Lithium, an emerging environmental contaminant, is mobile in the soil-plant system

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Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Master of Applied Science

How Mobile is Lithium in the Soil-Plant System?: Lithium, an emerging environmental contaminant, is mobile in the soil-plant system

by

Rohith Chowdary Yalamanchali

Lithium (Li) is the ligtest of the alkali metals and reportedly present in soil at concentrations of 20-30 mg kg^{-1}. World demand of Li is increasing at 8% per annum, driven primarily through the use of Li-ion batteries. There is a lucna of information on the behaviour of Li in the soil-plant system. I aimed to measure the concentrations of Li in New Zealand soils and pastures, the sorption of added Li to soil and the uptake of Li by food and fodder species. New Zealand soils and pasture Li concentrations were determined in a field trial. Batch experiments were used to measure the sorption of Li by the Templeton Silt Loam (TSL) at various pH values. I grew rye grass (*Lolium perenne*), beetroot (*Beta vulgaris*), broccoli (*Brassica oleracea*), carrot (*Daucus carota*), leek (*Allium porrum*), lettuce (*Lactuca sativa*), radish (*Raphanus sativus*), spinach (*Spinacia oleracea*), corn (*Zea mays*), tomato (*Solanum lycopersicum*), and courgette (also called zucchini - *Cucurbita pepo*) in the TSL limed to a pH of 6.2. Sunflower (*Helianthus annuus*) was in soils spiked with 0, 10 & 30 mg kg^{-1}Li. Mature plants were disected and the portions were analysed separately.

Lithium concentrations in soils from around New Zealand ranged from 0.08 mg kg^{-1} to 92 mg kg^{-1}. The highest Li concentrations were found in soils with high clay content. Most endogenous Li in soil is insoluble and hence unavailable to plants. However, when exogenous Li is added to soil, there is only limited sorption of Li. Lithium sorption increased with increasing soil pH and decreased with increasing Li concentrations. Compared to other cations in soil, Li is mobile and may leach into receiving waters or be taken up by plants. When grown in uncontaminated soil, the Li concentrations in the edible portions of various plant uptake of Li differed by two orders of magnitude. Salt tolerant plants, namely *B. vulgaris* and *S. oleracea* took up the most Li, while seed and fruit crops had the

lowest Li concentrations. Pasture grass (*L. perenne*) had the highest bioaccumulation factor for Li of any of the plants tested. When Li was added to soil, there were few differences in the uptake or tolerance of this element between species. At a soil concentration of just 5 mg kg^{-1}, the plants took up several hundred mg kg^{-1} Li into the leaves with no reduction in biomass. At such high Li concentrations, only a small amount of plant material would need to be consumed to exceed the Tolerable Daily Intake for Li. Lithium appears to be a phloem immobile element, with the highest concentrations occurring in the older leaves and the lowest concentrations occurring in the seeds or fruits. Therefore, planting fruit or seed crops on Li-contaminated soil may reduce the risk posed to human health. Future work should focus on the release of Li from electronic waste and industrial sources. Lithium mobility and plant uptake could be tested in tropical environments where informal e-waste reprocessing occurs.

Keywords: Lithium ion; toxicity; vegetables; Templeton Silt Loam; e-waste; human health; Tolerable Daily Intake; phytoaccumulation; phytotoxicity

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Chapter 1

Introduction

Lithium (Li) is the lightest solid, with a density of 0.53 g cm^{-3} (Merian, 1991). Of all the alkali metals, Li has the smallest atomic and ionic radius. Lithium can exist as two naturally occurring isotopes, ^{6}Li and ^{7}Li, which are present in the environment in a proportion of 8% and 92% respectively (Merian, 1991; Jain, 2002). Lithium occurs in numerous other minerals and was named after the Greek lithos, stone, because of its presence, in trace amounts, in virtually all rocks (Schrauzer, 2002). Lithium is found in trace amounts in all soils primarily in the clay fraction, and to a lesser extent in the organic soil fraction, in concentration ranging from 7 to 200 mg/g (Schrauzer, 2002; Aral and Vecchio-Sadus, 2008). The main Li minerals that are used commercially can be divided into three groups: silicates (spodumene-$LiAlSi_2O_6$,petalite-$LiAlSi4O_{10}$); micas (lepidolite-$[Li,Al]_3[Al,Si]_4O_{10}[F,OH]_2$, zinnwaldite-$[Li,Al,Fe]_3[Al,Si]_4O_{10}[F,OH]_2$ and phosphates (mainly amblygonite - [Li,Na]Al[F,OH] (Aral and Vecchio-Sadus, 2008). Lithium minerals are mined around the world in Zaire, Zimbabwe, Western Australia, Canada, Russia and China.

Lithium is a stable cation. In biological systems it does not participate in redox reactions. However, Li reacts with cyclic polyethers, polypeptides and proteins. Lithium has a high charge density resulting in it being the most polarising of all the alkali metals and explaining its tenancy to form covalent bonds (Wiberg *et al.*, 2001). Lithium can also be used as a reducing agent, such as in the Birch reduction route in the manufacture of methamphetamine (Makino *et al.*, 2005).

Lithium is a non-essential element for life (Leonard *et al.*, 1995; Lenntech, 2007; Aral and Vecchio-Sadus, 2008). However, Li affects the regulation of neurotransmission (Merian, 1991) and is therefore used in the treatment of bipolar disorder (Li, 2004). Even though Li is not considered an essential element for life, a link has been found between low Li intakes and altered behaviour such as aggressiveness in humans, indicating that some Li intake may be beneficial (Dawson, 1991; Schrauzer, 2002). The human body contains approximately 0.03 mg Li /kg with the Li being distributed in all organs and tissues (Merian, 1991). Lithium is toxic to humans in high doses (15-20 mg/l blood concentrations) and causes nausea, visual impairment, kidney damage, coma and cardiac arrest (Wiberg *et al.*, 2001) .

1.1 Concentrations of Li in soil

Lithium is released into soil from sedimentary rocks through weathering processes (Chan *et al.*, 1997; Aral and Vecchio-Sadus, 2008). The topsoil usually contains less Li and it has been suggested that this may be due to interactions with plant roots (Merian, 1991). The clay fraction of soil contains significantly more Li than the organic soil fraction (Schrauzer, 2002) as clay minerals concentrate Li (Horstman, 1957; Ashey, 1973; Starkey, 1982; Anderson *et al.*, 1988). It has been suggested that Li may be located internally within clay minerals in ditrigonal cavities (Anderson *et al.*, 1988). Soils with a high salt content usually contain Li concentrations greater than 200 mg kg^{-1} (Merian, 1991).

Anderson *et al.* (1988) investigated the distribution of Li in different horizons of Bonifay soils. They concluded that total and exchangeable Li indicates that the concentration of these forms of Li increases with depth and also that the top 60 cm of soil contains the least Li .The increase in Li concentration with depth is probably because clay minerals concentrate Li through isomorphous substitution in which structural cations present in the tetrahedral & octahedral sheets in clay minerals may be substituted by cations with a similar charge. Clay minerals are generally found at a greater depth in the mineral horizons, which are usually situated below the organic horizons.

1.2 Natural Li sources

Lithium is incorporated in clay minerals and is also lightly fixed by organic matter; therefore the Li content of soil is controlled more by conditions of soil formation than by its initial content in parent rocks (Kabata-Pendias and Pendias, 1992; Greger, 2004).

Millot *et al.* (2010) studied the behaviour of Li and its isotopes during weathering in the Mackenzie Basin, Canada and they concluded that dissolved Li is essentially derived from silicate weathering (Millot *et al.*, 2010). Table 1.1 shows the natural concentrations of Li in different natural environments. As Li is bound in the silicate matrices, the only pathway it can enter soil solution is through weathering of the sources, which is a slow process and therefore there is little risk of Li toxicity. However, Li toxicity may occur in areas such as Salar de Uyuni in Bolivia, where high evaporation has resulted in the concentration of soluble Li salts in surface soils.

Table 1.1 Natural concentrations of Li and alkali metals (adapted from Scott & Smith, 1987)

Source	Li (mg kg^{-1})	Na (mg kg^{-1})	K (mg kg^{-1})
Whole earth	2	4800	640
Earth crust	26	28000	26000
Igneous rock (basic)	13	19000	7500
Shale	62	9600	26000
Sandstone	15	7000	14000
Limestone	12	2000	3000
Sea water	0.17	11000	1300
Soil	26	6200	14000

1.3 Anthropogenic sources

Consumers routinely dispose of Li batteries in municipal solid waste (NEMA, 2001; Aral and Vecchio-Sadus, 2008). Lithium batteries have a life cycle of two to three years or 300 to 500 charge cycles before needing to be replaced (Tektronix, 2012). Countries have different regulations on proper disposal of Li ion batteries. Used batteries are explicitly mentioned by the EU regulations on wastes (EU Directives 91/156 and 91/689), this being included in the European community plan for waste management and a sustainable development. However, these regulations mainly refer to 'conventional' batteries and thus the safe disposal of spent Li batteries is an issue which is not yet properly addressed (Contestabile *et al.*, 2001).

In New Zealand there is no set law to recycle Li-ion batteries. The Ministry For the Environment website states that "there are different rechargeable battery types, including nickel cadmium (NiCd), nickel-metal hydride (NiMH), and Li-ion. These batteries may contain hazardous metals and should be recycled wherever possible (MFE, 1998). Other Li sources include manufacturing wastes (from aluminium, glass and ceramic production) and Li grease run off from roads (from vehicles).

Table 1.2 Anthropogenic uses of Li based compounds into soil per annum (adapted from Anderson,2011)

Type of use	% use	Tonnes used	Comments
Aluminum processing	2%	535	Used in alloys for aerospace equipment in the range 1-2% (w/w%)
Chemicals	6%	1605	nano-Li used in concrete floor treatments
Air treatment	6%	16052	Used in aqueous solution as a stable, chlorofluorocarbon-free absorption medium.
Other	10%	2675	Used for synthesis of organic compounds & as drying agents
Pharmaceuticals	11%	2942	Treatment of bipolar disorder.
Lubricants	11%	2942	Lithium grease more tolerance to high temperatures.
Batteries	22%	5885	Li-ion batteries have a longer shelf life and more charges
Glass & Ceramic production	32%	8560	Strengthens the glass and ceramic and avoids thermal shock.

1.3.1 Biosolids

Sewage treatment in NZ generates between 700,000 and 1,000,000 tonnes of biosolids annually (MFE, 2002). In NZ, biosolids are used as a soil amendment in production forest and on agricultural land as they can improve fertility (Gibbs, 2003). The importance of biosolids as a soil amendment may grow in response to increasing disposal costs for the biosolids and increased costs of fertilisers (FAO, 2008; Simmler, 2012).

Biosolids can potentially be a source of Li into soil. Lithium carbonate used for the treatment of manic disorder, is reported to be excreted through urine by kidneys (Aral and Vecchio-Sadus, 2008). The amount of Li from biosolids that enters soil has not been quantified.

1.3.2 Lithium interactions in soil

Lithium displays anomalous behaviour compared to the other elements in its group, due to its small size and high charge density, but shares some properties with Na (Wiberg *et al.*, 2001). The mechanism by which Li attaches to soil particles is not well researched. Most metals interact with soil particles via cation exchange, and possibly specific adsorption, depending on the properties as shown in Table A.3.

New Zealand soils are geologically young and therefore less weathered than soils from Europe and North America. Variably charged oxide minerals form an important constituent of NZ soils whereas the soils overseas are dominated by permanently charged clay minerals. Furthermore, the organic matter content is relatively high compared to similar soil in most other countries (Simmler, 2012).

By comparing the properties of Li with the other basic cations, one would expect that the Li^+ ion is likely bind to cation exchange sites in soil. The selectivity of cations binding to exchange sites on soil colloids is proportional to the charge/hydrated radius quotient. Lithium has the lowest charge/hydrated radius quotient (Table 1.3) of any metal, thus indicating that Li may have a low binding affinity to soil colloids.

Table 1.3 Charge/ Hydrated radius quotients of Li and the major cations in soil

Element	Ionic radius (Å)	Hydrated Radius (Å)	Charge/hydrated radius
Li^+	0.60	3.40	0.29
Na^+	0.95	2.76	0.36
K^+	1.33	2.32	0.43
Mg^{2+}	0.65	4.67	0.43
Ca^{2+}	0.99	3.21	0.62

1.3.3 Cation exchange

Most of natural Li is concentrated in the clay fraction and only a low proportion is available in an exchangeable form for cation exchange. That Li will probably have a low relative tendency to bind to cation exchange sites is supported by a study which found that exchangeable Li was less than 0.09% of the total effective Cation Exchange Capacity (CEC) and less than 0.1% of total Li present in soil. This is an insignificant portion of the total exchangeable cations and total Li present in soil (Anderson *et al.*, 1988).

