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# **BORON DYNAMICS AND AVAILABILITY IN *PINUS RADIATA* PLANTATION**

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A thesis presented in partial fulfilment of the requirements  
for the degree of

**Doctor of Philosophy**

in

Soil Science



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New Zealand

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# Abstract

*Pinus radiata* is an important forest species in New Zealand. Over 89 % of the country's plantation forestry area is planted with *P. radiata*. The species makes a major contribution to New Zealand's \$3.1 billion to GDP and the plantation area is projected to increase to 2.5 million hectares by 2025. Research work to date has recognised that soil B deficiency is a major issue in many forestry plantation areas. Edaphic and environmental conditions such as the coarse texture pumacious soils planted with radiata in the Central North Island, and summer drought condition in some areas, further aggravate B deficiency in New Zealand soils.

Boron deficiencies in *P. radiata* lead to growth defects in afflicted plants and a deterioration in wood quality and market value. The primary objective of this thesis was to assess the impact of ulexite, a slow-release B fertiliser, on the bioavailability of soil B, plant B uptake, and the relative effect of B application rate on plant growth and soil microbial activity. A further objective was to compare the rate of B adsorption to seven benchmark soils collected from the North Island of New Zealand. The purpose of the work was to propose a long-term slow-release fertiliser management solution for radiata pine forestry that may mitigate the economic damage caused by B deficiency in this important primary production sector.

Soil was collected from Taupo, the major *P. radiata* planting district in the Central North Island of New Zealand, and used to establish glasshouse studies with *P. radiata* at Massey University in Palmerston North. Plants for this research were obtained from the Forest Research Institute (SCION) in Rotorua, New Zealand. Two growth experiments were conducted. The second of these compared the B dynamics of a fast-growing and slow-growing clone of *P. radiata*.

The background concentration of B in this soil (less than 0.5 mg/kg calcium chloride extractable B) is low, and B fertiliser application induced a soil response. Results showed that the concentration of plant-available B (extracted using hot 0.02 M CaCl<sub>2</sub>) significantly increased with B application. Boron application at the highest level (32 kg/ha) led to a build-up of soil B to a critical toxicity level with the subsequent appearance of toxicity symptoms in plants.

Application of B resulted in rapid B uptake as shown by an increase in B concentration in all plant parts (needle, stem and roots), but with the greatest rate of increase in needles. The percentage distribution of B throughout the plant showed that B distribution was influenced by B application treatments. The root to needle B ratio is used in this work as an index of B transfer from source to sink parts of a plant. Results showed that under deficient and toxic soil B concentrations (defined through the CaCl<sub>2</sub> extractable B concentration), B was restricted to source tissues. However, B application at the rate of 4 kg/ha enabled B to move to sink parts including the new emerging needles. Regardless of clone and B treatment, needles, particularly older needles, were the main site of B accumulation followed by roots and stem. The B concentration in needles of Clone 37 was higher than in Clone 18 and this result reflects a higher demand of B for the faster growing Clone 37 relative to Clone 18.

Application of B affected *P. radiata* growth in terms of height, diameter and plant dry weight. Plants responded positively to B application over a range of fertiliser treatments (8-16 kg/ha) leading to sufficiency in soil as quantified through increases in the plant growth parameters plant height and dry weight. Boron application improved plant physiology as quantified by photosynthesis in this study. Results showed that photosynthesis positively responded to B application up to 8 kg/ha, however a further increase in B application resulted in a decline in photosynthetic activity.

Results from a B fractionation study showed that the plant unavailable residual-B fraction was the major form of B in the Taupo soil. With B fertiliser application the concentration of readily-available B increased proportionally to the B application rate. This increase in readily-available B demonstrates the importance of using B fertiliser to provide for a long-term increase in plant-available soil B for *P. radiata* plantations on the Taupo soil.

Soil microbial and microbiological properties also responded to B application. Soil dehydrogenase activity, an index of microbiological activity in soil, showed a concentration gradient from the bulk to rhizosphere soil. Regardless of clone there was approximately a three-fold higher dehydrogenase activity in the rhizosphere soil compared to the bulk soil. Maximum dehydrogenase activity was recorded by a B application at 4-8 kg/ha in both clones with a decrease in activity at higher rates.

Regardless of the radiata clone used, mycorrhizal colonisation increased with B application. However, for both clones the maximum mycorrhizal infection on roots was recorded for a B application rate of 2-4 kg/ha.

A B adsorption study performed using seven benchmark soils collected from around the North Island showed that B adsorption increased in all soils with the concentration of B in equilibrium solution. Langmuir and Freundlich isotherms modelled B adsorption in all seven soils. Further studies showed that B adsorption corresponded to pH in solution and linearly increased up to pH 9 and reduced thereafter.

The results from this study demonstrate the importance of B fertiliser to *P. radiata* plantation forestry. Both plant and microbiological parameters are affected by both low and excess levels of soil B. Therefore, it is suggested that a B application rate in the range of 4-8 kg/ha is optimal for plant growth and will have no harmful effect on soil microbiological parameters. In contrast, B application at the rate of 16 kg/ha is toxic to both plants and soil microbes and will lead to inhibitory effects on activity and growth.

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**Raza Ullah Khan**

**Palmerston North, 2012**

# **Dedication**

I dedicate this thesis to my late Great Grandmother



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# Chapter 1

## Background and Research Objectives

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### 1.1 Background

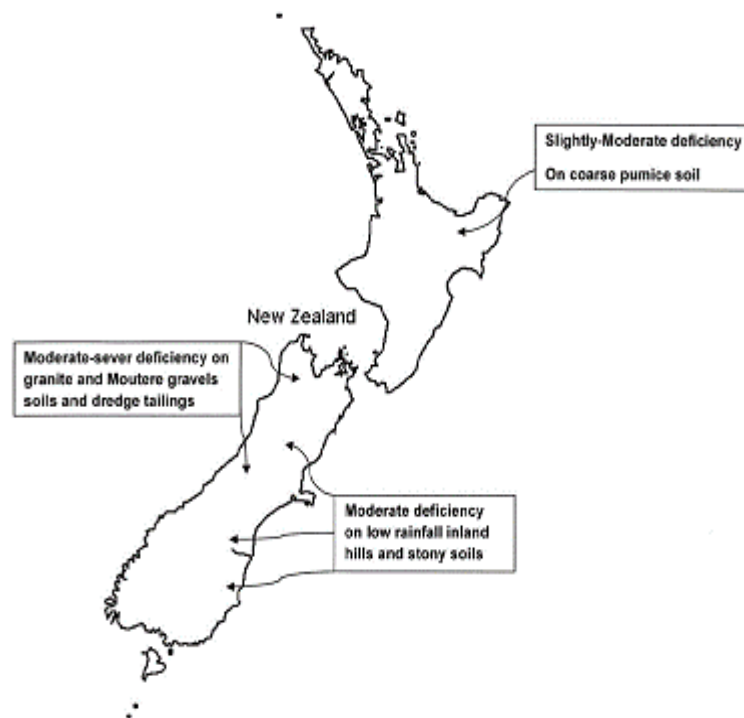
*Pinus radiata* (*P. radiata*), is native to the Pacific north-west region of North America where it has a distribution localised along the California coast and the two islands of Guadalupe and Cedros off the Mexico coast. The total area of native distribution is approximately 7000 ha at an altitude between 600-1200 m above sea level with the largest area of distribution occurring on the Monterey Peninsula (Hegan, 1993). The species has been introduced as a timber tree in vast areas of New Zealand, Australia, Chile, South Western Europe and South Africa (Kral, 1993; Little, 1980; Richardson, 1998), and *P. radiata* has become the most important exotic softwood tree in the Southern Hemisphere largely because of its rapid growth and high rate of production at cheap cost (Ryle, 1962). *Pinus radiata* can today be found planted on 2.6 million ha of the Earth's surface outside its native habitat, with Chile accounting for 37.2 % of this area, followed by New Zealand (30.8%), Australia (19.6%) and Spain ( $\leq 10\%$ ) with less than 2% in South Africa and Italy (Turner and Lambert, 1986b).

Introduced in 1859 to New Zealand (Hegan, 1993), *P. radiata* is currently planted on 1.78 million ha of land making up approximately 89% of the country's exotic plantation forest. The main areas of *P. radiata* forestry in New Zealand are the Central North Island (544,532 ha), followed by Northland (202,286 ha) in the North Island and Otago (215,145 ha) and Marlborough (171,675 ha) in the South Island (NZFOA, 2011).

Forestry contributes approximately 3.1 billion dollars annually to the New Zealand economy through export earnings, accounting for about 10% of New Zealand's total produce exports (MAF, 2010/2011). According to future plantation plans nearly 2.5 million hectares of land are to be brought under *P. radiata* plantation by 2025 (Payn et al., 1998).

In New Zealand extensive *P. radiata* plantations occur on coarse-textured pumaceous soils (Hewitt, 1993) of the Central North Island, and the Moutere gravels near Nelson. Both of

these areas are considered to have relatively low levels of soil B (Figure 1.1). Boron deficiency was first recognised and documented in New Zealand in 1962. Earlier research (Madgwick et al., 1988; Will, 1985) showed B to be the major nutrient constraint in *P. radiata* forestry, and described B deficiency as one of the most common micronutrient deficiency problems in New Zealand and Eastern Australia (Hill and Lambert, 1981; Snowden, 1982). Environmental conditions such as water stress (Lambert and Ryan, 1990) particularly during summer droughts, further contribute to B deficiency in *P. radiata* plantations.



**Figure 1.1 Major areas of boron deficiency in New Zealand after Will (1985)**

Boron deficiency has been associated with a number of growth defects such as leader tip dieback, multi-leadership, and the yellowing of needle tips (Dell and Malajczuk, 1994; Will, 1985). Coarse-texture and a low organic content of soil are proven factors to cause B deficiency (Gupta, 1979a; Shorrocks, 1997), while environmental factors such as low rainfall elevate B deficiency in marginally deficient areas (Lambert and Turner, 1977).

## 1.2 Use of B fertilisers in *Pinus radiata*

Boron fertiliser applications have been reported to alleviate B deficiency and as a result increase plant growth (Hopmans and Clerehan, 1991), and improve tree shape by controlling multi-leader growth. Most field trials (Hopmans and Clerehan, 1991; Hopmans and Flinn, 1984) have used highly soluble B fertilisers such as borax and sodium borate providing only short-term protection against B deficiency (Knight et al., 1983). An initial increase in B uptake followed by a rapid decline to a new stable level (Knight et al., 1983), low soil B retention of the applied fertiliser, (Ryan, 1989) and only passive diffusion of B to plant roots (Raven, 1980), are all factors that suggest the need for a long-term solution to B fertility that involves the use of slow-release B fertiliser.

Slow-release fertilisers such as ulexite (Table 1.1) have been considered a strong candidate for the management of B deficiency in *P. radiata* plantations (Hunter et al., 1990b) particularly in low rainfall areas (Olykan et al., 1995). According to Olykan et al. (2008) 44% and 24% of residual fertiliser B was found to remain in the top 0-20 cm of the soil depth 4 years after application of ulexite, at the Balmoral Forest in Canterbury, New Zealand, at rates of 8 kg B/ha and 32 kg B/ha application, respectively. The long residual effect of such B fertiliser is ascribed to the low solubility of B in slow-release fertiliser (Hunter et al., 1990b). Although slow-release B fertilisers have been generally prescribed by many New Zealand forest companies to correct B deficiency in *P. radiata* plantations, little information is available on the effect of different rates of slow-release B fertiliser, on both above-ground and below-ground growth and physiological aspects of this species, and on soil microbiological activities. The fate and the dynamics of the added fertiliser B in the soil have also not been studied in detail.



**Table 1.1 The chemical and physical properties of ulexite**

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Empirical Formula	(NaCaB <sub>5</sub> O <sub>6</sub> (OH) <sub>6</sub> ·5(H <sub>2</sub> O))
ICSD Name	Sodium calcium borate hydroxide hydrate
Composition	%
B <sub>2</sub> O <sub>3</sub>	42.97
Na <sub>2</sub> O	7.65
H <sub>2</sub> O	25 Mohs
Hardness	13.5
Specific gravity	1.955 g/cm <sup>3</sup>
Color & transparency	White, colorless. silky, transparent
Water (H <sub>2</sub> O)	35.6

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Sources: Webmineral.com; Mehmet, 2009

### **1.3 Objectives of the research study**

This research thesis aimed to increase the knowledge of B dynamics and availability when ulexite, a slow-release B fertiliser, is applied to *P. radiata*.

The specific objectives of this research are:

1. To examine the impact of increasing application rates of ulexite on the concentration of plant-available soil B under glasshouse conditions
2. To evaluate the effect of variable ulexite application rates on plant B concentration, and the growth and physiological responses of *P. radiata* under glasshouse conditions
3. To assess the response of mycorrhizae and soil dehydrogenase activity to different levels of B applied as ulexite under glasshouse conditions

4. To investigate the relationship between the plant-available soil B concentration and plant B concentration under glasshouse conditions
5. To compare the response of two *P. radiata* clones to the application of ulexite under glasshouse conditions
6. To determine the chemical distribution (fractionation) of B in glasshouse soils treated with ulexite.

Research towards these Objectives is described in this thesis in three chapters that represent three phases of laboratory and greenhouse research.

Chapter two presents a detailed overview of the current state of knowledge of B in the environment, with special consideration of the importance of B to forestry, and on the analysis of B in soil and plant.

Chapter three presents the results of an initial greenhouse study where the effects of different B rates on plant growth, plant B uptake, mycorrhizae colonization, plant available soil B and soil dehydrogenase activities are presented.

Chapter four presents the results of a follow-on greenhouse study where 2 diverse clones of *P. radiata*, having different growth rates, were exposed to different rates of B to determine how the nutrient influences plant growth and related physiological parameters such as plant photosynthesis, stomata conductance, plant available soil B, and soil dehydrogenase activities.

Chapter five presents the results of a laboratory study where two experiments were conducted; assessment of B adsorption onto seven soils collected from around the North Island of New Zealand, and assessment of the influence of pH on B adsorption to six of these soils.

In Chapter six the important aspects of these three experiments are considered and discussed in the context of B fertility management for *P. radiata* forestry.

# Chapter 2

## Review of Literature

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### 2.1 Introduction: An overview

Boron (B) is the only non-metallic element in Group 13 of the Mendeleev Periodic Table and shares a chemical resemblance with carbon. The importance of B as an essential plant nutrient was first established in 1923 by Warrington and now it is reported as the most deficient plant micronutrient worldwide (Shorrocks, 1997). Though plants vary in B need, there exists a narrow range between toxicity and deficiency, and plants can develop symptoms of both deficiency and toxicity in the same growing period (Reisenauer et al., 1973). Boron is ascribed to metabolic, structural and non-structural, physiological and biochemical roles in plants (Marschner, 1995).

Considered to be the most mobile micronutrient, B in the form of boric acid, the most common species of B in soil solution, is not involved in oxidation, reduction or volatilization reactions. Plants respond to B in soil solution, and the concentration of available B is strongly controlled by B adsorption reactions in the soil. Factors such as pH, texture, organic matter, temperature, moisture content and plant species influence B adsorption, and thereby, plant B uptake. Coarse textured soils prone to leaching as a result of excessive rainfall in humid climate are generally associated with B deficiency (Goldberg, 1997). Conversely, in an arid and semi-arid climate, naturally high soil B levels, salinity, a water balance that precludes net drainage, and high concentrations of B in irrigation water are all factors that result in B toxicity (Gupta et al., 1985; Nable et al., 1997).

This chapter reviews the literature on B occurrences, dynamics and availability in the terrestrial environment with emphasis on *P. radiata* plantations. Common methods for B analysis are considered in this review. Consequently, based on this review, important knowledge gaps were identified, and these have laid a framework for the research embodied within this thesis.

## 2.2 Aqueous chemistry of B

Boron is the first member of the third group of the periodic table of the elements, possessing an atomic number of five and valence number of 3<sup>+</sup>. Boron exists in two very common forms in soil, namely boric acid B(OH)<sub>3</sub> and the borate anion B(OH)<sub>4</sub><sup>-</sup>. Both B(OH)<sub>3</sub> and B(OH)<sub>4</sub><sup>-</sup> exist as monomeric forms at low concentration but polymeric forms such as tetra borate (B<sub>4</sub>O<sub>7</sub><sup>-2</sup>) occur in concentrated solution. Boric acid is a weak Lewis acid that accepts an electron rather than donating one (Parfitt, 1979) and has a tendency to adsorb hydroxyl ions (from water at higher pH) forming the tetrahedral, tetrahydroxy borate anion, B(OH)<sub>4</sub><sup>-</sup> (Equation 2.1). The negative log of the first acidity constant (*pKa*) for this reaction is approximately 9.25 (Shriver and Atkins, 1994), yielding the borate anion.



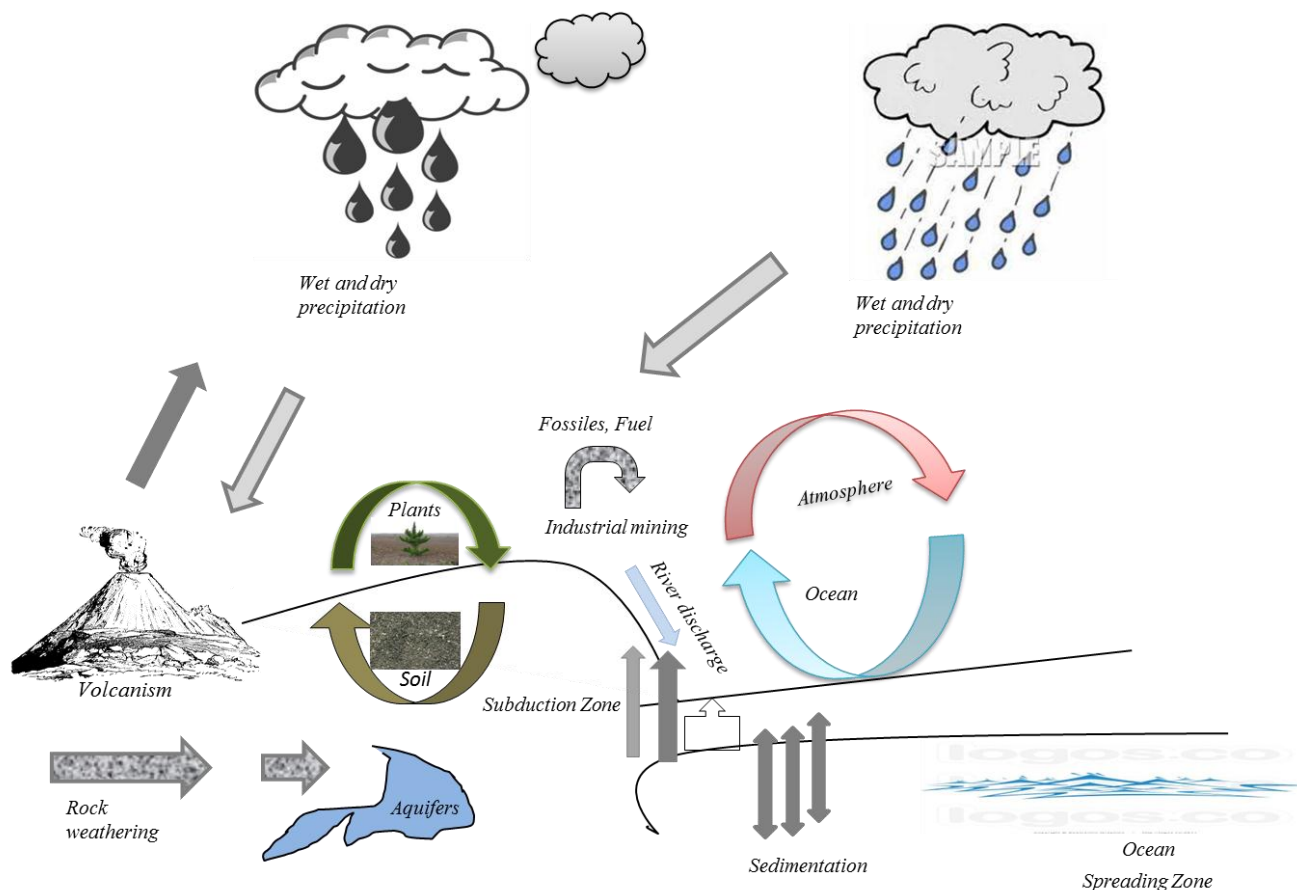
The solubility of boric acid B(OH)<sub>3</sub> increases from 2.52 g/100 ml at 0 °C to 27.53 g/100 at 100 °C. As a non-charged molecule, B(OH)<sub>3</sub> does not interact with charged soil colloids (limited non-specific adsorption) and is therefore, highly mobile in soil solution (Shorrocks, 1997). Soil colloids can however, retain boric acid through adsorption (specific adsorption) to organic matter, clay minerals and metal hydroxides such as Fe and Al hydroxides. The stability of these complexes decreases as B(OH)<sub>4</sub><sup>-</sup> becomes the dominant species in soil solution at pH > 9. Adsorbed B can be released back into soil solution from organic matter and clay minerals through microbial action (Hue et al., 1988).

The electronic configuration of B is [2s<sup>2</sup>, sp], but this element has the potential to form three covalent bonds using a trigonally hybridized sp<sup>2</sup> orbital. The resultant molecules are planar with a bond angle of 120 degree. Boron tends to form covalent rather than ionic bonds in different B complexes because of its high ionization potential. This prevents the formation of the B<sup>+</sup> cation (Stewart, 1988).

## 2.3 The occurrence of B on earth

Boron is placed within subgroup III of the Periodic Table of the Elements and therefore shares properties with both metals and non-metals (Bohnsack and Albert, 1977). Boron is present throughout the ecosystem at varying concentrations; 5-10 mg/kg in rocks (Shorrocks, 1997), 1-10 mg/L in sea water and 0.0028-0.028 mg/L in river water (Power and Woods, 1997), while another study reported B to be at a concentration of around 4.5 mg/L in the ocean (Lemarchand et al., 2000). Kot (2009) claims that B in the environment is primarily derived from the weathering of rocks and minerals. Chemical and mechanical weathering is estimated to contribute  $0.043 \times 10^9$  kg B and  $0.15 \times 10^9$  kg B to the environment each year respectively. However, Fogg and Duce (1985) reported that volcanic emissions and particulate B attached to sea salt aerosol are the major mechanisms for boron distribution around the globe, followed by weathering and anthropogenic processes. Park and Schlesinger (2002) suggested that volcanic emissions contribute  $0.022 \times 10^9$  kg B/year, almost 100  $\times$  less than the figure reported by Fogg and Duce (1985) ( $2.1 \times 10^9$  kg B/year). According to Argust (1998) the major sources and reservoirs of B in the biosphere are; the continental and ocean crusts, ocean, ground water, ice, coal deposits, biomass and groundwater.

Boron occurs in the Earth's crust mainly in the form of silicate minerals (borosilicate). The crystalline form of these minerals changes as a function of temperature. High temperature borosilicate contain anhydrous borate (e.g. tourmaline), whereas hydrous borate forms in low temperature minerals (e.g. borax ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ), ulexite ( $\text{Na}_2\text{Ca} [\text{B}_5\text{O}_6 (\text{OH})_6] \cdot 5\text{H}_2\text{O}$ ) and colemanite ( $\text{Ca}_2 [\text{B}_6\text{O}_{11}] \cdot 5\text{H}_2\text{O}$ ). Rain and snow are major scavenging agents to remove B out of the atmosphere (present at a concentration of 0.2-300  $\mu\text{g}/\text{L}$  in precipitation). Elevated concentrations of B in ground water can result naturally through the leaching of B out of parent materials, but also through the presence of anthropogenic contaminants such as Naperborate which finds use as a detergent (Vengosh et al., 1994). Park and Schlesinger (2002) described anthropogenic activities such as mining, biomass burning and fossil fuel combustion as factors that contribute significantly to global B cycling. Three major cycles; those of the soil-plant terrestrial ecosystem, the marine ecosystem and the atmosphere-ocean/land interface (Figure 2.1) influence B flux into environment.



**Figure 2.1 Schematic diagram of B turnover in the environment after Kot (2009)**

The terrestrial soil-plant ecosystem includes drainage of B from the soil to aquifers and surface water (Argust, 1998), and B discharge from rivers to the ocean, and via soil-bearing aerosols (Park and Schlesinger, 2002) (Soil bearing aerosols are defined as dispersion of B from soil to oceans and rivers via tiny liquid droplets in the atmosphere). In the marine ecosystem, carbonate, silica and deep sea organic material leads to the co-precipitation of B with biogenic carbonate materials and deep sea burial. Boron sorption onto organic-rich surfaces found within the marine environment leads to B enrichment of sedimentary materials (e.g. shale, clays and carbonate rocks) (Goldberg and Glaubig, 1986). The atmosphere-ocean/land interface leads to flux of B into the atmosphere in the form of sea salt aerosols, whereas the counter flux from atmosphere to the ocean and land is through wet and dry precipitation (Kot, 2009).

Kovda (1973) defined several mechanisms for B cycling within the soil: biological cycles (litter and needle decomposition in forest ecosystems), humus biosynthesis, clay formation and illuviation. As a result of rock weathering under the conditions of pH and redox potential

that are expected within soil, several anions of B, such as  $\text{BO}_2^-$ ,  $\text{B}_4\text{O}_7^{2-}$ ,  $\text{H}_2\text{BO}_3^-$ , and  $\text{B}(\text{OH})_4^-$ , can all be found in soil solution at elevated concentration (0.067-3 mg/L) (Kabata-Pendias and Pendias, 2001). Each of these anions has simple soil chemistry (Goldberg, 1997) and can be subjected to leaching under humid conditions, but will concentrate in the surface soil in arid and semiarid regions. The concentration of B in soil solution is controlled by adsorption reactions with soil colloid surfaces, and these reactions are of primary importance in dictating the availability of B for plant growth. Major factors that control B adsorption in soils and plant availability include soil solution pH, soil texture, moisture, temperature, and crop-specific species, growth stage and root morphology.

Boron exists in the form of boric acid at common soil pH (5.5-7.5); an un-dissociated, neutral molecule and the preferred B species for plant absorption from soil solution (Camacho-cristóbal et al., 2008; Greenwood, 1973; Hu and Brown, 1997) either by passive uptake (Brown and Shelp, 1997) or active uptake (Stangoulis et al., 2010). The soil-plant interface is affected by the role of B in root formation (Dell and Huang, 1997). Sufficient plant-available B enhances ectomycorrhizae (ECM) colonization (Lehto, 1994) due to a positive link between plant B and root carbohydrate content, subsequently leading to increase rates of root C exudation (Atalay et al., 1988).

## **2.4 Biogeochemistry of Boron: An Overview**

Boron is widely but not uniformly distributed in the terrestrial environment with average of 15 mg/kg and 42 mg/kg B in earth crust and worldwide respectively (Kabata-Pendias and Pendias, 2001). Sedimentary rocks particularly shales (100 mg/kg) have higher B contents than igneous rocks. Krauskopf (1972) classified B containing mineral as hydrous, anhydrous and complex borosilicate minerals. Both anhydrous and complex borosilicates such as tourmaline are high temperature minerals, whereas hydrous borates are low temperature minerals. Weathering of B containing rocks gives borate in to the soil solution, dominantly in the form of  $\text{B}(\text{OH})_3$  (Evans and Sparks, 1983).

Boron movement in the soil, follows the water flux, in cool humid soil, B leached down soil horizon, leading low B (1-2  $\mu\text{g/g}$ ), while B concentration on soil surface in warm humid or

arid to semi-arid range from 10 to 40  $\mu\text{g/g}$  (Aubert and Pinta, 1977; Kabata-Pendias and Pendias, 2001).

In soil, B is the most mobile of all micronutrients. B in water soluble B form ranges from 2-100 mg/kg with general range of 7-80 mg/kg, however water soluble B varies with soil parameters (Liu Zheng et al., 1983). Soil B availability depends on soil texture, pH, and soil moisture content. Boron concentration is usually higher in clay and loamy soils than in sandy soils (Gupta, 1968a). Liming of acid soil renders B unavailable B deficiency is most prevalent in humid region, not only due to leaching but also induced by drought. Under drought conditions release of B held by organic matter through microbial decomposition decreases leading to unavailability of B. Detailed description of soil properties affecting B availability and dynamics in soil is presented in section 2.5.

Boron retention is greater on sesquioxides than on clay. Lindsay (1972) reported B adsorption on Fe and Al oxides governing its solubility in soils. Soil organic matter (SOM) adsorbed more B than mineral soil constituents and OM has greater effect on B mobility and availability particularly in acid soils (Davies and White, 1981; Kabata-Pendias and Pendias, 2001). Adsorption mechanism such as oxy and hydroxy bonding, surface coating, and incorporation into interlayer or structural positions of aluminosilicates predominate in acid and neutral soils (Kabata-Pendias and Pendias, 2001).

Though deficient micronutrient in most of the soils, B present in excess in arid or semi-arid and overfertilised soils (Kabata-Pendias and Pendias, 1992). Use of water discharge from coal mines for irrigation purposes creates B contamination problems in irrigated agriculture (Craw et al., 2006).

## **2.5 Soil factors affecting B availability**

Table 2.1 summarises the soil properties that control B availability in soil. The effects of these properties on B availability are discussed later in this section. A sound understanding of these properties leads to better assessment of occurrences of B deficiency in all soils, including those that support forestry (Stone, 1990).



**Table 2.1 Soil properties affecting B availability in soil**

Soil property	B availability	References
Texture	Less available in coarse than fine texture soils, adsorption increases with increasing clay content and is correlated to clay minerals (kaolinite, montmorillonite, and chlorite)	(Adriano, 1986; Goldberg, 1997; Keren et al., 1981)
pH	As soil solution pH increases, B tends to be present as the borate ion and its availability to plant decreases.	(Goldberg, 1997; Gupta, 1993a; Lehto, 1995)
Organic matter	Boron availability is influenced by organic matter content. Adsorption on humus increases with increasing pH (maximum near to 9) and decreases with any further increase in pH.	(Hou et al., 1994; Keren et al., 1981)
Fe, Mn and Al oxy-hydroxides	Significantly related with Al-oxides. Adsorption increases both on Al and Fe oxides, as pH increases; maximum at pH 6 to 8 for Al and pH 7 to 9 for Fe oxides. Boron associated to Mn oxides shows increased B availability to plant  Affinity order: Al-oxides > Fe oxides due to higher surface area of Al-oxides and lower affinity for Mn oxides. Competing ions (silicates, sulphate, phosphate and oxalates) decrease B adsorption on oxides	(Goldberg and Glaubig, 1986; Takano et al., 2002)
Calcium carbonates	Liming increases B fixation by raising soil solution pH, CaCO <sub>3</sub> also acts as a surface for adsorption.	(Keren and Bingham, 1985; Keren et al., 1981)

### 2.5.1 pH

Soil solution pH shows an inverse relationship with the plant-available B concentration in soil. Significant correlations have been found between the soluble B content of a soil and soil solution pH (Keren et al., 1981). Boron adsorption onto soil particles has been found to be solution pH dependent (Goldberg, 1997), increasing over the range of pH 3 to 9 (Adriano, 1986; Carlson and Adriano, 1993; Keren et al., 1985; Lehto, 1995), but decreasing in the range of pH 10 to 11.5 (Keren et al., 1981). This manifests as a general inverse correlation between B adsorption and soil solution pH over the pH range required for plant growth (Evans, 1987; Okazaki and Chao, 1968). Boron, therefore, becomes less available to plants with increasing solution pH, and consequent this can result in B deficiency symptoms with the application of lime.

The soluble B content of arid zone soils ranging in pH from 6 to 8 has been shown to be significantly correlated with solution pH (Keren et al., 1981). Soil B adsorption, as measured by hot water extraction, has been shown to be positively correlated with pH for acid soils (pH < 7), and negatively correlated with pH for basic soils having a pH greater than 7, at a 95 % level of significance (Berger and Truog, 1945).

While studying B adsorption onto soil as a function of pH, Bingham et al. (1971) reporting on two allophanic soils from Mexico, Schalscha et al. (1973) reporting on three allophanic soil from Chile, and Goldberg and Glaubig (1986) reporting on 8 soil series from California US, all found that B adsorption increased over the pH range of 5 to 8.5 exhibiting peak adsorption for the pH range 8.5 to 10, and decreased for the pH range 10 to 11.5.

Liming helps increase B adsorption to soil through increasing pH and hence increases the abundance of  $B(OH)_4^-$  species available in soil. As pH increases further, the  $B(OH)_4^-$  concentration increases rapidly, with a proportional increase in B adsorption. Further increase (pH > 9) results in an increased  $OH^-$  concentration relative to  $B(OH)_4^-$ . Presence of free  $OH^-$  could be the reason for low B adsorption at alkaline conditions as the  $OH^-$  ion preferentially complexes to soil adsorption sites over the borate ion (Keren and Mezuman, 1981).

### 2.5.2 Parent material

The B concentration of soil parent material (Table 2.2) has a major influence on the B concentration derived from such rocks. Granite-derived soils often have low plant-available B (0.07-0.15 mg water soluble mg B/kg rock) when compared to soil derived from basalt (0.25-0.35 mg/kg) and some sedimentary rocks (0.50 mg B/kg) (Park and Park, 1966). Soils derived from granite and other igneous rocks, gneiss and sandstone are generally regarded as having low total and water soluble-B, while those derived from loess and shale contain more B (Liu Zheng et al., 1983).

### 2.5.3 Liming

Like other trace elements, B availability in soil is affected by liming. Boron deficiency is likely to be induced through liming acid soils due to increased B adsorption at higher pH (Reisenauer et al., 1973). However, the effect of lime is more than a simple result of increased pH, and increased adsorption can be effected by the calcium ion (Keren and Bingham, 1985). The mechanism explaining B deficiency following lime application is ascribed to the formation of insoluble borates. The process where B monomers chemically react to form a three dimensional polymer network chain is called polymerization. The formation of polymerizable calcium metaborates in soil has been proposed to occur following lime application; calcium metaborates (Figure 2.2) are thought to possess endless chain structures capable of polymerization.

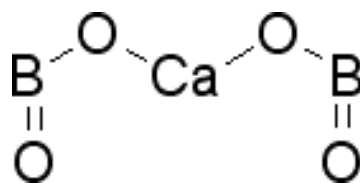
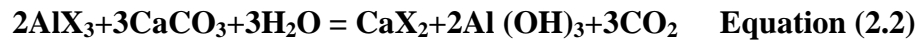


Figure 2.2 Ca-metaborates

The main reaction (Equation 2.2) occurring through the application of lime to acid soil, involves the replacement of exchangeable Al and hydroxyl-Al cation by calcium on soil colloid surfaces leading to the precipitation of Al(OH)<sub>3</sub> (Hatcher et al., 1967).



Where 'X' represents the exchange site. Precipitated Al(OH)<sub>3</sub> has an important role in B adsorption. In addition to the chemical function of Al(OH)<sub>3</sub>, the increased surface area in the soil affected through the formation of Al(OH)<sub>3</sub> is also an important factor which explains the greater B adsorption capacity of soils rich in Al hydrous oxides such as pumice or allophanic soils.

Other factors such as soil moisture status (Berger, 1949), the nature of the crop, and the length of time since application (Calba et al., 2004) all affect the extent of lime-induced B deficiency in soil.

**Table 2. 2                      Boron concentration (mg/kg) in major rock types after Shorrocks (1997)**

<b>Class of rock/mineral</b>	<b>type of rock/mineral</b>	<b>B (mg/kg)*</b>
Igneous	Basic: gabbro, basalt	5-20
	Intermediate: diorite	9-25
	Acid: granite, rhyolite	10-30
Metamorphic	Gneiss	10-30
Sedimentary	Shale	120-130
	Sandstone	30
Clay minerals	Muscovite	10-500
	Biotite	1-6
	Illite	100-2000 or greater
	Montmorillonite	5-200
	Kaolinite	10-30
	Chlorite	50 or lower

\*(Kabata-Pendia and Pendias, 1992)

#### **2.5.4      Soil texture and clay minerals**

Kubota et al. (1948) reported greater movement of B in sandy soil than in heavier textured soils; B leaching from the top 20 cm of sandy soils has been readily observed (Wilson et al., 1951). Coarse textured, sandy soils are therefore more prone to B deficiency (Fleming, 1980; Gupta, 1968b). A significant and positive correlation has been observed between clay content and the native B concentration in soil, clay content and the adsorbed B concentration in soil (Elrashidi and O'Connor, 1982; Keren et al., 1981) as well as clay content and maximum B adsorption (Goldberg and Glaubig, 1986), particularly for clay minerals such as kaolinite,

montmorillonite, and chlorite (Goldberg, 1997). Incorporation of B into the interlayer of aluminosilicates is one of the three mechanisms that can facilitate B fixation in acidic or neutral soil (Kabata-Pendias and Pendias, 2001).

While studying the effect of texture on water soluble B, Gupta (1968) found that greater amounts of hot water-soluble B were present in fine textured than in coarse textured soils, and that this could be ascribed to the fact that more B is adsorbed into clay and therefore less is subject to leaching.

