic acid and aluminium in natural waters have largely been ignored and nothing is known about the influence of these reactions on aluminium toxicity to aquatic fauna and flora. It was therefore an aim of this study to investigate aluminium/silicic acid reactions in dilute solutions, and to assess the acute toxicities of the resultant Al/Si species to salmon and compare the toxicities to that of uncomplexed aluminium. It was hoped that Al/Si interactions would provide a basis for the amelioration of acute aluminium toxicity in acidified natural waters.

1.3 Aluminium Toxicity Amelioration

Many past and current acid water mitigation programmes have or are monitoring the effect of neutralisation on surface water aluminium concentrations (Schreiber and Hartman 1987, Brocksen et al 1987, Davis and Goldstein 1987, Dalziel et al 1987, Tervet and Harriman 1988).

Greatest success in reducing the labile, monomeric aluminium fraction has been achieved from catchment liming with fine-grain calcite, (Davis and Goldstein 1987, Dalziel et al 1987, Tervet and Harriman 1988), whereas the direct addition of calcite to surface waters could initially result in an increase in the labile, monomeric aluminium fraction (Brocksen et al 1987), reduced mitigatory efficacy during snowmelt episodes (Davis and Goldstein 1987), and reacidification on the cessation of liming, (Porcella 1987).

The use of pH control alone to ameliorate aluminium toxicity requires that the pH remains above pH6.0, (Skogheim et al 1986), to reduce toxicity due to cationic aluminium species, and below pH7.0, (Skogheim et al 1987), to prevent toxicity due to the aluminate anion, $Al(OH)^-_{4(aq)}$. This fine balance will be very difficult to achieve and maintain in natural waters. A closer look at aluminium toxicology in acidified natural waters could suggest alternative methods of aluminium solubility

control and hence aluminium toxicity amelioration.

To reduce the biological availability of aluminium, and hence effect its amelioration, the soluble aluminium fraction has to be reduced and/or rendered non-toxic. A reduction in the soluble level, particularly in acid milieu, would require a change in solubility control and an increased rate of achievement of the equilibrium state. The detoxification of the soluble aluminium fraction, whilst not necessarily reducing its absolute value, would require a rapid and stable complexation of one (or more) of the monomeric aluminium species, equilibrium shifts would ensure that complete amelioration was achieved. It was proposed that silicic acid might effect one or both of the aforementioned effects. This proposition was tested in the present study and the results compared with more traditional methods of acid/aluminium amelioration.

It was hoped that the results would help characterise a novel and economically acceptable amelioration agent for acidified waters, particularly those rich in aluminium.

CHAPTER TWO: THE DETERMINATION OF ALUMINIUM

2.1 A Review of the Accepted Methodologies

Analytical methods for the measurement of aluminium in appropriate solvents are numerous and diverse. Brief synopses of the principles governing the more commonly employed methods are detailed below.

2.1.1 Colourimetric Spectrophotometry

These techniques are based upon the spectrophotometric measurement at a specified wavelength of a characteristically coloured complex formed by the reaction between aluminium and a specific chromogene. The most widely used colourimetric methods are based on the reaction of aluminium with ferron, (8-hydroxy-7-iodoquinoline-5-sulphonic acid; Davenport 1949, Rainwater and Thatcher 1960, Smith 1971), or pyrocatechol violet (3,3', 4'-trihydroxyfuxone-2"-sulphonic acid; Anton 1960, Wilson and Sergeant 1963, Dougan and Wilson 1974, HMSO 1979).

Both of these methods can be used to discriminate total and monomeric aluminium fractions with only limited sample pre-treatment (Driscoll 1980, Müller 1983, Seip *et al* 1984) and lend themselves to automation for both field and laboratory use (Henriksen and Bergmann-Poulsen 1975, Røgeberg and Henriksen 1985). Disadvantages of these

techniques include their susceptibility to interference effects, requiring the addition of masking agents, and the time intensive nature of the analytical procedure. Detection limits are usually of the order of 10-20μgl^{-1} and precision $\pm$ 3-5μgl^{-1}.

2.1.2 Instrumental Neutron Activation Analysis (INAA)

This comparatively new method of aluminium determination in biological samples involves the bombardment of a sample with neutrons and the measurement of the radioactivity induced by nuclear reactions. In this process, for which no chemical processing is required, ^{27}Al forms the radioactive ^{28}Al nuclide. Advantages of this technique include excellent sensitivity, relative independence from matrix effects and interference and reduced sample pre-treatment. A problem of INAA is the need to correct for fast neutron reactions on P which also produce ^{28}Al (Ehman et al 1983, Savory and Wills 1986, Courtijn et al 1987). The facilities required for this method of aluminium determination, one of which is a nuclear reactor, often render this technique prohibitive.

2.1.3 Nuclear Magnetic Resonance (NMR)

Though not universal in its application ^{27}Al NMR spectroscopy is used selectively in the determination of aluminium. In particular when aluminium is involved with

metal chelation agents such as desferriferrioxamine, (Garrison and Crumbliss 1987) and organic acids such as oxalic acid (Sjoberg and Ohman 1985). ^{27}Al NMR has also been used to determine free aluminium ions, ($Al(H_2O)_6^{3+}$) in natural soil solutions (Schierl 1985). Whilst selectively it is a proven method of aluminium measurement the high detection limits ($\sim$1000μgl^{-1}) preclude its general use.

2.1.4 Inductively Coupled Plasma Emission Spectrometry

Atomic emission spectrometry using a nitrous oxide acetylene flame is widely used for gross aluminium determination. However, it is unsuitable for the measurement of trace quantities i.e. $\mu gl^{-1}/\mu gkg^{-1}$. The flame is not hot enough to provide the energy requirement for total aluminium ionisation and this energy deficit is overcome through the use of inductively coupled argon plasma as an excitation source. In this technique ionised argon can reach temperatures of 4000°C. The high temperature eliminates matrix problems and reduces most chemical interferences. A significant interference however is caused by calcium which increases the aluminium background, raising the detection limits. Precision is generally good, ranging from $2\mu gl^{-1}$, (Lichte et al 1980) to $10\mu gl^{-1}$ (Schramel et al 1980).

As with INAA, cost of instrumentation can prove prohibitive (Savory and Wills 1986).

2.1.5 Atomic Absorption Spectrometry (AAS)

As with atomic emission spectrometry, flameless AAS is better equipped for trace aluminium determinations. Flameless AAS or electrothermal atomic absorption spectroscopy is currently the most widely chosen method of measuring total aluminium in biological samples (Gitelman and Alderman 1986). The advantages of this method include: (i) little, if any, requirement for sample pre-treatment; (ii) only small sample volumes are required (2-100 µl); (iii) very low detection limits ($0.5-5.0 ugl^{-1}$) with very high precision, and (iv) very few interferences and/or matrix effects. A disadvantage of this method is that only total aluminium can be reliably measured.

The use of GFAAS in studies of aluminium solubility and speciation requires carefully defined pre-treatment of the sample. Electrothermal atomic absorption spectroscopy was used to determine aluminium in this study.

2.2 Electrothermal Atomic Absorption Spectroscopy

2.2.1 General Principles

An in-depth study of graphite furnace atomic absorption spectroscopy (GFAAS), with particular reference to clinical requirements, is provided by Gitelman and Alderman (1986). The present study was directed towards the use of GFAAS to determine aluminium in dilute, aqueous environments.

The basis of the technique is that a known volume of sample is placed in a graphite tube and atomised electrothermally in the light path of a hollow cathode lamp. Aluminium is then measured as a function of the light absorbed. Aluminium was measured using a Perkin-Elmer 2280 AAS incorporating a Perkin-Elmer HGA-400 furnace.

Several analytical programmes for aluminium determination are published (Craney et al 1986, Sullivan et al 1987, Woolfson and Gracey 1987). However, rarely can identical programs be successfully applied to different spectrometers and laboratory conditions (Perkin-Elmer, pers. comm.). A specific analytical program based on known standards had, therefore, to be developed for the present study.

2.2.2 The Aluminium Program

2.2.2.1 Introduction

A known volume of sample is injected automatically, as a flat bead, onto the wall of a graphite tube. This tube is positioned in the optical beam of an atomic absorption spectrometer (Figure 2.1). Water-cooled electrical contacts at either end of the tube allow the controlled heating of the tube. Heating is initiated by passing an electric current through the tube, the degree of heating being a function of the amount of current provided. Four distinct heating phases are recognised.

(i) Drying - the sample is dried carefully without spattering. Spattering of the sample bead may result in a reduced aluminium signal.

(ii) Pre-treatment or Char stage - the temperature of the tube is raised so as to remove as much volatile material as is possible without losing any of the aluminium to be analysed.

(iii) Atomisation - the temperature is raised rapidly to the atomisation temperature range of aluminium. The aluminium is reduced to its ground state and the atoms are held in the light path of the spectrometer. The

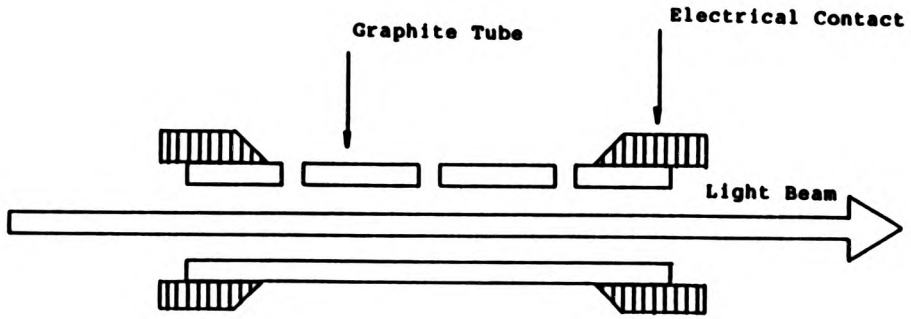


Figure 2.1. Schematic representation of the graphite tube in the optical beam.

atoms are maintained in the light path for up to 5 seconds, longer periods enhancing sensitivity.

Aluminium atoms are detected by their selective absorption of light of a pre-set wavelength, emitted by a hollow cathode lamp containing an aluminium filament.

(iv) Cleaning Stage - the tube is rapidly heated to a high temperature to remove any residues in the tube that could interfere with the next sample.

Throughout the heating stages, except during the reading of the aluminium absorption, oxidation of the tube is prevented by the through-flow of an inert gas, usually argon. This gas also helps in the removal of volatiles during the charring stage.

Every cycle of the analytical program has a number of variables, each will individually and synergistically affect the aluminium signal. The following series of experiments methodically outlines the optimisation of the analytical conditions.

Table 2.1 outlines typical instrumental parameters for aluminium determination. Those associated with the spectrophotometer are standard and widely accepted, those describing the furnace operation are variable and require optimising.

Table 2.1 Recommended HGA Analytical Conditions for Aluminium

Atomic Absorption Spectrophotometer	Graphite Furnace
Aluminium Hollow Cathode Lamp	Argon Purge with Interrupt
Wavelength: 309.3nm	Drying Stage: 100-400°C
Slit Width: 0.7A nm	Char Stage : 1400-1700°C
Tube Type : Pyro.Coated	Atomisation Temperature : 2400-2700°C
Background Correction	

2.2.2.2 Spectrophotometer Instrumental Parameters

Aluminium Hollow Cathode Lamp

A single element lamp operating at 20mA current provides the narrow line spectrum required for measuring aluminium close to the detection limits.

Wavelength

The cathode lamp is used at a wavelength of 309.3 nanometers, the most sensitive line for aluminium.

Slit Width

A slit width of 0.7A nanometers will give optimum analytical sensitivity, the best signal to noise ratio and good linearity for working curves. Wider slit widths will reduce noise,

though at the expense of sensitivity and linearity.

Background Correction

High levels of background absorbance can be reduced using background correction. Spectral interferences of this type are very common and the use of background correction is recommended.

Peak Height Absorbance

The absorbance produced during aluminium atomisation could be read directly as absorbance or as peak height in mm, the peaks being produced on a Perkin-Elmer R100A rapid response undamped recorder. The validity of each method was tested, (Table 2.2 and Figure 2.2), and both methods shown to be equally satisfactory. The convenience of peak height analysis made it the chosen method of interpreting aluminium absorbance.

2.2.2.3 The Graphite Furnace and Automatic Sampler

The Graphite Tube

Interferences due to the graphite tube occur either because of vapour-graphite reactions, often resulting in thermally stable metal carbides (Matsusaki 1987), or vapour absorption by the porous graphite (Perkin-Elmer 1977). Careful

Table 2.2 Comparison of absolute absorbance and peak height (mm) as indices of aluminium concentration with respect to the linearity of response. $\bar{x}$-mean, SD-standard deviation, n=9. r^2x100%-correlation coefficient.

	Aluminium Signal			
Al.Signal Index / [Aluminium] $\mu g l^{-1}$	Absorbance		Peak Height mm	
	$\bar{x}$	SD	$\bar{x}$	SD
0	0.0	0.0	0.0	0.0
10	0.050	0.000	20.7	0.5
20	0.091	0.001	38.3	0.9
30	0.141	0.001	58.0	1.6
40	0.219	0.001	83.7	0.5
50	0.255	0.000	100.0	0.0
60	0.316	0.001	125.7	0.5
70	0.352	0.003	145.7	0.5
80	0.397	0.000	167.0	0.8
90	0.444	0.001	188.0	0.0
100	0.484	0.001	211.7	0.5
r^2 x 100%	99.6		99.9	

Table Footnote: The absorbances of a range of known aluminium standards, $(Al(NO_3)_3$. Spectrosol. BDH Ltd)., were measured as both a digital absorbancy readout and peak height on a recorder. The linearity of the absorbance response to increasing aluminium concentrations as measured by both methods were compared.

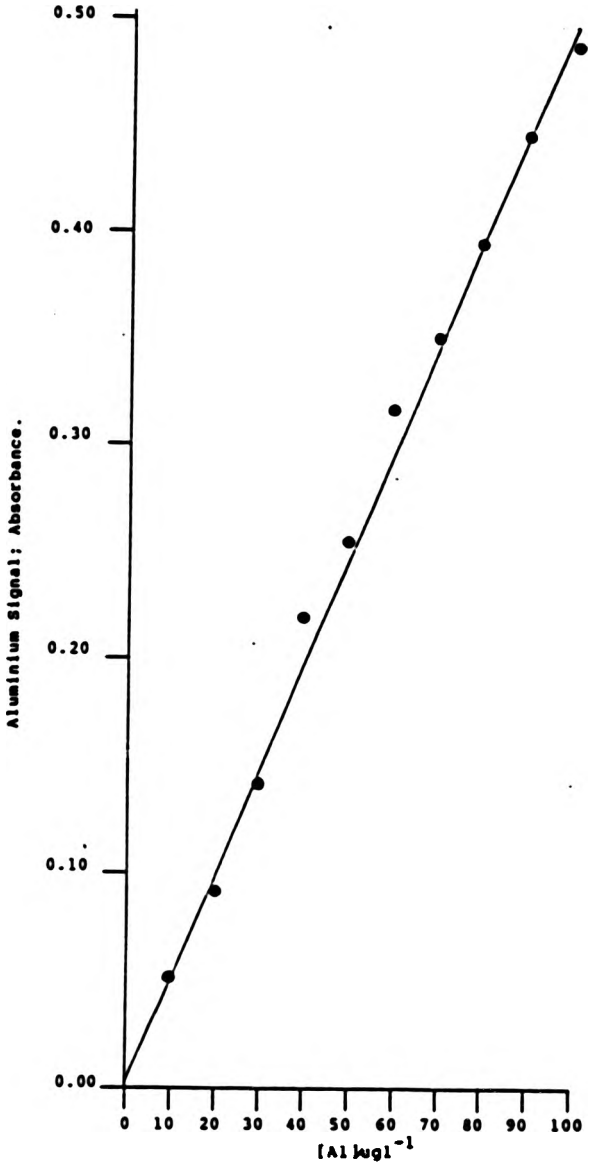


Figure 2.2a. The aluminium signal, measured as absolute absorbance, produced by known aluminium standards.
Mean values are plotted, n = 3.

Line plotted by regression: Y=0.0049x+0.003, r = 0.998. ($P < 0.01$).

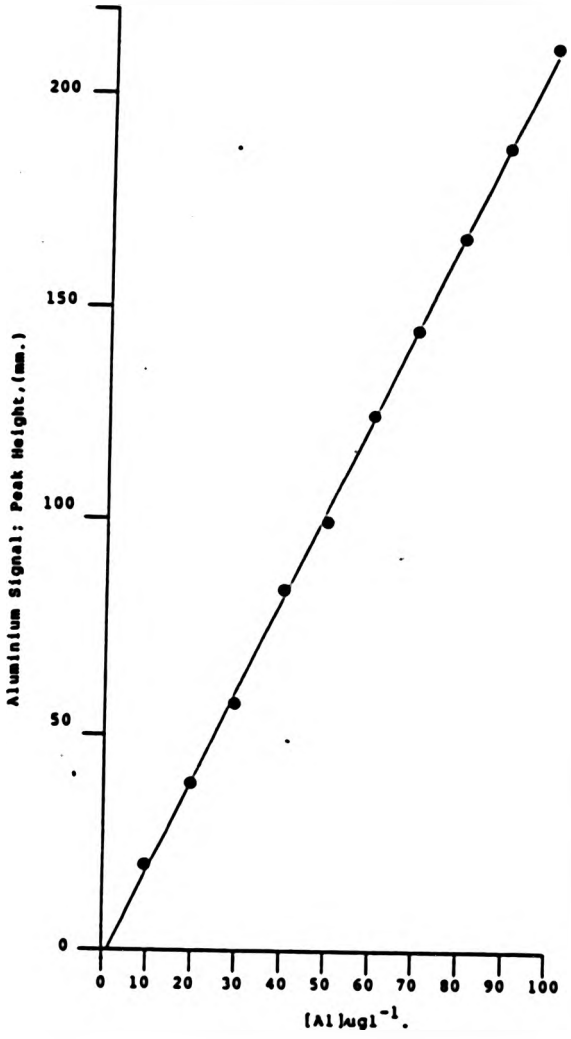


Figure 2.2b. The aluminium signal, measured as peak height, mm, produced by known aluminium standards. Mean values are plotted, n = 3.

Line plotted by regression: Y=2.12x - 2.44. r = 0.999. ($P < 0.01$).

deposition of a pyrolitic layer on the inner surface of a graphite tube isolates the vapour phase from the graphite and hence reduces tube interference effects. Pyrolytically coated graphite tubes (Perkin-Elmer Ltd.) were used for all aluminium determinations.

The Purge Gas

The purge gas prevents oxidation of the tube during heating. It should be completely inert with respect to the determinant. L'vov (1978) reported that aluminium should be determined in an argon atmosphere, not in nitrogen, because aluminium forms strong monocyanides in the presence of N_2. Aluminium sensitivity is improved two-fold in Ar as compared to N_2 (Fernandez and Manning 1971, Sturgeon et al 1976). High purity argon (99.98% minimum) (BOC Ltd.) was used for all aluminium determinations.

The Heating Cycle

(i) Drying Stage: Drying the injected sample is achieved without any spattering through two distinct stages. The tube is first warmed for 5 seconds at 80°C, before ramping the temperature up to 130°C, where it is held for 30 seconds to achieve complete drying. Initial warming of the tube reduces the potential of sample spattering, thereby reducing the potential for loss of aluminium signal.

(ii) Char Stage: The removal of impurities, with or without associated interferences, is achieved during this stage. The critical temperature is defined as the highest temperature possible without subsequent loss of sample. Table 2.3 and Figure 2.3 show the result of a study to define the optimal charring temperature. A charring temperature of 1500°C was used for all aluminium determinations. Prior to charring, the tube is heated to 500°C to improve the reproducability of the aluminium signal within replicates. A final ramp up to 1500°C for just 5 seconds ensures complete impurity removal and prepares the recorder for the absorbance signal reading stage. By the end of the char stage the aluminium in the sample will probably have been converted to the oxide, Al_2O_3 (Styris and Redfield 1987).

(iii) Atomisation Stage: The conversion of all the aluminium present in the sample to aluminium atoms in the ground state occurs during atomisation. The optimal temperature produces the maximum aluminium signal, (Table 2.4, Figure 2.4). The rapid achievement of this critical temperature, less than 1 second, ensures congruent atomisation of the aluminium and maximum absorbance. Thermal dissociation of condensed phase aluminium oxide is responsible for atomisation (Styris and Redfield 1987). During atomisation the purge gas flow is interrupted whilst the absorbance is read. An atomisation temperature of 2650°C was used for all aluminium analyses.

Table 2.3 The effect of char temperature, °C, on the aluminium signal at two aluminium concentrations. $\bar{x}$ - mean, SD - standard deviation, n = 9. Atom. temp.= 2650°C, SV = 30µl, PF = 300ml. Ar $min.^{-1}$

Aluminium absorbancy of two known standards was compared over a range of char temperatures.

	Aluminium Signal mm			
Al.Standard μgl^{-1} / Char Temp.°C	20		40	
	$\bar{x}$	SD	$\bar{x}$	SD
1200	93.7	1.3	178.0	1.6
1250	100.3	1.3	182.3	0.9
1300	107.0	1.4	185.3	0.5
1350	108.3	0.5	186.7	0.5
1400	109.3	1.3	188.3	1.3
1450	109.7	0.5	189.7	0.9
1500	110.7	0.5	191.0	0.0
1550	108.3	0.9	187.3	0.5
1600	105.0	1.4	184.3	0.5
1650	97.0	1.6	179.7	0.9
1700	72.7	1.7	163.7	2.6

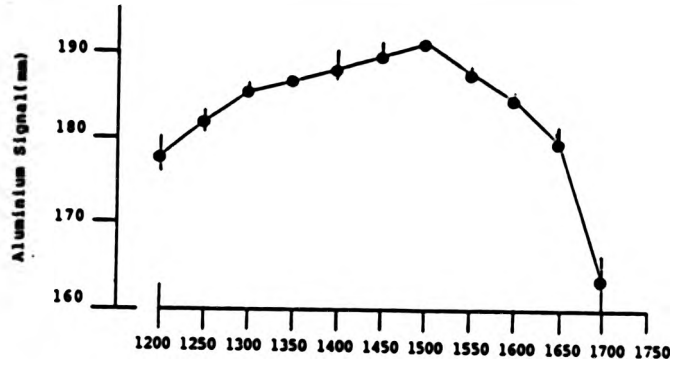


Figure 2.3a. $40\mu gl^{-1}$.

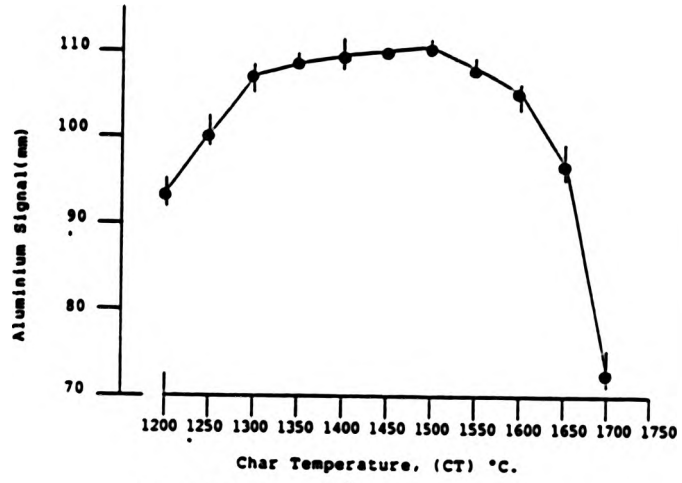


Figure 2.3b. $20\mu gl^{-1}$.

Figures 2.3a-b. The optimisation of the char temperature at the optimum atomisation temperature, 2650°C, for two known aluminium standards. Sample volume, (SV)=30µl, purge gas flow, (PF)=300ml. AR $min.^{-1}$. Mean values are plotted, bars indicate the range, n = 9.

Table 2.4 The effect of different atomisation temperatures, °C, on the aluminium signal produced by the atomisation of two known aluminium standards. $\bar{x}$-mean, SD-standard deviation, n=9. Char temp.(CT)= 1500°C, SV=30ul, PF=300ml Ar min^{-1}.

Aluminium absorbancy of two known standards was compared over a range of atomisation temperatures.

Al.Standard μgl^{-1} / Atomisation Temp °C	Aluminium Signal mm			
	20		40	
	$\bar{x}$	SD	$\bar{x}$	SD
2300	92.3	0.5	148.7	1.9
2350	94.7	1.7	158.7	0.5
2400	98.7	0.5	162.7	0.5
2450	102.3	0.9	169.0	0.8
2500	104.7	1.3	171.7	0.5
2550	106.7	0.9	175.3	0.9
2600	109.7	0.5	180.3	0.5
2650	111.3	0.9	185.0	0.0
2700	106.0	0.8	173.0	1.6

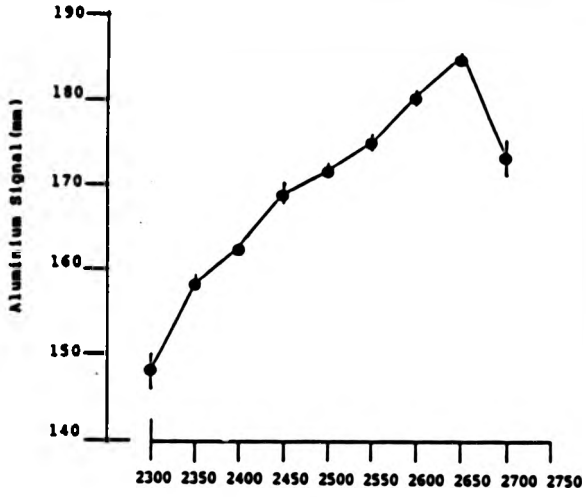


Figure 2.4a. 40ugl^{-1}.

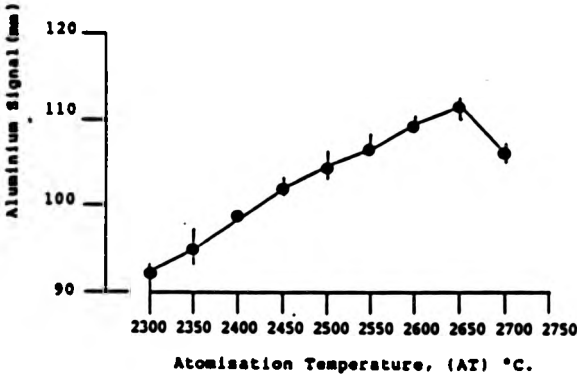


Figure 2.4b. 20ugl^{-1}.

Figures 2.4a-b. The optimisation of the atomisation temperature at the optimum char temperature, 1500°C, for two known aluminium standards. SV = 30ul, PF= 300ml Ar min.$^{-1}$ Mean values are plotted, bars indicate the range, n = 9.

(iv) Cleaning Stage: The maximum temperature to which pyrolitically coated graphite tubes can be heated without damage to the tube and coating is 2700°C. To ensure that all sample has been removed from the tube and hence reduce contamination of future samples by carry-over, the graphite tube is rapidly heated to 2700°C at the end of the heating cycle.

The Purge Gas Flow and the Sample Volume

The purge gas flow (measured in ml Ar $min.^{-1}$) is a critical component of the aluminium program. Optimal flow is defined by both high absorbances and a linear response to increasing aluminium concentrations.

Sample volume affects many different steps in the analytical program. For example, large volumes require a comparatively longer drying period. Large volumes improve precision though at the expense of replication in an unbalanced program. Optimal values for purge gas flow, (ml Ar $min.^{-1}$) and sample volume (µl) were determined (Tables 2.5, 2.6 and 2.7 and Figures 2.5, 2.6).
A purge gas flow of 300ml Ar $min.^{-1}$ combined with a sample volume of 30µl produced maximum absorbance signals and an excellent linear response to increasing aluminium concentration.

Table 2.5 The effect of increasing purge gas flow on the linear response between aluminium concentration in a standard and the resultant signal. $\bar{x}$-mean, SD-standard deviation, n=9. AT=2650°C, CT=1500°C, SV=20ul.

[Aluminium] $\mu g l^{-1}$ \ Purge Flow mlAr Min^{-1}	Aluminium Signal mm					
	100		200		300	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
0	0.0	0.0	0.0	0.0	0.0	0.0
10	8.7	0.5	9.7	0.5	10.0	0.0
20	29.3	0.9	21.7	0.9	18.7	0.5
30	35.7	0.5	35.7	0.5	31.7	0.5
40	81.0	0.8	51.7	0.5	50.7	0.5
50	102.7	0.5	73.3	1.3	69.0	0.8
60	120.7	0.5	85.7	0.5	86.0	0.0
70	148.3	1.3	110.7	0.5	101.0	0.8
80	149.3	1.7	119.7	1.3	119.7	0.5
90	166.3	2.6	137.0	0.0	136.7	0.5
100	168.3	1.3	149.3	0.9	146.3	1.3

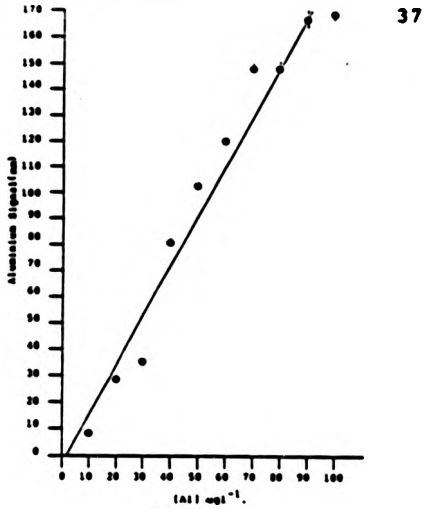


Figure 2.5a. Purge Flow: 100ml Ar min.$^{-1}$.
Line fitted by regression: Y = 1.91X - 3.46, r = 0.983

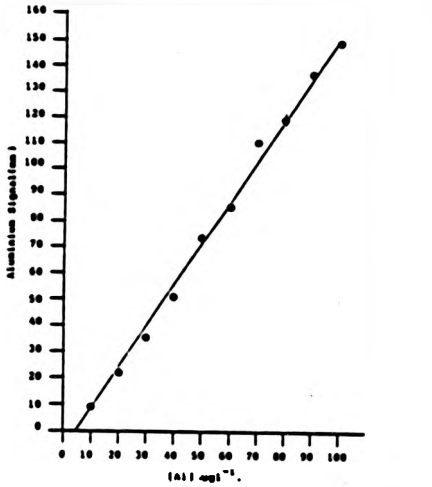


Figure 2.5b. Purge Flow: 200ml Ar min.$^{-1}$.
Line fitted by regression: Y = 1.38X - 0.58, r = 0.997

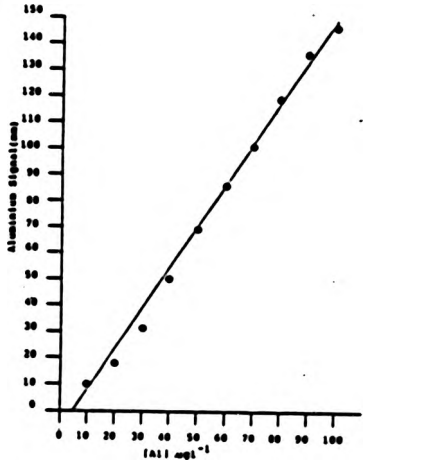
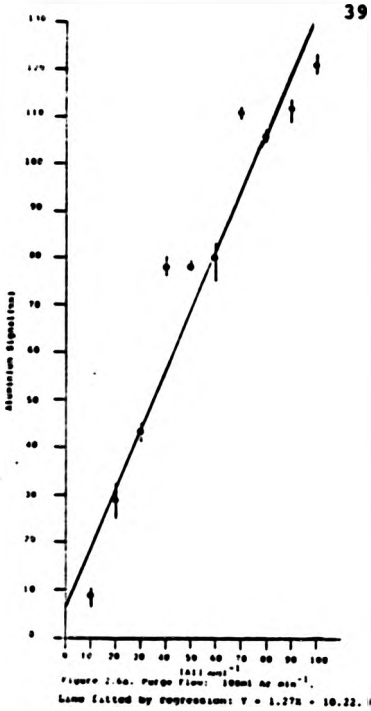


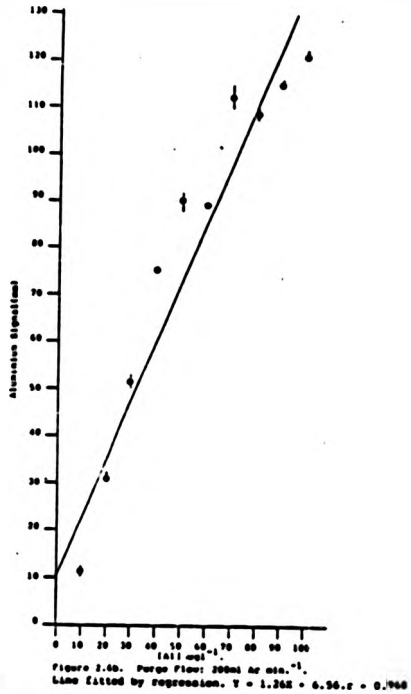
Figure 2.5c. Purge Flow: 300ml Ar min.$^{-1}$.
Line fitted by regression: Y = 1.16X - 7.90, r = 0.997

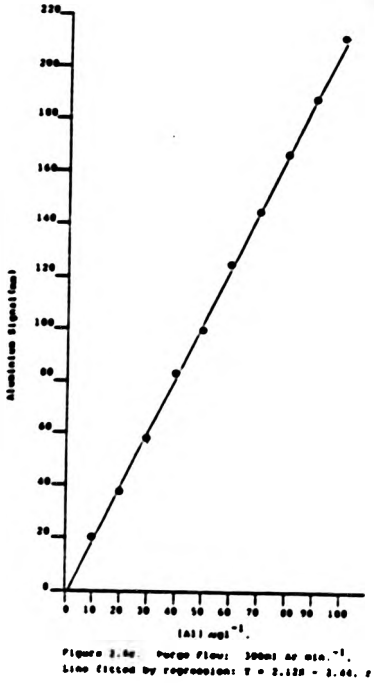
Figures 2.5a-c. The effect of increasing purge gas flow on the linearity and absolute value of the aluminium signal for known aluminium standards. AT=2650°C, CT=1500°C, SV=20µl. Mean values are plotted, bars show the range, n = 9.

Table 2.6 The effect of increasing purge gas flow on the linear response between aluminium concentration in a standard and the resultant signal. $\bar{x}$-mean, SD-standard deviation, n=9. AT=2650°C, CT=1500°C, SV=30ul.

	Aluminium Signal mm					
Purge Flow mlAr Min^{-1} / [Aluminium] ugl^{-1}	100		200		300	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
0	0.0	0.0	0.0	0.0	0.0	0.0
10	8.3	1.3	11.7	0.5	20.7	0.5
20	29.0	2.9	31.7	0.5	38.3	0.9
30	43.0	1.6	51.3	1.3	58.0	1.6
40	78.0	1.6	75.7	0.5	83.7	0.5
50	78.3	0.9	90.0	1.6	100.0	0.0
60	79.7	3.4	89.7	0.5	125.7	0.5
70	111.3	0.9	112.0	2.2	145.7	0.5
80	106.3	0.9	109.3	0.9	167.0	0.8
90	111.7	1.9	115.7	0.5	188.0	0.0
100	121.0	1.6	121.7	0.5	211.7	0.5







Figures 2.6a-c. The effect of increasing the purge gas flow on the linearity and absolute value of the aluminium signal for known aluminium standards.
AT=2650°C,
CT=1500°C,
SV=30µl.
Mean values are plotted, bars show the range, n = 9.

Table 2.7 Correlation coefficients, $r^2 x100\%$, describing the synergistic effects of sample volume and purge gas flow on the response of the aluminium signal to aluminium concentration. High coefficients describe the most linear response.

Purge Gas Flow $_{ml Ar\ min.^{-1}}$ / Sample Volume μl	100	200	300
20	96.63	99.40	99.40
30	93.70	93.51	99.92

2.2.3 Interferences

Interferences with the determination of aluminium by electrothermal atomic absorption spectroscopy are well documented (Persönn et al 1977, Matsusaki et al 1979, Manning et al 1982). Chloride is known to produce a negative interference attributed to the formation of aluminium chloride. Aluminium chloride is volatile, vapourisation occurring at ~300°C, thus aluminium is lost during the drying and char stages of the heating cycle. Similarly if chloride is present during the atomisation stage, the aluminium signal may be diminished because of the high association energy of aluminium chloride, which will reduce the number of aluminium atoms entering the ground state (Persönn et al 1977). Perchloric acid will also interfere with the aluminium signal, precluding its use as a digestant for biological material (Julshamn et al 1978). A reduction in the aluminium

signal was reported on the addition of 216 $\mu g l^{-1}$ sulphuric acid to an aluminium sample (Craney et al 1986), this effect being exacerbated by the tube age. Tube interference effects were also noted by Carrondo et al 1979 and Slavin et al 1981, necessitating the re-running of standards periodically.

In this study, standard curves were prepared from chloride, sulphate and nitrate salts of aluminium. The results are shown in Table 2.8 and Figure 2.7. The $Al(NO_3)_{3(aq)}$ standard solution gave the best linear response, as expressed by the correlation coefficient, $r^2 x100\%$, and at an aluminium concentration of 60 $\mu g l^{-1}$ had a significantly higher signal (t-test: $P < 0.05$), than the chloride standard and a higher signal, though not significantly so, (t-test: $P > 0.05$) than the sulphate standard. Consequently $Al(NO_3)_{3(aq)}$ was used both to prepare standards used in aluminium analyses and in the subsequent aluminium speciation and toxicity studies, (Chapters 5 and 6).

2.2.4 Matrices for Aluminium Determinations

A matrix defines the sample conditions under which aluminium analysis takes place. Generally the function of a matrix is to maximise the aluminium signal without loss of linearity or within-sample replication. Commonly employed matrices are: (i) magnesium nitrate, this retards the vapourisation of aluminium, thus permitting a higher char temperature without loss of signal (Slavin et al 1981), Slavin et al 1982, Leung and Henderson 1983).

Table 2.8 The effect of the nature of the standard aluminium salt on the nature of the standard curve. $\bar{x}$-mean, SD-standard deviation, n=9, r^2x100%-correlation coefficient.

	Aluminium Signal mm					
Aluminium Standard Salt / [Aluminium] μgl^{-1}	$AlCl_3$		$Al(NO_3)_3$		$Al_2(SO_4)_3$	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
0	0.0	0.0	0.0	0.0	0.0	0.0
10	7.9	0.1	13.6	0.9	10.4	0.6
20	29.8	1.1	36.1	0.1	31.1	3.1
30	46.9	1.4	54.9	0.4	58.6	1.6
40	62.2	0.8	69.2	0.2	62.2	2.2
50	71.3	0.5	83.3	0.7	76.4	1.2
60	82.1	2.2	93.7	0.4	89.7	1.9
r^2x100%	98.7		99.0		97.4	

Table Footnote: Identical aluminium standards were prepared from three distinctly different aluminium salts. The calibration curves for each salt were compared.

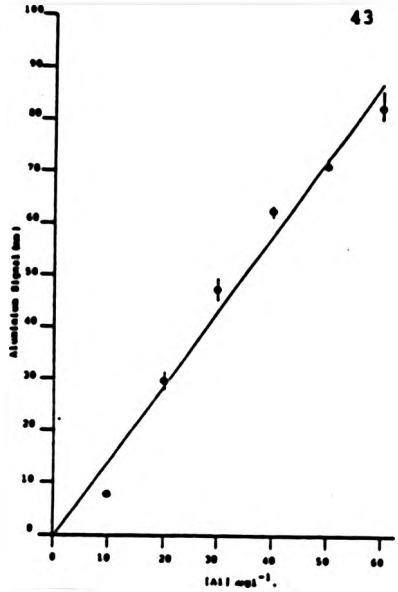


Figure 2.7a. $AlCl_3$.
Line fitted by regression: Y = 1.08E + 1.10. r = 0.994

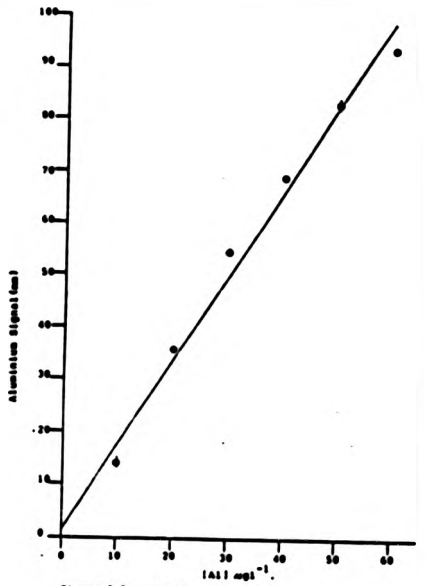


Figure 2.7b. $Al(NO_3)_3$
Line fitted by regression: Y = 1.02E + 1.51. r = 0.995

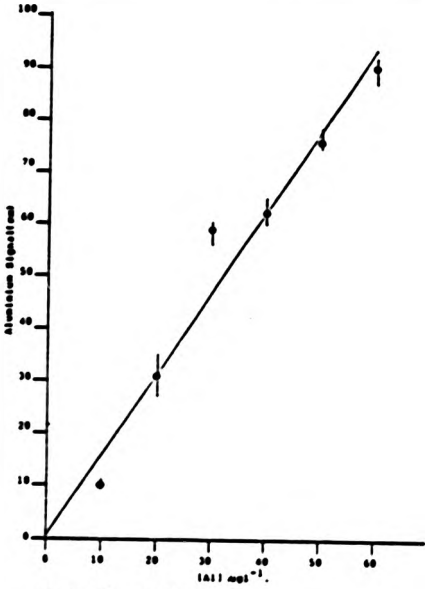


Figure 2.7c. $Al_2(SO_4)_3$.
Line fitted by regression: Y = 1.16E + 0.61. r = 0.997

Figures 2.7a-c. The effect of the nature of the standard aluminium salt on the linearity of the corresponding calibration curve. Mean values are plotted, bars indicate the range, n = 9.

(ii) phosphoric acid (Craney et al 1986), again this allows the use of a higher char temperature (May and Brumbaugh 1982), and delays the onset of atomisation in the high temperature stage (Eaton and Holcombe 1983);
(iii) ammonium hydroxide, which assists in the removal of chloride by the formation of volatile ammonium chloride (Pegon 1978); (iv) sample dilution with high purity water, to reduce the sample aluminium concentration to fall within the linear section of a standard curve, and (v) nitric acid to improve the homogeneity of aluminium within a sample.

The application of both magnesium nitrate and phosphoric acid was studied, however, both of these additions, used as high purity reagents (Aristar:BDH Ltd.), had an enhancement effect on blank and low aluminium solutions. The preferred matrix was HNO_3 (Aristar:BDH Ltd.) a prerequisite for analysis of total aluminium (see later). The criteria used to determine the proportion of acid to sample required were the smallest volume that would give the maximum signal that was stable with time. The results outlined in Table 2.9. suggested a matrix modifier of 1 or 2% (V/V) HNO_3(Aristar:BDH Ltd.). Both proportions of acids showed insignificant (t-test:$P \geq 0.05$) effects of time on the aluminium signal. To help enhance the life of the graphite tubes a matrix of 1% (V/V) HNO_3 (Aristar: BDH Ltd.) was chosen.