Table 1.3 shows the different properties of the basic cations present in soils along with Li. The charge/hydrated radius ratio quotient shows that Ca has the highest affinity to bind to soil particles, followed by Mg and K. Lithium has the lowest affinity for CEC sites, indicating that unless specific adsorption occurs, Li will be more mobile than the other major cations in soil.

1.3.4 Lithium in plants

Lithium is taken up by all plant species, and although it appears not to be crucial for proper growth and development, stimulation of plant growth has been observed (Aral and Vecchio-Sadus, 2008). Lithium in plants and animals interacts with Na and K and with enzymes requiring Mg (Aral and Vecchio-Sadus, 2008; Hawrylak-Nowak *et al.*, 2012). Competitive interaction between Ca and Li absorption by plant roots has been observed. This indicates that significant levels of Li in soil may have an influence on Ca uptake and cycling in plants (Epstein, 1960; Bradford, 1966; Anderson *et al.*, 1988). Plant uptake of Li is greater in acidic soils than in alkali soils. This is probably because soil acidity corresponds to an increase in the solubility of Li and plant Li levels are correlated with the concentration of these elements in soil (Schrauzer, 2002; Lenntech, 2007; Aral and Vecchio-Sadus, 2008). At lower soil pH, there is a reduced negative surface charge, resulting in lower binding sites

available for cations. The higher solubility of the elements will result in greater plant uptake of these elements which will subsequently result in greater plant uptake of Li.

Lithium accumulating plants belong to the Asteraceae and Solanaceae families. The concentration of Li in plants usually ranges from 0.2 mg kg^{-1} to 30 mg kg^{-1}. The Li tolerances of a range of plants are displayed in Table A.8. In high concentrations, Li may be toxic to plants (Tölgyesi, 1983; Vetter, 2005).

Symptoms of Li toxicity in plants include: necrosis along leaf margins, subsequent interveinal chlorosis and leaf abscission (Bingham *et al.*, 1964). However, plants that are exposed to low concentrations of Li may display improved productivity, an increase in yield, faster maturation and an increased resistance to disease (Anderson *et al.*, 1988).

Hawrylak-Nowak et al., (2012) grew *H. annuus* and *Z. mays* in different concentrations of Li nutrient solution with the aim of determining the uptake and influence of Li on monocots (*Z. mays*) and dicots (*H. annuus*). They concluded that exposure of *H. annuus* and *Z. mays* to increasing concentrations of Li (0–50 mg Li dm^{-3}) in a nutrient solution induced changes in biomass, leaf area and photosynthetic pigment accumulation, as well as levels of lipid peroxidation.

1.3.5 Entry of Li into food chain

"Chaney (1980) introduced the concept of the "Soil-Plant Barrier" for consideration of potential toxicity to the food chain if trace elements are applied to soils" (Chaney, 1990). Chaney's concept highlights the observations that for many contaminants in soil do not pose a risk to herbivores through plant uptake. Elements depending on the solubility in soils are divided into four groups according to the soil-plant barrier concept as shown in Table 1.4. It is unknown what group Li would be included in. However, I hypothesise that it will not belong in Group 1, since the literature indicates that Li is relatively mobile in soil.

Table 1.4 Differentiation of elements according to soil-plant barrier concept. (Adapted from Chaney, 1990)

	Solubility in soil	**Availability to plants**	**Translocation in plants**	**Examples**
Group 1	highly insoluble	not phytoavailable	no absorption and translocation	Cr & Ti
Group 2	moderately soluble	available	absorbed by roots but not translocated to shoots	Pb, As & Hg
Group 3	moderately soluble	high phytoavailablity	phytotoxicity protects food chain	B, Cu, Ni &Zn
Group 4	moderately soluble	high phytoavailability	readily translocated into the shoots. No food chain protection	Cd, Se & Mo

1.4 Aims & Objectives

I hypothesised that exogenous Li is relatively mobile in soil and therefore may be relatively available for plant uptake compared to other elements.

I aimed to evaluate the likely environmental fate of elevated Li in the environment specifically sought.

The main aims of this project were to determine:

- The Li concentrations in a range of New Zealand soils
- The solubility of Li as a function of soil pH and Li concentration
- The relative uptake of Li by selected plant species and their sensitivity to this element
- The distribution of Li is various plant organs.
- To determine which group Li belongs to, in Chaney's soil-plant barrier concept

Chapter 2

Background

2.1 World demand for Li

World annual consumption of Li has increased from 100 tonnes per annum in the 1900 s to more than 70,000 tonnes per annum in 2002 (Ebensperger *et al.*, 2005). Anderson E.R, (2011) stated that demand of all Li based products increased by 7-8 % per annum during the years 2002-2007. Due to recession between 2008- early 2010, the demand has decreased by 6%. The increased number of Li based projects in late 2010 has helped the recovery of the Li industry from recession and predicted demand growth from 2010 through to 2020 is said to be 8% per annum as shown in Figure 2.1 (Anderson, 2011). The consumption and demand of Li has increased significantly and is still on the rise with new technologies. Due to the high use and disposal of Li based products; it is probable that Li is now an emerging environmental contaminant.

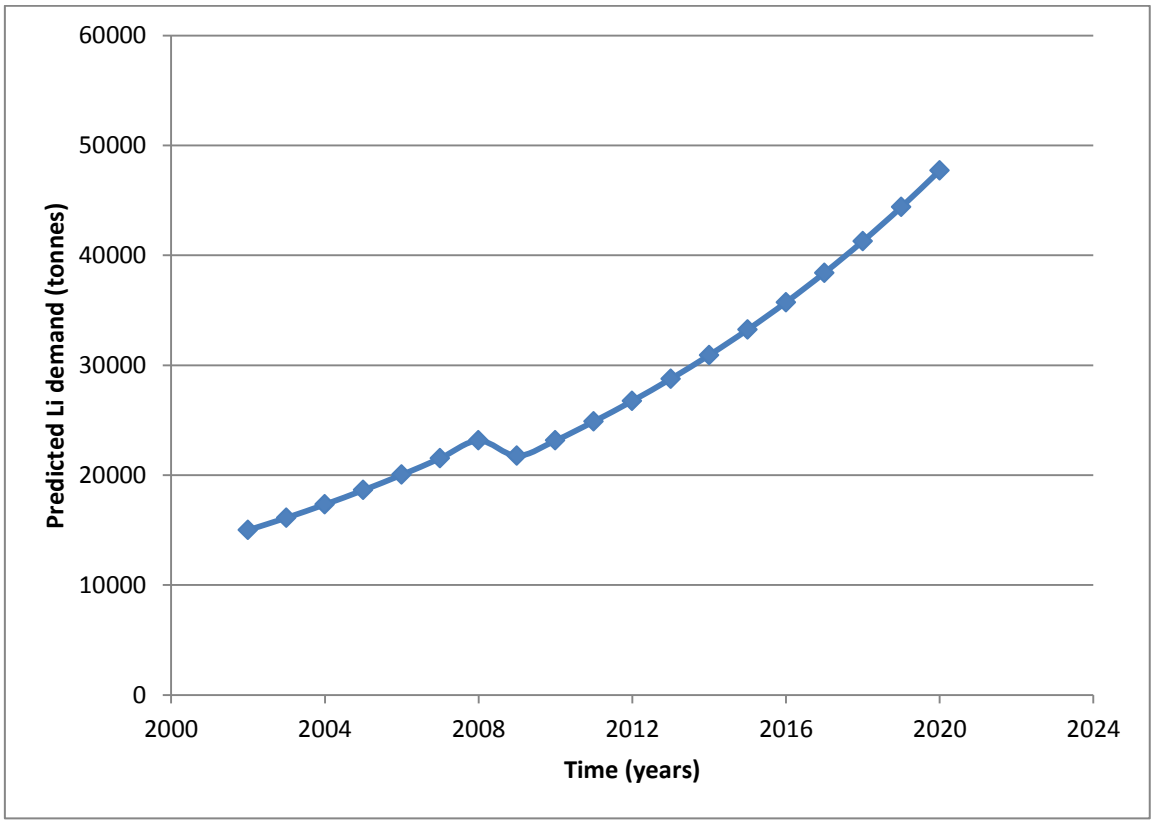


Figure 2.1 Shows the Li demand for the period of 2002-2011 and the prediction of demand until 2020. (Adapted from Anderson, 2011)

2.2 Uses of Lithium

Lithium is used in different industries of the modern world. The mainly noticed products that humans interact with are the portable devices with Li-ion batteries such as cell phones, laptops and many other electronic devices. Along with these Li is used in manufacturing, medicinal, automotive and ceramic industries (Table 1.2).

2.2.1 Glass, Ceramics & Aluminium processing

Glass, ceramic and aluminium refining industries are the major users of Li. Introduction of Li into the processing can not only save energy needed for melting the materials, but also give glass and ceramic products strengthening properties. The addition of Li in petalite to glass and ceramic batch compositions results in substantial energy savings by lowering the melting temperature and accelerating the melting process. Lithium is also a key ingredient in the production of zero-expansion (thermal shock resistant) glass, clay cookware, and glazes.

Lithium is also used in the potlines in electrolytic refining of aluminium to substantially reduce the electrical costs of the process. Lithium metal, when added to aluminium, is also used to create a light strong aerospace alloy (Le Couteur, 2011).

2.2.2 Lithium-ion batteries

The most common Li based products are rechargeable batteries, which can be long lasting and have the best size versus power density ratio. Lithium batteries are lightweight, compact and possess high energy density, excellent shelf life, long-term reliability, and high rate capability over a broad temperature range. These types of batteries are disposable (primary) batteries that have Li metal or Li compounds as the anode. Depending on the design and chemical compounds used, Li cells can produce voltages from 1.5 V to about 3.7 V, over twice the voltage of ordinary zinc-carbon battery or alkaline battery (NEMA, 2001).

Lithium batteries are widely used in modern portable consumer electronics, including iPods, iPhone, cameras, camcorders, and cell phones. The diminished battery size versus power aspect of the Li batteries has been credited with the smaller size of electronics (ILC, 2011).

2.2.3 Medicinal

Lithium was first introduced into the medical profession in the mid-1800s as a cure-all for many common illnesses (bi-polar disorder in particular). Lithium salts, especially the carbonate (Li_2CO_3) and acetate ($LiCH_3COO$) are extensively used in the treatment of manic-depressive disorders. Excretion of

Li is predominantly by the kidney and approximately 80% of Li is reabsorbed by the proximal renal tubule and 20% is excreted in the urine (Aral and Vecchio-Sadus, 2008).

Lithium is the main component in treatment of bipolar disorder. Lithium based batteries also find application in many long-life, critical devices, such as artificial pacemakers and other implantable electronic medical devices. These devices use specialized Li-iodide batteries designed to last 15 or more years (ILC, 2011).

2.2.4 Automotive industry

Since World War II, Li has essentially replaced Na in lubricants, resulting in waterproof greases (Le Couteur, 2011). Lithium soap is mixed with mineral oils as a thickening agent and therefore giving the mineral oils high resistance to extreme temperatures, non-corrosive and other properties shown in Table A.2 makes this the best lubricating agent that is widely used in the automotive industry. Lithium grease is a multi-purpose lubricant that provides superior lubrication and protection against corrosion. As a lubricant, it reduces metal-to-metal friction and wear while retaining it consistency over a wide temperature range. Also as a corrosion inhibitor, it prevents water and external contaminants from oxidizing metals (www.mgchemicals.com, 2012).

2.3 General soil sorption processes for Li

2.3.1 Cation exchange

Cation exchange is the process in which positively charged cations can bind to negatively charged soil surfaces. In most non acidic soils, the basic cations occupy 80 % or more of the exchange sites and the strength of affinity of cations with soil colloids are dependent on their charge. The strength at which cations are held to soil colloids in increasing order is: monovalent ions (e.g. Na^{+} and K^{+}), divalent ions (e.g. Ca^{2+} and Mg^{2+}) and finally trivalent ions (e.g.Al^{3+} and Fe^{3+}) (McLaren and Cameron, 1996).

Cations with a greater charge have a higher affinity to bind to soil colloids, due to a greater electrostatic attraction between the cation and the negatively charged soil colloid. Soil colloids are usually negatively charged due to isomorphous substitution in which cations with higher charges are replaced by cations with lower charges (McLaren and Cameron, 1996).

The charge of cations is not the only variable that affects cation exchange, as the strength of affinity is also influenced by the size of the hydrated radii of cations. Cations that have larger hydrated radii are held less tightly than cations with smaller hydrated radii. This is due to the shielding action of the water molecules surrounding the cations, which diminishes the strength of the charge of the cations,

subsequently reducing the electrostatic attraction between the cations and the soil colloids (McLaren and Cameron, 1996).