Under the pH condition of most soils, B exists in solution as the uncharged boric acid species. This means that electrostatic interactions are not responsible for B adsorption, and, therefore, retention onto soil surfaces or within the similar matrix occurs by mechanisms other than non-specific adsorption. For example, Goldberg et al. (2007) suggested that lowered point zero charge (PZC) as B adsorbed onto Fe oxides and Al oxy-hydroxides provides evidence of inner-sphere B adsorption. In the pH range found in most common soils, clay-rich soils with illite (2:1 layer) adsorbed more B than kaolinite or montmorillonite (2:1), with kaolinite (1:1 layer) adsorbing the least (Hingston, 1964). This observation was directly attributed to the surface area of clay minerals in the order illite > montmorillonite > kaolinite. The fixation mechanisms; specific adsorption to oxy and hydroxyl functional groups, surface coating, and interlayer incorporation into aluminosilicates dominate in acid soils and alkaline soils (pH >9), whereas the co-precipitation of B-Ca hydroxides and B-Mg hydroxides occurs in arid soils.

### **2.5.5 Soil organic matter**

The organic matter content of a soil (SOM) is an important factor affecting B availability in soil. There have been reports showing significant correlations of organic C with native soil B and hot water soluble B (El-Mogazi et al., 1988; Keren et al., 1981; Miljkovic et al., 1966), and adsorbed B and B adsorption maxima<sup>1</sup> (Keren et al., 1981). Other factors such as the degree of organic matter decomposition and the moisture status of the soil will also affect B availability. The higher content of SOM in surface soils results in a greater concentration of sorbed B in surface horizon over subsoil horizon. Organic matter may also affect the

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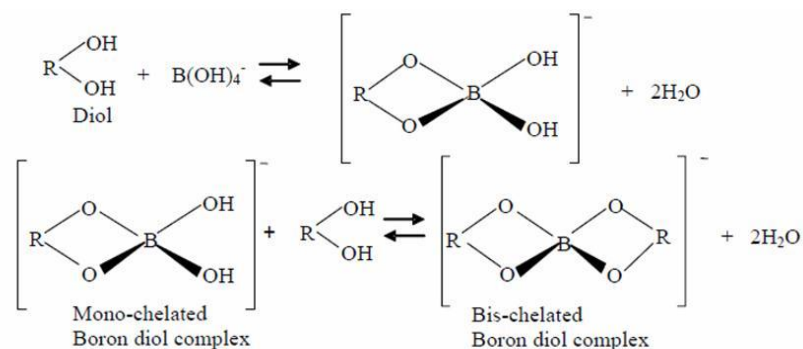
<sup>1</sup> at high equilibrium concentration the surface of an adsorbent becomes saturated, and reaches a maximum called maximum adsorption

interaction between soluble B and pH. Miljkovic et al. (1966) correlated soluble B with an interaction between pH and soil organic matter. They described a variable increase in soluble B associated with an increase in pH for a soil with high organic matter, but limited increase for a soil with low organic matter.

The bonding between B and organic matter increases in dominance and strength with an increase in the number of receptive OH<sup>-</sup> groups, as may occur with an increase in organic matter decomposition. Boron in the form of boric acid will adsorb onto organic matter by way of specific adsorption (Gu and Lowe, 1990) which may occur via ligand exchange or chemisorption (Bais et al., 2006; Bowden et al., 1980; Entry et al., 2002). Yermiyaho et al. (1988) reported that ligand exchange could be a possible mechanism for B sorption by organic matter.

Both boric acid and the borate anion complex through *cis*-diol linkages to small soluble organic molecules (Figure 2.3) facilitated by  $\delta$ -hydroxyl carboxylate groups (Dannel et al., 2000). Such compounds include sugar and sugar derivatives, both of which are constituents of hemicellulose in the cell wall. Thellier et al. (1979) reported that the *cis*-diol complex accounts for the major proportion of the total boron content in the cell wall of higher plant. Microbial breakdown of these complexes can release B back into the soil solution (Hue et al., 1988).

Borlan (1964) reported that a low soil to water ratio (less soil and more water) helped release more B from organic matter, paving the way for more B to be released under moist conditions than under dry conditions.



**Figure 2.3 Formation of a B-diol complex after Huettl (1976)**

Organic matter is considered to be more influential on B availability in acid soils as compared to basic soils, where pH and available calcium play a key role in B sorption (or resorption) onto the soil matrix (Berger and Truog, 1945).

### **2.5.6 Soil water content**

Plant stress brought about through drought affects the incidence and severity of B deficiency more than for any other micronutrient. Decreased B availability is observed as soil dries, and consequently plants develop deficiency symptoms (Fleming, 1980). The possible explanation for this observation is likely to be the reduced amount of available B in the subsoil encountered by the plant as it extracts moisture from a lower depth during a dry season (Fleming, 1980). Another reason for drought-induced B deficiency could be restricted mineralization and subsequently limited availability of organically-bound B in soil (Evans and Sparks, 1983). As soil B is often concentrated in the surface soil, drying of the surface layer could have significant impact on restricting the supply of B to a plant's meristem and therefore retard movement of B in the phloem.

Scott et al. (1975) reported that B diffusivity decreases with decreasing water content as a result of reduced soil solution mobility, and an increase in the diffusion path length due to drying. While investigating the effect of the soil to moisture ratio on soil B adsorption, Mezuman and Keren (1981) noted that soil B adsorption increased with increasing soil to solution ratio, while in another study, Gupta (1968b) found no effect on soil B adsorption by changing soil moisture content from 50% to 100 % of field capacity, equivalent to variation of a soil to solution ration of about 6:1 to 3:1. Alternate wetting and drying cycles increase the amount of B fixation (specific adsorption of B into clay lattice) by a kaolinite and montmorillonite soil (Biggar and Fireman, 1960). In the Biggar and Fireman study (1960), the rate of increased fixation was greatest during the first wetting and drying cycle but continued to increase slightly even after five cycles. The effect of drying became more pronounced as an increased concentration of B was added to the experimental system. Boron deficiency symptoms in *P. radiata* plantations are predominant observed during the dry season (Lambert and Ryan, 1990; Lambert and Turner, 1977), and, as has been established, low soil-water contents during dry periods lead to an inhibition of B release from decomposing soil organic matter.



### **2.5.7 Soil salinity**

Mehrotra (1989) showed that an antagonistic relationship exists between applied B and the sodium adsorption ratio (SAR) of irrigation water. Increasing levels of soil salinity have been shown to decrease B concentrations in chick pea (*Cicer aritinum*) and this effect was accentuated at higher B levels (Yadav et al., 1989). Though the mechanism of B-salinity interrelation is not clear (Kot, 2009), B responds antagonistically to salinity. Holloway and Alston (1992) reported increasing salinity corresponds to decreasing B uptake in wheat, and that B uptake was reduced in the presence of  $\text{Cl}^-$  (Yermiyahu et al., 2008). An explanation for the resulting low foliar B concentration may correspond to reduced water uptake due to high soil salinity (Ferreya et al., 1997). In sodic soil, gypsum application converts readily soluble Na-metaborates to less soluble Ca-metaborates, hence ameliorates an excess of B in soil (Bhumbla and Ckhabra, 1982).

Recently Grieve and Poss (2000) found an association between high soil B and high salt concentration in arid and semi-arid irrigated areas. Paliwal and Mehta (1973a) and (1973b) studied the interactive effect of B and salinity and suggested that paddy-rice germination was not affected by B application at the rate of 40 mg/kg where non-saline water was used. However, where salinity was apparent, the tolerance of the seeds was reduced such that a tolerance concentration of only 4.5 mg B/kg was apparent.

### **2.5.8 Summary: Boron adsorption and fixation in Soil**

Boron in soil exists in three status; as labile species in soil solution, less labile species adsorbed (non-specific) on phyllosilicate clay minerals (Goldberg and Glaubig, 1985; Su and Suarez, 1995), and non-labile species which are specifically adsorbed on oxides and oxy hydroxides minerals, organic complexes (Gu and Lowe, 1990) and on carbonate minerals (Goldberg and Forster, 1991). According to Goldberg et al. (1993b), B adsorption on Al and Fe oxides minerals represents ligand exchange with reactive surface  $\text{OH}^-$  groups. In this non-electrostatic attraction the form of exchanged B is boric acid,  $\text{B}(\text{OH})_3$ . This type of exchange involves displacement of  $\text{OH}^-$  (as  $\text{H}_2\text{O}$ ) from the surface by adsorbed B species (Evans and

Sparks, 1983; Toner and Sparks, 1995). Under conditions of normal soil pH (5-8) the possible mechanisms for adsorption of B to soil surfaces include anion exchange, precipitation of insoluble borates with sesquioxides, sorption of molecular boric acid, formation of organic complexes, and fixation of B onto in the clay lattice (Goldberg, 1997). Fleming (1980) reported that B rendered unavailable by application of lime to acid soil is historically called “B fixation”.

Generally, these are all examples of a non-electrostatic interaction of boric acid with the soil. The charged borate ion shows poor retention to soil surfaces through electrostatic attraction due to the repulsion of the negatively charged ion from a negatively charged soil surface at pH above 8, the pH above which borates become favoured in solution. The overall amount of B available to plants will depend upon the weathering of nearly insoluble B mineral reserves and also on the rate of B cycling that is a function of plant decomposition in soil. However, on a smaller time-scale, plants must rely on B released from hydroxylated mineral surfaces and SOM.

## **2.6 Environmental factors**

### **2.6.1 Sun light intensity**

Light intensity is a prime factor affecting the mineral nutrition of higher plants. Rapid plant growth under high light intensity induces B deficiency. Therefore, B deficiency or toxicity has an association with particular times of the year such as during summer. Hunter et al. (1990b) found more common and severe occurrences of B deficiency in *P. radiata* during summer on the East coast of New Zealand. Cakmak et al. (1995) and Noppakoonwong et al. (1993) reported abrupt B deficiency in sunflower seedlings and increased B requirements in black gram (*Vigna mungo*) exposed to both shaded and unshaded conditions. The reported differential response of dry matter production to B under both conditions could be due to high light intensity which was possibly due to a period of sustained high growth rate. Cakmak and Römheld (1997) suggested that B deficiency depressed phenol metabolism leading to cellular phenol accumulation under conditions of high light intensity.

## **2.6.2 Rainfall and moisture**

Boron generally exists in soil in a non-ionic form, and is subject to leaching as it is released from soil minerals or B fertilisers. This phenomenon explains why soil in high rainfall areas often develops B deficiency. In arid and semi-arid areas B is likely to concentrate on soil surfaces leading to the development of soil toxicity (Keren and Bingham, 1985).

The effect of moisture on B availability appears to be more pronounced than for some other elements. The phenomenon of B deficiency in plants during drought periods may be partially associated with a decrease in B mobility to roots (Kluge, 1971), and boric acid polymerization (Figure 2.2; Section 2.5.3). A reduction in the quantity of soil solution during a drought, as demonstrated by reduced mass flow, leads to reduced diffusion rate and limited transpiration flow and these may be the causative factors of B deficiency, despite the presence of an adequate supply of available B in the soil. Boron-diol complexes (Figure 2.3; section 2.5.5) may be affected by moisture, as during times of inadequate moisture, breakdown of these complexes by microbes can slow down, affecting B availability (Evans and Sparks, 1983).

Boron deficiency is generally found in dry soils where summer or winter drought is severe. This may explain why most B deficient districts in New Zealand are low rainfall areas (Will, 1985) such as the rain shadow of the Southern Alps with the worst deficiency symptoms observed in drought years (Hunter et al., 1990).

## **2.6.3 Temperature, humidity and transpiration rate**

Increasing soil temperature can lead to greater B fixation; however this result may be because of an interactive effect with the soil (Fleming, 1980). There exists scant and contradictory information regarding the effect of temperature on B availability. For example, Biggar and Fireman (1960) found a decrease in B fixation of about 30% for montmorillonitic soils and about 15% for a kaolinitic soil when temperature was increased from 25 to 45 °C. While in contrast, Bingham et al. (1971) found a slight increase in B adsorption as the temperature of an amorphous Mexican soil was increased from 10 to 40 °C.

The observed differences could be attributed to a difference in mineral composition of the soils used in each study. A conceptual model proposed by Conant et al. (2011) identified that the soil organic matter-mineral (SOM-M) interactions vary with temperature. As temperature increases, both low and high affinity adsorption to SOM-M surfaces increases. However, covalently bounded soil organic mineral interactions show no response with an increase in temperature. In crystalline mineral soils B adsorption decreases as a function of temperature in the range of 10-40°C (Goldberg et al., 1993) with a corresponding slight increase for amorphous soils over this temperature range (Bingham et al., 1971).

Low temperature restricts water transport through roots (Wan et al., 1999) and influences net photosynthesis. Day et al. (1991) reported that net photosynthesis in *P. taeda* seedlings gradually declined as temperature dropped (24 °C to 10 °C), and this in turn is likely to affect B uptake. Bowen (1972) reported a direct relationship between light intensity and B absorption, and an inverse relationship between relative humidity and B absorption in sugarcane (*Saccharum officinarum*) seedlings. Similarly, increasing duration and light intensity has been shown to result in increased water and B uptake by barley seedlings (Oertli, 1994). Transpiration rate plays a significant role in plant B uptake (Hu and Brown, 1997) and reported environmental affects influencing B absorption can be largely explained by changes in transpiration rate (Raven, 1980). For example, reduced humidity increases transpiration and consequently increases B absorption, while increased temperature creates water vapour deficits and thereby increases transpiration and B absorption. An increase in B adsorption with an increase in temperature can manifest as B deficiency associated with dry summer conditions. This phenomenon could be due to interactive effect of soil temperature with soil moisture (Goldberg, 1997).

#### **2.6.4 Distance from sea and sea spray**

Rainfall in coastal areas can contribute a significant amount of B to soil due to higher levels of B dissolved in rain water generated through evaporation over sea than over land. Data from the west coast of Sweden shows that this region receives 25 to 40 g B/ha/year as compared to less than 2 g B/ha/year in northern Sweden far from the sea (Wikner, 1986). It has been reported that the effect of seawater-derived B can lead to annual concentration increases in the soil of 4-5 mg B/kg, and that soils with minimal maritime influence can be more susceptible to B deficiency (Shorrocks, 1997; Stone, 1990). Sea spray B deposition in New Zealand

varies from 50 g/ha/year in exposed coastal regions to 5 g B/ha/year in inland areas (Blakemore, 1953). To put this figure into context, the average B accumulation in *P. radiata* biomass over 30 years of rotation would be in the order of 30 to 50 g/ha/year (Madgwick et al., 1988). This means that sea spray can provide 100% of B nutrient requirement for pine forestry in some coastal areas.

## **2.7 Sources of B**

### **2.7.1 Anthropogenic sources of soil B**

Fogg and Duce (1985) reported coal combustion and agricultural burning to be the most important anthropogenic sources of B. These authors reported that B is removed from the atmosphere by precipitation, particulate dry deposition, and through gaseous absorption by the land and sea. The data of Fogg (1983) support the belief that the sea is the net sink for atmospheric gaseous B.

### **2.7.2 Boron fertiliser**

Solubor ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O} + \text{Na}_2\text{B}_{10}\text{O}_{16} \cdot 10\text{H}_2\text{O}$ ) and foliarex ( $\text{Na}_2\text{B}_8\text{O}_{13} \cdot 4\text{H}_2\text{O}$ ) are commonly used as B fertilisers; both are very soluble and can be toxic if applied near to germinating seed. Other B-fertilisers, as shown in Table 2.3 are borax (11.3% B), sodium tetraborate ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 5\text{H}_2\text{O}$ ) (14% B), Na-pentaborate ( $\text{Na}_2\text{B}_{10}\text{O}_{16} \cdot 10\text{H}_2\text{O}$ ) (18% B), solubor ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 5\text{H}_2\text{O} + \text{Na}_2\text{B}_{10}\text{O}_{16} \cdot 10\text{H}_2\text{O}$ ) (20% B), and boric acid  $\text{B}(\text{OH})_3$  (17% B). Sherrell (1983) reported that in New Zealand, Na-borate, as borax ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ) or fertiliser borate ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 5\text{H}_2\text{O}$ ,  $\text{Na}_2\text{B}_4\text{O}_7$ ), are commonly used to control B deficiency in alfalfa. Other borate minerals used include colemanite and datolite which show medium and low solubility respectively.

Keeping in view the narrow range of B sufficiency that is bounded by deficiency and toxicity in plants slow release fertilisers such as ulexite are the most common B sources for use in long-term forestry plantations. Ulexite ( $\text{NaCa B}_5\text{O}_6(\text{OH})_6 \cdot 5\text{H}_2\text{O}$ ) (0.5% soluble in water at

20 °C) contains 30%-42%  $B_2O_3$  (total B content is 13.5%), and is considered to release B slowly as compared to other sources of B (Mehmet, 2009; Vengosh 1994).

The primary mode of application of B fertiliser is by soil application, although some  $B(OH)_3$  and solubor is applied as a foliar spray. Factors which affect B availability from fertiliser include the application method, as well as the source and constituents of the applied B fertiliser. One of the main focuses in fertiliser application is to control the potential for B leaching from the soil. As B is taken up by plants in a non-ionic form there is limited potential for adsorption of B from soil solution to soil colloids. Gupta and Cutcliffe (1978) found that on the Podzol soils of Eastern Canada, about 62% of applied fertiliser B was not recovered (using hot water extraction of soil) from the surface 15 cm of soil 5 months after broadcast application and they attributed this to leaching of B. Similarly, in another study, leaching from the 0-60 cm horizon was shown to alleviate toxicity otherwise associated to high B application in an oilseed rape-rice rotation system in southeast china (Wang et al., 1999). However, Barrow (1989) reported that borate sorption occurs on variable charge surfaces which are heterogenous but partially defined by the electric potential of surface.

**Table 2.3****Boron compounds, used as fertiliser after Mortvedt and Woodruff (1993)**

<b>B source</b>	<b>Chemical formula</b>	<b>Water solubility</b>	<b>% B</b>
Borax	$\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$	Soluble	11.3
Boric acid	$\text{B}(\text{OH})_3$	Soluble	17.5
Anhydrous borax	$\text{Na}_2\text{B}_4\text{O}_7$	Soluble	21.5
Fertiliser borate	$\text{Na}_2\text{B}_4\text{O}_7 \cdot 5\text{H}_2\text{O}$	Soluble	14.3-14.9
Solubor	$\text{Na}_2\text{B}_8\text{O}_{13} \cdot 4\text{H}_2\text{O}$	Very soluble	20.5
Colemanite	$\text{Ca}_2\text{B}_6\text{O}_{11} \cdot 5\text{H}_2\text{O}$	Slightly soluble	15.8
Ulexite	$\text{NaCa B}_5\text{O}_6(\text{OH})_6 \cdot 5\text{H}_2\text{O}$	Slightly soluble	13.3
B-frits	Boric oxide glass	V. slightly soluble	2.0-11.0

### **2.7.3 Irrigation water**

The use of irrigation water with a naturally high concentration of B is an important factor that contributes to high soil B levels in some parts of the world (Chauhan and Powar, 1978). In arid and semi-arid areas, B toxicity is commonly ascribed either to parent materials, or to the use of high B irrigation water (Nable and Paull, 1991). Understanding crop and soil interactions is important where irrigation water has high B (Nable et al., 1997). Irrigation of land containing soil with a high B affinity will lead to the build-up of large amounts of adsorbed B in the soil. However, continuous irrigation is likely to exceed soil adsorption capacity and will subsequently reduce crop yield as a result of excess B in soil solution that can become toxic (James et al., 1982).

A Boron concentration in irrigation water in excess of 1.5 mg/L results in a significant decrease in the yield of both wheat and pea (Chauhan and Powar, 1978). Depending on the crop species, Keren and Bingham (1985) suggested that a concentration of 0.3-4 mg B/L in irrigation water could be a safe limit for most of crop and vegetable species. Another study (Bingham et al., 1985) found that wheat grain yield reduced as the B concentration in irrigation water increased above 0.3 mg/L.

Boron levels in water depend on the climate and location of the water table, and the geochemical properties of the material through which groundwater is flowing. Based on a B concentration that corresponds to plant tolerance, irrigation water can be categorized into five classes (Table 2.4); excellent, good, fair, poor and unsuitable.



**Table 2. 4                      Classification of irrigation water based on the tolerance of plant species to the B concentration in solution**

Classification	Sensitive plant	Semi-tolerant plant	Tolerant plants
	B concentration (mg/L)		
Excellent	< 0.3	< 0.6	< 1.0
Good	0.4-0.6	0.7-1.3	1.0-2.0
Fair	0.7-1.0	1.4-2.0	2.1-3.0
Poor	1.1-1.3	2.1-2.5	3.1-3.8
Unsuitable	> 1.3	> 2.5	> 3.8

Note: B tolerant crop plants: Alfalfa, beet, cotton, tomato and oats. B moderately tolerance plant: Barely, cabbage, corn and turnip. B sensitive plant: Avogadro, bean, graphs, lemon and wheat (Mass, 1987)

Any B that builds-up in the rhizosphere as a result of irrigation of high B water at a rate that exceeds plant B requirements must be leached to ensure successful crop production (Ayars et al., 1990).

#### **2.7.4 Leaching**

Because of its non-ionized nature under conditions of normal soil pH (4-7), once B is released from soil minerals it is subject to rapid leaching from soil. Since B follows water flux (Kabata-Pendias and Pendias, 2001) leaching is the main mechanism which triggers B deficiency in high rainfall, and cool humid climates. Page and Cooper (1955) reported that leaching losses from acid, sandy soils account for as much as 85 % of the applied B after irrigation of the soil profile with 12.5 cm of water. Reisenauer et al. (1973) reported that B

movement is less rapid in heavy textured soils because of increased fixation (specific adsorption) by clay particles.

In humid regions, a surface horizon that has been leached of soluble salt generally contains residual available B that is held largely by the organic fraction of the soil colloids. This helps prevent B from leaching out of the rhizosphere.

### **2.7.5 Municipal compost, biosolids and industrial effluents**

The B concentration in municipal and industrial water may be significantly high (Kot, 2009). Municipal composts can contain substantial amounts of B (Brinton et al., 2008). Purves and Mackenzie (1974) reported that application of municipal composts at the rate of 5 to 10 t/ha can cause soil B contamination and phyto-toxicity. Municipal solid waste (MSW) produced by industries can therefore negatively affect plant growth due to an increase in tissue B levels (Brinton et al., 2008). However, Zupancic and Zupancic (2007) reported a beneficial effect of B-enriched municipal landfill leachate on plant growth. Presumably in this second example, the soil and these plants both were B deficient.

Biosolids contain macro and micronutrients including B. According to New Zealand Water and Waste Association (NZWWA, 2003) around 250 public wastewater treatment plants treat 80 % of domestic sewage in the country. Wastewater and solids associated with sewage treatment and disposal represent a possible source contamination of soil with inorganic elements (Berrow and Webber, 1972). According to a national study on the composition of sewage sludge in New Zealand, the B composition of raw, aerobic, anaerobic and pond sediments is reported to be 11, 21, 20 and 17 mg B/kg respectively showing the extent of B concentration in various types of sludge (Ogilvie, 1998). The use of borates and polyborates in detergents (around 12% of total consumption) as buffering, softening and bleaching reagents, generally accounts for the presence of B in sewage effluents and sludge. Vengosh et al. (1994) reported Na-perborate [ $\text{NaBO}_3 \cdot 4\text{H}_2\text{O}$ .PBS1], used in washing power to be a significant source of B contamination.

## **2.7.6 Coal fly ash**

Carlson and Adriano (1993) described fly ash as a source of toxic elements including B. Application of fly ash to soil can result in poor plant growth as a result of increased soil B concentration (Aitken and Bell, 1985). Toxic B concentrations in excess of 120 mg/kg have been reported in canola leaves grown on soil amended with fly ash (B = 6.7 mg/kg) at a rate of 625 t/ha. Lower rates of ash application to soil can however, promote plant growth where natural levels of soil B are low. The optimum rate for canola yield is more likely to be in the order of 25-36 t/ha (Manoharan et al., 2010; Yunusa et al., 2008).

## **2.8 Boron in soils: total vs. bioavailable concentration**

### **2.8.1 The total soil boron (TSB) status of soil**

Swaine (1955) reported that the total B concentration of normal soil ranges from 2 to 100 mg/kg, with an average value of 30 mg/kg. The total B concentration of soil depends largely on the soil's parent material. The highest B values occur in arid saline soils. Fine textured soils in humid climatic zones typically have B ranging from 30-60 mg/kg, whereas sandy soils often have B concentrations as low as 2-6 mg/kg (Whetstone et al., 1942).

Consideration of the global distribution of B shows that China has a higher total B concentration in different soil groups (4-145 mg/kg) than other countries (Liu Zheng et al., 1983). Ilin and Anikina (1974) reported that in Russia, B concentrations are higher in the soils of the dry steppe, semi-desert, and desert zones compared to the central regions. In these high soil B areas concentrations between 200 to 400 mg/kg are not uncommon. Gupta (1968) reported that soil from Eastern Canada has total B ranging from 45 to 124 mg/kg. The range of soil B concentrations around the world is clearly large.

The most common method used for the determination of TSB is fusion by  $\text{Na}_2\text{CO}_3$  (Bingham, 1982). However, total B is generally an unreliable index of B availability in soil, and therefore, extractable or plant available B is used for the diagnosis of deficiency and toxicity.

### **2.8.2 Bioavailable or Plant-available Boron**

As an index of bioavailable B in soil, extractable-B rather than total B, is commonly used for diagnostic purposes (Adriano, 2001). Reported plant-available B can be categorised based on climatic zone. Temperate, boreal and humid tropical regions generally contain the lowest range of plant available B (1-2 mg/kg) while arid and semiarid region soils show average to high water-soluble B concentrations (10-40 mg/kg) (Aubert and Pinta, 1977).

Extraction techniques are used to estimate B in the labile pool which constitutes B in soil solution and that is readily available for plant uptake. Different extraction solutions such as hot water or a weak electrolyte solution ( $\text{CaCl}_2$ ) are used to assess this readily soluble B and the magnitude of total soil B that is plant available. These extraction techniques are summarized in Table 2.5, and the hot water extraction and hot 0.02 M  $\text{CaCl}_2$  extraction methods are considered in detail in this review.

**Table 2.5 Extraction techniques used to assess plant available B in soil after Nable et al. (1997)**

Method	Comments of authors	Reference
Hot water soluble (HWS) reflux 1:2 soil-water	Good for assessing plant available-B. Widely used; good prediction of deficient levels; estimates plant-available B; site specific; does not indicate total soil B	(Berger and Truog, 1940; Bingham, 1982)
Saturation extracts	Comparable with solution B; B tolerance for plant based on this test, only a portion of adsorbed B goes into solution must be related to field moisture conditions.	U.S. Salinity Laboratory Staff (1954); (Gupta et al., 1985)
CaCl <sub>2</sub>	Good for non-specifically adsorbed-B. Indicates plant available-B at a variety of pH levels; does not indicate total soil B	(Aitken and McCallum, 1988; Spouncer et al., 1992);
NH <sub>4</sub> /DTPA	Used to determine availability of many nutrients in one extract.	(Handreck, 1990)
NH <sub>4</sub> -Oxalate (pH 3.75)	Solubilises crystalline and non-crystalline oxy hydroxides from soil; poor correlation to plant uptake of B.	(DeEndredy, 1963; Schwertmann, 1964)
Acidified NH <sub>2</sub> -HCl	Good for separating B associated with Mn minerals from Fe minerals; poor correlation with plant level of B for calcareous soil	(Chao, 1972; Cox and Kamprath, 1977)
Mannitol exchangeable-B	Estimates non-specifically and specifically adsorbed forms; assess regenerative power of soil for B	(Rhoades et al., 1970)

### **2.8.2.1 Hot water extractable B (HWEB)**

According to Berger and Truog (1939) this method involves refluxing soil for 5 minutes with boiling water using a soil: water ratio of 1: 2. Wear (1965) reported HWEB to be the best indicator of B availability to plants. According to Berger and Truog (1940) HWEB constitutes < 5% of the total B content of soil and even less for soils exposed to humid climates (Sillanpää and Vlek, 1985), while soil from arid and semi-arid regions contain from 5 % to 16 % of total B in the water-soluble form (Aubert and Pinta, 1977). Gestring and Soltanpour (1984) found the HWEB concentration of Colorado soils to vary between 0.10 to 6.5 mg/kg and over this range no deficiency or toxicity symptoms in alfalfa were observed during a greenhouse study. Focusing on HWEB concentrations in different countries, El-seewi and Elmalky (1979) reported an average value of 1.3 mg/kg in Egypt, Gupta (1968b) reported a range from 0.38 to 4.67 mg/kg in Eastern Canada; and Archer (1980) reported an average value of 1.0 mg/kg in England and Wales. Gupta (1968b) positively related HWEB to the organic matter content of the soil. Berger (1949) reported hot water-ext. B concentrations relevant to the growth performance of various crops (Table 2.6).

While simple, the method has a number of drawbacks, and has been reported to suffer from uncontrolled variation in extraction time and temperature (Lambert et al., 1980), and a lack of correlation with crop response (Gestring and Soltanpour, 1987). The non-standard reflux time in literature ranges from 5 minutes (Berger and Truog, 1939), 10 minutes (Aitken et al., 1987; McLaren et al., 1990) to 30 minutes (Spouncer et al., 1992) making datasets difficult to relate (Novozamsky et al., 1990). Dispersion of soil particles during boiling (McGeehan et al., 1989) and possible resorption during cooling are additional problems (Gupta, 1967). It would seem likely that the final concentration of water-soluble B will be affected by the length of the reflux step.

**Table 2.6 Optimum\* hot-water extractable B levels in soil for the growth of various crop species**

<b>&lt; 0.1</b>	<b>0.1-0.5</b>	<b>&gt; 0.5</b>
<b>(mg B/kg)</b>		
Small grain	Tobacco	Apple
Corn	Tomato	Alfalfa
Soybean	Lettuce	Clovers
Pea and bean	Peach	Beets
Strawberry	Pear	Turnip
Potato	Cheery	Asparagus
Flax	Olive	Radish
	Cotton	Celery
	Sweet potato	Rutabaga
	Peanut	

\*Gupta (1985)

Furthermore, although HWEB appears to be a suitable index of the concentration of B in soil that is plant-available (Bingham, 1982), studies suggest a poor correlation between HWEB and plant response to B fertiliser application (Sims and Johnson, 1991) .

### **2.8.2.2 Hot CaCl<sub>2</sub> extractable B**

To avoid the problems inherent to the hot water extractable B technique, weak electrolytes such as CaCl<sub>2</sub> can be used to extract B. A key problem with this technique is colouring of the extract solution due to organic matter which can enhance absorbance compared to actual absorbance and therefore overestimate the B concentration in the extraction (Dible et al., 1954; Gupta, 1979b; Parker and Gardner, 1981). Colour interference due to organic matter can be

minimized using black charcoal (Gupta, 1979b). The use of a low concentration of CaCl<sub>2</sub> (for example 0.01 M) has also been advocated to mitigate colour interferences. This has the added benefit of flocculating clay particles that can affect the accuracy of spectroscopic determinations. Adams et al. (1991) reported the total, hot water and 0.02 M CaCl<sub>2</sub> extractable B concentration of several Canterbury soils from New Zealand and found good agreement between the concentration of B extracted by both techniques (Table 2.7).

**Table 2.7 Total, hot water and 0.02 M CaCl<sub>2</sub> extractable B in Canterbury after Adams et al. (1991)**

<b>B form</b>	<b>Concentration (mg/kg)</b>
Total Soil B	37-90
Hot water extractable B	0.32-2.67
Hot 0.02 M CaCl <sub>2</sub> extractable B	0.30-3.0

Both hot water and hot 0.01 M CaCl<sub>2</sub> extraction methods have been found to equally well describe sunflower growth (Aitken et al., 1987). However, Bell (1999) reported that although a similar amount of B was extracted by both hot water and 0.01 M CaCl<sub>2</sub> at low B levels, the two techniques were not likely to extract the same amount of B at higher extractable B concentrations (> 0.5 mg/kg). The difference could be caused by high clay content, and longer refluxing time (10 min. for 0.01 M CaCl<sub>2</sub> versus 5 min. for hot water extraction). Flocculation of colloidal materials and immediate filtration could add to the difference (Wikner, 1986). Spouncer et al. (1992) reported that hot 0.01 M CaCl<sub>2</sub> extracted twice as much B as hot water from soils with a pH range from 4.2-7.8. Comparative analysis of literature describing extraction techniques generally shows that soil extraction using hot 0.01 M CaCl<sub>2</sub> is the superior method to assess plant available B in soil. Qualities such as simplicity, minimal interferences and good correlation with plant uptake support this relative judgement.

The readily-soluble B fraction is a good index for the soil B status of agriculture crops, particularly annual crops and vegetables, but makes up a small portion of total soil B (Xu et



al., 2001). However, in the context of this study, long-lived pine plantations that generally mature over two or three decades may require different indicators to sustain long-term B availability. Understanding other B fractions may indicate how much each fraction contributes to the B cycle and to the potential availability of B in forest soils.

### **2.8.3 The effect of depth on the water extractable B concentration of soil**

El-seewi and Elmalky (1979) reported that total B was usually high in the top 30 cm, and occasionally high in the next 30-60 cm soil layer. A decrease in extractable B with increasing depth is ascribed to decreasing organic and increasing Fe and Al oxide contents deep in the soil (Sarkar et al., 2008). Adriano (2001) reported that HWSB in saturation extracts<sup>2</sup> was more or less evenly distributed. Similarly, Awad and Mikhael (1980) found that B (1.8 to 5.7 mg/kg) was practically evenly distributed throughout the 0-90 cm of a soil collected from the Egypt Western desert. In another series of studies (Wang et al., 1997; Wang et al., 1999) redistribution by leaching out of the 0-60 cm soil layer mitigated B toxicity in an oilseed rape-rice rotation in southeast China.

## **2.9 Analytical techniques for B determination in solution**

Various analytical methods are used to determine plant-available B in soil. The methods vary depending on the soil type, plant species and climatic condition. Major techniques include colorimetric methods, fluorimetric, inductively coupled plasma-optical emission spectrometry (ICP-OES), and inductively coupled plasma-mass spectrometry (ICP-MS). The colorimetric method commonly suffers from interferences due to sample colour (Lovatt, 1985) and nitrate complexes in solution prepared through HNO<sub>3</sub> acid digestion (Berger and Truog, 1939). It has been suggested that such interferences can be minimised by the use of thioglycolic acid (Zarcinas, 1995).

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<sup>2</sup>The extract is obtained by filtering a soil saturated paste using a buchner funnel and vacume flask

### 2.9.1 Interpretation of soil B level

Soil capacity to supply B in proportion to plant needs is controlled by soil texture, mineralogy, pH and organic matter. As B reacts more strongly with clay than sand in soils (Keren et al., 1984), B deficiency is corrected by higher fertiliser addition to sandy soils in comparison to clay-rich soils. The critical range of extractable B level in alkaline soils is greater than in acid soils, and is associated with a higher proportion of  $B(OH)_4^-$  in soil solution (Bell, 1997). Therefore, a higher concentration of B must be maintained in the soil solution of alkaline soils relative to acid soils. Bell (1997) reported a higher critical range of hot-water extractable B (0.32-0.38 mg/kg) for wheat grown on an alkaline clay soil relative to the same crop grown on a loam soil (0.12-0.15 mg/kg).

Snowdon (1982) reported a concentration range of 0.2-0.25 mg hot-water extractable B/kg as the critical level for deficiency and 0.15-0.32 mg/kg hot-water extractable B as the critical marginal range for *P. radiata* and compared this level to a range of other species (Table 2.8 ). There is clearly a large overlap between these ranges, attesting to the difficulty of stating critical values for a single species across a range of soil and environmental conditions.

**Table 2. 8 Critical B concentration in soil for different crops and vegetables using hot water extractable B after Bell (1999)**

Soil test	Crop	Depth (cm)	Critical concentration (mg/kg)			Reference
			Deficient	Marginal	Toxic	
Hot water	Black Gram ( <i>Vigna mungo</i> )	0-25		0.08-0.13		(Bell et al., 1990)
Hot water	Broccoli, Brussels Sprout, and Cauliflower ( <i>Brassica oleracea</i> )			0.28-0.34		(Gupta and Cutcliffe, 1973)
Hot water	Kiwi fruit ( <i>Actinidia chinensis</i> )	0-15			0.5, 1.3 strongly toxic	(Smith and Clark, 1989)
Hot water	Lucerne ( <i>Medicago sativa</i> )	0-15	0.23-0.36	0.34	0.36-0.84	(Haddad and Kaldor, 1982)
Hot water	Peanut ( <i>Arachis hypogaea</i> )	0-10		0.14-0.16		(Bell, 1999)
Hot water	Pine ( <i>P. radiata</i> )	0-7.5	0.2-0.25 severe	0.15-0.32		(Snowdon, 1982)
Hot water	Sunflower ( <i>Helianthus annuus</i> )	0-15	0.03-0.14	0.14		(Aitken et al., 1987)
		0-15	0.12-0.14	0.14	0.17-0.93	(Aitken et al., 1987)
Hot water	Wheat ( <i>Triticum aestivum</i> )			0.12-0.15		(Rerkasem et al., 1988; Rerkasem et al., 1989)
Hot water				0.32-0.38		(Gupta and Cutcliffe, 1973)

## 2.10 Soil dehydrogenase activity and B

Dehydrogenase activity is caused by a broad group of endocellular enzymes in soil involved in transferring electrons and hydrogen from substrates to electron acceptor during the

oxidation of organic compounds (Chander and Brookes, 1991; Skujins, 1978). During this process 2, 3, 5-triphenyltetrazolium chloride (TTC) is reduced to triphenylformazan (TPF) under anaerobic condition, where TTC acts as electron acceptor in the this reaction Equation 2.3



Dehydrogenase activity is an index of microbiological activity in soil (Skujins, 1973) and has been used in different environments to quantify biological activity. Beyer et al. (1992) explicitly associated dehydrogenase to biotic activity in soil.