Table 2.9 The use of different concentrations of concentrated nitric acid (Aristar,BDH Ltd.) as a matrix modifier over a wide pH, on the measured aluminium concentration of a 50 μgl^{-1} Al standard solution. $\bar{x}$-mean, SD-standard deviation, n=9.

	Measured Aluminium Concentration μgl^{-1}							
%HNO_3 / pH	0		0.5		1.0		2.0	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
3.0	49.0	2.2	48.7	0.5	51.0	0.0	51.0	0.0
4.0	45.0	3.3	46.7	0.5	48.7	0.5	48.7	1.3
5.0	42.0	2.5	45.3	1.3	47.0	0.0	47.7	0.5
6.0	34.7	2.9	43.0	1.4	47.3	0.5	47.0	0.0
7.0	38.0	3.7	43.7	0.5	47.7	0.5	48.3	0.9
8.0	37.7	1.9	44.7	0.5	50.7	0.5	50.0	0.0
9.0	42.7	2.6	46.0	0.0	52.3	0.9	52.0	0.8

At low concentrations of aluminium, $\lesssim 20\ \mu g l^{-1}$, the use of a matrix of 1% (V/V)HNO_3 (Aristar:BDH Ltd.) was found to significantly increase the aluminium signal above the level expected from contamination alone (Table 2.10). The results suggest that precision may be reduced at low aluminium concentrations.

Table 2.10 Interference effects of a matrix of 1% concentrated nitric acid (Aristar:BDH Ltd.) on the measurement of low concentrations of aluminium. $\bar{x}$ - mean, SD - standard deviation, n = 9.

Transformed data (Arcsine).

	[Aluminium] $\mu g l^{-1}$		% Signal Enhancement	
Al Standard $\mu g l^{-1}$ \ %HNO_3	1.0		1.0	
	$\bar{x}$	SD	$\bar{x}$	SD
5.0	8.7	0.9	73.3	18.9
10.0	12.3	0.9	23.3	9.4
20.0	24.3	0.9	21.7	4.7
30.0	31.7	0.9	5.3	3.3
40.0	40.3	0.5	0.8	1.2
50.0	50.0	0.0	0.0	0.0

Sample dilution was necessary to confidently measure high, ($\gtrsim 100\ \mu g l^{-1}$), concentrations of aluminium. The standard curve was non-linear above aluminium concentrations of $100\ \mu g l^{-1}$, (Table 2.11, Figure 2.8).

Table 2.11 The effect of increasing aluminium standards on the linearity of the signal/concentration relationship. $\bar{x}$ - mean, SD - standard deviation, r - correlation coefficient, n = 9.

Aluminium Standard $\mu g l^{-1}$	Aluminium Signal mm		
	$\bar{x}$	SD	r
0	0.0	0.0	-
10	10.3	0.5	-
20	19.0	0.0	-
30	30.0	0.0	-
40	41.7	0.5	-
50	50.7	0.5	-
60	63.0	0.0	-
70	72.0	0.0	-
80	83.7	0.5	-
90	89.7	0.5	-
100	103.7	0.9	0.999
150	123.7	0.9	0.985
200	139.3	1.7	0.968
250	151.3	1.3	0.956
300	160.7	2.1	0.948
350	168.7	1.3	0.943
400	173.7	1.7	0.937
450	177.0	2.5	0.931
500	182.3	2.1	0.926

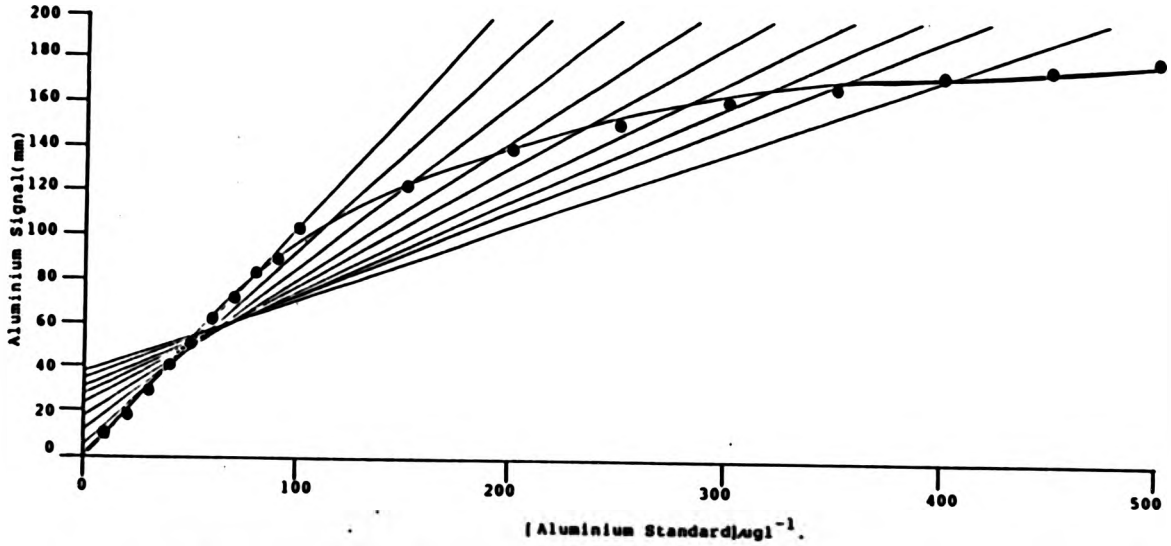


Figure 2.8 Aluminium calibration curve at increasingly high aluminium standard concentrations. The response is curvilinear. The plotted slopes indicate the effect of assuming a linear response above 100ugl-1. Mean values are plotted, n = 9. Lines fitted by regression, (Table 2.10).

The loss of linearity is not adequately conveyed by the correlation coefficient alone. Lines of "best fit" elegantly quantify the result of assuming a linear response for aluminium levels above 100 $\mu g l^{-1}$ (Fig.2.8). Linearity in this aluminium range (0-100 $\mu g l^{-1}$) has been noted by other workers for the electrothermal determination of aluminium, (Gardiner et al 1981, Bettinelli et al 1985, Woolfson and Gracey 1987).

Additional error was found when samples were diluted more than five-fold. The general effect of high sample dilutions was an underestimation of the aluminium content of the sample (Table 2.12). Some of this loss of aluminium could be attributed to experimental error associated with preparing the diluents.

Table 2.12 Interference effects of various dilution factors on the measured aluminium concentration of a 100 $\mu g l^{-1}$ standard. $\bar{x}$-mean, SD-standard deviation, n=9. Transformed data (Arcsine).

Dilution Factor	Measured [Al] $\mu g l^{-1}$		% Error	
	$\bar{x}$	SD	$\bar{x}$	SD
1.0	100.0	0.0	0.0	0.0
2.0	99.7	0.5	- 0.3	0.5
5.0	99.3	0.5	- 0.7	0.5
10.0	85.7	0.9	-14.3	0.9
20.0	79.0	1.6	-21.0	1.6
50.0	72.3	2.5	-27.7	2.5

2.2.5 Conclusion

The application of electrothermal graphite furnace atomic absorption spectroscopy to the determination of aluminium in dilute aqueous environments requires careful consideration of many operational and non-operational criteria. It is essential that the analytical methodology is critically defined and is thereafter used as standard in replicate analyses of aluminium. The analytical program designed for the determination of aluminium in the experiments in this research is outlined in Table 2.13.

2.3 Simple Aluminium Speciation

2.3.1 Introduction

Electrothermal atomic absorption spectroscopy does not differentiate between aluminium species in aqueous solution. Studies on aluminium solubility and speciation therefore require the selective pre-treatment of samples before GFAAS.

Studies of aluminium speciation in natural waters are very numerous (for example: Campbell *et al* 1983, Lazerte 1984, Driscoll 1984, Seip *et al* 1984, Miller and Andelman 1986, Litaor 1987). Suggested schemes are often multi-factorial and time-intensive, and are so designed to deal with the heterogeneity of natural waters.

Table 2.13 Analytical Program for the GFAAS Determination of Aluminium.

Element: Aluminium Al — Matrix 1% HNO_3 (20% HNO_3 Digest)

Instrumental Parameters

Inst. Model: Perkin-Elmer 2280 AAS

Wavelength: 309.3 nm

Spectral Bandwidth: 0.7A nm

Light Source: Cathode lamp

Current: 20mA

Background Correction?: Yes

Readout Parameters

Linear Response Range: 0-100$\mu g l^{-1}$

Integration Time: 3 seconds

Signal Index: Peak Height mm

Recorder: R100A (P-E)

Speed: 20mm$Min.^{-1}$ Range 5-10mV.

HGA Parameters

HGA Model: HGA 400 (P-E)

Graphite Tube: Pyro. coated

Purge Gas: Argon; 300ml Ar $min.^{-1}$

Autosampler Model: AS40 (P-E)

Sample Aliquot: 30 μl

Sample Introduction

Automated: Yes

Replicates: 3 (Min.)

Standard Soln. Spectrosol $Al(NO_3)_3$.

Keyboard Entries

Step	1	2	3	4	5	6	7
Temp.°C	80	130	500	1500	1500	2650	2700
Ramp(s)	5	10	10	10	1	0	1
Hold(s)	5	30	10	15	5	5	3
Read.						✓	
Record						✓	
Baseline					✓		
Purge Flow						Stop	

Such schemes are largely impractical when large numbers of aluminium determinations are to be carried out. For the purpose of the present experiments, a simple scheme for discriminating aluminium fractions was determined. The scheme concentrated on three main fractions:

(i) Total aluminium, i.e. acid-digestible aluminium in an untreated sample;

(ii) Soluble or filtered aluminium, i.e. all aluminium not retained by vacuum filtration through a 0.04 µm membrane (Ultipor N66:Pall); and

(iii) Exchangeable aluminium, i.e. all aluminium retained by a strongly acid cation exchange resin (Amberlite IR 120 Na^+ BDH Ltd.).

The application and characteristics of this scheme were tested with respect to pH effects on aluminium hydroxide chemistry at room temperature.

2.3.2 Total Aluminium

The total aluminium fraction equates to the aluminium measured by GFAAS in a sample matrix of 1% (V/V) HNO_3 (Aristar: BDH Ltd.), that has been allowed to digest for a minimum of 5 minutes. Experiments showed that increasing the period of digestion had no significant effect (t-test: $P \geq 0.05$) on the aluminium recovery from dilute solutions. Other

authors are not in general agreement about the length of the digestion period. Driscoll (1984) recommends acidification of the sample to pH 1.0 and 1 hour digestion, whilst Seip et al (1984) acidify to pH ca 1.4 and leave samples to digest for 24 hours. Different waters will require specific treatment according to the prevalent forms of aluminium. It is important that the treatment is defined as part of the speciation scheme.

2.3.3 Soluble or Filtered Aluminium

The use of membrane filtration to determine the soluble aluminium fraction was examined in detail as described below.

Identical solutions of aluminium, prepared from $Al(NO_3)_3 \cdot 9H_2O$ (Aristar:BDH Ltd.) salt and double-distilled water were filtered under vacuum, through membranes of different pore diameters, in a closed poly-carbonate filter apparatus, (Sterifil: 47mm Millipore). Membrane filters of pore diameters, 0.45, 0.20, 0.10 and 0.04um, (100% nylon; Ultipor N66, (47mm): Pall), were washed in 5% (V/V) HNO_3 (Aristar:BDH Ltd.) and then double-distilled water, prior to use.

Samples of total, (pre-filtered solutions) and soluble, (not retained by membrane filtration) aluminium were determined for the different pore sizes by GFAAS. Results were expressed as the proportion of aluminium not retained

by the respective membrane.

The results (Table 2.14, Figure 2.9), clearly show that the amount of aluminium in the soluble fraction was a function of membrane characteristics. A larger proportion of the total aluminium was retained by progressively smaller membrane pore diameters. This effect was most obvious at extremes of pH on either side of the theoretical point of minimum aluminium solubility (ca pH6.0, Roberson and Hem 1969), and in particular towards pH9.0.

The method by which aluminium is retained by a membrane is often a balance between stearic effects, where the membrane acts solely as a sieve, and adsorption, whereby particles are held on the membrane surfaces. Thus smaller particles than the membrane pore diameter may be trapped within the labyrinth of the membrane depth by adsorption. Stearic effects are easily accountable, whilst adsorption is largely impossible to quantify.

The membrane filters used in this study were absolute rated, and thus the spread of pore diameters was very narrow. It is possible, therefore, that stearic effects were responsible for the differences in retention. The membrane filters used are characterised as having a slight electronegative charge at neutral pH. If it is assumed that this charge will become progressively more positive under acid conditions and more negative under alkaline

conditions, then this effect and the additional consideration of increased material surface area for adsorption of the lower pore diameter membranes will explain some of the differences in retention of the membrane filters tested. Research into the adsorptive effects of membrane filters has revealed significant reductions in filterable aluminium fractions, however, these effects are independent of pore diameter for synthetic solutions (Goenaga et al 1987). Increasing the solution pH exacerbated these effects and it has been established that increasing the pH will increase the extent of adsorption of hydrolysable metal ions (James 1971).

This experiment has shown that whilst a thorough explanation of the differences in aluminium retention from synthetic solutions by membranes of different pore diameters, cannot be assumed, the use of this method of aluminium fractionation should be clearly defined in terms of the type and size of membrane filter used. For the purpose of these studies, soluble or filtered aluminium is defined as all aluminium not retained by a 0.04 µm membrane filter, (Ultipor N66: Pall).

Table 2.14 The effect of membrane pore diameter on the percentage solubility of aluminium in a sample with change in pH. $\bar{x}$-mean, SD-standard deviation, n=9. (Initial [Al] = 200$\mu g l^{-1}$) Transformed Data (Arcsine).

pH	Membrane Filter Rating µm							
	0.04		0.10		0.20		0.45	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
3.0	79.7	0.5	100.0	0.0	100.0	0.0	100.0	0.0
4.0	48.7	0.9	90.7	1.3	91.3	1.7	96.0	1.4
4.5	38.7	0.5	55.0	1.6	74.0	2.2	76.3	0.9
5.0	30.7	0.9	47.3	1.3	54.3	2.5	58.0	2.2
5.5	17.3	0.5	33.3	0.5	39.7	0.9	42.3	1.3
6.0	6.7	0.5	15.3	1.7	16.3	0.5	16.7	1.7
6.5	8.3	0.5	9.0	0.0	10.0	0.8	14.7	0.5
7.0	22.3	0.9	25.7	0.5	27.3	1.7	32.3	1.9
8.0	35.3	0.9	38.7	0.5	51.3	0.9	74.7	1.3
9.0	43.3	1.3	58.0	1.4	80.3	0.5	99.0	1.4

2.3.4 Exchangeable Aluminium

Exchangeable aluminium was defined as that fraction of aluminium retained by a cation-exchange resin, (Amberlite IR120 Na^+ BDH Ltd.) after the filtration of a known aluminium solution through the resin. It was measured as the difference between the total and the non-exchanged fraction.

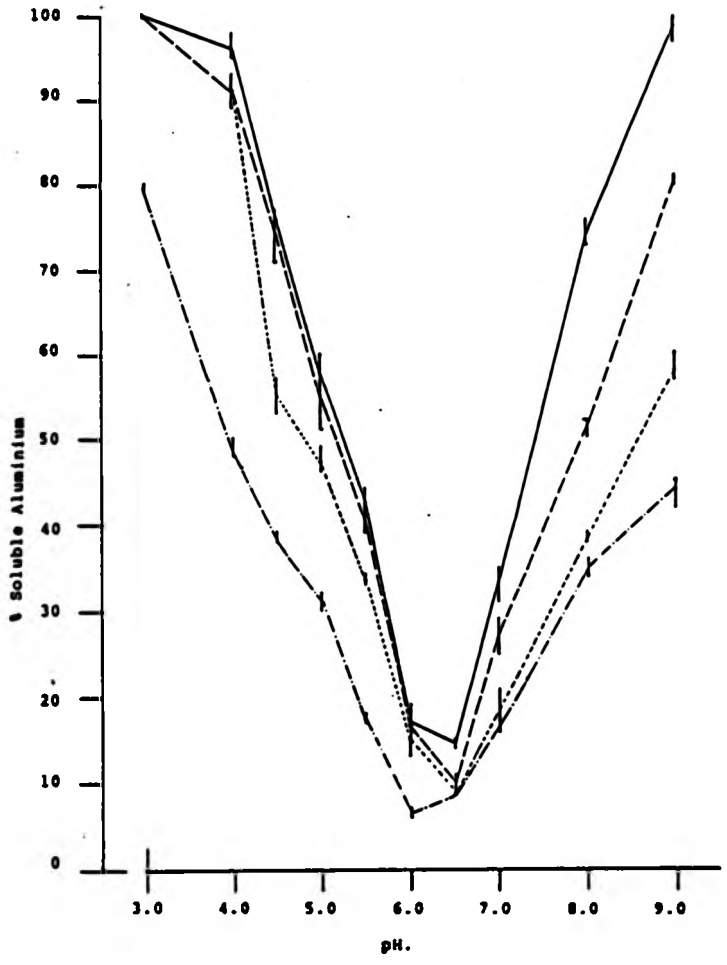


Figure 2.9. Aluminium solubility curves determined using membrane filters of 4 characteristic pore diameters, 0.45um, ——, 0.20um, ---, 0.10um, ... , and 0.04um,-.-.-. Mean values are plotted, bars indicate the range, n = 9.

A determining factor in what exchange took place was the residence time i.e. the time available for exchange to occur. Where resin columns are used, residence time can be a function of the length and diameter of the column. In this study a column diameter of 5mm was used and an optimal dry weight of resin was determined experimentally. A range of dry weights, 0.50 to 4.00g, were tested such that the residence time would increase in proportion with the increased resin weights. The resin weights were tested with undersaturated solutions of known aluminium content and pH. The criteria describing the optimum weight of resin were maximum exchange at pH3.0 and minimum exchange at pH6.0 and 9.0. Pre-treatment of the exchange column was important.

The resin was slurry-packed using double distilled water. The column was then flushed with a solution of sodium chlorite (5% W/V), adjusted to the pH of the sample, until the influent and effluent pH values were similar, ($\pm$ 0.2 units). The column was then flushed again with double-distilled water before use. The above procedure using sodium chlorite and double-distilled water was carried out after every sample. Each individual volume of resin was replaced after 3 replicate samples.

The results obtained and outlined in Table 2.15 indicated that 2.00g was the optimal dry weight of resin. This

Table 2.15 The effect of different dry weights of cation exchange resin (Amberlite IR-120 Na^+ BDH Ltd.) and pH on the exchangeable aluminium fraction. $\bar{x}$ - mean, SD - standard deviation, n = 9.

The original aluminium solution was 100 $\mu g l^{-1}$, and was undersaturated with respect to amorphous $Al(OH)_3$ at each pH level tested.
The results were expressed as % of total exchanged. (Arcsine transformation).

pH	Weight of Cation Exchange Resin (grammes)									
	0.50		1.00		2.00		3.00		4.00	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
3.0	64.7	3.9	78.3	1.7	84.3	0.9	64.3	2.5	58.3	2.5
6.0	24.7	2.1	21.3	0.9	6.7	0.9	22.0	2.2	33.7	2.5
9.0	2.7	0.9	0.3	0.5	0.0	0.0	0.7	0.9	5.7	1.7

gave a column height, on generation, of 50mm and a residence time of 30 seconds.

In all exchangeable aluminium determinations carried out in this thesis the volume of solution used was a constant ($35cm^3$) and the methodology was as described above.

There are many reports on the use of cation exchange resins in aluminium speciation. The resin can be used in columns, (e.g. Driscoll 1984, Goenaga et al 1987) or in stirred suspensions, (e.g. Campbell et al 1983, Litaor 1987). The residence time can be increased to look at the rate of exchange of aluminium and this may be used, providing a constant pH is maintained, to define both rapidly and slowly exchangeable species. These will include the free hexa-aqua trivalent species, the di- and monovalent hydroxy-aluminium species and aluminium bound in kinetically labile complexes where the binding is weaker than the aluminium-Amberlite complex. Exchangeable aluminium as defined by the columns used throughout these experiments will be of the rapidly exchangeable type, mainly cationic monomers and weakly bound aluminium complexes.

The term, exchangeable aluminium, should be adequately defined in respect of the type of resin used and the procedural details that accompany its use.

2.4 General Discussion

The analytical methods described were developed specifically to address the conditions under which the research was carried out, for example the facility of GFAAS was applied directly to the measurement of low concentrations of aluminium in dilute, aqueous and mainly synthetic waters. Similarly, the scheme developed to study simple aluminium speciation took into account both the practicalities of a large number of samples and the security of a "known quantity" in respect of the various solvents employed. The use of synthetic solutions, derived from stock salt solutions, precluded the additional speciation effects of other aluminium complexing ligands such as fluorides and organics thereby improving the possibility of the development of cause and effect relationships with the remaining chemistry.

The intention was to clearly define the basic experimental methodology and thus facilitate the interpretation of results derived from its use.

2.5 Summary

The use of graphite furnace atomic absorption spectroscopy was optimised for aluminium determinations in artificially controlled synthetic milieu. In conjunction with well-defined sample pre-treatment methodologies, GFAAS could be used to determine three distinct aluminium fractions; total-Al, filtered-Al and exchangeable-Al (Figure 2.10).

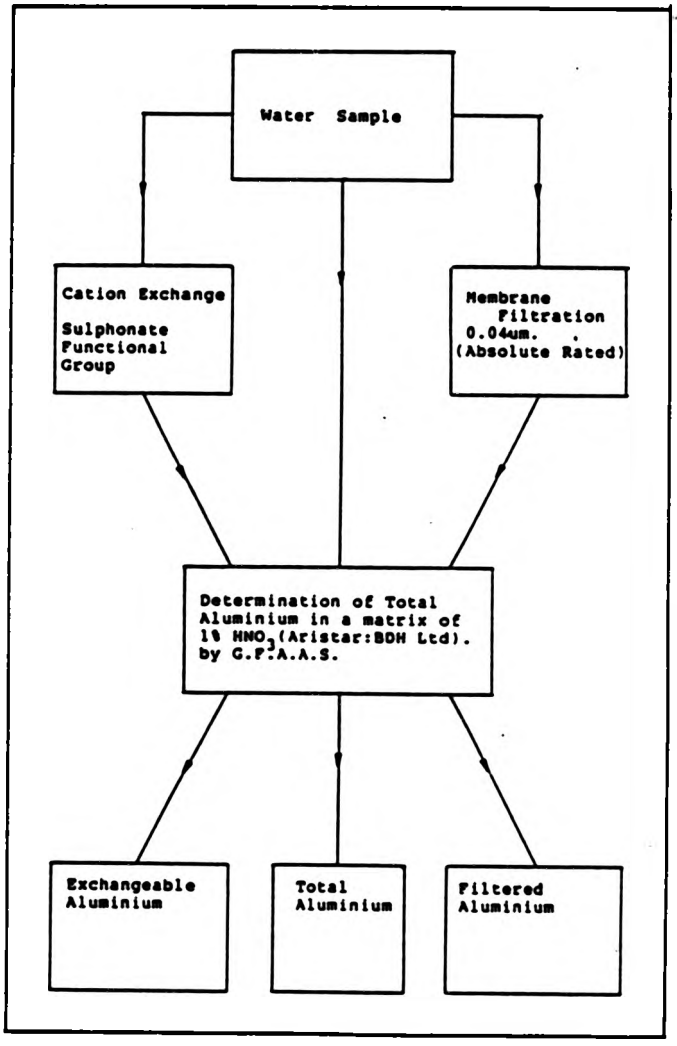


Figure 2.10. Simple flow diagram describing the procedures involved in defining the 3 distinct aluminium fractions.

CHAPTER THREE: AMELIORATION STRATEGIES FOR ALUMINIUM IN ACID WATERS

3.1 Introduction

The term amelioration describes the improvement of the water quality of acid waters. It represents a fuller description of acid water treatment than the often used term neutralisation. This term describes the formation of water and thus a change in pH to 7.0 and is an inadequate description of the physical and chemical processes involved in the detoxification of acid water.

The treatment of acid waters to improve their quality for aquaculture is not a new concept and was practised in Norway as early as the 1920s. Limestone filters were used to neutralise acid waters in hatcheries (Dahl, 1926, 1927; Sunde 1926) and were generally successful in raising pH by a unit and improving the survival of salmon eggs and fry. By the mid 1930s 80 per cent of all Norwegian hatcheries employed limestone filters (Bakke 1939). The amelioration of acid waters has continued (Torgersen 1934, Somme 1942) and over the past twenty years has become increasingly important in alleviating the many problems associated with acid rain.

However, amelioration strategies to date, the great majority of which have involved the large-scale additions

of limestone to surface waters and catchments, have been developed solely on the basis of the reduction in acidity of surface waters and with no specific attempt to address the major toxicant of acid waters, aluminium (Exley and Phillips 1988).

The mitigation of aluminium toxicity in acid waters can be achieved by reducing the biological availability of the aluminium. In the past this has been achieved through pH-induced reductions in the soluble aluminium fraction, (e.g. Rosseland et al 1986, Skogheim et al 1987). The primary control of aluminium solubility in acid waters during neutralisation will be the crystalline aluminium hydroxide, gibbsite, the least soluble common hydroxide phase (Hem 1968a, May et al 1979). For aluminium levels to approach the solubility defined by gibbsite, a period of equilibration is required and this may represent a substantial period of time (for example up to a month in certain water conditions (May et al 1979)), particularly if a degree of aluminium immigration into the water is sustained. Thus the soluble aluminium fraction, under aluminium hydroxide control could remain high for a long period during neutralisation and is prone to increase should the water pH fall slightly or rise above neutral. The nature of the soluble aluminium fraction is defined by aluminium hydrolysis equilibria, (equations 1-4), and the proportion of each species present is a function of the pH (Martin 1986);

(1) $Al^{3+} + H_2O \rightleftharpoons AlOH^{2+} + H^+$

(2) $AlOH^{2+} + H_2O \rightleftharpoons Al(OH)_2^+ + H^+$

(3) $Al(OH)_2^+ + H_2O \rightleftharpoons Al(OH)_3 + H^+$

(4) $Al(OH)_3 + H_2O \rightleftharpoons Al(OH)_4^- + H^+$

The octahedral, hexahydrate, $Al(H_2O)_6^{3+}$ will dominate at $pH < 5.0$ and the tetrahedral $Al(OH)_4^-$ at $pH \geq 6.2$, a mixture of species will exist from $5.0 < pH < 6.2$.

These soluble monomeric species are responsible for acute aluminium toxicity in natural waters, (the nature of this toxicity is discussed in Chapter 6). The amelioration of aluminium toxicity could be more effectively achieved by changing the solubility control rather than changing the pH per se.

The longer term success of an ameliorative strategy will depend on the stability of the biologically unavailable aluminium form with respect to water quality changes, and in particular pH fluctuations.

The ideal amelioration compound would:

1) Effect the rapid removal of biologically available aluminium from the water column such that the resultant insolubles and corresponding solid phase were non-toxic and stable with respect to fluctuations in the water quality.

2) Increase the water pH towards the range 6.0 to 7.0 and donate sufficient buffering capacity to sustain this pH level, $\pm$ 0.5 pH units. This pH regime is both suitable for salmonid populations, (Alabaster and Lloyd 1980), and will ensure minimal concentrations of soluble aluminium species, (Hem 1968a).

In seawater, aqueous silica controls the solubility of aluminium (Willey 1975a, 1975b), which rarely exceeds $10 \mu g l^{-1}$, (Sackett and Arrhenius 1962, Goldberg 1965). The geology of catchments draining into acidified waters will dictate that these waters will be low in $Si_{(aq)}$ (Farmer 1986).

This thesis postulates that the replenishment of the aqueous silica concentration of acidified natural waters will reduce aluminium toxicity to salmonids through a change in solubility control from that of a hydroxide phase to that of an aluminosilicate phase.

This theory was first tested through the comparison of two existing, calcium carbonate and calcium hydroxide,

and one potential, calcium silicate, amelioration compounds.

All of these compounds are readily available, relatively inexpensive and share the same major cation, $Ca^{2+}_{(aq)}$, thereby reducing potential cationic effects on their comparative amelioration efficacy, (see Davison and House 1988),). In addition, calcium carbonate is historically the foremost compound used in acid water amelioration, (Exley and Phillips 1988), calcium hydroxide is a compound in general and increasing use today, (Slavin 1986), and calcium silicate is a novel and as yet untested alternative to the carbonate and hydroxide salts.

This chapter will cover the application of the ameliorating agents, both in a field study, ($Ca(OH)_{2(s)}$ only), and in the laboratory and will aim to characterise the ideal compound for the amelioration of aluminium in acid waters.

3.2 Kenmure Rainbow Trout Farm: A Study of the Use of Calcium Hydroxide in Acid Water Amelioration

3.2.1 Introduction

Kenmure is a rainbow trout farm located close to Castle Douglas in south-west Scotland. The farm pumps water from Loch Ken, (ca 115,200m^3/day), and this water supply is consistently acidic and high in metals,

in particular aluminium, manganese and copper. To reduce the potential toxicities of these conditions, (see Exley and Phillips 1988), to the farmed fish, the influent supply to the farm is dosed continuously with calcium hydroxide, $Ca(OH)_{2(s)}$. Kenmure Fishery offered an excellent opportunity to investigate the effectiveness of this dosing strategy in a practical field situation. The aim of the present study was to assess the strategy in terms of the buffering of acidity, the reduction of the concentration of biologically available metals and the maintenance of adequate calcium and magnesium levels.

3.2.2 Materials and Methods

Sampling was carried out over a 14 month period from April 1987 to May 1988. $Ca(OH)_{2(s)}$ was dosed, dry, from a hopper to a mixing chamber served by the influent farm supply. A propellor ensured good mixing and the resultant slurry fed the farm supply. The rate at which the hopper served the mixing chamber was manually adjusted according to the pH of both the pre- and post-lime water. The weight of $Ca(OH)_{2(s)}$ used was recorded each week and mean weekly doses determined for each month.

Water samples were taken in acid-washed polyethylene bottles twice weekly, (Monday p.m., Friday a.m.) from each of three sample points (Figure 3.2.0):

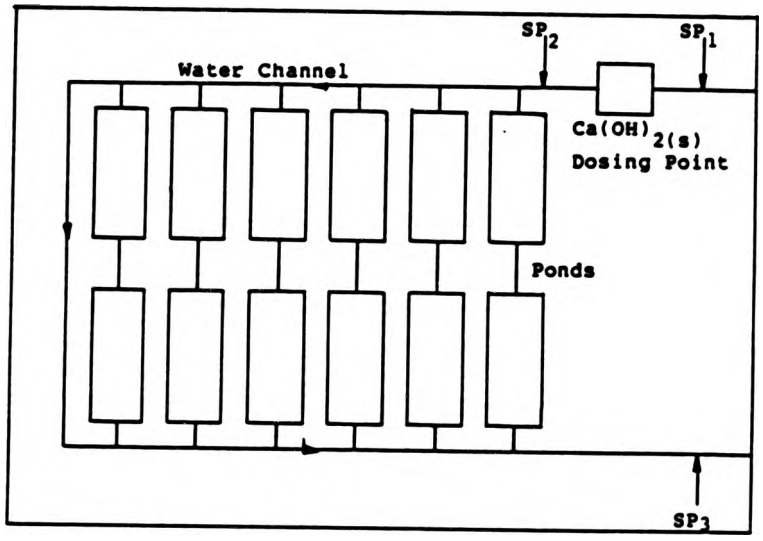


Figure 3.2.0. Schematic representation of the layout of the ponds and sampling points, (SP1 → 3), at Kenmure Fishery.

1) Pre-lime; untreated Loch Ken water prior to entering the farm.

2) Post-lime, directly after liming and prior to entering the culture ponds.

3) Outflow waste water leaving the farm complex.

Samples were stored at 4°C in the dark and sent to Stirling for analysis. The delay between sampling and analysis was variable, usually of the order of 2-7 days. The significance of the delay was that it precluded representative fractionation studies on the metals in the sample. Thus all metal analyses were recorded as total content in each sample.

In addition, pH and water temperature were recorded on the farm at each sample point and at the time of sampling qualitative observations on the weather and on the health of the fish were also recorded and generalised states determined for each month. Fish health was recorded as a fish health index, (Table 3.2.1), and the index was derived from both the stressed state of the fish and the frequency which stressed fish were observed each month.

In the laboratory, sample pH was recorded, (P.W.9409 Digital Recorder: Philips), and the sample was then acidified, 1% V/V HNO_3 (Aristar:BDH Ltd.), to reduce adsorptive effects with the sample bottle and prepare the sample for metal analysis.

Aluminium, manganese and copper were analysed by graphite furnace atomic absorption spectroscopy as total metal only. Aluminium was determined using the program described in Chapter Two and programs for copper and manganese were derived from literature figures (Perkin-Elmer Ltd.).

Total calcium and magnesium were measured by flame atomic absorption spectroscopy after methods described by Golterman et al (1978).

During October 1987, 2 year old harvest trout were analysed for tissue aluminium. The analytical technique is described elsewhere, (section 6.2.3.4).

3.2.3 Results

3.2.3.1 General Weather Conditions

Precipitation, and hence the wet deposition of air-borne pollutants, was heaviest during the autumn and winter months, September to March (Table 3.2.1). Significant snowfall was evident during January and February, whilst the spring and early summer of 1987 were exceptionally dry (Table 3.2.1).

Table 3.2.1 The monthly weather conditions and general health status of the fish during the 14 month study at Kenmure Fishery.

Month	General Weather	Fish Health
April 1987	Dry	3
May	Dry	2
June	Dry	2
July	Dry and Stormy	4
August	Dry and Stormy	1
September	Dry/Wet	4
October	Wet and Stormy	4
November	Dry and Stormy	3
December	Dry/Wet	1
January 1988	Wet and Snow	3
February	Dry and Snow	5
March	Dry	2
April	Wet	2
May	Dry	1

Table Footnote: Qualitative Fish Health Scale:

1. - no apparent signs of stress.
2. - occasional signs of stress.
3. - many signs of stress.
4. - stress and occasional deaths.
5. - stress and many deaths.

3.2.3.2 Water Temperature

The water temperature varied seasonally with maximum, (ca 17.0°C) and minimum, (ca 3.5°C), peaks in July and December respectively, (Table 3.2.2, Figure 3.2.1). There were also considerable fluctuations in water temperatures during certain months, particularly during spring and summer, (Figure 3.2.1).

3.2.3.3 Calcium Hydroxide Dosage

$Ca(OH)_{2(s)}$ dosage was highest during the summer months and most variable during the spring and summer, (Table 3.2.3). Mean additions of $Ca(OH)_{2(s)}$ throughout the year were equivalent to the addition of between 0.9 and 1.8 mg of calcium per litre of inflow water.

3.2.3.4 pH Control

Correlations between the water pH and the seasons/weather were not obvious though the influent pH was noticeably consistently lower during the summer and early autumn of 1987 (Table 3.2.4, Figure 3.2.2), and this may be related to the weather conditions earlier in the same year where a dry spring and early summer were followed by stormy, wet weather in the late summer and early autumn, (Table 3.2.1).

Table 3.2.2 The mean monthly water temperatures and temperature range for Kenmure Fishery between April 1987 and May 1988. n = 8.

	Temperature °C	
Month	Mean	Range
April 1987	8.0	4.5-13.0
May	11.5	10.0-13.0
June	13.5	12.0-15.5
July	17.0	15.0-19.5
August	15.0	14.0-16.0
September	13.5	11.0-16.0
October	9.0	8.0-11.0
November	6.5	4.0-8.0
December	3.5	2.0-5.0
January 1988	4.0	3.0-5.0
February	4.0	2.5-5.0
March	5.5	4.0-7.5
April	8.5	8.0-10.0
May	13.0	10.5-15.0

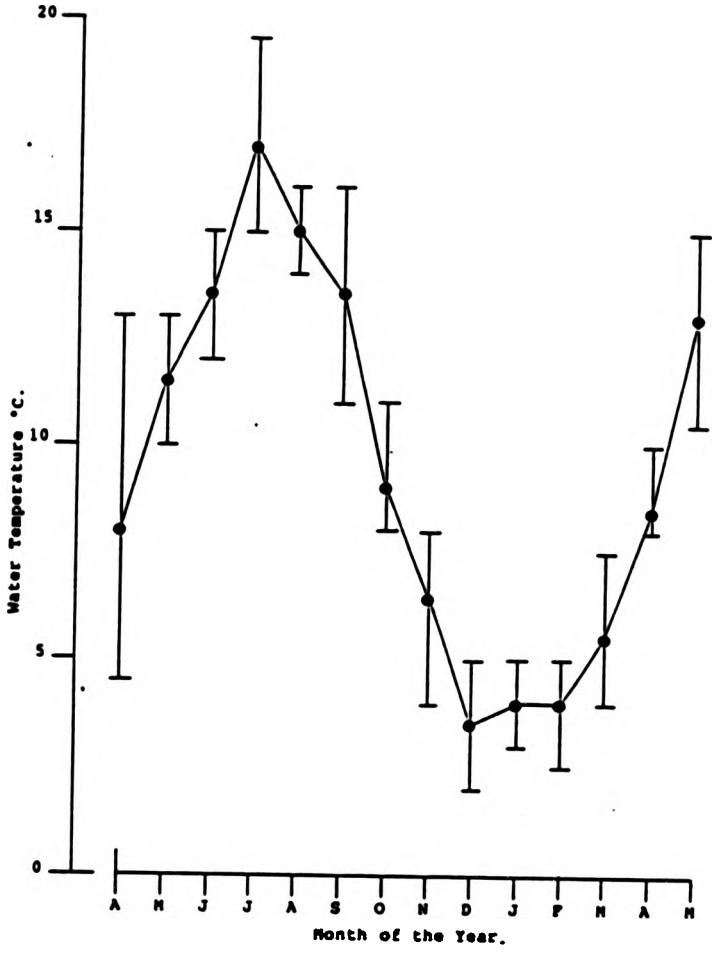


Figure 3.2.1. The water temperature profile for Kenmure Fishery for the period between April 1987 and May 1988. Monthly mean values are plotted, bars indicate the range, n = 8.

Table 3.2.3 Mean weekly dose and range of $Ca(OH)_{2(s)}$ used per month between April 1987 and May 1988 at Kenmure Fishery. n = 4.

Lime Dosage	Mean: $Kgwk^{-1}x10^{3}$	Range: $Kgwk^{-1}x10^{3}$	Mean: KgM^{-3}
Month			
April 1987	1.0	0.9-1.3	$1.24x10^{-3}$
May	0.7	0.4-0.9	$0.87x10^{-3}$
June	1.2	0.6-1.8	$1.49x10^{-3}$
July	1.2	0.9-1.4	$1.49x10^{-3}$
August	1.2	0.9-1.4	$1.49x10^{-3}$
September	1.4	1.2-1.8	$1.74x10^{-3}$
October	1.3	1.2-1.4	$1.61x10^{-3}$
November	1.3	1.2-1.4	$1.61x10^{-3}$
December	1.1	1.0-1.2	$1.36x10^{-3}$
January 1988	0.9	0.9-1.2	$1.12x10^{-3}$
February	0.9	0.8-1.0	$1.12x10^{-3}$
March	0.7	0.6-0.9	$0.87x10^{-3}$
April	0.7	0.6-0.8	$0.87x10^{-3}$
May	0.0	0.0	0.0

Table 3.2.4 Measured pH levels, mean and range, at each sample point for each month between April 1987 and May 1988, at Kenmure Fishery. n = 8.

Sample Point / Month	Inflow/Pre-Lime		Inflow/Post-Lime		Outflow	
	Mean	Range	Mean	Range	Mean	Range
April 1987	6.07	5.65-6.45	6.71	6.43-7.10	6.30	6.16-6.50
May	5.74	5.46-6.05	6.56	6.27-7.16	6.14	5.91-6.33
June	5.83	5.65-6.29	6.40	6.10-6.74	5.87	5.42-6.32
July	6.00	5.85-6.38	6.33	5.97-6.92	6.20	6.10-6.53
August	5.84	5.56-6.30	6.77	6.46-7.16	6.08	5.70-6.40
September	5.75	5.28-6.33	6.89	6.73-7.29	6.47	6.34-6.50
October	5.71	5.09-6.34	6.92	6.45-7.67	6.40	6.15-6.52
November	6.10	5.86-6.50	7.04	6.75-7.40	6.40	6.30-6.57
December	6.22	5.92-6.50	6.99	6.75-7.51	6.46	6.30-6.70
January 1988	5.98	5.56-6.28	6.61	5.95-7.40	6.47	6.38-6.53
February	6.18	5.98-6.50	6.93	6.64-7.22	6.46	6.36-7.20
March	6.08	5.88-6.25	7.01	6.80-7.40	6.39	6.25-6.56
April	6.22	6.01-6.40	6.53	6.03-7.09	6.34	6.22-6.40
May (No Lime)	6.57	6.38-6.73	n.a.	n.a.	6.39	6.19-6.78

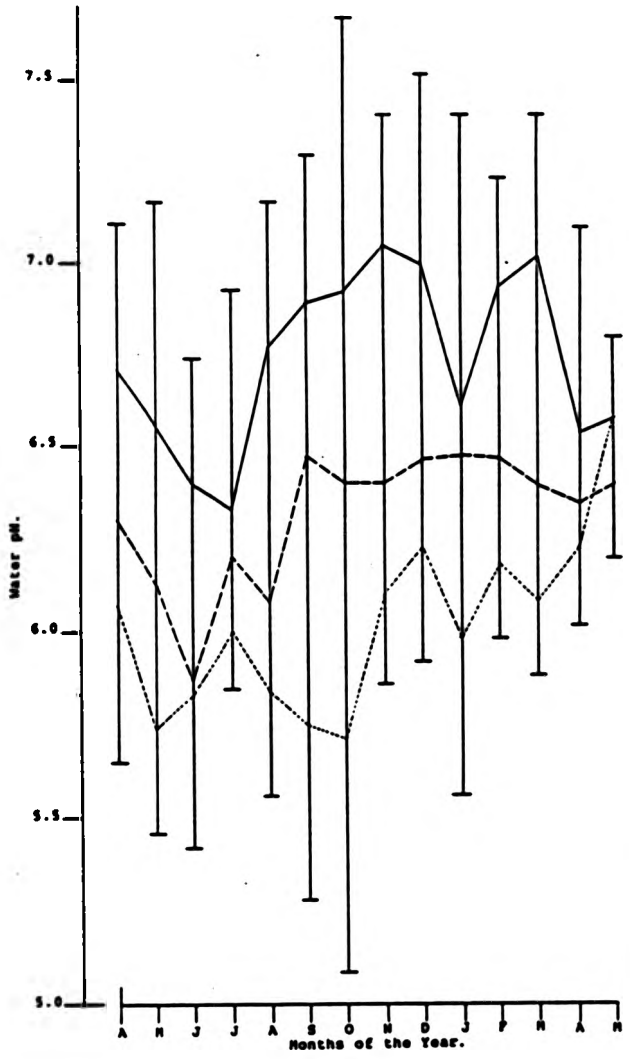


Figure 3.2.2. The water pH at the pre-lime ----, post-lime, ——, and outflow, ---, sample points, during the 14 month study at Kenmure Fishery. Monthly means are plotted, n = 8. Bars indicate the full range, n =24.