2.3.2 Specific adsorption

Adsorption is the process by which ions in soil solution form bonds with soil colloids with the ions accumulating on the surface of the soil colloids. The bonds which are formed between ions and soil colloids are chemical bonds such as ionic or covalent bonds (McLaren and Cameron, 1996). Two modes of chemical adsorption are distinguished. The free ion can sorb, along with the surrounding hydration water via ion-dipole interactions and electrostatic forces. This mode of adsorption is referred to as outer sphere, ion exchange or non-specific adsorption and is generally considered reversible.

The second mode of adsorption is termed inner sphere, chemisorption or specific adsorption. Here, the free ion loses the hydration water and forms chemical bonds (coordinative or other types) with solid surface functional groups (Simmler, 2012). Occlusion, which is the formation of an iron or aluminium coating around ions bound to soil colloids may occur following specific adsorption (McLaren and Cameron, 1996).

With regard to specific adsorption, Li is the most weakly bound of the alkali metals (Tanji and Wallender, 1990). A study found that the presence of calcium chloride ($CaCl_2$) significantly enhanced Li adsorption. However, the mechanisms that promoted the fixation were not identified (Davey and Wheeler, 1980; Anderson *et al.*, 1988). The presence of magnesium chloride ($MgCl_2$) also enhanced Li adsorption, but again the mechanisms explaining why this occurred were not identified (Anderson *et al.*, 1988).

2.3.3 Using Li as a tracer of soil contamination

Lithium is usually bound in the minerals as trace impurities in other compounds, especially feldspars and micas, or adsorbed onto soil solid phase, especially illites (Scott and Smith, 1987; Sparks and Helmke, 1996).

Due to the minimal health and regulatory concerns associated with Li, it is relatively inexpensive and easy to analyse, has a low background level, and most importantly shows a significant but low degree of sorption. Lithium can be considered a generic reactive tracer that does not necessarily mimic the behaviour of any pollutants of concern, but has the desirable property of providing interpretable reactive-solute field responses in reasonable amounts of time. Therefore Li has been used to investigate the movement of trace impurities in soil (Claridge *et al.*, 1999; Anghel *et al.*, 2002).

2.4 Bioaccumulation coefficient (BC)

The uptake of metals by plants following wet and dry deposition is considered as one of the most critical interactions between the atmosphere and vegetation (Rao and Dubey, 1992). When plants are used as bioindicators, the measured uptake of metals may give an indication of their availability in the soil (Bjerre and Schierup, 1985). In addition, the use of plants as bioindicators provides an estimation of their contribution to the food chain (Kovacs and Podani, 1986; Brault *et al.*, 1994).

2.5 Potential mechanisms of plant Li uptake

Water and dissolved minerals, including metals such as Li, are taken up by plants from the soil solution through the epidermis. The epidermis is a single layer of cells covering the root. Root hairs enhance the uptake process as they increase the surface area of the epidermal cells. The uptake of these nutrients is driven by passive transport through diffusion or mass flow. Once passed the epidermis the solutes enter the apoplast, which describes the cell wall continuum (Campbell and Reece, 2002).

Solutes cross the cortex of the root either through the apoplastic or the symplastic lateral transport route. In the apoplastic route solutes travel across organs via the cell wall continuum while in the symplastic route solutes enter one cell through the plasma membrane and move across organs via the cytosolic continuum. For the apoplastic pathway, movement to the stele and vascular tissue is restricted by the endodermis including the Casparian strip, a belt made up of suberin, a waxy material that is impervious to water and dissolved minerals. To enter the vascular tissue for upward transport in the stele, solutes in the apoplast must enter a cell through the selective plasma membrane to cross the Casparian strip while solutes in the symplast have already crossed a selective plasma membrane and can therefore directly pass the endodermis (Fig. 2.2) (Campbell and Reece, 2002).

The symplastic pathway plays a key role in the transport of most nutrients. Solutes either enter the symplast at the rhizodermis and the root hairs or at the endodermis. In the symplast, solutes move from cell to cell through plasmodesmata. They connect neighbouring root cells in a complex structure. The transport of any compound through the plasma membranes is facilitated by transporter proteins. There are three known kinds of transporter proteins: (1) primary active transporters (pumps), (2) secondary active transporters or coupled transporters and (3) passive transporters. For primary transporters solute transport is directly coupled to the hydrolysis of an energy substrate such as ATP or pyrophosphate. With the secondary transporters the electrochemical gradient generated by (mostly) hydrogen ions is used to transport a solute either in

the same (symport) or the opposite (antiport) direction. Passive transporters catalyse the movement of solutes down their electrochemical gradient through a variety of uniports and channels.

In the vascular tissue of the stem, solutes other than essential nutrients and water are transported from the roots to the shoots, leaves and reproductive organs. The plant's vascular system comprises the phloem and the xylem. In the xylem metals and water are transported upwards through bulk flow, driven by the tension caused by transpiration. In the phloem organic compounds, such as sucrose made in mature leaves, and some minerals are transported to the roots and other non-photosynthetic parts of the shoot system such as developing leaves and fruits (Campbell and Reece, 2002).

From the xylem solutes enter the leaf cell apoplastic spaces and are then transported across a plasma membrane via cation channels and transporters to enter the symplasts where they are distributed to the required cells (Longnecker and Robson, 1993; Welch and Norvell, 1999; Schrauzer, 2002). As the xylem transport is driven by transpiration, solutes released from the xylem, accumulate in the sites of highest transpiration, which are often not the sites of highest demand for nutrients.

Phloem transport is driven by bulk flow to sites of lower internal pressure, which means sites that act as solute sinks. During long distance transport in the vascular tissue metals are exchanged between the xylem and the phloem. Nutrients can be redistributed within the plant from older tissues to young tissues with high nutrient demand through phloem transport. The phloem transports nutrients to areas of high demand, which are either utilization sinks such as root tips, shoot apices and stem elongation zones or storage sinks (Engels *et al.*, 2012).

Lithium's transportation patterns in plants is not known and no published knowledge on whether Li is transported through xylem (apoplastic pathway) or through the phloem (symplastic pathway). But by taking other cations of similar physical and chemical properties it may be speculated that Li would enter the root via the apoplastic pathway and be primarily transported in the xylem, with limited phloem mobility.

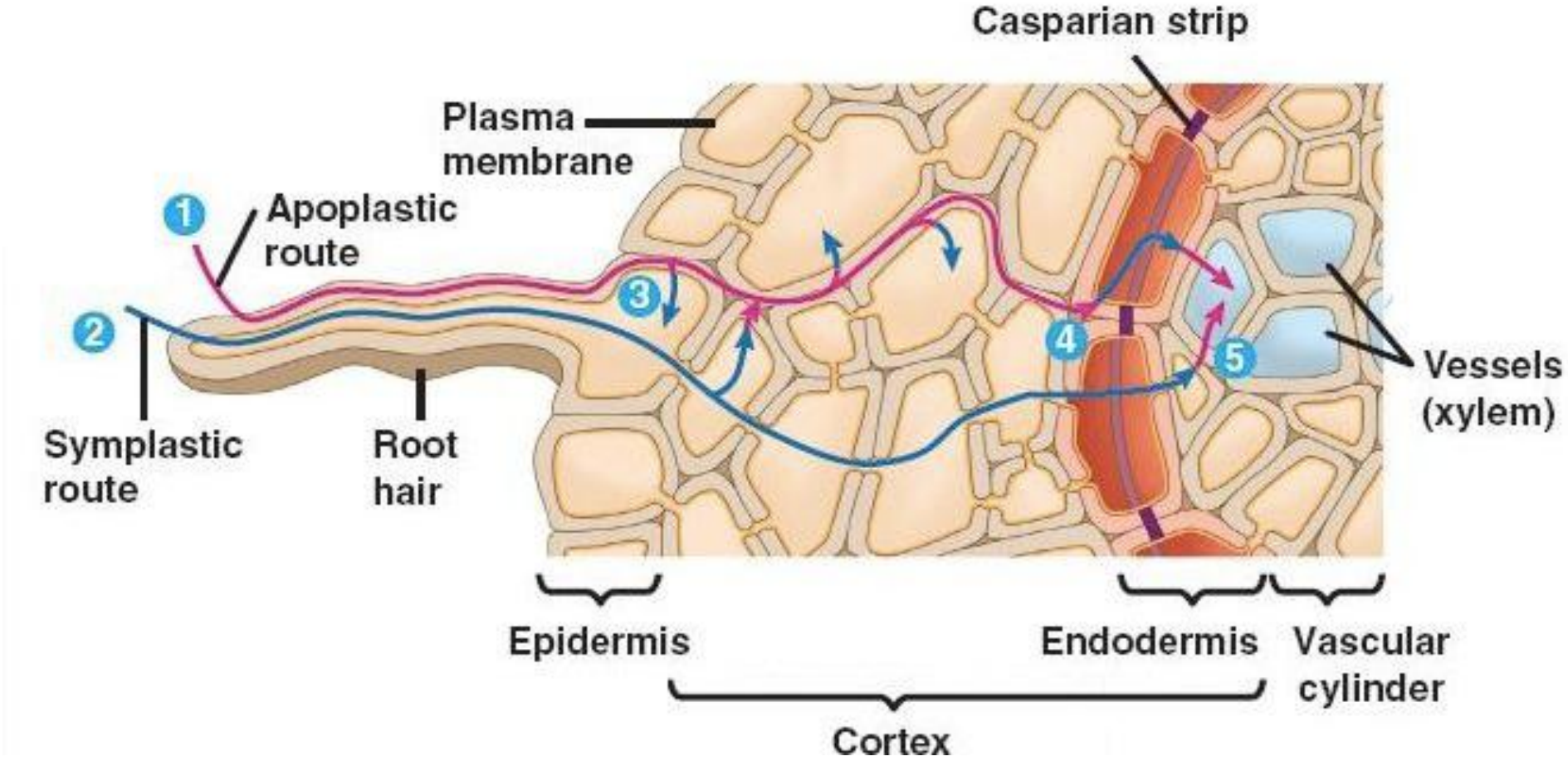


Figure 2.2 The uptake of water and minerals (e.g. Li) by plant roots and the lateral transport to the vascular tissue. Solutes are transported in roots through the apoplastic (1) or the symplastic (2) route; they may change from the apoplastic to the symplastic route during their transport (3). To enter the xylem (5) metals must cross the endodermis (4) and the Casparian strip, which act as barriers (Campbell and Reece, 2002).

Chapter 3

Methods and materials

3.1 Survey of Li in New Zealand soils and pastures

Soil and pasture samples were collected from various farms around New Zealand (Fig. 3.1). After removing herbage and any surface litter, soil was collected using a 7.5 cm soil corer. Pasture was harvested using scissors ca. 1 cm from the growing points to avoid soil contamination. Pasture samples were thoroughly washed in deionised water. Pasture and soils were dried at 105^{o}C until a constant weight was obtained. The samples were stored in sealed plastic bags for chemical analyses.

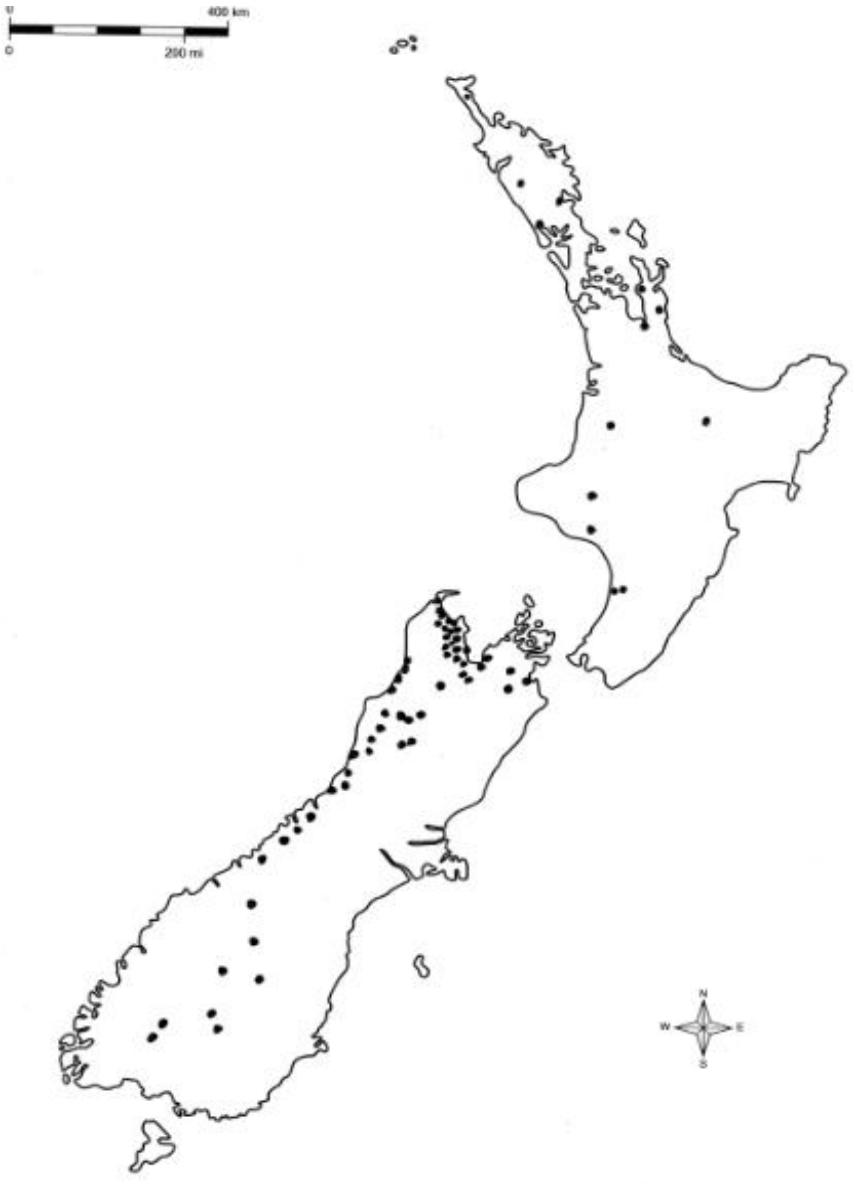


Figure 3.1 Sampling locations around New Zealand

3.2 Soil collection and pretreatment

The soil for the experiment was collected from Lincoln university dairy farm located on Springston Rolleston road (coordinates: -43.636, 172.438) as shown in Fig. 3.2. The top 7.5 cm of the TSL soil after leaf litter had been removed was collected and transported to field service centre, located on Lincoln university campus.