Serdar et al. (2011) recently reported a positive correlation between B application and soil dehydrogenase activity, where a soil application of B at a rate of 3 kg/ha produced a significantly higher dehydrogenase activity (267  $\mu\text{g}$  TPF/g soil in 24 h) relative to the control treatment of no applied B. The influence of applied B was greater in the upper profile (0-30 cm) of the soil relative to the lower soil profile (30-60 cm). Boron application at rates greater than 3 kg/ha reduced soil microbial population,  $\text{CO}_2\text{-C}$  production and the abundance of general enzyme activity in the soil. This study elaborated the significant role B plays in the functioning of the soil biological environment.

## **2.11 Boron fractionation**

Trace elements, including B, which can exist in the solid phase of soil particles, can be studied using chemical fractionation. Chemical fractionation involves the use of extractants capable of extracting specific chemical forms (fractions) of trace elements from soil constituents either by single extraction (Hou et al., 1996) or sequential extraction (Tessier et al., 1979). Sequential extraction schemes commonly use 3-8 sequential extractions that are used according to an order of decreasing reactivity to the solid phase of the soil. In addition to simplicity, sequential extraction schemes help understand the origin, mode of occurrence, mobility, and biological and physiochemical availability of trace elements in solid samples (Jin et al., 1987). Sequential extraction schemes generally partition trace elements into one of five operationally defined geochemical fractions: readily soluble and exchangeable; specifically sorbed; oxide-bound; organic bound; and residual.

A variety of sequential extraction schemes have been developed to evaluate various fractions of trace elements including B in soil (Hou et al., 1994; Jin et al., 1987; Tessier et al., 1979; Tsadilas et al., 1997). Hou et al. (1994) developed a sequential extraction scheme for B based on pre-existing schemes for trace elements (Table 2.9) (Chao and Sanzalone, 1984; Jin et al., 1987; Tessier et al., 1979).

### **2.11.1 Exchangeable or readily-soluble B (solution plus non-specifically sorbed)**

The fraction of B that is soluble or weakly sorbed, or retained onto the soil surface by weak electrostatic interactions, is called the exchangeable or readily-soluble B fraction. Boron in all other fractions must transform into this fraction before being taken up by plants. An aqueous electrolyte solution such as dilute  $\text{CaCl}_2$  (0.01 M) (Novozamsky et al., 1989) is used to displace sorbed B from clay and other variable charged surfaces either by anion exchange or mass reaction (Hou et al., 1994). The necessary qualities of a solute include a constant electrolyte concentration, a similar ionic strength to the salt concentration in soil solution, and a relative flocculating power that will control dispersion of soil colloids (e.g. the  $\text{Ca}^{2+}$  ion which will facilitate flocculation) (Houba et al., 1996; Houba et al., 2000).

**Table 2.9 Modified sequential extraction scheme after Hou et al. (1996)**

<b>Step</b>	<b>Fraction</b>	<b>Extractant</b>	<b>Condition; extraction procedure</b>
1	Readily soluble (solution plus non-specifically adsorbed)	0.01 M CaCl <sub>2</sub>	0.50 g soil, 10 ml extractants; 16 hours shaking
2	Specifically adsorbed	0.05 M KH <sub>2</sub> PO <sub>4</sub>	Residue of step 1 shaken for 1 h with 10 ml extractant
3	Oxide bound	0.2 M acidic NH <sub>4</sub> -oxalate	Residue of step 2 shaken for 4 h with 10 ml extractant
4	Organically bound	0.02 M HNO <sub>3</sub> ; H <sub>2</sub> O <sub>2</sub> (30%)	Residue of step 3 heated for 2 h with 3, 5 ml of first two extractants; reheated with 5 ml of third extractant, shaken and centrifuged
5	Residual	Aqua regia	Residue from step 4 heated with 10 ml aqua regia, diluted and filtered

### **2.11.2 Specifically-sorbed B**

This fraction of soil can be ascribed to B specifically adsorbed to either Fe, Mn or Al hydrous oxides or to carbonate. (McBride, 1981; Jackson, 1958). A dilute solution of  $\text{KH}_2\text{PO}_4$  (0.05 M) is commonly used as an effective extractant to displace B from these exchangeable sites.  $\text{KH}_2\text{PO}_4$  has been identified as an effective extractant as phosphate reduces B adsorption over a wide range of pH (5.2-10.6) in soil (Barrow, 1989).

A substantial amount of B can be associated with carbonate sediments in soil. Jackson (1958) reported that > 90 % of total carbonate can be extracted using NaOAC along with HOAC at pH 5. Extraction of soil with these chemicals can therefore determine the content of soil B that is bound to carbonate (Tessier et al., 1979).

### **2.11.3 Iron and manganese oxides-bound B**

Boron occluded within Fe and Mn oxides is defined as the oxide-bound fraction of soil B. These oxides act as strong scavengers of trace elements including B (Arunachalam et al., 1996; Krishnamurti et al., 1995), and will coat a variety of mineral surfaces. Reagents such as Na-dithionite, Na-citrate, and Na-bicarbonate buffered at pH 7.3 are used to extract the total free iron content of soil (Mehra and Jackson, 1960). Both crystalline and amorphous oxyhydroxides can be dissolved using these reagents. Other reagents such as acidified  $\text{NH}_4$  oxalate at pH 3 has been proposed to extract amorphous Fe oxides when the extraction is conducted in the dark (Hou et al., 1996; LeRiche and Weir, 1963; Schwertmann, 1964).

### **2.11.4 Organically-bound B**

The organically-bound soil fraction describes B found in association with various organic materials including living organisms and detritus (humates, fulvates) as a result of specific adsorption (Gu and Lowe, 1990; Van Duin et al., 1985). Boron from this fraction is released by the use of strong oxidising agents;  $\text{H}_2\text{O}_2$ , NaOCl, Na or K pyrophosphate. Various functional groups such as the carboxyl, hydroxyl, and phenyl terminal units of humic substances are responsible for the B-organic complex. Established protocols have

used a combination of dilute  $\text{HNO}_3$  and 30 %  $\text{H}_2\text{O}_2$  and  $\text{NH}_4$  acetate (Tessier et al., 1979) to release B from organic matter. Ammonium acetate (3.2 M) during this process is preferentially used to prevent reabsorption or precipitation of metal ions released from organic surfaces.

### **2.11.5 Residual**

Boron held within primary (tourmaline) and secondary (colemanite) minerals is described as residual B. Residual B is generally associated with silicates (Jin et al., 1987). Soil digestion with strong acids such as HF is necessary to dissolve the silicate structure and thereby release B into solution (Chao and Sanzalone, 1989; Tessier et al., 1979; Zarcinas and Cartwright, 1987). Residual B accounts for the major portion of total soil B. Xue et al. (2001) reported the Residual-B fraction to comprise from 87.4 % to 99.7 % of the total B in 13 Chinese soils, and that the magnitude of this fraction is not generally well related to the concentration of plant-available B in soil (Jin et al., 1987; Tsadilas et al., 1994). However this B fraction may become available in the long term as the minerals which contain B are weathered. This form of B is not easily lost by leaching.

In New Zealand, limited studies such as that conducted by Olykan et al. (1995) have reported on the B fractionation in soil under *P. radiata* plantations. In Olykan's study, B was applied at a rate of 7.5 kg/ha which, relative to the control, resulted in a significant increase in soil B held by Al and Fe oxides. However, there was no report of any increase in the concentration of organic bound and residual B in the soil.

## **2.12 Boron interaction and availability influenced by other elements**

### **2.12.1 Interaction in Soil**

Boron interacts with other nutrients in soil. Boron in borate forms sorbed onto clay in preference to other anions (such as  $\text{Cl}^-$  and  $\text{NO}_3^-$ ) (Kabata-Pendias and Pendias, 2001)



where appropriate charge exists on the soil surface for this to occur. The ions  $\text{Cl}^-$ ,  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  have little influence on B adsorption to clay surfaces (Jasmund and Lindner, 1973). Wójcik (2000) reported increased B availability and uptake through the use of N fertilisers (as calcium nitrates and ammonium nitrates) on a coarse-textured B-deficient soil, and suggested that nitrates increased the soil solution B concentration by inhibiting B sorption onto Fe and Al oxides. This effect can be explained in the context of other anions (e.g.  $\text{H}_2\text{PO}_4^-$ ) which are known to reduce B adsorption on Fe and Al oxides and hence increase availability in soil solution (Goldberg et al., 1996).

A soil B-Ca interrelationship is often reported (Kabata-Pendias and Pendias, 2001). In calcareous soil Ca serves as an important sink for B adsorption (Goldberg, 1997). Evan (1987) suggested that the formation of insoluble Ca-borate (Figure 2.2; section 2.43) as a possible mechanism to explain the retention of B in Ca-rich soil. Aluminium also has an inter-relationship with B. Aluminium present in acidic soil (pH 4-4.5) as  $\text{Al}(\text{OH})_3$  shows a chemical similarity to  $\text{B}(\text{OH})_3$  inside a plant (Kochian, 1995). It is suggested that Al toxicity induces B deficiency through substitution of B for Al during plant uptake (Blevins, 1987). Boron provides protective role against excess Al and help root penetration in acidic soil having with high Al subsoil (Lenoble et al., 1996a; Lenoble et al., 1996b)

## **2.13 Importance of forestry in New Zealand**

Forestry is an important sector of the New Zealand economy. It constitutes around 2.8% of the country's GDP through the export of good quality wood and wood-related products. Around 1.1% of the global and 8.8% of the Asia-Pacific forest product trade is ascribed to New Zealand (NZFOA, 2011). Of New Zealand's total geographic area, pastures and arable land share 11.8 million hectare (44%), followed by natural forests on 6.5 million hectares (24%). Plantation forestry covers 1.8 million hectares (7% of the total land use area). Of this final figure, 70% is located in the North Island and the remaining 30% is in the South Island (MAF, 2010/2011).

*Pinus radiata* (*P. radiata*) is the main species composing 89% of plantation or exotic forest in New Zealand. The Central North Island is the main plantation site followed by Northland, Nelson and Marlborough respectively. Characteristics such as strength,

versatility, suitability for preservative treatment, good painting, gluing, machinability and excellent finishing/sanding properties provide justification for the dominance of *P. radiata* in the New Zealand forestry sector.

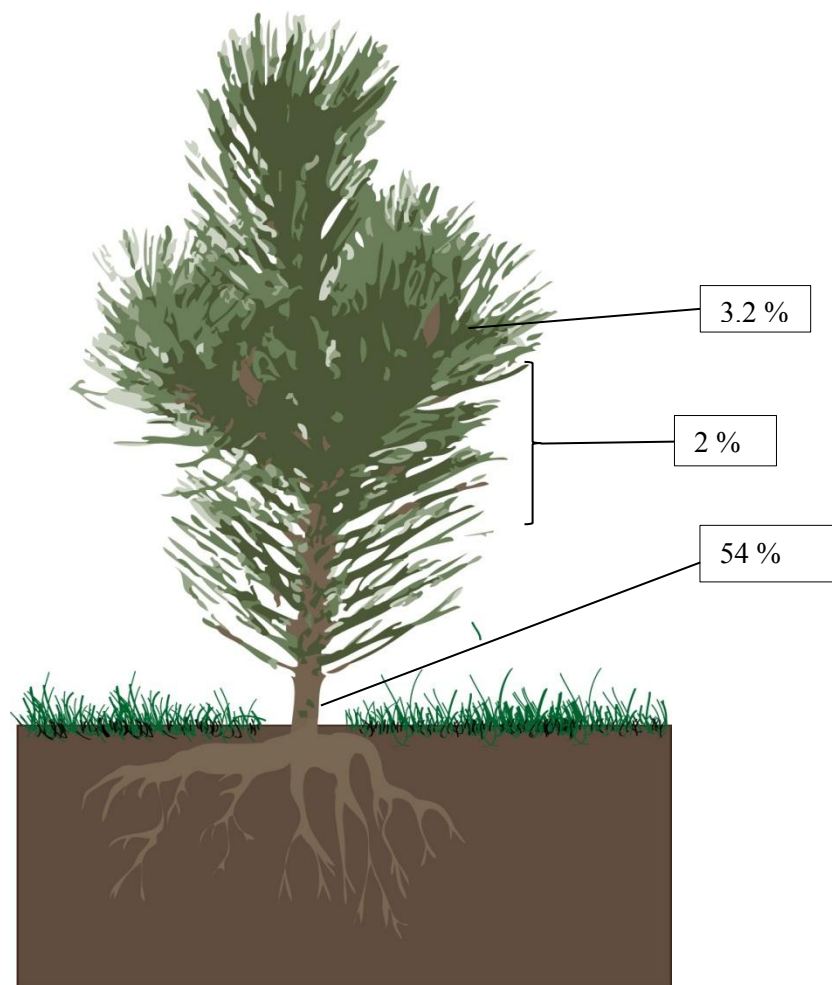
By the year 2025 plantation forest primarily composed of *P. radiata* is projected to cover 2.5 M ha area (Payn et al., 1998). This increase in forestry plantation is likely to trigger demand for nutrients. Boron deficiency symptoms reported for *P. radiata* plantation on coarse textured soils of the Central North Island and gravel soils near Nelson (Hunter et al., 1990b) emphasize the importance of B management in *P. radiata* forest plantations.

## **2.14 Boron cycling in *Pinus radiata* forest ecosystem**

The flux of B into forest ecosystems occurs by way of fertilisation, the weathering of soil parent material containing B, and through atmospheric deposition of particulate B attached to water, snow, dust or salt. Depending on climatic conditions, newly deposited B may be adsorbed by soil exchangeable colloids such as Fe and Al oxides, and thereby made unavailable to plants (Keren et al., 1985), or drain into the groundwater, river to sea. A major factor contributing to the B re-deposition to land is through sea spray (Wikner, 1983).

In a forest ecosystem where B input is low, cycling of B through the closed forest ecosystem preserves the total amount of B in cycle (Aphalo et al., 2002; Tamminen and Saarsalm, 2004). Boron is released from needle and stem litters by decomposition by microorganisms, adsorbed to organic matter, released back into soil solution and then taken up by new plant growth. Boron taken up by plants is partitioned into different components according to physiological requirements (Figure 2.4). The distribution of aged needle B concentration shows a decreasing gradient from the lower to upper part of the tree crown (4.9 to 4.3  $\mu\text{g/g}$  respectively) for an un-fertilised tree, but an increasing gradient for a fertilised tree (22.1 to 42.1  $\text{mg/kg}$ ) Hopmans (1991). On average, pine forests contain approximately 1.23 kg B/ha within the above-ground biomass for a canopy of 13-year old *P. radiata* trees. More than half (54%) is retained in stems, 21 % in foliage, 13.5 % in dead branches, 8.2% in live branches, and 3.2% in cones (Figure 2.4) (Madgwick et al., 1988). A high amount of B is removed from the forest ecosystem by

lumbering activities (Lehto et al., 2010) as the B in wood and bark (30% and 56%) is not available for decomposition and release back into the soil system.



**Figure 2.4** Boron partition in *P. radiata* after Madgwick et al. (1988)

## 2.15 Boron nutrition of forest trees

### 2.15.1 Boron functions in plants

Boron is involved in a variety of plant functions from membrane structural integrity (Cakmak and Römheld, 1997), to a wide range of physiological and biochemical process. For example:

- Borate-sugar complexes appear to play significant roles in both short-and long-term sugar transportation in higher plants (Duggar, 1983). The works of Brown and Hu (1996) and Bellaloui et al. (1999) suggest increased sorbitol-B complex formation and an increasing role of sorbitol in B uptake and transport in sorbitol-producing plant species.
- Auxin and phenol are accumulated when B is deficient in plant tissues. Lovatt (1979) suggested an involvement of B in indole acetic acid (IAA) metabolism, lignification and xylem differentiation, with increased IAA and phenol accumulation (in meristematic tissues) associated with leaf necrosis in B deficient plants (Mengal and Kirkby, 1982). Boron deficiency can inhibit the production of *cis*-diol complexes, and thereby enhance phenol biosynthesis and polyphenol oxidase activity in cells. Increased polyphenol oxidase activity will in-turn trigger the production of caffeic acid, quinone then superoxide radicles which will damage cell membranes.
- Boron is thought to regulate the intake of water into plant cells and the balance of water inside a cell (Wallace, 1961). One of the most important functions of B is to provide strength to cell wall structure and to facilitate cell wall function (O'Neill et al., 2001; O'Neill et al., 2004). The existence of B cross-linked pectin polysaccharides through borate-diol bonding of rhamnogalacturonan II (RG-II) in plant cell walls demonstrates the importance of B to plant growth (O'Neill et al., 2001). A higher B requirement in dicotyledonous plants is attributed to the presence of *cis*-diol compounds such as pectin and polygalacturonans in the cell wall (Loomis and Durst, 1992).
- Boron may affect the deposition of cell wall materials by altering membrane properties (Goldbach and Amberger, 1986). Recently research has found that B may play a role in

cross-linking glycoproteins in plant membrane and in the stabilization of membrane microdomains (Wimmer et al., 2009)

- Although B is assumed to be not required by fungi (Bohnsack and Albert, 1977), enhanced root carbohydrates, root colonization with vesicular arbuscular, ectomycorrhiza (Ramon et al., 1990) and levels of IAA in mycorrhizal roots (Mitchell et al., 1986) are all observed after B application suggesting a key role of B in the symbiotic host-microbe relationship. Boron is known to play a crucial role in N<sub>2</sub> fixation by Rhizobium and Frankia (Bolaños et al., 2004), and heterocystous Cyanobacteria (Bonilla et al., 1990).

### **2.15.2 The effect of boron on wood quality**

There have been reports to show that *P. radiata* wood quality, milling behaviour and cell wall thickness can all be linked to the B status of the tree. For viable timber production, B fertilisation of forest plantations is a necessity (Tollenaar, 1969). Möttönen et al. (2003) reported a higher carbon content of mature Norway spruce timber supplied with B fertiliser which suggests an important role of B in wood composition..

### **2.15.3 Boron uptake and plant requirements**

Brown and Shelp (1997) categorised plants into two groups; plant species with restricted<sup>3</sup> B mobility, and species with significant<sup>4</sup> B mobility. For species with restricted B mobility in the phloem, water serves as the translocation agent through xylem tissues and B uptake is proportional to concentration and water flow. For B-mobile species, B mobility is related to the presence of sorbitol, and species with a higher sorbitol and related sugar alcohol species content show higher B mobility (Brown and Hu, 1996). Stangoulis et al.

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<sup>3</sup> In plant species where B is phloem immobile the nutrient cannot be remobilized from old organs to new shoots and so is ultimately trapped at the end of the transpiration stream

<sup>4</sup> In plant species where polyols (sugar alcohols such as sorbitol and mannitol) are abundant, B as complexed with these sugars and will show a high degree of phloem mobility

(2010) shows that sucrose can also play a role in B phloem transport. Plants uptake B primarily as boric acid, a neutral and un-dissociated B molecule that is transported passively across the root membrane (in proportion to the concentration gradient) (Brown and Shelp, 1997) due to the high permeability coefficient of boric acid to the lipid bilayer (Raven, 1980). Once across the root membrane, B is transported in the xylem as an un-dissociated molecule (Greenwood, 1973) as a function of transpiration (Hu and Brown, 1997).

Boron deficiency and toxicity symptoms in both young and mature tissues (Marschner, 1995) traditionally suggested that B is not involved in translocation, and that its distribution via the xylem in plants is related to a loss of water through the transpiration stream triggered by mass flow (Shelp et al., 1995). The reported observation and theory generally sets the hypothesis of passive diffusion across the lipid bilayer under the condition of adequate B supply only. However, this hypothesis has been recently reported as being no longer true (Miwa and Fujiwara, 2010). Boron retranslocation from older or mature leaves to young growing leaves under controlled supply suggests active B diffusion in plants (Shelp, 1988). Furthermore, two transporters for B in *Arabidopsis thaliana* (BOR1 and NIP5; 1) responsible for efficient B transport across the plasma membrane indicate an active diffusion mechanism in plant under limited B supply. Takano et al.(2002) concluded that these transporters are responsible for the movement of boric acid through the xylem under conditions of B deficiency.

Recently, Dannel et al. (2000) grew sunflower (*Helianthus annuus*) under controlled conditions at 25°C both at low B (1 M) and high B (100 M) fertiliser treatment. A higher concentration of boric acid tracer (10 B) in the xylem exudates under low B concentration (1 M) suggests active transport of B against concentration gradients. This observation suggests that boric acid can be transported against the concentration gradient under certain environmental conditions. Active B transport under conditions of low B supply have also been reported in charophyte algae (Stangoulis et al., 2010).

## **2.16 Boron deficiency**

### **2.16.1 Symptoms of B deficiency**

The structural integrity of cell wall membranes, physiological function such as carbohydrate metabolism and phenol accumulation, and general metabolic activity, are all affected by B deficiency (Brown et al., 2002; Parr and Loughman, 1983). Boron deficiency is recorded as causing anatomical, physiological and biochemical changes in a range of plants (Shelp et al., 1995).

The characteristic features of B deficiency in *P. radiata* include leader tip dieback (Figures 2.5-2.7) and tree malformation (Dell and Malajczuk, 1994; Will, 1985), termination of apical growth (Hopmans and Clerehan, 1991), and emerging multi-leaders (Lambert and Ryan, 1990). All of these factors will lead to a decline in both wood quality and volume.



**Figure 2.5** Leader dieback



**Figure 2.6** Severe dieback affecting whole plant



**Figure 2.7** Shoot and tip dieback

*Source: Scion-Next generation biomaterials, Christchurch, New Zealand*



## 2.16.2 Boron fertiliser effect on tree growth

Several studies have been conducted in New Zealand and Australia to investigate the effect of different B fertiliser rates and sources on *P. radiata* growth and foliar B concentration (Table 2.10). Results have shown that the response of *P. radiata* to B fertilisation is increased foliar B concentration, height and an alleviation of B deficiency symptoms (Hopmans and Clerehan, 1991; Hopmans and Flinn, 1984). In New Zealand, Olykan et al. (2008) reported a residual effect of B fertiliser to be increased soil and foliar B concentration and tree growth, improved tree form by way of a reduction of leader dieback, and increase height growth. Saarsalmi and Tamminen (2005) reported that B application in *P. radiata* cured growth disorder and improved tree height but not tree diameter.

With the exception of a few studies such as those conducted by Olykan et al. (1995) and Olykan et al. (2008), most growth studies have used highly soluble-B fertiliser sources (borax, Na-borate) which give only short-term protection against B deficiency (Knight et al., 1983). Hunter et al. (1990b) compared the effect of B on *P. radiata* growth using both soluble and slowly-soluble B fertilisers such as Colemanite and ulexite, but at only one B application rate (6 kg/ha).

The use of highly-soluble, fast-release B fertiliser can lead to issues such as rapid uptake followed by a stabilisation period (2-3 years) (Aronsson, 1983; Knight et al., 1983), high rates of leaching (within 2 years) (Lambert and Ryan, 1990), and poor soil retention in soils having low clay contents (Ryan, 1989).

In a recent study Olykan et al. (2008) reported the importance of slow-release B fertiliser in *P. radiata* plantation soil management under the conditions of two diverse climates in New Zealand. These authors reported that the use of ulexite as a B fertiliser improves foliar B concentration over a long time (2.3-5.4 years), and suggested a B application rate of 4-8 kg/ha to be economically viable for *P. radiata*.

**Table 2. 10**                      **Trials on *P. radiata* using different sources and rates of B fertiliser**

<b>Location</b>	<b>Trial conditions</b>	<b>Results</b>	<b>References</b>
Balmoral and Taupo, NZ; field trial on juvenile trees	4 B treatments (0, 4, 8, 16, 32 Kg B/ha as ulexite) x 2 weed control practices (WC) x 8 <i>radiata</i> genotypes	Increase in foliar and soil B (hot 0.01 M CaCl <sub>2</sub> ) concentration 3.5 to 5.4 years after ulexite application. Growth response to B depended on WC and rainfall.	(Olykan et al., 2008)
Ashley Forest, North Canterbury, NZ;	0 and 7.4 kg B/ha as ulexite and N (at 0 and 400 kg/ha) as urea were applied to 4-year old <i>P. radiata</i> trees.	B alone significantly increased above-ground tree biomass, and combination with N increased the B content of current, 1-year old needles.	(Olykan et al., 1995)
Koetong, Victoria Australia; field trial on 1-year old trees	Variable B rates (0, 50, 100 and 150 kg B/ha as borax) to young <i>P. radiata</i> plants	Maximum foliar B concentration increased (5 to 40, 80 and 110 µg/ g) over first 10 months then decreased to 25 µg/g after 2 years and remained constant to 6 years.	(Hopmans and Clerehan, 1991)

<i>East coast, NZ</i> ; field trial on 2-year-old trees	Different B fertilisers (Na- Borate, Ca-borate (colemanite), Ca-borate 2-5 mm chips, Na-Ca-borate (ulexite) fine-ground and Na-Ca borate 2-5mm chips) applied @ 6 kg B/ha	8-14 mg/kg foliar B for control; 70 mg/kg (nearly toxic level) for Na-borate; and 20 mg/kg for ulexite and colemanite (Hunter et al., 1990b)
Central north Island trace elements data	Data compilation on trace elements status of <i>P. radiata</i>	Foliar B concentration in 1 year old pine of 12 mg/kg increased marginally as needles aged (1-2 years) then remained constant. As tree aged the B concentration in woody tissues, stem bark and live branches declined. (Madgwick, 1990)
Koetong, Victoria Australia	Variable B rates (0, 50, 100, 150 kg B/ha as borax) to young pine plantation	Marked increase in foliar B led to alleviation of B deficiency symptoms. B at 50 kg/ha adequate to correct B deficiency (Hopmans and Flinn, 1984)

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## 2.17 Boron and rhizosphere effect

The rhizosphere refers to the part of the soil that surrounds and is in direct contact with living plant root mass, and is influenced by root activities (Hartmann et al., 2008). Although difficult to differentiate from bulk soil, the rhizosphere extends away from plant roots over a distance from a few micrometres to several cm depending on whether a nutrient is soluble (such as  $\text{NO}_3^-$ ) or poorly soluble (such as for phosphate and the potassium ion) (Gregory, 2006), with poorly soluble nutrients being affected by the rhizosphere over only very small distances (Hübel and Beck, 1993). Several factors such as reduced pH (Dieffenbach and Matzner, 2000) and the presence of trace element (metal) complexes with root exudates distinguish the rhizosphere soil from the bulk soil. The nature and extent of the rhizosphere depends on soil chemical and physical properties (structure, water content, particle size and buffering capacity), plant properties (root morphology, mycorrhizae colonization) (Jakobsen et al., 1992), nutrient status and plant physiological status (Neumann et al., 2000).

Nutrient transport at the soil-root interface is a function of soil physical and chemical properties, microbial activity, plant requirement and the apparent mechanisms of root uptake. Mobile nutrients present at high concentration in soil solution (such as  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$ ) reach the root surface through mass flow as a result of root water uptake and transpiration. Soil accumulation of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  will occur where movement of these nutrients in soil solution exceeds the rate of plant uptake (Youssef and Chino, 1987), or where salts such as  $\text{CaSO}_4$  precipitate on the surface of roots. The concentration of many poorly-soluble macronutrients and micronutrients (such as  $\text{K}^+$ ,  $\text{H}_2\text{PO}_4^-$  and  $\text{NH}_4^+$ ) can be rapidly depleted in the rhizosphere (Lloyd and Farquhar, 1996). Plant-effected chemical and physical changes of soil properties in the rhizosphere strongly influence the uptake of these nutrients (Marschner et al., 1986).

The pH of the rhizosphere may be different by one-two units from bulk soil due to an imbalance in cation/anion uptake which leads to a net release of  $\text{H}^+$  or  $\text{OH}^-$  into the rhizosphere, as well as the excretion of organic acids and other ion exudates. Rhizosphere acidification has been reported to readily occur in response to P deficiency in plants (Hedley et al., 1982).

While studying structural changes in a rhizosphere microbial community exposed to an increased B and sodium chloride concentration, Nelson and Mele (2007) found both an

indirect and direct effect on microbial counts by root exudates and microbial toxicity respectively. Recently, Ibekwe et al. (2010) reported an interaction of salinity-B-pH on bacterial diversity in the rhizosphere of cucumber, and concluded that population changes induced by stress in the form of salinity-B-pH may be first indicator of potential B nutrient imbalance.

## **2.18 Boron role in Mycorrhizae**

Mycorrhizae are a symbiotic relationship between the majority of plant species and fungi. In this relationship the host plant sustains the fungi with soluble carbon in return for increased capacity for water retention and nutrient availability from the soil. Mycorrhizal associations are widespread covering 83 % of dicotyledonous and 79 % of monocotyledonous plant species, and 100 % of Gymnosperms (Wilcox, 1991). Based on how the fungal mycelium relates to root structure, mycorrhizae are divided into two major groups: endomycorrhizae where the fungi live within the cortical cells of the plant and grow intercellularly and ectomycorrhizae (ECM) where fungi live outside the plant root cells and grow into the soil. Ectomycorrhizae occur primarily on woody and some herbaceous plants, and are characterised by features such as an interwoven mantle of hyphae around the root surface (mantle) or by hyphae penetrating into the root intercellular space of the cortex forming a network of fungal mycelium.

The factors which influence the formation and function of mycorrhizas include soil physical properties (composition, moisture, temperature), chemical properties (pH, cation exchange capacity) and anthropogenic activities (soil compaction, and contaminants) (Entry et al., 2002). In addition to the core benefit of increased nutrient availability, the relationship is also helpful in promoting the improved functioning of the soil-plant water relationship, will enhance resistance to pathogens (Bais et al., 2006), and increase the uptake of elements with a slow rate of diffusion such as P, Cu and Zn (Lambert et al., 1980).

Under conditions of B deficiency, rates of sugar translocation to roots have been shown to decline (Venter and Currier, 1977), altering the auxine and indole acetic acid concentration in roots (Bohnsack and Albert, 1977). Studies have shown that B can increase carbohydrate exudation by roots, improving root colonization by ECM (Dixon et al., 1989). Boron nutrition has been linked to increased vesicular arbuscular mycorrhizae (VAM) in vegetable and rough

lemon seedlings (Dixon et al., 1989), forage crops; red clover and alfalfa (Lambert et al., 1980).

Conifer trees have a lower B requirement than cotyledon species (Marschner, 1986) but a positive response between root growth, ECM colonization and B fertilisation has been reported for *P. ehinata* (Mitchell et al., 1987), while root growth and ECM formation are influenced by B deficiency in Norway spruce (Lehto, 1994). A number of commercially important forest trees such as Douglas-fir (Hung and Trappe, 1987) and Norway spruce (Lehto, 1994) have been studied for the effect of B on mycorrhizae. While *P. radiata* is known to have an ECM fungi-root association (Comerford and Skinner, 1989; Skinner and Bowen, 1974), no studies that investigate the influence of B on mycorrhizas appear in the scientific literature.

## **2.19 Boron role in Photosynthesis**

Plant photosynthesis activity has been reported to suffer under conditions of both B deficiency and toxicity. Decreased photosynthesis capacity (Kastori et al., 1995), directly or indirectly induces structural and functional damage (El-Shintinawy, 1999). Lovatt et al. (1984) and Sotiropoulos et al. (2002) reported a decline in photosynthetic activity induced by B toxicity in squash and kiwifruit respectively. The effect of B on photosynthesis and gas exchange in apple (Wojcik et al., 2008), citrus (*Citrus sinensis*) (Sheng et al., 2010) and kiwifruit (*Actinidia deliciosa*) (Sotiropoulos et al., 2002) as well as Jack pine (*Pinus banksiana*) (Apostol and Zwiazek, 2004) has been studied. However, no such research appears in the literature for the effect of B on photosynthesis in *P. radiata*.

## **2.20 Boron Interaction with other nutrients in Plants**

In plants supplied with B, a declining N concentration in developing needles could be attributed to a dilution of N in the increasing biomass (Lehto and Mälkönen, 1994). Boron is associated with nitrogen metabolism in plants. Reduced nitrate reductase (NR) activity in B deficient plants results in  $\text{NO}_3^-$  accumulation (Kastori and Petrović, 1989), whereas adequate B leads to an increase in NR activity in higher plants (Shelp, 1988; Shelp, 1990).

Li et al. (1989) reported an antagonistic relationship between B and K in rape (*Brassica napus*); the B concentration decreased with an increase in K concentration, and at the maximum B concentration the K/B ratio was established as 1000:1. Increased B accumulation in radish (*Raphanus sativus*) (Tanaka, 1967), and cotton (*Gossypium spp.*) (Snyder et al., 1993) has been reported as soil P levels decrease. A moderate increase in B uptake has been reported for *P. radiata* through addition of P to the soil. In contrast, P application has been shown to reduce B availability and thereby ameliorate B toxicity in calcareous soils (Gunes, 2000). Barley plants subjected to a low level of Zn in soil but high P fertilisation have been shown to accumulate B to toxic level when the available concentration of B in soil is increased (Graham et al., 1987). Following on from this observation, Zn fertilisation has been identified as a mechanism to alleviate B toxicity by reducing plant B accumulation (Güneş et al., 1999). These results suggest a partial protective role of Zn against B toxicity (Gunes et al., 2000; Güneş et al., 1999).

Liming increases the Ca:B ratio of plant tissues, and conversely soil having a high water-soluble B concentration would be expected to effect a low Ca:B ratio in plant tissues (Adriano, 2001). Normal plant growth is expected if a balance exists between the uptake and tissue concentration of Ca and B (Kot, 2009). Both B and Ca play significant roles in cell wall structure, and accumulation of one to a greater proportion results in exclusion of the other (Turan et al., 2009). An increase in the Ca concentration of the upper leaves of a B deficient tomato plant was attributed to growth inhibition effected by B deficiency (Yamauchi et al., 1986).

In one study, increased Ca concentration in plant roots but decreased concentration in leaves has been attributed to poor Ca solubility and restricted xylem translocation under conditions of B deficiency (Artes and Ruiz, 1987). Kabata-Pendias and Pendias (2001) also reported a B-Mg synergetic interrelationship. In contrast, the B-Si couple in soil is reported to have an antagonistic interrelationship as both elements in soil solution (as anions) compete for adsorption sites on soil colloids. Lenoble et al. (1996a) reported that root inhibition attributed to Al was reduced with an increase in the B concentration in nutrient solution. In an example for another heavy-metal, low Cu uptake was observed in B deficient alfalfa (Lambert et al., 1980).

## **2.21 Boron deficiency and toxicity across the globe**

### **2.21.1 Global deficiency trends**

With the exception of some regions such as the dry lands of South Australia (Cartwright et al., 1986), the West coast of Malaysia (Shorrocks, 1964), the Andes foothills in northern Chile (Carlson and Adriano, 1993) and the Searle Lake area of California (Chesworth, 1991), soil concentrations of B are deficient for plant growth in many forestry and agricultural areas of the world. Soils having a plant-available B concentration of less than 0.5 mg/kg (using hot  $\text{CaCl}_2$  extraction) are regarded as deficient, and apparent deficiency occurs for all major geographical areas and for particular soil groups (Table 2.11). Liu et al. (1980) reported that soil in large areas of south and east China, south of the Yangtze River are B deficient (hot water soluble B < 0.25 mg/kg). Clay rich Acrisols south of the Yangtze, and soil developed from loess and calcareous alluvium of the Yellow River are particularly B deficient. Acrisols in Assam, northern Karnataka, and Luvisols in Tamil Nadu and Kerala are B deficient soils in India. Acrisols formed mainly on acid igneous rock in African countries are also B deficient. In most Scandinavian countries (Finland, Sweden, and Denmark) and Northern Europe, soil developed from acid igneous and metamorphic rocks is B deficient. In South America parts of Brazil and Chile are B deficient. In the USA, States along the Atlantic Coast and Gulf of Mexico, the Pacific Northwest and the Great Lake region are B deficient (Shorrocks, 1997).

It is evident from Table 2.11 that certain soil group; Acrisols, Podzols, Andosols, Arenosols and Lithosols, Luvisols are the predominantly B deficient soils. According to FAO/UNESCO statistics more than 100 million ha of Acrisols are spread over different geographical areas such as east and south Asia, central Africa, Australia and the Pacific Islands (FAO/UNESCO, 1978). The main features of such soils are high weathering (Gupta, 1979a), and coarse texture with low base exchange capacity. As these soils have mostly developed under humid conditions, mobile elements like B have been generally leached out of the root zone (Harmsen and Vlek, 1985).

Podzols primarily sustain extensive coniferous forest plantations. Such soils present in the Scandinavian countries, Australia, and northeast Canada are B deficient. Bingham et al. (1971) reported strong adsorption of B by allophane in soil derived from volcanic ash, and associated B deficiency due to such adsorption in Chile, Colombia, Ecuador, Japan, New Zealand and



USA. Excessive leaching and illuviation of clay in Arensols and Luvisols has resulted in B deficiency in these soils around the globe (Shorrocks, 1997).

## **2.21.2 Global toxicity trends**

Boron toxicity, like B deficiency, is a global phenomenon reported across the world at locations such as South Australia (Cartwright et al., 1986), parts of Victoria Australia (Hobson et al., 2006), Western Australia (Brennan and Adcock, 2004), the region of Sicily in Italy (Moody et al., 1988), and Western Canada (Apostol and Zwiazek, 2004; Nable et al., 1997). Boron toxicity is associated with low yields in many forests of Australia, North Africa, and West Asia that have alkaline and saline soils, low rainfall and therefore low rates of nutrient leaching from soil (Camacho-cristóbal et al., 2008).