The pH of the inflow, pre-lime pH, was generally pH5.5 to pH6.5, though was not as low as might have been expected for the area, (Table 3.2.4, Figure 3.2.2). The immediate effect of $Ca(OH)_{2(s)}$ addition was to raise the pH by between 0.7 to 0.9 pH units. The pH of the water would then fall to about pH 6.4 at the outflow sampling point. Whilst both the pre- and post-lime pH were consistently variable, the outflow pH remained fairly constant, particularly during the autumn and winter months. This correlates quite closely with the low and less variable water temperatures during these months and suggests that while the addition of $Ca(OH)_{2(s)}$ was seen to successfully control the pH of the farm water, the control may be derived more from the water temperature than the pH of the influent supply.

3.2.3.5 Calcium

The calcium concentration in the farm inflow was ca 2.5mgl^{-1}, and would not be expected to be limiting with respect to fish growth, (Rodgers 1984).The calcium level was supplemented by the addition of $Ca(OH)_{2(s)}$ and the difference between the pre- and post-lime levels were equivalent to the actual dosage, (Table 3.2.3). The post-lime calcium level was equivalent to 70-90% of the calcium added above the pre-lime level whilst the calcium level recorded in the outflow was generally equivalent to more than the calcium added on $Ca(OH)_{2(s)}$ addition (Table

Table 3.2.5 Measured calcium levels, mean and range, each month at each sample point during the 14 month study at Kenmure Fishery. n = 8, units = mgl^{-1}.

Sample Point / Month	Inflow/Pre-Lime		Inflow/Post-Lime		Outflow	
Calcium mgl^{-1}	Mean	Range	Mean	Range	Mean	Range
April 1987	2.4	1.7-3.3	3.4	2.9-4.8	3.8	3.3-4.7
May	2.6	1.9-3.9	3.3	2.8-4.3	3.7	2.8-4.4
June	2.3	1.7-2.8	3.3	2.6-4.1	3.6	2.1-5.2
July	2.4	1.1-3.0	3.7	2.9-4.3	4.0	3.3-4.2
August	2.6	0.8-3.3	3.9	1.6-5.9	4.4	3.4-6.3
September	2.5	2.3-2.7	3.4	3.1-3.7	3.7	3.2-4.1
October	2.7	2.1-3.0	4.2	3.5-5.2	6.8	5.9-7.5
November	2.7	2.3-3.3	4.3	3.8-5.3	5.7	3.1-7.5
December	4.9	2.5-6.1	6.2	4.7-9.1	6.9	5.5-8.1
January 1988	4.5	3.7-4.9	5.3	3.7-5.7	9.1	7.7-9.9
February	3.2	1.1-4.3	4.2	5.5-6.2	6.0	3.2-7.5
March	2.9	1.7-4.1	3.9	3.1-5.2	3.9	3.1-4.3
April	2.5	0.8-3.1	3.5	2.9-6.9	7.0	5.6-8.2
May (No Lime)	2.2	1.1-2.5	n.a.	n.a.	2.4	1.6-3.0

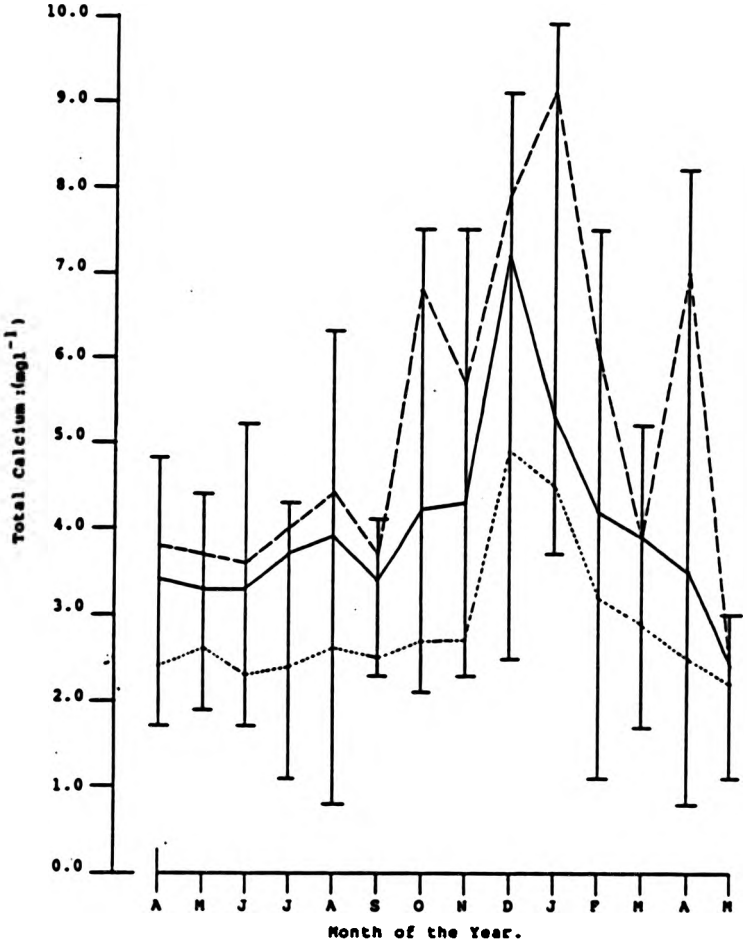


Figure 3.2.3. Calcium concentrations at the pre-lime,..... post-lime, ——, and outflow, ---, sample points during the 14 month study at Kenmure Fishery. Monthly means are plotted, n = 8. Bars indicate the full range, n = 24.

3.2.5, Figure 3.2.3). This was particularly true during the autumn and winter months and suggested that sedimented calcium resulting either from undissolved $Ca(OH)_{2(s)}$ or precipitated calcium products was dissolving in the water column, perhaps during conditions when calcium dissolution would be enhanced such as cold, acidic water.

3.2.3.6 Magnesium

The magnesium level in the inflow to the farm was ca $1.0mgl^{-1}$ and was adequate with respect to both salmonid growth and survival, (Alabaster and Lloyd 1980). Magnesium levels within the farm showed fluctuations of $\pm$ $0.6mgl^{-1}$ and were largely independent of $Ca(OH)_{2(s)}$ addition, (Table 3.2.6, Figure 3.2.4), though were generally lower during the winter months. Wide intra-month variations in magnesium were evident during August and March and these correlate quite closely to periods of heavy rain and snowmelt respectively, (Table 3.2.1).

3.2.3.7 Aluminium

Aluminium levels in the influent supply to the farm were both high and variable, (Table 3.2.7, Figure 3.2.5). In the absence of high concentrations of aluminium complexing agents, the measured levels of total aluminium, $150-300ugl^{-1}$, would be expected to be acutely toxic to rainbow trout, (Salmo gairdneri), at pH less than 6.0 (Exley and Phillips 1988).

Table 3.2.6 Measured magnesium levels, mean and range, each month at each sample point during the 14 month study at Kenmure Fishery. n = 8, units = mgl-1.

Sample Point / Month	Inflow/Pre-Lime		Inflow/Post-Lime		Outflow	
Magnesium mgl^{-1}	Mean	Range	Mean	Range	Mean	Range
April 1987	1.2	0.9-1.6	1.2	1.1-1.6	1.2	1.0-1.5
May	1.1	1.1-1.2	1.2	1.1-1.6	1.2	1.1-1.6
June	1.1	1.0-1.3	1.0	1.0-1.1	1.1	0.8-1.2
July	1.7	1.4-1.8	1.6	1.3-1.8	1.6	1.5-1.9
August	1.5	1.0-2.0	1.4	0.9-1.8	1.4	0.9-1.9
September	1.2	1.1-1.5	1.2	1.0-1.4	1.2	1.0-1.4
October	1.2	0.9-1.3	1.2	1.0-1.4	1.0	0.8-1.1
November	0.9	0.8-1.1	0.8	0.7-0.8	0.8	0.7-0.8
December	1.0	0.9-1.1	1.0	0.9-1.1	1.0	0.8-1.1
January 1988	1.1	1.0-1.2	1.1	1.0-1.1	0.8	0.6-1.0
February	0.9	0.9-1.0	0.9	0.7-1.1	1.0	0.8-1.1
March	0.7	0.6-0.9	2.0	1.8-2.2	0.9	0.7-1.1
April	1.1	1.0-1.3	1.1	0.9-1.2	1.1	1.0-1.5
May (No Lime)	0.9	0.6-1.1	n.a.	n.a.	0.9	0.8-1.0

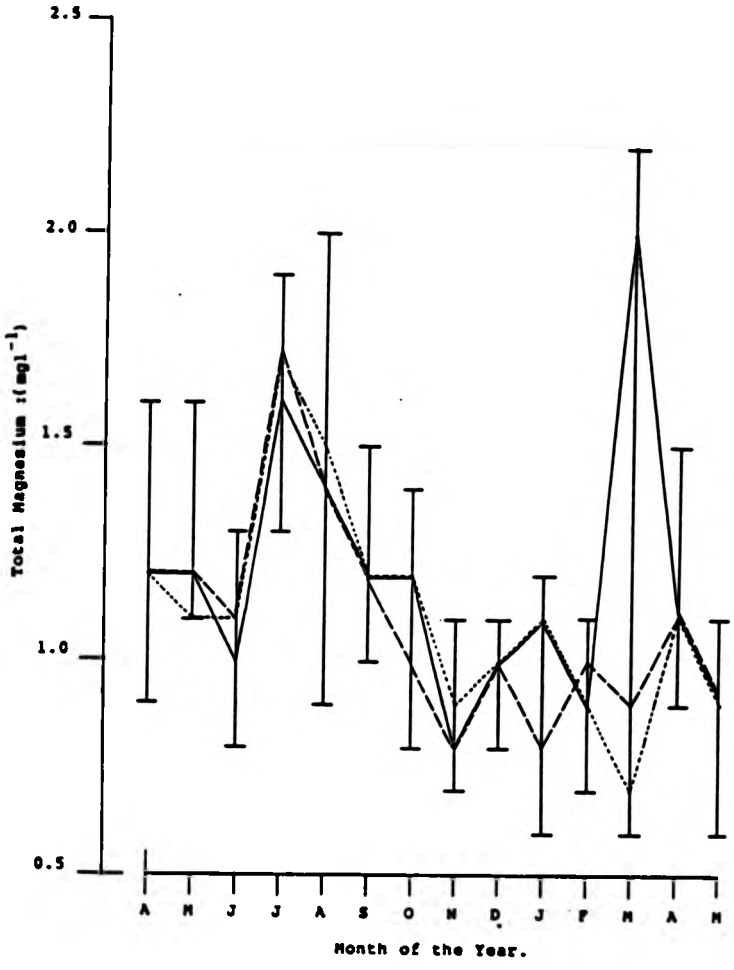


Figure 3.2.4. Magnesium concentrations at the pre-lime,------, post-lime, ——, and outflow ---, sample points during the 14 month study at Kenmure Fishery. Mean values plotted, n = 8. Bars indicate the full range, n = 24.

Table 3.2.7 Measured aluminium levels, mean and range, each month at each sample point during the 14 month study at Kenmure Fishery. n = 8, units = $\mu g l^{-1}$.

Sample Point / Month	Inflow/Pre-Lime		Inflow/Post-Lime		Outflow	
Aluminium $\mu g l^{-1}$	Mean	Range	Mean	Range	Mean	Range
April 1987	165	130-190	200	150-240	198	120-250
May	195	130-230	201	150-235	208	120-250
June	163	130-230	174	120-240	194	135-235
July	207	140-300	216	150-300	264	200-300
August	233	160-300	257	180-302	246	140-310
September	303	277-313	311	291-322	319	290-341
October	256	160-300	258	150-270	258	180-300
November	253	201-305	263	200-310	328	270-511
December	239	208-252	251	211-263	255	231-263
January 1988	247	200-299	258	233-301	279	231-311
February	291	188-327	299	192-343	319	200-341
March	251	201-259	262	209-283	281	233-295
April	201	137-247	210	150-247	200	139-212
May (No Lime)	171	123-185	n.a.	n.a.	191	124-211

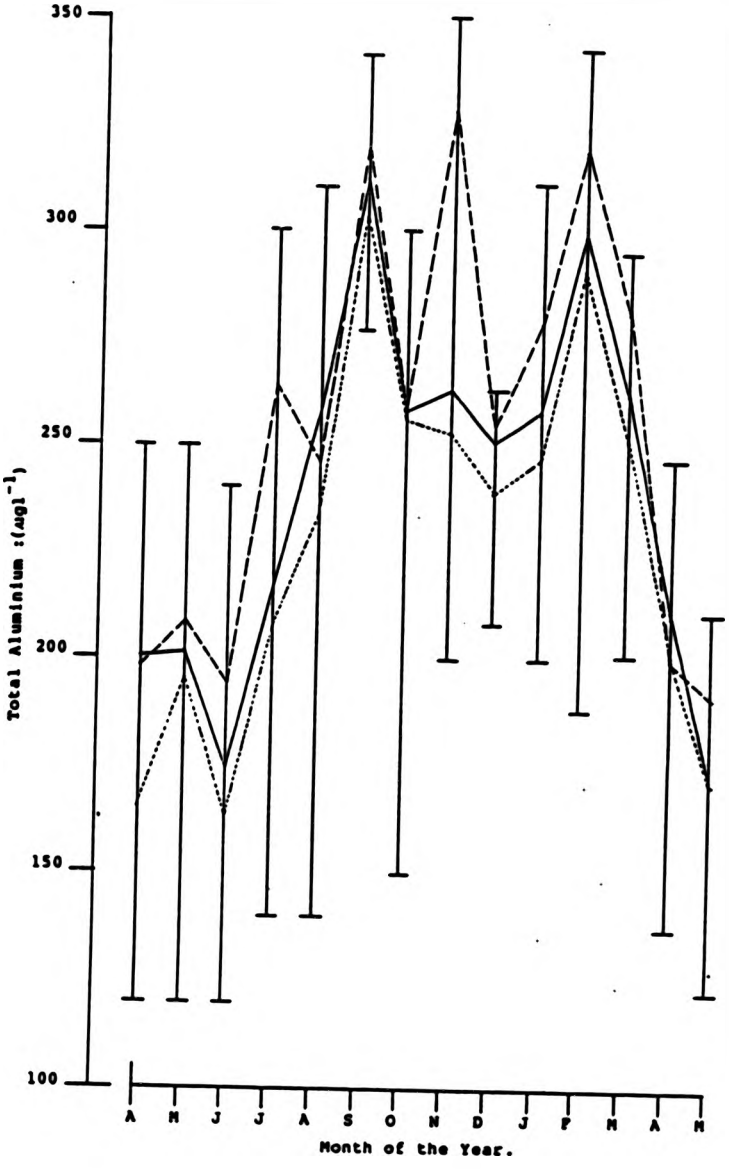


Figure 3.2.5. Aluminium concentrations at the pre-lime,..... , post-lime ——, and outflow,- - - - sample points during the 14 month study at Kenmure Fishery. Mean values are plotted, n = 8. Bars indicate the full range, n = 24.

The addition of $Ca(OH)_{2(s)}$ increased the total concentration of aluminium, the post-lime aluminium level consistently exceeding the pre-lime level by 2-30μgl^{-1}, (Figure 3.2.5). In addition the outflow aluminium levels generally exceeded the post-lime levels by up to 65μgl^{-1}, an indication perhaps that further dissolution of aluminium from lime particles was occurring and/or aluminium was both deposited and subsequently released from the pond sediments. Aluminium levels peaked during the autumn and winter months and in particular during September and February, months corresponding to periods of heavy rain and snowmelt respectively, (Table 3.2.1).

3.2.3.8 Tissue Aluminium

The analysis of the tissues of apparently, i.e. visibly unstressed, healthy rainbow trout (2 years old ca 1Kg weight), revealed significant accumulations of aluminium (Table 3.2.8). The largest accumulations were located in brain, (range: 123-191 μgg^{-1}), and gill tissue (range 100-159 μgg^{-1}), though aluminium was measured in all tissues sampled.

The tissue digestion technique, (Chapter Six), determined only total aluminium in a known mass of tissue and was therefore unable to predict the form of the accumulated aluminium or the nature of the association of the aluminium with the tissue.

Table 3.2.8 Aluminium accumulations ($\mu g g^{-1}$ dry weight) in the tissues of live rainbow trout from Kenmure Fishery. n = 8.

Tissue	Mean	Range
Brain	160	123-191
Liver	83	42-91
Gonad	21	16-25
Gills	114	100-159
Muscle	48	12-75

3.2.3.9 Manganese

The manganese concentrations found in the inflow to the farm, ca 50-100$\mu g l^{-1}$, would not be acutely toxic to rainbow trout, though might confer some sub-lethal gill damage at pH ca 5.0 (Exley and Phillips 1988). $Ca(OH)_{2(s)}$ addition removed manganese from the water column (Table 3.2.9 and Figure 3.2.6) presumably through the precipitation of the $Mn^{2+}_{(aq)}$ ion (see Howells 1984). Manganese levels did show a seasonal trend, generally being higher during the autumn and winter months. The manganese level peaked during October and February and during the latter the precipitation of manganese was greatly reduced, a suggestion, (though not vindicated by the pH measurements taken), perhaps of more acid water during this period.

Table 3.2.9 Measured manganese levels, mean and range, each month, at each sample point during the 14 month study at Kenmure Fishery. n = 8, units = $\mu g l^{-1}$

Month \ Sample Point	Inflow/Pre-Lime		Inflow/Post-Lime		Outflow	
Manganese $\mu g l^{-1}$	Mean	Range	Mean	Range	Mean	Range
April 1987	45	27-62	53	40-77	58	42-72
May	82	65-125	57	47-67	72	52-145
June	73	62-82	62	54-70	56	47-66
July	69	30-101	61	23-92	58	24-92
August	74	17-110	69	22-114	53	11-74
September	91	76-130	72	54-102	64	53-82
October	103	59-121	85	61-101	79	61-95
November	93	69-99	81	59-102	80	61-92
December	84	73-89	75	42-90	71	44-78
January 1988	78	69-103	77	52-84	76	47-83
February	102	97-153	100	75-121	93	64-125
March	63	27-69	51	36-65	49	42-55
April	42	12-79	41	32-55	37	29-45
May	31	10-41			19	15-26

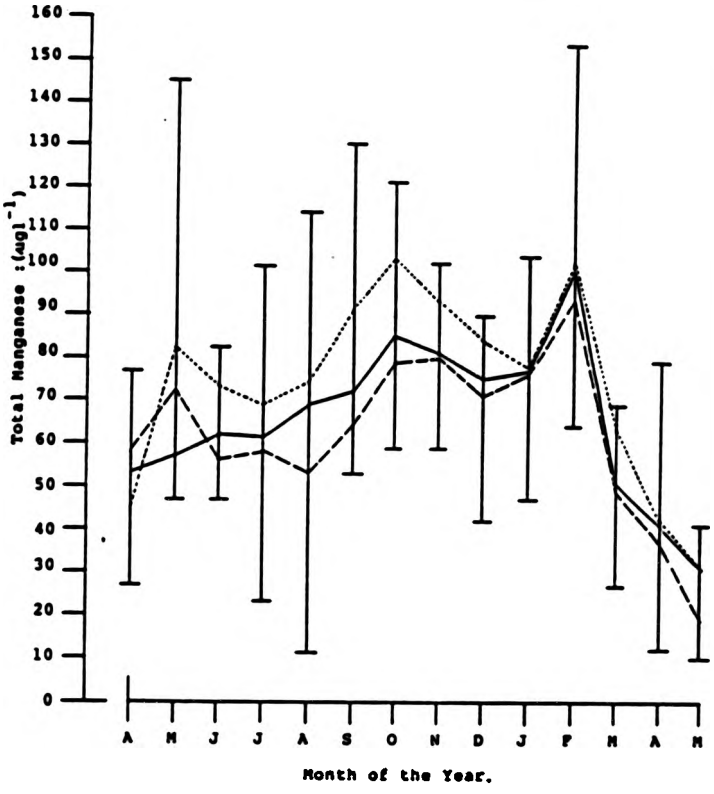


Figure 3.2.6. Manganese concentrations at the pre-lime,-----, post-lime, ——, and outflow,---, sample points during the 14 month study at Kenmure Fishery. Monthly means are plotted, n=8. Bars indicate the full range, n = 24.

3.2.3.10 Copper

The level of copper in the influent supply to the farm was high, range 0-20μgl^{-1} (Table 3.2.10), and was, on occasion, (June and August), above the suggested toxicity threshold of 7μgl^{-1}, (Pagenkopf 1983, Lauren and McDonald 1985). Whilst copper levels throughout the farm, (Table 3.2.10) were unlikely to be acutely toxic, they were likely to contribute to the overall stressed state of the fish (Exley and Phillips 1988). $Ca(OH)_{2(s)}$ addition had no consistent effect on the total copper levels, (Table 3.2.10, Figure 3.2.7). No seasonal trend for copper was evident though prominent peak levels were recorded during August and February.

3.2.3.11 Fish Health

Mortalities were lower than would have been predicted by water quality conditions per se, (Table 3.2.1). The only deaths directly attributable to the water chemistry were during February 1988, and, as is discussed later, were correlated with increased levels of metals and in particular aluminium. Mortality during July, September and October 1987 were attributed to the bacterial disease, enteric redmouth, (ERM).

Fish showed visible signs of stress for up to 80% of the year. Most typical were inappetance and general lethargy. Stressed fish showed little if any fright response and

Table 3.2.10 Measured copper levels, mean and range, each month at each sample point during the 14 month study at Kenmure Fishery. n = 8, units = $\mu g l^{-1}$.

Sample Point / Month	Inflow/Pre-Lime		Inflow/Post-Lime		Outflow	
Copper $\mu g l^{-1}$	Mean	Range	Mean	Range	Mean	Range
April 1987	2.5	1.0-6.0	2.5	1.5-4.5	3.0	1.5-4.5
May	2.5	1.0-5.5	1.5	1.0-3.0	2.0	1.5-3.0
June	3.0	1.0-8.0	3.0	0.0-8.0	2.0	0.0-9.0
July	2.5	1.0-3.0	2.0	0.0-4.0	2.5	0.0-6.0
August	5.5	1.5-20.0	4.5	1.5-14.0	3.0	1.5-6.0
September	3.0	2.0-3.5	2.5	1.5-3.5	4.0	2.0-7.0
October	2.5	0.0-3.5	2.5	1.5-3.0	3.0	1.0-5.0
November	4.5	1.5-6.0	3.0	0.0-4.0	3.5	1.0-5.0
December	1.3	0.0-2.0	1.3	1.0-2.5	1.3	0.0-2.5
January 1988	1.0	0.0-1.5	0.0	0.0-0.5	1.5	0.0-5.0
February	4.0	2.5-5.0	3.5	1.0-4.0	4.5	2.0-5.0
March	1.5	0.0-1.5	2.0	0.0-2.5	1.5	1.0-2.0
April	2.5	1.0-4.0	3.5	2.5-5.0	3.5	1.5-6.0
May (No Lime)	1.0	0.0-1.0	n.a.	n.a.	2.5	0.0-3.0

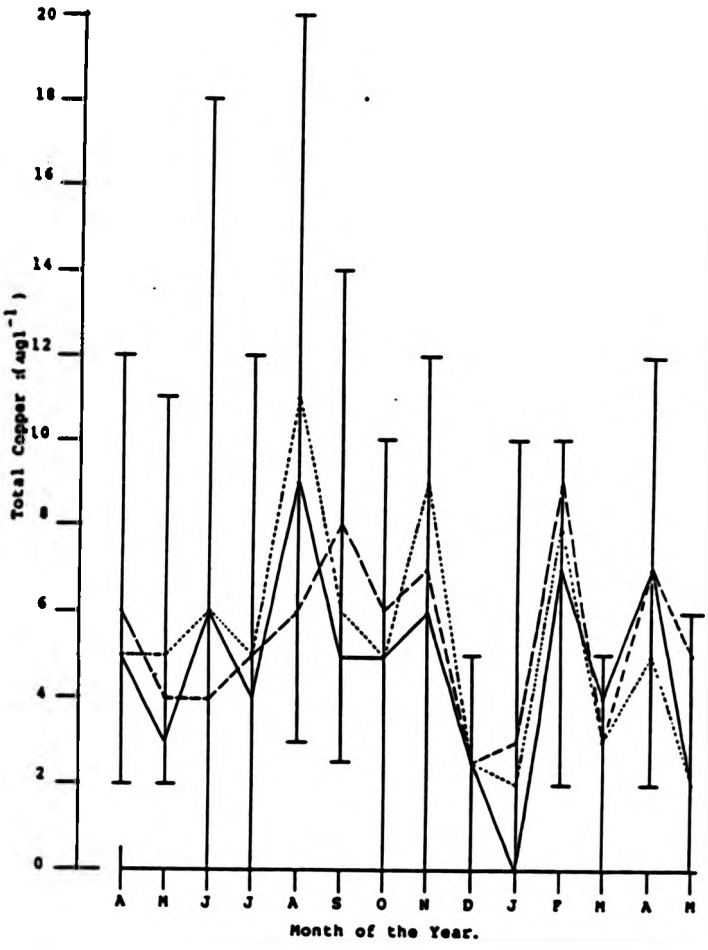


Figure 3.2.7. Copper concentrations at the pre-lime,....., post-lime, ——, and outflow,----, sample points during the 14 month study at Kenmure Fishery. Monthly means are plotted, n=8. Bars indicate the full range, n = 24.

tended to swim high up in the water column, often at the surface. A spasmodic swimming action was observed in fish close to death. These stress criteria were used to construct a fish health index (Table 3.2.1). Increased levels of stress could generally be correlated with adverse monthly weather conditions.

3.2.4 Discussion

Calcium hydroxide addition appeared to successfully control the pH of the influent supply to Kenmure Fishery, (Table 3.2.4, Figure 3.2.2), though did not abolish acid-related stress, (Table 3.2.1). Water temperature was found to be a significant influence on $Ca(OH)_{2(s)}$ dosage and necessitated higher doses during the warmer spring and summer months to effect the required increase in the influent supply pH. This can be attributed to the reduced solubility, and hence dissolution, of $Ca(OH)_{2(s)}$ at higher water temperatures. The increased levels of calcium recorded in the outflow water with respect to both pre- and post-lime calcium levels suggested that calcium was deposited in the culture ponds sediments and may then be available as an alternative source of alkalinity, to that already dissolved in the water column, for the buffering of acidity, (Table 3.2.5, Figure 3.2.3).

Although the pH was controlled and may have reduced fish mortalities, the fish remained stressed for much of the

year, (Table 3.2.1).

From all the water quality information available it can be surmised that the aluminium level was the most likely precursor of both sub-lethal and lethal responses. The rainbow trout Salmo gairdneri, is the most sensitive salmonid to acid/aluminium stress, (Exley and Phillips 1988). Assuming that strong aluminium complexing ligands such as $F^-_{(aq)}$ and dissolved organic carbon, (DOC), were negligible, as found at Kenmure by D'Anjou (1988), the predominant monomeric aluminium species present at the recorded pH levels of the farm water could be represented by an equilibrium between the dihydroxy cation, $Al(OH)^+_{2(aq)}$, and the aluminate anion, $Al(OH)^-_{4(aq)}$. These species are unlikely to be acutely toxic however sufficient acidic aluminium ions, e.g. $Al^{3+}_{(aq)}$, and $AlOH^{2+}_{(aq)}$, would be present to confer a degree of physiological acclimation to aluminium exposure on the culture fish, (Wood et al 1988b, 1988c). Physiological adaptation will reduce the sensitivity of the fish to subsequent aluminium exposure and might explain the lower than predicted mortalities on the farm.

Evidence in favour of a degree of physiological adaptation to aluminium was provided by the tissue digestion analyses, (Table 3.2.8). The occurrence of aluminium in all tissues analysed and in particular the brain and gill suggested biologically available aluminium was present in the farm supply though rarely at sufficient levels to be acutely toxic.

Comparative studies on tissue aluminium levels are rare, however, similarities exist between this study and that of Buergel and Soltero (1983), where the gill was shown to be the principal site of aluminium accumulation in live rainbow trout and also Berg and Burns (1984), where the brain was shown to be the major tissue for aluminium accumulation in a wide range of live fish. The significance of the aluminium accumulations with respect to toxicity is unknown, however aluminium associations at the gill surface are correlated with acute aluminium toxicity in salmon fry (Chapter Six).

Acute toxicity would have occurred at Kenmure if the high total aluminium levels had coincided with a fall in the farm water pH. Such an event might help explain the mortalities suffered during the month of February 1988. During this month snowfall during January and early February began to melt, (Table 3.2.1) and the water temperature fluctuated about a low monthly mean, ca 4.0°C (Figure 3.2.1). Wide variations of calcium, unrelated to the $Ca(OH)_{2(s)}$ dosage, were recorded and suggested calcium dissolution from the sediments (Figure 3.2.3). Manganese concentrations peaked and were not reduced on $Ca(OH)_{2(s)}$ addition, an indication perhaps of increased water acidity (Figure 3.2.6). The copper levels also peaked for only the second time during the study period (Figure 3.2.7). Aluminium levels rose to a winter peak during

February and the greatly enhanced levels recorded in the outflow (Figure 3.2.5), were evidence of aluminium dissolution from the pond sediments.

Measured pH levels at this time were not low (Figure 3.2.2), however, the extensive mortalities suffered during this period (Table 3.2.1) combined with the aforementioned water quality observations would suggest that the sampling procedure may have missed the fall in pH, the duration of which need not have extended beyond several hours.

It was likely that the events described were associated with the mechanics of snowmelt (Elgmork et al 1973, Johanessen et al 1980), whereby a large pulse of acid water had passed through the farm (the $Ca(OH)_{2(s)}$ dosing system having undercompensated) disrupting chemical equilibria and irreversibly intoxicating the fish population through a rapid increase in the biologically available aluminium fraction.

This event suggests that the manual dosing of $Ca(OH)_{2(s)}$ was not a sufficiently flexible amelioration strategy to cope with such events as snowmelt and perhaps stormflow.

Calcium hydroxide has been used elsewhere in acid/aluminium amelioration. Dickson (1983) succeeded in reducing the total aluminium levels in a fish farm by

dosing 100m above the farm complex. As in the present study, Dickson (1983) also recorded seasonal variations in metal concentrations, (Al, Cu, Mn), with maxima occurring during the winter and in connection with snow-melt, and found that $Ca(OH)_{2(s)}$ failed to ameliorate acid-related mortalities particularly in the late winter.

3.3. A Comparison of Three Basic Calcium Salts as Amelioration Agents for Aluminium Toxicity

The criteria used to compare the three compounds, calcium hydroxide, $Ca(OH)_{2(s)}$, calcium carbonate, $CaCO_{3(s)}$, and calcium silicate, $CaO/SiO_{2(s)}$, were:

(i) Neutralisation Value - their ability to buffer acidity,

(ii) Aluminium Insolubilisation Capacity - their ability to insolubilise aluminium over a wide and environmentally significant pH range.

Each compound was of an industrial grade, (ICI plc), and had an estimated particle size of $\lesssim$100um. Laboratory studies investigated the saturation solubility, i.e. the maximum weight of compound that will dissolve in a known volume of solvent, the neutralisation efficacy, i.e. the rate at which and extent to which the compound will reduce the acidity of a solvent, and the effect of the addition of a saturating weight of each compound on the solubility of aluminium in a solvent.

3.3.1 Solubility

3.3.1.1 Materials and Methods

The solubility of each test compound was determined between pH3.0 and 7.0 and at 5°C (pH3.0 only), and 25°C, in three water-based solvents.

(i) Double-distilled water,(D.D.),

(ii) 500mgl^{-1} $Al(NO_3)_3 \cdot 9H_2O$(Aristar:BDH Ltd.) in double-distilled water, (AR),

(iii) Organic-rich (Humic and Fulvic acids), streamwater, (OR).

One gram of test compound was added to a litre volume of each treatment, contained in an acid-washed polyethylene bottle. The solution was then adjusted to the required pH using $NaOH_{(aq)}$ or $HNO_{3(aq)}$ (Aristar:BDH Ltd.). Solutions were stirred continuously for 24 hours prior to vacuum filtration through 0.45um membrane filters, (Ultipor N66, Pall Inc.). The residue was dried to a constant weight and subtracted from the original weight of compound added, (ca 1g), to give the quantity of agent lost to solution.

Table 3.3.1. The solubility in mgl^{-1} of each amelioration compound in three solvent types and over the pH range of 3.0 to 7.0 at 25°C. $\bar{x}$ - mean, SD - standard deviation. n = 3.

Compound	Solvent pH	3.0		4.0		5.0		6.0		7.0	
Solubility mgl^{-1}	Solvent Type	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Calcium Hydroxide $Ca(OH)_{2(s)}$	Double Distilled	803.3	9.4	670.0	14.1	613.3	4.7	526.7	16.9	416.7	9.4
	Aluminium Rich	793.3	9.4	696.7	4.7	573.3	18.9	530.0	0.0	413.3	4.7
	Organic Rich	740.0	14.1	683.3	4.7	600.0	24.5	513.3	4.7	416.7	4.7
Calcium Carbonate $CaCO_{3(s)}$	Double Distilled	60.3	1.7	14.7	2.1	9.7	0.5	8.0	1.4	9.0	1.4
	Aluminium Rich	50.7	0.5	14.0	0.0	14.0	3.6	9.7	1.2	3.3	0.5
	Organic Rich	61.0	1.4	23.7	1.9	19.3	0.5	7.3	0.9	9.3	1.7
Calcium Silicate $CaO/SiO_{2(s)}$	Double Distilled	88.7	0.9	39.3	3.3	35.0	2.8	29.0	0.8	26.0	1.4
	Aluminium Rich	49.7	0.9	39.0	2.5	29.7	1.9	28.7	0.5	26.7	0.9
	Organic Rich	72.3	2.5	34.0	1.4	33.0	1.4	28.7	1.2	28.7	0.5

Table 3.3.2.The influence of a lower solvent temperature on the solubility of each compound, in each solvent type at pH3.0. $\bar{x}$-mean, SD - standard deviation. n =3.

Compound	Solvent Temp.°C	5		25	
Solubility mgl^{-1}	Solvent Type	$\bar{x}$	SD	$\bar{x}$	SD
Calcium Hydroxide $Ca(OH)_{2(s)}$	Double Distilled	1610.0	14.1	803.3	9.4
	Aluminium Rich	1696.7	4.7	793.3	9.4
	Organic Rich	1580.0	0.0	740.0	14.1
Calcium Carbonate $CaCO_{3(s)}$	Double Distilled	129.3	0.5	60.3	1.7
	Aluminium Rich	162.7	2.4	50.7	0.5
	Organic Rich	180.0	2.2	61.0	1.4
Calcium Silicate $CaO/SiO_{2(s)}$	Double Distilled	190.0	1.4	88.7	0.9
	Aluminium Rich	229.0	1.6	49.7	0.9
	Organic Rich	209.7	0.9	72.3	2.5

Table 3.3.3.Solubility ratios for $CaCO_3$:CaO/SiO_2:$Ca(OH)_2$over the pH range of 3.0-7.0.

pH / Temp.°C	3.0	4.0	5.0	6.0	7.0
25°	1:1:14	1:3:41	1:3:45	1:4:64	1:5:72
5°	1:1:11	-	-	-	-

The solubilities were expressed as weight i.e. mgl^{-1} rather than molar indices. Molar solubility is a better comparative index, (specifically when chemical reactions are to be balanced), however, assuming the molar weight of each compound was as per the molecular formula, i.e. $Ca(OH)_{2(s)}$ ca 74g, $CaCO_{3(s)}$ ca 100g, $CaO/SiO_{2(s)}$ ca 116g, the weight solubility could be adequately used on a comparative basis. On an applied basis, i.e. dosing programs for use in the field, weight is an appropriate measure of solubility.

3.3.1.2 Results and Discussion

(i) Effect of Solvent Type

$Ca(OH)_{2(s)}$ was universally more soluble, (ca 10-fold), than either $CaCO_{3(s)}$ or $CaO/SiO_{2(s)}$, (Tables 3.3.1, 3.3.2, and 3.3.3,Figures 3.3.1a-c and 3.3.2).Solubility was potentiated for each agent ($Ca(OH)_{2(s)}$ ca 1.6-1.7gl^{-1}, $CaCO_{3(s)}$ ca 0.13-0.18gl^{-1} and $CaO/SiO_{2(s)}$ ca 0.19-0.23gl^{-1}), at a solution pH of 3.0 and solution temperature of 5°C.

Generally the solvent type had little influence on the saturation solubility of each compound. Solvent type had no significant, (t-test: $P \geq 0.05$), influence on $Ca(OH)_{2(s)}$ solubility, regardless of solution pH and temperature (Tables 3.3.1,3.3.2).$CaCO_{3(s)}$ solubility was significantly, (t-test: $P < 0.05$), lower in AR than DD or OR at pH3.0 and

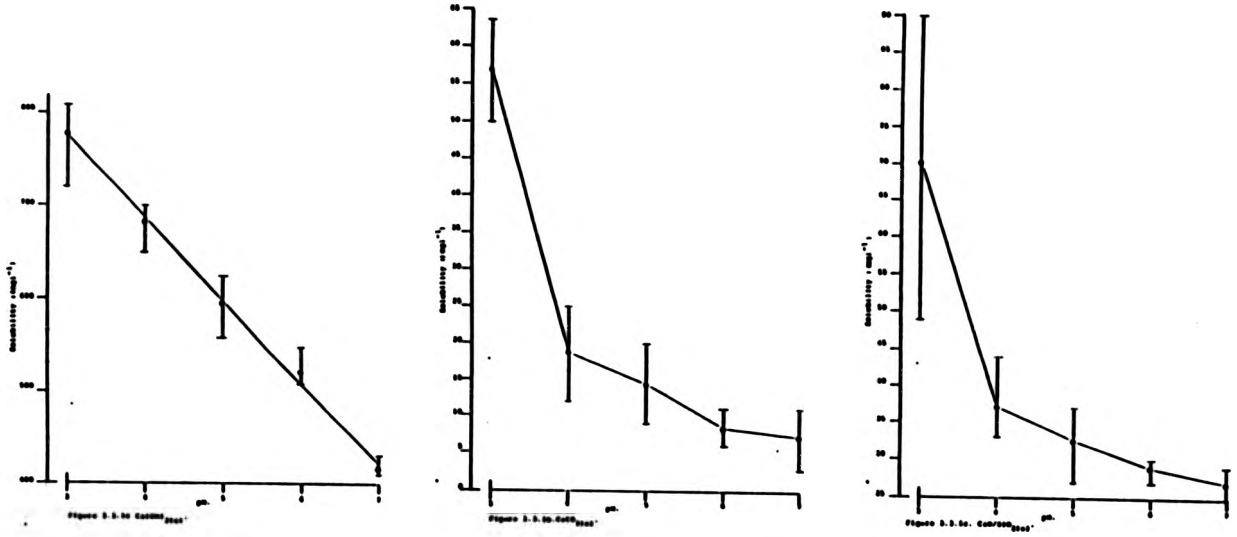


Figure 3.3.1a-c. The effect of increasing pH on the saturation solubility of each amelioration compound. This effect was largely independent of the solvent type and the mean values and ranges from all three solvent types are shown, n = 9.

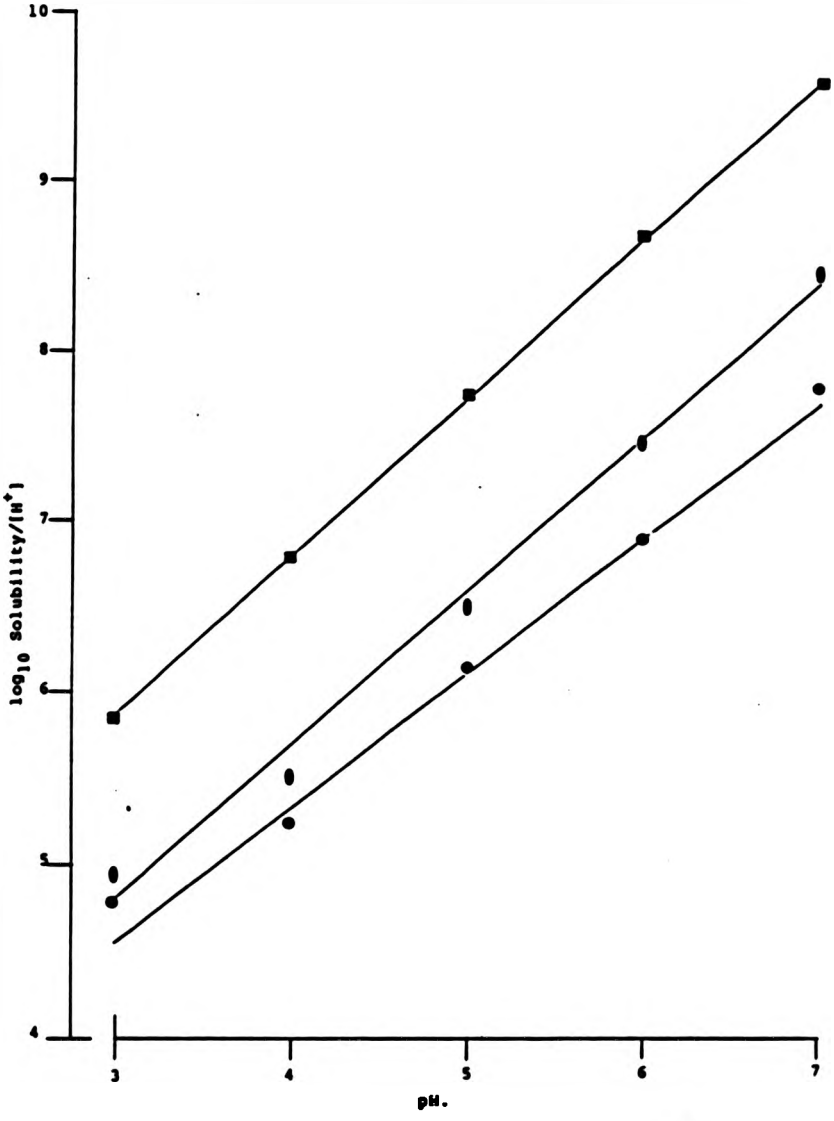


Figure 3.3.2. The relationship between the $[H^+]$ and the solubility of $Ca(OH)_{2(s)}$ ■, $CaO/SiO_{2(s)}$ ▮ and $CaCO_{3(s)}$ ●. Mean values are plotted, n = 9.