Figure 3.2 Soil collection site for the experiment (source: google maps)

I used the Templeton Silt Loam (TSL) for my experiments. The TSL is a recent soil that has formed from river sediments that are now above the current floodplain. These soils have developed in fine textured sediments, forming a medium to free draining soil with moderate moisture retention. (Officer *et al.*, 2004).

3.3 Greenhouse experiments

3.3.1 Soil preparation

Agricultural grade lime was added to bring the pH of the TSL soil from initial pH of 5.0 to 6.2 .The details of the pH experiments are shown in Table A.4. Two Li complexes $LiNO_3$ and LiCl were used as the sources of Li for this experiment. Different concentrations (0, 1, 3, 10, 30 & 100 mg kg^{-1}) were made up by mixing different weights (calculated on the basis of % Li in the Li complexes) of crystals with 250 ml deionised water (as shown in Table A.6). The range of Li solutions along with agricultural grade lime (at 3% w/w ratio) was mixed with soil using a concrete mixer (Fig. 3.3). The soil with $LiNO_3$ and lime was potted into fifteen 2.5 litre pots (and three 7.5 litre pots per treatment (only three

concentrations (0, 10 & 30 mg kg^{-1}) were tested for *H. annuus*. The soil with LiCl and lime was potted into 6 pots per treatment. The pots were then moved to a greenhouse. Sub samples of the soils with Li concentrations and lime were collected; oven dried (~105^{o} C) and passed through a 2 mm sieve for $Ca(NO_3)_2$ extractions and pseudo-total elemental analysis.

Figure 3.3 The concrete mixer used for soil preparation and 7.5 litre pots after soil mixing

3.3.2 Natural Li uptake by selected plants:

Selected plant species were grown in unspiked TSL to determine the range of natural Li concentrations. Twelve species, replicated three times were grown in 36 two-litre cylindrical plastic pots filled with TSL to a depth of 10 cm. The pots were placed in a greenhouse at Lincoln University, New Zealand and left for two weeks before planting. The vegetables analysed include broccoli (*Brassica oleracea* L. var "Shogun"), carrot (*Daucus carota* L. var "Egmont Supreme"), and lettuce (*Lactuca sativa* L.) leek (*Allium porrum* (L.) J. Gay), radish (*Raphanus sativus* L. var "Champion"), spinach (*Spinacia oleracea* L. var "New Zealand"), corn (*Zea mays L*. var. rugosa), tomato (*Solanum lycopersicum* L. var "Russian Red"), courgette (*Cucurbita pepo* L. var "Blackjack"), beetroot (*Beta vulgaris*), rye grass (*Lolium perenne* L.) and sunflower (*Helianthus annuus* L.). All plant material was sourced from Oderings Nursery, 20 West Coast Road, Yaldhurst, and Christchurch, New Zealand. *Daucus carota*, *R. sativus*, *S. oleracea*, *Z. mays* L and *C. pepo* were grown from seed, whereas, *B. oleracea, A. porrum* and *S. lycopersicum* were grown from seedlings. Three seeds were planted per pot. After emergence, seedlings were thinned out to one per pot. Pots were arranged in a randomised block design.

Plants were harvested upon maturation of the edible portions. The edible portions of the plants were excised. The root vegetables (*R. sativus*, *B. vulgaris* and *D. carota*) were peeled. The fresh weight of the edible portions was determined. Both the edible portions and the residual material was washed

thoroughly with deionised water and placed in a drying cabinet at 105 ^{o}C until a constant weight was obtained. Samples were ground and stored in an airtight container until chemical analyses.

3.4 Plant uptake and tolerance of Li:

2.5 litre pots were used for smaller plants (*B. vulgaris* ($LiNO_3$), *L. sativa* ($LiNO_3$), *B. nigra* (LiCl) and *L. perenne* ($LiNO_3$ & LiCl) and the 7.5 litre pots were used for *H. annuus* ($LiNO_3$) (Note: Li complexes used for testing the plants were shown in brackets beside each plant type) . *Lolium perenne* (50 seeds/pot), *B. vulgaris* (5 seeds /pot), *B. nigra* (5 seeds /pot), *L. sativa* (4 seeds /pot) and *H. annuus* (3 seeds /pot) were introduced into the pots on 19/09/2011. After the plants were visible they were thinned to one plant per pot (with the exception of *L. perenne*). The plants were grown in a green house where they were maintained with regular watering and removal of weeds. On 22/09/2011, the plants were organised into a randomised block design.

Figure 3.4 Showing the pot trial setup for the project

On 19/12/ 2011, when the edible portions of the plants were mature, they were harvested using scissors ca. 1 cm from the soil surface. Root crops were harvested as described below. The harvested plants were thoroughly washed in deionised water and transferred into pre labelled paper bags and oven dried for at 105^{o}C until constant weight was obtained. The dry plant tissue was ground into a fine powder and stored in labelled plastic bags.

The *B. vulgaris* leaves and bulbs were harvested, leaves were washed & dried and the bulbs were peeled, washed and dried separately. The *H. annuus* plants were divided into six samples – bottom leaves, middle leaves, top leaves, shoots, flower and roots (as shown in Figure B.1).

3.5 Batch experiments:

3.5.1 Calcium nitrate extraction

0.05 M $Ca(NO_3)_2$ at a ratio of 1:6 (soil: $Ca(NO_3)_2$) was used for this part of the experiment. The solution was made up using $Ca(NO_3)_2$ (BDH AnalaR $Ca(NO_3)_2x4H_2O$) and de-Ionised water. This procedure was used to determine extractable Li from soils. This involved weighing out 5 grams of air-dried soil sieved to < 2mm into a centrifuge tube and addition of 30 ml 0.05M $Ca(NO_3)_2$ solution. The mixture was shaken end-over-end for two hours, followed by ten minutes centrifuge at 1200 rpm. The supernatant was then filtered through a Whatman 52 filter paper (pore site 7 µm) into a 30 ml vial and stored in a refrigerator for further analysis.

3.5.2 Extractable Li at different pH's and different Li concentrations

Five grams of the originally collected soils (without lime) were weighed into vials and 30 ml of 0.05M $Ca(NO_3)_2$ is added to the soil. The original pH was 4.3. Different pH (2.8, 4.3, 5.2, 6.5 & 7.4) was adjusted using HNO_3 and KOH (as shown in Table A.7). The concentration of Li that can be extracted at different pH values was done by spiking the concentrations using the pre made $LiNO_3$ solutions. Reagent blanks were prepared for all treatments. The tubes with the spiked Li ,$Ca(NO_3)_2$ and soil mixture were shaken end-over-end for two hours, followed by centrifuging for 10 minutes at 1200 rpm. The supernatant was filtered through a Whatman 52 filter paper (pore site 7 µm) into a 30 ml vial and stored in a refrigerator and analysed for extractable Li concentrations.

3.6 Chemical analyses

3.6.1 Pseudo-total elemental analysis - plant tissue sample preparation

Some 0.3 ($\pm$ 0.05) grams of the dried and finely ground plant samples were accurately weighed using a weighing paper and transferred into 10 mL plastic tubes. Five (5) ml of concentrated HNO_3 was dispensed into the tubes and were then left in a fume hood overnight to eliminate the excess HNO_3. The tubes were then sealed using a rubber stopper and a lid and processed through a microwave digestion method; in which, the samples were digested for 40 minutes (20 minutes to increase the temperature to 175^{o}C and 20 minutes holding the samples at 175^{o} C). After the microwave digestion of samples, 15 ml of MilliQ water was added to the tubes; the digested solution was then transferred and stored in a 30 ml vials for analysis.

3.6.2 Pseudo-total elemental analysis- soil sample preparation

The pseudo analysis for the soils involved a similar procedure to that of plant tissues, 0.5 g of air dried and sieved soil was digested in 15 ml HNO_3 and 1ml (H_2O_2). Upon microwave digestion procedure, the samples were filtered through Whatman 52 filter papers into 30ml pre labelled vials for analyses.

Concentrations of Li along with Al, As, B, Cd, Cr, Cu, Fe, K, Mn, Ni, P, Pb, and Zn for soils and plants were determined using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES Varian 720 ES). Wageningen reference soil (ISE 921) and plant (IPE 100) material were analysed for quality assurance. Recoverable concentrations were 91% - 108% of the certified values. The detection limit for Li in solutions was 0.001 mg L^{-1}. For plants and soils, which were digested using nitric acid, the detection limit was 0.05 mg kg^{-1}.

3.7 Data Analysis:

All the data from the experiment was stored in the form of Microsoft Excel 2010. Minitab® 16 was used for ANOVA with Fisher's Least-Significant-Difference post-hoc test to compare means. The level of significance was 0.05.

Chapter 4

Results & Discussion

4.1 Lithium concentrations in different soils and pastures of New Zealand

The Li concentrations in NZ soils ranged from 0.08 mg kg^{-1} to 92 mg kg^{-1}. Table 4.1 shows that soils with high clay content had significantly higher Li concentrations. This is consistent with previous work that has reported higher Li concentrations in the clay fraction (Schrauzer, 2002) .In contrast, sandy soils had lower Li concentrations.

Table 4.1 Mean [Li] (mg kg^{-1}) dry weight for NZ pasture soils. Values in brackets are the standard error of the mean. Values with the same letter are not significantly different at the 5% level.

Texture	N	Mean Soil (SE)^{grouping}	N	Mean Li *L.perenne* (SE)^{grouping}
Silty clay loam	7	48.8 (11.1)^{a}	5	1.4 (0.6)^{a}
Clay loam	15	24.9 (4.3)^{b}	6	1.2 (0.2)^{a}
Silt loam	9	24.3 (4.4)$^{b\,c}$	6	1.8 (0.7)^{a}
Loam	17	21.6 (2.7)$^{b\,c}$	9	1.8 (0.6)^{a}
Sandy loam	13	11.7 (2.6)^{c}	7	1.7 (0.6)^{a}
Sandy clay loam	4	10.9 (3.2)$^{b\,c}$	2	0.9 (0.6)^{a}

One possible explanation for these findings is that clays have a higher Li concentration is that the pseudo-total analyses that I performed does not access the Li bound deep within silicate matrices. It has been suggested that Li may be located internally within clay minerals in ditrigonal cavities (Anderson *et al.*, 1988). Small particles, i.e. clays, have a large surface area: volume ratio. Therefore, more Li may dissolve from the surface of such particles compared to the larger silt and sand particles. This hypothesis could be tested by dissolving the soils with hydrofluoric acid, which breaks down silicate matrices.

Pasture concentrations ranged from 0.5 mg kg^{-1} – 6.1 mg kg^{-1}. Pasture concentrations were some tenfold magnitude lower than soil concentrations. Unlike the soil Li concentration, there were no significant differences between the pasture concentrations from different soil types.

Table 4.2 shows the correlations between Li in soils and plants with various other soil and plant parameters. There was a strong positive correlation between Li concentration and clay content of the soil and negative correlation with sand content of the soil. Aluminium, B, Fe, K, Mg, Mn and Zn were strongly and positively correlated with Li soil concentrations.

Pasture Li was significantly negatively correlated with soil Mg. Potentially, Mg may compete with Li for binding sites at the plant roots, thereby reducing uptake. This may be due to the reported chemical similarities between Li and Mg (Hawrylak-Nowak *et al.*, 2012).