Total soil and plant-available B is generally high in arid and semi-arid regions where leaching is very limited. Factors such as high B in parent material, irrigation with high B concentration water, and over fertilisation with mineral fertilisers including B are held responsible for developing B toxicity (Gupta et al., 1985)

### **2.21.2.1 Boron toxicity symptoms**

A high plant tissue B concentration is likely to trigger a range of physiological and growth problems for a plant. Boron toxicity leads to characteristic symptoms such as a reduction in leaf chlorophyll, reduced CO<sub>2</sub> fixation, termination of cell wall expansion and a reduction in leaf surface area (Loomis and Durst, 1992; Lovatt and Bates, 1984). As B moves along the transpiration stream it accumulates in the margins and tips of older leaves, and produces the classical conifer B toxicity symptoms of chlorosis and

**Table 2.3** Reported soil boron deficiency across the main FAO/UNESCO soil groups of the world after Shorrocks (1997)

Continent	Region	Soil group
Asia	China, South and North of Yangtze, NE China	Acrisols (Ultisols)
		Lithosols/Cambisols(Lithic Inceptisols)
		Lithosols/Luvisol (Lithic Fluvents)
		Lithosols/Cambisols(Lithic Inceptisols)
	Korea	Acrisols (Ultisols)
	Thailand, NW	Cambisols (Inceptisols)
	India NE	Acrisols (Ultisols)
		Luvisols (Alfisols)
		Arenosols (Psamments)
	West Africa	Nigeria, Benin
Ivory Coast, Chad		Acrisols (Ultisols)
		Luvisols (Alfisols)
	Ferralsol (Oxisols)	
Central Africa	Zambia, Zimbabwe, Malawi	Luvisols (Alfisols)
		Ferralsol (Oxisols)

Scandinavia and North Europe	Denmark, Germany, Norway,	Podzol (Spodosols)
	Sweden, Finland, USSR	Luvisols (Alfisols)
North America	USA, Atlantic, SE coastal plains	Acrisols (Ultisols)
		Podzol (Spodosols)
	USA, Pacific NE	Luvisols (Alfisols)
	USA Great Lake	Podosols(Spodosols)
		Luvisols (Alfisols)
	Canada, Atlantic	Podosols(Spodosols)
	Canada, NE	Podosols(Spodosols)
South America	Brazil	Ferralsol (Oxisols)
		Acrisols (Ultisols)
	Chile	Andosols (Andepts)

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necrosis at needle tips and older leaves (Bennett, 1993; Eaton, 1944). Boron under excess soil supply is therefore passively distributed within the plants (as described in section 2.15.3).

Gupta (1993b) established a critical tissue value for B toxicity for many plants (Table 2.12). However, due to the steep concentration gradient that is observed within leaf tissues (Nable et al., 1990), and the associated uneven distribution of B in leaf material that can be expected (Sotiropoulos et al., 2002), setting general critical values at which B in plants becomes toxic is a problematic issue. For example in wheat and barely a wide range (10-130 mg B/kg) makes it hard to set a critical toxicity level (Nable et al., 1997). Gupta et al. (1985) reported

that B toxicity will be apparent at a leaf B concentration exceeding 200 mg/kg although sensitive crops may show toxicity at a B concentration much lower than 200 mg/kg. Nable et al. (1997) reported that in species where leaf biomass acts as a sink for B, plants can accumulate as much as 250 mg B/kg (dry weight). Some plants have a high physiological requirement for B. For example, celery has a sufficient range of 68-432 mg/kg (Table 2.12). Under conditions where extreme soil toxicity may be apparent, the leaf B concentration may exceed up to 700-1000 mg/kg. At such a high concentration, plant growth will be significantly retarded. Mehmet et al. (2004) reported that some plant species such as *Gypsophila sphaerocephala* can retain a considerable amount of B in above ground parts ( $2093 \pm 199$  mg/kg) compared to a low concentration ( $51 \pm 11$  mg/kg) in roots. Such plants have been classified as B hyperaccumulators.

**Table 2. 4 Deficient, sufficient, and toxic levels of B in a variety of plants after Gupta (1993b)**

		Dry matter B (mg/kg)		
Plant	Plant part	Deficient	Sufficient	Toxic
Carrot	Mature leaf lamina	< 16	32-103	175-307
Celery	Leaflets	20	68-432	720
Tomato	Mature leaf	< 10	30-75	> 200
Potato	Fully developed leaf	< 15	21-50	> 50
Bean	43-days old plants	-	12	>160
Cauliflower	Whole plant	3	12-23	-
Cucumber	Mature leaves	< 20	40-120	> 300
Wheat	Boot stage	2.1-5.0	8	> 16
	Straw	4.6-6.0	17	> 34
Oats	Boot stage	1.1-3.5	6-15	> 35
	Straw	3.5-5.6	14-24	> 50
Barley	Boot stage	1.9-3.5	10	> 20
	Straw	7.1-8.6	21	> 46
<b>Conifers</b>				
<i>Pinus caribaea</i>	Needle	< 4	5-18	
<i>Pinus ponderosa</i>	Needle		20-31	
<i>Pinus radiata</i> nursery	Needle			
6 yrs.		8		
2 yrs.		10-12		
				101
<i>Pinus radiata</i>	Needle	4-5	10-36	
18-20 yrs.				
<i>Pinus sylvestris</i>	Needle		32	480
20 yrs.				

## 2.22 Structure of thesis

The results from three experiments are presented in this thesis in six chapters sequenced to address the objectives of the work. The structure of thesis is thematically shown as Table 2.13. The introductory chapter (Chapter 1) is followed by a review of literature (Chapter 2) which has provided detailed information on the edaphic and environmental factors that affect B dynamics and its availability to plant, and a definition of the problem of B deficiency around the world with specific reference to *P. radiata* plantations in New Zealand.

**Table 2.5 Chapter structure of thesis**

Chapter theme	Contents
1. Introduction	Background information, overview and objectives of the research
2. Review Literature	Review of earlier research findings, and identification of gaps in present understandings pertaining to plant, soil and microorganism relationships with B
3. Response of <i>Pinus radiata</i> to slow release boron fertiliser	Plant growth, dry weight and net photosynthesis, soil B concentration and soil microbiological indicators discussed
4. Comparative response of two clones of <i>P. radiata</i> to boron fertiliser	Growth response of two diverse <i>P. radiata</i> clone were evaluated in response to different levels of B fertiliser. Plant B uptake, soil B concentration, mycorrhizal colonization and dehydrogenase activities discussed
5. Boron adsorption in soils	Seven benchmark soils were selected and a B adsorption study was carried out using both Langmuir and Freundlich isotherms. The response of B adsorption to varying pH in the equilibrium solution is discussed
6. Overall conclusions	Key results summarised and discussed along with the need for possible follow-up research work

Chapter 2 highlights the importance of *P. radiata* to the New Zealand economy and the relevance of adequate knowledge of B nutrition to this tree species. Currently, B nutrient management involves the use of fast-release and highly-soluble B fertilisers which impart a short-term solution to B deficiency issue in *P. radiata* plantation. Only limited work has been undertaken to address B deficiency problems in the long term with slowly soluble and slow-release B fertilisers.

Chapter 3 provides information on the use of different levels of B supplied using the slow release fertiliser ulexite on *P. radiata* growth under glass house conditions. The influence of applied B was evaluated on soil concentration, plant B status, plant B uptake and distribution, root needle B relations, and plant growth and physiological parameters such as photosynthesis and stomata conductance. However, B affects below-ground as well as above-ground biological activity. The review of literature in Chapter 2 revealed that scant information is available on soil-microbe B interactions. To address this knowledge gap further research was performed in Chapter 3, to assess the response of soil microbial and microbiological properties to B using the two indices of soil dehydrogenase activity and ectomycorrhizae density.

Boron in soil exists in different forms ranging from readily-soluble B to that associated with highly unavailable soil fractions. Review of the literature in Chapter 2 showed how the relative distribution of these fractions affects the plant available (readily soluble) form of B in soil. Chapter 3 and Chapter 4 both describe the fractionation of B in a Taupo soil after *P. radiata* harvest. Chapter 4 describes a second glasshouse study investigating the relative growth of two *P. radiata* clones of diverse growth habits exposed to different levels of B following an experimental design comparable to that used in Chapter 3. Plant B uptake, B distribution in plant, photosynthesis, photosynthesis light response curve, soil B concentration and microbiological parameters were investigated in Chapter 4.

Soil B adsorption is considered an important mechanism influencing plant B availability to plants and is influenced by soil properties such as pH, texture and organic matter. Chapter 5 describes a laboratory study performed to quantify B adsorption on seven benchmark soils selected from the North Island. Langmuir and Freundlich adsorption isotherms were used to model B adsorption. A second laboratory study was also performed which involved B testing the adsorption rate of B to six soils, at varying levels of pH in the equilibrium solution.

Chapter 6 discusses the overall conclusions arising from this doctorate research work. In Chapter 6 the findings of the research are summarised and areas for future research are identified. The objective of the chapter was to derive a safe dose of B fertiliser that satisfies both plant nutrition and the environmental requirements of soil microbiology.

## 2.22 Research Scope

*Pinus radiata* is an important exotic forest species, covering around 89 % of the New Zealand forest. The export of *Pinus radiata* wood and other related products fetch handsome amount of money (\$3.1 billion) to New Zealand GDP and source of employment. However, the problem of B deficiency is a serious issue in such plantation. Edaphic factors like coarse textured pumaceous soil coupled with seasonal conditions such as dry summer and drought further aggravate the situation. As a result of B deficiency, *P. radiata* suffers a lot, resulting in growth defects such as multi leadership, shoot dieback hence declining forestry export potential of New Zealand forestry sector.

The detail commentary of literature in this chapter established the importance of B fertiliser in *Pinus radiata* plantation. It has been found that use of B fertiliser helps ameliorate soil B deficiency and keep plant B deficiency away along with improving plant growth and physiology. However, application of B fertiliser in radiata plantation mostly confined to 'slow release' fertilisers such as Na-borate, and borax. As the chemistry of such fertilisers give short term benefits and plant experiences B deficiency again after couple of years. Hence undermine the used of fast release B fertiliser as an ineffective option.

Keeping in mind the soil and climatic conditions that govern the B availability to plant, slow release fertiliser such as ulexite has been recommended so far, very limited works have been done on slow release B fertiliser and mainly focus on above ground plant B uptake. Use of slow release B fertilisers has potential to sustain adequate B nutrition for the entire rotation of *P. radiata*.

This study was undertaken with main objectives to look at B dynamics and availability in plant, soil and microbes interface. For this purpose ranges of ulexite were applied.

For this purpose two glasshouse trials along with one laboratory studies on B adsorption studies were carried out. In the first glasshouse trial *P. radiata* clone were given with different rates of ulexite to assess their effect on plant B uptake, soil B concentration, soil



dehydrogenase activities and mycorrhizae. Ulexite application rates were revisited and refined based on the findings of first glasshouse trial with inclusion of two *P. radiata* clones with diverse growing behaviours. In the second glasshouse trial plant and soil B concentration along with dehydrogenase activities both in rhizosphere and bulk soils and mycorrhizae were studied.

In B adsorption studies seven soils collected from North Island of New Zealand, to assess for adsorption capacity and suggest need for slow release B fertiliser use on *P. radiata* grown on such soils. In the second part of the adsorption studies, B adsorption on selected soils was evaluated under the dynamics of solution pH. The study reflects on the importance of liming practice in radiate plantation grown on these soils. As literature review in chapter 2 discussed the detail reason of liming induced B deficiency, the results from the study would of significant importance in areas where farmers and tree growers practicing liming acid soil and renders B unavailable for plant.

Overall, the research studies reported in this thesis provided detail background information about the importance of *P. radiata* and the problems of B deficiency found in association with such plantation. The thesis discussed the issue of use of fast release B fertiliser and prospects of slow release fertiliser with emphasis on plant soil and soil microbes. It was found that slow release B fertiliser can significantly deals with problem of B deficiency in long run.

## Chapter 3

# Response of *Pinus radiata* to slow release boron fertiliser

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This chapter has been published in the following refereed journal and conference papers:

1. **Khan, R., Anderson, C.W.N., Loganathan, P., Xue, J and Clinton, P.E** 2012 Response of *Pinus radiata* D. Don to boron fertilisation in a glasshouse study. Communication in Soil and Plant Analysis 43(10), 1412-1426
2. **Raza U. Khan., Christopher W.N. Anderson., Paripurnanda Loganathan., Jianming Xue., and Peter Clinton** Response of *Pinus radiata* (D. Don) to boron fertilisation under greenhouse conditions 2010. 19<sup>th</sup> World Congress of Soil Science, Soil Solutions for a Changing World, 1-6 August 2010, Brisbane, Australia.

### 3.1 Introduction

The prevalence of B deficiency as the most dominant micronutrient problem for the New Zealand plantation forestry sector was clearly defined in Chapter 2. Coarse-textured pumice soils of the Central North Island and the Moutere gravels near Nelson are extensively utilised for *P. radiata* forestry, but these are also soils with a proven B deficiency.

In *P. radiata*, B deficiency triggers severe growth defects such as the death of terminal buds, multi-leader emergence, sub-optimal tree form and shape and reduces shoot height (Hunter et al., 1990a; Will, 1985). The consequent bushy appearance and multiple-leaders of a deficient tree reduce both wood volume and quality (Hopmans and Clerehan, 1991; Olykan et al., 1995).

Several studies have investigated the response of *P. radiata* to B fertiliser application. However, in most of these trials, only short-term relief against deficiency symptoms is afforded due to the use of highly soluble and fast-release B fertilisers (Aronsson, 1983; Hunter et al., 1990a; Knight et al., 1983). It has proven difficult to achieve an optimum long-term foliar B concentration through a single application of fast-release B fertiliser (Hopmans and Clerehan, 1991). A rapid decline of foliar B concentration following an initial increase in uptake (Aronsson, 1983), a general low level of retention of B by soils (Ryan, 1989; Wikner, 1983) and passive diffusion by roots (Raven, 1980) are factors that emphasise the need for slow-release B fertilisers. Therefore, slow release B fertilisers have been recommended for sustaining adequate B nutrition in *P. radiata* for an entire rotation (Hunter et al., 1990b). Olykan et al. (2008) reported that 44 % and 24 % of residual B was held in the top 0-20 cm soil depth 4 years after application of ulexite, a slow-release B fertiliser, at the Balmoral Forest in Canterbury, New Zealand at rates of 8 kg B/ha and 32 kg B/ha respectively. The long residual effect of slow-release B fertiliser is attributed to the slow release of B in the soil due to the low solubility of the fertiliser (Hunter et al., 1990b). Although slow-release B fertilisers have been generally prescribed by many New Zealand forest companies to correct B deficiency in *P. radiata* plantations, little information is available on the effect of different rates of slow-release B fertiliser on both above-ground and below-ground growth and physiological aspects of this species, and on soil microbiological activities. These parameters were investigated in the current chapter, by way of a glasshouse trial where *P. radiata* was grown on a Central North Island plantation soil, amended with five rates of ulexite fertiliser.

## **3.2 Materials and Methods**

### **3.2.1 Experiment design and plant growth conditions**

A pot experiment was conducted under glasshouse conditions at the Plant Growth Unit of Massey University in Palmerston North by applying five levels of B to 9-month old Monterey pine (*P. radiata*, D. Don) cuttings grown in 6 L plastic containers with internal dimensions of 26.5 cm diameter × 21 cm height. Five B levels (i.e. treatments) were calculated from the field application rates of 0, 4, 8, 16 and 32 kg B/ha based on the pot surface area, and applied to the pots at the rates 22, 44, 89 and 178 mg B/kg soil respectively. The B fertiliser used was

ulexite ( $B_5CaH_{16}NaO_{17}$ ), a forest-grade slow-release granular B-fertiliser obtained from Scion, Rotorua (Table 3.1). Each B treatment was replicated five times and there were 25 pots in total.

**Table 3.1 Chemical composition of ulexite**

Empirical formula	$B_5CaH_{16}\cdot NaO_{17}$
ICSD*	Sodium calcium borate hydroxide hydrate
$B_2O_3$ (%)	31.5
$Na_2O$ (%)	7.65
$CaO$ (%)	13.83
$H_2O$ (%)	35.55

\*Inorganic Crystal Structure database

Ulexite (2-3 mm grain size) was applied separately to two soil levels (depths) in each pot (B is applied at two split depths primarily to avoid toxicity as it could accumulate somewhere in root zone due to small size of granules). Half of the calculated fertiliser dose was evenly placed on the soil surface once the pots were filled to the 3L mark. Additional soil was then added to the 4.5 L mark before the remaining fertiliser was spread over the soil surface. The soil weight per pot was equivalent to 5 kg of collected soil.

The soil used in this experiment was collected from Taupo forest in the Central North Island of New Zealand (38°55'S, 175°55'E). The soil is a Waipahihi sand derived from water-sorted tephra and classified as an Orthic Pumice Soil (Hewitt, 1998) under the New Zealand soil classification system, and a Vitrand in the US soil taxonomic classification. The field soil was passed through a stainless steel sieve with 2-mm openings before potting. Climatic and selected soil properties relevant for the field location are summarised in Table 3.2.

**Table 3. 2          Climatic conditions and selected properties of Taupo soil used in this study**

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Mean annual rainfall (mm)	1616
Mean annual temperature (°C)	11.9
Mean annual sunshine hours	1965
Soil type	Waipahihi sand
NZ classification	immature
(Hewitt, 98)	Orthic Pumice soil
Soil texture	
Sand (%)	34
Silt (%)	45.5
Clay (%)	20.5
Total N (g /100 g)	0.20
Total C (g /100 g)	3.4
Total C/N	17
Bray P (mg/ kg)	20
Hot 0.01 CaCl <sub>2</sub> ext. B (µg/g)	0.30
Pseudo total-B (mg/kg)	27
CEC (meq/100 g soil)	21.5

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Note: total C and N determined using a LECO furnace., P according to the method of Bray and Kurtz (1945), B by 0.01 CaCl<sub>2</sub> extraction, and Pseudo total-B by aqua regia digestion followed by ICP-MS analysis

Nine-month old uniform cuttings of a commercial radiata pine clone were obtained from Forest Genetics CellFor Ltd, Rotorua. Each pot was planted with one cutting in mid July 2008 and all pots were arranged in a randomized complete block (RCB) design. Pots were irrigated regularly to maintain soil moisture at 80 % of field capacity. The glasshouse temperature was partially controlled, and varied at 20-29 °C (day) and 10-17 °C (night) during the experiment period. Temperatures were lowest during the winter months at the start of trial and highest during the summer months. The experiment was run for 210 days and the plants were harvested in February 2009.

### 3.3 Measurement and chemical analysis

#### 3.3.1 Needle net photosynthesis rate, plant growth and dry weight

Net photosynthesis rate was measured three days before harvesting of trees in each pot using a CIRAS-2 portable photosynthesis system (PP Systems, Hitchin, UK) equipped with a standard 2.5 cm-diameter cuvette and a halogen light unit mounted above the cuvette. During the measurement, the relative humidity, temperature and light intensity inside the cuvette were maintained at 80%, 20 °C and 1600  $\mu\text{mole/m}^2/\text{s}$  PPFD (Photosynthetic Photon Flux Density) respectively. Two youngest mature fascicles (i.e. 6 needles) were selected from the upper crown portion of each plant for measurement of net photosynthesis at controlled  $\text{CO}_2$  (375 ppm) between 9 am and 12 midday. Needle surface leaf area (LA) was calculated as below (Equation 3.1)

$$\text{LA} = \text{D} (\pi+n) \times (\text{L} \times \text{fn}) \quad \text{Equation (3.1)}$$

Where D, n, L and fn stand for fascicle diameter, number of needles per fascicle, length of cuvette and number of fascicles, respectively.

The tree height and ground line diameter were measured just before harvesting. All plants were harvested at day 210 by collecting needle, stem and root samples separately for each replicate pot. Needle and stem samples were harvested following the coding system presented in Table 3.3. Roots from each pot were carefully collected and washed after collecting rhizosphere soil samples. The fresh weights of needle stem and root samples were recorded before samples were rinsed in deionised water and then dried at 65 °C until constant weight. Needle and root samples were ground using a cyclotech herbage mill. Stem samples were first coarsely ground using a knife mill then finely ground using a cyclotech herbage mill. The ground samples were used for plant B analysis.

**Table 3.3 Description of codes used for needle and stem analysis**

<b>Code</b>	<b>Description</b>
NI	The initial (original) needles associated with <i>P. radiata</i> seedlings at the time of planting
NCM	Current-year mature needle (needles which emerged after planting and which matured prior to harvest)
NCI	Current-year immature needle (needles which were immature at time of harvest)
SI	The initial (original) stem and branches of the seedlings at the time of planting
SC	Current-year stem and branches which developed during the experiment

### **3.3.2 Soil sampling and preparation**

Soil samples (bulk and rhizosphere) were collected from each pot for measurement of soil dehydrogenase activity (fresh samples), and hot CaCl<sub>2</sub>-extractable soil B concentration (dry samples). To separate rhizosphere soil samples as described in the following section from bulk soil, the stem of each plant was carefully removed from the pot to ensure minimal disturbance of the root system, then shaken in a paper bag. The soils in the paper bags were collected as rhizosphere soils, which were stored in a refrigerator (4 °C) until the analysis of dehydrogenase activities. The bulk soil samples were air-dried, ground and sieved (2 mm) for the analysis of the CaCl<sub>2</sub>-extractable soil B.

### **3.3.3 Analysis of soil boron**

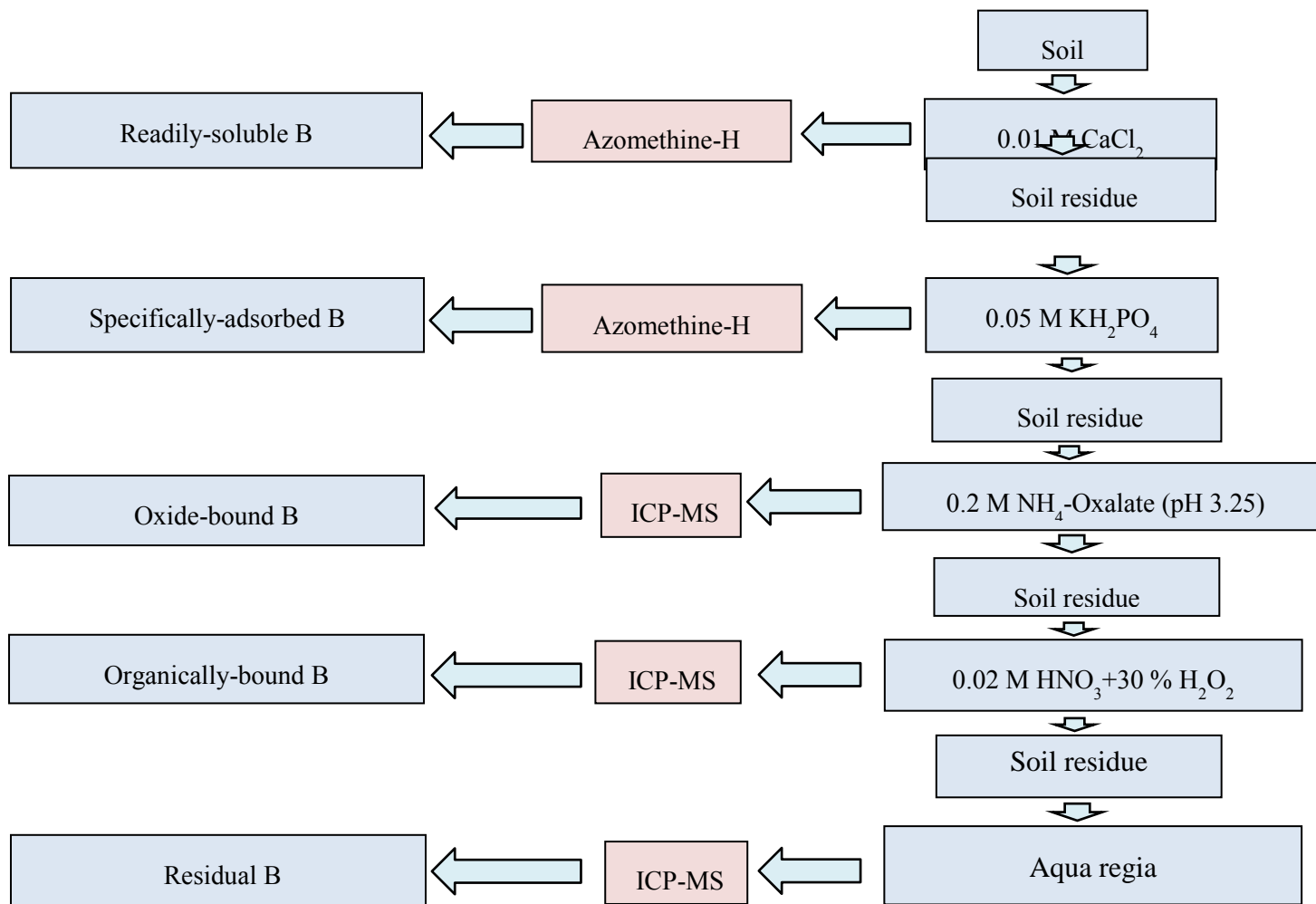
Sub samples of ground soil were extracted with hot  $\text{CaCl}_2$  to determine plant-available soil B according to the modified method of Parker and Gardner (1981). Ten g of soil was placed into a 100 ml conical flask fitted with an air refluxing tube along with 20 ml of 0.02 M  $\text{CaCl}_2$  and 0.2 g charcoal black, boiled on a hot plate for 5 minutes then filtered through Whatman-42 paper into a plastic tube. A calibration curve was constructed using standard solutions of varying concentration prepared from 1000  $\mu\text{g/g}$  CertiPur boric acid stock solution. The concentration of B in the extracts was determined according to Azomethine-H method reported for plant B analysis by mixing a 4 ml aliquot with buffer masking solution (4 mL) before Azomethine-H reagent (1 mL) was added. The preparation was stirred on a vortex mixer then left to settle for 1 h to allow for the development of colour. The intensity of the developed colour was measured using a spectrophotometer at 420 nm.

Pseudo total-B (Table 3.2) was determined by ICP-MS after digestion of 1 g soil in 10 ml aqua regia at 150 °C. For quality control two certified reference soil materials, CRM 051-050 USA (B  $11.8 \pm 6.19$  mg/kg) and NCS DC 73321 China (B  $23 \pm 3$  mg/kg) were run in parallel with the test samples as standard reference soil samples for quality control. The mean values were found to be 8 mg/kg and  $20 \pm 1$  mg/kg for CRM 051-050 USA and NCS DC 73321 China within the range of 68 % and 92 % of the certified total values respectively, but within the range of certified concentration.

### **3.3.4 Boron fractionation using a sequential extraction procedure**

Boron fractionation studies were carried out using a modified sequential extraction scheme based on that of Hou et al. (1996), Jin et al. (1987) and Tessier et al. (1979) as used by Datta et al. (2002). The details of this modified scheme are presented as reported in Figure 3.1. Every extraction was conducted with triplicate samples.





**Figure 3.1** Schematic chart of the modified sequential extraction scheme adopted for this study after Datta al. (2002)

#### **3.3.4.1 Readily soluble B**

A sub sample of homogenised soil (1 g) was accurately weighed into a 50 ml polyethylene centrifuge tube.  $\text{CaCl}_2$  (20 mL of 0.01 M) was added and the tubes were rotated in an end-over-end shaker at 25 °C for 16 h, centrifuged at 15000 rpm for 30 min (Hou et al., 1994) and then passed through a 0.45  $\mu\text{m}$  Millipore filter. The extracts were analysed for readily soluble B using the modified Azomethine-H method (Parker and Gardner, 1981).

#### **3.3.4.2 Specifically adsorbed B**

$\text{KH}_2\text{PO}_4$  (20 ml of 0.05 M) was added to the soil residue from the first step and the resulting suspension shaken for 1 h on an end-over-end shaker, centrifuged at 15,000 rpm for 30 minutes, and then passed through a 0.45  $\mu\text{m}$  Millipore filter. The extracts were analysed for specifically adsorbed-B using the modified Azomethine-H method (Parker and Gardner, 1981)

#### **3.3.4.3 Oxide bound B**

The soil residue from step 2 was re-suspended in 20 ml of 0.2 M acidic  $\text{NH}_4$ -oxalate (pH 3.25), shaken for 4 h on an end-over-end shaker, centrifuged at 15,000 rpm for 30 min. and then passed through a 0.45  $\mu\text{m}$  Millipore filter. Boron in solution (oxide bound) was determined using inductively coupled plasma mass spectroscopy (ICP-MS), as the yellowish to reddish brown colour of the extracts (Fe and organic matter in solution) interfered with spectrophotometer reading using the Azomethine-H method.

#### **3.3.4.4 Organically bound B**

To extract organically-bound B, the soil residue from step 3 was subjected to a mixture of reagents that could oxidise the soil organic matter, thereby releasing organically-bound B into solution. Three ml of 0.02 M  $\text{HNO}_3$  and 5 ml of 30 %  $\text{H}_2\text{O}_2$  (pH 2) was first added to the residue. The suspension was heated at 85 °C for 2 h with occasional agitation, before a further 3 ml of 30 %  $\text{H}_2\text{O}_2$  was added. The suspension was heated again at 85 °C with occasional agitation before 5 mL of 3.2 M ammonium acetate (made up in 20 %  $\text{HNO}_3$ ) was added. The extract was made to 20 ml with deionised water and then shaken for half an hour, centrifuged

at 15,000 rpm for 30 min. and passed through a 0.45  $\mu\text{m}$  Millipore filter. Boron in solution (the organically bound fraction) was determined using ICP-MS.

#### **3.3.4.5 Residual B**

The soil residue from step 4 was transferred into a 100 ml Teflon tube containing 20 ml of aqua regia and heated to 150 °C on glycerol bath for 15 minutes. The extract was diluted to 20 ml and analysed for residual B by ICP-MS.

#### **3.3.5 Soil dehydrogenase activity**

Soil dehydrogenase activity (DHA), an indicator for the metabolic activity of microorganisms in the soil, was colorimetrically measured after incubation of soil sub-samples with 2, 3, 5-triphenyltetrazolium chloride (TTC). As a result of microbial DHA in soil, water-soluble colourless TTC is reduced to water insoluble 2, 3, 5- triphenyletetrazolium formazan (TFP) that has a red colour. In this study the method of Chander and Brookes (1991) was used, where 3 mL of 3 % TTC and 0.1 g  $\text{CaCO}_3$  were added to 5 g of fresh soil and incubated for 24 h at 28 °C. The red colour TFP formed by the end of the reaction was extracted with methanol and measured at an absorbance of 485 nm on a spectrophotometer. The colour and thus intensity of absorbance is proportionate to the microbiological activity in the soil.

#### **3.3.6 Mycorrhizae Scoring**

Plant roots were washed with tap water and subsamples were selected randomly for estimation of ectomycorrhizae colonization (EMC). Roots were fixed in FAA (Formaldehyde, acetic acid solution) (70 % ethanol, formaldehyde, and acetic acid in 90:5:5 ratio by volume), and washed three times with distilled water before staining. Root segments were scored for mycorrhizae using the method of Giovannetti and Mosse (1980), this method has been used for studying vasicular arbuscular and ectomycorrhiza infection (Brundrett and Abbott, 1994). Root samples were dipped into 10 % KOH in McCartney containers. It was noticed that KOH solution turns yellow showing the release of tannins from roots into KOH

solution. The container was then autoclaved for 30 min., washed three times in distilled water followed by a second autoclave in 10 % KOH for 15 min. Samples were then acidified (5 % HCl for 1 min.) to facilitate retention of the stain, washed three times with distilled water, and stained overnight in a solution of trypan blue made up with 0.2 g of trypan blue in 1 L glycerol, 950 ml water and 50 ml acetic acid. Fine stained root segments (n=10) of equal length (1 cm) were cut using scissors from each root system and mounted on a microscope parallel to each other. The presence (+) or absence (-) of extra-mycelium hyphae was recognised by a small thread emerging from the main root segment and counted as the slide was moved. Counting was performed using a Nikon polarizing microscope at 400 × magnification.

### **3.3.7 Plant Boron**

The B concentration was determined in ground samples of plant organs (needle, shoot and root), following the standard method of dry ashing and colorimetric analysis by Azomethine-H (Gaines and Mitchell, 1979; Wolf, 1974). A subsample of biomass (0.5 g) was dry ashed in a porcelain crucible using a muffle furnace at 600 °C for 1 h. The ashed sample was allowed to cool then wetted by adding 5 drops of deionised water and 10 ml of 0.36 N H<sub>2</sub>SO<sub>4</sub>. The reaction mixture was allowed to sit, with occasional stirring, for 1 h at room temperature before filtering.

For quality control purpose samples of grass (B = 9.73 mg/kg) and wheat straw (B = 3.07 mg/kg), obtained from the international plant analytical exchange program (IPAEP) of Wageningen University, The Netherlands, were run as reference plant materials in parallel with the unknown samples for quality control. The mean B concentrations found for the reference plant materials were  $9.7 \pm 1$  mg /kg for the grass sample and  $3.4 \pm 0.01$  mg/kg for wheat straw, values which agree to within 99.7 % and 90.2 % of the reported mean values for these plant reference materials respectively.

### **3.4 Data analysis**

Statistical tests were performed using SAS (SAS 9.1.2, 2004, SAS Institute, Cary, NC). The General Linear Model function (GLM) was used for analyses of all variables (plant height, diameter, dry weight, plant B, soil B and dehydrogenase activity) to test the B treatment effect. Least significant difference (LSD) tests were used to distinguish among means ( $P \leq 0.05$ ). Polynomial regression was performed between hot 0.02 M CaCl<sub>2</sub> extractable-B and needle B concentration using Microsoft Excel.

### **3.5 Results and Discussion**

#### **3.5.1 Plant growth, dry weight, and net photosynthesis rate**

Plant height responded positively to B fertiliser up to a treatment of 8 kg/ha, and then decreased slightly at higher B rates. Plant height was significantly greater at 8 kg/ha than for the control (Table 3.4). All rates of B application significantly increased stem diameter when compared to the control. However, the maximum stem diameter was obtained at a B application of 4 kg/ha. Diameter at the highest B rate of 32 kg/ha was significantly lower than that at the optimal rate of 4 kg/ha, but still greater than the diameter for the control treatment. This indicates that stem diameter was more responsive and sensitive to B application rates than height. The maximum dry weight (DW) was obtained at 8-16 kg B/ha. However, at the highest B application rate a reverse trend of decreasing DW was apparent to a level that was equivalent to the control and the 4 kg/ha application rate. A statistically significant B effect was observed on the shoot to root biomass ratio. The shoot: root biomass ratio increased as the B application rate increased to the treatment maximum (32 kg/ha).

Boron application up to 4-8 kg/ha increased the net photosynthesis (Net Pn) rate in the current-year mature needles when compared to the control. However, B rates greater than 8 kg/ha reduced the needle photosynthesis to the level of the control treatment (Table 3.4). In this study, the underlying mechanisms for reduced net photosynthesis cannot clearly be linked to B deficiency and toxicity. However, B deficiency has been reported to reduce plant

photosynthetic capacity (Han et al., 2008; Kastori et al., 1995; Zhao and D.M, 2002) by causing decreases in CO<sub>2</sub> assimilation and photosynthetic enzyme activities (Han et al., 2008), stomata conductance (Han et al., 2008), Hill reaction activity/photosynthetic O<sub>2</sub> evolution (Kastori et al., 1995; Sharma and Ramchandra, 1990), and energy transfer from PSII to PSI (Goldbach et al., 1991). It is also suggested that B deficiency can reduce photosynthesis indirectly through feedback-regulation by excessive accumulation of starch and hexoses in B-deficient leaves (El-Shintinawy, 1999; Han et al., 2008). Boron toxicity has been observed to reduce the photosynthesis of young squash plants as a result of decreased leaf chlorophyll concentration, leaf area, stomata conductance and CO<sub>2</sub> fixation (Lovatt and Bates, 1984).

**Table 3. 4**      **Effect of B application rates on plant height, stem diameter, dry weight, shoot root biomass weight and net photosynthesis**

<b>Treatments (kg B/ha)</b>	<b>Plant height (cm)</b>	<b>Plant diameter (mm)</b>	<b>Dry wt. (g)</b>	<b>Shoot :root dry weight ratio</b>	<b>Net Pn (<math>\mu\text{mole/m}^2/\text{s}</math>)</b>
0	39.25 b	8.85 c	26.75 b	1.17 ab	5.94 b
4	43.28 ab	10.0 a	30.8 b	1.15 ab	10.98 a
8	47.2 a	9.70 ab	32.5 ab	1.12 b	11.46 a
16	44.35 ab	9.50 ab	37.3 a	1.19 ab	6.90 b
32	44.5 ab	9.46 b	28.8 b	1.61 a	6.60 b

*\*Values in columns followed by different letters are significantly different ( $P \leq 0.05$ )*

Pn              Net photosynthesis rate

### 3.5.2 Plant B concentration and percentage B distribution in plant organs

Boron treatment had a highly significant effect ( $P \leq 0.005$ ) on foliar/needle B concentration, which increased with increasing B rates in all three needle classes (Table 3.5). The control treatment needle B concentrations (6-11  $\mu\text{g/g}$ ) were lower than the critical value of 12  $\mu\text{g/g}$  reported by Turner and Lambert (1986a) and Hopman et al. (1991) for adult *P. radiata*, indicating B deficiency. The highest needle B concentrations (ca. 280 mg/kg) were observed for the 32 kg B/ha treatment, and were in the range of critical values for B toxicity as expected when the needle concentration is above 250 mg B/kg (Gupta et al., 1985). This could be responsible for the yellowish needle tips and reduced plant growth for this treatment (Figure 3.2). For a wide variety of plant species, the typical visible symptom of B toxicity is leaf burn-chlorosis and/or necrotic patches, often at the margins and tips of older leaves (Bennett, 1993; Bergmann, 1992; Nable et al., 1997).