As the pH becomes less acid the solubility actually increases with respect to the available $[H^+]$.

pH7.0 (Tables 3.3.2,3.3.3). $CaCO_{3(s)}$ solubility was significantly higher, (t-test: $P < 0.05$), in OR than DD and AR at pH4.0 and pH5.0 (Table 3.3.1). $CaO/SiO_{2(s)}$ solubility was significantly reduced, (t-test: $P < 0.05$) in AR as compared to both DD and OR at pH3.0 (Table 3.3.2). The solvent effects on compound solubility are explained in terms of solute/solvent interactions.

Solubility in DD was a close reflection of true solubility as all solutes were derived from the dissolving amelioration agent. For AR and OR the additional solvent-associated solutes could give rise to anomalous results. For example, the solubility of $CaO/SiO_{2(s)}$ in DD was determined by the dissolution of the component species of $CaO/SiO_{2(s)}$ in equilibrium with an insoluble phase. $CaO/SiO_{2(s)}$ solubility in AR was, however, a product of several equilibria since dissolution products were free to react with solvent-associated solutes, i.e. aluminium. If the products of these interactions were less soluble than the amelioration compound, precipitation would occur. The methodology used in this study to measure solubility may not discriminate between the potential insoluble fractions and this would explain the reduced solubility of $CaO/SiO_{2(s)}$ in AR at pH3.0 with respect to both DD and OR (Table 3.3.2). Under certain conditions, (see below), OR would be expected to increase solubility above predicted levels. Increased solubility in OR was found for calcium carbonate at pH4.0 and pH5.0. Humic substances will bind and adsorb dissolving species,

for example, $Ca^{2+}_{(aq)}$, thereby removing them from their respective equilibria and promoting the further dissolution of the insoluble phase to restore equilibrium. The organic removal of cations will exhibit saturation kinetics, (Pott et al 1985), and will increase solubility when conditions are favourable for binding/adsorptive processes.

The nature of the solvent could be important with respect to amelioration compound solubility and should be considered in a dosing model.

(ii) Effect of Solution pH

The solubility of each compound was a function of the solution pH and was potentiated at acid pH, (Tables 3.3.1, 3.3.2, 3.3.3). $Ca(OH)_{2(s)}$ solubility was linearly related to pH in the range 3.0-7.0 (Figure 3.3.1a), whereas $CaCO_{3(s)}$ and $CaO/SiO_{2(s)}$ solubility was exponentially related, the solubility fell more rapidly at lower pH, (Figures 3.3.1b and 3.3.1c.) The solubility of each compound, with respect to $[H^+]$ increased at more alkaline pH (Figure 3.3.2). The solubilising efficacy of $[H^+]$ or ability of $[H^+]$ to enhance dissolution on each compound was linearly related to pH, (r: $P < 0.01$), and not significantly different (b: $P > 0.05$), between compounds. Solubility was exponentially related to $[H^+]$ particularly with respect to $Ca(OH)_{2(s)}$.

The relative solubilities of $Ca(OH)_{2(s)}$, $CaCO_{3(s)}$ and

$CaO/SiO_{2(s)}$ were pH dependent, (Table 3.3,3). The higher solubility of $Ca(OH)_{2(s)}$ was exacerbated at more alkaline pH relative to $CaCO_{3(s)}$, but remained unchanged relative to $CaO/Sio_{2(s)}$. The relative solubilities of $Ca(OH)_{2(s)}$ and $CaO/SiO_{2(s)}$ were not significantly different, (t-test: $P \geq 0.05$) in the pH range of 3.0 to 7.0.

The saturation solubility of each compound was reduced at more alkaline pH, however, the reduction was not attributable to $[H^+]$ per se. The rate of change of solubility with pH was similar for $Ca(OH)_{2(s)}$ and $CaO/SiO_{2(s)}$ and much reduced for $CaCO_{3(s)}$. (Figure 3.3.2). Thus the efficacy of $CaCO_{3(s)}$ as an amelioration agent, (or more specifically, a supplier of alkalinity) was reduced more rapidly at increasing pH than either $Ca(OH)_{2(s)}$ or $CaO/SiO_{2(s)}$. The influence of $[H^+]$ on compound solubility was critical and should be an important criterion in the development of a dosing strategy.

(iii) Effect of Solution Temperature

The solubility of each amelioration compound was increased at a solution temperature of 5°C (Tables 3.3.2, 3.3.3). The increase for $Ca(OH)_{2(s)}$, (ca 100%) was independent of solvent type however $CaCO_{3(s)}$ solubility was significantly higher (t-test: $P < 0.05$) in OR and AR (both ca 200%) than in DD (ca 100%), and solubility increases for $CaO/SiO_{2(s)}$ in DD, OR, and AR were ca 100%, ca 200% and ca 360% respectively.

The true solubility, (i.e. DD only), for each compound at pH3.0 and 5°C was increased by ca 100% and was probably a reflection of the exothermic nature of the dissolution process.

Additional increases in solubility of $CaCO_{3(s)}$ and $CaO/SiO_{2(s)}$ in OR and AR were probably due to temperature effects on each of the competing equilibria and hence solubility controls. The relative solubilities of the three compounds were unchanged at 5°C with respect to 25°C and this suggested that temperature effects on solubility (particularly in DD), were not compound specific. The solution temperature was an important factor in determining the solubility of each compound and should be considered when evaluating dosing agents and strategies. Ignorance of temperature effects could result in problems of under- or over-dosing.

3.3.2 Neutralisation Efficacy

3.3.2.1 Materials and Methods

The efficiency of neutralisation of each compound in each solvent type was studied at a solution temperature of 25°C and initial solution pH of 3.0. Efficiency was measured in terms of the dry weight of compound required to effect a known pH change over a known time interval and the rate of change of pH induced by a known weight of amelioration compound.

(i) Weight-Induced pH Change

Litre volumes of each solvent, i.e. DD, OR, and AR, were adjusted to pH3.0 using $HNO_{3(aq)}$ (Aristar:BDH Ltd.), and stirred gently using magnetic stirrers. The solution pH was raised to pH7.5 during a 1 hour period through the periodic additions of dry amelioration agent. The minimum addition of agent required to effect the pH change 3.0 to 7.5, in a maximum period of 1 hour, was recorded.

(ii) Rate of Change of pH

A known weight of amelioration agent, standardised to account for solubility differences between the compounds at pH3.0 and 25°C, and sufficient to give a practical reaction rate, ($Ca(OH)_{2(s)}$ ca 28mg, $CaCO_{3(s)}$ ca 363mg, $CaO/SiO_{2(s)}$ ca 303mg), was added to a litre volume of slowly-stirring double-distilled water, previously adjusted to pH3.0 with $HNO_{3(aq)}$ (Aristar:BDH Ltd.). Standardisation was achieved by using compound weights in the proportions outlined in the solubility ratios of the three compounds at pH3.0 and 25°C (Table 3.3.3). The pH was monitored continuously and the time required for the known weight of amelioration agent to increase the pH in 0.5 unit increments was recorded.

3.3.2.2 Results and Discussion

(i) Weight-Induced pH Change

The solvent type had a significant (t-test: $P < 0.05$) influence on the weight of compound added to effect the required pH change (Table 3.3.4). Generally:

$$OR \gtrdot \gtrdot AR \gtrdot DD$$

where $\gtrdot$ implies: required a greater weight of compound per unit change in pH than. An exception was $CaO/SiO_{2(s)}$ addition to DD and AR, where the weights added were not significantly, (t-test: $P \gtrsim 0.05$), different from one another. The differences evident in all other treatments were a reflection of the relative buffering capacities of the solvents. Aluminium and aquatic organics are buffers in natural acidified waters, (Exley and Phillips 1988), and whilst mole per mole aluminium is the stronger buffer, the estimated greater concentration of humic substances in OR relative to available aluminium in AR would confer a higher buffering capacity on OR. This greater buffering potential was reflected in the high quantities of each agent used to bring about the neutralisation reaction in OR. The insignificant, (t-test: $P \gtrsim 0.05$), effect of AR relative to DD, for $CaO/SiO_{2(s)}$ addition was indicative of the abolition of the buffering capacity of AR, perhaps due to complex solute interactions.

Table 3.3.4 The dry weight (mg) of each amelioration compound required, per litre of solvent, to effect the pH change from 3.0 to 7.5, in three distinct solvent types. $\bar{x}$ - mean, SD - standard deviation, n = 3.

Solvent Type \ Compound	$Ca(OH)_{2(s)}$		$CaCO_{3(s)}$		$CaO/SiO_{2(s)}$	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Double Distilled	9.7	0.5	8.7	0.5	5.7	0.9
Aluminium Rich	31.3	1.7	106.7	9.4	6.0	0.8
Organic Rich	129.3	1.9	583.3	4.7	323.3	9.4

The weights of both $CaCo_{3(s)}$ and $CaO/SiO_{2(s)}$ required to effect each neutralisation within 1 hour were representative of heavily saturated solutions with respect to AR, ($CaCO_{3(s)}$ only) and OR (Table 3.3.1). This finding was probably a reflection of their reduced rates of neutralisation with respect to $Ca(OH)_2$ and/or differences in the short-term chemistry (ca 1 hour) of their solute interactions as compared to the longer term chemistry (24 hours) associated with the saturation solubilities. Filtration of solutions from this study at equilibration of the pH at 7.5 would have revealed practical information in this respect.

In summary, the nature of the solvent was important in defining the amount of amelioration agent required for neutralisation within a specific time period.

(ii) Rate of Change of pH

$Ca(OH)_{2(s)}$ effected the most rapid pH change, (3.0 to 7.0), ca 4 times the rate of $CaCO_{3(s)}$ and ca 3 times the rate of $CaO/SiO_{2(s)}$ (Table 3.3.5, Figures 3.3.3a-c, 3.3.4). The times required by each agent to effect the pH change of 3.0 to 7.0 ($\bar{x}$ = 97.3, 326.3 and 249.7 seconds for $Ca(OH)_{2(s)}$, $CaCO_{3(s)}$ and $CaO/SiO_{2(s)}$ respectively (Table 3.3.5)), were significantly, (t-test: $P \not< 0.05$), different from one another.

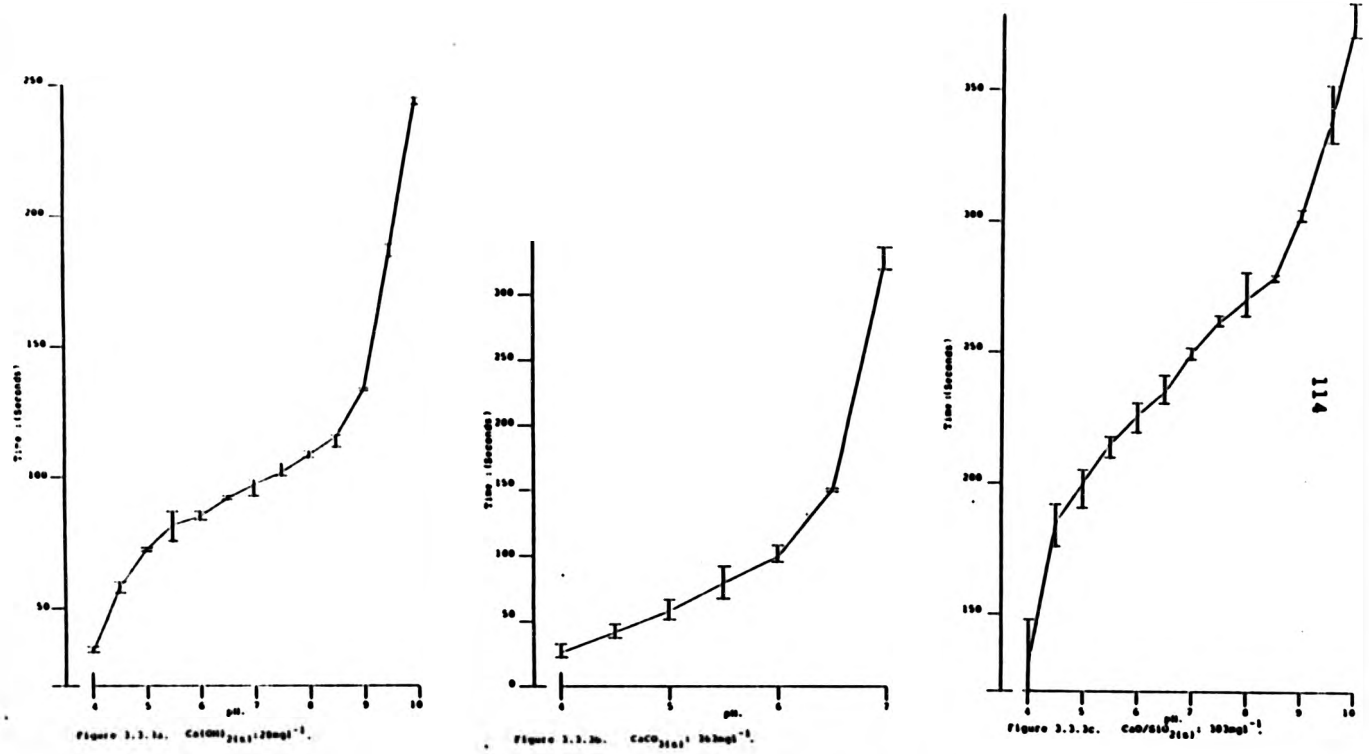
For each compound, the rate at which neutralisation was effected, $\frac{d[H^+]}{dt}$, (Figure 3.3.4), was reduced at increasing pH. This reduction in rate of neutralisation at more alkaline pH was greater for $CaCO_{3(s)}$ than $Ca(OH)_{2(s)}$ and $CaO/SiO_{2(s)}$.

$Ca(OH)_{2(s)}$ showed the fastest rate of neutralisation (steepest line gradient) and the lowest rate of reduction of this neutralisation efficacy at increasing pH levels. $CaO/SiO_{2(s)}$ had a similarly low reduction of neutralisation rate, however, the rate of neutralisation was significantly, (t-test: $P < 0.05$), lower than $Ca(OH)_{2(s)}$.

$CaCO_{3(s)}$ showed the lowest neutralisation efficacy in respect of both the rate of neutralisation and the reduction of this rate at increasing pH.

Table 3.3.5.The time to effect a change of the pH, in seconds, on addition of an excess of each amelioration compound to acidified double-distilled water at 25°C. $\bar{x}$-mean, SD-standard deviation, n = 3.

pH \ Compound	$Ca(OH)_{2(s)}$		$CaCO_{3(s)}$		$CaO/SiO_{2(s)}$	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
3.0	T=0	n.a.	T=0	n.a.	T=0	n.a.
4.0	34.3	0.9	26.7	4.1	131.3	12.0
4.5	58.0	1.6	41.7	4.5	184.7	6.6
5.0	72.3	0.5	56.7	6.0	199.7	5.6
5.5	81.7	4.5	78.7	10.0	215.0	3.6
6.0	85.3	1.2	99.7	5.9	226.0	4.5
6.5	92.3	0.5	152.3	0.9	234.7	4.5
7.0	97.3	1.7	326.3	6.2	249.7	1.7
7.5	102.3	1.9			262.3	1.9
8.0	109.3	0.9			270.7	7.3
8.5	114.7	1.9			279.0	0.8
9.0	133.0	0.0			302.7	1.7
9.5	186.7	1.7			338.7	9.5
10.0	243.7	0.9			375.3	6.9



Figures 3.3.3a-c. The time taken for a known weight of each amelioration compound to effect a given change in pH, in the pH range of 3.0 to 10.0. Mean values are plotted, bars indicate the range, n = 3.

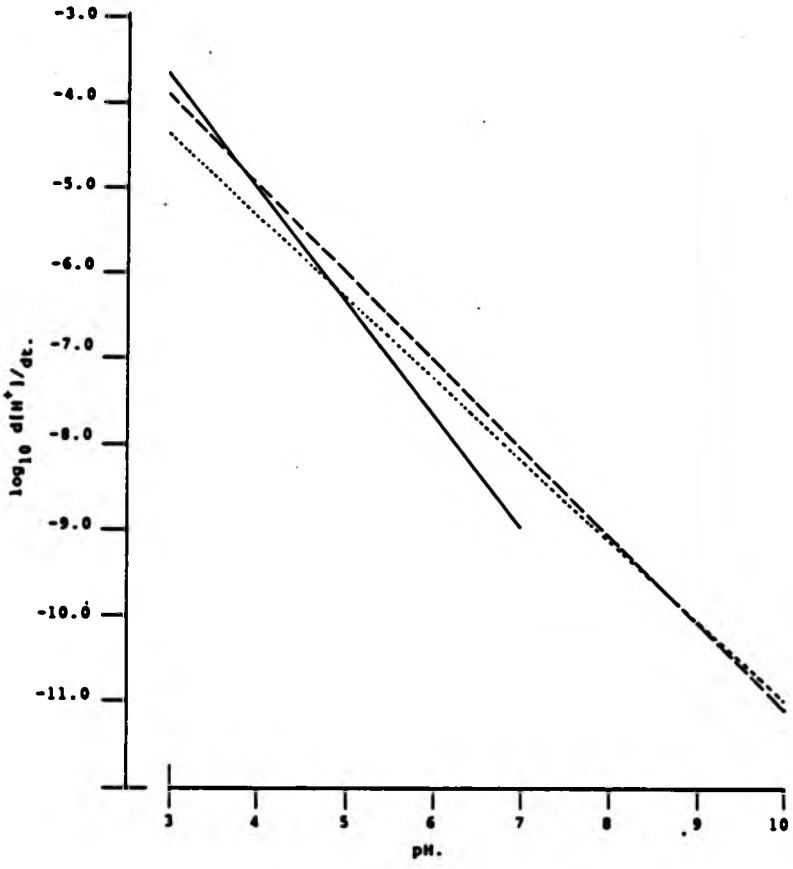


Figure 3.3.4. The rate of change of pH, expressed as the buffering of $H^+_{(aq)}$ per unit of time, induced by $Ca(OH)_2$, ----, (Y= -1.04x -0.70, r = -0.988), $CaCO_{3(s)}$, ——, (Y= 1.32x +0.32, r = -0.991), and $CaCO/SiO_{2(s)}$, ····· , (Y= -0.95x -1.53, r = -0.991), as a function of increasing pH, n = 7.

The rate at which acid water can be neutralised and the capacity of an amelioration agent to sustain the neutralisation are both integral components of successful acid water amelioration and will be important considerations in developing mitigative procedures.

3.3.3 Aluminium Insolubilisation

3.3.3.1 Materials and Methods

The effect of a saturated solution of each amelioration agent, ($Ca(OH)_{2(s)}$ ca $750mgl^{-1}$, $CaCO_{3(s)}$ ca $35mgl^{-1}$, $CaO/SiO_{2(s)}$, ca $45mgl^{-1}$), on aluminium solubility in the pH range 3.0 to 9.0 was tested in the following manner. A 1 litre volume of saturated solution was prepared in a $500ugl^{-1}$ $Al(NO_3)_3.9H_2O$ (Aristar:BDH Ltd.) solvent at 25°C. The solution pH was adjusted to the required level, (3.0 to 9.0 in increments of 0.5pH units), using $NaOH_{(aq)}$ and $HNO_{3(aq)}$ (Aristar:BDH Ltd.), and a $50cm^3$ sample was removed on stabilisation of the pH for a minimum of 5 minutes for total and soluble aluminium analysis. Details of fractionation and aluminium analysis are given in Chapter Two.

3.3.3.2 Results and Discussion

The presence of both $Ca(OH)_{2(s)}$ and $CaCO_{3(s)}$ resulted in characteristic V-shaped aluminium solubility curves with

soluble fraction minima in the pH range of 6.0-6.5 and solubility rising steeply on both acid and alkaline sides of these minima, (Table 3.3.6,Figure 3.3.5). $CaO/SiO_{2(s)}$ reduced aluminium solubility at all pH levels $\geq$4.0, with respect to both $Ca(OH)_{2(s)}$ and $CaCO_{3(s)}$, and this effect was exacerbated in the pH range 6.5 to 9.0.

The curves for $Ca(OH)_{2(s)}$ and $CaCO_{3(s)}$ were typical of aluminium solubility as predicted by its hydroxides, gibbsite and amorphous $Al(OH)_3$. The soluble aluminium fraction on the acid side of minimum solubility, ca pH$<$6.0, will be characterised by cationic, monomeric, aluminium and aluminium-hydroxy species whereas at pH$\geq$ 6.0, the anionic monomer, aluminate, $Al(OH)^-_{4(aq)}$, will predominate. The reduced aluminium solubility on $CaO/SiO_{2(s)}$ addition was indicative of a change in aluminium solubility control from the hydroxide phase to an aluminosilicate phase. This change in solubility phase could be the result of silicic acid (from $CaO/SiO_{2(s)}$ dissolution) interactions with hydroxy-aluminium species (Farmer 1986, Lou and Huang 1988), and in particular the aluminate anion (Chappell and Birchall 1988) at alkaline pH.

Neither $Ca(OH)_{2(s)}$ or $CaCO_{3(s)}$ reduced aluminium solubility with respect to pH-predicted levels. $CaO/SiO_{2(s)}$ effected a rapid insolubilisation of aluminium at pH$\geq$ 4.0 and presents the first evidence of an amelioration agent specifically reducing the biological availability (i.e. the soluble fraction) of aluminium in an acidified water.

Table 3.3.6.Aluminium solubility with respect to the presence of each amelioration compound over the environmentally significant pH range of 3.0 to 9.0. $\bar{x}$-mean, n = 9. Figures show % Al total. Arcsine transformation.

Compound / pH	$Ca(OH)_{2(s)}$		$CaCO_{3(s)}$		$CaO/SiO_{2(s)}$	
	$\bar{x}$	Range	$\bar{x}$	Range	$\bar{x}$	Range
3.0	91.7	91-92	91.7	89-93	91.7	89-95
3.5	83.7	83-85	80.0	80-81	91-3	88-93
4.0	74.0	71-77	63.0	62-65	59.3	59-60
4.5	61.3	60-62	52.0	51-53	41.0	40-42
5.0	51.7	51-52	41.0	39-43	25.7	25-26
5.5	26.0	25-27	34.3	33-35	13.3	11-15
6.0	6.3	5-9	8.7	6-10	9.7	7-10
6.5	21.7	21-23	25.7	25-26	3.7	3-4
7.0	30.3	29-31	34.7	33-36	3.0	1-5
7.5	36.7	35-38	57.0	53-60	1.7	2-3
8.0	44.3	43-45	79.3	79-80	1.7	1-3
8.5	60.7	61-62			1.3	2-3
9.0	89.7	88-91			3.0	1-5

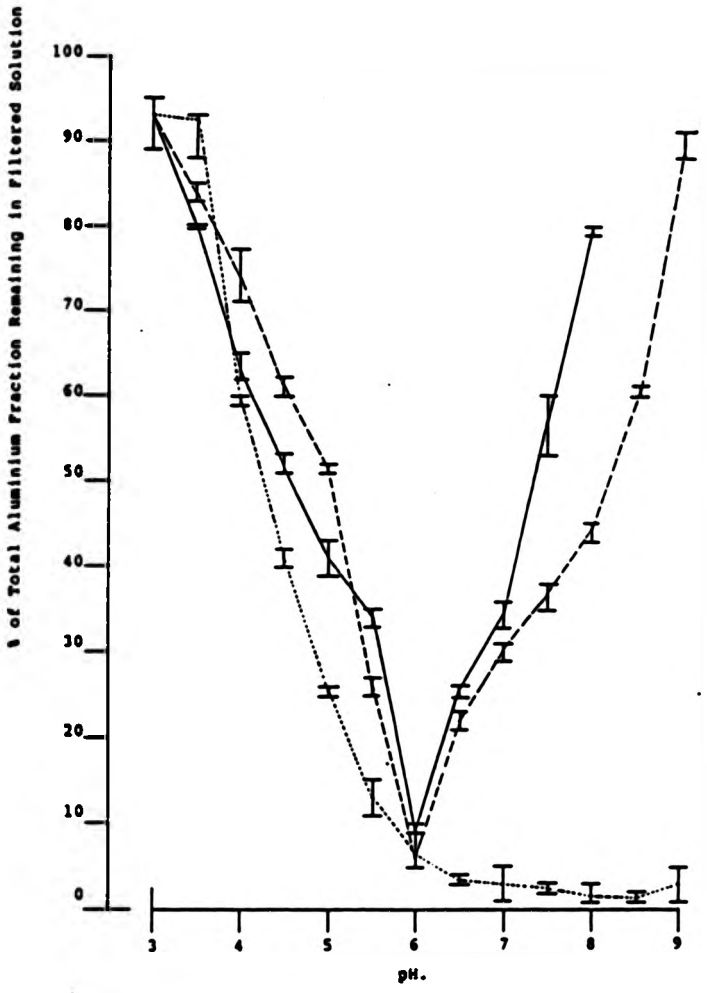


Figure 3.3.5. The aluminium solubility curves in the presence of an excess of, $Ca(OH)_{2(s)}$, ----, $CaCO_{3(s)}$, ——, and $CaO/SiO_{2(s)}$, ------ , at pH of 3.0 to 9.0. Mean values are plotted, bars indicate the range, n = 9.

3.4 General Discussion

All three compounds were able to provide effective acid buffering and the high neutralisation efficiencies of both $Ca(OH)_{2(s)}$ and $CaO/SiO_{2(s)}$ increased their potential for pH overcompensation whereby aluminium solubilisation would be enhanced at alkaline pH. In contrast the ability of $CaCO_{3(s)}$ to provide alkalinity was rapidly lost in less acid environments reducing the possibility of severe pH overcompensation. This advantage of $CaCO_{3(s)}$ has to be weighed against its much reduced rate of acid buffering which may undercompensate the pH in the short-term.

Each of the compounds will provide additional calcium, considered important in aluminium toxicity amelioration, (Brown 1983), and provide solid phase calcium buffers which may replenish the alkalinity of the water in the event of reacidification.

The use of calcium salts in acid water amelioration may actually limit the potential carbonate alkalinity because of the low solubility of calcium carbonate, (Davison and House 1988). Neutralisation using the corresponding sodium salts, ($NaOH_{(s)}$, $Na_2CO_{3(s)}$), was shown to provide twice the available alkalinity of the calcium salts. The efficacy of solid phase calcium buffers in providing additional calcium and alkalinity may also be severely restricted by the inactivation of

the solid phase surface. This is particularly true in organic rich waters where adsorption of organics onto the solid surface will rapidly restrict the available buffering capacity to the alkalinity in solution, (Sverdrup 1983, 1984).

In the present study, only $CaO/SiO_{2(s)}$ induced the rapid insolubilisation of aluminium, with respect to hydroxide solubility control, and the resultant insolubles were stable over a wide pH range. $Ca(OH)_{2(s)}$ and $CaCO_{3(s)}$ had no effect on aluminium solubility control with respect to hydroxide.

Amelioration strategies for acidified waters are very numerous, (Exley and Phillips 1988), however, none have targeted aluminium in their mitigative approach. This study has shown that the rapid insolubilisation of aluminium over a wide pH range can be achieved through the use of a silicic acid-releasing compound. Previous studies using seawater and shellsand filters as neutralisation agents, (Rosseland and Skogheim 1986, Leivestad et al 1987), have inadvertantly recorded ameliorative effects on aluminium toxicity to salmonids beyond those expected from simple base additions. The amelioration was not explained in terms of reductions in the labile aluminium fractions and was attributed to "increased ionic strength" effects. Both seawater and shellsand would be expected to release silicic acid, $Si(OH)_4$, and it would not therefore be

unreasonable to suggest that the silicic acid was the precursor of the observed amelioration of aluminium toxicity.

3.5 Summary

The development of an amelioration strategy for aluminium toxicity in acidified waters requires careful consideration of both the solute, (amelioration agent), and the solvent, (the acid water). Solute/solvent interactions will define the success of an amelioration strategy. The pH, the water temperature and the chemical nature of the acid water will influence these interactions and should be considered.

Aluminium insolubilisation over a wide pH range was achieved using a silicic acid-releasing amelioration compound, ($CaO/SiO_{2(s)}$). If the aluminosilicates formed during the insolubilisation of aluminium are non-toxic, a silica based amelioration compound could provide some of the answers to problems associated with base additions alone, problems highlighted in the study at Kenmure rainbow trout farm.

CHAPTER FOUR: THE DETERMINATION OF SILICON

4.1 Review of Analytical Methodology

Silicon is found as monomeric, dimeric and polymeric species and insoluble and colloidal sub-micron, particulates in natural waters, (Iler 1979). As silica, $SiO_{2(s)}$, it is often the principal solute of natural waters and occurs predominantly as silicic acid, ($Si(OH)_4$), (Farmer 1986). As such, the analytical determination of silicon usually infers the soluble fraction only and the potential influence of particulate silicon, (e.g. clay particles), is removed by filtration prior to analysis.

4.1.1 Spectrophotometric Determination of Silicon

Two common techniques for measuring silicon are based on the absorbance of a molybdenum blue complex. Ammonium molybdate will react with soluble silicon species, (monomeric and dimeric silicic acids and silicates), under controlled acid conditions to give yellow molybdosilicic acid. Reduction by either, ascorbic acid or ANSA will give the molybdenum blue complex.

(i) Ascorbic Acid Reduction Method

The addition of ascorbic acid to molybdosilicic acid yields

a silicomolybdenum blue complex the absorbance of which can be determined spectrophotometrically.
Range, (without dilution), 0-10mgl^{-1}. Detection limit, 30µgl^{-1}.

(ii) ANSA Reagent Reduction Method

The addition of 1-amino-2-naphthol-4-sulphonic acid, (ANSA), yields a heteropoly molybdenum blue complex.
Range, (without dilution), 0-500µgl^{-1}. Detection limit, 2-4µgl^{-1}.

Total silicon can be measured by both methods following extensive pre-treatment of the sample to convert all forms of silicon into molybdate reactive silicon, (HMSO 1980).

Both methods are time intensive although an automated system can be used for analysis of large numbers of samples.

4.1.2 Atomic Absorption Spectroscopy, (AAS)

Very little literature on this methodology is available, though graphite furnace AAS is extensively used to measure low levels of silicon, (Perkin-Elmer 1984). Discussion concerning the type of purge gas, (Ar or N_2), and graphite tube, (coated or uncoated), is inconclusive, (Manning and Fernandez 1970, Thompson et al 1975, and Cedergren 1980). Potential interferences include chloride, sulphate,

dichromate, selenium and lanthanum, (Lo and Christian 1977, Frech and Cedergren 1980). Recommended charring and atomisation temperatures are ∠1350°C and 2600-2650°C respectively, (Perkin-Elmer 1984).

Graphite furnace atomic absorption spectroscopy was the chosen analytical method for total silicon determination in this research.

4.2. The Determination of Silicon by Graphite Furnace Atomic Absorption Spectroscopy

4.2.1 Introduction

The principles of g.f.a.a.s. were described earlier (Chapter Two), and are equally relevant to the analysis of silicon. The method provided a means of rapidly analysing a large number of samples with only limited sample pre-treatment. Instrumental and operational criteria were taken from the literature and combined with knowledge ascertained during the development of the aluminium program to provide a working program for the analysis of total silicon.

4.2.2 The Silicon Program

The program used for the analysis of total silicon is shown in Table 4.3. It was necessary to optimise both the

pre-treatment and atomisation temperatures and optimum temperatures were 1150°C and 2650°C respectively (Table 4.1, Figure 4.1 and Table 4.2, Figure 4.2). The program provided adequate observed precision, $\pm$ 5.0ugl^{-1} in a working, linear range of 0-500ugl^{-1}. Samples were diluted to measure concentrations greater than 500ugl^{-1}.

The final program was used to determine total silicon in both water samples, (matrix of 1% (V/V)HNO_3 (Aristar:BDH Ltd.)) and acid digests, (matrix of 10% (V/V)HNO_3 (Aristar:BDH Ltd.)).

4.2.3 Comparison with Colourimetric Method

The validity of the Si program and g.f.a.a.s. methodology was tested through comparison with the molybdate reactive methodology (Table 4.4). Both standard solutions (Spectrosol: BDH Ltd.) and field samples were compared. In both cases standards/samples were passed through 0.45µm membrane filters, (Ultipor N66: Pall) for particulate removal, before analysis by the respective methodology.

The two methods were comparable and did not give significantly different, (t-test: $P \geq 0.05$) mean values for either standards or field samples (Table 4.4).

Table 4.1 Optimisation of the Char (pre-treatment) Temperature. AT = 2650°C, SV = 30ul, PG = 300 mlArmin^{-1}. n = 9. SD = standard deviation.

Char Temp. °C	Silicon Signal mm	
	Mean	SD
1000	162	2.5
1050	167	1.2
1100	172	0.5
1150	178	0.0
1200	172	0.9
1250	141	3.1

Table Footnote: Silicon standard was 100ugl^{-1} (Spectrosol:BDH Ltd.)

Table 4.2 Optimisation of the Atomisation Temperature, °C. PT = 1150°C, SV = 30ul, PG = 300 mlArmin^{-1}. n = 9. SD = standard deviation.

Atomisation Temperature °C	Silicon Signal mm	
	Mean	SD
2500	156	2.2
2550	168	1.9
2600	172	0.9
2650	172	0.0
2700	160	2.5
2750	154	2.2
2800	145	1.2

Table Footnote: Silicon standard was 100 ugl^{-1} (Spectrosol:BDH:Ltd.)

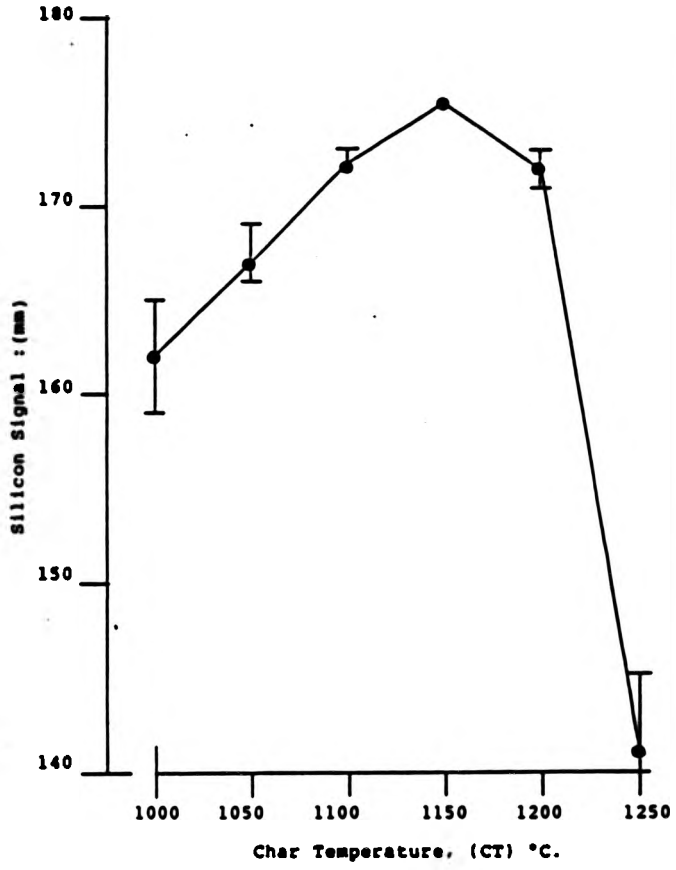


Figure 4.1. The optimisation of the char temperature, CT, at the optimum atomisation temperature, 2650°C. Mean values are plotted, bars indicate the range, n = 9. The optimum temperature, (1150°C), gave both a high absorbance signal and good replication. The rapid fall after 1200°C was indicative of substantial sample loss during charring.

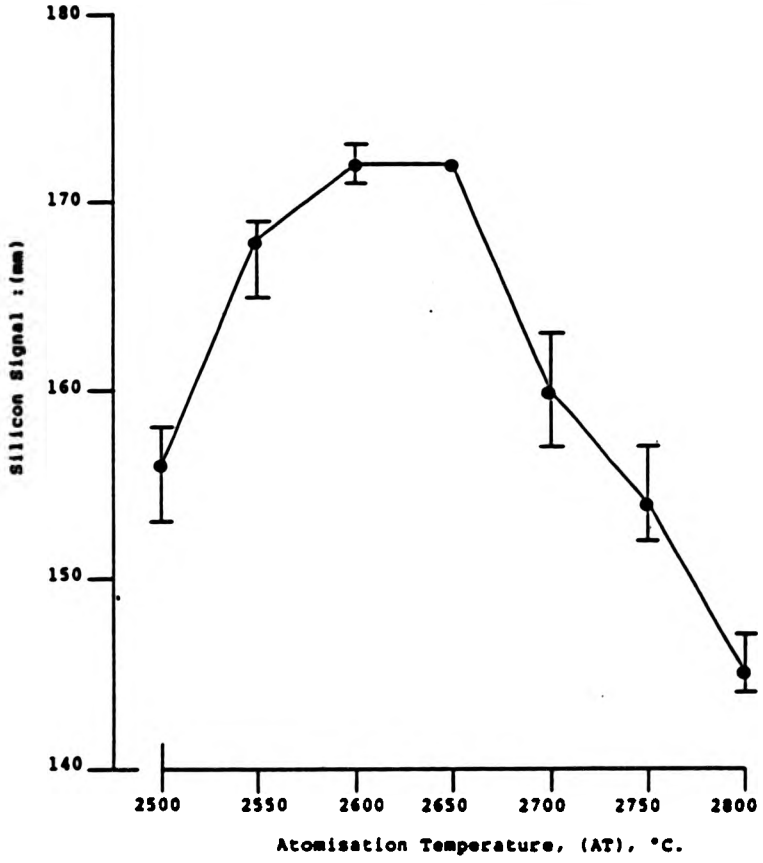


Figure 4.2. The optimisation of the atomisation temperature, (AT), at the optimum char temperature, 1150°C. Mean values are plotted, bars indicate the range, n = 9.
The optimum temperature was 2650°C, which gave both the mean maximum absorbance signal and good replication.

Table 4.3 Analytical Program for the GFAAS Determination of Silicon.

Element: Silicon Si Matrix 1% (V/V)HNO_3(Aristar: BDH Ltd.)

Instrumental Parameters

Model: 2280 AAS (P-E)

Wavelength, nm: ca 252

Bandwidth nm: 0.2A

Light Source: Cathode

Current, mA: 40

Background Correction: Yes

Readout Parameters

Range: 5-500ugl^{-1}

Peak Height:Time (S).3

Recorder:5mV-range

Speed - 20mm/min^{-1}

HGA Parameters

Model HGA 400 (P-E)

Tube: Uncoated

Sample Vol.(ul):30

Purge Flow: 300mlAr min.$^{-1}$

Replicates: 3 (Min).

Sampler: AS40 (P-E)

Standard Soln;

Si (Spectrosol)

Keyboard Entries

Step	1	2	3	4	5	6	7
Temp.°C	80	130	500	1150	1150	2650	2700
Ramp(S)	5	10	10	10	1	0	1
Hold(S)	5	30	10	15	5	5	3
Read						✓	
Record						✓	
Baseline					✓		
Stop Flow						✓	

Table 4.4 A Comparison of the Colourimetric and GFAAS Methods of Measuring Silicon in both Standard Solutions and Field Samples, (n=3).

Sample	Colourimetric μgl^{-1}		GFAAS μgl^{-1}	
Units μgl^{-1}	Mean	SD	Mean	SD
200ugl^{-1} standard	194	2.2	199	0.5
500ugl^{-1} standard	490	4.5	497	0.5
1000ugl^{-1} standard	990	0.9	1001	2.9
Field 1	1467	12.8	1497	5.4
Field 2	1385	11.1	1407	5.0
Field 3	1359	11.0	1360	7.8

4.3 Summary

An analytical procedure was developed for measuring total silicon in both synthetic and natural waters. The use of g.f.a.a.s. allowed a large number of samples to be determined within a reasonable time period, (ca 100 samples per day). The program was also successfully used to determine total silicon in acid digests, (10% (V/V)HNO_3 (Aristar:BDH Ltd.)).

CHAPTER FIVE: THE EFFECTS OF pH, TOTAL SILICON CONCENTRATION AND EQUILIBRATION PERIOD ON SIMPLE ALUMINIUM SPECIATION

5.1 Introduction

In pure water, aluminium exists as complexes of aluminium, water and hydroxide groups. The relative proportions of aluminium products and species are governed by the absolute aluminium concentration, the age of the solution and the pH of the solution. These controlling factors are not discrete and interact synergistically to describe simple aluminium speciation and solubility at any one time.

In saturated solutions, in the absence of all other complexing ligands, aluminium solubility is controlled by aluminium hydroxides, $(Al(OH)_3)_{(s)}$. The stable crystalline phase of $Al(OH)_{3(s)}$ is the mineral gibbsite. The solubility of $Al(OH)_{3(s)}$ with respect to solid gibbsite is described by the equation:

$$Al(OH)_3(\text{gibbsite}) + 3H^+ \rightleftharpoons Al^{3+} + 3H_2O \qquad (1)$$

$$K_{sol.} = \left[Al^{3+}\right] / (H^+)^3 = 10^{9.2} \quad \text{(Martin 1986)}$$

Equilibrium with respect to gibbsite is only slowly achieved and rarely occurs within biological systems. To account for this slow rate of equilibration with respect to the crystalline phase of $Al(OH)_{3(s)}$ the control of aluminium solubility can be described in terms of non-crystalline or amorphous $Al(OH)_{3(s)}$.

$$Al(OH)_3(\text{amorphous}) + 3H^+ \rightleftharpoons Al^{3+} + 3H_2O \qquad (2)$$

$$K_{sol.} = \left[Al^{3+}\right] / (H^+)^3 = 10^{10.7} \quad \text{(Martin 1986)}$$

Amorphous $Al(OH)_3$ can be up to 100-fold more soluble than gibbsite, (Figure 5.1).

The soluble phase of aluminium in pure water, either in equilibrium with the solid hydroxide phase or in unsaturated solution is pH and age dependent. Aluminium is present as monomeric, dimeric and polymeric species. In dilute solutions the monomeric species predominate and their form is pH dependent, (Figures 5.2a, 5.2b). In acid solution at pH$\lesssim$5.0, the hexa-aqua trivalent aluminium species, $Al(H_2O)_6^{3+}{}_{(aq)}$ is the most numerous and as the pH increases above pH 5.0, the hydrolysis products of Al^{3+} begin to predominate.