Lithium concentrations in pasture were positively correlated with Al and Fe concentrations. This is consistent with some of the plants' measured Li concentrations in plants arising from dust particles on the leaves that may not have been removed, even following extensive washing (Robinson *et al.*, 2008).

Table 4.2 Correlation matrices between soil Li Vs soil texture, soil Li Vs Li pasture and soil Li Vs other elements

Elements	Soil Li and soil properties	Pasture Li and soil properties	Pasture Li and other elements in pasture
Sand	-0.53 (S**)	0.05 (NS)	
Silt	0.36 (S)	0.03 (NS)	
Clay	0.43 (S**)	-0.14 (NS)	
pH	0.06 (NS)	-0.04 (NS)	
Al	0.59 (S**)	-0.10 (NS)	0.90 (S**)
B	0.65 (S**)	0.17 (NS)	-0.02 (NS)
Ca	0.12 (NS)	0.16 (NS)	-0.16 (NS)
Cu	0.13 (NS)	0.04 (NS)	-0.002 (NS)
Fe	0.56 (S**)	0.09 (NS)	0.90 (S**)
K	0.56 (S**)	0.20 (NS)	-0.14 (NS)
Mg	0.68 (S**)	-0.23 (S)	-0.23 (NS)
Mn	0.45 (S**)	0.07 (NS)	0.06 (NS)
Mo	0.15 (NS)	0.21 (NS)	0.21 (NS)
P	0.08 (NS)	-0.07 (NS)	-0.07(NS)
S	-0.22 (S)	0.08 (NS)	0.08 (NS)
Zn	0.60 (S**)	-0.15 (NS)	-0.15 (NS)

4.2 Lithium sorption by soil:

Table 4.3 shows the total and soluble concentrations of Li and the major cations in the TSL. The degree of sorption is indicated by the value of K_D (sorbed/solution concentration coefficient). Compared to the major cations and plant nutrients, Li in the TSL has a K_D value that is over an order of magnitude greater than P, which is considered an immobile element in most soils (Lajtha and Schlesinger, 1988)**.** This indicates that the endogenous Li in these soils is likely to be highly unavailable for plant uptake.

Table 4.3 The properties of Templeton Silt Loam on Lincoln university (adapted from (Knowles *et al.*, 2011)

	Total	**Extractable**	**K_D**
pH	5.6	n/a	n/a
C (%)	2.0	n.d	n.d
N (%)	0.18	n.d	n.d
CEC (cmol kg^{-1})	12.5	n/a	n/a
Li (mg kg^{-1})	31.8	0.004	7950
Mg (mg kg^{-1})	855	16.6	52
K (mg kg^{-1})	1401	14.0	100
Na (mg kg^{-1})	136	44.9	3
Ca (mg kg^{-1})	3005	n.d.	n.d.
P (mg kg^{-1})	518	0.71	729
S (mg kg^{-1})	193	8.5	23

Fig. 4.1 shows the sorption of Li by soil (note that the endogenous Li was excluded for the calculations of K_D values shown in Fig. 4.1) as a function of pH. The values of K_D in this study (tested with spiked Li concentrations of 1,3,10 & 30 mg/kg) ranged from 0.13-3 (Fig. 4.1). These values are much lower than K_D values typically reported for other metals. For comparison, the K_D values for Cu, Ni, and Zn in this soil were Cu: 358, Ni: 82 and Zn: 55 respectively. This indicates that added Li is likely to be relatively mobile in soil. There was a sharp contrast between the soil's ability to sorb soluble Li that is added to the soil, compared to the low solubility of the soils endogenous Li present in the silicate phase.

With respect to pH and the concentration of Li added, the sorption of this metal by soil followed a pattern that is consistent for most cationic elements in soil. Lithium sorption increased as pH increased. Increasing the concentration of Li in solution resulted in relatively less sorption, presumable due to saturation of exchange sites. My results indicate that the behaviour of Li in soil is consistent with that of a non-specifically adsorbed cation.

The CEC of the TSL was 12.5 $_c$mol/kg. This is in the low-medium range of NZ soils. It is likely that high-fertility soils and peat soils that can have a CEC > 40 $_c$mol/kg may retain more Li (as shown in Table A.11)**.**

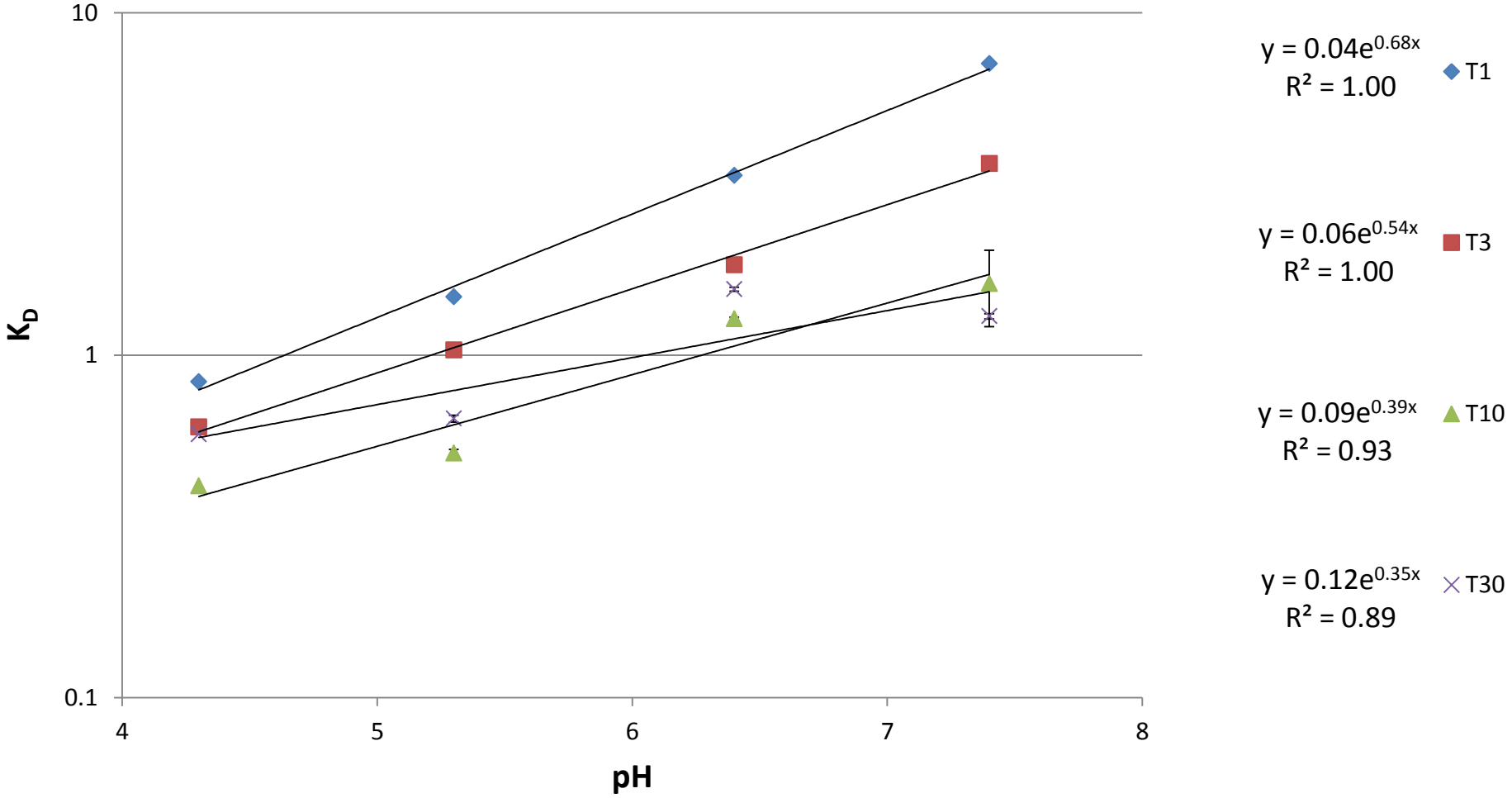


Figure 4.1 K_D as a function of pH at varying Li concentrations. Error bars represent the standard error of the mean (n=3). The background Li in the soil was 25 (mg kg^{-1}).

4.3 Lithium uptake by food & fodder species

Figure 4.2 shows that the Li concentrations in edible plants grown in the TSL, differed by 2 orders of magnitude, ranging from <1 mg kg^{-1} – 1.8 mg kg^{-1}. The results indicate that, as with other elements, the plant concentration was highly species dependent.

Generally, Li concentrations were higher in the leafy portions of the plants, with bulbs and seeds being significantly lower. *B. vulgaris* bulbs were an exception, having higher Li concentrations than *B. vulgaris* leaves.

Vetter (2005) reported that dicots take up more Li than monocots. In my study, *L. perenne* (a monocot), had the highest Li concentration. Interestingly, the Li uptake by *L. perenne* in this experiment was higher than that found in the NZ pasture survey. This may be due to variation in species composition because the survey of NZ pastures contained a variety of species, which may have had different Li uptake profiles. The Li concentration in the TSL was 31.8 mg kg^{-1} (Table 4.3), which was towards the higher end of the soils in the NZ pasture survey (Table 4.2).

My results indicate that Li may be contained in silicate matrices (Table 4.1). If *L. perenne* can open up these matrices then it may accumulate more Li. Therefore, high Li concentrations in *L. perenne* could have been influenced by the high tendency of Si accumulation in grasses. The other monocots in this experiment, *Z. mays* and *A. porrum* had the lowest Li concentrations. In the case of *Z. mays,* it was the cobs, rather than the leaves that were analysed. Likewise, only the lower portions of the leaf were analysed in *A. porrum*.

Of the common food sources for humans *B. vulgaris* had the highest Li concentration followed by *S. oleracea* and *L. sativa*. With the exception of *L. perenne,* this pattern of accumulation is similar to that reported by Gartler, (2010) for Zn and Cd in the same species. As both of these elements are relatively phloem immobile, my results indicate that Li may be primarily transported in the xylem, being deposited in the leaves, which are the major water sink.

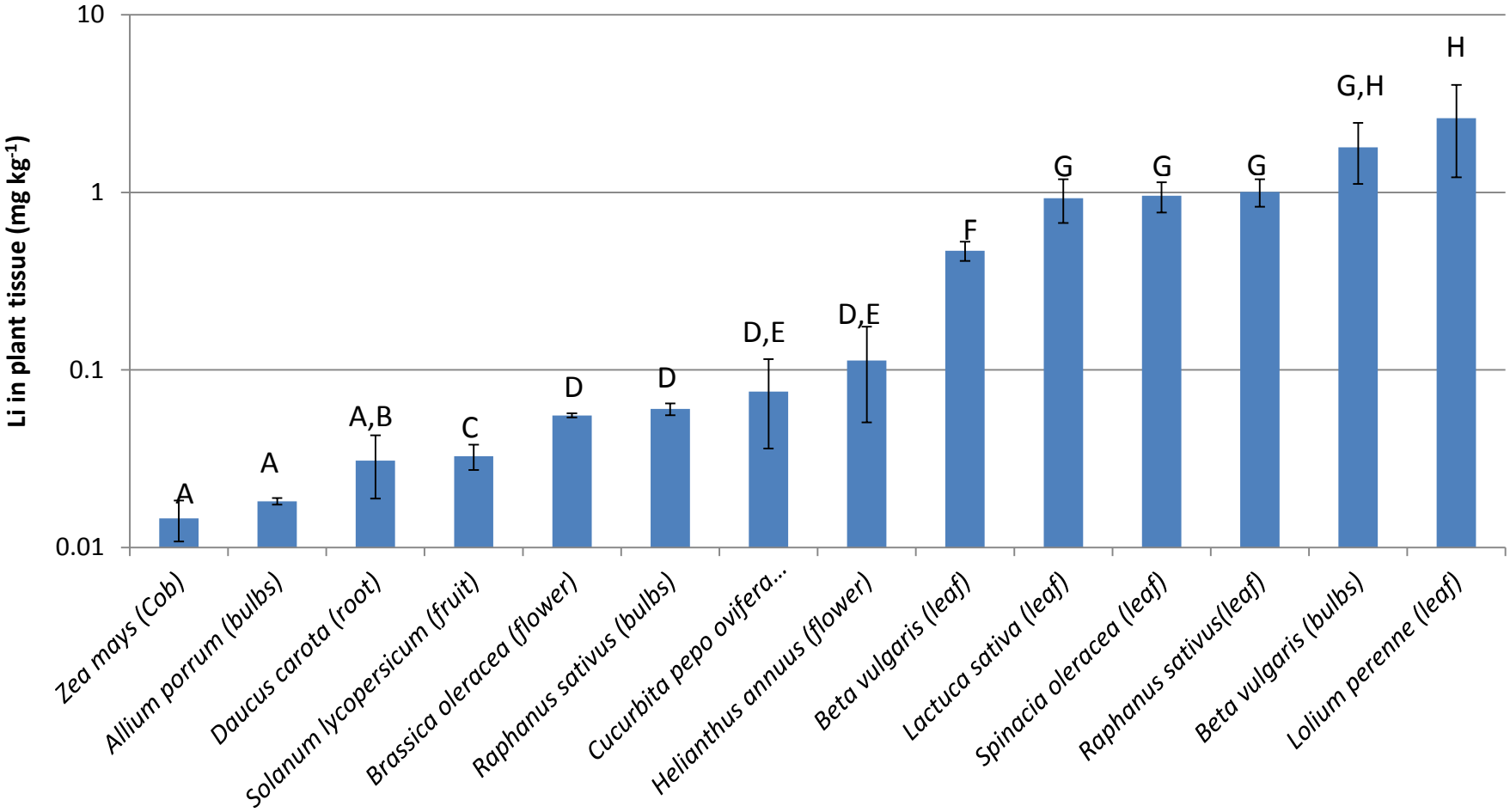


Figure 4.2 Li concentration in edible portion of food and fodder species from control Templeton Silt Loam soil. Error bars represent the standard error of the mean (n=3)

Table 4.4. Bioaccumulation coefficients (plant / soil concentration quotient) for Li and other selected elements in the plants tested. Values are reported using both the total and $Ca(NO_3)_2$ extractable soil concentrations. Note that I could not determine soluble Ca in the extract.