**Figure 3.2** Yellowing tips at high B dose (32 kgB/ha) in *P. radiata* needles

The magnitude of the needle B concentration (91.5 mg/kg) found at the lowest application rate (i.e. 4 kg B/ha) in this study could be attributed to the young age of the *P. radiata* plants, the dense growth of roots in a confined soil volume, and no leaching loss of the slow-release B fertiliser under glasshouse conditions. The study indicates that the control treatment was B deficient and that the highest B rate was excessive or toxic, as reflected in the needle B concentration.



**Table 3.5 Effect of B application rates on B concentration in plant components (needle, stem and root)**

Treatment (kg B/ha)	Plant B concentration (mg/kg)					Root needle B ratio
	Needle			Stem		
	NI	NCI	NCM	SI	SC	
0	16.9 e	20.4 e	13.16 d	9.4 d	14.4 d	0.23 a
4	94.6 d	84.0 d	96.0 c	12.3 c	20.0 c	0.05 d
8	221.2 c	143.4 c	193.4 b	15.2 b	22.2 c	0.07 c
16	252.8 b	252.8 b	273.2 a	15.8 b	43.6 b	0.07 c
32	272.8 a	285.0 a	279.4 a	24.86 a	68.4 a	0.10 b

NI	The initial needles associated with <i>P. radiata</i> seedlings at the planting time	SI	The initial (original) stem and branches of the seedlings at the time of planting
NCM	Current-year mature needle (needles which emerged after planting and which matured prior to harvest)	SC	Current-year stem and branches which developed during the experiment
NCI	Current-year immature needle (needles which were immature at time of harvest)		

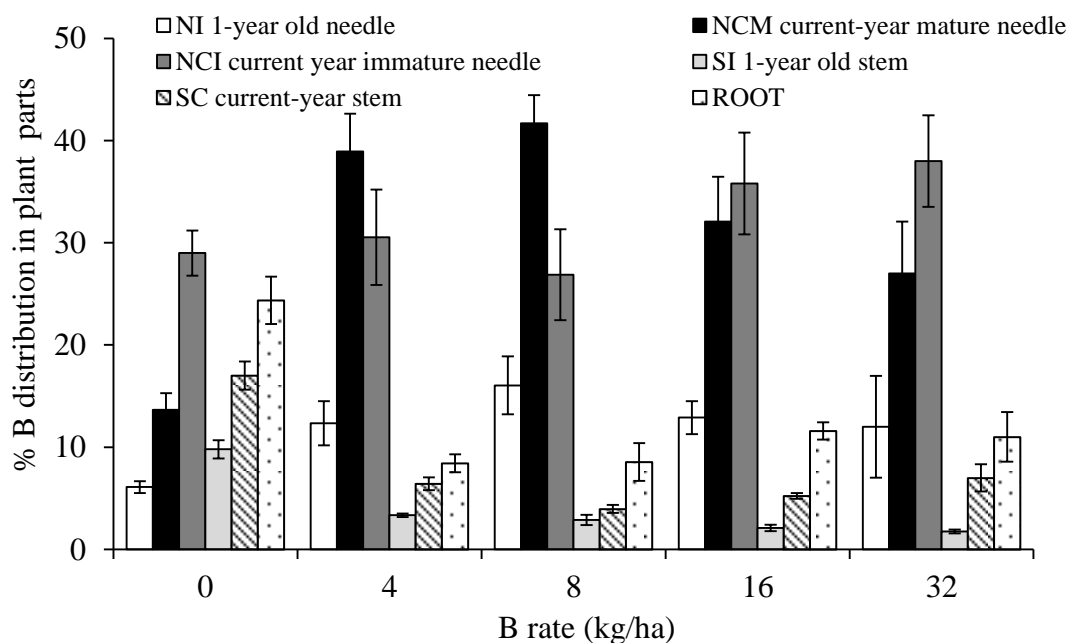
The B concentrations in stems and roots (Table 3.5) also increased with increasing rates of B fertiliser. However, the B concentrations were much lower in stems than in needles and roots for all B treatments, implying an uneven distribution of absorbed B among different plant organs. This agrees with previous observations that B is primarily accumulated and distributed at the end of the transpiration stream (i.e. leaves) in most species through xylem transportation (Brown and Shelp, 1997; Raven, 1980). The lower B concentration in the 1-year old stem (relative to the current-year stem) was due to the dilution of B by the pre-existing biomass before B treatment.

The root to needle concentration ratio is an index of B transfer from roots to shoots. A high ratio indicates restricted transfer whereas a low ratio indicates active transfer to vegetative organs in plants. This study demonstrates that root transfer of B to shoots was significantly restricted in the control treatment, but was promoted at lower rates of B (Table 3.5). Similar trends have been observed for B in navel orange (Sheng et al., 2009), Norway spruce (Möttönen et al., 2003) and canola (Asad et al., 2002). Likewise, a considerably lower B ratio in storage root to old leaves has been reported in an earlier study with sufficient B supply (Shelp and Shattuck, 1987). Restriction of B transfer was also noticeable at the treatment with 32 kg B/ha relative to 4-16 kg/ha treatment. The restriction of B transfer in the control treatment and at the highest B application rate could be, at least partially, related to the damage of vascular tissues caused by B deficiency or toxicity (Dell and Huang, 1997; Nable et al., 1997) .

Percentage distribution of B (% B) in different plant organs (needles, stems and roots- Figure 3.3) identified needles to be the major sink (52 %-85 % B accumulation) depending on the B application level. Boron accumulation in current-year stem relative to 1-year old stems increased as B application rates increased. These findings are consistent with the important role of B in stem formation and elongation through cell division and cell elongation (Dugger, 1985) and subsequently in lignification of the xylem (Lewis, 1980).

There was a gradual increase in B concentration for both NCI and NCM as the B application rate increased. On average, the B concentration in NCI and NCM increased 4-7 fold with the optimum B application rate of 4 kg/ha. As the B application rate increased needle B concentration accordingly increases. However, at the highest B application rate (32 kg/ha), the B concentration in both age group needle classes remained similar (285 and 280 mg/kg).

Of the total plant B that was accumulated in needles, compared to the mid-range B rate treatment (4-8 kg/ha), B was relatively more distributed into newly growing needles (NCI), stems (SI and SC) and roots under limited B supply (0 kg/ha), but into newly growing needles (NCI) and roots only under excessive B supply (16-32 kg/ha). This indicates that both B deficiency and toxicity changed B distribution within plants.



**Figure 3.3 Effect of B application on the percentage B distribution in plant parts**

### 3.5.3 Plant available soil B and soil dehydrogenase activities

The plant-available soil B concentration as determined by hot 0.02 M CaCl<sub>2</sub> extraction of the soils (CaCl<sub>2</sub>-B) increased with increasing rates of B fertiliser (Table 3.6). CaCl<sub>2</sub>-B in the control soils was 0.3 mg/kg which is approximately the same as the critical B deficiency value of 0.25 mg/kg reported for *P. radiata* growth (Snowdon, 1982), and less than the 0.5 mg/kg hot water soluble-B deficiency level reported by Ryan et al. (1998). With increasing B rate, soil B did increase showing that an increasing supply of B from the fertiliser increased the plant-available pool of B. However, high B application rates (e.g. 32 kg B/ha) resulted in the build-up of soil B to toxic level (>14.05 mg/kg) as evidenced by yellowish needle tips and reduced growth, classical B toxicity symptoms (Gupta, 1993a). Cartwright et al. (1986) reported sub-soil B toxicity (CaCl<sub>2</sub> ext.-B >15 mg/kg) in southern Australia.

Soil dehydrogenase activity, an index of soil microbial metabolic activity, was significantly reduced ( $p \leq 0.05$ ) at B rates greater than 8 kg/ha (Table 3.6). This implies that high rates of B fertiliser could be toxic to some soil microorganisms sensitive to added B. Trace element toxicity effects on soil microbial activity (Bääth, 1989) and dehydrogenase activity (Jeyakumar et al., 2008) have been reported previously, and recently there has appeared a single study (Serdar et al., 2011) on the influence of B on dehydrogenase activity in soil. A key aim of the current experiment was to obtain more information on the effect of varying slow-release B fertiliser concentrations on soil dehydrogenase activity under *P. radiata* forestry.

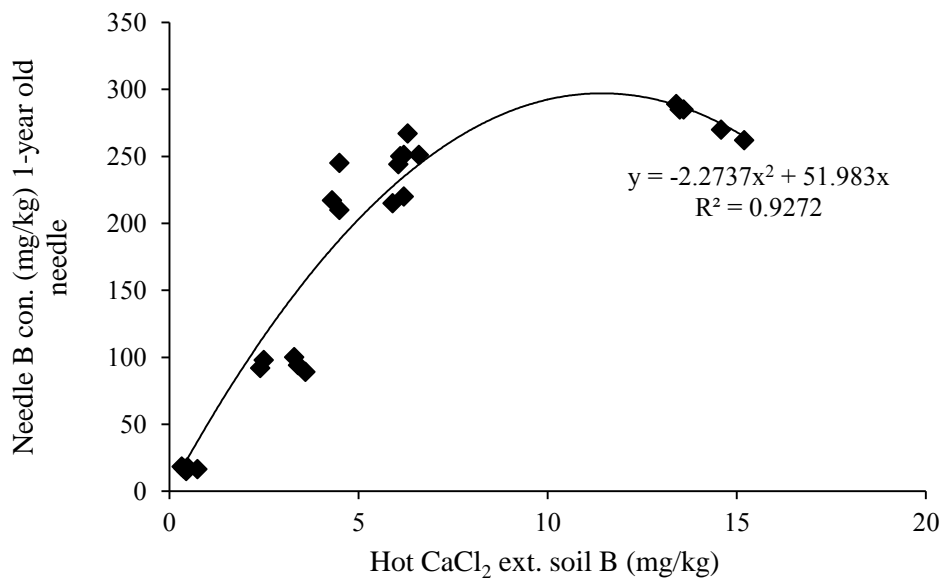
**Table 3.6 Effect of B application rates on CaCl<sub>2</sub> ext. soil B and Soil dehydrogenase activities**

<b>Treatment (kg/ha)</b>	<b>CaCl<sub>2</sub> ext. soil B (mg/kg)</b>	<b>Soil dehydrogenase activities (µg TPF/g oven dry soil)</b>
0	0.30 e	402.09 a
4	3.04 d	437.8 a
8	5.39 c	437.3 a
16	5.95 b	277.5 b
32	14.05 a	189.6 b

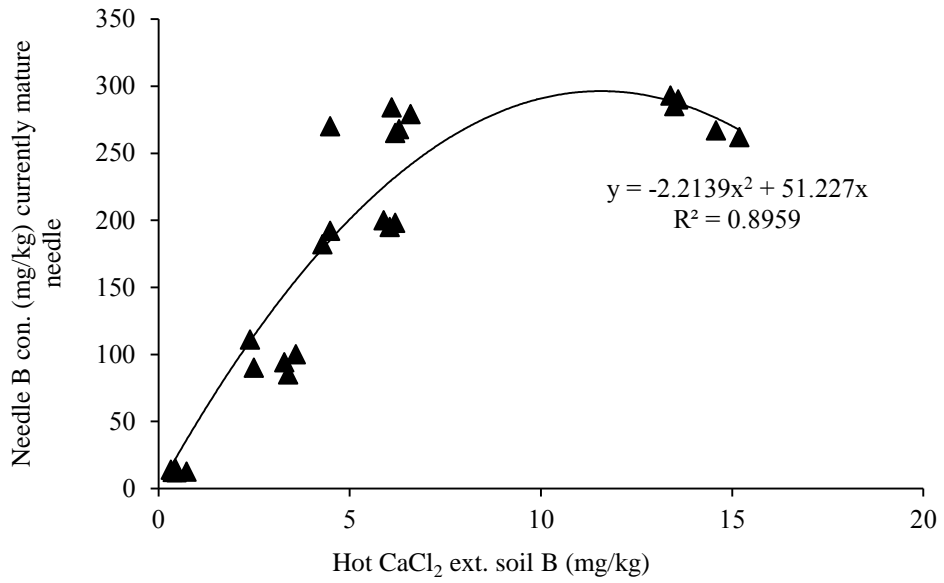
*\*Values in columns followed by different letters are significantly different ( $P \leq 0.05$ )*

### 3.5.4 Relationship between the CaCl<sub>2</sub> extractable soil B and the plant B concentration

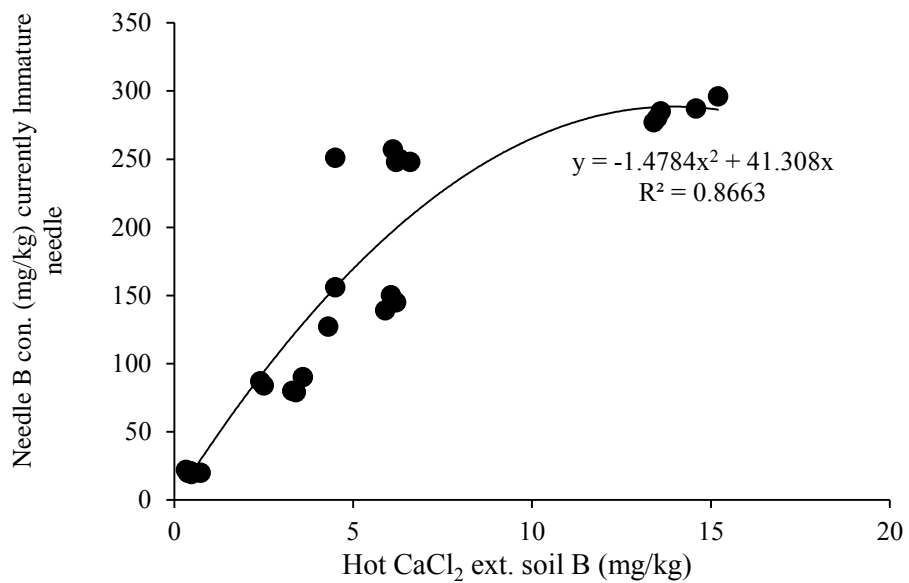
Soil extraction with 0.02 M hot CaCl<sub>2</sub> is considered to be the best index of plant-available soil B, having a sound relationship between extractable B and plant B concentrations (Aitken et al., 1987). In the current study CaCl<sub>2</sub>-B had a significant correlation with the B concentration in different age needles of *P. radiata* ( $R^2 > 0.90$ ,  $N = 25$ ;  $y = a + bx + cx^2$ ). Of the different age needles, year old needles had the highest coefficient of determination ( $R^2 = 0.92$ ) showing this age class of needles to be the best indicator of the B diagnostic status in young *P. radiata* (Figure 3.4; Figure 3.5; Figure 3.6). The CaCl<sub>2</sub>-B concentration corresponding to the critical needle concentration of 8-12 mg/kg reported in literature (Braekke, 1983), obtained from the relationship of soil B concentration with needle B concentration, appears to be around 0.3-0.5 mg/kg which is very similar to the critical hot water soluble B value of 0.25 mg/kg reported by Snowdon (1982) for B deficiency in *P. radiata*. More accurate determination of this critical value was not possible because only a few data points are available in the region of this critical concentration; the majority of the data points are above 2 mg/kg.



**Figure 3.4 Relationship between hot CaCl<sub>2</sub> extractable soil B concentration and the B concentration in 1-year old needles**



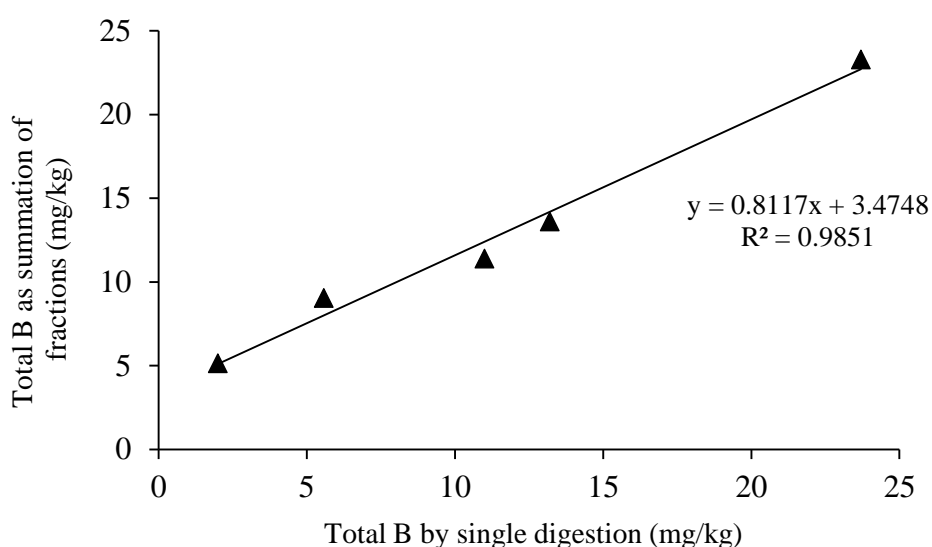
**Figure 3.5 Relationship between hot CaCl<sub>2</sub> extractable soil B concentration and the B concentration in currently mature-needles**



**Figure 3.6 Relationship between hot CaCl<sub>2</sub> extractable soil B concentration and the B concentration in current-immature needles**

### 3.5.5 Boron fractionation in soil

Agreement of the total soil B concentration determined through single digestion with that determined as a sum of fractions was good at high concentration, but less accurate at low concentration (Figure 3.7). Poor agreement at the control and 4 kg/ha treatment is likely due to an interfering effect caused by other ions masking total B recovery. This effect was more apparent at a low concentration of B in soil.



**Figure 3.7 Relationship between sum of all B fractions concentration and the total metal concentration determined by aqua regia digestion**

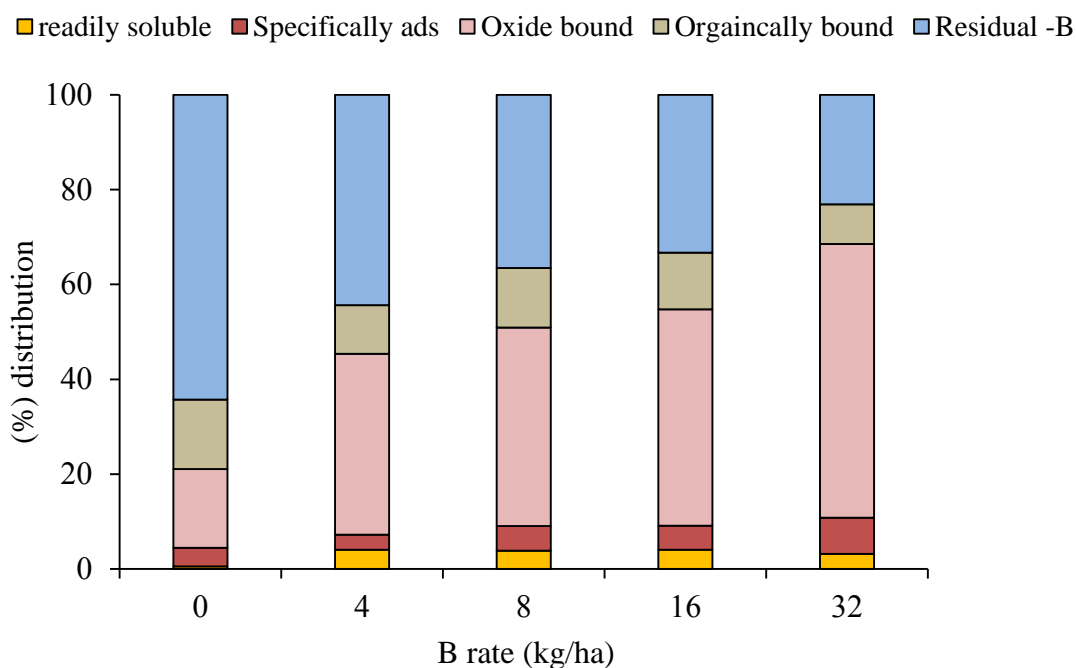
This study demonstrated that the readily-soluble B fraction (non-specifically adsorbed-B) made up between 0.05% and 4% of the total soil B (as quantified by a sum of all fractions) (Figure 3.8). Tsadilas et al. (1994) reported that readily-available B constitutes < 3% of the total soil B, while Gupta (1968b) reported > 4 % of total B shared by readily-soluble B. On average > 16 %-57%, 4 %-8% and 8 %-14% of total soil B was present as oxide-bound B, specifically adsorbed, and organically-bound B respectively. The major pool (23%-64%) of total soil B comprised residual-B which does not correlate with the plant available B fraction (Jin et al., 1987). Most studies have confirmed residual-B to be the major pool of the soil B making up to 91 %-99 % of total B (Datta et al., 2002; Hou et al., 1996). Xu et al. (2001)

reported that residual-B fraction constituted 87.4%-99.7% of the total soil B in 13 Chinese soils studied. The percentage of total soil B associated with the residual fraction and organic fraction decreased at higher treatment levels with a corresponding increase of B associated with the more available fractions.

It is evident that compared to other fractions, non specifically-adsorbed B comprises only a limited content of total B (3.5 %), suggesting that only a small quantity of this fraction can be held in soil solution due to immediate demand for B uptake by plants. Gupta et al. (1985) reported that plants primarily respond to B in soil solution. Keren et al. (1985) reported non specifically-adsorbed B to be the most readily-available B fraction to plant. As cited in section 2.5, besides soil factors, environmental factors such as precipitation and drought play a significant role in regulating the readily-available B concentration through mass flow. Keeping in mind the precipitation data relevant to the forestry site from where the soil was collected (Table 3.2), it is important to consider changes in the available B fraction to ensure that these meet plant B needs. The results of the current study suggest that B fertiliser application has potentially increased the most readily- available B fraction in soil. A similar trend of increasing bioavailable B with increasing B fertiliser has been reported elsewhere (Hou et al., 1996). In the current study the oxide-bound B fraction was the second largest constituent (16 %-57 %) of total soil B, indicating the important role played by oxides and oxyhydroxides in B adsorption.

The distribution of B between the five fractions follows the order residual B > oxide- bound B > organic-bound B > specifically-adsorbed B > non-specifically-adsorbed B. In this study there was an observed change in the relative content of the most available fraction of soil B (labile B or non-specifically adsorbed-B) and the most unavailable fraction of soil B (non-labile B or residual-B). As the rate of applied B fertiliser increased, more and more of the non specifically-adsorbed B become part of the soil B pool, but at the same time the relative content of residual-B decreased. These findings show the importance of B fertilisation not only on the relative distribution of each B in each soil fraction, but also on the absolute concentration of B (as plant uptake of B depends on the readily-available B concentration) associated with the most plant available B fraction.

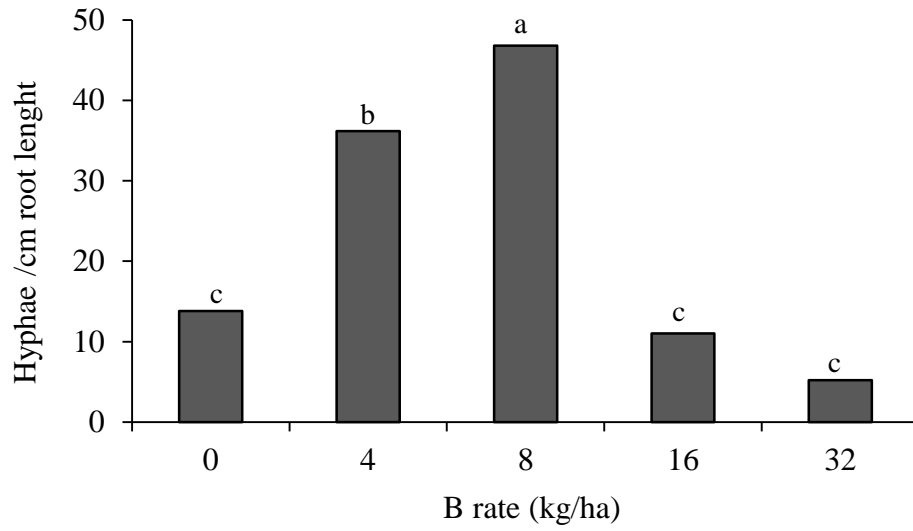




**Figure 3.8 Percentage distributions of B fractions in soil after B fertiliser application**

### 3.5.6 Mycorrhizae colonization in *Pinus radiata*

Increasing the B application rate significantly increased mycorrhizae colonization of the *P. radiata* roots as quantified by an increase in hyphae up to a rate of 8 kg/ha. However, fertilisation above this rate showed a reverse trend with a reduction in hyphae above 8 kg/ha (Figure 3.9). The current research indicates that B at higher rates (16-32 kg/ha) could be toxic to mycorrhizae. Recently, Jeyakumar et al. (2010) reported a decrease in mycorrhiza hyphae density of poplar trees as the Cu level of a biosolids-amended soil increased under glasshouse conditions. The declining density of mycorrhiza hyphae in this study at higher B level could be explained either in the context of an absence of tolerant mycorrhiza species reported by Leyval et al. (1997), or a differential response of existing mycorrhiza species to supply adsorbed B onward to the host plant or to immobilise this B within mycelium (Lehto et al., 2004). Mitchell et al. (1990) reported a positive response of mycorrhiza to B fertiliser application through increasing fungi colonization of roots, leading to improved plant nutrient uptake potential. Similarly, Möttönen et al. (2001) reported that B fertilisation improved plant nutrient uptake via establishing ectomycorrhiza colonization on plant roots.



**Figure 3.9** Mycorrhizae hyphae counts of *P. radiata* roots as affected by the B fertiliser application rates. Bars with different letters shows significant difference at  $p \leq 0.05$

### 3.6 Conclusions

The concentration of plant-available soil B recorded for the control treatment was below the critical soil B level ( $< 0.5$  mg/kg), but this increased with each consecutive rate of B application. However, B application at the maximum rate (32 kg/ha) increased the  $\text{CaCl}_2$  extractable soil B concentration ( $> 14$  mg/kg) close to the reported critical level of toxicity in soil (i.e.  $> 15$  mg/kg). The plant B concentration at the treatment rate of 32 kg/ha exceeded 280 mg B/kg, leading to observed toxicity in needle tips which appeared chlorotic and necrotic. The associated reduction in green surface area could be associated with low photosynthetic capacity as a function of the high B application. The data in this chapter show that plant photosynthesis capacity was detrimentally affected above a treatment rate of 8 kg/ha, but there was an apparent increase in net photosynthesis rate of *P. radiata* at treatments less than 8 kg/ha. The current study has shown that there is a negative relationship between B toxicity and photosynthesis in *P. radiata*.

Plant growth parameters such as height and diameter positively responded to B application in this study. Maximum plant height was obtained at a B application rate of 8 kg/ha, whereas maximum diameter was obtained at 4 kg/ha B application. Maximum dry weight was obtained across a range of B application rates (8-16 kg/ha)

The root to needle B ratio is regarded as an important index to quantify the translocation of B from roots to growing shoots such as needles. In this study it was observed that B application for shoot B acquisition at a treatment rate of 4 kg/ha led to lowest ratio and was thus the optimum rate of fertiliser application. At treatment levels above 4 kg/ha the root to needle ratio increased, although even at 32 kg/ha the ratio was lower than the control. This study showed B at 4kg/ha to be the most efficient B rate. Boron applied at this rate satisfied plant B demand in new growing plant tissues; B was translocated from roots to above-ground plant parts such as shoot and ultimately to needle.

Boron fractionation results showed that B fertiliser application resulted in a gradual increase in the plant available form of the nutrient. However, the major pool of soil B was recognized to be residual B, an unavailable form of B in soil. As cited earlier, Xue et al. 2001 reported that 87.4% to 99.7% of total soil B was present in the residual B fraction which is not directly available for plant uptake.

Boron deficiency in the soil used in this study as quantified by the low concentration of plant available B in the control also detrimentally affected mycorrhizae colonization of *P. radiata*. Boron fertilisation increased colonization of the plant roots. However, this study also showed that root colonization by mycorrhizal infection significantly declined as the B application rate exceed a treatment rate of 16 kg/ha. These finding suggests that a B rate above this level could be detrimental for mycorrhizae growth.

Similarly, soil dehydrogenase activity, a benchmark environmental test of soil microbial activity, shows a declining trend as the B application exceeds 8 kg/ha.

Overall, these results show that B application at an experimentally determined optimum rate will not only improve *P. radiata* growth in term of height and diameter, but will also significantly increase needle net photosynthesis as well as soil microbial activity and ecomycorrhizal root development.

## Chapter Four

# Comparative responses of two clones of *Pinus radiata* to boron fertiliser

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### 4.1 Introduction

The results of the first pot experiment (Chapter 3) confirmed that soil-applied ulexite (a slow-release boron (B) fertiliser) could stimulate the growth of a radiata pine clone at low rates of application (4-8 kg B/ha) but will reduce net photosynthetic rate and diameter growth at higher rates (16-32 kg B/ha). However, Chapter 3 provided no insight into any differences that might be apparent between radiata pine clones with contrasting growth parameters in their responses to soil-applied slow-release B fertiliser, and similarly provided no insight into the underlying mechanisms responsible for such different responses. Could radiata pine clones with higher growth rate be more susceptible to B deficiency than clones with slow growth rate? A better understanding of the differential responses of radiata pine clones to a range of B application rates under greenhouse conditions could provide insight into the underlying mechanisms responsible for the different responses between clones, and provide useful information for B nutritional management at clonal nurseries and plantation forestry areas of New Zealand.

This second pot experiment was therefore conducted to test the differential responses of two radiata pine clones with contrasting growth rates to slow-release B fertiliser. It was hypothesised that the radiata pine clone with the higher growth rate would be more susceptible to B deficiency and has a higher B requirement for optimum growth. The specific objectives are detailed as follows:

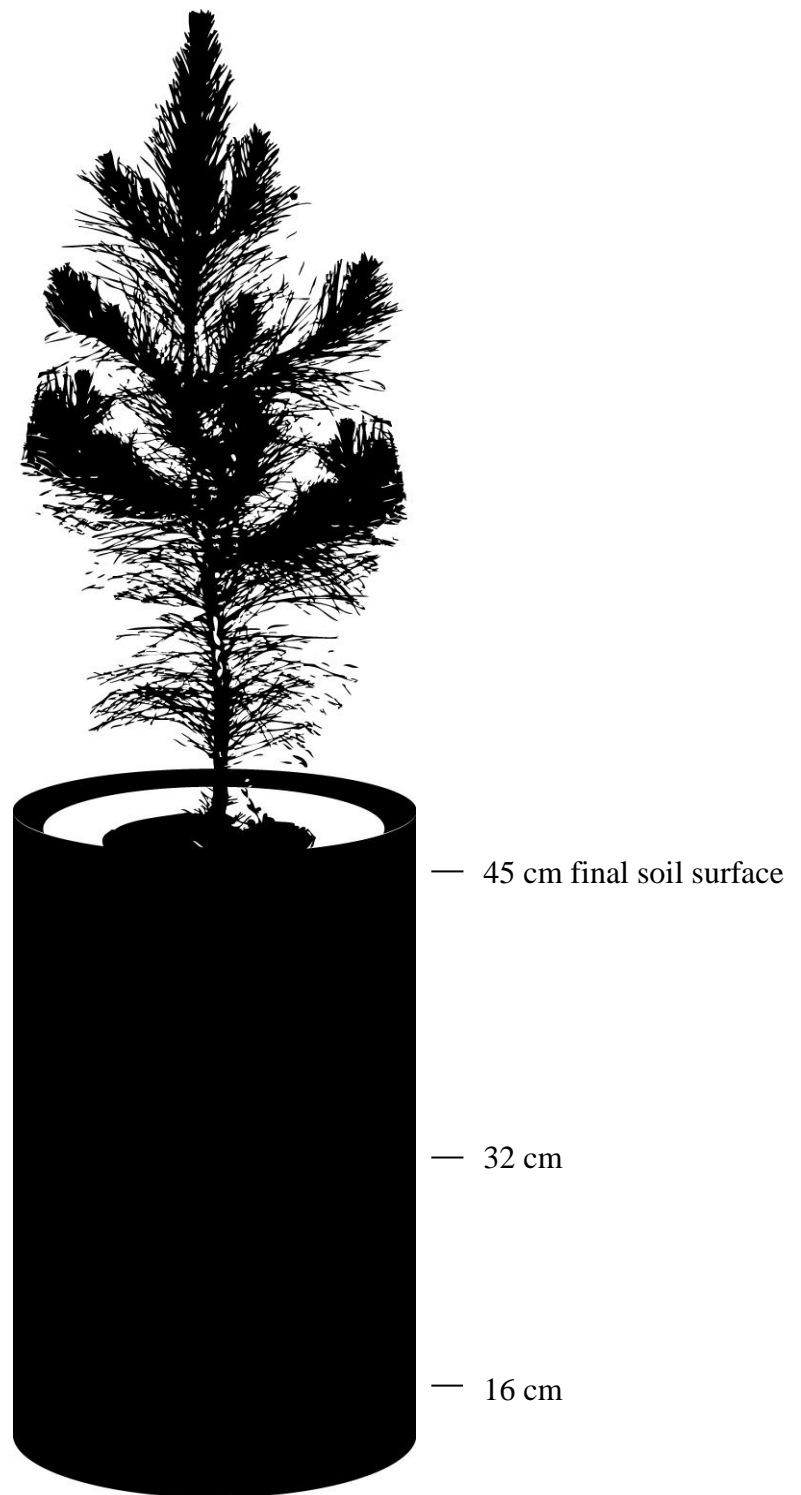
1. To investigate plant growth (e.g. height, diameter, fresh and dry weight, etc.) responses of two radiata pine clones to variable B application rates;

2. To determine the B requirements for plant growth of two radiata pine clones;
3. To compare the B accumulation and distribution of two radiata pine clones;
4. To investigate the effect of B application rates on soil biological properties and plant physiological processes of the two radiata pine clones.

## 4.2 Materials and methods

### 4.2.1 Experimental design and plant growth conditions

This pot experiment was a factorial of five B application rates × two radiata pine clones (Clone 18 and Clone 37) with six replicates per treatment per clone. Each of sixty PVC base-sealed pots (195 mm diameter × 395 mm height) were filled with 7 kg air-dried soil collected from Taupo forest in the Central North Island. The soil is Waipahihi sand derived from water sorted tepra, and is classified as pumaceous. Environmental, physical and chemical properties of the soil were listed in Table 3.2. Five B rates equivalent to 0, 2, 4, 8 and 16 kg B/ha (i.e. 0.058, 0.117, 0.23 and 0.46 g ulexite per pot) were applied along with a basal dose of N at 50 kg/ha (as calcium ammonium nitrate), P at 30 kg/ha (as single superphosphate) and Mg at 30 kg/ha (as epsom or kiesserite) with actually application rates based on the pot surface area. Boron was applied in the form of ulexite ( $B_5CaH_{16}NaO_{17}$ ), a forest grade slow-release granular B fertiliser. Around 1/3 of the total ulexite treatment (as granules) was evenly spread over the soil surface of each pot at three depths as the pots were filled: 16 cm, and 32 cm, where 0 cm is defined as the base of each pot (Figure 4.1). The final filled depth of each pot was 45 cm. Nitrogen and magnesium was evenly applied as a single dose to the surface of the filled pots. Pots were periodically irrigated with distilled water to maintain water contents at 80% field capacity during the entire experiment. Uniform ramets of two *P. radiata* clones (Clone 18, a relatively slow growing clone, and Clone 37, a relatively fast growing clone) sourced from Forest Genetics Ltd, Rotorua were transplanted in mid-September, 2009. All sixty pots were arranged in a randomised complete block design (Figure 4.2).



**Figure 4.1** Schematic diagram showing the placement of ulexite at different soil depths in each experimental pot



**Figure 4.2** Layout of the pot trial in glasshouse

## **4.2.2 Measurement and chemical analysis**

### **4.2.2.1 Plant growth, total fresh and dry weights**

Plant height and stem basal diameter were measured biweekly after establishment of the pot trial, with the final measurement at harvesting. Plants were harvested following the coding system described in Table 4.1 and recorded for fresh weight. Roots from each pot were carefully collected and washed after collecting the rhizosphere soil samples. Plant materials (needle, stem and root) were dried at 65 °C after rinsing with deionised water, and then measured for dry weights before being ground with a Cyclotech herbage mill for chemical analysis.