The relative proportions of each species at any one pH are governed by the proton affinity of each species with respect to water. The successive deprotonations of aluminium bound water molecules are described by the following equations and equilibrium constants (Martin 1986):

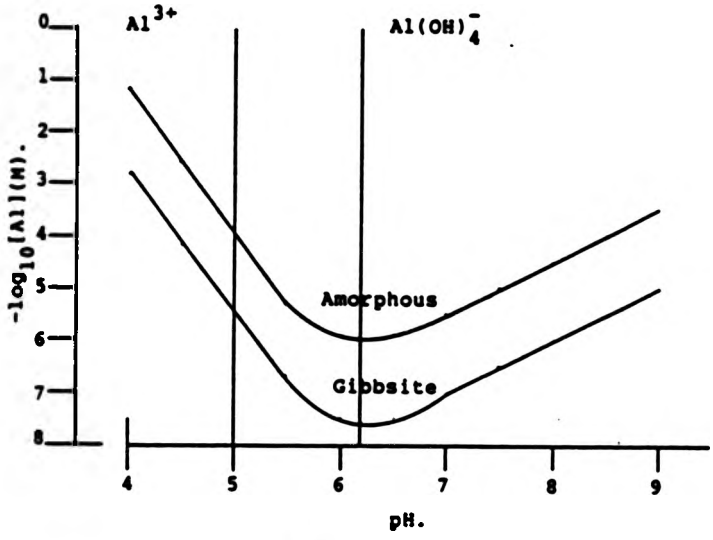


Figure 5.1. Negative logarithm of the total molar concentration of aluminium allowed by amorphous and crystalline $Al(OH)_{3(s)}$ against pH. $Al^{3+}_{(aq)}$ is the dominant ion below pH5.0 and $Al(OH)^-_{4(aq)}$ predominates above pH6.2, the minimum solubility point for both curves (Martin 1986).

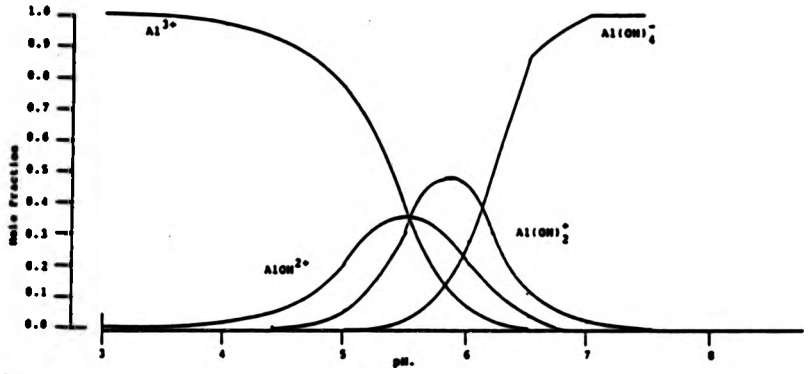


Figure 5.2a. Distribution of soluble monomeric aluminium ion species in aqueous solutions, (I=0.16M), (Martin, 1986).

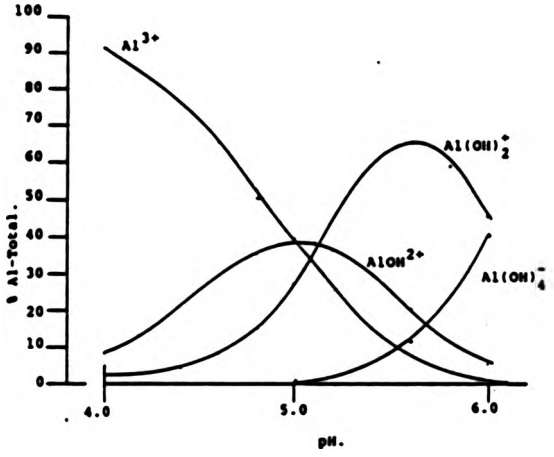


Figure 5.2b. Distribution of soluble monomeric aluminium ion species in aqueous solutions, (I=OM), (Johnson et al 1981).

$$Al^{3+} + H_2O \rightleftharpoons AlOH^{2+} + H^+$$

$$K = (H^+) \cdot \left[AlOH^{2+}\right] \Big/ \left[Al^{3+}\right] = 10^{-5.5} \quad (3)$$

and

$$AlOH^{2+} + H_2O \rightleftharpoons Al(OH)_2^+ + H^+$$

$$K = (H^+) \cdot \left[Al(OH)_2^+\right] \Big/ \left[AlOH^{2+}\right] = 10^{-5.6} \quad (4)$$

and

$$Al(OH)_2^+ + 2H_2O \rightleftharpoons Al(OH)_4^- + 2H^+$$

$$K = (H^+)^2 \cdot \left[Al(OH)_4^-\right] \Big/ \left[Al(OH)_2^+\right] = 10^{-12.1} \quad (5)$$

Between the monovalent cationic and monovalent anionic species an uncharged $Al(OH)^\circ_{3(aq)}$ species is postulated.

$$Al^{3+} + 3H_2O \rightleftharpoons Al(OH)^\circ_{3(aq)} + 3H^+$$

$$K_H = (H^+)^3 \cdot \left[Al(OH)^\circ_{3(aq)}\right] \Big/ \left[Al^{3+}\right] = 10^{-15.00} \quad (6)$$

(Smith and Hem 1972)

However, evidence suggests that only negligible concentrations of this uncharged soluble species will be present even at minimum solubility. High concentrations of hydroxy aluminium species (1M) and/or significant ageing of aluminium stimulates polymerisation, (Hem 1968a). The simplest polymer is the dimeric, $Al_2(OH)_2(H_2O)_8^{4+}$, cation (Johansson 1963), the polymerisation resulting via the

formation of a double OH bridge between adjacent aluminium ions, (Thomas and Tai 1932, Hsu and Bates 1964). The tendency of aluminium species to polymerise is enhanced as the ratio of aluminium bound hydroxide to aluminium increases. For example, for the monomeric species; $Al(OH)^{-}_{4(aq)} \rightleftharpoons Al(OH)^{\circ}_{3(aq)} \rightleftharpoons Al(OH)^{+}_{2(aq)} \rightleftharpoons AlOH^{2+}_{(aq)} \rightleftharpoons Al^{3+}_{(aq)}$, the free solvated $Al^{3+}_{(aq)}$ cation shows the least tendency for polymerisation.

The coexistence of aluminium and silicon in solution has been poorly reported. Research to date has focussed largely on their interactions in soil environments, (Farmer 1986, Bache 1986), and/or their interactions with clays (Lou and Huang 1988). Interactions in synthetic solutions of similar nature to physiological environments have come under recent investigation (Birchall and Chappell 1988a, Chappell and Birchall 1988), and may be relevant to the etiology of Alzheimer-related conditions, (Birchall and Chappell 1988b).

Soil waters are often rich in soluble silica ($Si(OH)_4$), the solubility of which may be in equilibrium with aluminosilicate minerals such as kaolinite, halloysite or montmorillonite (Paces 1978, Lindsay 1979).

The mineral stability diagram (Figure 5.3) shows that, for the minerals in question, aluminium solubility decreases as the silicon concentration increases (Lindsay 1979). By

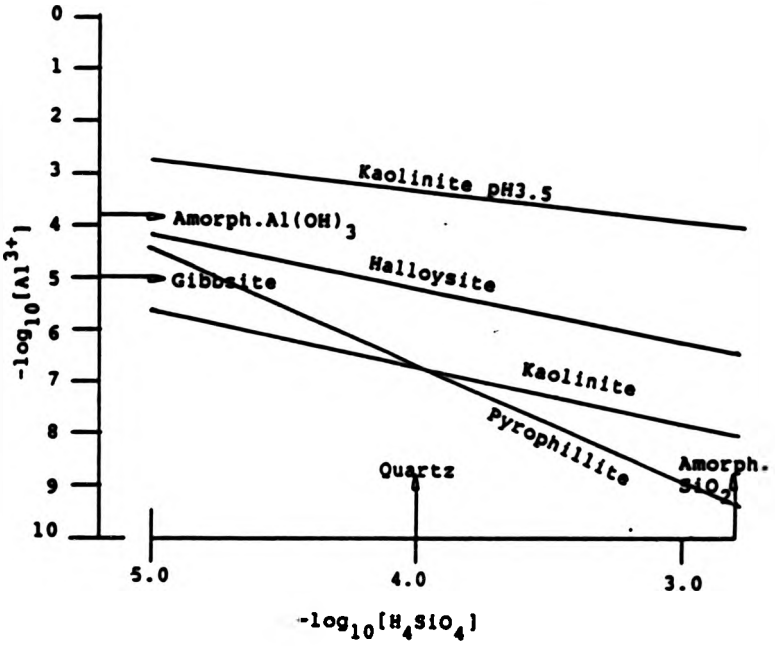


Figure 5.3. Reduction in soluble $[Al^{3+}]$ by soluble H_4SiO_4, in equilibrium with some aluminosilicate minerals at pH4.5. The line for Kaolinite at pH3.5 shows the effect of changing the pH (Bache, 1986).

way of contrast, the discovery of the mineral imogolite, $(OH)_3Al_2O_3SiOH$ (Farmer et al 1983), and in particular proto-imogolite, has suggested that aluminium will be labilised in soil and surface waters in the form of soluble-silica complexes. These species may be similar to the hydroxy-aluminosilicate ions recently described by Lou and Huang (1988).

The existence of aluminosilicate phases in dilute environments and low absolute concentrations of aluminium and silicon has not been previously determined. The existence of such phases could have significant implications for aluminium toxicity in dilute acid waters. The following experiments investigated more closely Al/Si interactions in dilute milieu and attempted to define the conditions for Al/Si complexation.

5.2 Materials and Methods

The solvent used throughout these experiments was double-distilled water and all solution temperatures were equilibrated to 25°C $\pm$ 1°C. pH was measured using a laboratory pH meter (Philips PW 9409) incorporating a glass electrode (Russell CWL) and was controlled through the manual additions of aliquots of $HNO_{3(aq)}$ and $NaOH_{(aq)}$ (Aristar:BDH Ltd.). Solutions were stirred using magnetic stirrers and pH equilibrium was judged to have occurred when the recorded pH remained stable, $\pm$ 0.02 unit,

for 5 minutes. Both aluminium and silicon were measured by graphite furnace atomic absorption spectrometry using programs described in Chapters Two and Four respectively.

Soluble or filtered aluminium was defined as all aluminium not retained when total aluminium solutions were passed under vacuum through membrane filters (Ultipor N66:Pall). The filters used were hydrophillic polymer membranes made from Nylon 66 cast onto an inert polyester substrate, and retained particles ca 0.04um in diameter. The kinetics of membrane filtration, including both adsorptive and stearic effects (see Chapter Two), and the absolute rating of this particular membrane filter, will dictate the filtration performance and particles much smaller than 0.04μm were likely to be retained.

Exchangeable aluminium was defined as the difference between the total aluminium concentration and the aluminium fraction not retained on a cation-exchange column, (Amberlite IR120 Na^+ BDH Ltd.). Two grammes of dry resin produced an active column 5mm in diameter, 50mm long and with a residence time of 30 seconds. The resin used was a gel type, strongly acidic resin with strongly aluminium binding sulphonate groups. Upon activation, with a solution of 5% (W/V) sodium chlorite (Technical:BDH Ltd.) the hydrated resin swelled and assumed a characteristic porosity (ca 0.05μm).

Sample aliquots were $50cm^3$, of which the first and last $20cm^3$ were discarded, for membrane filtration and $35cm^3$,

of which the first and last $10cm^3$ were discarded, for cation exchange. Stock solutions, ($10mgl^{-1}$), were freshly prepared from $Al(NO_3)_3 \cdot 9H_2O$ (Aristar:BDH Ltd.) and Na_4SiO_4 (reagent grade: Alfa) and were used to prepare test solutions, (1 litre), in acid washed polyethylene bottles with screw lids. pH was manually adjusted (see earlier) and after the stabilisation of pH sample aliquots were taken for immediate fractionation and aluminium analysis. Remaining solutions were aged for 168 hours at constant pH and then sampled as above. The Al/Si treatments investigated, all for both 0 and 168 hours equilibration, are shown in Table 5.1.

The total aluminium level used in all treatments, ($200 \mu gl^{-1}$) reflected an environmentally significant concentration of total aluminium found in acidified waters. Where data are expressed as proportions or percentages an arcsine transformation was used to evaluate the mean $\pm$ 1 standard deviation. This accounted for non-normal distribution of data at either extreme of the percentage/proportion scale.

5.3 Results

Data from all experiments were used to compare the measured soluble and exchangeable aluminium fractions as models for theoretical aluminium solubility. Measured solubility was compared with up to three known solubility controls, amorphous $Al(OH)_{3(s)}$, gibbsite $Al(OH)_{3(s)}$ and

Table 5.1 The Al/Si Treatments Investigated in this Study

A * denotes an investigated treatment.

Treatment Units: ugl^{-1}	Si = 0 Al = 200	Si = 100 Al = 200	Si = 200 Al = 200	Si = 400 Al = 200	Si = 1000 Al = 200	Si = 2000 Al = 200	Si = 3000 Al = 200	Si = 4000 Al = 200
Si:Al ratio	0:1	1:2	1:1	2:1	5:1	10:1	15:1	20:1
pH								
3.0	*	/	*	/	*	/	/	*
4.0	*	/	*	/	*	/	/	*
4.5	*	/	*	/	*	/	/	*
5.0	*	*	*	*	*	*	*	*
5.5	*	*	*	*	*	*	*	*
6.0	*	*	*	*	*	*	*	*
6.5	*	*	*	*	*	*	*	*
7.0	*	/	*	/	*	/	/	*
8.0	*	/	*	/	*	/	/	*
9.0	*	/		/	*	/	/	*

a predicted aluminosilicate phase, imogolite, $((HO)_3Al_2O_3SiOH)$, (J. Chappell, pers. comm.).

5.3.1 Aluminium Solubility with Respect to pH and Equilibration

The aluminium solubility/pH profile was characteristically V-shaped with a minimum in the pH range of 6.0 to 6.5, (Table 5.2, Figure 5.4). Lower than predicted (theoretical) soluble aluminium at acid pH (3.0-4.5), can be attributed to peculiarities of the cation-exchange and membrane filtration methodologies, (Chapter Two), and at alkaline pH, (7.0 to 9.0), the unsuitability of cation-exchange as a model for aluminium solubility in neutral and alkaline milieu.

In the pH regime where the aluminium concentration, ($200\mu g l^{-1}$), was oversaturated with respect to either or both, aluminium hydroxide phases, (5.0 to 8.0), aluminium solubility was akin to that described by amorphous $Al(OH)_{3(s)}$. Ageing, (168 hours), emphasised this control, (Table 5.3, Figure 5.5), as equilibration with respect to amorphous $Al(OH)_{3(s)}$ was approached from undersaturation. Solubility as measured by cation-exchange in acid milieu was consistently higher than that measured by membrane filtration. Retention of $Al(OH)_{3(aq)}$ and/or dimeric/polymeric aluminium species in aged solutions by the sulphonate ion exchange resin could explain this effect.

Table 5.2 Soluble and Exchangeable Aluminium Fractions as a Function of pH, Zero and Excess Si(aq) and Zero Equilibration Period.
Mean value for n = 3.

Treatment Solution		200ugl^{-1}Al. 0Si			200ugl^{-1}Al.4000ugl^{-1}Si		
pH	Al.Fraction	[Al]ugl^{-1}	[Al](M)	$-\log_{10}$[Al]	[Al]ugl^{-1}	[Al](M)	$-\log_{10}$[Al]
3.0	Soluble	161	5.96×10^{-6}	5.225	139	5.13×10^{-6}	5.290
	Exchangeable	167	6.19×10^{-6}	5.209	143	5.31×10^{-6}	5.275
4.0	Soluble	100	3.70×10^{-6}	5.431	73	2.69×10^{-6}	5.570
	Exchangeable	99	3.68×10^{-6}	5.434	115	4.27×10^{-6}	5.369
4.5	Soluble	73	2.69×10^{-6}	5.570	57	2.10×10^{-6}	5.679
	Exchangeable	63	2.35×10^{-6}	5.629	127	4.69×10^{-6}	5.329
5.0	Soluble	59	2.20×10^{-6}	5.658	191	7.09×10^{-6}	5.149
	Exchangeable	65	2.39×10^{-6}	5.621	96	3.56×16^{-6}	5.449
5.5	Soluble	32	1.19×10^{-6}	5.926	198	7.33×10^{-6}	5.135
	Exchangeable	69	2.54×10^{-6}	5.595	89	3.28×10^{-6}	5.484

cont'd...

Table 5.2 cont'd...

Treatment Solution		200ugl^{-1}Al. 0Si			200ugl^{-1}Al.4000ugl^{-1}Si		
pH	Al.Fraction	[Al]ugl^{-1}	[Al](M)	-log$_{10}$[Al]	[Al]ugl^{-1}	[Al](M)	-log$_{10}$[Al]
6.0	Soluble	11	4.22×10^{-7}	6.374	113	4.17×10^{-6}	5.380
	Exchangeable	9	3.19×10^{-7}	6.497	75	2.76×10^{-6}	5.559
6.5	Soluble	14	5.19×10^{-7}	6.285	9	3.19×10^{-7}	6.497
	Exchangeable	14	5.19×10^{-7}	6.285	23	8.40×10^{-7}	6.077
7.0	Soluble	43	1.61×10^{-6}	5.794	18	6.67×10^{-7}	6.176
	Exchangeable	13	4.96×10^{-7}	6.304	55	2.02×10^{-6}	5.694
8.0	Soluble	63	2.35×10^{-6}	5.629	94	3.48×10^{-6}	5.458
	Exchangeable	9	3.19×10^{-7}	6.497	16	5.93×10^{-7}	6.227
9.0	Soluble	87	3.24×10^{-6}	5.490	142	5.26×10^{-6}	5.279
	Exchangeable	0.6	2.22×10^{-8}	7.653	2	7.41×10^{-8}	7.130

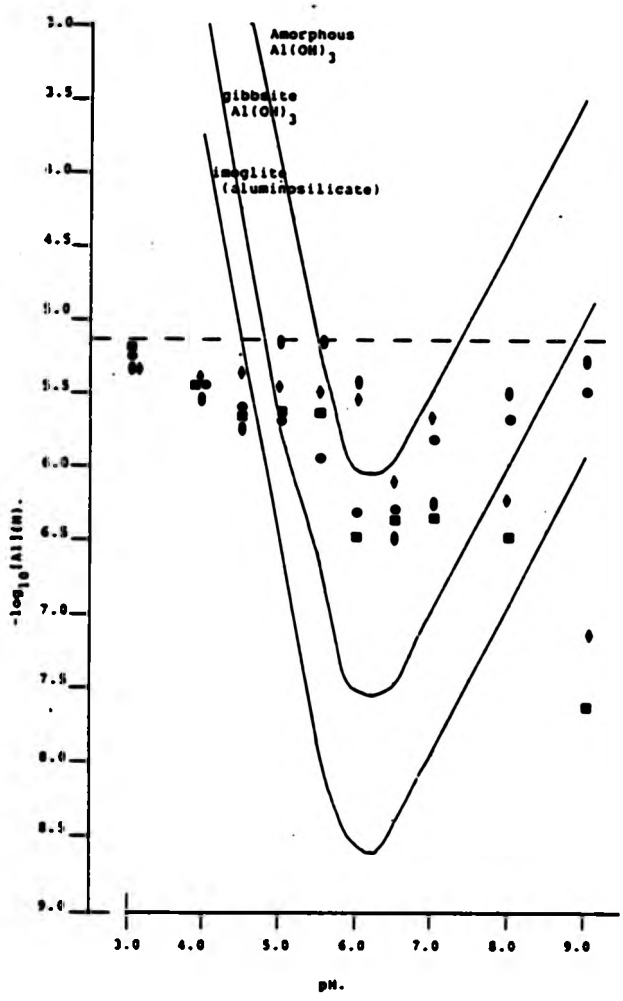


Figure 5.4. Aluminium solubility, (Al-exchangeable and Al filtered), with respect to zero and excess Si(aq), zero equilibration period, pH and compared to three known solubility controls. ● Al-filtered/zero Si(aq), ■ Al-exchangeable/zero Si(aq), ◆ Al-filtered/4000 $\mu g l^{-1}$ Si(aq), ◆ Al-exchangeable/4000 $\mu g l^{-1}$ Si(aq) ----- ≡ 200 $\mu g l^{-1}$ Al-total.
Mean values are plotted, n = 3.

Table 5.3 Soluble and Exchangeable Aluminium Fractions as a Function of pH, Zero and Excess Si(aq) and 168 Hours Equilibration Period. Mean value for n = 3.

Treatment Solution		$200ugl^{-1}$Al. 0Si			$200ugl^{-1}$Al.$4000ugl^{-1}$Si		
pH	Al.Fraction	$[Al]ugl^{-1}$	[Al](M)	$-\log_{10}[Al]$	$[Al]ugl^{-1}$	[Al](M)	$-\log_{10}[Al]$
3.0	Soluble	141	$5.21x10^{-6}$	5.283	175	$6.50x10^{-6}$	5.187
	Exchangeable	161	$5.95x10^{-6}$	5.226	141	$5.21x10^{-6}$	5.283
4.0	Soluble	19	$6.89x10^{-7}$	6.162	70	$2.59x10^{-6}$	5.586
	Exchangeable	101	$3.73x10^{-6}$	5.429	143	$5.31x10^{-6}$	5.275
4.5	Soluble	11	$3.93x10^{-7}$	6.406	65	$2.39x10^{-6}$	5.621
	Exchangeable	99	$3.65x10^{-6}$	5.437	157	$5.83x10^{-6}$	5.234
5.0	Soluble	32	$1.19x10^{-6}$	5.926	195	$7.24x10^{-6}$	5.140
	Exchangeable	99	$3.65x10^{-6}$	5.437	101	$3.76x10^{-6}$	5.425
5.5	Soluble	31	$1.13x10^{-6}$	5.946	82	$3.04x10^{-6}$	5.518
	Exchangeable	70	$2.59x10^{-6}$	5.586	137	$5.06x10^{-6}$	5.296

cont'd...

Table 5.3 cont'd...

Treatment Solution		200ugl^{-1}Al. 0Si			200ugl^{-1}Al.4000ugl^{-1}Si		
pH	Al.Fraction	[Al]ugl^{-1}	[Al] (M)	-log$_{10}$[Al]	[Al]ugl^{-1}	[Al] (M)	-log$_{10}$[Al]
6.0	Soluble	41	1.53×10^{-6}	5.814	53	1.98×10^{-6}	5.704
	Exchangeable	110	4.07×10^{-6}	5.390	121	4.47×10^{-6}	5.350
6.5	Soluble	37	1.36×10^{-6}	5.868	27	9.85×10^{-6}	6.006
	Exchangeable	93	3.46×10^{-6}	5.461	157	5.87×10^{-6}	5.237
7.0	Soluble	45	1.68×10^{-6}	5.774	40	1.48×10^{-6}	5.829
	Exchangeable	110	4.07×10^{-6}	5.390	148	5.48×10^{-6}	5.261
8.0	Soluble	63	2.32×10^{-6}	5.635	71	2.62×10^{-6}	5.583
	Exchangeable	3	9.63×10^{-8}	7.016	95	3.50×10^{-6}	5.455
9.0	Soluble	81	3.01×10^{-6}	5.521	64	2.37×10^{-6}	5.625
	Exchangeable	0.0	0.0	-	16	5.93×10^{-7}	6.227

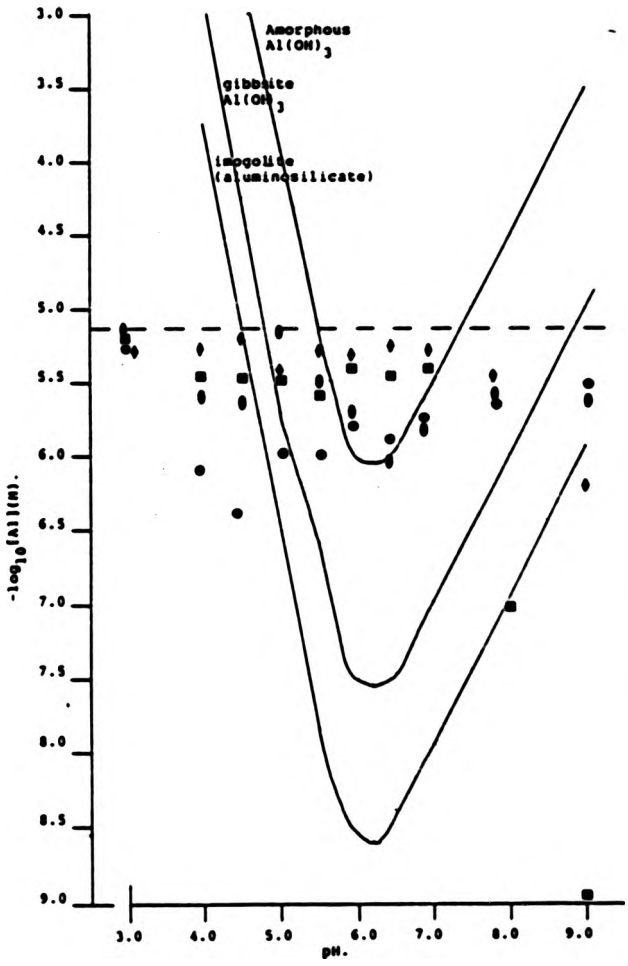


Figure 5.5. Aluminium solubility, (Al-exchangeable and Al-filtered) with respect to zero and excess Si(aq), 168h equilibration, pH and compared to three known solubility controls. ● Al-filtered/zero Si(aq), ■ Al-exchangeable/zero Si(aq), ▲ Al-filtered/4000 $\mu g l^{-1}$ Si(aq), ◆ Al-exchangeable/4000 $\mu g l^{-1}$ Si(aq). ---- = 200 $\mu g l^{-1}$ Al total.
Mean values are plotted, n = 3.

5.3.2 Effect of Excess Si(aq) Addition

Aluminium solubility control in the presence of an excess of silicic acid, ($3x10^{-3}$M), will change from a hydroxide phase to a less soluble aluminosilicate phase, such as imogolite, (Farmer 1986), (Tables 5.2, 5.3, Figures 5.4, 5.5). At silicic acid concentrations less than $3x10^{-3}$M aluminium and silicon mixtures will exist as both stable and metastable complexes of imogolite and/or other aluminium and aluminosilicate species.

The addition of a molar excess of Si(aq) to aluminium (ca 19:1 Si:Al), at pH $\geq$4.5 generally increased aluminium solubility with respect to $Al(OH)_{3(s)}$, (Figures 5.4, 5.5), an effect that was exacerbated in aged solutions. Solubility with respect to known solubility controls was lost and this effect was emphasised by both cation-exchange and membrane filtration. This effect of Si(aq) on aluminium solubility was examined further.

5.3.3 Aluminium/Si(aq) Interactions in Dilute Milieu

Al/Si interactions in moderately acid/alkaline synthetic solutions of very low ionic strength were studied with respect to pH at a constant Si:Al molar ratio and with an increasing Si:Al molar ratio at constant pH. With the additional criterion of ageing these widely overlapping strategies helped to define criteria for Al/Si interactions.

Data, (Tables 5.4a-h), were compared with theoretical $Al(OH)_{3(s)}$ solubility controls, (Figures 5.6a-h), and several general trends were observed. In the presence of an excess of Si(aq) ($\geq 200\mu gl^{-1}$), aluminium solubility control switched from a state of under to oversaturation with respect to $Al(OH)_3$ (amorphous). An increase in the [Siaq] above $2000\mu gl^{-1}$ exacerbated the increase in solubility at pH $\leq$ 5.5 and slowly reversed the "solubilisation" effect at near neutral pH, (6.0-8.0).

The solubilising effect of Si(aq) was emphasised by the exchangeable aluminium level and in particular at lower Si(aq) levels, (up to ca 10:1, Si:Al molar ratio).

Ageing increased the proportion of soluble aluminium and in particular at low [Si(aq)],(Si:Al molar ratios $\leq$ 5:1).

When aluminium solubility at constant pH and increasing [Si(aq)] was studied, (Figures 5.7a-d), further effects on aluminium solubility were evident. These effects were exacerbated by the method of determination of soluble aluminium.

(i) Membrane Filtration

The rapid solubilisation of aluminium with respect to $Al(OH)_{3(s)}$ and in the presence of increasing [Si(aq)] was not linear at low pH, (5.0-5.5), and tended to observe a

Tables 5.4a-h. Aluminium Solubility, as Defined by Filtration and Cation Exchange, with respect to pH, Si(aq) Concentration and Equilibration Period. Mean Value for n = 3.

Table 5.4a. 0 $\mu g l^{-1}$ Si(aq)

	Soluble Aluminium Fraction(M)				Exchangeable Aluminium Fraction(M)			
	0 Equilibration Period		168 hours Equilibration Period		0 Equilibration Period		168 hours Equilibration Period	
pH	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]
5.0	2.20×10^{-6}	5.66	1.19×10^{-6}	5.92	2.39×10^{-6}	5.62	3.68×10^{-6}	5.43
5.5	1.19×10^{-6}	5.92	1.13×10^{-6}	5.95	2.54×10^{-6}	5.60	2.59×10^{-6}	5.59
6.0	4.22×10^{-7}	6.38	1.53×10^{-6}	5.82	3.19×10^{-7}	6.50	4.07×10^{-6}	5.39
6.5	5.19×10^{-7}	6.29	1.36×10^{-6}	5.87	5.19×10^{-7}	6.28	3.46×10^{-6}	5.46
7.0	1.61×10^{-6}	5.79	1.68×10^{-6}	5.77	4.96×10^{-7}	6.30	4.07×10^{-6}	5.39
8.0	2.35×10^{-6}	5.63	2.32×10^{-6}	5.63	3.48×10^{-7}	6.46	9.63×10^{-8}	7.02

Table 5.4b $100 \mu g l^{-1}$ Si(aq)

	Soluble Aluminium Fraction(M)				Exchangeable Aluminium Fraction (M)			
	0 Equilibration Period		168 hours Equilibration Period		0 Equilibration Period		168 hours Equilibration Period	
pH	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]
5.0	2.05×10^{-6}	5.69	2.57×10^{-6}	5.59	2.54×10^{-6}	5.60	3.46×10^{-6}	5.46
5.5	6.89×10^{-7}	6.16	1.09×10^{-6}	5.96	2.69×10^{-6}	5.57	2.37×10^{-6}	5.63
6.0	5.49×10^{-7}	6.26	1.36×10^{-6}	5.87	1.98×10^{-6}	5.70	3.93×10^{-6}	5.41
6.5	4.44×10^{-7}	6.35	1.43×10^{-6}	5.84	8.37×10^{-7}	6.08	3.70×10^{-6}	5.43
7.0	n.a.		n.a.		n.a.		n.a.	
8.0	n.a.		n.a.		n.a.		n.a.	

Table 5.4c $200\mu gl^{-1}$ Si(aq)

	Soluble Aluminium Fraction (M)				Exchangeable Aluminium Fraction (M)			
	0 Equilibration Period		168 hours Equilibration Period		0 Equilibration Period		168 hours Equilibration Period	
pH	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]
5.0	2.37×10^{-6}	5.63	2.99×10^{-6}	5.53	2.72×10^{-6}	5.57	2.81×10^{-6}	5.55
5.5	8.37×10^{-7}	6.08	1.26×10^{-6}	5.90	2.91×10^{-6}	5.54	4.10×10^{-6}	5.39
6.0	6.89×10^{-7}	6.16	1.13×10^{-6}	5.95	2.84×10^{-6}	5.55	4.00×10^{-6}	5.40
6.5	5.70×10^{-7}	6.24	1.26×10^{-6}	5.90	1.26×10^{-6}	5.90	4.15×10^{-6}	5.38
7.0	9.11×10^{-7}	6.04	6.89×10^{-7}	6.16	1.36×10^{-6}	5.87	5.53×10^{-6}	5.26
8.0	1.65×10^{-6}	5.78	7.93×10^{-7}	6.10	7.93×10^{-7}	6.10	1.87×10^{-6}	5.73

Table 5.4d $400\mu gl^{-1}$ Si(aq)

	Soluble Aluminium Fraction (M)				Exchangeable Aluminium Fraction (M)			
	0 Equilibration Period		168 hours Equilibration Period		0 Equilibration Period		168 hours Equilibration Period	
pH	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]
5.0	3.31×10^{-6}	5.48	3.63×10^{-6}	5.44	3.21×10^{-6}	5.49	3.26×10^{-6}	5.49
5.5	1.04×10^{-6}	5.98	1.33×10^{-6}	5.89	3.53×10^{-6}	5.45	4.32×10^{-6}	5.36
6.0	8.89×10^{-7}	6.05	1.33×10^{-6}	5.89	2.99×10^{-6}	5.53	4.39×10^{-6}	5.36
6.5	5.93×10^{-7}	6.23	1.63×10^{-6}	5.79	1.76×10^{-6}	5.76	4.00×10^{-6}	5.40
7.0	n.a.		n.a.		n.a.		n.a.	
8.0	n.a.		n.a.		n.a.		n.a.	

Table 5.4e $1000\mu gl^{-1}$ Si (aq)

	Soluble Aluminium Fraction (M)				Exchangeable Aluminium Fraction (M)			
	0 Equilibration Period		168 hours Equilibration Period		0 Equilibration Period		168 hours Equilibration Period	
pH	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]
5.0	3.98×10^{-6}	5.40	3.76×10^{-6}	5.43	4.05×10^{-6}	5.39	3.26×10^{-6}	5.49
5.5	1.50×10^{-6}	5.82	1.53×10^{-6}	5.81	4.32×10^{-6}	5.36	5.13×10^{-6}	5.29
6.0	1.36×10^{-6}	5.87	1.36×10^{-6}	5.87	4.20×10^{-6}	5.38	5.04×10^{-6}	5.30
6.5	6.67×10^{-7}	6.18	1.50×10^{-6}	5.82	2.30×10^{-6}	5.64	4.00×10^{-6}	5.40
7.0	5.41×10^{-7}	6.27	1.50×10^{-6}	5.82	1.73×10^{-6}	5.76	5.24×10^{-6}	5.28
8.0	7.93×10^{-7}	6.10	2.37×10^{-6}	5.63	1.70×10^{-7}	6.77	2.42×10^{-6}	5.62

Table 5.4f 2000$\mu g l^{-1}$ Si(aq)

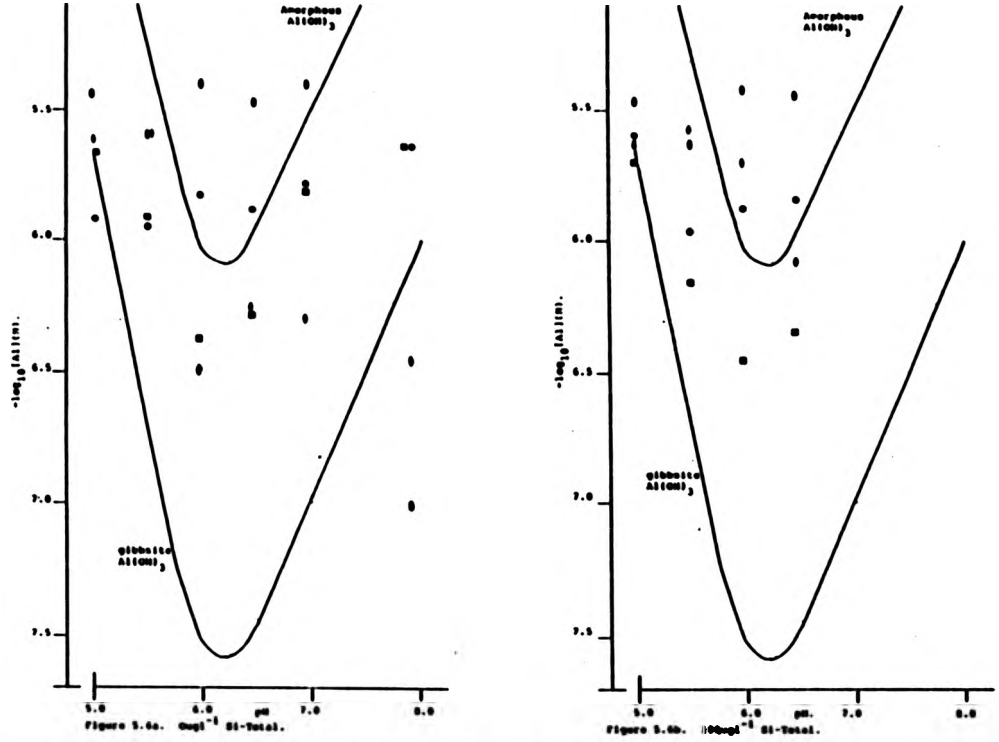
	Soluble Aluminium Fraction (M)				Exchangeable Aluminium Fraction (M)			
	0 Equilibration Period		168 hours Equilibration Period		0 Equilibration Period		168 hours Equilibration Period	
pH	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]
5.0	4.61×10^{-6}	5.34	4.69×10^{-6}	5.33	3.87×10^{-6}	5.41	3.65×10^{-6}	5.44
5.5	4.47×10^{-6}	5.35	2.37×10^{-6}	5.63	3.46×10^{-6}	5.46	5.09×10^{-6}	5.29
6.0	2.15×10^{-6}	5.67	1.76×10^{-6}	5.76	2.64×10^{-6}	5.58	5.13×10^{-6}	5.29
6.5	8.37×10^{-7}	6.08	1.19×10^{-6}	5.93	1.19×10^{-6}	5.93	4.15×10^{-6}	5.38
7.0	n.a.		n.a.		n.a.		n.a.	
8.0	n.a.		n.a.		n.a.		n.a.	

Table 5.4g 3000μgl^{-1} Si(aq)

	Soluble Aluminium Fraction (M)				Exchangeable Aluminium Fraction (M)			
	0 Equilibration Period		168 hours Equilibration Period		0 Equilibration Period		168 hours Equilibration Period	
pH	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]
5.0	5.80×10^{-6}	5.24	5.85×10^{-6}	5.23	3.76×10^{-6}	5.43	3.68×10^{-6}	5.43
5.5	6.74×10^{-6}	5.17	2.61×10^{-6}	5.58	3.24×10^{-6}	5.49	5.11×10^{-6}	5.29
6.0	3.06×10^{-6}	5.51	1.76×10^{-6}	5.76	2.57×10^{-6}	5.59	5.13×10^{-6}	5.29
6.5	6.44×10^{-7}	6.19	9.85×10^{-7}	6.01	8.67×10^{-7}	6.06	4.27×10^{-6}	5.37
7.0	n.a.		n.a.		n.a.		n.a.	
8.0	n.a.		n.a.		n.a.		n.a.	

Table 5.4h 4000$\mu g l^{-1}$ Si(aq).

	Soluble Aluminium Fraction (M)				Exchangeable Aluminium Fraction (M)			
	0 Equilibration Period		168 hours Equilibration Period		0 Equilibration Period		168 hours Equilibration Period	
pH	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]	[Al]	$-\log_{10}$[Al]
5.0	7.09×10^{-6}	5.15	7.24×10^{-6}	5.14	3.56×10^{-6}	5.45	3.76×10^{-6}	5.42
5.5	7.33×10^{-6}	5.13	3.04×10^{-6}	5.52	3.28×10^{-6}	5.48	5.06×10^{-6}	5.30
6.0	4.17×10^{-6}	5.38	1.98×10^{-6}	5.70	2.76×10^{-6}	5.56	4.47×10^{-6}	5.35
6.5	3.19×10^{-7}	6.50	9.85×10^{-7}	6.01	8.37×10^{-7}	6.08	5.80×10^{-6}	5.24
7.0	6.67×10^{-7}	6.18	1.48×10^{-6}	5.83	2.02×10^{-6}	5.69	5.48×10^{-6}	5.26
8.0	3.48×10^{-6}	5.46	2.61×10^{-6}	5.58	5.93×10^{-7}	6.23	3.50×10^{-6}	5.46



Figures 5.6a-h. Aluminium solubility, (Al-exchangeable and Al-filtered), with respect to pH, Si(aq) concentration, equilibration period and relative to the solubility curves for both amorphous and gibbsite $Al(OH)_3$. ■ Al-filtered/zero equilibration, ● Al-filtered/168 h. equilibration, ♦ Al-exchangeable/zero equilibration, ◊ Al-exchangeable/168 h. equilibration. Mean values are plotted, n = 3.

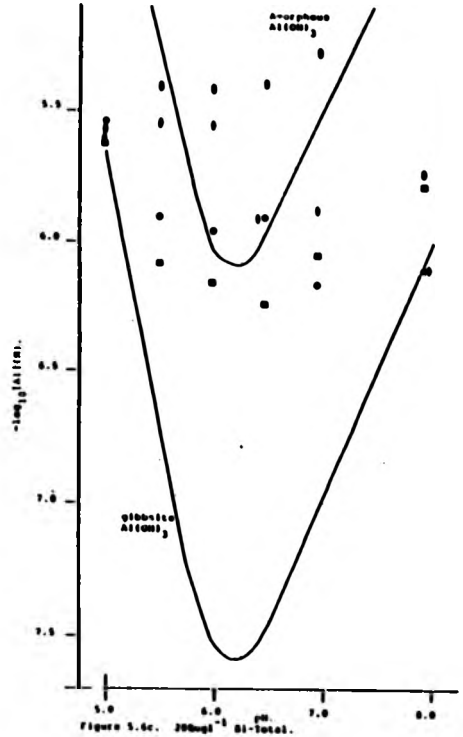


Figure 5.6c. 200mgl^{-1} Si-Total.

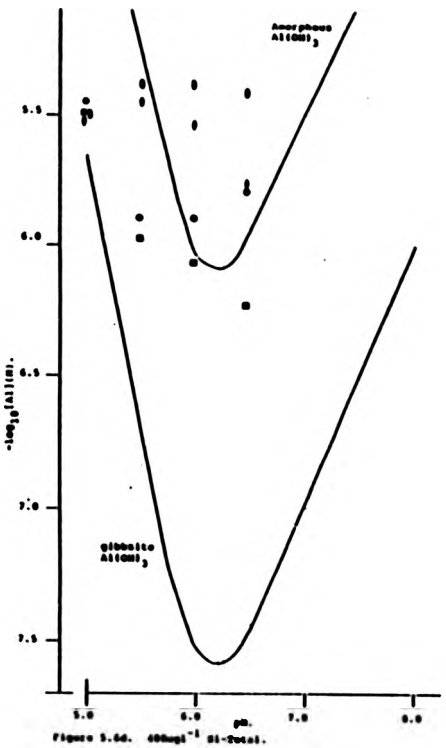


Figure 5.6d. 400mgl^{-1} Si-Total.