Bioaccumulation coefficients calculated using total Li						
	Li	**Ca**	**Mg**	**Na**	**K**	**B**
Zea mays	0.000	0.6	1.5	0.4	15	2.4
Allium porrum	0.001	0.7	0.6	5.1	5.3	0.6
Daucus carota	0.001	0.7	0.4	15	8.9	1.9
Solanum lycopersicum	0.001	0.2	0.7	1.8	14	1.0
Brassica oleracea	0.002	1.9	1.1	4.9	11	4.1
Raphanus sativus **(bulbs)**	0.002	0.8	1.0	19	21	2.6
Cucurbita pepo	0.003	0.2	1.2	0.4	6.2	1.1
Beta Vulgaris **(leaves)**	0.015	3.9	2.3	113	22	11
Lactuca sativa	0.029	2.3	1.3	13	26	2.9
Spinacia oleracea	0.031	3.5	1.7	89	21	3.3
Raphanus sativus **(leaves)**	0.031	8.9	1.6	29	19	4.4
Beta Vulgaris **(bulbs)**	0.056	0.3	0.8	2.2	4.8	1.2
Lolium perenne	0.082	1.6	1.3	9.6	13	1.8
Bioaccumulation coefficients calculated using extractable Li						
Zea mays	3.8	n.d.	147	1.5	2845	192
Allium porrum	4.5	n.d.	63	21	982	52
Daucus carota	7.8	n.d.	45	60	1639	153
Solanum lycopersicum	8.3	n.d.	71	7.4	2664	78
Brassica oleracea	14	n.d.	106	20	2095	330
Raphanus sativus **(bulbs)**	15	n.d.	96	79	3860	211
Cucurbita pepo	20	n.d.	123	1.5	1142	85
Beta vulgaris (leaves)	118	n.d.	225	462	4153	848
Lactuca sativa	233	n.d.	131	53	4714	235
Spinacia oleracea	243	n.d.	170	363	3958	263
Raphanus sativus **(leaves)**	250	n.d.	155	120	3482	358
Beta vulgaris **(bulbs)**	448	n.d.	81	9.0	885	93
Lolium perenne	655	n.d.	134	39	2462	148

Tables 4.4 shows the Bioaccumulation Coefficients (BC), defined as the plant/soil concentration quotient, for Li, B and the major soil cations in the food and fodder species. The bioaccumulation coefficient gives a measure of the plant's ability to accumulate an element in the above ground

portion. I have calculated the BC using both the total and extractable [$Ca(NO_3)_2$] soil concentrations as the denominator. Table 4.4 shows that compared to the B and the major cations, the bioaccumulation coefficients calculated with the total soil Li concentration are some two orders of magnitude lower than for the other elements. This is consistent with the observation that the endogenous Li in the soil has a low solubility compared to other elements (Table 4.1). However, when the extractable Li was used to calculate the BC, the values for Li were of a similar magnitude to those of the major cations. Therefore, given the low-capacity of the soil to sorb exogenous Li, I would hypothesise that any added Li would be readily taken up by plants and thence enter the food chain.

4.4 Lithium uptake by *L. perenne, B. nigra, B. vulgaris and L. sativa*

Fig. 4.3 shows Li concentrations in a *L. perenne, B. vulgaris, B. napus* and *L. sativa* as a function of Li concentration in the soil, added both as the nitrate and the chloride. Lithium uptake into plants increased in proportion to the soil Li concentration up to a soil concentration of 5 mg kg^{-1}, with little difference between nitrate and chloride (Fig. 4.3 a). At soil concentrations of 5 mg kg^{-1} and above, there was a plateau of Li in plant tissue at ca. 1000 mg kg^{-1}. Fig. 4.3 b shows a similar pattern for the other species. All the plants follow remarkably similar Li uptake responses (Fig. 4.3). At this soil Li concentration, the bioaccumulation coefficient of the plants was >200. These results indicate that plants offer no food chain protection for any Li that is added to the soil.

Although *B. vulgaris* bulbs had the highest Li concentration in the control soils, there was no significant correlation with increased Li concentrations in soil and the plant tissue. *B. vulgaris* is a relatively salt tolerant plants (Shanno and Grieve, 1999). It is possible that their high tolerance of Na confers tolerance to increased soil Li. Lithium may be transported via the same uptake mechanism as Na into the plants. The Li concentrations in *B. vulgaris* (leaves) increased with increasing soil concentrations; the *B. vulgaris* bulbs had relatively low Li concentration with increasing soil concentrations.

Table 4.5 Showing the vegetables tolerance to salinity (adapted from (Shanno and Grieve, 1999))

Slighty sensitive	**Moderately sensitive**	**Highly sensitive**
Brassica oleracea	*Lactuca sativa*	*Daucus carota*
	Spinacia oleracea	*Raphanus sativus*
	Beta vulgaris	
	Brassica nigra	

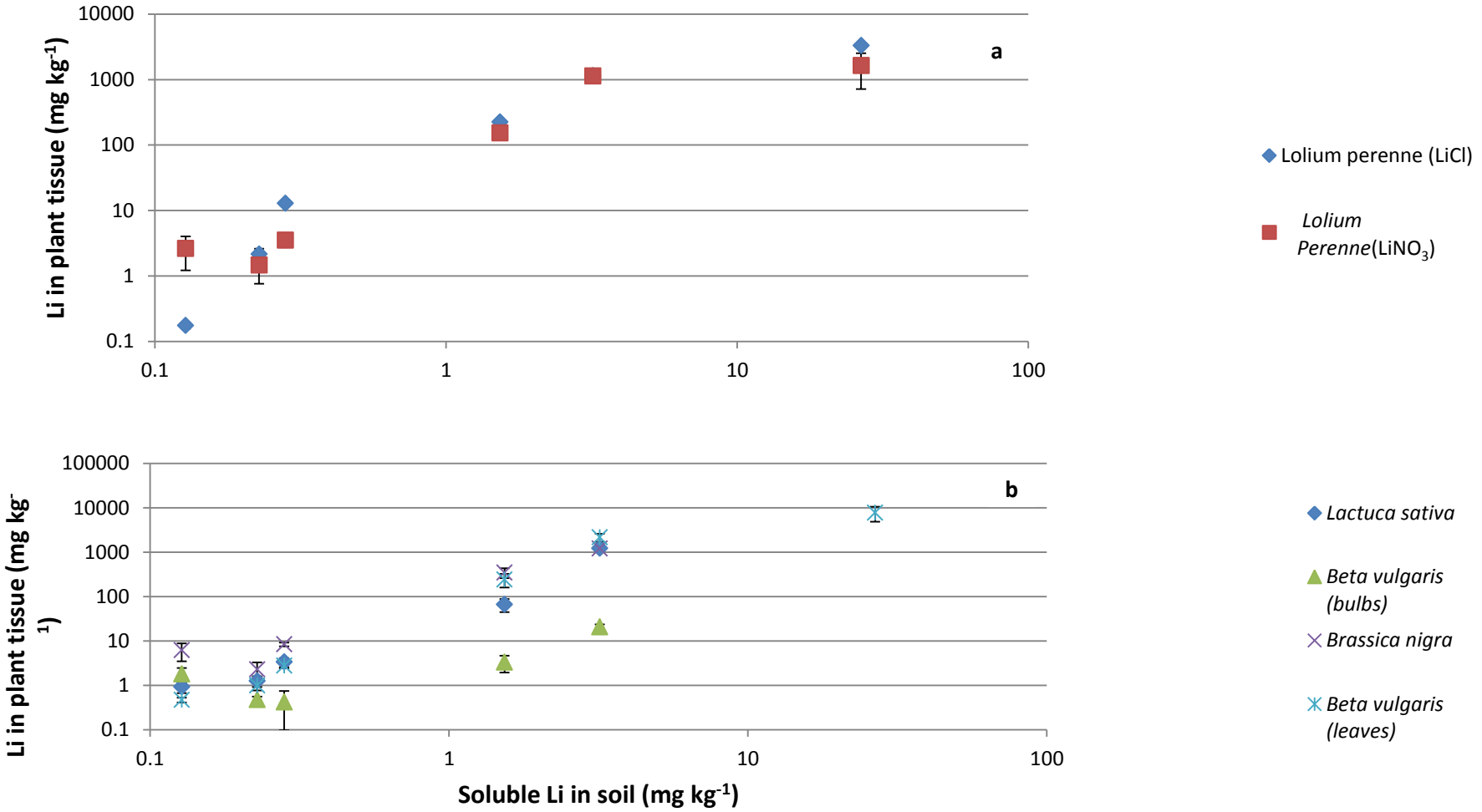


Figure 4.3 (a) Li uptake in *L. perenne*, (b) Li uptake by *B. nigra*, *B. vulgaris* & *L. sativa*. Error bars represent the standard error of the mean (n=5)

4.5 Toxicity thresholds of *L. perenne*, *B. nigra*, *B. vulgaris* and *L. sativa*:

Figure 4.5 shows the biomass of *L. perenne* as a function of the foliar Li concentration. The biomass production of *L.perenne* was unaffected by Li concentrations in the leaves of <1000 mg kg^{-1} dry weight (Fig 4.5 a). This concentration was achieved when just 5 mg kg^{-1} Li was added to the soil (Fig. 4.5). For *B. vulgaris*, *L. sativa* and *B. nigra* (Fig. 4.5 b), the toxicity thresholds were somewhat lower (ca. 500 mg kg^{-1}) again achieved at a soil concentration of ca. 5 mg kg^{-1} added Li. Above these toxicity thresholds, the plants progressively developed chlorosis with necrotic spots appearing on the leaf margins and leaf tips (Fig.4.4). This is similar to the toxicity symptoms of B and Na (Brownell and Crossland, 1972; Brown and Hu, 1996).

Comparing the uptake and toxicity of Li in molar terms, 1000 mg kg^{-1} Li is to Na and Cd concentrations of 2,000 mg kg^{-1} and 1.5% respectively, which in the case of Cd has never been reported. This indicates that Li is comparatively non-toxic to plants.

The extraordinarily high effective concentration of Li required to reduce growth indicated this element is unlikely to seriously perturb biochemical or physiological function. Toxicity of Li at these high concentrations may be due to changes in osmotic balance rather than direct toxicity. *Beta vulgaris* TIs were markedly different to the other species, which may reflect different allocation of Li between plants parts associated with the salt tolerance of this species.

Less Li is mobilised to flowers and fruits compared to leaves as shown in Figures 4.5 (a and b). This indicates that, in Li contaminated soils, there is unlikely to be reduction in productivity. In terms of the soil-plant barrier concept by Chaney (1983), phytotoxicity of Li is unlikely to offer any food chain protection.