**Table 4.1 Description of codes used for needle and stem sampling and analysis**

<b>Code</b>	<b>Description</b>
NI	The initial (original) needles associated with <i>P. radiata</i> seedlings at the time of planting
NCM	Current-year mature needles (Needles which emerged after planting and matured prior to harvest)
NCI	Current-year immature needle (Needles which were immature at time of harvest)
SI	The initial (original) stem and branches of the seedlings at the time of planting
SC	Current-year stem and branches which developed during the experiment

#### **4.2.2.2 Photosynthesis measurement**

Net photosynthetic rate ( $P_n$ ) and stomata conductance were measured in two youngest mature fascicles (Code NCM-6 needles) selected from the upper crown portion using a CIRAS-2 portable photosynthesis system (PP Systems, Hitchin, UK) equipped with a standard 2.5 cm-diameter cuvette between 9 am and 12 midday on the 10-20 Aug, 2010. Cuvette conditions were maintained at a photosynthetic photon flux density (PPFD) of 1600  $\mu\text{mol}/\text{m}^2/\text{s}$ , relative humidity 80%, and a leaf temperature of 20 °C. Needle surface leaf area (LA) was calculated using Equation 4.1 below:

$$\mathbf{LA = D (\pi+n) \times (L \times fn)} \quad \mathbf{(Equation 4.1)}$$

Where  $D$ ,  $n$ ,  $L$  and  $f_n$  stand for fascicle diameter, number of needles per fascicle, length of cuvette and number of fascicles, respectively.

Light response curves were also determined using a CIRAS-2 portable photosynthesis system (PP Systems, Hitchin, UK) in the youngest mature fascicles of the upper crown portion. Light response curves were determined over a range of light intensity (1000, 600, 400, 200, 100, 50, 20 and 0  $\mu\text{mol}/\text{m}^2/\text{s}$  PPFD) and at a constant  $\text{CO}_2$  level (370  $\mu\text{mol}/\text{mol}$ ).

#### **4.2.2.3 Soil sampling and preparation**

Soil samples (rhizosphere, non-rhizosphere and bulk) were collected for the determination of  $\text{CaCl}_2$ -extractable soil B concentration and dehydrogenase activity. Non rhizosphere soil samples were collected from three different locations at 0-10 cm, and 10-20 cm using a micro-auger, mixed to make a composite sample, and analysed for depth dependent  $\text{CaCl}_2$ -extractable soil B concentration. In order to separate rhizosphere soil from bulk soil, root bound soil was carefully dug out and shaken vigorously in a paper envelope until soil adhered to roots detached. The soil collected this way was defined as rhizosphere soil and was put into a plastic envelope and properly labelled. A subsample of fresh bulk and rhizosphere soil samples was stored in the fridge (4 °C) and analysed later for dehydrogenase activity. A second sub sample of bulk and rhizosphere soil was air-dried, ground and sieved for the analysis of  $\text{CaCl}_2$ -extractable soil B. Residual soil after plant harvest was mixed and a bulk sample representative of the pot volume was used for analysis.

#### **4.2.2.4 Soil boron analysis**

Sub samples of all sampled soils were extracted with hot 0.02 M  $\text{CaCl}_2$  to determine plant-available soil B according to the modified method of Parker and Gardner (1981). Ten g of soil was placed into a 100 ml conical flask fitted with an air refluxing tube along with 20 ml of 0.02 M  $\text{CaCl}_2$  and 0.2 g charcoal black, boiled on a hot plate for 5 minutes, then filtered through Whatman-42 paper into a plastic tube. A calibration curve was constructed using standard solutions of varying concentration prepared from 1000 mg/kg CertiPur boric acid

stock solution. The concentration of B in the extracts was determined according to the colorimetric method (Bingham, 1982), in which B forms a stable complex with Azomethine-H at pH 5.1. The solution pH was buffered with ammonium acetate and glacial acetic acid. After 1 h of colour development, absorbance was read at 420 nm. Boron fractionation was carried out as described in Chapter 3.

#### **4.2.2.5 Soil dehydrogenase activity determination**

Soil dehydrogenase activity (DHA), an indicator for the metabolic activity of microorganisms in the soil, was colorimetrically measured after incubation of soil sub-samples with 2, 3, 5-triphenyltetrazolium chloride (TTC). As a result of microbial DHA in soil, water-soluble colourless TTC is reduced to water insoluble 2, 3, 5-triphenyletetrazolium formazan (TFP) that has a red colour. In this study the method of Chander and Brookes (1991) was used, where 3 mL of 3% TTC and 0.1 g CaCO<sub>3</sub> were added to 5 g of both bulk and rhizosphere fresh soils separately, and incubated for 24 h at 28 °C. The red colour TFP formed by the end of reaction was extracted with methanol and measured at an absorbance of 485 nm on a spectrophotometer. The colour and thus intensity of absorbance is proportional to the microbiological activity in the soil.

#### **4.2.2.6 Mycorrhizal colonisation assessment**

Plant roots were isolated from soil residues and fine roots were cut-off the main root using scissors for ectomycorrhizal (ECM) colonisation assessment. The excised root segments were fixed in FA (70% ethanol, formaldehyde and acetic acid at a 90:5:5 ratio by volume) and washed thrice with distilled water before staining. Root samples were cleaned in 10 % KOH solution in an autoclave for 30 min, washed in distilled water three times followed by a second autoclaving in 10% KOH for 15 min. The re-sterilised samples were subsequently soaked in 5% HCl for 1 min., washed three times in distilled water, and stained overnight in trypan blue composed of 0.2 g trypan blue in 1 L glycerol, 950 ml water and 50 ml acetic acid. Fine root segments (n=10) of equal length (1 cm) were cut by scissors from each root system and mounted on a microscope parallel with each other to visually quantify for the

presence (+) or absence (-) of extra-mycelium hyphae. The mycorrhizal colonisation was expressed as hyphae counts per cm root.

#### **4.2.2.7 Plant boron analysis**

The B concentration was determined for ground samples of plant organs (needle, shoot and root) following the standard method of dry ashing and colorimetric analysis with Azomethine-H (Gaines and Mitchell, 1979; Wolf, 1974). A subsample of biomass (0.5 g) was dry-ashed in a porcelain crucible using a muffle furnace at 600 °C for 1 h. The ashed sample was allowed to cool then wetted by adding 5 drops of deionised water and 10 ml of 0.36 N H<sub>2</sub>SO<sub>4</sub>. The reaction mixture was allowed to sit, with occasional stirring, for 1 h at room temperature before filtering. For B analysis, a 4 ml aliquot was mixed with buffer masking solution (4 mL) and Azomethine-H reagent (1 mL) stirred on a vortex mixer then left to settle for 1 h to allow for the development of colour. The intensity of the developed colour was measured using a spectrophotometer at 420 nm.

#### **4.2.3 Quality control parameters**

To provide analytical quality assurance for soil B determinations, two certified reference soil materials, CRM 051-050 USA (B 11.8 ± 6.19 mg/kg) and NCS DC 73321 China (B 23 ± 3 mg/kg), were run in parallel with the test samples as standard reference soil samples for quality control. The mean values were found to be 8 ± 3 mg/kg for CRM 051-050 USA and 20 ± 1 mg/kg for NCS DC 73321 China, within the range of 68 % and 92 % of the certified total values respectively.

To provide similar quality assurance for plant B determination, two reference plant materials, grass (B = 9.73 mg/kg) and wheat straw (B = 3.07 mg/kg), obtained from the international plant analytical exchange program (IPAEP) of Wageningen University, The Netherlands, were run as reference plant materials in parallel with the unknown samples for quality control. The mean B concentrations found for the reference plant materials were 9.7 ± 1

mg/kg for the grass sample and  $3.4 \pm 0.01$  mg/kg for wheat straw, values which agreed to within 90 % of the reported mean values for these plant reference materials.

#### **4.2.4 Data analysis**

Two way analysis of variance (ANOVA) was conducted to test main effects (B treatment and Clone) and the interaction of B treatment  $\times$  clone with plant growth and photosynthesis parameters (stem height, diameter, total fresh and dry weight, root dry weight, shoot root dry weight ratio, net photosynthetic rate and stomata conductance), plant B concentration (needle, stem and root B concentration), soil  $\text{CaCl}_2$  extractable B concentration, and microbiological parameters (dehydrogenase activity and ECM colonisation). All statistical determinations were made for the parameters measured at harvest. All statistical analyses were carried out by using SAS 9.1 (SAS Institute, Cary, NC, 2004). Duncans multiple range test (DMRT) was used to determine the differences among means at a 95 % probability level ( $p < 0.05$ ).

### **4.3 Results and discussion**

#### **4.3.1 Plant growth**

At the time of harvest, there were significant main effects of B and clone and their interaction for most growth parameters of radiata pine (Table 4.2 a, b). Overall, based on plant height and total fresh weight, Clone 37 grew faster than Clone 18, and different B rates had different effects on plant height and dry weights except stem basal diameter and root biomass. The significant interaction of B by clone indicates differential growth responses of the two radiata pine clones to different rates of B fertiliser.

**Table 4.2 a** *P* values for main and interactive treatment effects for plant height stem basal diameter, fresh and dry weights of radiata pine at harvest

Factors	Plant height	Stem basal diameter	Total fresh weight	Total dry weight	Shoot dry weight	Root dry weight
B	< <b>0.0001</b>	0.1959	< <b>0.0001</b>	< <b>0.0001</b>	< <b>0.0001</b>	0.1567
clone	< <b>0.0001</b>	0.3936	< <b>0.0001</b>	< <b>0.01</b>	< <b>0.01</b>	0.8527
B × clone	< <b>0.05</b>	0.6601	< <b>0.01</b>	< <b>0.001</b>	< <b>0.001</b>	0.6514

*P* < 0.05 shown in bold

**Table 4.2 b** *P* values for main and interactive treatment effects for dry weights of plant parts and the shoot to root dry weight ratio of radiata pine at harvest

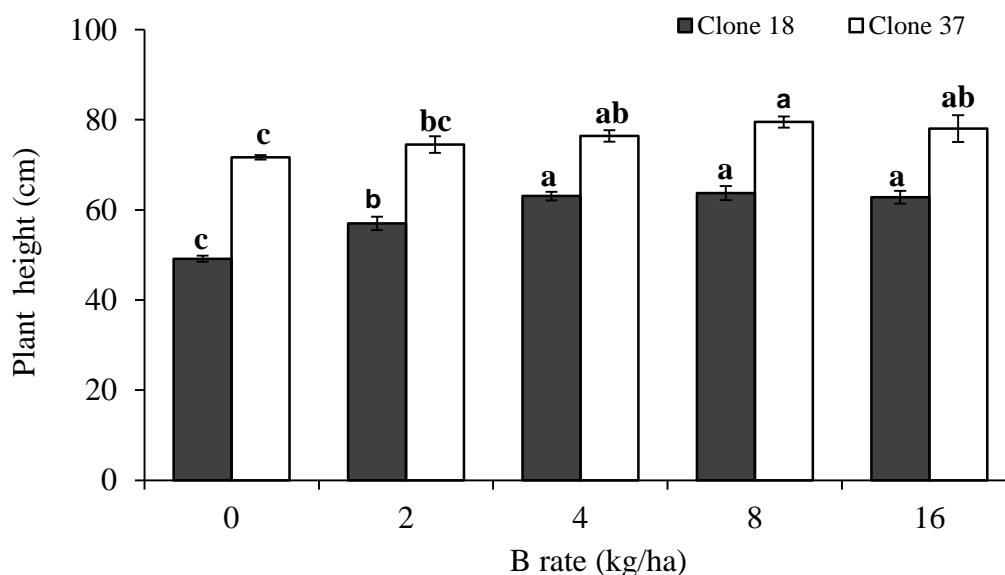
Factors	Dry weight of NI needles	Dry weight of NCM needles	Dry weight of NCI needles	Dry weight of SI and SC stems	Shoot/root ratio
B	< <b>0.05</b>	< <b>0.0001</b>	< <b>0.0001</b>	< <b>0.0001</b>	< <b>0.0001</b>
clone	0.0898	< <b>0.001</b>	< <b>0.01</b>	< <b>0.0001</b>	<b>0.0003</b>
B × clone	0.3390	0.2349	< <b>0.001</b>	< <b>0.0001</b>	< <b>0.0001</b>

*P* < 0.05 shown in bold

- NI The initial needles associated with *P. radiata* seedlings at the planting time
- NCM Current-year mature needle (needles which emerged after planting and which matured prior to harvest)
- NCI Current-year immature needle (needles which were immature at time of harvest)
- SI The initial (original) stem and branches of the seedlings at the time of planting
- SC Current-year stem and branches which developed during the experiment

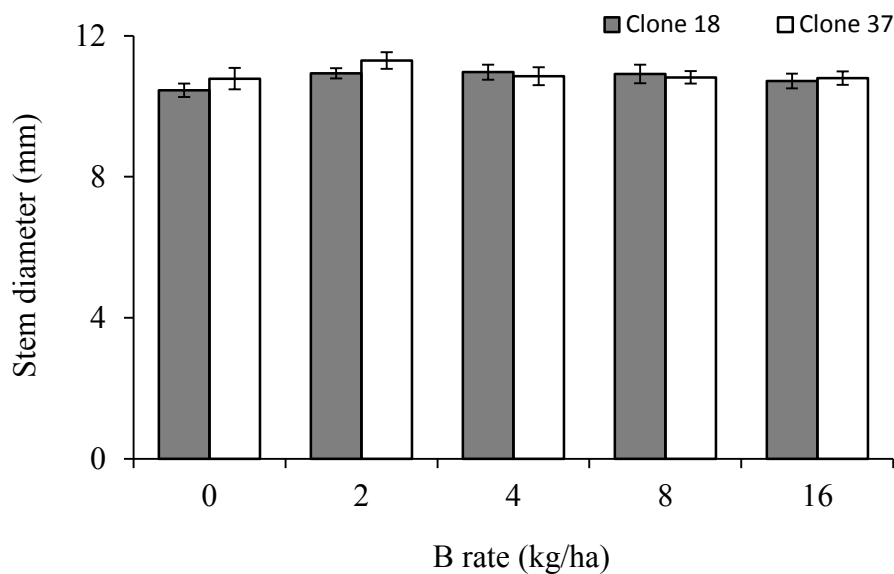
#### 4.3.1.1 Plant height, stem diameter, total fresh and dry weights

For Clone 18, plant height was significantly greater under B treatment relative to the control. For Clone 37, B treatment from 4 kg/ha increased plant height relative to the control. For Clone 37 the optimal rate of B fertiliser for plant height was 8 kg/ha, with this treatment showing a significant increase in plant height over the 2 kg/ha treatment. For Clone 18, there was a significant increase in plant height from 2 kg/ha to 4 kg/ha B, but no further increase with increasing B application. Compared with the control treatment, the absolute value of plant height for Clone 18 was increased by 16-30% when B was applied at rates of 2-8 kg/ha respectively, and by 27% at the rate of 16 kg/ha. For Clone 37 an increase of plant height between 4 and 11% was observed for B applied at 2- 8 kg/ha, and by 9% at 16 kg/ha (Figure 4.3). The results from this study are in agreement with a previous study on differential height growth responses of two navel orange cultivars to both low and high (excess) B treatment under greenhouse conditions (Sheng et al., 2009).



**Figure 4.3** Plant height responses of two radiata pine clones to different rates of B fertiliser. Means for each clone with different letters are significantly different ( $p < 0.05$ )

In contrast to plant height, there was no statistically significant increase in stem diameter for either clone as a function of the B application rate (Figure 4.4). This finding is in agreement with a previous study (Hopmans and Flinn, 1984), where plant height was reportedly increased by application of borax but no effect was noticed on plant diameter. Under conditions of minimal to low B deficiency, diameter growth in conifers generally is relatively unaffected, but height growth may be reduced even when there is no loss of apical dominance (Lehto et al., 2010; Saarsalmi and Tamminen, 2005; White and Krause, 2001).

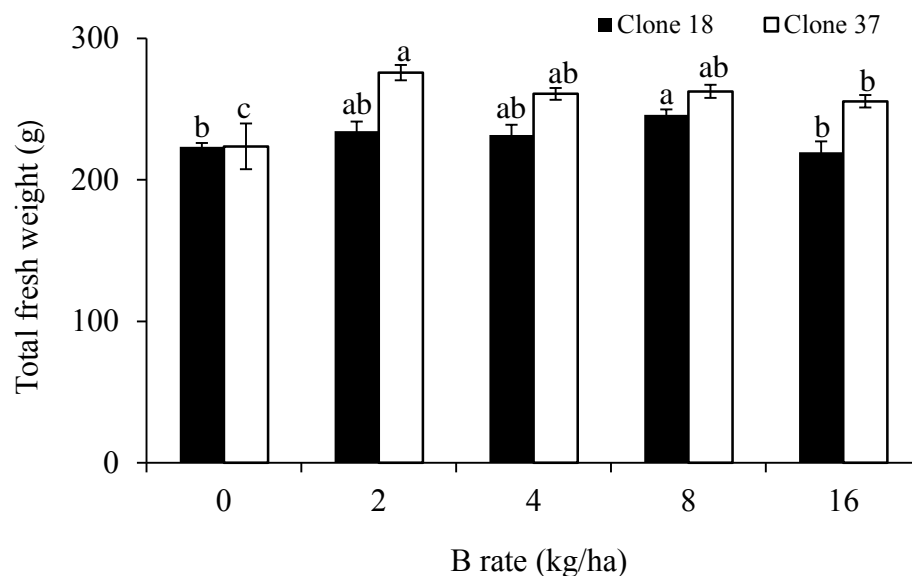


**Figure 4.4** Stem diameter response of two radiata pine clones to different rates of B fertiliser

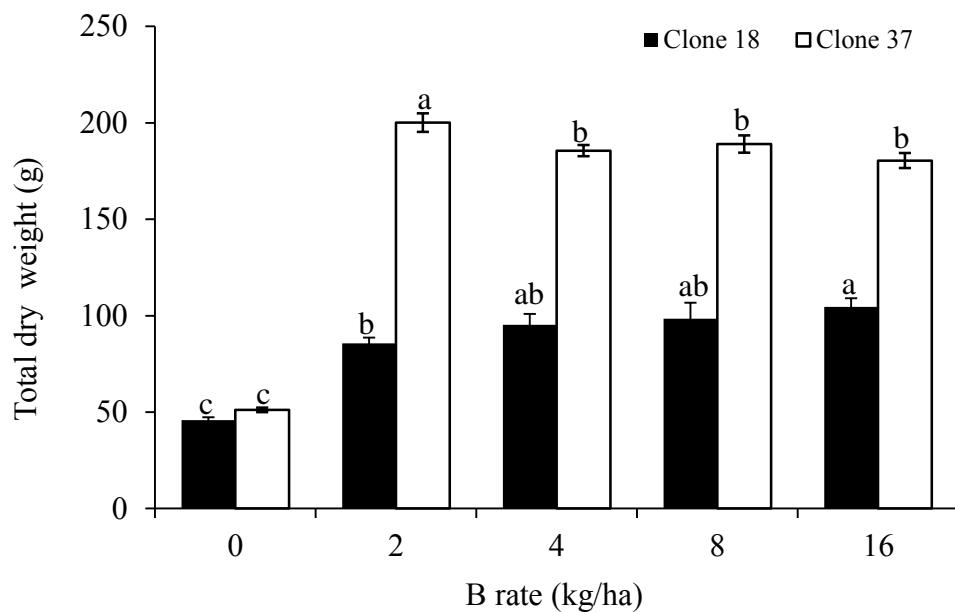
Boron application had a significant effect on total fresh weight yield for both clones (Figure 4.5). Compared to the control treatment, a weight gain of 5-10 % was observed when B was applied at 2-8 kg/ha and 3% at 16 kg/ha for Clone 18, although the increase was only significant for the treatment of 8 kg/ha. By contrast, the maximum gain in fresh weight for Clone 37 was obtained for a B application of 2 kg/ha (24%) and then the gain reduced to an increase of 16-17 % over the control at rates of 4-8 kg/ha and to 13% at the highest rate of 16 kg/ha. A significant increase in fresh weight was recorded in Clone 37 for all B treatments relative to the control. Similarly, total dry weight was also significantly increased by B application, with the maximum dry weight obtained at 16 kg B/ha (104.7 g/plant) for Clone 18 and at 2 kg B/ha (200 g/plant) for Clone 37 (Figure 4.6). The increased dry mass



yield for Clone 37 between the 0 and 2 kg/ha B treatments was very marked (4-fold increase). The fresh and dry weight results further indicate clonal differences in response to the soil-applied B fertiliser. Clone 37 was more responsive to lower rates of B but was also more susceptible to the inhibition of growth at higher B rates as a significant reduction in yield was recorded as a function of increasing B treatment. Clone 18 required a higher application rate of B for its optimum growth and was relatively less stimulated by all rates of B relative to Clone 37. Genotypic variation in growth response to B supply has been reported previously in navel orange cultivars (Sheng et al., 2009) and eucalyptus Clones (José et al., 2009).



**Figure 4.5** Total fresh weight response of two radiata pine clones to different rates of B fertiliser. Means for each clone with different letters are significantly different ( $p < 0.05$ )



**Figure 4.6** Total dry weight response of two radiata pine clones to different rates of B fertiliser. Means for each clone with different letters are significantly different ( $p < 0.05$ )

#### 4.3.1.2 Dry weights of plant parts and shoot to root dry weight ratio

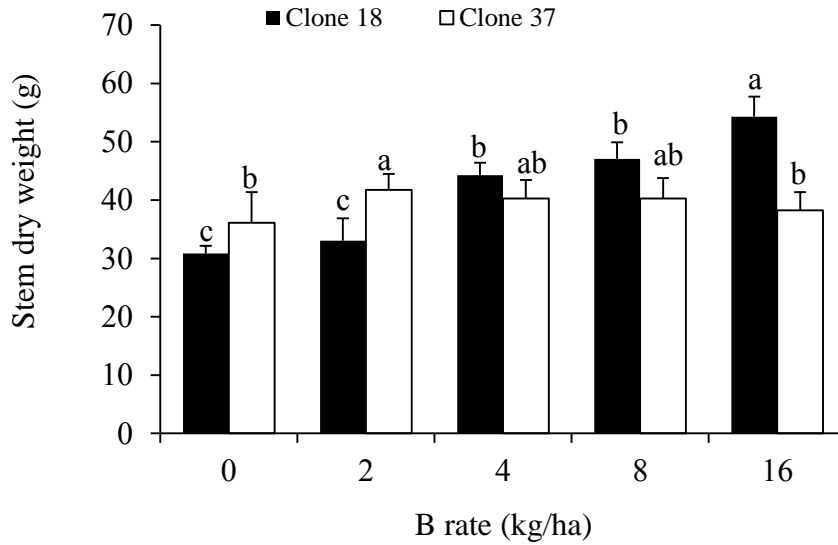
Clone 37 exhibited a faster growth rate than Clone 18 across all B treatments, as evidenced by greater dry weights of NCM and NCI needles, and stem biomass (Table 4.3; significant difference between clone main effects). Application of B generally increased the dry weight of each measured plant tissue component for both clones. However, the optimum rate of B fertiliser for the maximum dry weight yield varied with different tissues (Table 4.3). For example, the dry weight of NI needles was increased at 4 kg B/ha, but reduced at 16 kg B/ha. By contrast, the dry weight of stems was increased with increasing B rates up to 16 kg/ha (Table 4.3). Similar to total dry weight, there was a significant interactive effect of B with clone for dry weight yield of NCI needles and stems (Table 4.2 b). This further confirms that there is a clonal variation in response to soil-applied B fertiliser. Based on the stem dry weight, Clone 18 required a higher application rate of B for its optimum growth but was

relatively less stimulated by the lower rates of B, while Clone 37 was also more responsive to lower rates of B but was also more susceptible to the inhibition of growth at higher B rates (Figure 4.7).

**Table 4.3** Effect of boron (B) rate and clone on the dry weight recorded for different age classes of needles and stems

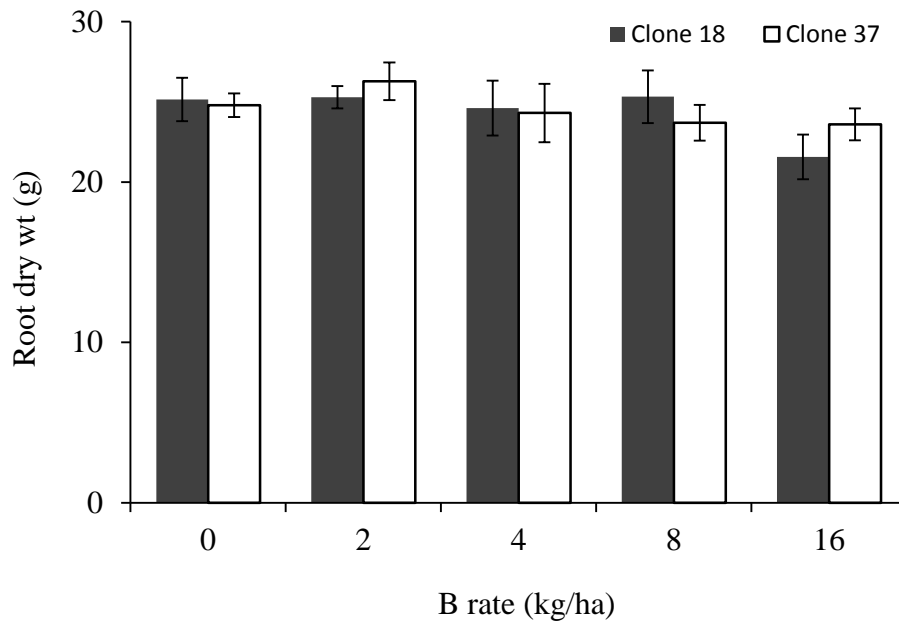
B levels	NI	NCM	NCI	Stems (SI + SC)
<b>Boron main effect</b>				
B0	2.76 b	20.7 b	7.15 c	33.5 d
B2	2.55 b	39.6 a	13.1 ab	37.4 c
B4	4.78 a	35.9 a	13.9 ab	42.3 b
B8	3.07 b	35.6 a	15.2 a	43.7 b
B16	2.21 b	41.4 a	12.2 b	46.3 a
<b>Clone main effect</b>				
Clone 37	2.62 a	39.7 a	13.2 a	41.9 a
Clone 18	3.53 a	29.6 b	11.0 b	39.3 b

NI	The initial needles associated with <i>P. radiata</i> seedlings at the planting time
NCM	Current-year mature needle (needles which emerged after planting and which matured prior to harvest)
NCI	Current-year immature needle (needles which were immature at time of harvest)
SI	The initial (original) stem and branches of the seedlings at the time of planting
SC	Current-year stem and branches which developed during the experiment



**Figure 4.7** Stem dry weight response of two radiata pine clones to different rates of B fertiliser. Means for each clone with different letters are significantly different ( $p < 0.05$ )

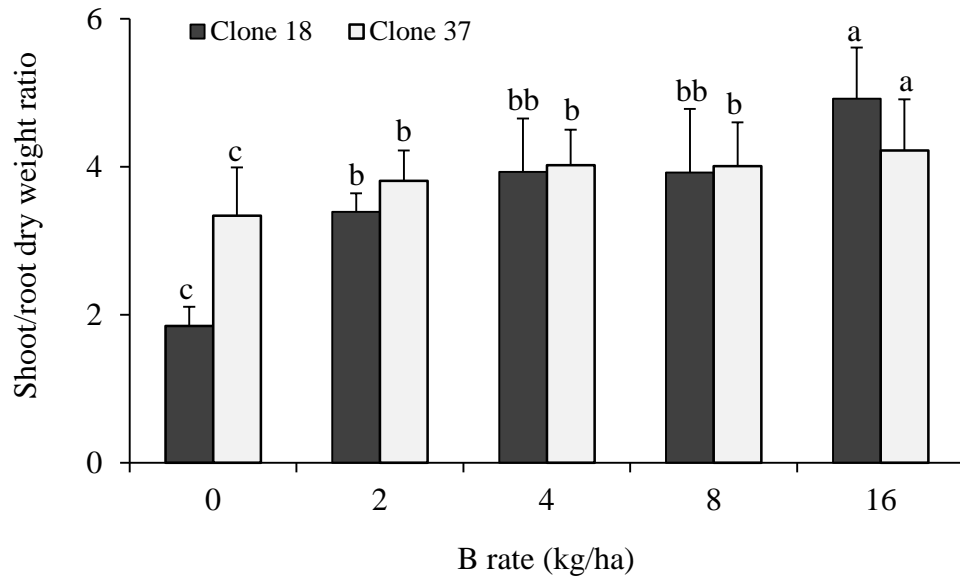
Similar to stem basal diameter, there was no significant effect of B treatment, clone or their interaction on root dry biomass production (Table 4.2 a). However, it appeared that the root dry weight of both clones was nominally (not significant at  $p < 0.05$ ) increased at 2 kg B/ha and then reduced at higher B rates, especially at 16 kg B/ha (Figure 4.8). For Clone 18 this reduction in root dry biomass relative to the control was significant a  $p < 0.05$ . It has long been known that B deficiency inhibits root elongation (Dugger, 1983; Marschner, 1995) and root growth is more sensitive to B deficiency than shoot growth (Dell and Huang, 1997). Root dry weight (Räisänen et al., 2007) or root tip number (Möttönen et al., 2001a, b) has been reported in literature to be reduced by low B well before macroscopic above-ground effects occur in Norway spruce seedlings (Lehto et al., 2010). The disagreement between this study and the referenced studies could be due to differences in the severity of B deficiency in the growth substrates used. In this study, plants suffered no to only marginal B deficiency as was evident from a lack of visual deficiency symptoms, and the non-significant increase in root dry biomass production with B fertiliser for both radiata pine clones (Figure 4.8). The lack of apparent deficiency will be further evidenced by plant B concentrations (Figures 4.13-4.15),



**Figure 4.8** Root dry weight responses of two radiata clones to different rates of B fertiliser

By contrast, there were highly significant effects of B and clone and their interaction for the shoot to root dry weight ratio (Table 4.2 b). The shoot to root dry weight ratio (S/R) was generally increased with increasing B rates for both clones (Figure 4.9). The lower S/R ratio in the control (without B applied) implies plant adaptive behaviour to cope with B deficiency stress. A low S/R ratio under B deficiency is likely to keep photosynthates partitioning restricted to roots as to avoid growth of new needle/foilage, a major sink of B, and hence improve B availability to current biomass (Mei et al., 2011). Deficit of water or nutrients influences plant growth by decreasing the S/R ratio and changing root architecture (Brouwer, 1962; Lopez-Bucio et al., 2003; Wilson, 1988). However, there was an apparent clonal variation in the response of the S/R ratio to different B rates, especially to low (0 kg/ha) and high (16 kg/ha) rates of application (Figure 4.9). The results indicate Clone 18 had greater acclimation (than Clone 37) to variability in soil B supply through an apparent ability to change its S/R ratio in response to the conditions of B fertility. Under the condition of low soil B supply (e.g. the control), Clone 18 might allocate more photosynthesis to the below-ground biomass, and in so doing, form a larger root system for B absorption from soil. This would result in a relatively greater response to lower rates of applied B. This response is highlighted in Figure 4.9, where the S/R ratio of Clone 18 increases from under 2 to

greater than 3 (difference of 1 unit), from the control to the 2 kg/ha treatment. The corresponding increase for Clone 37 is approximately 0.2. A similar mechanism has been reported previously for the different responses of plant genotypes to water and nutrient stresses (Kulkarni and Phalke, 2009).



**Figure 4.9** Shoot root dry weight ratio response of two radiata clones to different rates of B fertiliser. Means for each clone with different letters are significantly different ( $p < 0.05$ )

#### 4.3.2 Net photosynthetic rate, stomata conductance and light response curve

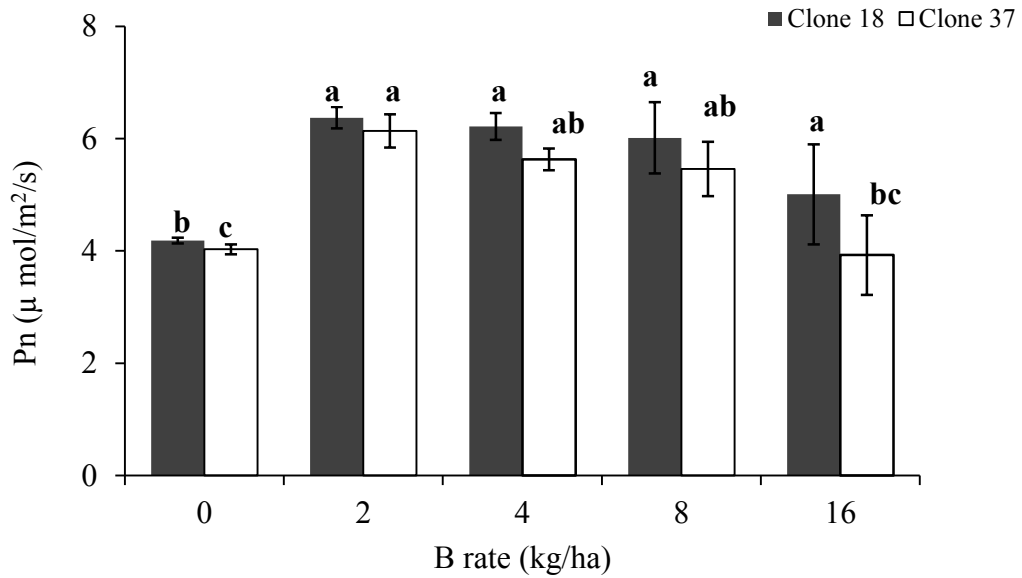
Boron and clone main effects were significant for net photosynthetic rate and stomata conductance, however the interactive effect of B and clone was not significant for these parameters (Table 4.4).

**Table 4.4** *P* values for main and interactive treatment effects on net photosynthetic rate of current-year needle and stomata conductance of radiata pine measured before harvest

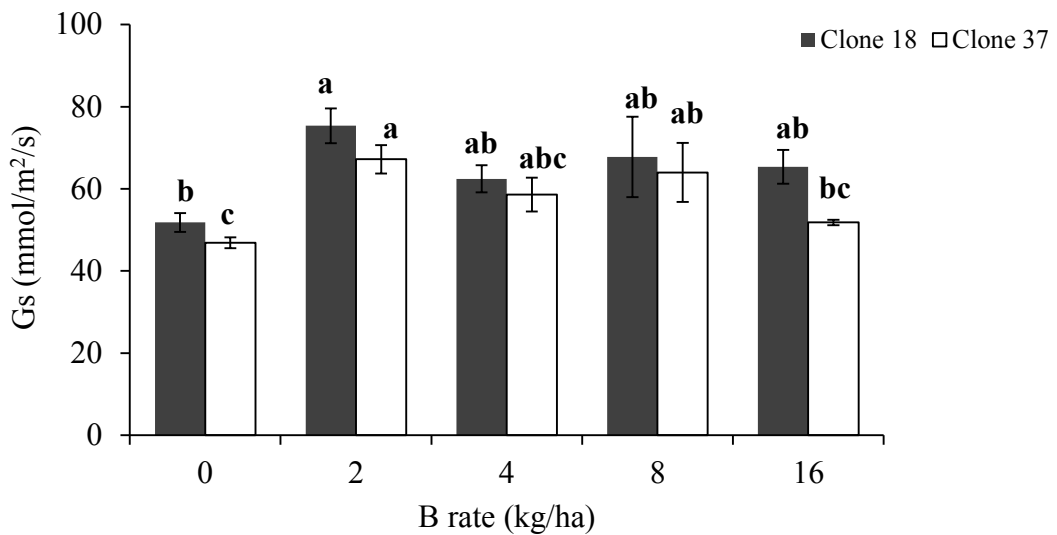
Factors	Net photosynthetic rate	Stomata conductance
B	< <b>0.0001</b>	< <b>0.005</b>
clone	< <b>0.05</b>	< <b>0.01</b>
B × clone	0.835	0.640

*P* < 0.05 shown in bold

Application of B generally increased the net photosynthetic rate in the current-year mature needles of both clones, which was greatest at 2 kg B/ha when compared to the control (Figure 4.10). For Clone 18, the net photosynthetic rate appeared to decline slightly when B was applied at a rate greater than 2 kg/ha, but there was no significant change across the B treatments. For Clone 37, the B rate of 16 kg/ha reduced the net photosynthetic rate relative to the optimal treatment (Figure 4.10). The results indicate clonal variation in the response of net photosynthetic rate to increasing rates of B fertiliser. Similarly, clonal variation was also found for the response of stomata conductance to B fertiliser application (Figure 4.11).