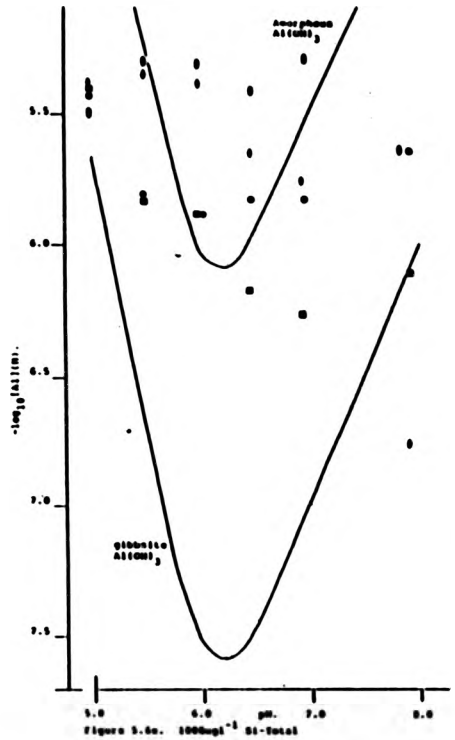


Figure 5.6a. 100$\mu g l^{-1}$ Si-Total

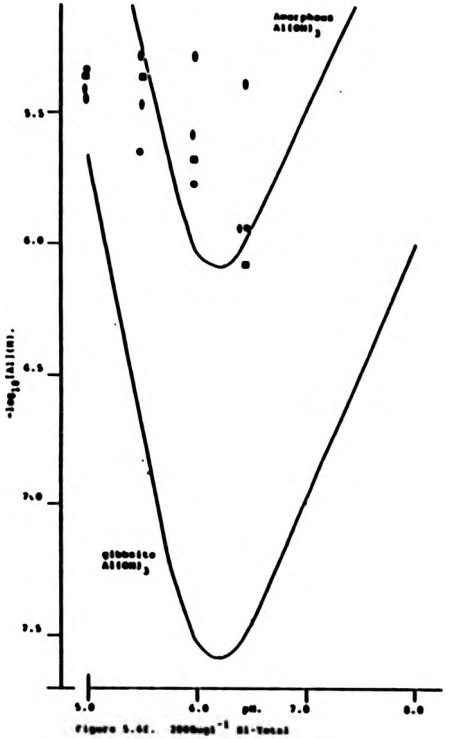


Figure 5.6b. 2000$\mu g l^{-1}$ Si-Total

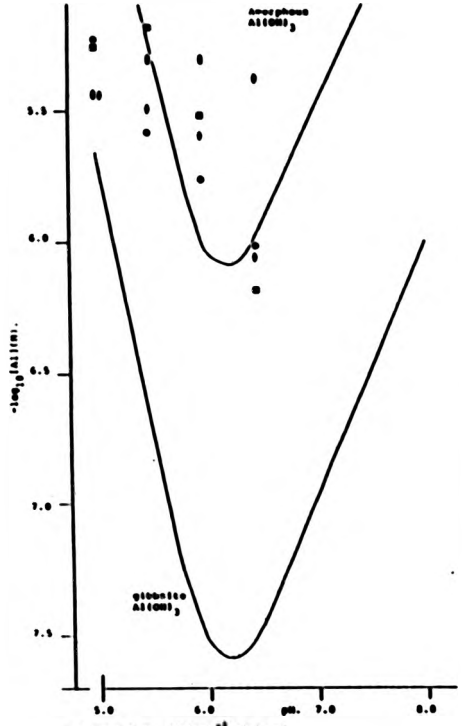


Figure 5.6g. 3000ugl^{-1} Si-Total

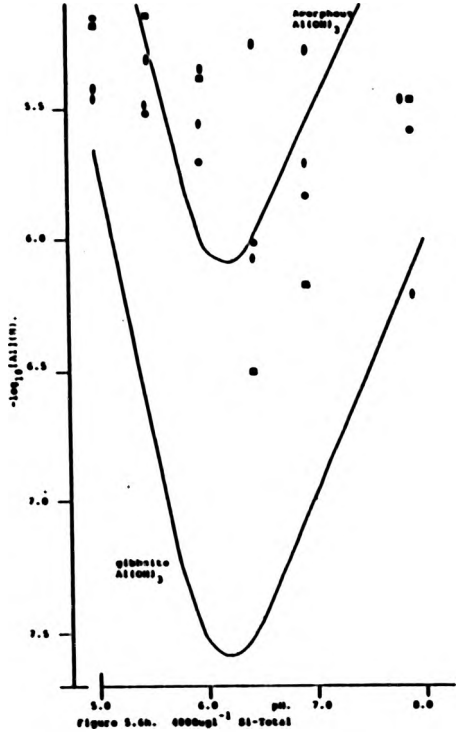


Figure 5.6h. 4000ugl^{-1} Si-Total

sigmoidal relationship, (Figures 5.7a, 5.7b). This effect was still evident at pH5.0 after 168 hours equilibration though was reduced at pH5.5 after the same time period. Si(aq) concentration had no apparent effect on aluminium solubility at pH6.5 even after 168 hours equilibration.

(ii) Cation-Exchange

A sigmoidal relationship between the soluble aluminium fraction and the [Si(aq)] was also evident, however, the extent of solubilisation was reduced, with respect to that measured by membrane filtration, above a critical [Si(aq)] range of 800-1200$\mu g l^{-1}$. The effect of ageing was to increase the soluble aluminium fraction overall and to reduce the effects of increasing [Si(aq)].

The results from both membrane filtration and cation-exchange together suggest that different aluminosilicate species/phases were formed and the nature of the Al/Si interactions were very much dependent on pH and the [Si(aq)].

5.4 Discussion

Aluminium solubility and speciation in natural waters has received much attention, particularly with reference to aluminium mobilisation in soils, (Lindsay 1979 , Young and

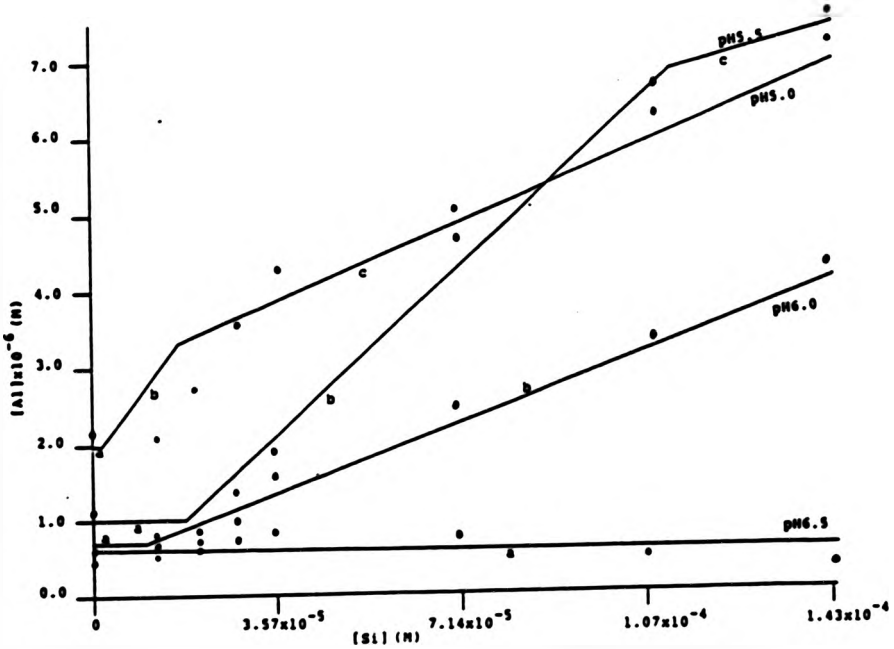


Figure 5.7a. Filtered Aluminium/Zero Equilibration.

Figures 5.7a-d. The effect of Si(aq) concentration and equilibration period on the levels of Al-exchangeable and Al-filtered at four different solution pH. Mean values are plotted, n = 9. Up to three distinct stages are evident, a, b, and c, and are probably reflections of discrete aluminosilicate phases differing in their charge, size and stability.

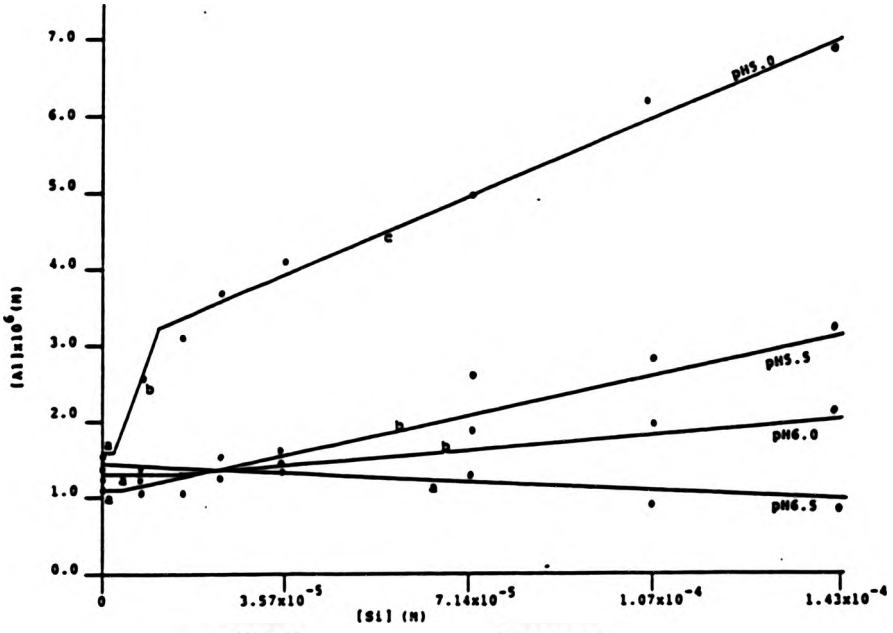


Figure 5.7b. Filtered Aluminium/168 Hours Equilibration.

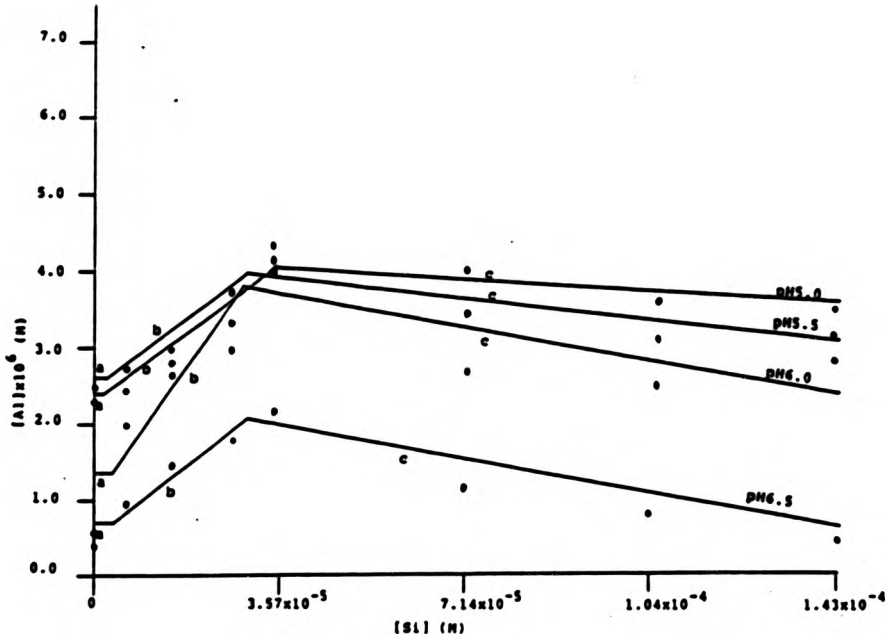


Figure 5.7c. Cation Exchangeable Aluminium/Zero Equilibration.

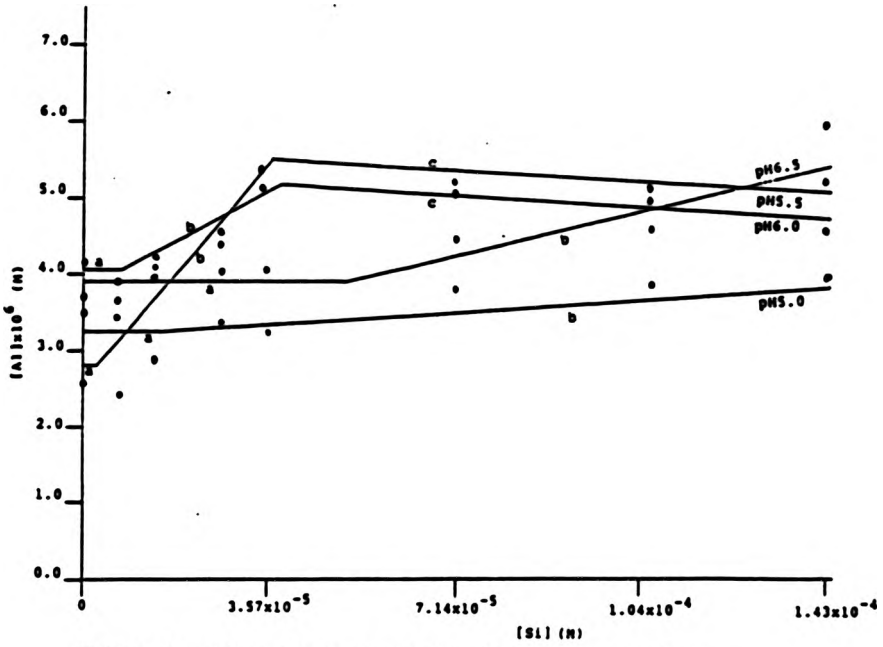


Figure 5.7d. Cation Exchangeable Aluminium/168 Hours Equilibration.

Bache 1985, Bache 1986, Farmer 1986, Arp and Ouimet 1986), and acidified water, (Lawrence et al 1986, Cronan et al 1986, Tam and Williams 1986, Tipping et al 1987, Goenaga and Williams 1988). Studies using synthetic water are also well documented, (Hem 1968a, Hem 1968b, May et al 1979, Hedlund et al 1987), however, few of these studies using synthetic waters can be related directly to aluminium chemistry in natural waters.

This study attempted to quantify aluminium solubility in dilute solution taking into account the pH, the time for equilibration and the addition of Si(aq).

5.4.1 The Experimental Method

An understanding of the methods used to determine aluminium solubility is critical when discussing the results. The filtered aluminium fraction will contain 99% of particles/species $\lesssim$ 0.04μm and probably 10-20% of particles in the size range of 0.04 to 0.06μm. Adsorptive effects, particularly at low pH, may reduce these proportions, (see Chapter 2).

The exchangeable aluminium fraction will include all particles/species in the order of 0.05μm in size that carry a net positive charge or are loosely bound/unstable species capable of releasing a cation to the strongly

binding sulphonate groups of the exchange resin. Both pH and the [Si(aq)] will effect the soluble aluminium fraction and the difference between the soluble fractions as expressed by either cation-exchange or membrane filtration will help to identify the nature of the soluble species present.

5.4.2 Aluminium Hydroxide Solubility

Aluminium solubility in pure water is controlled by both amorphous and crystalline $Al(OH)_{3(s)}$. The most common of these are $Al(OH)_{3(gibbsite)}$ and $Al(OH)_{3(amorphous)}$. Solubility curves for these phases were determined from data taken from Martin (1986), (Figures 5.4, 5.5, 5.6a-h).

$$Al(OH)_{3(s)}\ (\text{gibbsite or amorphous}) + 3H^{+}_{(aq)} \rightleftharpoons Al^{3+}_{(aq)} + 3H_2O_{(l)} \quad (1)$$

$$Ksp\ (\text{solubility product}) = \left[Al^{3+}\right] / 10^{-3pH} = 10^{9.2}(\text{gibbsite}) \quad (2)$$

$$= 10^{10.7}(\text{amorphous})$$

$$\left[Altot\right] = Ksp \times 10^{-3pH} \times {}^{1}/_{\gamma} \quad (3)$$

where ${}^{1}/_{\gamma}$ is a constant at any one pH and is determined from:

$${}^{1}/_{\gamma} = 1 + \left[10^{-5.5} / 10^{-1pH}\right] + \left[10^{-11.1} / 10^{-2pH}\right] + \left[10^{-23.2} / 10^{-4pH}\right] \quad (4)$$

The curves predict a minimum aluminium solubility around pH6.2 with approximate linear increases in solubility on either side of the pH range $5.0 \leq pH \leq 7.0$.

The aluminium solubility data from this present study largely mimicked the predicted levels though equilibration, (168 hours), moved the point of minimum solubility from pH 6.0-6.5 to a pH minimum around 5.5. The reduced solubility of aluminium in acid milieu was a consistent but inexplicable occurrence.

The low levels of exchangeable aluminium at near-neutral and alkaline pH were indicative of charge reversal around minimum aluminium solubility, (pH6.2). Hydroxide fuelled hydrolyses will allow the monomeric aluminate anion, $Al(OH)^-_{4(aq)}$, to dominate in alkaline solution, and this species will not be retained on the exchange resin.

Aluminium solubility control at zero equilibration was intermediate with respect to both hydroxide phases, (Figure 5.4). Equilibration, (168 hours), increased aluminium solubility and equilibrium with respect to $Al(OH)_3$ (amorphous) was approached from undersaturation (Figure 5.5).

The results suggest that aluminium solubility in dilute aluminium solutions of very low ionic strength is controlled by the amorphous and not the crystalline

$Al(OH)_{3(s)}$ phase.

5.4.3 Al/Si Interactions

Aluminosilicates will form over a wide pH range from the aqueous interactions of aluminium and silicon, (Farmer et al 1983, Lou and Huang 1988), to effect, at equilibrium, a reduction in aluminium solubility with respect to $Al(OH)_3$ (gibbsite), (Lindsay 1979), (Figure 5.3). This change in aluminium solubility control was evident for the aluminosilicate mineral imogolite, (Figures 5.4, 5.5), the solubility curve for which was computed from theoretical data, (J. Chappell, pers. comm.), (Figure 5.10). Farmer (1986) predicted that a minimum silicic acid concentration of $1000 \mu g l^{-1}$ was needed for imogolite and/or protoimogolite to form.

In this study aluminosilicate-like species were formed at Si:Al molar ratios of ≥ 1.0, ($\geq 200 \mu g l^{-1}$, Si-total). Aluminosilicate formation increased aluminium solubility over a wide pH range and in particular in the range of pH5.0-6.0, (Figure 5.8). Aluminium solubility remained high even after an equilibration period of 168 hours (Figure 5.9), suggesting that equilibrium with respect to the aluminosilicate solid phase was only slowly achieved. This was particularly true at lower pH, ($<$ pH5.0), and lower [Si(aq)], ($< 1000 \mu g l^{-1}$), (Figures 5.6a-h).

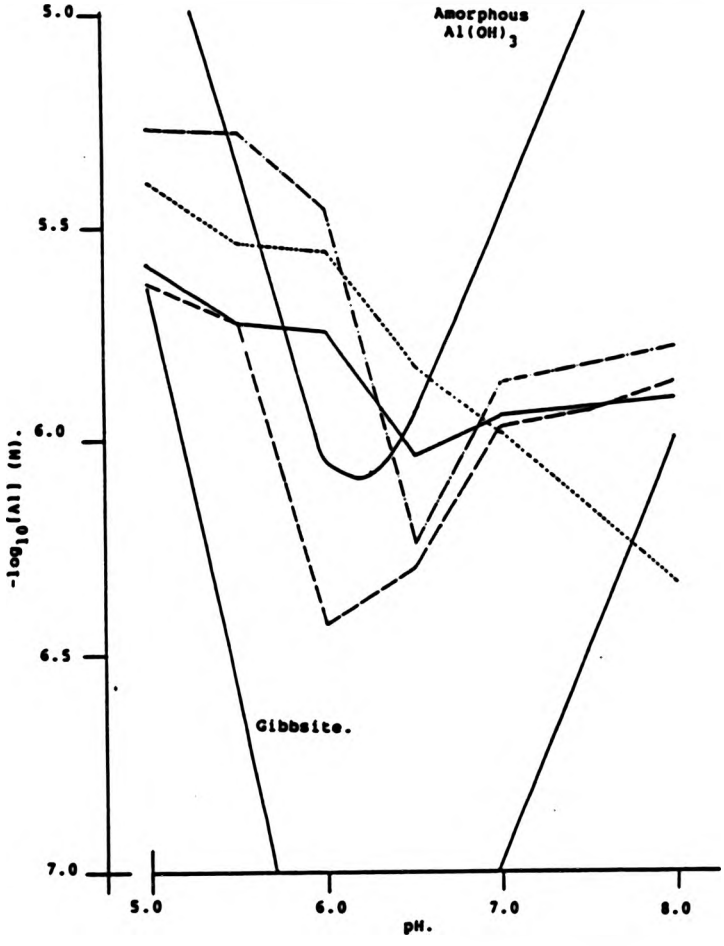


Figure 5.8. Aluminium solubility, (determined from the mean of the Al-exchangeable and Al-filtered), for various Si(aq) concentrations, 0μgl^{-1}, ---, 200μgl^{-1}, ——, 1000μgl^{-1}, ... and 4000μgl^{-1} -.-.-.-. and an Al-total level of 200μgl^{-1} in the pH range of 5.0 to 8.0 and after zero equilibration period at each pH. Mean values are plotted, n = 6.

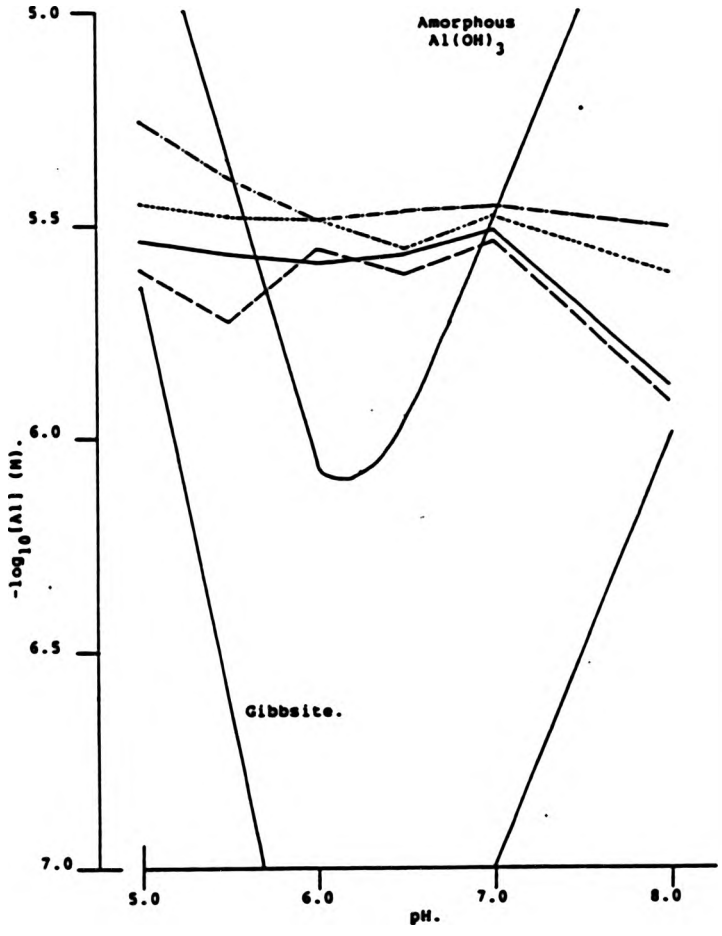


Figure 5.9. Aluminium solubility, (determined from the mean of the Al-exchangeable and Al-filtered), for various Si(aq) concentrations, $0ugl^{-1}$, ----, $200ugl^{-1}$ ——, $1000ugl^{-1}$------ , and $4000ugl^{-1}$ -.-.-.-, and an Al-total level of $200ugl^{-1}$ in the pH range of 5.0 to 8.0 and after 168 h. equilibration at each pH. Mean values are plotted, n = 6.

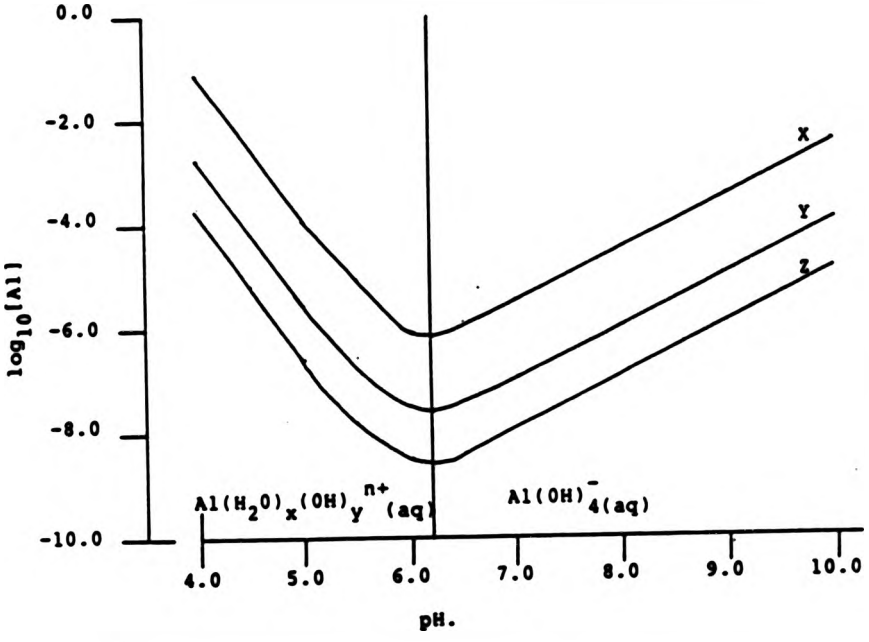


Figure 5.10. Effect of silicic acid, X = ca 1.0ugl^{-1}, Y = ca 1400ugl^{-1}, Z = ca 84,000ugl^{-1}, to lower aluminium solubility.
(Reproduced by kind permission of J. Chappell).

There was good evidence that the nature of the aluminosilicate species formed was influenced by the pH and the [Si(aq)]. Probable aluminosilicate species were characterised by their size, charge and stability and these properties were identified from the result of cation-exchange and membrane filtration studies.

At low [Si(aq)], ($\angle$ ca 1000μgl^{-1}), the aluminosilicate species formed rapidly in the presence of an excess of Si(aq) ($\triangle$200μgl^{-1}), and effected a rapid solubilisation of aluminium. The species were unstable with respect to cation-exchange and were retained as aluminium and/or hydroxy-aluminosilicates on the exchange resin. The species were retained by membrane filtration particularly at pH 5.0 and this was probably indicative of a loose, disorganised and highly variable structure.

Stable aluminosilicates were formed at [Si(aq)] $\triangle$ 1000μgl^{-1} and pH $\triangle$5.0. These species had a tighter, more organised structure and were not retained by membrane filtration, particularly at low pH. There was evidence that they were retained as aluminosilicates on the exchange resin again particularly at low pH where they probably had a net positive charge. This charge was reduced at more alkaline pH which may have allowed some aggregation of species and hence reduced the filtered aluminium fraction at higher pH.

In alkaline solution an increase in filtered aluminium and sharp reduction in exchangeable aluminium would suggest that the stable aluminosilicate species carried a net negative charge and would not, therefore, form aggregations, thereby increasing the filtered aluminium fraction, and also would not be retained on a cationic exchange column.

Four principal aluminosilicate species could be identified:

(i) Unstable hydroxy-aluminosilicates probably existing in equilibrium with Al hydroxy complexes.

(ii) Stable, positively charged aluminosilicates, very slow to come out of solution because of their charge.

(iii) Stable, neutral, aluminosilicate species, capable of some aggregation and the first to come out of solution as equilibrium is approached from over-saturation.

(iv) Stable, negatively charged, aluminosilicates slow to approach equilibrium because of their charge.

The nature of the Al/Si interaction is probably via the aluminate anion, $Al(OH)^-_{4(aq)}$, (Chappell and Birchall 1988, Birchall and Chappell 1988a). Whilst only present at nanomolar concentrations at acid pH,almost instantaneous

shifts in aluminium hydrolysis equilibria will ensure a rapid aluminium solubilisation and hence rapid production of an aluminosilicate species. The concentration of Si(aq) will determine the stability and form of the species and the solution pH will influence any charge carried by the species.

The mechanisms of simple aluminosilicate formation are thought to involve the displacement of a proton from an aluminium-co-ordinated OH group by silicic acid, (Hem et al 1973). Subsequent polymerisations will result in stable aluminosilicate structures such as imogolite which is described as a tube, (ca 0.002um outer diameter, 0.001um inner diameter), whose wall consists of a single continuous gibbsite sheet with the inner hydroxyl surface of the gibbsite replaced by O_3SiOH groups, (Cradwick et al 1972). The structure has the empirical formula, $(HO)_3Al_2O_3SiOH$, (Farmer et al 1983). Proto-imogolite is the non-dialysable precursor of imogolite, (Farmer and Fraser 1982) and is probably similar to the aluminosilicate species identified in this study.

Silicic acid is a very weak acid (pKa 9.8), and will only interact with basic metal ions. Reactions with monomeric aluminium species are, therefore, likely to be with $Al(OH)^-_{4(aq)}$, however at acid pH (where $Al(OH)^-_{4(aq)}$ is present at only very low levels) reactions with $Al(OH)^+_{2(aq)}$ and possibly $AlOH^{2+}_{(aq)}$ cannot be discounted.

In these studies the presence of silicic acid, up to ca 4000μgl^{-1}, did not reduce aluminium solubility in the pH range of 3.0 to 9.0. Aluminium solubility was increased through the formation of aluminosilicate species. The implications of these Si/Al interactions for aluminium toxicity to Atlantic salmon, Salmo salar L. are investigated in Chapter 6.

5.5 Summary

Silicic acid will complex aluminium in dilute solution over a wide pH range. The nature of the resultant aluminosilicate was dependent on both the pH and the silicic acid concentration. The relative proportions of aluminium and aluminosilicate species changed with time for equilibration. $Al(OH)^{-}_{4(aq)}$ was the most likely monomeric aluminium species to bind silicic acid to give stable aluminosilicate structures similar to the proto-imogolite structures suggested by Farmer and Fraser (1982).

CHAPTER SIX: THE AMELIORATION OF ALUMINIUM TOXICITY BY SILICIC ACID

6.1 Introduction

The toxic nature of aluminium in biological systems is a product of its solution chemistry. The amelioration of aluminium toxicity involves the manipulation of the chemistry to abolish or reduce its biological availability. This part of the study investigated the acute toxicity of aluminium to Atlantic salmon and its short-term (96 hours) amelioration using silicic acid.

In Chapter Five, silicic acid was shown to interact with the monomeric aluminium hydroxy species, and in particular the aluminate anion, $Al(OH)^{-}_{4(aq)}$, to different degrees of stability. The aim of the present work was to determine the relative toxicities of the results of these Al/Si interactions and compare them with the toxicities of uncomplexed aluminium species.

6.2 Materials and Methods

All toxicity trials were carried out in a constant temperature room maintained at 15°C $\pm$ 0.5°C. Analytical procedures were carried out in a water quality laboratory at room temperature, (ca 25°C).

6.2.1 Preliminary Investigations into the use of Silicic Acid to Ameliorate Aluminium Toxicity to Atlantic Salmon, Salmo salar.

Short exposure, (12 hours), static tests were used to test the hypothesis that silicic acid would ameliorate acute aluminium toxicity. Five treatments were set up in acid-washed 10 litre perspex aquaria (Table 6.2.1). Ions were added as $Ca(NO_3)_2.4H_2O$, $Mg(NO_3)_2.6H_2O$ and $Al(NO_3)_3.9H_2O$ (All Aristar: BDH Ltd.). Silicon was added as Na_4SiO_4 (Reagent: AlFa).

Solutions were equilibrated to pH5.0, and 20 fish, ($\bar{x}$ = 0.80g, range $\pm$ 0.15g), were introduced into each treatment. Water temperature was maintained at 15°C $\pm$ 0.5°C and solutions were aerated gently throughout the exposure period.

All mortalities were noted and death was characterised by a cessation of the opercular beat. Dead fish were removed immediately.

6.2.2 The Through-Flow System

Static toxicity tests are unsuitable for longer-term aluminium toxicity studies. The need for constant environmental conditions and replicate homogeneity within treatments and the minimisation of additional stressors

Table 6.2.1 Nominal concentrations of Ca^{2+}, Mg^{2+}, total Al and total Si in each of the treatments 1 to 5.

Composition / Treatment	$Ca^{2+}_{(aq)}$ mgl^{-1}	$Mg^{2+}_{(aq)}$ mgl^{-1}	Al_t mgl^{-1}	Si_t mgl^{-1}
1. Control	2.00	0.25	0.0	0.0
2. No $Si_{(aq)}$	2.00	0.25	0.50	0.00
3. Low $Si_{(aq)}$	2.00	0.25	0.50	0.50
4. Med.$Si_{(aq)}$	2.00	0.25	0.50	2.50
5. High $Si_{(aq)}$	2.00	0.25	0.50	10.00

such as handling and fright, precludes the use of static systems. Where chemical speciation is integral to the trial, a static system may soon misrepresent the important equilibria, perhaps through the build up of concentration gradients in poorly mixed solutions.

These types of consideration are particularly pertinent to studies involving aluminium and especially in oversaturated solutions. It is not possible to fully mimic 'natural' water conditions in a controlled environment dosing a synthetic water, however, the use of a flow through system does help to achieve optimal test conditions with a good degree of stability over time.

The following discussion covers each individual aspect of the system used in these studies and the rationale behind their use. A schematic representation of the through-flow system is presented in Figure 6.1

6.2.2.1 The Synthetic Water

The experiments were performed using a synthetic water, (Ca^{2+} ca 2mgl^{-1}, Mg^{2+} ca 0.25mgl^{-1}, Na^{+} ca 1mgl^{-1}, K^{+} ca 0.1mgl^{-1}, conductivity, ca 40μScm^{-1}, temperature ca 15°C and pH ca 5.00) derived from the multistage treatment of Stirling mains tapwater.

Tapwater was run into reservoir A (see Figure 6.1) and

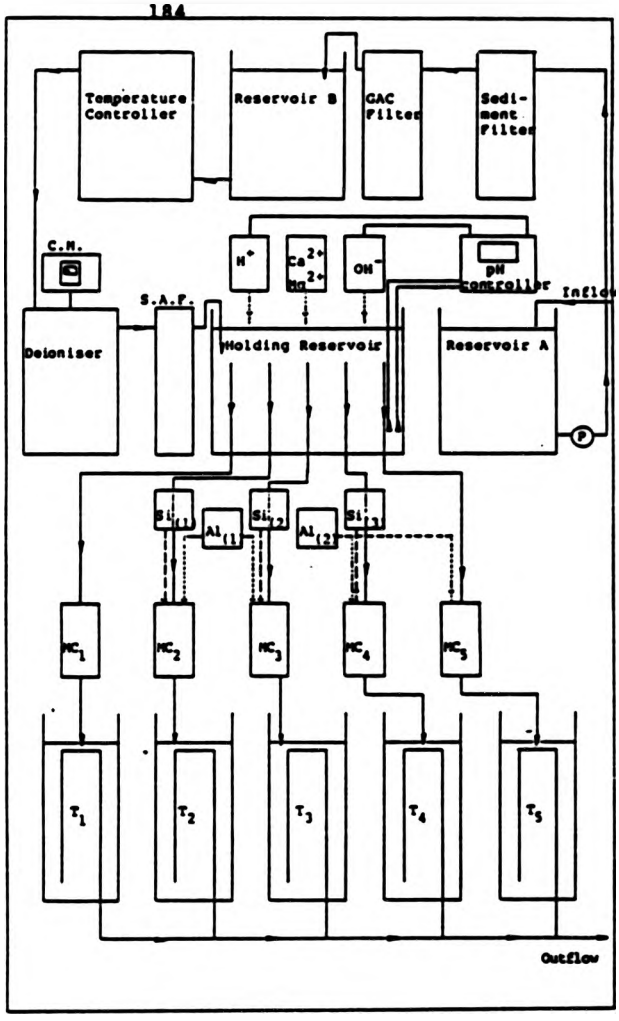


Figure 6.1. Diagramatic representation of the through-flow system used in the toxicity studies. C.M.-conductivity meter, S.A.F. - silicic acid filter, P-pump, Si(1-3) - silica stock solutions, Al(1-2) - aluminium stock solutions, MC(1-5) - mixing chambers, T(1-5) - treatment tanks,

—▶—, water flow and direction,

-----▶, chemical flow and direction.

aerated vigorously to aid the removal of residual free chlorine. The water was then pumped through a sediment/granular activated carbon combined filter for the removal of particulates (> 1um in diameter), free chlorine, trace organics and some inorganic species. The water was then collected in reservoir B where aeration was continued as a precautionary measure. The water was then pumped through a custom built temperature controller (Swan Environmental Ltd.) which maintained outflowing water at 15°C ± 0.5. Water was then passed through a mixed bed deioniser (Seradest SF4000:BDH Ltd.) producing product water with a conductivity less than $0.5 uScm^{-1}$, and then finally a silicic acid filter, (BDH Ltd.) which reduced total Si levels to less than $5 \mu gl^{-1}$ before being collected in the holding reservoir. The holding reservoir was made from acid washed, high density polyethylene, completely covered in an insulating layer of polystyrene and of 200 litres capacity.

The reservoir water was then dosed, using a peristaltic pump, (202U/AA:Watson-Marlow Ltd.) with calcium and magnesium from a 4 litre stock solution, $25.5gl^{-1}$ $Ca(NO_3)_2 \cdot 4H_2O$ (Aristar:BDH Ltd.) and $5.75gl^{-1}$ $Mg(NO_3)_2 \cdot 6H_2O$ (Aristar:BDH Ltd.) made up in double-distilled water, to give a final calcium concentration of ca $2mgl^{-1}$ and a final magnesium concentration of ca $0.25mgl^{-1}$ in the make-up water. An automatic pH controller, (EIL 9143:Kent Inst. Ltd.) maintained the synthetic water pH at 5.00 ± 0.05 through

the additions of 0·1M NaOH (Aristar:BDH Ltd.) and 0·1M HNO_3 (Aristar:BDH Ltd.). These acid-base titrations were responsible for introducing both Na^+ ca $1.0mgl^{-1}$ and K^+ ca $0.1mgl^{-1}$, to the synthetic treatment water.

The water in the holding reservoir was well mixed through the use of controlled aeration.

6.2.2.2 The Treatments

The synthetic water was dosed at $0.16\ lmin^{-1}$ from the holding tank to each of the treatment tanks using a combination of a constant head and gravitational flow. Flow rates were equivalent to 2 litres of water per gram of fish per day. The flow was considered optimal with respect to the oxygen requirements of the experimental salmon fry (Sprague 1969). The water replacement time for each treatment tank was 12 hours for 90% replacement, (about 30 hours for 99% replacement). This value was in agreement with values previously recommended for salmonid toxicity trials (Alabaster and Abram 1965).

Before entry to the treatment tanks, the synthetic water percolated through an acid washed perspex mixing chamber filled with silicone rubber chips. Aluminium and/or Si solutions were dosed peristaltically into these chambers to allow mixing of the two or three fractions before they entered the treatment tanks.

Aluminium was dosed as aluminium nitrate, $Al(NO_3)_3 \cdot 9H_2O$, (Aristar:BDH Ltd.) from a stock solution ($0.96gl^{-1}$) adjusted to pH5.0 and aged for 168 hours prior to use. During ageing and dosing, aluminium solutions were stirred continuously, though not aggressively, using magnetic stirrers. Silicon was dosed as sodium orthosilicate, Na_4SiO_4, (reagent grade: AlFa) from stock solutions, ($4.5gl^{-1}$, $1.1gl^{-1}$ and $0.2gl^{-1}$) made up freshly and adjusted to pH5.0 before use. $Si_{(aq)}$ stock solutions were stirred during dosing.

The five treatment tanks were made of high density polyethylene, acid washed before use and insulated with polystyrene. Each held 60 litres of treatment water, a swinging arm outlet controlling the level. Gentle aeration in each tank improved mixing and helped to maintain optimal levels of dissolved oxygen of $\geq 10mgl^{-1}$. From the treatment tanks all water went to waste. The nominal ionic composition of the treatment tanks 1 to 5 is shown in Table 6.1.

6.2.3 Experimental and Sampling Procedures

The flow through system was run for 48 hours continuously before any fish were introduced into the treatment tanks. Physical and chemical parameters were measured during this time to ensure that the complete system was operating efficiently. Acceptable levels of stability were usually achieved within 24 hours and maintained within acceptable limits thereafter.

Table 6.1 Predicted nominal values for the measured physical and chemical parameters in each treatment tank.

Tank/Treatment / Parameter	1 Control	2 High[$Si_{(aq)}$]	3 Med.[$Si_{(aq)}$]	4 Low[$Si_{(aq)}$]	5 Al only
Aluminium $\mu g l^{-1}$	0.0	200.0	200.0	200.0	200.0
Silicon $\mu g l^{-1}$	0.0	4000.0	1000.0	200.0	0.0
Calcium $mg l^{-1}$	2.0	2.0	2.0	2.0	2.0
Magnesium $mg l^{-1}$	0.25	0.25	0.25	0.25	0.25
Sodium $mg l^{-1}$	1.0	1.0	1.0	1.0	1.0
Potassium $mg l^{-1}$	0.1	0.1	0.1	0.1	0.1
pH	5.00	5.00	5.00	5.00	5.00
Temperature °C	15.0	15.0	15.0	15.0	15.0

6.2.3.1 The Experimental Fish

Experiments were carried out on Atlantic salmon, Salmo salar, fry (mean weight 1.00g, range ± 0.25g), obtained two weeks prior to experimentation from Howietoun fish farm. All fish had completed yolk sac resorption and had successfully made the transition from endogenous to exogenous feeding.

During the first week after collection the fish were held in synthetic water of the same ionic composition as the control treatment tank, (Table 6.1), at pH6.50-7.00. Thereafter the pH was gradually reduced to and maintained at pH5.00 ± 0.10, through the manual additions of dilute acid or base as appropriate. The salmon were acclimated to these conditions for up to 168 hours before the onset of experimentation. At the beginning of each trial, 120 salmon were introduced in a randomised manner, (Sprague 1969), into each of the treatment tanks.

6.2.3.2 Sampling

Water samples were taken every 12 hours for aluminium, silicon and pH and every 24 hours for calcium, magnesium, sodium, potassium and total ammonia. Samples were taken in acid-soaked polyethylene bottles and collected from the outflow of each tank to avoid the unnecessary disturbance of the salmon. Because sampling of the salmon involved stress and disturbance it was reduced

to a practical minimum. Dead fish were recorded and removed every 12 hours. Live and/or moribund fish were sampled for digestion and histological analyses also every 12 hours. Dead salmon are known to decompose rapidly and consequently were not used for digestion or histological analyses. Moribund and healthy salmon were killed by a sharp flick to the head. Whole salmon were washed, briefly, in 5% (V/V) HNO_3 (Aristar:BDH Ltd.), using a distilled-water bottle, to remove surface adsorbed Al and/or Si, packed individually in polyethylene sachets and frozen.

The anterior portion of sampled fish was stored in 10% phosphate-buffered formalin for future histological preparation and analysis.

6.2.3.3 Sample Treatment and Analysis

(i) pH

Sample pH was measured with a digital meter with built in temperature compensation, (PW9409: Philips) and a standard glass electrode, (CWL:Russell pH Ltd.).