Figure 4.4 Showing the *H.annuus* leaves at the start of the experiment and at the harvest

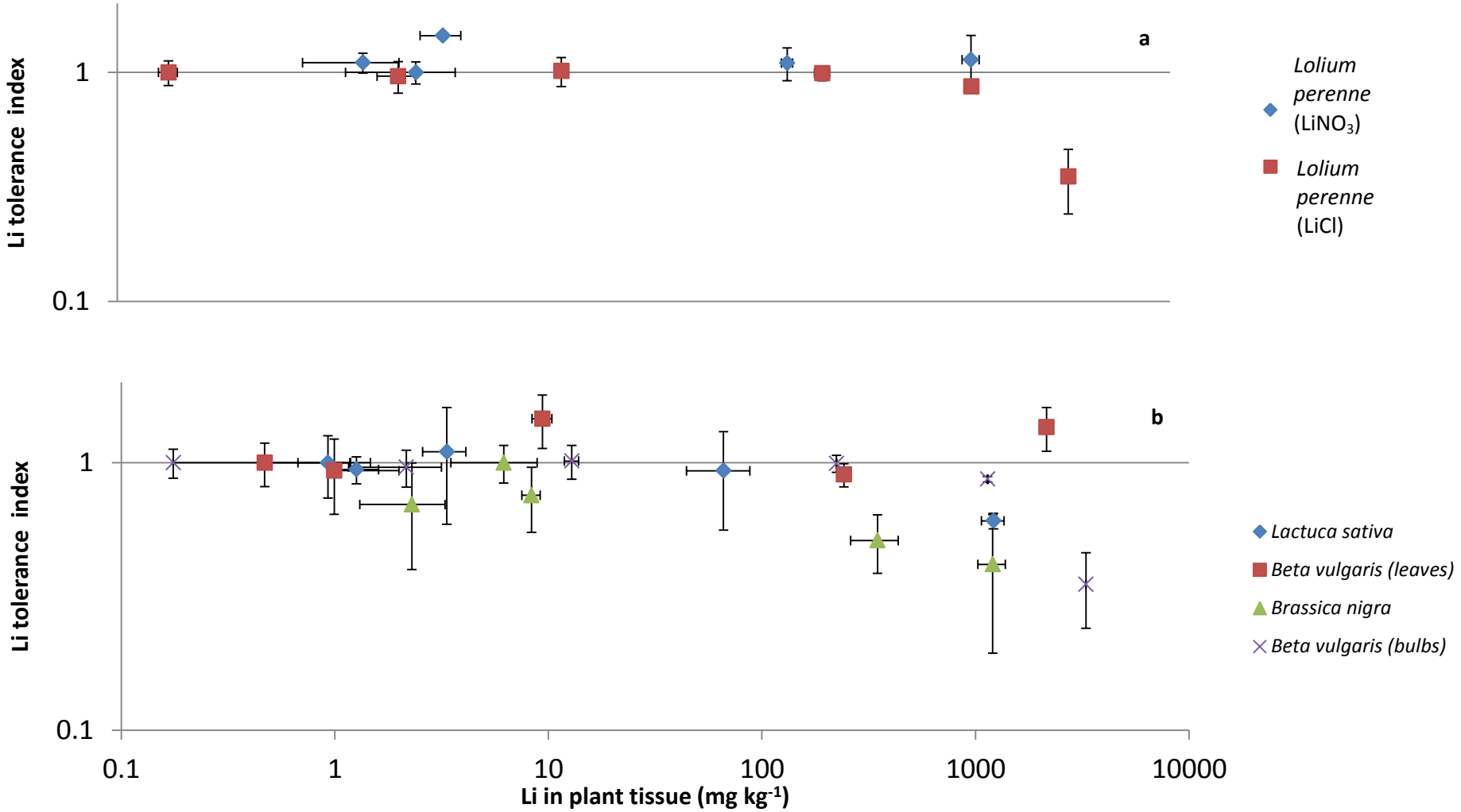


Figure 4.5 (a) Li toxicity threshold *Lolium perenne*, (b) Li toxicity threshold: *Brassica nigra, Beta vulgaris & Lactuca sativa*. Error bars represent the standard error of the mean (n=5)

4.6 Lithium's influence on other elements in plant tissue (s):

Table 4.6 shows a correlation matrix of Li concentrations in plants versus other essential elements, using fisher *p* value as shown in Table A.9.

In *L. perenne*, increasing Li concentrations resulted in decreased uptake of B, Mg, Mn and Mo. The negative correlation of Mg and B may be the result of competition between Li and these elements for transporters in the plant roots – or possibly inhibition of these transporters by Li. A positive correlation with Ca, Fe, K and Zn concentrations was also observed. Again, this may be due to the effect of Li on transporters activity in the plant roots. It is unlikely that the 5 – 30 mg kg^{-1} Li would have affected the soil's ability to bind plant nutrients because Li is comparatively weakly-bound and Li concentrations were comparatively low.

Lithium concentrations in *L.perenne* could have been influenced by Si. Silicon can enhance the uptake of Li by plants, as both of these elements have been reported to have a positive correlation in rats (Kielczykowska *et al.*, 2008). Silicon is known to be able to effectively mitigate various abiotic stresses such as Mg, Al and heavy metal toxicities, and salinity, drought, chilling and freezing stresses (Liang *et al.*, 2007). The concentration of Si is high in grasses (0.3–1.2%), which need Si for their structure (Greger, 2004).

Table 4.6 Correlation matrix of Li in plants versus essential elements in plants

Element	*L. perenne*	*L. sativa*	*B. vulgaris,* leaves	*B. vulgaris*, bulbs
B	-0.84 (S**)	-0.68 (S**)	0.06 (NS)	0.31 (NS)
Ca	0.58 (S**)	0.19 (NS)	0.31 (NS)	0.09 (NS)
Cu	0.07 (NS)	0.72 (S**)	0.21 (NS)	-0.37 (NS)
Fe	0.49 (S*)	0.32 (NS)	0.30 (NS)	-0.15 (NS)
K	0.52 (S*)	0.32 (NS)	-0.66 (S**)	0.26 (NS)
Mg	-0.78 (S**)	0.48 (S)	0.69 (S**)	0.28 (NS)
Mn	-0.64 (S**)	0.02 (NS)	0.54 (S*)	0.47 (S)
Mo	-0.80 (S**)	0.00 (NS)	0.10 (NS)	0.16 (NS)
P	-0.19 (NS)	0.52 (S*)	-0.25 (NS)	-0.44 (S)
S	0.14 (NS)	0.27 (NS)	0.19 (NS)	0.02 (NS)
Zn	0.44 (S)	0.71 (S**)	0.72 (S**)	0.72 (S**)

4.7 The partitioning of Li in *H. annuus*

Figures 4.5 (a) and (b) show Li in different plant tissues of *H. annuus* grown in soil containing 10 mg kg^{-1} [1.5 mg kg^{-1} soluble Li] and 30 mg kg^{-1} [3.2 mg kg^{-1} soluble Li] spiked soils (Figure 4.6 (a and b)). The average Li concentrations in the above-ground portions of the plants were 27 and 756 mg kg^{-1} respectively.

At the lower soil concentration, Li showed a distribution profile typical for a phloem immobile element with highest concentrations occurring in the major water sinks, namely the leaves. The older leaves, which have transpired more water, had higher Li concentrations could be due to prolonged Li uptake. At the higher soil concentrations, there were fewer differences between the plant organs. This again indicates that there are saturations or a threshold Li concentration of ca. 1000 mg kg^{-1}. The flowers had the lowest concentration and the concentration in the plant tissues increased with increasing soil Li concentrations. The biomass production of the *H. annuus* grown in the lower soil concentration was not significantly different from the control (data not shown), indicating that Li was not phytotoxic at these concentrations. However, at the higher soil concentration, the plants exhibited necrosis on the lower leaves (Fig. 4.4 and Fig. 4.6).

Table 4.7 shows the correlation of selected elements in *H. annuus* to Li. Boron, Ca, Fe, Na and Sr were significantly and positively correlated with Li. These elements are reportedly phloem immobile and taken up via the apoplastic pathway in the roots (Hayes and Reid, 2004).These results, along with the observation that leafy food and fodder crops tended to have the highest Li concentrations (Fig. 4.2), indicate that on Li contaminated soils, seed or fruit crops such as *Z. mays*, *H. annuus*, and *S. lycopersicum*, may pose a lower risk to humans and animals, than leafy vegetables such as *S. oleracea* and *L. sativa*.

Table 4.7 Correlation matrix of Li vs other elements of above ground tissue of *H .annuus* grown in soil with Li concentration of 10 mg kg^{-1}

Element	Correlation Li concentration
B	0.90 (S**)
Ca	0.61 (S)
Cu	-0.41 (NS)
Fe	0.58 (S)
K	0.46 (NS)
Mg	0.49 (NS)
Mn	0.24 (NS)
Mo	0.18 (NS)
Na	0.68 (S*)
P	-0.48 (NS)
S	0.27 (NS)
Sr	0.85 (S**)
Zn	0.24 (NS)

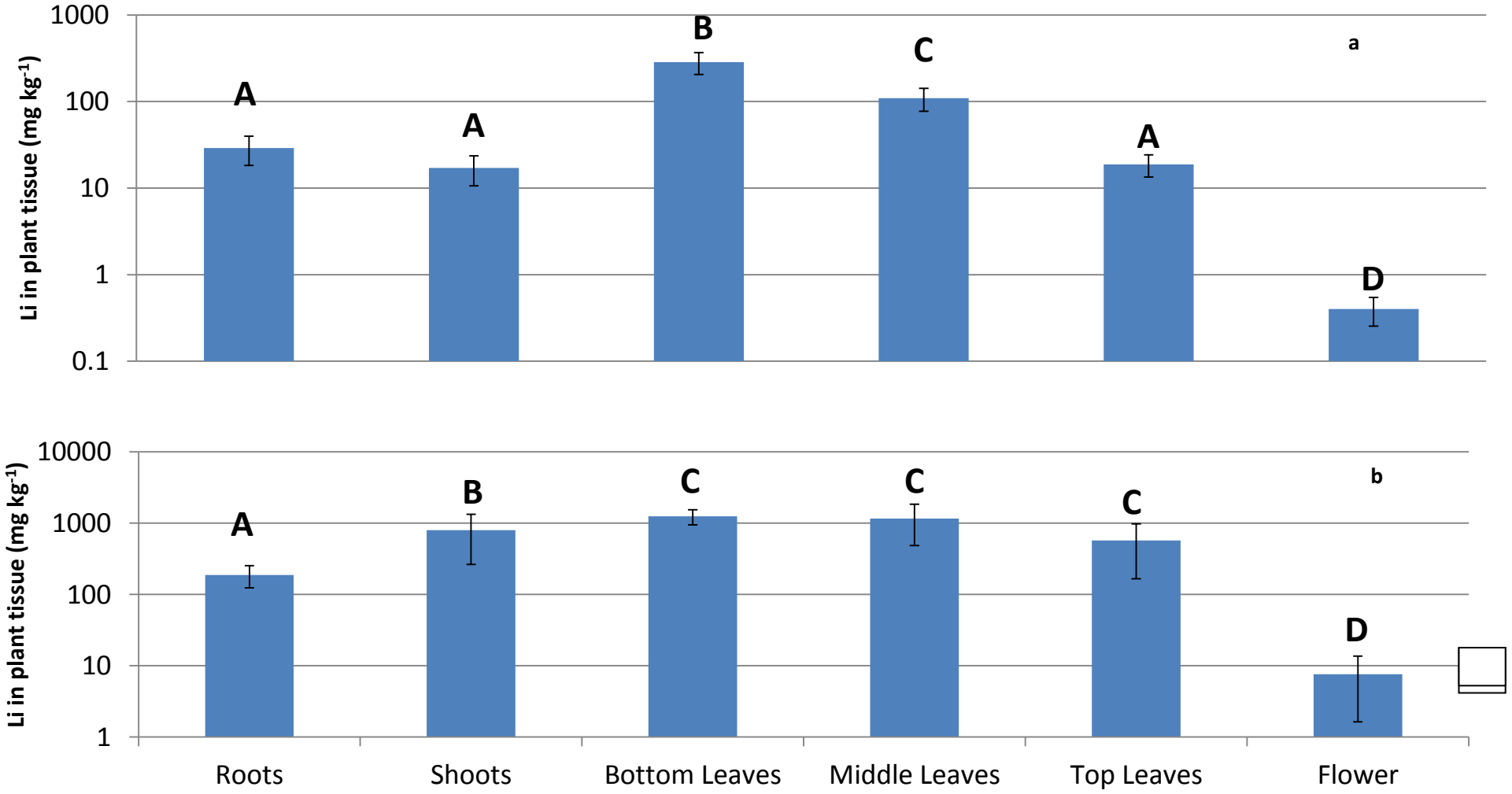


Figure 4.6 (a) Li concentrations in different parts of *H. annuus* at treatment of 10 mg kg^{-1}; (b): Li concentrations in different of *H. annuus* parts at treatment of 30 mg kg^{-1}. Error bars represent the standard error of the mean (n=3)

4.8 Potential threat posed by plant uptake of Li

The potential threat posed by plant uptake of Li to humans and livestock depends upon the plant concentration, the toxicity of Li to humans or animals and the amount of plant material consumed. Tables 4.7 and 4.8 show the amount of food or fodder required to exceed the Tolerable Daily Intake (TDI) for Li for both a nominal case, i.e., the plant growing on a non-contaminated soil, and a worst-case scenario, with the maximum non-toxic plant concentration

The Tolerable Daily Intake (TDI) of Li is reported as 0.02 mg kg^{-1} day^{-1} (EPA, 2007). Using this value, the calculations for the amount of fresh and dry weights needed for a 70 kg adult to exceed the TDI were calculated. Table 4.8 shows that, when grown on uncontaminated soil, there is virtually no chance that humans would be exceed the TDI for Li by consumption of vegetables. Even *B. vulgaris* bulbs, which had the highest Li concentration, would need to be consumed in excess of 5 kg per day (f.w.) to exceed the TDI. Clearly, in this nominal case, Li from vegetable consumption poses no risk to human health.