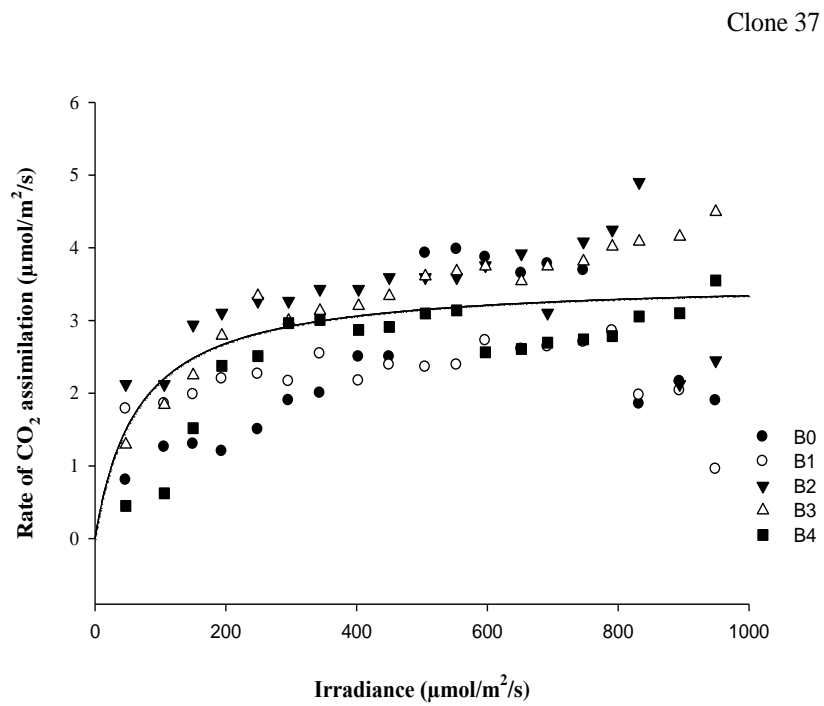
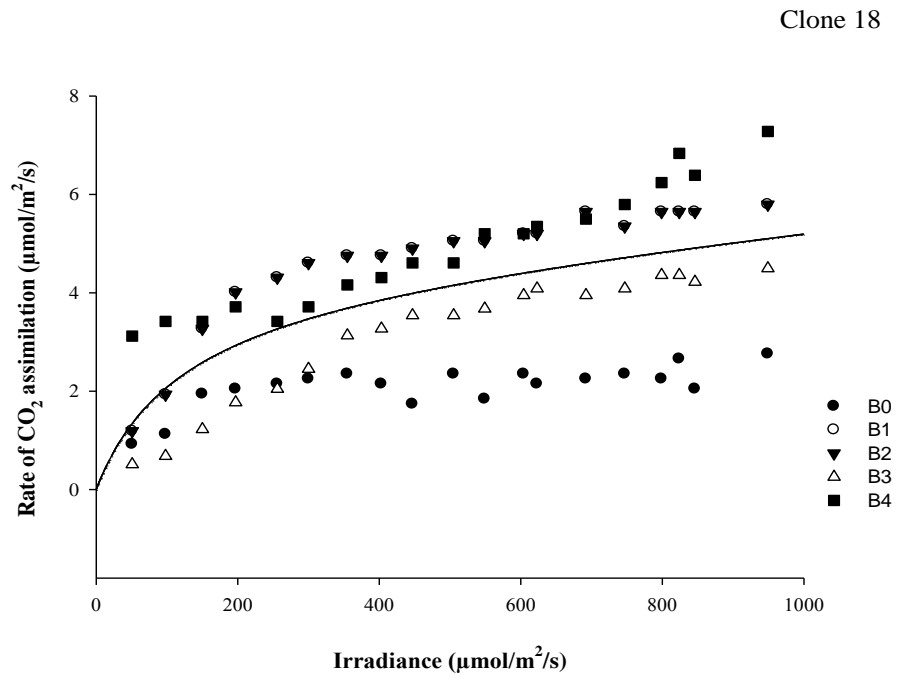
**Figure 4.10** Effect of B application rates on net photosynthetic rate in current year needles (Pn) of Clone 18 and 37. Means for each clone with different letters are significantly different ( $p < 0.05$ )



**Figure 4.11** Effect of B application rates on stomata conductance of Clone 18 and 37. Means for each clone with different letters are significantly different ( $p < 0.05$ )



Boron application also affected the photosynthetic light response curve for both clones (Figure 4.12). It appeared that the differences in net photosynthetic rate effected by B fertilisation commenced at a light intensity of about 400  $\mu\text{mol}/\text{m}^2/\text{s}$  and became more obvious at higher light intensities (irradiance). For both clones, the net photosynthetic rate was lower in the control than in all B treatments at light intensities of 400-1000  $\mu\text{mol}/\text{m}^2/\text{s}$ . The net photosynthetic rate was at a maximum at 8 kg B/ha for Clone 18, but at 2 kg B/ha for Clone 37 (Figure 4.12).



**Figure 4.12** Photosynthetic light response curves of Clone 18 and 37, as affected by B application rates

In this study, the underlying mechanisms for reduced photosynthesis cannot clearly be linked to B deficiency and toxicity. However, B deficiency has been reported to reduce plant photosynthetic capacity (Han et al., 2008; Kastori et al., 1995; Zhao and D.M, 2002) by causing decreases in CO<sub>2</sub> assimilation and photosynthetic enzyme activities (Han et al., 2008), stomata conductance (Han et al., 2008), Hill reaction activity/photosynthetic O<sub>2</sub> evolution (Kastori et al., 1995; Sharma and Ramchandra, 1990), and energy transfer from PSII to PSI (Goldbach et al., 1991). It is also suggested that B deficiency can reduce photosynthesis indirectly through feedback regulation by excessive accumulation of starch and hexoses in B-deficient leaves (El-Shintinawy, 1999; Han et al., 2008), or through reduced photosynthetic leaf area and chlorophyll contents (Sotiropoulos et al., 2002) and reduced stomata pore, transpiration and water potential (Sharma and Ramchandra, 1990), as low numbers of stomata openings result in a decline of photosynthesis under B deficiency (Kastori et al., 1995) and under Zn deficiency (Sharma et al., 1994). Boron toxicity has been observed to reduce the photosynthesis of young squash plants as a result of decreased leaf chlorophyll concentration, leaf area, stomata conductance and CO<sub>2</sub> fixation (Lovatt and Bates, 1984). The reduction of CO<sub>2</sub> assimilation in citrus leaves by excessive B is probably caused by a combination of factors such as oxidative damage, reduced photosynthetic enzyme activities and impaired electron transport capacity (Han et al., 2009). The improvement in photosynthesis response as B application rate increased in the current study emphasizes the indirect role of B in plant physiology such as net photosynthesis rate.

### **4.3.3 Boron concentration and distribution in plant organs**

#### **4.3.3.1 Plant B concentrations**

The main effect of B treatment was significant for needle (all classes), stem and root B concentrations, while the main effect of clone was only significant for B concentrations in NI and NCI needles. The interactions of B with clone were significant for the B concentrations of NI and NCM needles, and root (Table 4.5).

**Table 4.5** *P* values for main and interactive treatment effects on needle, stem and root B concentrations (< 0.05 shown bold)

Factors	NI	NCM	NCI	SI	SC	Root
B	<0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.01	< 0.0001
Clone	< 0.001	0.1267	< 0.01	0.2777	0.4920	0.2008
B × clone	< 0.01	< 0.05	0.4651	0.65062	0.4871	< 0.005

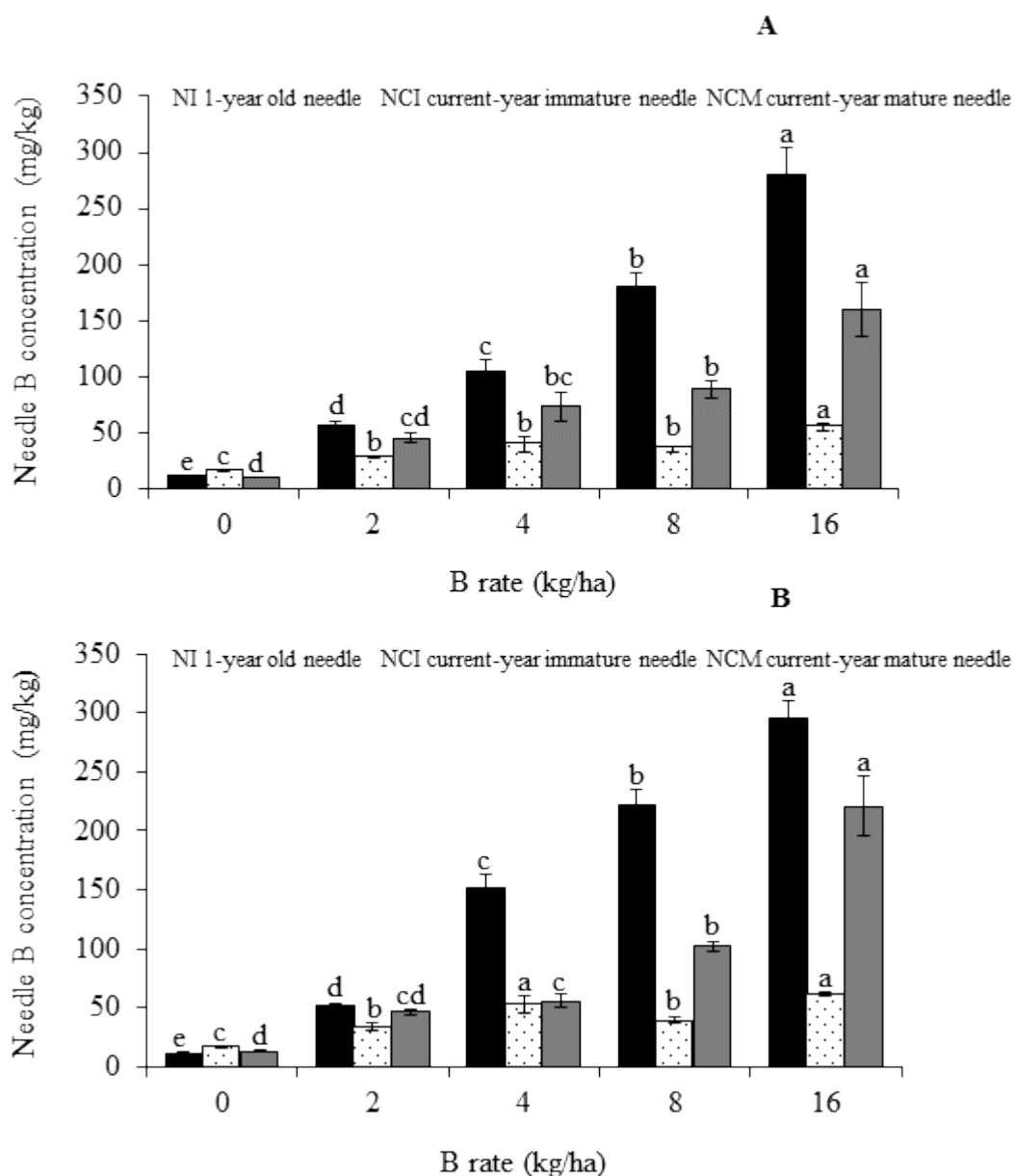
NI	The initial needles associated with <i>P. radiata</i> seedlings at the planting time
NCM	Current-year mature needle (needles which emerged after planting and which matured prior to harvest)
NCI	Current-year immature needle (needles which were immature at time of harvest)
SI	The initial (original) stem and branches of the seedlings at the time of planting
SC	Current-year stem and branches which developed during the experiment

Clone 37 generally had a higher needle B concentration (averaged across all B treatments) than Clone 18, especially NI needles (146 vs. 113 mg B/kg). This implies that Clone 37, which was relatively fast growing, might have a greater B requirement for its growth. Increasing B application rates in the soil linearly increased the B concentration (averaged across both Clones) in all plant parts in the following order: NI > NCM > NCI > root > SC > SI (Figure 4.13-15), indicating that basal or older needles (e.g. NI) were the largest sinks of B accumulation in plant organs for both clones. This is in agreement with a previous study on B concentration and distribution within trees of two citrus genotypes (Papadakis et al., 2003), and supports the general idea of B as an immobile plant nutrient. However, such a statement on mobility is not true if the data is considered in more detail.

In the current study, the B concentration in NI and NCM needles for the control treatment in both clones (approximately 12 mg/kg) (Figure 4.13) was at the critical value of 12 mg/kg reported by Turner and Lambert (1986a) and Hopmans and Clerehan (1991) for adult *P. radiata*, indicating marginal B deficiency. For the control treatment, the B concentration

(averaged across both clones) in the upper needles (NCI, 16 mg B/kg) was, however, significantly higher than that in the basal needles in both clones (mean NI, 11 mg B/kg). This, in fact, suggests preferentially translocation of B under the control conditions to the upper-younger needles to support growth where the plant is under conditions of marginal B deficiency. In this scenario (where no B is applied) B is a relatively mobile nutrient. This agrees with a previous study on B accumulation and distribution in two navel orange cultivars (Sheng et al., 2009). In the current study the highest B concentration (279 mg/kg, averaged across both clones) was observed in NI needles at the 16 kg B/ha treatment, and was in the range of critical values for B toxicity predicted to occur above a needle concentration of 250 mg B/kg (Gupta et al., 1985). Onset of B toxicity, as evidenced by a visible yellowing of needle tips, accounted for the reduced growth of the NI needle biomass component and the whole plant at this treatment (Table 4.5). The significant interaction of clone and B treatment apparent for needle classes NI and NCM indicates a clonal variation in needle B response to different rates of soil-applied B. Clone 37 overall had a higher B concentration in NI and NCM needles than Clone 18, and the differences between the two clones became larger at higher B application rates (Figure 4.13). This could be responsible for the different dry weight responses of the two clones to the applied B rates (Figure 4.6), explaining why Clone 37 was more responsive (than Clone 18) to lower rates of B but was more susceptible to the inhibition of growth at higher B rates. In effect, Clone 37 more readily accumulated B to a toxic level than Clone 18, leading to a more apparent and rapid biomass response of B at both low (positive) and high (negative) rates of treatment.

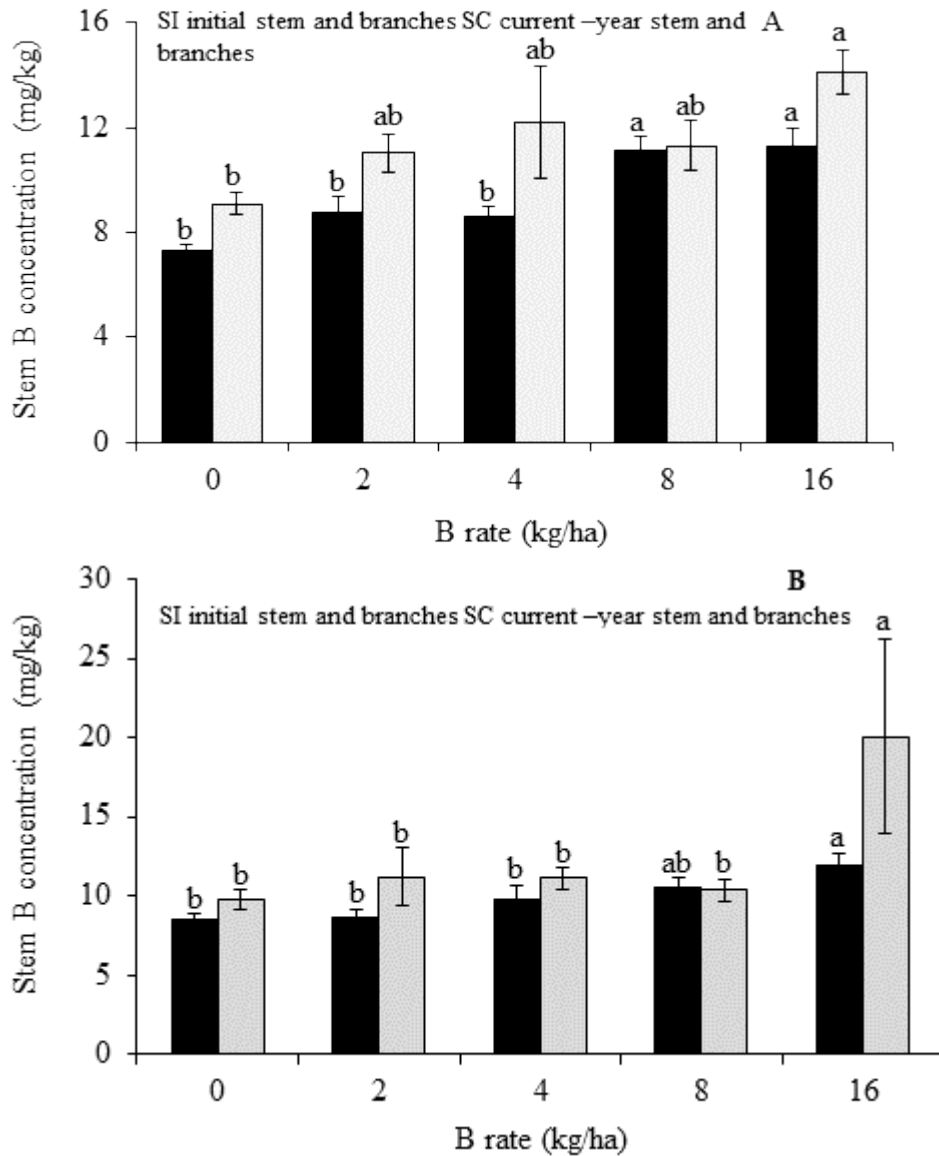
The B concentrations in stems (Figure 4.14) and roots (Figure 4.15) for both clones increased linearly with increasing rates of B fertiliser. However, the B concentrations were much lower in stems than in needles and roots for all B treatments, implying an uneven distribution of absorbed B among different plant organs. This agrees with previous observations that B is primarily accumulated and distributed at the end of the transpiration stream (i.e. leaves) in most species through xylem transportation (Brown and Shelp, 1997; Raven, 1980).



**Figure 4.13** Boron concentrations in one year-old (NI), currently mature (NCM), and currently immature (NCI) needles of Clone 18 (A) and Clone 37 (B). Means for each clone with different letters are significantly different ( $p < 0.05$ )

The much lower B concentrations in stems (than needles) accounted for the positive growth response of stem dry weight to application rates of B up to 16 kg/ha (Table 4.3). The lower B concentration in the 1-year old stem (than the current-year stem) was due to the dilution of

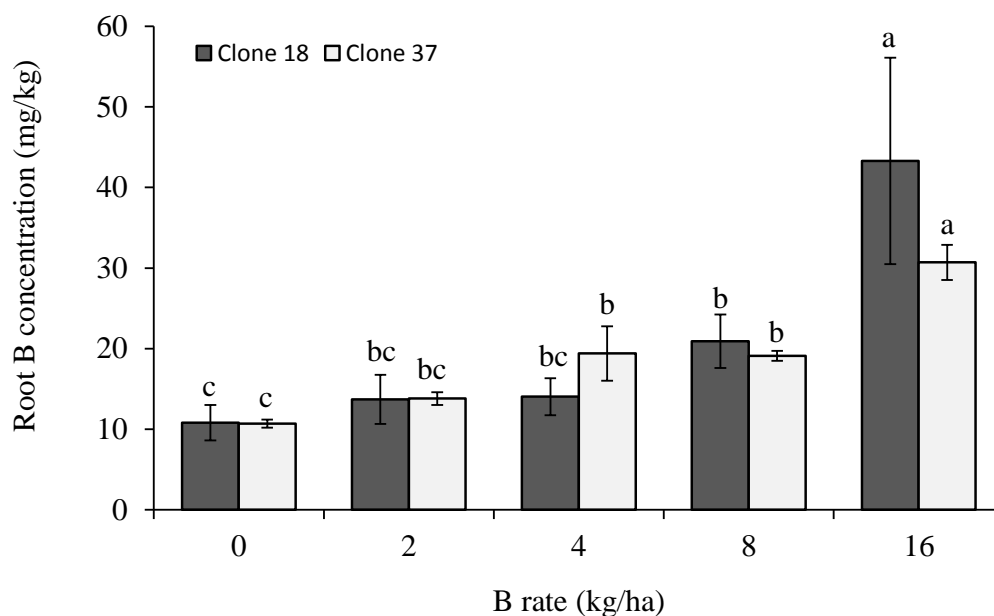
B by the pre-existing biomass before B treatment. The proportional distribution of B in stem tissues emphasizes its role in the biosynthesis of lignin (Lewis, 1980).



**Figure 4.14** Stem B concentrations in one year-old stem (SI), and current-year stem (SC) in Cone 18 (A) and Clone 37 (B). Means for each clone with different letters are significantly different ( $p < 0.05$ )

The significant interaction effect of clone and B treatment for the root B concentration indicates clonal variation in the root B concentration in response to different rates of soil-

applied B. Clone 18 recorded a higher concentration of B in roots than Clone 37 at higher B application rates, especially at 16 kg B/ha (Figure 4.15).



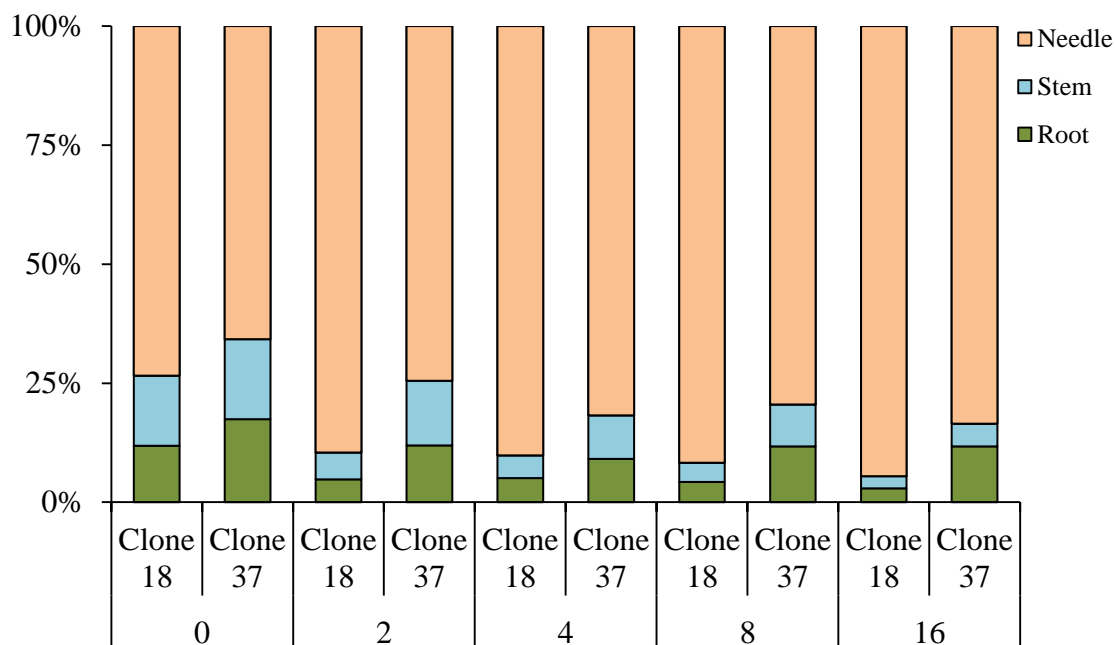
**Figure 4.15** Root B concentrations of Clone 18 and 37, as affected by B application rates. Means for each clone with different letters are significantly different ( $p < 0.05$ )

#### 4.3.3.2 Percentage B distribution

Percentage B distribution (%) in all plant parts (root, stem and needle) of the two clones was greatly influenced by the B treatment rate (Figure 4.16). Needles were found to be the most dominant sink for B accumulation (82% of total B), regardless of clone and B treatment. For both clones, the percentage of total plant B found in needles increased, but decreased in stems and roots as the B application rate increased from 0 to 16 kg B/ha (Figure 4.16). These results suggest that the translocation of B from root to shoot was restricted under the condition of low soil B supply which was progressively overcome with increasing application of B fertiliser supporting the classification of B as an immobile nutrient. This is despite the earlier observation that B under the same condition is preferentially distributed from old to younger needle. This finding confirms previous observations of more B held by



roots under low B supply in Norway spruce (Möttönen et al., 2001), navel orange (Sheng et al., 2009) and canola (Asad et al., 2002) than in aerial biomass. Although similar patterns of % B distribution in plant parts were found for both clones, the magnitude of change in percentage B distribution at different B application rates was larger in Clone 18 than Clone 37 (Figure 4.16). For example, the percentage B distribution in needles increased from 73% at 0 kg B/ha to 90% at 2 kg B/ha for Clone 18, but from 66 % to 75 % at the corresponding treatments for Clone 37 (Figure 4.16). Across all B treatments, Clone 37 retained more B in roots than Clone 18 (12% vs. 6%), and allocated less B into needles (77% vs. 87%). The B distribution results account for the greater susceptibility of Clone 37 to B deficiency and the greater growth response to B fertiliser relative to Clone 18.

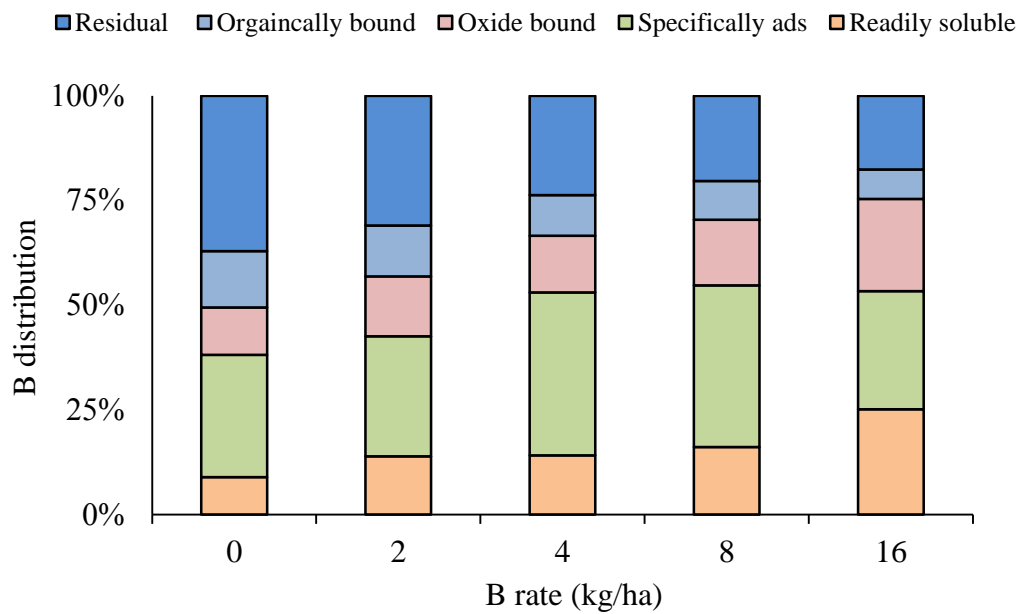


**Figure 4.16** Percentage B distribution in plant parts of two *P. radiata* clones, as affected by B application rates

#### 4.3.4 The distribution of B in soil

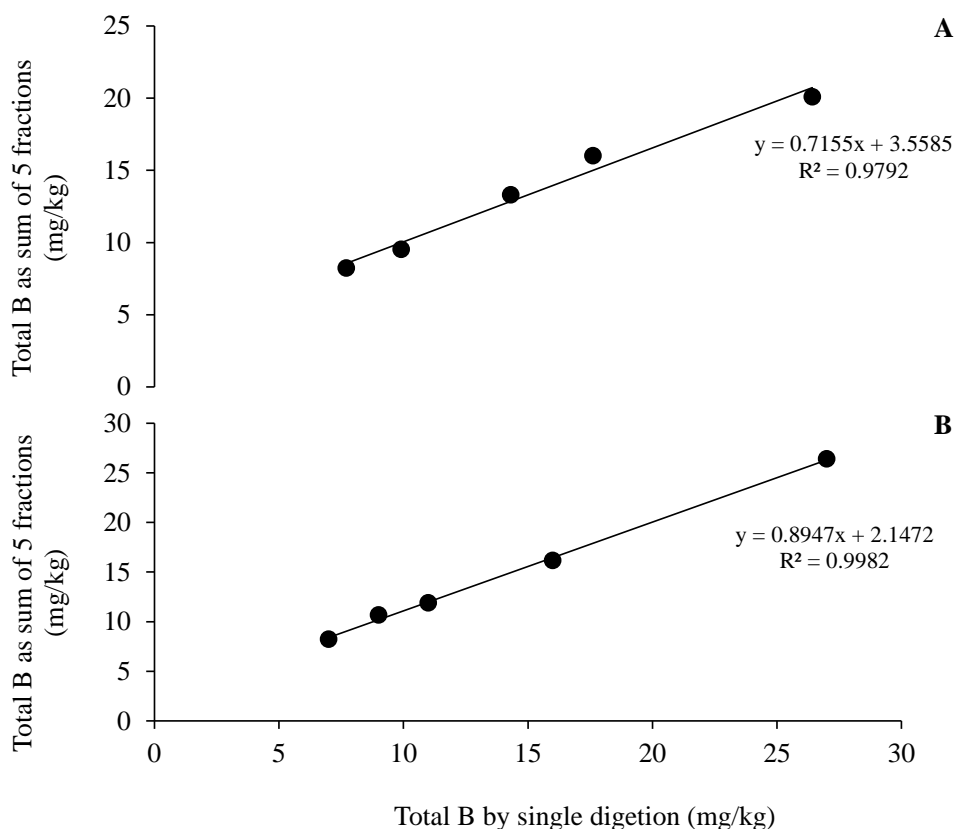
##### 4.3.4.1 Boron fractions

Results from the soil B fractionation study show that the total percentage of B in the readily-soluble form (non specifically-adsorbed B) increased as the B fertiliser application rate increased, with a concomitant decrease in B associated with the residual phase (Figure 4.17). When compared with the control treatment, an increase of up to 3-fold in the percentage of soil B in the plant available phase (i.e. readily-soluble B) was observed as the applied treatment increased to 16 kg B/ha. The amount of B associated with the residual phase declined from 37% to 17% over the same treatment range. This residual B has been shown to not correlate with the level of plant available B in soil (Jin et al., 1987) .



**Figure 4.17 Percentage distribution of B within each of five defined B fractions in soil as a function of B treatment rate**

Correlations between the total soil B concentration determined by single digestion and the sum of five B fractions were strong for soil samples collected under both Clone 18 ( $R^2 > 0.979$ ) and Clone 37 ( $R^2 > 0.998$ ) (Figure 4.18).



**Figure 4.18 Relationship between the sum of five B fractions and total soil B by single digestion at harvest time for (A) Clone 18 and (B) Clone 37**

#### 4.3.4.2 Plant available soil B

The main effects of B and clone and their interactions were significant for plant available soil B as determined by hot 0.02 M  $\text{CaCl}_2$  extraction ( $\text{CaCl}_2\text{-B}$ ) at each of the samples depths of 0-10 and 10-20 cm (Table 4.6). However, only the main treatment effect of B was significant for the  $\text{CaCl}_2$ -extractable B concentration in the bulk soil sampled from the entire pot after harvest. The  $\text{CaCl}_2\text{-B}$  concentration increased with the increasing rate of B fertiliser, regardless of clone and soil depth (Figures 4.19-4.21). This indicates that an increasing supply of B from the fertiliser increased both the relative amount (percentage; Figure 4.16) and the concentration of plant-available soil B. The  $\text{CaCl}_2\text{-B}$  concentration in the control soil was within a range of 0.10-0.25 mg/kg, which is lower than the critical B deficiency value of 0.25 mg/kg reported for *P. radiata* growth (Snowdon, 1982), and lower than the hot water soluble-B deficiency level of 0.5 mg/kg reported by Ryan et al. (1998). For both soil layers

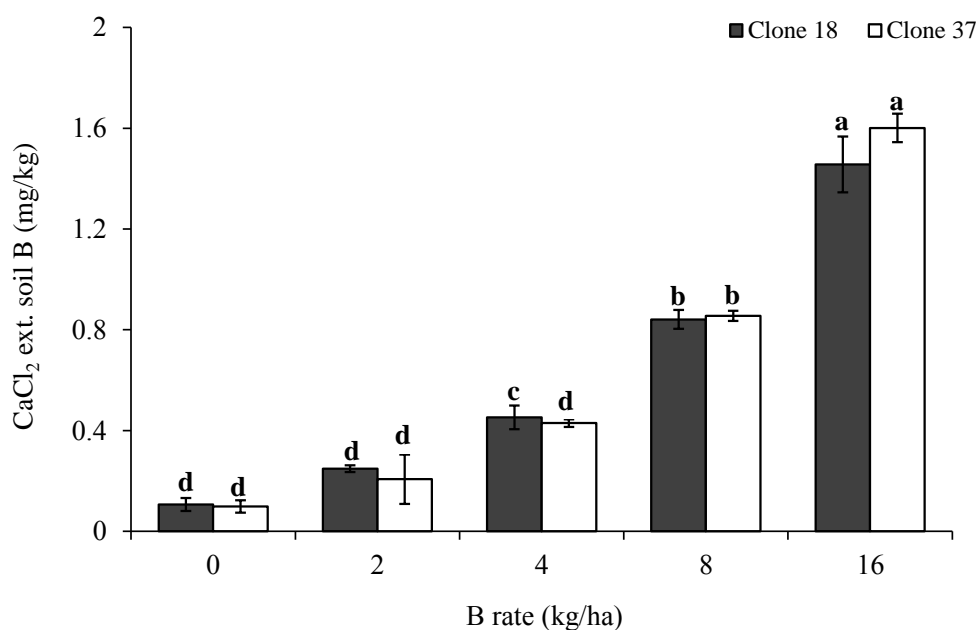
(i.e. 0-10 and 10-20 cm), the  $\text{CaCl}_2\text{-B}$  concentrations were lower in the soil with Clone 37 than with Clone 18, especially for the treatments with 0 and 2 kg B/ha. This implies that Clone 18 might have a greater ability than Clone 37 to facilitate the release of soil or ulexite B for plant uptake. Or it may simply be due to greater B demand for one clone over another. The lower shoot/root ratio (i.e. a relatively larger root system) of Clone 18 relative to Clone 37 at the 0 and 2 kg B/ha treatments (Figure 4.9) could be a result of an acclimatory mechanism of Clone 18 to explore more soil volume and increase B availability in the soil. However, further study will be needed to examine the differences in clonal mechanisms to increase B availability in soil (e.g. through root exudation). Such study would provide further insight into the below-ground response of these clones and the link between plant genotype and soil B availability.

The  $\text{CaCl}_2\text{-B}$  concentration in the depth of 0-10 cm was consistently higher than that in the 10-20 cm for both clones (Figures 4.20-4.21). This may be a function of greater uptake of B by roots below 10 cm depth, or increased dissolution of the ulexite fertiliser into soil solution in the surface soil layer, or greater fractionation of B to a non-labile phase with depth.

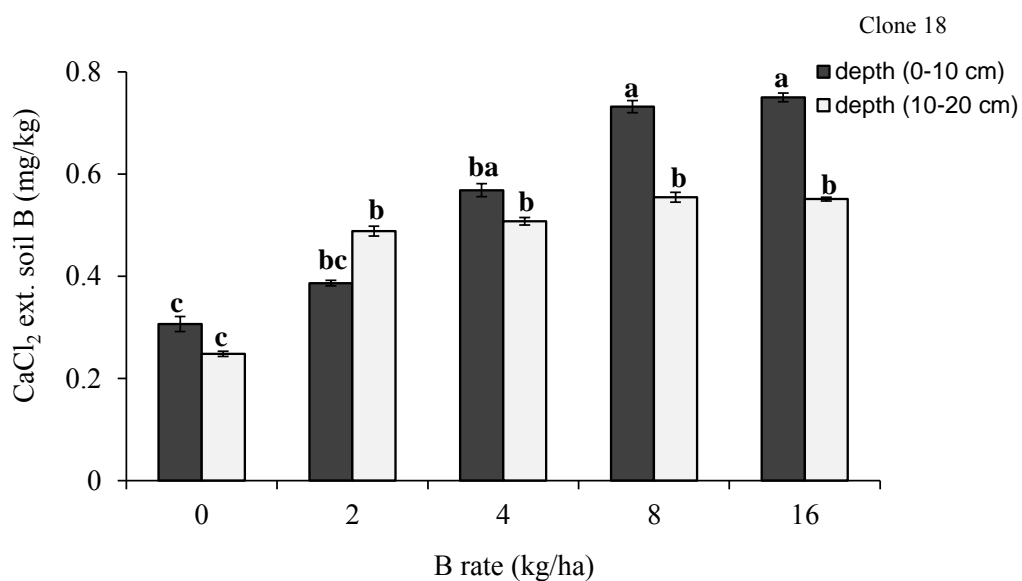
**Table 4.6** *P* values for main and interactive treatment effects on the plant available soil B concentration extracted from different soil depths

Factors	Plant available soil B concentration (mg/kg)		
	Whole (bulk) pot soil	0-10 cm	10-20 cm
B	< 0.0001	< 0.0001	< 0.0001
clone	0.5514	< 0.0001	< 0.0001
B × clone	0.2738	< 0.0001	< 0.0001

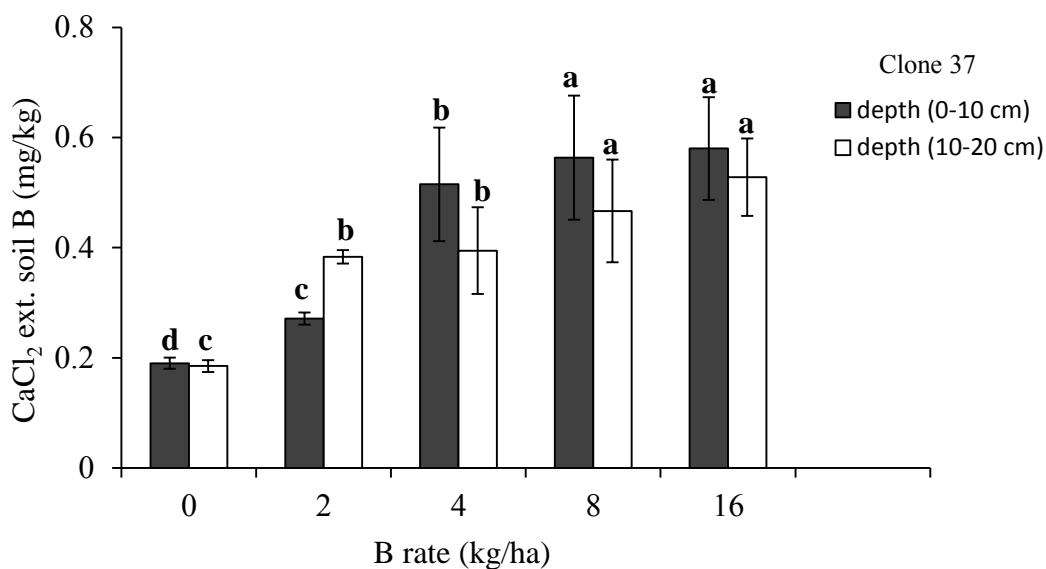
*P* values < 0.05 shown bold



**Figure 4.19** CaCl<sub>2</sub>-extractable B concentration in soil at the time of harvesting Clone 18 and Clone 37. Means for each clone with different letters are significantly different (*p* < 0.05)



**Figure 4.20** CaCl<sub>2</sub>-extractable B concentration in soil under Clone 18 at two sampling depts (0-10 and 10-20 cm) at the time of harvest. Means for each depth with different letters are significantly different ( $p < 0.05$ )



**Figure 4.21** CaCl<sub>2</sub>-extractable B concentration in soil under Clone 37 at two sampling depts (0-10 and 10-20 cm) at the time of harvest. Means for each depth with different letters are significantly different ( $p < 0.05$ )

### 4.3.5 Soil dehydrogenase activity and mycorrhizal colonisation

#### 4.3.5.1 Soil dehydrogenase activity

Dehydrogenases are essential components of the enzyme systems of microorganisms. Soil dehydrogenases are biologically synthesised enzymes, and play a significant role in the biological oxidation of soil organic matter. Their activity can therefore be used as an indicator of biological redox systems and as a measure of microbial metabolic activity in soil (Chander and Brookes, 1991; Skujins, 1973). The main effects of B treatment and clone and their interactions were significant on soil dehydrogenase activity measured in both rhizosphere and non-rhizosphere soils (Table 4.7).