(ii) Aluminium

Total aluminium was fractionated to give filtered and exchangeable aluminium, (see Chapter 2 for details).

After fractionation samples were stored in acid-washed polyethylene bottles in a matrix of 1% (V/V) HNO_3, (Aristar:BDH Ltd.) at 4°C. The acid matrix was successful in reducing sample losses through adsorption. Total Al was measured by graphite furnace atomic absorption spectroscopy using the program described in Chapter 2.

(iii) Silicon

Samples were pre-filtered through 0.45µm membrane filters, (Ultipor,N66.Pall) to remove particulates and then stored in acid-washed polyethylene bottles in a matrix of 1% (V/V)HNO_3 (Aristar:BDH Ltd.) at 4°C. Total silicon was determined by graphite furnace atomic absorption spectroscopy using the program described in Chapter 4.

(iv) Calcium, Magnesium, Sodium and Potassium

Samples were stored in acid-washed polyethylene sample bottles, in a matrix of 1% (V/V) HNO_3 (Aristar:BDH Ltd.) at 4°C. Total levels of each were determined using atomic absorption flame spectroscopy as described in Golterman et al (1978).

(v) Total Ammonia

Samples were filtered through 0.45µm membrane filters, (Ultipor, N66.Pall) to remove particulates and then stored frozen in acid-washed polyethylene bottles.

The total ammonia content of thawed samples was determined colorimetrically using a modification of the salicylate-dichloroisocyanurate reaction in the presence of nitro-prusside, (Crooke and Simpson 1971) and automated with an autoanalyser (Technicon, Autoanalyser II).

6.2.3.4 Tissue Digestion and Analysis

The whole body content of both aluminium and silicon in the salmon fry was determined by the following method. The whole salmon were thawed and excess water removed with an absorbant paper. Fish were then dried slowly at 50°C to constant weight. Slow drying, 24-48 hours, at low temperature reduced the probability of Al and/or Si loss due to inorganic and/or organic volatiles. The dry mass, of known constant weight, (usually about 0.2g) was then digested in teflon bombs using $5cm^3$ of concentrated HNO_3 (Aristar: BDH Ltd.), and moderate heating on a hot plate for one hour. The sealed teflon bombs greatly reduced sample loss due to acid fuming and prevented sample contamination. The digestant was allowed to cool, before dilution with double-distilled water to a total sample volume of $50cm^3$. The samples were then stored for analysis in acid-washed polyethylene bottles at 4°C.

Both aluminium and silicon in the samples were determined by graphite furnace atomic absorption spectroscopy using the programs cited earlier (Chapters 2 and 4).

6.2.3.5 Histology and Microphotography

The anterior half, dorsal fin to head, of each sampled fish was fixed in 10% phosphate-buffered formalin, for a minimum of 24 hours. Samples could be successfully stored in this manner for up to one year. The specimens were then dehydrated using a series of graded alcohol solutions, cleared in chloroform and impregnated with molten wax.

After mounting the specimens in wax blocks, 5um sections were cut on a microtome and mounted on glass slides ready to be stained.

A standard haemotoxylin stain was modified to specifically locate aluminium in or on fish tissues. A traditional form of this extensively used stain incorporates high concentrations of aluminium to act as a mordant and bind haematein, (oxidised haematoxylin), to the tissue. If aluminium is omitted from the stain format then aluminium on or in the tissue under investigation will act as the mordant and bind haematein.

The stain stock solution was made from 2.00g of haematoxylin (Anhydrous, certified) and 0.20g of sodium iodate (Analar grade:BDH Ltd.) dissolved in one litre of double-distilled water and buffered to ca pH6.0 using 0·1M $NaOH_{(aq)}$ (Aristar: BDH Ltd.). The resultant solution had a rich amber colour

and stained aluminium purple. The stain was readily oxidised in air and fresh stain was made up for each use.

Slides were immersed in the stain for one hour, unstained material took up a straw yellow colouration and aluminium stained purple.

This stain has also been used to locate aluminium by Havas (1986) though on fresh, unprepared tissues. Havas (1986) showed that the stain would also colour iron, reddish-brown, lead, grey and copper, pink.

Several counterstains were tried with the haematoxylin stain to enhance the tissue structures,however all the stains tried masked the aluminium staining.

Photomicrographs of the stained and unstained tissues were taken using light microscopy (Orthomat; Zeiss) employing a 50 ASA colour slide film (Ektachrome 50; Kodak).

6.2.3.6 Biometrical Analysis

The following statistical analyses were performed in the evaluation of the statistical significance of the results.

(i) The F-test, two-tailed, was used to test the significance of differences between two variances.

F = larger variance/smaller variance

Significance was tested at P = 0.05, ($F_{0.025}$).

(ii) Where variances of means were not significantly different, statistical comparisons between treatment groups with the same number of observations were made using:

"Students" t-test for unpaired data.

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{S(\bar{x}_1 - \bar{x}_2)}}$$

$\bar{x}_1$ and $\bar{x}_2$ are the mean values of treatment groups.

S - standard deviation of the difference between these means.

$$SD = \frac{x^2 - (\Sigma X)^2/n}{n-1} \qquad n = \text{no. of observations.}$$

(iii) The product momentum correlation coefficient, r, was calculated as an index of y variation with x and judged against a significance level of P = 0.05.

$$r = \frac{\Sigma(x-\bar{x}) \cdot (y-\bar{y})}{\sqrt{\Sigma(x-\bar{x})^2 \cdot \Sigma(y-\bar{y})^2}}$$

(iv) Probit analysis was used in the determination of ET_{50}, (Colquhoun 1971).

(v) Confidence limits were determined for regression lines, (95%).

$$CL\ (\hat{Y}_i) = \bar{Y} + bx_i \pm (t0.05 \cdot Sy \cdot Xi).$$

The statistical treatments used are given in many standard texts (Li 1964, Clarke 1980, Sokal and Rolfe 1987).

6.3 Results

6.3.1 Static Toxicity Trial

Mortalities were recorded in treatment 2. (No $Si_{(aq)}$) and treatment 3 (Low $Si_{(aq)}$)(Table 6.3.1). Salmon fry in the remaining treatments, 1. (Control), 4. (Medium $Si_{(aq)}$) and 5. (High $Si_{(aq)}$), showed no visible signs of stress during the 12 hour exposure period.

Silicic acid was found to abolish the acute toxicity of aluminium at pH5.0, and the concentration of silicic acid was a critical factor in the amelioration.

6.3.2 The Flow-Through Toxicity Study

6.3.2.1 The Experimental Conditions

Near nominal levels of all physical and chemical water quality

characteristics were achieved in each treatment for the duration of the trial (Table 6.1). Ca, Mg, Na, K, (Table 6.2) and Si, (Table 6.4) concentrations were approximately constant and did not vary significantly (t-test $P \geq 0.05$), with respect to time within treatments (all parameters) or across treatments (excepting Si). Nominal Si levels were not achieved in treatments 2 and 3, however, Si levels in all treatments were significantly, (t-test: $P < 0.01$) different from each other.

Water temperature and pH were constant ($\bar{x} = 15 \pm$ range, 1°C and $\bar{x} = 4.95 \pm$ range 0.05) both within and across treatments for the duration of the trial (Table 6.5). Treatment tank total aluminium levels were generally sub-nominal, (Tables 6.3 and 6.6). Excepting treatment 2 and the control treatment 1, across treatment total aluminium levels were not significantly different, (t-test: $P \geq 0.05$), over the trial period. The mean total aluminium in treatment 2 was significantly, (t-test: $P < 0.05$), higher than the mean total aluminium levels in treatments 4 and 5 (Table 6.3). Within all treatments, mean total, filtered and exchangeable aluminium fractions were significantly, (t-test: $P < 0.05$) different from each other. Across treatments, exchangeable -Al was the highest in treatment 2, (high Si treatment), and lowest in treatment 5 (no Si treatment).

Filtered-Al was lowest in treatment 2, (range 6-21 $\mu g l^{-1}$) compared with treatments 3, 4 and 5, (range 12-30 $\mu g l^{-1}$).

Table 6.2 Determined ionic composition of the test water in the experimental tanks over the 96 hour exposure period. n = 60.

Parameter	Concentration mgl^{-1}	
	Mean	Range
Calcium $Ca^{2+}_{(aq)}$	2.14	1.95 - 2.39
Magnesium $Mg^{2+}_{(aq)}$	0.45	0.21 - 0.51
Potassium $K^{+}_{(aq)}$	0.25	0.10 - 0.45
Sodium $Na^{+}_{(aq)}$	5.65	1.95 - 9.91
Total Ammonia	0.50	0.37 - 0.59

Table 6.3.1 The cumulative mortalities of salmon fry in treatments 1 to 5 during the 12 hour exposure period.

Exposure Hours / Treatment	1	2	3	4	5	6	7	8	9	10	11	12
1.Control	0	0	0	0	0	0	0	0	0	0	0	0
2.No $Si_{(aq)}$	0	1	2	5	6	10	20					
3.Low $Si_{(aq)}$	0	0	1	3	7	10	15	16		19	19	20
4.Med.$Si_{(aq)}$	0	0	0	0	0	0	0	0	0	0	0	0
5.High $Si_{(aq)}$	0	0	0	0	0	0	0	0	0	0	0	0

Table 6.3 Mean levels of each aluminium fraction for each treatment during the 96 hour exposure period. $\bar{x}$ - mean, SD - standard deviation, n = 81.

		Aluminium Concentration $\mu g l^{-1}$		
Treatment	Al Fraction	$\bar{x}$	SD	Range
1.Control	Total	23.2	2.0	21 - 26
	Exchangeable	5.4	1.0	4 - 7
	Filtered	5.1	0.9	4 - 7
2.High $Si_{(aq)}$	Total	192.6	21.8	172 - 234
	Exchangeable	162.4	24.1	132 - 211
	Filtered	12.8	5.2	6 - 21
3.Med.$Si_{(aq)}$	Total	181.2	17.0	165 - 221
	Exchangeable	135.1	12.6	118 - 165
	Filtered	25.9	3.1	21 - 30
4.Low $Si_{(aq)}$	Total	162.8	9.7	150 - 180
	Exchangeable	110.7	7.9	100 - 123
	Filtered	19.6	3.7	12 - 23
5. Al only	Total	168.6	15.9	155 - 212
	Exchangeable	41.4	7.7	32 - 55
	Filtered	20.4	2.5	16 - 24

Table 6.4 Mean levels of total silicon in each treatment tank during the 96 hour exposure period. $\bar{x}$ - mean, SD - standard deviation, n = 81.

Treatment	Total Silicon Concentration μgl^{-1}		
	$\bar{x}$	SD	Range
1. Control	18.7	2.9	13 - 24
2. High $Si_{(aq)}$	2615.1	225.1	2392 - 3111
3. Med.$Si_{(aq)}$	699.5	23.1	666 - 746
4. Low $Si_{(aq)}$	153.3	6.4	144 - 165
5. Al only	17.0	1.6	13 - 20

Table 6.5 Mean pH and temperature levels in the treatment tanks during the 96 hour exposure period. n = 30.

	pH		Temperature °C	
Treatment	Mean	Range	Mean	Range
1.	4.98	4.95 - 5.01	15.0	15.0
2.	4.96	4.92 - 4.98	15.0	14.5 - 15.5
3.	4.93	4.90 - 4.97	14.5	14.0 - 15.0
4.	4.94	4.91 - 4.99	15.0	14.0 - 15.5
5.	4.94	4.92 - 5.00	14.5	14.5

Table 6.6 Mean levels of each aluminium fraction in each trestment at 12 hour intervals during the 96 hour experimental period, $\bar{x}$ - mean, SD - standard deviation, n = 9.

Exposure Period (Hrs.)		0		12		24		36		48		60		72		84		96	
Treatment	Al.Fraction μgl^{-1}	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
1. Control	Total	21	1.3	21	1.3	21	1.4	21	0.5	25	1.6	25	0.5	24	0.9	25	0.9	26	0.9
	Exchangeable	4	0.5	6	1.4	6	0.5	5	0.5	7	0.5	6	1.3	6	0.9	4	0.0	5	1.7
	Filtered	6	0.0	5	0.5	4	0.5	5	0.0	5	1.7	7	0.0	5	0.5	4	0.5	5	0.5
2. High Si(aq)	Total	172	2.2	234	5.0	231	4.9	184	2.8	182	1.9	186	4.1	185	0.5	176	3.4	183	0.5
	Exchangeable	132	0.9	199	1.3	211	1.6	160	1.6	158	0.5	153	3.3	153	1.3	147	2.4	149	0.5
	Filtered	6	0.5	7	0.5	9	1.2	10	0.5	11	0.5	14	2.2	17	0.9	20	0.8	21	0.5
3. Med. Si(aq)	Total	221	0.8	183	1.7	172	6.2	169	3.1	165	2.8	199	6.5	169	3.9	177	4.3	176	1.9
	Exchangeable	139	1.6	118	2.2	127	3.3	126	3.6	132	0.9	165	4.3	130	2.2	142	1.4	137	3.3
	Filtered	21	0.5	22	0.9	23	1.9	25	1.7	26	0.9	28	0.9	29	1.4	29	0.5	30	0.5
4. Low Si(aq)	Total	180	2.2	154	2.5	152	0.0	150	0.9	157	2.6	165	2.6	164	0.5	170	0.5	173	0.5
	Exchangeable	112	1.4	101	2.1	105	3.4	100	0.9	108	0.9	108	5.0	119	1.7	120	0.9	123	1.7
	Filtered	12	0.5	15	0.5	17	0.5	21	0.5	22	1.9	22	0.9	23	2.8	22	0.9	22	0.0
5. Al only	Total	212	2.8	162	0.9	163	0.5	170	1.3	168	2.9	155	4.3	164	2.4	159	2.4	164	0.9
	Exchangeable	43	1.3	34	0.9	32	0.9	36	0.5	37	0.9	37	5.4	49	2.8	50	1.4	55	0.0
	Filtered	19	0.5	16	0.5	17	0.9	21	1.4	22	1.4	21	1.7	22	0.5	22	0.9	24	1.9

The results emphasised Al/Si complexation in treatments 2, 3 and 4.

The exposure period was a determinant of aluminium speciation within treatments (Table 6.6, Figures 6.2,a-d). Total-Al was reduced or remained constant with time in all treatments. Falls in the total aluminium level during the exposure period were not significant ($r:P \triangle 0.05$) and were attributed to adsorptive processes with the salmon and other surfaces. Exchangeable-Al increased with respect to the total-Al level in each treatment and over time. This effect was most prominent in treatments 4, ($r: P < 0.05$) and 5, ($r:P < 0.05$) and was particularly emphasised after 60 hours. In all treatments, filtered Al increased significantly, ($r:P < 0.01$) relative to total-Al and exposure time. This effect was exacerbated by increasing levels of Si.

6.3.2.2 Toxicity

Aluminium, (treatment 5; $\bar{x}$ = 169ugl^{-1} total-Al), was acutely toxic at pH5.0 to salmon fry (Table 6.7, Figure 6.3c), the median survival time (ET_{50}) for which was 21.5 hours $\pm$ 9 hours, (95% CL). Mortality was particularly high 24-36 hours after initial exposure. During this interval 61% of the total population died. The presence of silicic acid ameliorated the toxicity,

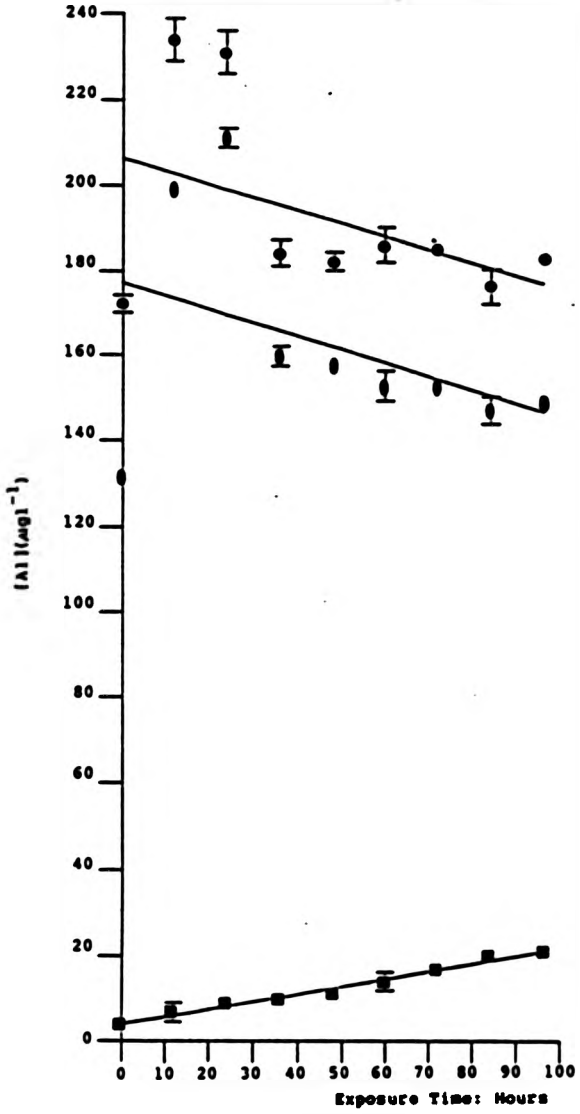


Figure 6.2a. Treatment Tank 2.

Figures 6.2a-d. The concentrations of total, ● exchangeable, ◐ and filtered, ■ aluminium, found in treatment tanks, 2, 3, 4 and 5 during the 96h experimental period. Mean values are plotted, bars show the range, n = 9.

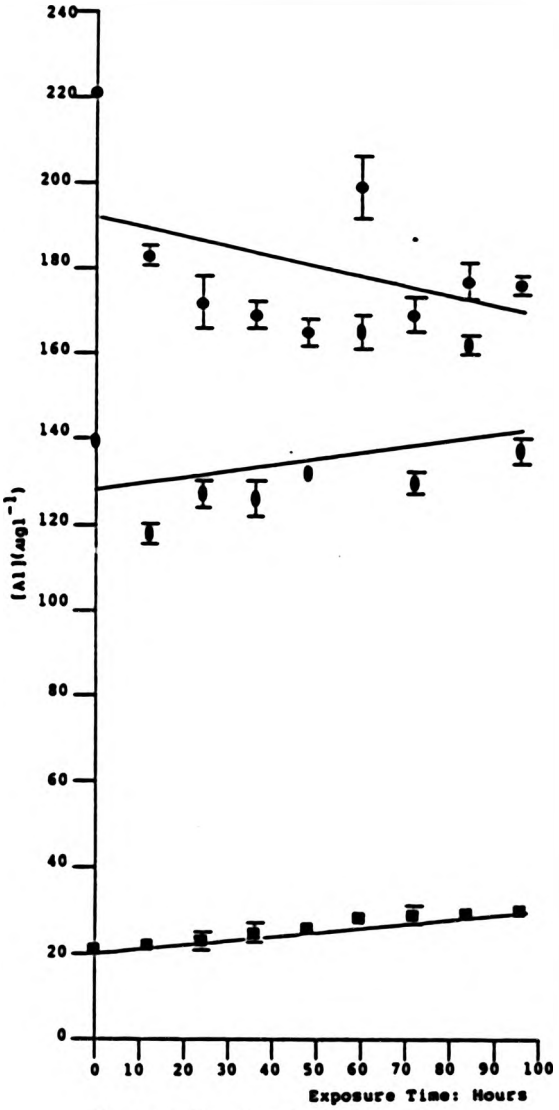


Figure 6.2b. Treatment Tank 3.

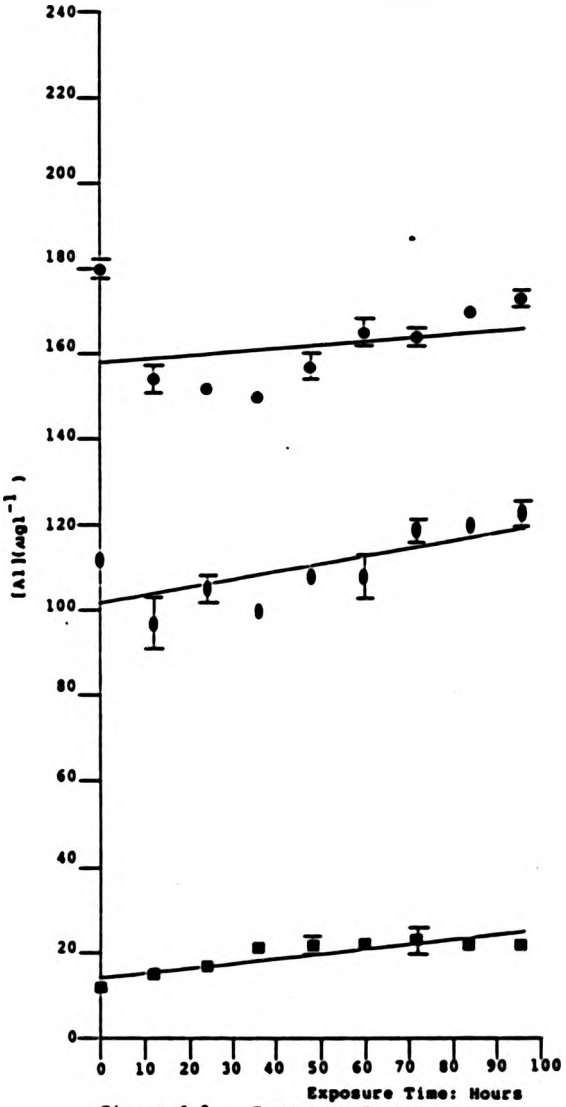


Figure 6.2c. Treatment Tank 4.

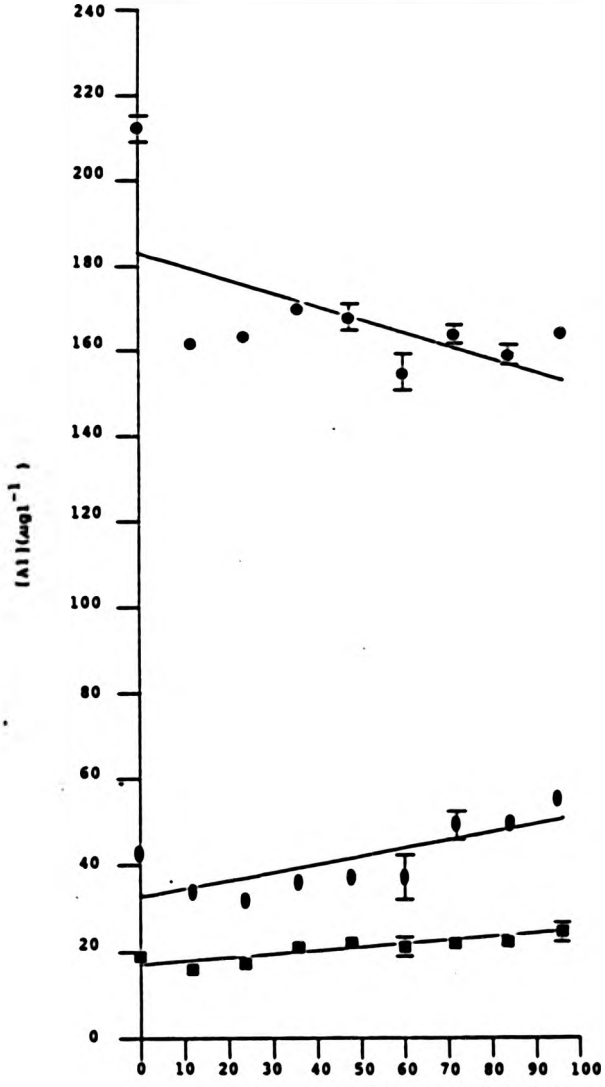


Figure 6.2d. Treatment Tank 5.

(Table 6.7, Figures 6.3a and 6.3b), the extent of the amelioration was dependent on the concentration of silicic acid. In treatment 2, (Total-Al $\bar{x}$ = 193 ugl^{-1}; Total-Si $\bar{x}$ = 2615ugl^{-1}), acute aluminium toxicity was abolished. No mortalities occurred during each of the three replicate trials. The presence of ca 700ugl^{-1} total-Si reduced the toxicity of ca 181ugl^{-1} total-Al (treatment 3), ET_{50} 29.1 hours $\pm$ 12 hours (95% CL) (Figure 6.3a). However, the amelioration was not significant, (t-test:$P \geq 0.10$), with respect to treatment 5. Again mortality was highest 24-36 hours after initial exposure during which interval about 58% of the total population died.

In treatment 4 (Figure 6.3b), ca 153ugl^{-1} total-Si reduced the toxicity of ca 163ugl^{-1} total-Al, ET_{50} 38.5 hours $\pm$ 10 hours (95% CL) significantly, (t-test: $P < 0.1$) with respect to treatment 5, but insignificantly (t-test: $P \geq 0.1$) with respect to treatment 3. Mortality was highest 24-36 hours after the initial exposure at around 39%, though the mortality was significantly (t-test:$P < 0.05$) less than in treatments 3 and 5 for the same time interval.

There were no deaths during each of the replicate trials in the control tank, treatment 1.

The results show that the presence of silicic acid can ameliorate acute aluminium toxicity at pH5.0 and 2.0mgl^{-1} Ca and that the degree of amelioration is dependent on both total-Al and total-Si concentrations.

Table 6.7 Mortalities of salmon in each treatment during the 96 hour exposure period. n = 3.

Exposure Interval (Hours)		0-12	12-24	24-36	36-48	48-60	60-72	72-84	84-96	N(%T)
Treatment	Mortality Index									
1.	No.Dead/Interval	No Mortality during the 96 Hour Exposure Period								0(0)
	% Died/Interval									
	Probit of % D/I									
2.	No.Dead/Interval	No Mortality during the 96 Hour Exposure Period								0(0)
	% Died/Interval									
	Probit of % D/I									
3.	No.Dead/Interval	2	22	69	18	9				120(100)
	% Died/Interval	1.7	18.6	71.9	66.7	100				
	Probit of % D/I	3.59	4.08	5.64	5.49	6.46				
4.	No·Dead/Interval	2	9	47	36	17	9			120(100)
	% Died/Interval	1.7	7.6	43.1	58.1	65.4	100			
	Probit of % D/I	3.59	3.76	4.80	5.24	5.45	6.46			
5.	No·Dead/Interval	6	34	73	7					120(100)
	% Died Interval	5.0	29.8	91.3	100					
	Probit of % D/I	3.69	4.41	6.21	6.46					

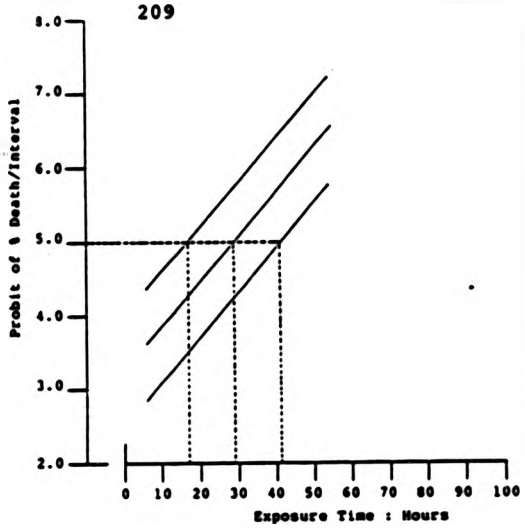


Figure 6.3a. An estimation of the median survival time (ET_{50}) of salmon in treatment 3, using the probit transformation and 95% confidence limits.
ET_{50} = 29.1h ± 12h. n = 3.

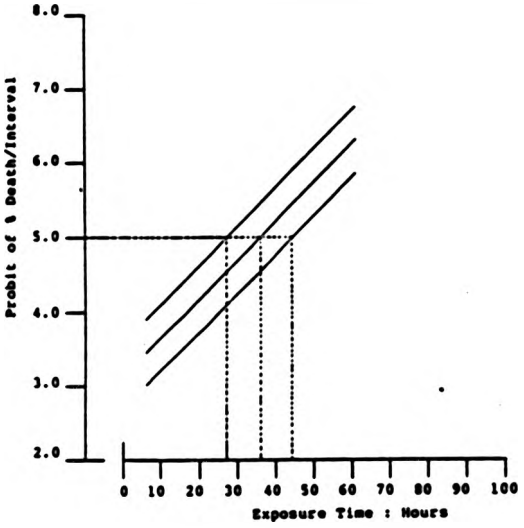


Figure 6.3b. An estimation of the median survival time (ET_{50}) of salmon in treatment 4, using the probit transformation and 95% confidence limits.
ET_{50} = 38.5h ± 10h. n = 3.

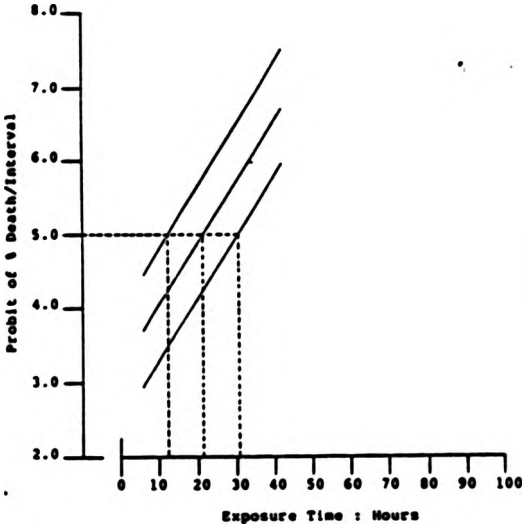


Figure 6.3c. An estimation of median survival time for treatment 5, using the probit transformation and 95% confidence limits. ET_{50} = 21.5h ± 9h. n = 3.

6.3.2.3 Tissue Digestion Analyses

Aluminium was found associated at above control treatment levels in salmon from treatments 3, 4 and 5 and the net increases of associated aluminium in salmon from each treatment were significant, (r: $P < 0.05$) with respect to the exposure period, rising to about 62, 88 and 42 $\mu g g^{-1}$ for treatments 3, 4 and 5 respectively, (Table 6.8, Figure 6.4). Aluminium levels in salmon from treatments 1 and 2 were very low, range: $\bar{x}$ = 4.29-19.33$\mu g g^{-1}$, and in treatment 2 did not vary significantly, (r: $P > 0.05$), from pre-exposure levels. In the control treatment aluminium associations increased significantly, (r: $P < 0.05$), from the pre-exposure levels rising to about 16$\mu g g^{-1}$ after 96 hours exposure. The rate of aluminium association with the salmon from treatments 3, 4 and 5, was most rapid during the first 12 hours of exposure and was 5.0, 3.3, and 9.8$\mu g g^{-1}$ hr^{-1} for treatments 3, 4 and 5 respectively. These projected rates of association correlate closely to the median survival times of the salmon in the respective treatment tanks, (Figure 6.5). After 12 hours exposure, tissue aluminium levels were reduced in treatments 3 and 5 and stabilised in treatment 4 and all assumed a level of about 40$\mu g g^{-1}$. Thereafter tissue aluminium levels in salmon from all treatments except treatment 2 continued to rise, though particularly so in salmon from treatment 4.

The association of Si with whole salmon during the 96h exposure period was significantly (r: $P < 0.05$) higher in

Table 6.8 Whole body aluminium levels, $\mu g g^{-1}$, of moribund (treatments 3, 4 and 5) and healthy fish (treatments 1 and 2) during the 96 hour experimental period. $\bar{x}$ - mean, SD - standard deviation. n = 9.

Exposure Hrs.	0		12		24		36		48		60		72		84		96	
Treatment	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
1. Control	5.15	0.29	5.07	0.17	9.17	2.09	14.68	3.19	5.70	1.89	7.88	0.77	19.33	3.41	18.53	2.28	15.46	3.55
2. High $Si_{(aq)}$	4.29	1.07	9.20	0.80	11.83	2.34	12.24	2.39	12.59	4.39	11.71	2.53	10.86	2.74	8.89	2.25	10.22	3.48
3. Med.$Si_{(aq)}$	5.17	0.51	59.27	4.13	46.03	11.72	52.45	11.63	55.59	7.55	61.87	12.83						
4. Low $Si_{(aq)}$	5.96	0.99	39.98	7.99	42.68	3.46	66.81	11.92	74.55	6.76	91.06	1.70	88.19	5.85				
5. Al only	5.11	1.15	118.28	15.10	41.45	10.64	55.84	10.24	41.89	4.27								

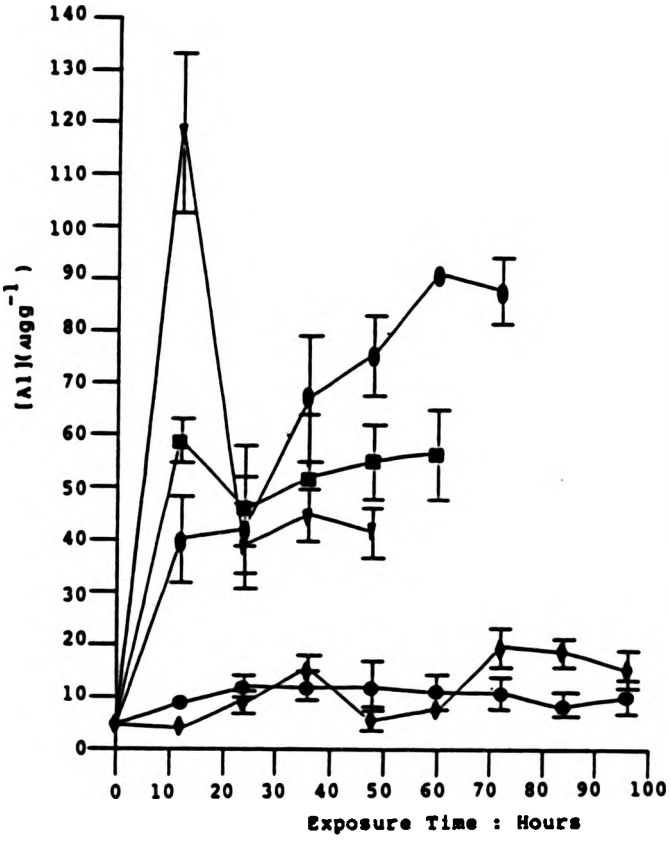


Figure 6.4. The whole-body composition of aluminium in salmon from treatments 1,◊ 2,● 3,■ 4,▯ and 5 ▼ during the 96h exposure period. Mean values are plotted, bars indicate the range, n = 9.

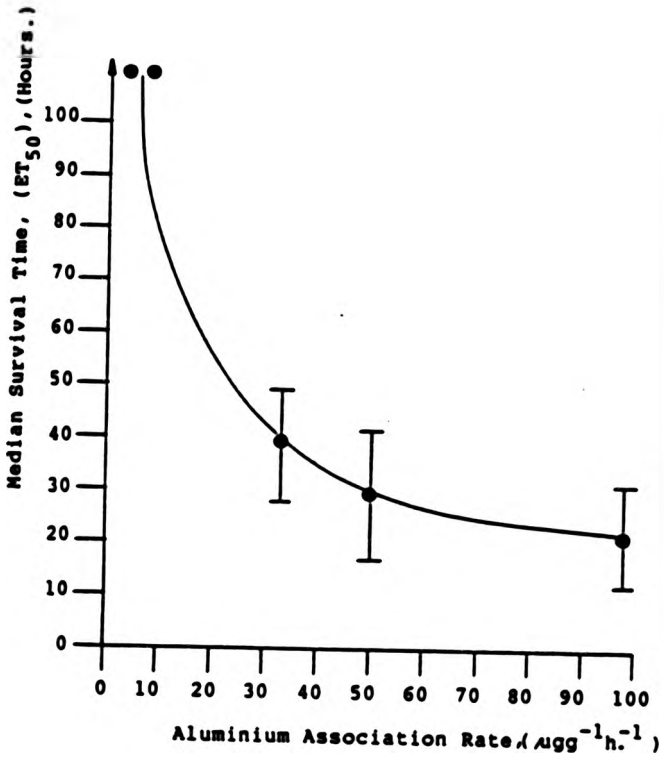


Figure 6.5. The relationship between acute aluminium toxicity and the rate of whole-body aluminium association during the first 12 hours of exposure. Mean values are plotted, bars indicate the 95% confidence limits, n = 3.

treatments 2, 3 and 4 than the control and treatment 5 (Table 6.9, Figure 6.6). The association of Si in salmon from treatments 2, 3 and 4 reached a plateau at about 23$\mu g g^{-1}$ for treatment 2 and 12-13$\mu g g^{-1}$ for treatments 3 and 4, after 36, 60 and 72 hours for treatments 3, 4 and 2 respectively. There was no evidence to suggest that Si was acutely toxic to the salmon fry.

6.3.2.4 Histological Analyses

The gill tissue of salmon from each treatment was examined histologically and photomicrographs of the results are shown in Figure 6.8a-j. The gill tissue of the control salmon (treatment 1) was typical of salmonid fry, (Morgan and Tovell 1973), straight primary lamellae with equally spaced secondary lamellae, (Figures 6.8a-b). There was evidence of interlamellar epithelial hyperplasia and some distal hypertrophy of epithelial cells of secondary lamellae. Chloride cells were evident in both the interlamellar spaces and on the secondary lamellae.

The effect of aluminium was dramatic in the absence of silicic acid (Figures 6.8c-d). All regular lamellar structure was lost and sloughed damaged cells were particularly evident. Primary lamellae were thickened by extensive hyperplasia and distinguishable secondary lamellae were highly vacuolated and mucous and chloride cells proliferated. The presence of low concentrations of silicic acid

Table 6.9 Whole body silicon levels, $\mu g g^{-1}$, of moribund (treatments 3, 4 and 5) and healthy fish (treatments 1 and 2) during the 96 hour experimental period. $\bar{x}$ - mean, SD - standard deviation, n = 9.

Exposure Hrs.	0		12		24		36		48		60		72		84		96	
Treatment	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
1. Control	2.47	0.60	1.60	0.93	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2. High $Si_{(aq)}$	2.51	0.19	2.87	0.82	7.27	2.58	11.98	1.93	16.88	1.32	19.35	0.89	22.82	0.69	23.13	1.52	21.82	3.27
3. Med.$Si_{(aq)}$	3.01	1.15	3.87	0.91	10.27	2.30	12.12	4.23	12.11	2.14	11.02	1.62						
4. Low $Si_{(aq)}$	2.55	0.41	3.69	0.60	6.56	2.24	8.11	1.16	10.85	3.50	13.19	0.67	13.02	2.32				
5. Al only	2.39	0.60	1.73	0.28	1.44	1.13	2.77	0.74	2.93	1.01								

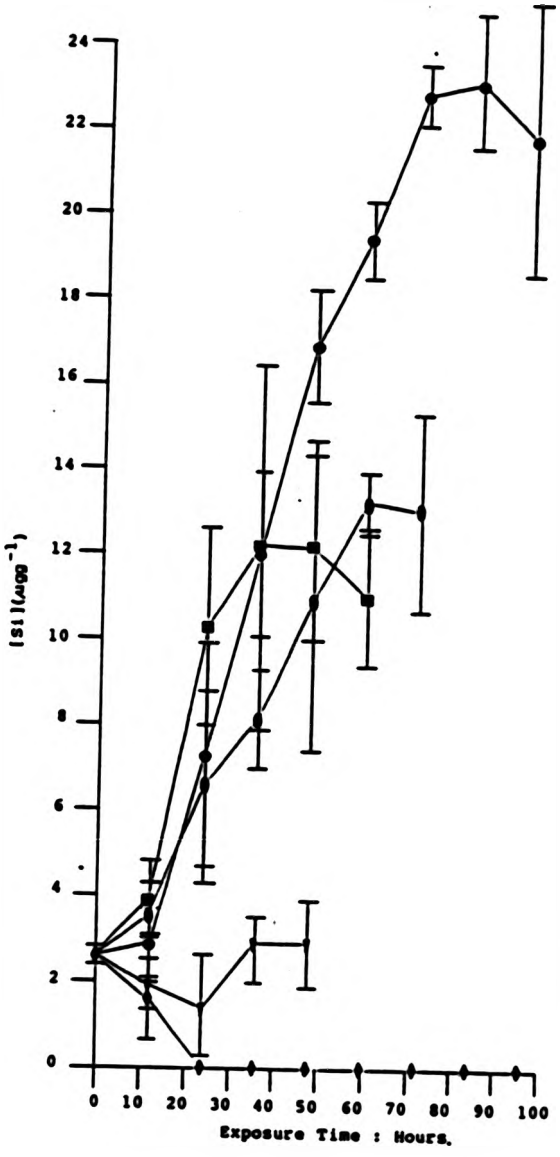


Figure 6.6. The whole-body composition of silicon in salmon from treatments 1, ◆ 2, ● 3, ■ 4, ◐ and 5 ▼ during the 96h exposure period. Mean values are plotted, bars indicate the range, n = 9.

Figure 6.8a. Section through the primary and secondary lamellae of the gill of salmon fry from the control treatment. pl - primary lamella, sl - secondary lamella, Mag.X250.

Figure 6.8b. Detail of primary and secondary lamellae of a salmon fry gill taken from the control treatment. cc - chloride cell, pc - pillar cell, mc - mucous coat, Mag.X1000.

Figure 6.8c. Section through the primary and secondary lamellae of the gill of a salmon fry taken from the Al-only treatment. pl - primary lamella, sl - secondary lamella, dh - distal hypertrophy, os - oedomatous separation, cc - chloride cell, mc - mucous cell, am - aluminium-stained sloughed material, Mag.X400.

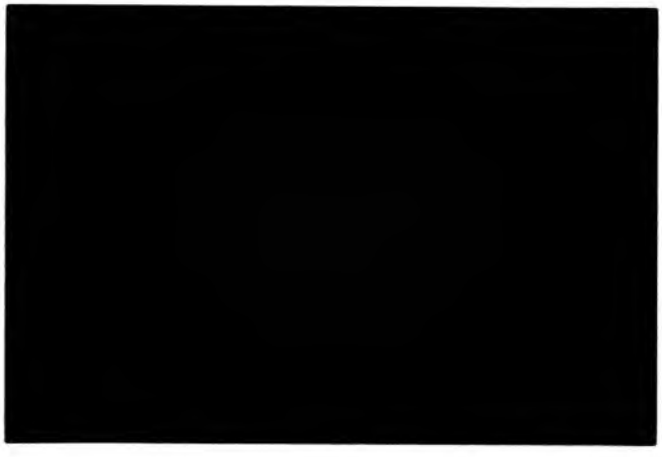


Figure 6.8d. Section through the secondary lamellae of gills taken from salmon fry from the Al-only treatment showing the complete lifting off of the lamellar epithelium. os - oedomatous separation, pc - pillar cell, Mag.X1000.

(treatments 3 and 4) ameliorated the effects of Al over comparative time periods (24 hours).

Characteristic effects evident in salmon from treatments 3 and 4 included fusion of secondary lamellae, (Figure 6.8e) distal hypertrophy of secondary lamellae, (Figure 6.8f), hyperplasia of the interlamellar cells and deterioration of the regular lamellar structure (Figures 6.8e,f).