Table 4.8 The amount of material (kg fresh weight) of different vegetables grown in non-contaminated soils that a 70 kg human would need to eat to exceed the Tolerable Daily Intake (TDI) for Li. Note that the average serving size, moisture content, and Li concentration can be found in Table A.13

	Amount required (kg) to be consumed to exceed the TDI for Li
B. vulgaris (bulbs)	5.5
S. oleracea	15
L. sativa	25
R. sativus (leaves)	31
B. vulgaris (leaves)	32
B. oleracea	212
C. p. ovifera	303
A. porrum	395
D. carota	499
R. sativus (bulbs)	596
S. lycopersicum	767

For a worst-case scenario (Table 4.9), where plants were grown on Li-contaminated soil (with no plant toxicity symptoms) consumption a moderate amount of plant material would result in humans exceeding the TDI for Li. Given the low toxicity of Li to plants, crops grown on Li-contaminated soil may show no toxicity symptoms, yet pose a risk to human health.

Table 4.9 The amount of material (kg fresh weight) of different vegetables grown in Li contaminated soils that a 70 kg human would need to eat to exceed the Tolerable Daily Intake (TDI) for Li. Note that the average serving size, moisture content, and Li concentration can be found in Table A.12

Plant type	Amount required (kg) to be consumed to exceed the TDI for Li
B. vulgaris **(leaves)**	0.06
B. vulgaris **(bulbs)**	0.04
L. sativa	0.35

4.8.1 Tolerable Daily Intake- cattle & sheep

I have calculated the amounts of dry matter required to exceed TDI's for a cattle beast (753 kg) and a sheep (23.8 kg). I made the assumption that the tolerance of sheep and cattle to Li is similar to that of humans. Of course, this may not be the case. To exceed the TDI, a cattle beast needs to consume 5.7 kg (d.w) of *L. perenne* grown in soils with natural concentrations of Li, whereas it only needs consume dry weight of 0.1 kg of *L. perenne* grown in soils with spiked Li concentrations. In case of the sheep, the dry weights of 0.18 kg of *L. perenne* (grown in non-contaminated soils) and 0.003 kg of *L. perenne* (grown in Li contaminated soils) are required to exceed the TDI. These results indicate that the normal Li consumption of livestock exceeds the TDI for humans. It is unclear, however, whether all of this Li is absorbed by the animal. An estimation of this could be made by measuring Li concentrations in faecal matter.

Chapter 5

Conclusions

Lithium concentrations in soils from around New Zealand ranged from 0.08 mg kg^{-1} to 92 mg kg^{-1}. The highest Li concentrations were found in soils with high clay content. Most endogenous Li is soil is insoluble and hence unavailable to plants. However, when exogenous Li is added to soil, there is only limited sorption of Li. Lithium sorption increases with increasing soil pH and decreases with increasing Li concentrations. Compared to other cations in soil, Li is mobile and may leach into receiving waters or be taken up by plants.

When grown in an uncontaminated soil, the Li concentrations in the edible portions of various plant uptake of Li differed by two orders. Salt tolerant plants (Table 4.5), namely *B. vulgaris* and *S. oleracea* took up the most Li, while seed and fruit crops had the lowest Li concentrations. Pasture grass (*Lolium perenne*) had the highest bioaccumulation factor for Li of any of the plants tested.

When Li is added to soil, there were few differences in the uptake or tolerance of this element between species. At a soil concentration of just 5 mg kg^{-1}, the plants took up several hundred mg kg^{-1} Li into the leaves with no reduction in biomass. At such high Li concentrations, only a small amount of plant material would need to be consumed to exceed the tolerable daily intake for Li.

Clearly, plants offer no food chain protection with regard to Li. Even minor soil contamination of a few mg kg^{-1} can result in plants taking up this element to hundreds of mg kg^{-1} in the leaves. By considering the results indicating the mobility and uptake by plants, it can be included in the Group 4 elements along with Cd, Se & Mo.

Lithium appears to be a phloem immobile element, with the highest concentrations occurring in the older leaves and the lowest concentrations occurring in the seeds or fruits. Therefore, planting fruit or seed crops on Li-contaminated soil may reduce the risk posed to human health.

Future research needs

My study investigated Li that has been spiked into soil using soluble Li salts. It is unclear how Li that is derived from grease or electronic waste would behave in soil. It is probable that the release of Li from such sources would be gradual and therefore exist in the plant root zone for a longer time than a soluble salt, which would presumably leach out within a few months. It would therefore be worthwhile to test soils and plants that may have become contaminated from such activities. Many e-waste reprocessing areas occur in tropical countries with distinct laterised soils. Such soils may have different Li retention properties that the Templeton Silt Loam used in my experiments.

Finally, the capacity of the human and animal gut to absorb plant-borne Li should be investigated. My results indicate that when grown on a contaminated soil, just a few grams of plant material would need to be consumed to exceed the tolerable daily intake for Li. However, not all of this Li may be absorbed by the gut and enter the bloodstream.

Appendix A

Tables

A.1 Characteristics of lithium. Adapted from :(Merian, 1991; Pratiyogita Darpan Group, 2002)

Characteristic	Measurement
Radii	0.074 nm
Atomic mass	6.94 gmol-1
Melting point	180.54^{o}C
Boiling point	1336 ^{o}C
Electronic configuration	$1s^2$, $2s^1$
Density	0.534 gcm-3

A.2 Physical properties of Li grease. Adapted from: http://www.mgchemicals.com/tds/tds-8461.pdf

Physical properties	Value
Base material	Mineral Oils
Filler	Lithium soap
Electrical class	Insulator
Colour	White / Amber
Viscosity	Cream, semi-solid
Specific gravity	0.90
Melting Point	185 ^{o}C
Autoignition temperature	210 ^{o}C
Boiling point	371 ^{o}C
Odor	None
Non- corrosive	Excellent
Aqueous solubility	Insoluble

A.3 Properties of Group I and Group II metals (source: Farrell,1999)

	Li	Na	K	Mg	Ca
Atomic radius (Å)	1.33	1.57	2.03	1.36	1.74
Ionic radius (Å)	0.60	0.95	1.33	0.65	0.99
Hydrated radius (Å)	3.40	2.76	2.32	4.67	3.21
Polarizing power z/r^2	2.80	1.12	0.56	4.70	2.05
Electronegativity	1.0	0.9	0.8	1.2	1.0

A.4 Showing the pH values before and after addition of lime to TSL

Reading 1	**pH without lime**	**pH with lime**
1	5.09	6.21
2	4.98	6.28
3	5.07	6.19
4	5.05	6.17
Average	**5.05**	**6.21**

A.5 Calculation of the grams of $LiNO_3$ required to make different concentrations Li

	Molecular weight(g/mol)
Li	6.94
$LiNO_3$(g/mol)	68.95

The % of Li in $LiNO_3$ is : 9.93
The % of Li in $LiNO_3$ is determined using :
M*wt* ($LiNO_3$) ÷ M*wt* (Li)

A.6 Showing the grams of $LiNO_3$ added to 250 ml de-ionised water for each of the treatment

Concentrations (mg (Li)/kg (soil)	mg/10 kg soil	mg of $LiNO_3$ needed / treatment	Grams of $LiNO_3$/250ml
0	0	0	0
1	10	99	0.37
3	30	298	1.12
10	100	993	3.73
30	300	2980	11.2
100	1000	9933	37.3

A.7 Showing the pH scheme and the acid/base added to the soil- calcium nitrate mixture to achieve the desired pH.

pH	Volume (µl) added	Acid/base added
3(2.8)	300	HNO_3(1:10)
4(4.3)	0	0
5(5.2)	100	2M KOH
6(6.5)	250	2M KOH
7(7.4)	400	2M KOH

A.8 Different plant type's tolerance to Li, adapted from (Bingham *et al.*, 1964)

Very sensitive	Sensitive	Slightly sensitive	Tolerant
Avocado	Grape	Cotton	Rhodesgrass
Soybean	Red kidney bean	Dallisgrass	Sweet corn
Sour orange	Tomato	Red beet	

A.9 Determination of significance of essential elements to Li concentrations

	L.perenne	*L.sativa*	*B. vulgaris* Leaves	*B.vulgaris*, bulbs	Significance to Li
	df = 25	df= 23	df= 24	df= 18	
p (0.1)	0.323	0.338	0.331	0.378	NS
p (0.05)	0.381	0.398	0.389	0.444	S
p (0.01)	0.445	0.507	0.497	0.561	S*
p (0.001)	0.597	0.619	0.608	0.679	S**

A.10 Moisture content of the vegetables, adapted from:(Bastin and Henken, 1997)

	Moisture content (%)	Standard error (%)
B. vulgaris **(leaves)**	90.8	0.38
B. vulgaris **(bulbs)**	85.9	0.30
B. oleracea	88.1	0.62
D. carota	90.9	0.17
C. pepo	93.9	0.40
A. porrum	80.5	0.57
L. sativa	93.9	0.4
R. savitus **(bulbs)**	96.1	0.24
R. savitus **(leaves)**	95.5	0.11
S. lycopersicum	94.4	0.15
S. ***oleracea***	90.4	1.08

A.11 Cation Exchange Capacity ratings of different soil types. Adapted from Knowles *et al*., 2011.

Rating	CEC (me/100g)	Comment
Low	5-12	Soil very low in organic matter. Typical of sandy soils
Medium	12-25	Pumice soils often in the range 13-18; lower fertility mineral soils in the range 15-25.
High	25-40	High fertility soils may be in the range 25-35. Also may have high clay content.
Very High	40 +	Values typically found in peat soils. Consolidated peats typically in range typically in range of 40-65; raw peat may be as high as 100.

A.12 Calculations of TDI's for vegetables grown in Li contaminated soils

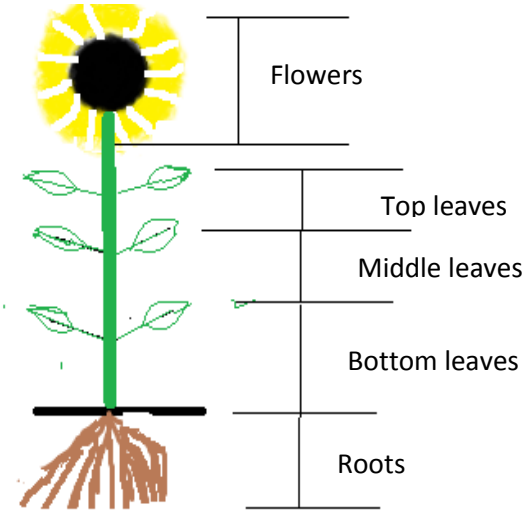
	Serving size (g)	Li (mg kg^{-1})	Moisture content (%)	Maximum concentration (F.W)	Amount required (kg) to Exceed TDI	Amount required (g) to Exceed TDI
B. vulgaris **(bulbs)**	100	224	85.9	31.5	0.04	44.4
B. vulgaris **(leaves)**	6	242	90.8	22.3	0.06	62.9
L. sativa	6	66	93.9	4.03	0.35	347.4

A.13 Calculations of TDI's for vegetables grown in non- contaminated soils

Vegetable	Serving Size (g)	Li (mg kg^{-1})	Moisture content (%)	Maximum concentration (F.W)	Fresh weight required (kg) to exceed TDI
B. vulgaris **(bulbs)**	100	1.792	85.9	0.253	5.54
S. ***oleracea***	30	0.956	90.4	0.092	15.26
L. sativa	6	0.928	93.9	0.057	24.73
R. savitus **(leaves)**	6	1.007	95.5	0.045	30.90
B. vulgaris **(leaves)**	6	0.469	90.8	0.043	32.44
B. oleracea	36	0.055	88.1	0.007	212.35
C. pepo	100	0.08	93.9	0.005	303.57
A. porrum	89	0.018	80.5	0.004	395.07
D. carota	61	0.031	90.9	0.003	499.47
R. savitus **(bulbs)**	85	0.060	96.1	0.002	596.35
S. lycopersicum	91	0.033	94.4	0.002	767.32

Appendix B

Figures



B.1 Schematic diagram showing the division of *H. annuus*

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