Gill lamellar structure of salmon from treatment 2 (high total Si) showed none of the disruptions previously attributed to aluminium (Figures 6.8g,h). They were, however, characterised by very large interlamellar mucous cells. Some hypertrophy of proximal secondary lamellar epithelial cells was also evident. The presence of a high concentration of silicic acid completely abolished the gross cellular disruptions of lamellar structure manifested during acute aluminium toxicity.

The modified haematoxylin stain showed aluminium closely associated with lamellar structures and damaged tissue of gills of salmon from treatments 3, 4, and 5. In treatment 5 (Figures 6.8c,i,j), aluminium was associated mainly with damaged sloughed epithelial cells and some adsorption/ binding at the gill surface. Associations with the gill epithelia were particularly evident in the more "intact" gills of salmon from treatments 3 and 4 (Figure 6.8e,f). Aluminium associations were located on both the filament

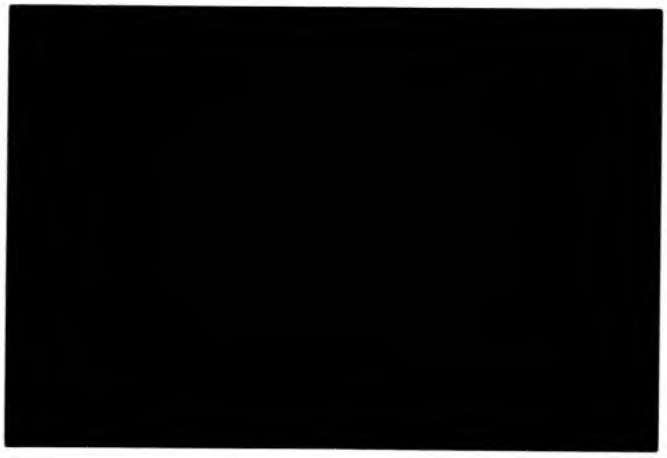


Figure 6.8e. Distal fusion of the secondary lamellae in a gill taken from a salmon fry subjected to high Al and an intermediate level of silicic acid. pl - primary lamella, sl - secondary lamella, mc - mucous cell, aa - aluminium association at gill epithelium, Mag.X1000.

Figure 6.8f. Distal hypertrophy of secondary lamellae in a gill taken from a salmon fry exposed to high Al and a low level of silicic acid. mc - mucous cell, am - aluminium-stained sloughed material, aa - aluminium association at the gill epithelium, Mag.X1000.

Figure 6.8g. Section through the primary and secondary lamellae of a gill taken from a salmon fry from the high Al and high silicic acid treatment. pl - primary lamella, sl - secondary lamella, mc - mucous cell, Mag.X400.

Figure 6.8h. Detail of primary and secondary lamellae of a salmon fry gill taken from the high Al and high silicic acid treatment. mc - mucous cell, ph - proximal hypertrophy, mco - mucous coat, Mag.X1000.

Figure 6.8i. Aluminium associations with both the interlamellar and secondary lamellae epithelia, Mag.X1000.

Figure 6.8j. Aluminium associations with lamellar epithelia and an interlamellar chloride cell. pl - primary lamella, sl - secondary lamella, cc - chloride cell, am - aluminium associated with sloughed material, Mag.X1000.

epithelium and the respiratory epithelium of the secondary lamellae. Aluminium associations with mucous and mucous cells were also evident on selected gill tissues. Aluminium was not positively located intracellularly, however in all sections the blood appeared to stain positively for aluminium.

Aluminium was not positively located either as surface adsorbed or intracellularly in gill tissue of salmon from the control treatment or treatment 2. The high levels of silicic acid present in treatment 2 appeared to have prevented aluminium associations at the gill surface.

Throughout all treatments aluminium was not stained associated with any other tissues except some evidence of surface adsorbed aluminium on bone associated with gills of salmon from treatments 3, 4 and 5.

6.4 Discussion

6.4.1 Acute Aluminium Toxicity

In this study, aluminium at an environmentally significant concentration (Wright and Gjessing 1976), about 169μgl^{-1} total-Al, was acutely toxic to salmon fry at pH5.0 and 2.0mgl^{-1} Ca. This acute toxicity was correlated to the concentration of biologically available aluminium. The nature of this critical aluminium fraction will be

determined by the solution chemistry. The use of a flow-through exposure system enabled the close control of both physical and chemical variants and afforded the assumption that in the aluminium-only treatment, (treatment 5) an aluminium hydroxide phase, such as gibbsite or amorphous $Al(OH)_{3(s)}$ would control aluminium solubility and thereby the biologically available fraction. At pH5.0, solubility with respect to gibbsite would predict a soluble aluminium level ca 60μgl^{-1} and amorphous $Al(OH)_{3(s)}$ ca 1000μgl^{-1}. The soluble fraction in the aluminium-only treatment (5) was in the range 32-55μgl^{-1} (Table 6.3). Gibbsite was the likely solubility control and this control was achieved by a prior ageing of the aluminium stock solutions for 168 hours at pH5.0. Tank aluminium conditions simulated stable water conditions and not the heterogeneity associated with storm flow events where amorphous $Al(OH)_{3(s)}$ is the main solubility control (Goenaga and Williams 1988). At pH5.0, the soluble aluminium fraction in this study was composed of the monomeric aluminium cations, $Al^{3+}_{(aq)}$, $AlOH^{2+}_{(aq)}$ and $Al(OH)^{+}_{2(aq)}$, probably in the proportions 35-70%, 25-35% and 5-30% respectively (see Johnson et al 1981 and Martin 1986).

The absence of aluminium complexing ligands in the aluminium-only treatment should have ensured that the aforementioned species accounted for the soluble aluminium fraction. However membrane filtration,0.04μm,

(Ultipor, N66; Pall) solubility data showed a soluble range of only 16-24μgl^{-1} (Table 6.3). The solubility discrepancy, 16-31μgl^{-1}, highlighted by the use of both exchangeability and filtration as definitions for the soluble fraction, could be explained by aluminium adsorption, onto water-borne particulates, (Goenaga et al 1987). For example, mucous-related substances could complex soluble aluminium, releasing the aluminium to the strongly acid sulphonate groups on the exchange resin but preventing passage of the aluminium through the membrane filter matrix. This adsorbed/bound aluminium fraction, which could account for up to 50% of the total soluble fraction, would only have been biologically available if (i) it could be substituted directly at the gill surface and/or (ii) it was in a dynamic equilibrium with the non-adsorbed soluble fraction. Thus, the soluble aluminium fraction responsible for acute aluminium toxicity in the aluminium-only treatment (5), was found in a probable concentration range of 16-55μgl^{-1}.

Acute aluminium toxicity was correlated with aluminium association/binding at the gill surface. The gill surface carries a net negative charge in acid milieu (Booth et al 1988). Binding constants predict that aluminium will displace Ca^{2+} and Na^{+} from respective binding sites and that aluminium and $H^{+}_{(aq)}$ will compete for weak acid anions at the gill surface (Tam and Williams 1986). The $Al^{3+}_{(aq)}$ species will show the greatest tendency to bind irreversibly at the gill surface at

pH5.0 and was the likely precursor of acute aluminium toxicity in this study. Previous studies have ascribed acute toxicity to the divalent, monomeric species, $AlOH^{2+}_{(aq)}$, (Helliwell et al 1983, Fivelstad and Leivestad 1984, Sadler and Lynam 1987), the rationale for this being the reduced toxicity of aluminium at low pH, $\leq$ 4.50, (Schofield and Trojnar 1980, Baker and Schofield 1982, Hutchinson et al 1987, Battram 1988, Booth et al 1988), and the use of theoretical aluminium hydrolysis equations that predict a predominance of the species at pH around 5.0 (Smith and Hem 1972, Johnson et al 1981, French 1985, Neal et al 1987). The predominant form of soluble monomeric aluminium species in the aluminium-only treatment (5), was likely to be $Al^{3+}_{(aq)}$, (Figures 5.2a, 5.2b), though the proportions will vary according to the solution molarity, (see Johnson et al 1981, Martin 1986). Greatly increased competition from $H^{+}_{(aq)}$ would predict an amelioration of aluminium toxicity at low pH.

Similar ambiguities surround the pH of the interlamellar water, (Dalziel et al 1987, Booth et al 1988, Wood et al 1988a, Wood and McDonald 1988), a criterion that could prove critical in defining acute aluminium toxicity. Present knowledge of aluminium toxicity to salmonids in acid water can be used to accurately predict the pH changes at the gill surface during acute and sublethal aluminium challenge. Two opposing equilibria, (see equations 1 and 2), in the blood, the gill epithelium cell and the mucous layer surrounding the lamellae,

will influence the interlamellar pH. At circum-neutral pH, these equilibria result in the acidification of the interlamellar water by up to 1 pH unit, (Wright et al 1986).

(1) $CO_2 + H_2O_{(l)} \underset{\text{anhydrase}}{\overset{\text{carbonic}}{\rightleftharpoons}} HCO^-_{3(aq)} + H^+_{(aq)}$ pKa 6.08 at 15°C (Boutilier et al 1985)

(2) $NH_3 + H^+_{(aq)} \rightleftharpoons NH^+_{4(aq)}$ pK 9.58 at 15°C (Thurston et al 1979).

Three important physiological occurrences are common to acute aluminium exposure in acid water; (i) an initial blood acidosis (Jensen and Weber 1987, Dalziel et al 1987, Wood et al 1988, Heming and Blumhagen 1988, Walker et al 1988), (ii) the inhibition of active Na^+/NH^+_4 exchange, (Dalziel et al 1985a, 1985b, Booth et al 1988, Wood et al 1988a), and (iii) some 25-40% reduction in carbonic anhydrase activity (Staurnes et al 1984). The initial combined effect of these responses to acute aluminium toxicity will be a very significant reduction in ammonia removal across the gill and some reduction in CO_2 dissolution at the gill surface. Thus the interlamellar pH in gills of salmon from the aluminium-only treatment, (5), would not have deviated from the treatment water pH, (pH ca 5.0) during the initial period immediately after exposure. Subsequently, the build up of excretory products in the blood e.g. NH^+_4 (Witters 1986) will reverse the blood acidosis and the concomitant NH_3 gradient will increase the passive efflux of NH_3, this gradient will be exacerbated

further by NH_4^+ trapping in the mucous layer, (Wright and Randall 1988). Increased efflux of NH_3, pK9.58, will cause a gradual alkalinisation of the interlamellar water, particularly in a poorly buffered acid milieu, as were the conditions in this study.

In the aluminium-only treatment of the present study acute aluminium toxicity was effected during the initial period of blood acidosis probably through $Al^{3+}_{(aq)}$ binding irreversibly to a threshold level (also suggested by Booth et al 1988) of critical anionic sites at the gill surface. Subsequent gill alkalinisation would then have increased competition for binding at the gill surface from the less toxic hydroxy-Al species, however, this would not ameliorate the acute response if the aforementioned toxicity threshold had been surpassed.

The results from the aluminium-only treatment, (5), suggest, therefore, that at pH5.0 a concentration of less than 38μgl^{-1} $Al^{3+}_{(aq)}$ was responsible for the acute toxicity to salmon fry.

These changes in the pH of the interlamellar water may explain the ameliorative effect of pre-exposure to sub-lethal levels of aluminium on the acute toxicity of aluminium. For example, a 10 week sub-lethal (pH5.2, 150μgl^{-1} total-Al), aluminium exposure was shown to abolish the acute toxicity of an aluminium challenge,

(pH4.8, 333μgl^{-1} total-Al), during a 72h exposure period (Wood *et al* 1988a, 1988b). The observation can be explained in terms of aluminium binding at the gill surface. The concentration of $Al^{3+}_{(aq)}$ during pre-challenge period (pH5.2, 150μgl^{-1} total-Al) would not have been sufficient to effect an acute response before the alkalinisation of the interlamellar water prevented further binding of $Al^{3+}_{(aq)}$ to the gill surface. The subsequent aluminium challenge (pH4.8, 333μgl^{-1} total-Al) was not sufficiently stressful to significantly reverse the alkalinisation of the interlamellar water and therefore any binding of $Al^{3+}_{(aq)}$ at the gill surface would not be sufficient to effect an acutely toxic response. This hypothesis could be easily tested either by using a higher total-Al concentration in the challenge test or by introducing an interim period between the sub-lethal exposure and the challenge during which the fish would be kept in circum-neutral water of zero aluminium content. The results of the present study would suggest that these small tests would show that the pre-conditioning to an aluminium challenge would either be insufficient to ameliorate a more rigorous aluminium challenge or lost completely due to the interim exposure to aluminium-free water.

If these assumptions are correct, the results of Wood *et al* (1988a, 1988b) lend considerable support to $Al^{3+}_{(aq)}$ being the most acutely toxic of the monomeric cationic aluminium species.

6.4.2 Acute Aluminium Toxicity Amelioration with Silicic Acid

The acute toxicity of aluminium, ca 193μgl^{-1} total-Al, was completely abolished by the presence of a large excess of silicic acid, ca 2600μgl^{-1} total-Si. The amelioration was not achieved through a reduction in the soluble fraction of aluminium *per se*, but a reduction in the biologically available aluminium fraction, and $Al^{3+}_{(aq)}$ in particular.

The presence of silicic acid in the concentration range of 150-2600μgl^{-1}, increased aluminium solubility in the high, (2), medium, (3), and low, (4), $Si_{(aq)}$ treatments to a level, (ca 100-211μgl^{-1}), beyond that predicted by gibbsite. As was observed for aluminium solubility in the aluminium-only treatment, (5), the membrane filtration solubility data for treatments 2, 3 and 4 suggested a considerably lower soluble aluminium fraction, (6-30μgl^{-1}), than the measured exchangeable aluminium fraction. The explanation for the discrepancies between the two "soluble" fractions, (range 94-181μgl^{-1}), can be found to a limited extent in particulate adsorption theory, however, size and charge characteristics of species within the soluble fraction may also be important. The presence of silicic acid promoted the rapid dissolution of $Al(OH)_{3(s)}$ (gibbsite) and abolished aluminium hydroxide solubility control. This change in solubility phase was an indication of Al/Si

complexation and would probably be described by the following equilibria:

(1) $Si(OH)_{4(aq)} + Al(H_2O)_5OH^{2+}_{(aq)} \rightleftharpoons$ Al/Si complex.(W)

(2) $Si(OH)_{4(aq)} + Al(H_2O)_4(OH)^{+}_{2(aq)} \rightleftharpoons$ Al/Si complex.(X)

(3) $Si(OH)_{4(aq)} + Al(H_2O)_2(OH)^{-}_{4(aq)} \rightleftharpoons$ Al/Si complex.(Y)

Silicic acid is a very weak acid, pKa ca 9.8, and was largely undissociated at pH5.0. The stability of an Al/Si complex would depend on the basicity, (Al:OH ratio), of available aluminium species and the concentration of free silicic acid in the milieu. At pH5.0, $Al(OH)^{-}_{4(aq)}$ will be preferentially bound by silicic acid, equilibrium 3. The theoretical concentration of $Al(OH)^{-}_{4(aq)}$ at pH5.0 will be low by comparison to the cationic aluminium species, probably less than 1% of the mole fraction, (Johnson et al 1981, Martin 1986). However, in terms of Avogadro's number, (6.0222×10^{23}), the number of atoms/particles that constitute one mole of any substance, there will still exist sufficient aluminate anions to effect rapid Al/Si complexation reactions.

In the high $Si_{(aq)}$ treatment, (2), equilibrium 3 above would have been dominant and all monomeric aluminium species would have been rapidly complexed, equilibrium shifts ensuring that Al/Si complex (Y) was instantaneously

the dominant form of solution aluminium. Al/Si complex (Y) was positively charged, it was retained by sulphate groups on a cation exchange resin, was no larger than around 0.05um and was probably a precursor of an insoluble aluminosilicate phase such as imogolite, (J. Chappell, pers. comm.). It was not acutely toxic to salmon fry and was not found associated with the gill surface.

Silicic acid amelioration of acute aluminium toxicity was dependent on the silicic acid concentration, and was not found to be statistically significant (t-test: $P \geq 0.05$) in the medium, (3) and low, (4) $Si_{(aq)}$ treatments, (range; 140-750ugl^{-1} total-Si). The $Si_{(aq)}$ enhanced dissolution of aluminium hydroxide (gibbsite) was evident in treatments 3 and 4, though at a significantly (t-test: $P < 0.05$) slower rate than in the high $Si_{(aq)}$ treatment (2), suggesting that Al/Si complexation was occurring in the lower $Si_{(aq)}$ environment of these treatments. It was likely that either: equilibrium 3 was unable to dominate in the lower $Si_{(aq)}$ treatments, (3 and 4) and Al/Si complexes were in equilibrium with Al-hydroxy species and/or the concentration of silicic acid in the milieu was not sufficient to induce the stable form of the aluminosilicate species. Aluminosilicate species similar to those identified in Chapter 5, (Si-Total $<$ 1000ugl^{-1}), are predicted for these lower $Si_{(aq)}$ treatments, probably in equilibrium with Al-hydroxy species. Some

amelioration of toxicity was evident in these lower $Si_{(aq)}$ treatments as indicated by median survival times (Figures 6.3a-c) and reduced rates of aluminium association during the first 12 hours of exposure (Figure 6.5). The extent of the early amelioration was not sufficient to prevent binding of $Al^{3+}_{(aq)}$ to critical sites on the gill surface to a supra-toxicity threshold level.

The solubilisation effect of silicic acid on aluminium solutions, the solubility of which was previously controlled by gibbsite, created a "paradox of aluminium toxicity", increasing solubility resulting in the reduced biological availability of the aluminium (Figure 6.7). A critical level of silicic acid defined acute and non-acute aluminium toxicity, somewhere in the range 750-2400μgl^{-1} total-Si, and this range corresponded closely to recent findings that the stable formation of hydroxy-aluminosilicate species will occur at silicic acid concentrations above about 1000-1100μgl^{-1} (Farmer 1986, Birchall and Chappell 1988a, Chappell and Birchall 1988).

6.4.3 Tissue Digests

Whole body tissue digestion analyses showed that aluminium was associated with salmon from all treatments and that the association, particularly with respect to the gills, (see histological results: section 6.3.2.4) was greatly increased in treatments acutely toxic to the salmon,

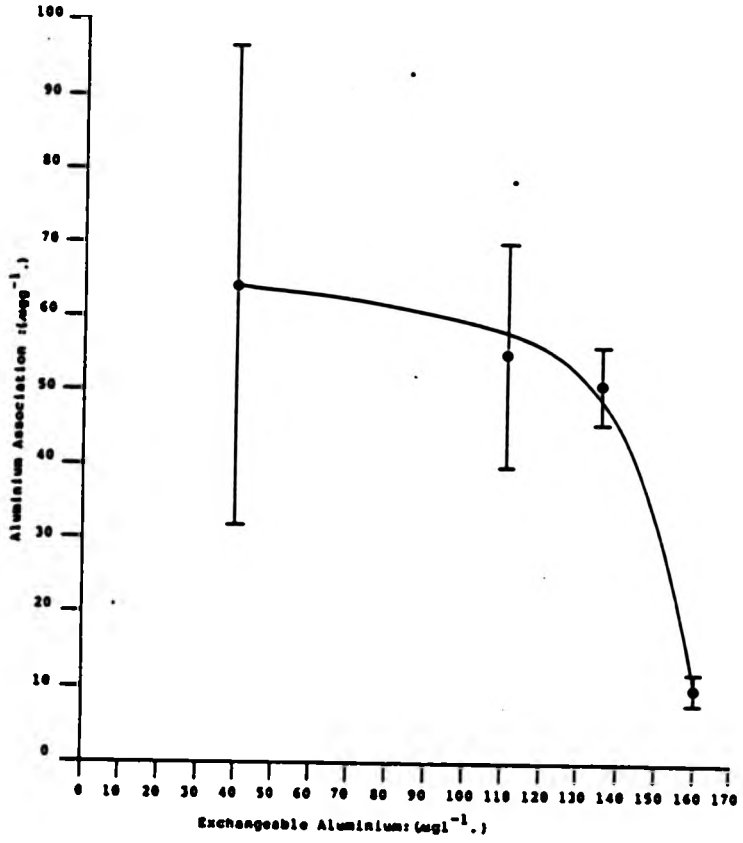


Figure 6.7. The Paradox of Aluminium Toxicity. In the presence of an increasing excess of total Si the exchangeable Al fraction increases and the whole-body aluminium association is reduced. Means and standard deviations for 48 hours exposure are plotted. n = 12.

(Table 6.8, Figures 6.4, 6.5). The results suggest that aluminium associations during the first 12 hours of exposure may have instigated the acute response and associations thereafter were secondary and related to the state of the gill and the proportion of uncomplexed aluminium in solution. After 24 hours exposure, aluminium associations with salmon from the lower $Si_{(aq)}$ treatments and the aluminium-only treatment, were very similar, around 40-50$\mu g g^{-1}$. Subsequent to this time aluminium associations did not fall below this range, and this concentration may have been representative of a critical threshold of irreversibly bound aluminium. A similar scheme whereby aluminium was predicted to have instigated an irreversible gill effect was recently proposed by Booth *et al* (1988). The gill aluminium levels recorded in their studies, 50-200$\mu g g^{-1}$ (converted from wet weight results using the formula: Dry Wt = Wet Wt/4.5, (Stoner *et al* 1984)), were generally in excess of the results of this study, 40-120$\mu g l^{-1}$. Booth *et al* (1988) used 100-300g brook trout *Salvelinus fontinalis*, which are generally accepted to be the least sensitive salmonid to acid/aluminium stress, (Exley and Phillips 1988). To study more closely the relationship between acute aluminium exposure and aluminium associations at the gill surface, sampling should be more frequent and sampled salmon could be used in recovery studies to ascertain whether or not a critical association of aluminium would lead to an irreversible acute response.

The whole fish levels of aluminium and silicon were not found to be related to each other. Silicon association was loosely correlated with the silicic acid concentration in each treatment. Because silicic acid is a small electrostatically neutral molecule it would be free to diffuse across the lamellar membranes of the salmon gill and into the plasma following a concentration gradient. It could have been possible that plasma silicic acid concentrations, normally in the range of $1000 \mu g l^{-1}$ for mammals, (Carlisle 1986), were reduced in the salmon during the acclimation period, and thereafter these reduced levels might have contributed to the overall toxic effect. Thus in the aluminium-only treatment, (5), the plasma silicic acid levels would have remained supressed and the subsequent aluminium toxicity was found to be acute. In the lower $Si_{(aq)}$ treatments, (3 and 4), the plasma silicic acid levels would have been raised but not sufficiently to significantly ameliorate the aluminium toxicity, whereas in the high $Si_{(aq)}$ treatment, (2), the plasma silicic acid levels would have been raised rapidly and might have contributed to the amelioration of the acute aluminium toxicity. These hypotheses raise the question whether silicic acid can control the biological availability of aluminium in the blood?

6.4.4 Histological Analyses

The gill effects, due to acute aluminium exposure observed in this study were characteristic of many previous

studies on acid/aluminium toxicity, (see Jagoe et al 1987, Exley and Phillips 1988, Tietge et al 1988, Evans et al 1988). The gross cellular changes identified in this study could have been largely the response of the salmon themselves, and may have represented attempts to adapt to the conditions. Hyperplasia may be an attempt to replace damaged cells and mucous cell proliferation an attempt to increase mucous production and thereby reduce the permeability of the gill surface. The hypertrophic response was probably not adaptive and may have reflected a breakdown in osmoregulation. The disruption of ion and water balance control was reflected in the increased vacuolation and subsequent lifting off and complete necrosis of the epithelia of the gill secondary lamellae. Such gross changes in the gill structure are not specific to aluminium and/or acid toxicity, (see Mallat 1985), however, the instigation of the observed effects probably are unique to aluminium and may be explained by binding of aluminium to the gill surface.

The adaptation of a modified haemotoxylin stain to locate aluminium in tissues was successfully achieved though does require further research to ascertain its quantitative usefulness. Aluminium was shown to be associated with the lamellae of gills of salmon from the lower $Si_{(aq)}$ and aluminium only treatments. Most importantly, the stain conclusively showed that the aluminium species in the high $Si_{(aq)}$ treatment, (2), were not associated

with the gill lamellae, or any other structure. It is not possible to be too specific about the location of aluminium on the gills of salmon from the lower $Si_{(aq)}$ and aluminium-only treatments, however, it was found covering the lamellar membranes, on sloughed damaged tissue and specifically with mucous and mucous cells. Conclusive evidence for the intracellular association of aluminium was not found, however, the observation that the filament capillaries stained positively for aluminium does suggest that this technique could be used to locate focal accumulations of aluminium, for example, in bone and brain tissue. There was no evidence of such accumulations in this study. Aluminium associations with gill tissue during acute aluminium exposure have been postulated by many previous workers, (Youson and Neville 1987, Exley and Phillips 1988, Booth et al 1988, Evans et al 1988), though none have demonstrated aluminium occurrence so conclusively as in this study. The use of this technique could be applied to further studies of aluminium ecotoxicology where the association of aluminium is considered an integral factor.

6.5 Summary

In a synthetic water at pH5.0, aluminium was acutely toxic to salmon fry. Acute toxicity was probably instigated by $Al^{3+}_{(aq)}$ binding at the gill surface. Acute aluminium toxicity was completely abolished in the presence of a large excess of silicic acid. Silicic acid appeared to

reduce the biological availability of aluminium through its complexation to give hitherto unrecognised soluble aluminium species. Tissue digestion and histological analyses lent further evidence to this hypothesis.

CHAPTER SEVEN: GENERAL DISCUSSION

Aluminium has been shown to be toxic to a wide diversity of living forms including man (see Taylor (ed) 1986), other mammals, (see Alfrey 1986), birds, (see Scheuhammer 1987), fish (O'donnell et al 1984, Exley and Phillips 1988), invertebrates (see Burrows 1977, EPA 1986), plants (see Jones and Bennett 1985, Ohki 1986), and micro-organisms, (Thurman and Gerba 1988). However it is a relatively newly discovered toxicant and was considered as a generally benign element as recently as 1974 (Sorenson et al 1974). Whilst the evidence to support the ecotoxicology of aluminium is currently overwhelming, the toxicological pathways implicated in the elevation of aluminium from a benign to a malignant metal remain poorly understood.

The present understanding of aluminium toxicity to fish is based largely on the symptoms related to exposure to the toxicant. The mechanisms of the toxicity are ill-defined and this area of research warrants further investigation. The toxicity of aluminium to fish is complicated because it can be both acute and chronic and it is likely that these toxicities are distinctly different from each other. This study was concerned with the acute toxicity of aluminium, though the results have much wider implications.

Aluminium is acutely toxic if it effects death within a short time period, usually less than 96 hours. Acute toxicity in fish is often the result of damage to respiratory and/or osmo- and iono-regulatory systems (Mount et al 1988a, Mount et al 1988b, Walker et al 1988, McDonald and Milligan 1988, Wood et al 1988a, Exley and Phillips 1988). It is likely that both of these integral physiologies are affected to some extent during acute aluminium toxicity and that both are manifested as severe gill damage, as was seen in this study and many previous studies (Jagoe et al 1987, Tietge et al 1988, Evans et al 1988, Exley and Phillips 1988).

Gill damage characteristic of acute aluminium toxicity, for example, hypertrophy, hyperplasia, necrosis and tissue sloughing is likely to be the result of both the direct effects of the toxicant and the physiological attempts of the fish to compensate for these direct effects. These compensatory responses, which may include cell proliferation and mucous production, form part of the secondary stress response, (Mazeaud et al 1977), and if unsuccessful will be exacerbated and may then contribute towards death. The method through which aluminium instigates these responses is still largely unknown and will form the basis of this discussion.

Acute aluminium toxicity in salmonids is induced through aluminium interactions at the gill surface (Booth et al 1988). The exact nature of these interactions is unknown though likely associations would be with calmodulin in mucous, (Haug and Weis 1986, Weis and Haug 1987), phospholipids in the apical cell membrane, (see Haug and Caldwell 1985), carboxylate and thiol ligands on membrane proteins (Haug and Caldwell 1985, Exley and Phillips 1988), nucleotides associated with ATPase pumps in the apical membrane (Ganrot 1986, Dryssen et al 1987), carbonic anhydrase in mucous (Staurnes et al 1984), and other anionic groups within mucous (Booth et al 1988). To effect an acute response the aluminium must occupy irreversibly a threshold level of critical sites on the lamellar membrane. The consequent secondary stress response will exacerbate the condition and death will result, probably from a combination of plasma-dilution, respiratory failure and the direct influence of aluminium on calcium homeostasis. The latter is of particular interest and has received little attention in respect of aluminium toxicity to fish. During acute aluminium toxicity, aluminium is found in the plasma, (Witters et al 1988). Aluminium in the plasma is known to increase the concentration of bound plasma calcium but greatly decrease the level of ionised calcium (Llach et al 1986, Mollah pers. comm.), with the result that the interstitial fluids particularly those associated with muscle tissues, may become hypocalcemic. The effect of hypocalcemia (and

concomitant aluminium associations with ATPase molecules in the muscle sarcoplasmic reticulum)on muscular contractions is probably muscular rigor. Such an effect was consistently evident in fish suffering from acute aluminium toxicity in these studies, and previous research has implicated aluminium in the inhibition of locomotory movement and power in fish (Ormerod et al 1987, Exley and Phillips 1988).

The irreversible, or only slowly reversible, binding of aluminium to gill binding sites will depend on the nature of the aluminium species, the nature of the complexing ligand and competition from other aqueous species (Tam and Williams 1986). Competition between metal species and $H^{+}_{(aq)}$ for binding with anionic groups will depend on the relative magnitudes of Km, (the association constant for the product), and the ratio of the hydrogen ion concentration $[H^{+}]$ and the association constant, (Ka), for the hydrogen ion and the anionic group. Thus, when pH $\geq$ pKa+1, binding will be a function of Km only, however when pH $<$ pKa+1, competition from hydrogen ions must be considered. The observation that the toxicity of a certain level of aluminium to fish can be reduced by lowering the water pH towards pH4.0, (Sadler and Lynam 1987, Ormerod et al 1987, Exley and Phillips 1988), can be explained in terms of increased competition from $H^{+}_{(aq)}$. This result infers that critical anionic groups on the membrane and to which aluminium may bind will have pKa

values ca $\geq$ 3.0. If this is true, this will exclude such anionic groups as HPO_3^- (pKa 1.5), $H_2PO_4^-$ (pKa 2.1) and SO_4^{2-}(pKa 2.0), as being critical in acute aluminium exposure. All of these groups may bind aluminium and the binding will be independent of $[H^+]$ over a wide pH range.

Irrespective of pH and pKa, the value of Km is generally related to the metal valency. Thus $M^{3+} \geq M^{2+} \geq M^+$, where $\geq$ implies a greater binding tendency of a metal for any given anionic group. Relating this to the potential binding in the aluminium only treatment (5), of the toxicity study, (see Chapter 6), aluminium, and in particular, $Al^{3+}_{(aq)}$, would show a greater tendency to bind at the gill surface than either $Na^+_{(aq)}$ or $Ca^{2+}_{(aq)}$. Competition from aluminium for $Ca^{2+}_{(aq)}$ and $Na^+_{(aq)}$ binding sites will be very great in acid solution (Tam and Williams 1986). At pH5.0 it is unlikely that $H^+_{(aq)}$ will be excluded as an active competitor for anionic groups on the gill membrane. Because of this competition from $H^+_{(aq)}$ binding tendency at the gill surface will reflect the difference between log Km and pKa. The greater the difference, the greater the binding tendency.

The effect of hydrolysis on the Km of aluminium for different anionic groups is not known, however valency differences between the hydrolysis products would predict that at pH5.0 only $Al^{3+}_{(aq)}$ could bind strongly to the weak acid anions at the gill surface.

The implication that $Al^{3+}_{(aq)}$ is the most likely precursor of aluminium associations at the gill surface tends to contradict present assumptions that $AlOH^{2+}_{(aq)}$ is the major toxic moiety in acute aluminium toxicity. As was discussed in Chapter Six, this assumption was based largely on studies that have shown aluminium toxicity to be reduced at pH$\leq$5.0 with respect to the pH range of 5.0-5.2. Similar studies showed reduced toxicity again at pH of 5.5 and above. I believe that if aluminium toxicity is potentiated at pH 5.0-5.2 this is more likely to be a function of the reduced competition from $H^{+}_{(aq)}$ at less acid pH than the increased $AlOH^{2+}_{(aq)}$ levels, and that the subsequent reduction of aluminium toxicity observed at increasing pH above 5.2 could be the result of aluminium hydrolysis reactions greatly increasing the proportions of the less toxic divalent and monovalent aluminium species with respect to the toxic hexa-aqua trivalent species. An additional factor complicating these arguments are the inconsistencies that exist in the literature concerning the relative proportions of each monomeric aluminium species present at any one pH. This thesis favours a compromise between the work of Martin (1986) , and Johnson et al (1981) (Figures 5.2a, 5.2b).

It is not, as yet, possible to predict the nature of the critical anionic groups at the gill surface, however, anions likely to bind $Al^{3+}_{(aq)}$ strongly at pH5.0 would

include bidentate organic carboxylates and some diester phosphates. Such groups, particularly carboxylates, are present on the acidic protein calmodulin (Weis and Haug 1987).

Calmodulin has been identified in the mucous surrounding the gill lamellae (Flik et al 1982), and is likely to be involved in membrane permeability (You-Han 1986). Activation of calmodulin requires $Ca^{2+}_{(aq)}$ binding at specific sites, (Haug and Weis 1986).

It is unlikely that aluminium will compete with calcium for these sites, (Martin 1986, Haug and Weis 1986), however, binding of aluminium by calmodulin will confer conformational changes of the protein that will inhibit its activation, despite calcium binding continuing though perhaps at non-specific sites (Siegel and Haug 1983).

This extracellular inactivation of calmodulin may be important in acute aluminium toxicity and should itself be inhibited in the presence of molar excesses of aluminium chelators, such as citrate, (Haug and Weis 1986).

Further understanding of acute aluminium toxicity and the biochemistry associated with this toxicity will come from knowledge of what ameliorates the toxicity. In this study evidence was presented for the complete abolition of acute aluminium toxicity at pH5.0 by silicic acid (Chapter Six).

If acute aluminium toxicity at low pH is the result of aluminium associations at the gill surface then the addition of a ligand/chelate that will prevent or reduce these associations should ameliorate the toxicity.

Silicic acid, $H_4SiO_{4(aq)}$ is a very weak acid, (pKa, ~9.8), and is highly undissociated in acid solution, it will however form complexes with aluminium in acid solution (Farmer et al 1983, Farmer 1986, Lou and Huang 1988, Birchall et al, submitted). The use of infra-red spectroscopy indicates that formation is via Si-O-Al bonds when aluminium reacts with monosilicic acid (Farmer et al 1978, Wada and Wada 1980). To date the existence of such complexes has only been verified at high silicic acid concentrations. Farmer (1986) predicts that the aluminosilicates, protoimogolite and imogolite, require a minimum silicic acid concentration of $1mgl^{-1}$ for formation. In this study, complexes of aluminium and silicic acid were identified over a wide pH range and at silicic acid concentrations above $200\mu gl^{-1}$.

The relative toxicities of these species at pH5.0 to Atlantic salmon Salmo salar fry, were investigated, (see Chapter Six) and it was found that the efficacy with which aluminium toxicity was ameliorated was dependent on the available silicic acid concentration.

Histological evidence,(see Figures 6.8a - j), showed that in the presence of high silicic acid concentrations, 2000-3000μgl^{-1}, the association of aluminium at the gill surface was abolished. Tissue digestion analyses verified this finding and together these techniques showed that during the 96 hour experiment the addition of an excess of silicic acid, 13:1, Si:Al molar ratio, rendered aluminium biologically unavailable.

For silicic acid to achieve this amelioration it must complex aluminium strongly. The complexation or binding must be more stable than is offered by anionic groups at the gill surface otherwise substitution of aluminium from the Al/Si complex would occur. Alternatively the Al/Si complex could have a very rapid rate of formation and very slow dissolution, thereby making substitution at the gill surface unlikely, particularly when an excess of silicic acid was present. Evidence from this study and other recent studies, (Birchall and Chappell 1988, Chappell and Birchall 1988), has shown that aluminosilicate species were retained on cation exchange resins employing sulphonates as the functional group. This stability with respect to sulphonate (HSO_3) suggests that the aluminosilicate species will also be stable with respect to the functional anionic groups at the gill surface. The aluminosilicate species were positively charged, as implied by their retention on a cation exchange resin, and might therefore be expected to bind anionic ligands at the gill surface. The total lack of binding observed

in this study indicates a very low Km for the aluminosilicate species, probably lower than that for competitive cations such as $Ca^{2+}_{(aq)}$ and $Na^{+}_{(aq)}$ and very low with respect to the ratio of $[H^{+}_{(aq)}]$ and the association constants, Ka, of the acid anions at the gill surface. Thus the aluminosilicate will not form stable complexes with gill surface anions in acid media.

The nature of the stable aluminosilicate species, formed at pH5.0 is unknown. Similar species identified very recently, (Chappell and Birchall 1988, Birchall and Chappell 1988) had molecular species Si:Al ratios of 0.25 to 0.50 and started to form at around pH5.0. These species were formed at higher molar concentrations of aluminium, ($2.7mgl^{-1}$) and silicic acid, ($14.0mgl^{-1}$) than the species in this study and in solutions of 0.01M NaCl, around 25 times the molarity of the test water used in the toxicology experiments in this study.

Hydroxy-aluminosilicate species will form in acid media, (Farmer et al 1979, Wada and Wada 1980). Lou and Huang (1988) showed their formation at low pH, ∠ pH4.36, from aluminium ($2.7gl^{-1}$) and monosilicic acid ($51.8mgl^{-1}$) however such conditions are unlikely in natural waters.

In this study the assumption was made that in dilute (I = 4-5mM), acid solutions the greater the basicity

of the aluminium species the more likely it would be to form a stable complex with silicic acid. Thus n molecules of $Al(OH)^{-}_{4(aq)}$ will combine with a single molecule of silicic acid to form a stable biologically unavailable "soluble" aluminium species. Amelioration of aluminium toxicity at pH5.0 will only be achieved through the rapid removal of $Al^{3+}_{(aq)}$ therefore at pH5.0, where the fraction of $Al(OH)^{-}_{4(aq)}$ species will be very low (less than 1% of the mole fraction), a critical concentration of silicic acid was required to both fuel the aluminosilicate formation reaction and ensure the stability of the resultant aluminosilicate species. This rapid reaction then promoted rapid hydrolysis of $Al^{3+}_{(aq)}$, $AlOH^{2+}_{(aq)}$ and $Al(OH)^{+}_{2(aq)}$ to provide further $Al(OH)^{-}_{4(aq)}$ to combine with silicic acid. The result was an almost instantaneous conversion of soluble aluminium and aluminium-hydroxy species to "soluble" aluminosilicate species. The main factors controlling the aluminosilicate formation reaction would be the concentrations of both $Al(OH)^{-}_{4}$ (i.e. pH) and silicic acid. The results of this study clearly show that at pH5.0, a critical concentration of silicic acid was controlling aluminium toxicity.

The results set a precedent for the hypothesis:

> "The major role of silicon in biology is as a detoxification agent for aluminium"
>
> (J.D. Birchall, pers. comm.)

This hypothesis can be examined further by looking closely at some of the suggested roles of silicon in biology.

Silicon has an essential role in biology and these roles have been demonstrated both in plants (Volcani 1981, Robinson and Sullivan 1987), and higher animals, (Carlisle 1986).

Whilst a dietary requirement for silicon has been demonstrated in rats and chicks, (Schwarz and Milne 1972, Carlisle 1972), much speculation has surrounded the nature of silicon essentiality (Birchall 1978, Birchall and Espie 1986), in higher animals. Silicon deficiency in rats and chicks resulted in severely retarded skeletal development (see Carlisle 1986), and reduced activity of prolyl hydroxylase (Carlisle 1984), an enzyme involved in collagen biosynthesis. However, the mechanisms through which silicon can exert this essentiality remain unknown. The reaction between silicic acid and metal ions, in particular, aluminium, is the likely precursor of silicon essentiality (Birchall and Espie 1986). For example, aluminium, in the absence of silicic acid will inhibit the aforementioned enzyme, prolyl hydroxylase, whilst pre-mixing of the aluminium with silicic acid, prevents the inhibitory effect of aluminium (Birchall and Espie 1986).

Focal distributions of aluminium in the brain have been associated with severe learning disabilities in mammals

(see Alfrey 1986), and pre-senile dementia of the Alzheimers type in humans (Krishnan et al 1988). Whilst the toxic nature of aluminium to the brain, and in particular in the etiology of Alzheimers disease, (Birchall and Chappell 1988b), has yet to be fully ascertained, the accumulation of aluminium in the brains of rats was reduced in rats fed supplemental silicon (Carlisle and Curran 1987, Carlisle et al 1988).

Silicon, probably as silicic acid, controls the biological availability of aluminium. If the Si to Al balance is tipped in favour of aluminium, as may occur during catchment acidification or renal dialysis therapy in humans, the toxic nature of aluminium will be manifested.

During catchment acidification an aluminosilicate phase may control aluminium solubility in soil pore water (Bache 1986, Litaor 1987), however, the geology of catchments susceptible to acidification predicts very low levels of siliceous materials (Farmer 1986). Both soil and surface waters in these areas will consequently have low concentrations of silicic acid (Farmer 1986), the precursor to the amelioration of aluminium toxicity. The results of this thesis have shown that if a threshold level of silicic acid, (pH dependent concentration), is maintained aluminosilicate formation will preclude the biological availability of aluminium.

The influence of other chelators of aluminium in natural waters is unknown. Competition for binding will exist from both organic (humic substances) and inorganic (phosphate, fluoride, sulphate, carbonate) ligands, and may influence Al/Si complexation. In synthetic solutions silicic acid will reduce the biological availability of aluminium, further research on Al/Si complexation in the presence of competitive ligands should reveal its efficacy as an amelioration agent in natural waters.

The studies that have made up this thesis have asked a great many questions concerning all aspects of aluminium ecotoxicology. They have also flaunted a controversial but unignorable solution: the ubiquitous relationship of aluminium and silicon in biology.

Fish, and in particular, salmonids, represent excellent experimental animals for aluminium toxicity studies. Through their enforced intimacy with their environment their uptake and turnover of aluminium is rapid and this factor precludes the use of unrealistic exposures to aluminium as often occurs in mammalian studies.

Perhaps the next logical step in aluminium toxicity to fish is to establish whether or not a longer-term toxicity to aluminium will occur from exposure to sub-lethal levels of aluminium, and whether or not the nature of the toxic

response is different to the acute response evident in this study. Would a silicon supplemented diet reduce aluminium toxicity? There is a suggestion today that aluminium availability to biological systems in human plasma is controlled by an aluminosilicate phase! (J. Chappell, pers. comm.).

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