**Table 4.7** *P* values for main and interactive treatment effects on soil dehydrogenase activities both in rhizosphere and non-rhizosphere soils, and on mycorrhizal colonisation

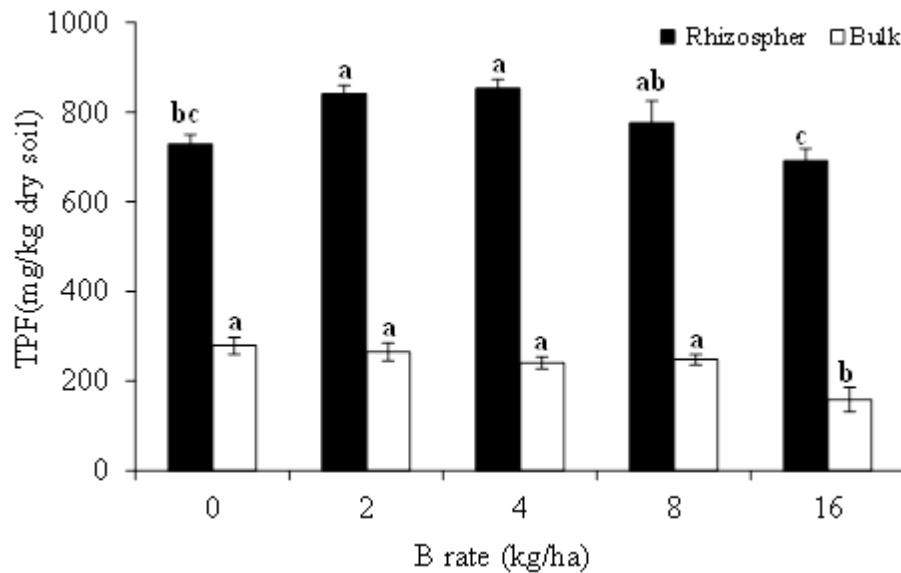
Factors	Soil dehydrogenase activity (mg TPF/kg oven dry soil)		Hyphae count (no/cm root length)
	rhizosphere	Non-rhizosphere	
B	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt; 0.0001</b>
clone	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	0.5121
B × clone	<b>&lt;0.05</b>	<b>&lt;0.01</b>	<b>&lt; 0.0001</b>

*P* values < 0.05 shown bold

NI	The initial needles associated with <i>P. radiata</i> seedlings at the planting time
NCM	Current-year mature needle (needles which emerged after planting and which matured prior to harvest)
NCI	Current-year immature needle (needles which were immature at time of harvest)
SI	The initial (original) stem and branches of the seedlings at the time of planting
SC	Current-year stem and branches which developed during the experiment



Compared to the control treatment, the dehydrogenase activity in the rhizosphere soil (averaged across both clones) was increased by 18-23% for B fertiliser treatments of 2-8 kg/ha, but was reduced at 16 kg/ha to a level the same as the control.



**Figure 4.22** Soil dehydrogenase activities (TPF) in bulk and rhizosphere soils of Clone 18, as affected by different rates of B fertiliser. Means for each soil with different letters are significantly different ( $p < 0.05$ )

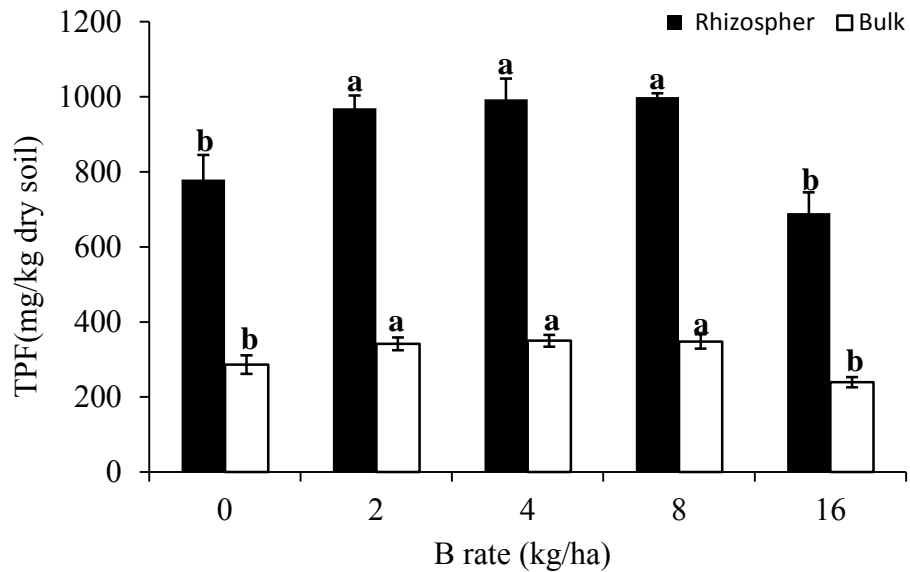
For all treatments, under both clones, the rhizosphere soil showed a greater level of dehydrogenase activity than the bulk soil. The rhizosphere soil under Clone 37 (886 mg TPF/kg) generally had a higher level of soil dehydrogenase activity (averaged across all B treatments) than that under Clone 18 (778 mg TPF/kg). The significant interactive effect of clone and B treatment presented in Table 4.7 indicates clonal variation in the response of dehydrogenase activity to B application rates in the rhizosphere soil. For Clone 18, the dehydrogenase activity in the rhizosphere soil increased as a function of B treatment up to 4 kg/ha and then decreased with further increases of applied B (Figure 4.22). For Clone 37, dehydrogenase activity increased with increasing B up to 8 kg/ha and then decreased at the rate of 16 kg B/ha to an activity the same as that recorded for the control treatment (Figure

4.23). For Clone 37, there was no difference in the dehydrogenase activity of the rhizosphere across the three treatment levels 2, 4 and 8 kg/ha.

The B and clone main effects on the dehydrogenase activity in the bulk soil were similar to those in rhizosphere soil when considered as an average across all treatments. There was clonal variation in the response of dehydrogenase activity in the bulk soil to increasing B application rate. For Clone 18, the dehydrogenase activity in the bulk soil showed a non-significant reduction with increasing B up to 8 kg/ha but a significant reduction in activity at the treatment level of 16 kg B/ha (Figure 4.22). For Clone 37 activity in the bulk soil was equally increased at rates of 2, 4 and 8 kg/ha relative to the control, but decreased to a level equal to the control at a treatment of 8 kg/ha (Figure 4.23).

The dehydrogenase results from the current study confirm the conclusions of a previous study where soil dehydrogenase activity increased through the application of low rates of B to soil, but was inhibited at higher rates of soil B (Serdar et al., 2011). A decrease in soil dehydrogenase activity at high soil B could be an indirect response of soil microbes to high B, as a result of the reduction of microbial diversity and richness at high concentrations of B in the rhizosphere (Nelson and Mele, 2007). Nelson and Mele (2007) proposed that B and salinity were more likely to affect rhizosphere microbial community structure indirectly through an effect on root exudation by plants rather than directly through microbial toxicity.

The increased dehydrogenase activity in rhizosphere soil relative to the bulk soil for each B treatment and both clones (Figures 4.22-4.23) is in agreement with a previous study by Kang and Freeman (2007), who reported that enzyme activities, including dehydrogenase, were higher in rhizosphere soil. However, the underlying mechanisms for the clonal differences in soil dehydrogenase activity in both the rhizosphere and bulk soils are not clear through the findings of the current study.

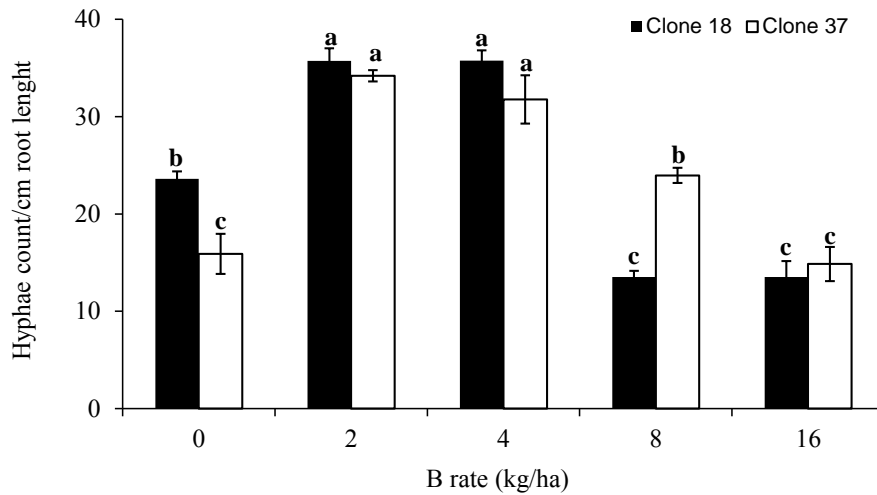


**Figure 4.23** Soil dehydrogenase activities (TPF) in bulk and rhizosphere soils of Clone 37, as affected by different rates of B fertiliser. Means for each soil with different letters are significantly different ( $p < 0.05$ )

#### 4.3.5.2 Mycorrhizal colonisation rate in *Pinus radiata*

The main effect of B treatment and the interactive effect of clone and B were significant for ectomycorrhizal colonisation rate of *P. radiata* roots (Table 4.7; hyphae count). When compared to the control treatment, the application of B at rates of 2-4 kg/ha increased the colonisation rate by around 75% across the two clones, but application of B at 16 kg/ha reduced colonisation by up to 30% (Figure 4.24). There was no significant difference in the ectomycorrhizal colonisation rate between the two clones at a treatment of 8 kg/ha. For Clone 18, the ectomycorrhizal colonisation rate increased with increasing B up to 4 kg/ha and then decreased considerably with any further increase of B fertiliser, while for Clone 37 hyphae count increased with increasing B up to 2 kg/ha and then decreased gradually with all further increases of applied B fertiliser (Figure 4.24). The positive response of ectomycorrhizal colonisation to an application of B at 2-4 kg/ha indicates that B deficiency in the control soil has affected the development of ectomycorrhizal fungal communities. This may be directly associated with the mycorrhizal fungal, or may be due to deficiency experienced by the plant that will indirectly affect the fungi. The negative response of ectomycorrhizal colonisation to higher B supply implies possible direct or indirect B toxicity

to ectomycorrhizal fungal communities at rates above 4 kg B/ha. The differential rate of hyphae count reduction between 4 and 16 kg B/ha indicates a differential response of mycorrhiza of the two clones to B level in the transition concentration from sufficiency to toxicity.



**Figure 4.24** Mycorrhizae hyphae count of two *P. radiata* Clones in response to different B application rates. Means for each clone with different letters are significantly different ( $p < 0.05$ )

An increase in ectomycorrhizal colonisation after B fertilisation has been reported in Norway spruce (*Picea abies*) grown in the field (Lehto, 1994) and in a growth room (Möttönen et al., 2001b), and for *Pinus echinata* seedlings (Mitchell et al., 1986; Mitchell et al., 1990; Mitchell et al., 1987). However, the underlying mechanisms for an enhancement in mycorrhizal formation through B fertilisation are not clear (Lehto et al., 2010). Possible mechanisms for enhanced mycorrhizal formation as a function of B fertilisation may be related to increased carbohydrate allocation to root as a result of increased indole acetic acid production (Lambert et al., 1980), or a reduction of the concentration of phenolic compounds in B-fertilised roots (Marschner, 1995; Sword and Garrett, 1994). However, the observed results may also be a simple function of a certain B requirement for the growth of mycorrhizal fungi (Lehto et al., 2010).

## 4.4 Conclusions

The Pumaceous soil used in this study had a low plant-available soil B concentration (quantified through  $\text{CaCl}_2$  extractable B), that was below the critical soil level for plant available B defined in literature ( $< 0.5 \text{ mg/kg}$ ). The concentration of  $\text{CaCl}_2$ -extractable B increased with increasing rates of B fertiliser, regardless of clone, at both soil depths (0-10 cm and 10-20 cm). Overall, *P. radiata* seedlings responded positively to low rates of B fertiliser (4-8 kg B/ha), but a growth reduction (e.g. height, fresh and dry matter) was observed at the highest rate used in this study (16 kg B/ha). This result supports the view that there is a narrow concentration range between B deficiency and toxicity in plants. Clonal variation was found in the response of the seedlings to different application rates of B, and this was particularly apparent for the dry weight yield of NCI needles and stem, whole-plant total dry biomass, and the shoot/root concentration ratio. Clone 37 (a relatively fast growing Clone of *P. radiata*) was more responsive to lower rates of B (up to 4 kg/ha) but was more susceptible to the inhibition of growth at higher B rates, while Clone 18 (a relatively slow growing Clone) required a higher B rate (c.a. 8 kg/ha) for its optimum growth but was relatively less stimulated by the lower rates of B treatment.

The mature needle B concentration of plant growing in the control soil (no B added) was close to the critical B deficiency concentration of 12 mg/kg for adult *P. radiata*, indicating marginal B deficiency. Boron was preferentially translocated to the upper immature needles under low B conditions, and this is presumably a physiological response to maintain the normal growth of young needles under conditions of limited B supply. Increasing rates of B fertiliser application to the soil linearly increased the B concentration in all plant parts in the following order: NI > NCM > NCI > root > SC > SI, indicating that basal or older needles (e.g. NI) were the largest sinks of B accumulation in plant for both clones. This supports the generally-accepted theory of B being an immobile plant nutrient but this may only be apparent for the scenario where plants are not B deficient. The B concentration in NI needles at 16 kg B/ha value was above the reported critical toxicity value of 250 mg/kg. There were clonal differences in the B concentrations in NI and NCM needles and roots, and in the percentage distribution of total plant B between plant parts. Clone 37 retained more B in roots and allocated less B to needles than Clone 18. This more apparent at the control treatment, implying more susceptible of this clone to B deficiency under conditions of low

soil B and made this clone more responsive to B fertilisation. Clone 18 had a relatively larger root system (as quantified through a lower shoot/root ratio) and therefore showed more efficient translocation of B into shoots. This accounts for an apparent lower relative reduction of growth of this clone under conditions of low soil B, and a lesser response to B fertilisation at the lower application rates relative to Clone 37.

Boron fertilisation enhanced needle photosynthetic rate, ectomycorrhizal colonisation of roots in *P. radiata* and soil dehydrogenase activity when applied at the rates of 2-8 kg/ha. However, higher rates had an inhibitory effect on these parameters. Fertiliser application rates at 2-8 kg/ha are therefore defined as optimal rates of B fertilisation for pumice soil with respect to physiological and soil biological parameters. Clonal differences were also apparent for needle photosynthetic rate, ectomycorrhizal colonisation rate and soil dehydrogenase activity in response to different rates of B fertiliser.

In conclusion, this study demonstrated that B applied at an experimentally determined optimum rate will not only improve *P. radiata* growth, but will also enhance needle photosynthesis, root mycorrhizal formation and soil microbial activity. However, different clones will require different rates of B fertilisation necessary to maximise their growth potential. The findings from this study have important implications on management strategies for B nutrition in *P. radiata* forestry.

# Chapter Five

## Boron adsorption in soils

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### 5.1 Rational of the chapter

The two glasshouse trials described in detail in chapter 2 and 3 established the importance of B fertiliser application in *P. radiata* plantation in New Zealand. The described research further confirmed the narrow range of B sufficiency, and evaluated how increasing the B application rate would effect the plant growth parameters height and diameter, and soil indices such as dehydrogenase activities and mycorrhizae population.

The soil used in both these studies was pumaceous Taupo soil collected from the Central North Island. This soil and area is well known for major radiata plantation in New Zealand. Detailed commentary on the physical and chemical properties of the soil was presented in chapter 2 of thesis.

As both of these studies were confined to the use of the Taupo soil only, the need to assess B behaviour in other important soils, whether they are subject to *P. radiata* planting or not, became apparent. For this purpose, Chapter 5 describes B adsorption studies on a range of soils collected from around the North Island of New Zealand. Although fertilisation is usually carried out on such soils, the extent to which B availability can be influenced by soil variables, and the subsequent effect on plant B availability in each of these was unclear at the outset of the study.

Boron adsorption studies were therefore carried out with an aim to investigate how soil properties could effect B adsorption for each soil, and to estimate and establish relationships between B adsorption parameters such as maximum adsorption and bonding energy constants with soil properties. The research underpinning Chapter 5 also provided information about liming and its effect on B availability. Liming induced B deficiency is a common consequence of silvicultural practice in acidic forest soils.

The work in this chapter emphasizes the importance of using B fertiliser to maintain a soil solution B concentration that will sustain *P. radiata* growth.

## 5.2 Introduction

Adsorption can be defined as the process of accumulation of a substance or material at an interface between a solid surface and the bathing solution (Spark, 1995). Stumm (1992) reported that adsorption can include solute removal from solution by a solid phase and attachment of the solute molecule to the solid surface. From the perspective of studies into plant nutrients, heavy metals, and pesticides residues in soil, adsorption can be considered one of the most important chemical processes that occur in a soil. Milmile et al. (2011) reported that the relationship between the quantity of adsorbate adsorbed onto an adsorbent and the concentration of dissolved adsorbate remaining in solution at equilibrium can be described by adsorption isotherms. Adsorption isotherms are therefore routinely used to describe the relationship between the equilibrium concentration of the adsorbate and the quantity and affinity of this adsorbate onto the surface of adsorbants. Adsorption isotherms are conducted at constant pH and temperature (Sohn and Kim, 2005). Sposito (1984) reported that the shapes of the isotherms could follow 4 different types; S, L, H and C (Table 5.1).

The B concentration in soil solution, which is the over-riding control over plant uptake of B, depends on B adsorption and desorption processes in soil. However, B adsorption and desorption not only depends on B concentration in soil solution but also on a number of soil properties such as pH, the type of exchangeable or adsorption sites present in the soil, soil mineralogy, other anions present in soil solution, organic matter, wetting/drying cycles and ionic strength (Keren and Bingham, 1985).



**Table 5.1 Four types of adsorption isotherm described by Sposito (1984) for soil**

Isotherm type	Features
S-type	slope of the relationship initially increases with adsorbate concentration followed by gradual decrease.
L-type (Langmuir)	exhibits decreasing slope as concentration increases due to non-availability of vacant sites
H-type	known for strong adsorbate-adsorbent interaction
C-type	Adsorptive ions distributed between an interfacial phase and bulk solution without specific bonding between adsorbent and adsorbate

### 5.2.1 Boron adsorption on clay minerals

Hingston (1964) reported that B adsorption to soil is influenced by the B species in solution ( $B(OH)_3$  and  $B(OH)_4^-$ ), the relative dominance of which is a function of pH. At pH less than 7,  $B(OH)_3$  dominates due to the low ionization constant ( $K = 5 \times 10^{-10}$ ) which is replaced by  $B(OH)_4^-$  as pH increases. Boron adsorption as  $B(OH)_4^-$  increases in the pH range from 5-10, but decreases at higher pH due to competition with  $OH^-$ .

The effect of pH on B adsorption to clay minerals can be summarized according to pH-dependant criteria. For pH less than 7,  $B(OH)_3$  is the predominant B species in solution. This species has a low affinity for clay surfaces and correspondingly, less B is adsorbed at this pH range. As the soil solution pH increases to about 9, the species  $B(OH)_4^-$  starts to dominate and B adsorption will increase rapidly. However, a further increase of pH to above 9 will increase the concentration of  $OH^-$  relative to  $B(OH)_4^-$  and B adsorption will begin to diminish due to competition for adsorption sites by the hydroxide ion. Maximum B adsorption onto different adsorbents therefore depends on the ratio of the affinity coefficient

of the three species;  $B(OH)_3$ ,  $B(OH)_4^-$  and  $OH^-$  (Stewart, 1988). In the case of Al hydrous oxide surfaces there is a sharp increase in B adsorption in the pH range 3-5, followed by a sharp decrease above pH 9. The mechanism of variable adsorption as a function of pH is the same for hydrous oxides as reported for crystalline clay minerals.

Keren and Mezuman (1981) proposed that B adsorption occurs on clay edges instead of on the planer surface of the clay minerals. At the edges of the tetrahedral silica and octahedral alumina sheets the crystalline structure is disrupted resulting in areas where the clay minerals expose ions that are not properly coordinated by structural oxygen. Increased B adsorption also corresponds to a decrease in clay particle size which is a direct function of broken clay edges (Keren and Talpaz, 1984). Keren and Gast (1983) reported that the hydrous oxides of Al, Fe and Mg will readily adsorb B with the hydrous oxide of Al showed the greatest affinity at pH 9.5 under the same environmental chemistry conditions (adsorption onto Al hydrous oxides is 7.5 times than that onto Ca-montmorillonite).

Boron can specifically adsorb to the clay minerals illite, montmorillonite and kaolinite. On a weight basis illite is the most reactive of these minerals followed by montmorillonite and kaolinite (Keren and Mezuman, 1981). Boron adsorption onto clay minerals shows a strong pH dependence; pH 5-8.5 for illite, and pH 7-9.5 for montmorillonite. In one detailed study, adsorption onto illite increased from 4.9 to 7.5  $\mu\text{mol/g}$  with a pH increase from 7.4 to 8.5, from 1 to 2.5  $\mu\text{mol/g}$  onto montmorillonite for the same pH increase, and from 1 to 1.49  $\mu\text{mol/g}$  onto kaolinite as the pH increased from 7.4 to 10 (Keren and Mezuman, 1981).

Keren (1982) reported that B adsorption on Ca-montmorillonite was maximum (4.5  $\mu\text{mol/g}$ ) at an ionic strength of 0.36 mmol/L in equilibrium solution.

Keren and Gast (1983) proposed three mechanisms for B adsorption onto soil surfaces; (1) simple exchange of  $B(OH)_4^-$  for  $OH^-$  (2) specific adsorption of  $B(OH)_4^-$  onto Fe and Al hydrous oxides to form polymers, and (3) the formation of borate-diol complexes (Figure 2.3; chapter 2). At high pH where the species  $B(OH)_4^-$  predominates in soil solution, soil surfaces have high net-negative charge consequently repel anions. The fact that B under these conditions will still adsorb to soil surfaces indicates the importance of binding mechanisms 2 and 3. Goldberg et al. (1996) show that B adsorbing sites are essentially specific to B, and are independent of competing anions such as phosphates and sulfates.

There is some evidence that B adsorption will vary as a function of electrical conductivity (EC), although this effect is not consistent across all pH values. Goldberg et al. (2008) studied B adsorption as a function of pH and electrical conductivity (EC), and reported that B adsorption was independent of EC at pH 3-9. However at pH greater than 9, B adsorption increased from solutions having a higher EC.

### **5.2.2 Boron adsorption on organic matter**

Organic matter plays a significant role in B adsorption and will affect the amount of plant-available B in soil. Gupta (1968b) reported a strong positive correlation ( $p < 0.001$ ) between organic matter content and the concentration of hot-water soluble B, as organic matter is a major source of available B in acid soils. Hue et al. (1988) studied Hawaiian soils while comparing two soils of identical mineralogy (both allophanic soils) at the same pH (5), reported increased B adsorption in Maile soil (Organic C = 87.6 g/kg) than Akaka soil (Organic C = 77 g/kg). Organic matter complexes B through dihydroxy and  $\alpha$  hydroxyl carboxylic functional groups (see Chapter 2 section 2.4.5), (Fleming, 1980; Parks and White, 1952). At a soil solution pH less than 7, conditions under which the B species  $B(OH)_3$  will predominate, most adsorption occurs onto hydroxyl and carboxylic groups of organic matter. However, at higher pH ( $pH > 7$ ),  $B(OH)_4^-$  replaces  $B(OH)_3$  and this species shows an affinity to *Cis*-diol groups of organic matter. Further increase in pH will increase the concentration of free  $OH^-$  in soil solution which will compete with B for adsorption sites or lead to the decomposition of B-diol complexes (Gu and Lowe, 1990) resulting in reduced B adsorption. The pH dependency of B on organic matter therefore follows that for adsorption on clay minerals. As relatively little B is adsorbed on the mineral fraction at low pH, organic matter serves to be one of the main sources of B in acid soils (Okazaki and Chao, 1968).

The role of organic matter in B nutrient cycling is two-fold. Organic matter adsorbs B from solution and makes it less available to plants. But B adsorbed to organic matter can also be mineralised, releasing B to solution and thereby increasing B availability. Boron released from organic matter upon mineralization remains either in soil solution or is adsorbed onto other soil colloidal surfaces (Gupta et al., 1985). The concentration of B in soil solution is important from the plant growth standpoint as plants respond to B only in soil solution

(Keren and Bingham, 1985). Organic matter holds a large part of total soil B, and the release of this nutrient depends on microbial decomposition of organic matter which is influenced by soil moisture conditions. Nonetheless, the process of B adsorption by organic matter dominates in comparison to B release from organic matter, and therefore organic matter can be regarded as a B sink rather than source.

Boron will also interact with organic ligands in soil solution forming various dissolved complexes (Gu and Lowe, 1990). Recently, Communar and Keren (2008) reported that both B species;  $B(OH)_3$  and  $B(OH)_4^-$  complexed with dissolved organic matter (DOM) in sewage effluent used for irrigation purposes. The stability of the B-OM complex is again a function of pH, reaching a maximum at pH 9.3.

Lemarchand et al. (2005) reported that carboxylic and phenolic functional groups present on dissolved organic matter (DOM) are strong  $B(OH)_3$  complexation sites. The pH dependency of the interaction of B with DOM, hydrous oxides and clay minerals is almost the same (Mezuman and Keren, 1981). However, the stronger adsorption affinity of organic matter provides for a stronger B-organic matter interaction relative to the interaction of B with clay minerals (Yermiyaho et al., 1988; Yermiyahu et al., 2001). This stronger affinity of organic matter towards B (Yermiyahu et al., 1995) can be ascribed to the outstanding capacity of organic matter to form B-organic matter complexes with both  $B(OH)_3$  and  $B(OH)_4^-$  across a wide range of pH. Boric acid-carboxyl and boric acid-hydroxyl complexes dominant at low soil solution pH (less than 7.8), but these are replaced by borate-hydroxyl complexes as pH increases from 7.8 to 9.5 (Communar and Keren, 2008). The strong B-organic matter interaction has been implicated in B deficiency in plants reported for soils with a high organic matter contents (Hue et al., 1988). Sharma et al. (2006) reported that organic matter addition to soil will increase B adsorption. Interestingly, complete removal of organic matter instead of decreasing adsorption has been reported to increase B adsorption. This may be explained through the exposure of Fe and Al hydrous oxides as organic matter is removed, and the subsequent availability of these surfaces for further B adsorption (Marzadori et al., 1991; Sarkar and Das, 1990).

### 5.2.3 Modelling boron adsorption

The relative distribution of B between solid and solution phases in soil can be investigated by using either Langmuir or Freundlich adsorption isotherm models (Datta and Bhadoria, 1999). Diana et al. (2010) reported Langmuir maximum adsorption values of 112.36 and 185.19 mg B/kg were found for the organo-mineral and mycorrhiza inoculum treatments, respectively, while Freundlich constants related to adsorption capacity (K-calculated by equation) were found to be 93.14 and 111.88 L/mg for the organo-mineral and wine distillation residues, respectively. The exchangeable cation  $\text{Na}^+$  was negatively correlated with the Langmuir maximum adsorption ( $r = -0.742$ ), Eadi-Hofstee maximum adsorption ( $r = -0.648$ ), and Freundlich  $X_m$  (Freundlich constant related to adsorption capacity calculated by equation) ( $r = -0.552$ ). The sodium ion as a monovalent cation is loosely held in soil by cation exchange processes explaining its antagonistic relationship with B (see Chapter 2). Arora and Chahal (2007) applied these three isotherms to B adsorption (1-100 mg B/L) to four soil samples, collected from arid and semi-arid zones having a pH range of 8.15-8.8, and reported that B adsorption increased as the solution B concentration increased, but that the percentage of B adsorbed decreased accordingly. Freundlich 'K' ranged from 0.247 to 0.684 mg/kg, and the Langmuir maximum adsorption ranged from 4.168-15.873 mg B/kg. This study confirmed a positive correlation between B adsorption and organic carbon content ( $r = 0.998$ ), clay content (0.99) and CEC ( $r = 0.90$ ).

In most studies, B adsorption has been satisfactorily described by Langmuir or Freundlich models. However, Gupta et al. (1985) reported deviation from the Langmuir isotherm at higher concentration. Evans and Sparks (1983) reported that for a B concentration greater than 30 mg/L the isotherm deviates from the expected model (Evans and Sparks, 1983). Such behaviour can be attributed to multisite B adsorption at high concentration (Hatcher et al., 1967). Elrashidi (1982) studied B adsorption on 10 soils from New Mexico and reported that seven soils confirmed to the Langmuir adsorption isotherm over a limited concentration range and all 10 soils followed a Freundlich adsorption isotherm over the entire concentration range tested. In this study, Langmuir maximum adsorption (b) ranged from 2.27-33.90  $\mu\text{g/g}$  soil, and the bonding energy 'K' ranged from 0.03-0.63 ml/ $\mu\text{g}$ , while the Freundlich bonding energy 'K' values ranged between 0.08-3.99.

Despite their use in modelling B adsorption, Langmuir and Freundlich isotherms can neither predict B adsorption as a function of pH nor take into account the nature of surface reaction (Gupta et al., 1985). Some researchers (Keren and Gast, 1981) have therefore chose to describe B adsorption using phenomenological adsorption equations (PAE) based on the assumption that  $B(OH)_3$ ,  $B(OH)_4^-$  and  $OH^-$  all compete for the same adsorption sites (Keren and Mezuman, 1981). In such a case the adsorption coefficient is determined using the total amount of adsorbed B versus the equilibrium B concentration.

Soil properties play an important role in soil B adsorption. Datta and Bhadoria (1999) studied 25 soil samples (0-25 cm) with a pH range of 4.99-6.38 collected from lateritic and alluvial tracts of southwest Bengal, India. Multiple regression analyses showed that 93 % of the variation in the amount of adsorbed B corresponded to CEC,  $Fe_2O_3$ , pH, organic C and clay contents. Soil properties influenced B adsorption in the order:  $Fe_2O_3 > clay > organic C > pH > CEC$  (correlation coefficient values of 0.59, 0.46, 0.37, 0.31 and 0.20, respectively). They associated B adsorption to silicate and Fe-Mg minerals as reported earlier (Evans and Sparks, 1983; Rhoades et al., 1970). Multiple regression equations showed that more than 80 % of variability in the Langmuir adsorption maximum (b) was attributed to CEC, pH, Organic C, and clay, whereas 76 % of variability in the Langmuir bonding energy (K) was associated to  $Fe_2O_3$  and clay, and 90 % of variation in the Freundlich 'K' ascribed to CEC,  $Fe_2O_3$  and clay content.

Alleoni and De Camargo (2000) studied B adsorption on five acidic soils collected from the state of Sao Paulo, Brazil (Rhodic Hapludox, Arenic Hapludox, and 3 Typic Hapludox). They found that  $CaCO_3$  enhanced B adsorption in all soils particularly those with a coarse texture. High correlation coefficients were found between adsorbed B and clay content ( $r = 0.79$ ), amorphous aluminium oxides content ( $r = 0.76$ ) and specific surface area ( $r = 0.73$ ). Bingham et al. (1971) conducted a B adsorption study on 4 Mexican soils formed on andesitic volcanic ash (classified as a Hydric Dystrandepsts in the US soil classification system) and 6 Hawaiian soils formed on basic volcanic ash (classified as Typic Hydrandepst in the US system). This study found a significant correlation between the amount of B adsorbed and the  $Al_2O_3$  content in both the Hawaiian ( $r = 0.94$ ), and Mexican ( $r = 0.74$ ) soils.

To investigate B adsorption behaviour in a range of important soils from the North Island of New Zealand, a laboratory study were carried out. The two most important adsorption

isotherms; Freundlich and Langmuir were used for this purpose. Soil chemical properties such as the concentration of crystalline and amorphous Fe oxide and Al oxy-hydroxides along with particle size distribution were used to explain the adsorption isotherm parameters maximum adsorption (b) and binding energy constant (k).

#### **5.2.4 Study objectives**

Objectives set for the studies in this chapter were to evaluate B adsorption on benchmark soils of New Zealand with special focus on soils under *P. radiata* plantation, to model B adsorption using Langmuir and Freundlich isotherms to investigate the influence of soil properties in controlling B availability, and to study the effect of varying pH on B adsorption in selected soils.

### **5.3 Materials and methods**

The soils used for this phase of the research study were collected from different locations in New Zealand (Table 5.2), and analysed by Dr. P Jeyakumar (Jeyakumar, 2010). The soil samples were collected from the soil surface horizon (0-10 cm) passed through a 2-mm stainless steel sieve while moist and then air-dried, homogenized, labelled, and stored for the analysis of different chemical and physical properties.

#### **5.3.1 Chemical analysis**

The total concentration of crystalline free Fe and Al oxides, amorphous Fe and Al oxy-hydroxides and exchangeable Fe and Al was extracted from soil sub samples using the dithionite-citrate Fe and Al extraction method described by Blakemore et al. (1987). The method followed the sequence of adding 50 ml of 22% Na citrate solution to 1 g soil in a centrifuge bottle then shaking overnight (16 h), followed by the addition of 5 drops of 0.2% surfloc, vigorous shaking and finally filtration through Whatman-42 filter paper. Iron and Al in the filtrate were analysed by atomic adsorption spectrophotometry (AAS).

Amorphous Fe and Al oxides were extracted by acid oxalate. In this method, 100 ml acid oxalate was added to 1 g soil in a 250 ml centrifuge bottle, shaken for 4 h (in the dark), and filtered through Whatman-42 filter paper. After dilution of the filtrates, the Fe and Al concentration in the filtrate were measured by AAS.

Extractable Fe and Al were determined by extraction as described by McLean et al. (1958), adding 50 ml 1 N  $\text{NH}_4\text{OAC}$  (pH 4.8) to 10 g soil in a beaker. The suspension was mixed well and laid to rest for 2 h. The solution was then filtered and diluted to 100 ml with  $\text{NH}_4\text{OAC}$  followed by mixing again and absorbance read on spectrophotometer at 535 nm wave length.



**Table 5.2 Description of the soil used in this study after Hewitt (1998)**

Soil Order	Occurrences	Site location	Properties			
			Physical	Chemical	Depth (cm)	NZ Land cover (%)
Recent Soil	Throughout New Zealand on young land surfaces (alluvial plains and unstable steep slope)	Manawatu	Weak soil development, and exposed to soil erosion	High base saturation, and low P retention capacity	0-10	6
Ultic red Soil	Most commonly in northern North Island, Wellington, Marlborough and Nelson districts.	Tokomoru	Slow permeability due to clayey sub-soil	Strongly acidic soils, High Al in B horizon Cause root inhibition. Medium-high CEC	0-10	3
Pallic Soil	Found in seasonally dry eastern parts of North and South Islands, and lower North Island (Manawatu)	Palmerston North	Characterized by droughty summer and moist winter	Low concentration of secondary Fe oxides, high base saturation. Low S, and organic matter contents	0-10	12
Gley Soil	Areas with high ground water-table or where seepage dominates	Carnarvon	Poorly drained, high groundwater-table and bulk density and shallow rooting depth	High organic matter contents, soil has segregated Fe and Mn oxides	0-10	3
Brown Soil	Most extensively occur where summer dryness is not common and where no water logging occurs in winter	Palmerston North	Stable topsoils	Low-moderate base saturation, illite and vermiculite clay minerals dominate	0-10	43
Allophanic Soil	Form from North Island volcanic ash, in climate where rainfall > 1000 mm	Taranaki	Low bulk density. Highly permeability and high water retention	Large affinity for P and low base saturation	0-10	5
Pumice Soil	On pumacious relatively young volcanic ash in the Central North Island, along the volcanic plateau. The extensive <i>P. radiata</i> plantations in Central North Island are mainly developed on Pumice	Taupo	Low clay contents (< 10 %), and low soil strength and high porosity	High to moderate P retention. Deficient of trace elements particularly B	0-10	7



The crystalline Fe and Al oxy-hydroxides concentration was determined by subtraction of the amorphous Fe and Al oxide and exchangeable Fe and Al concentrations from the dithionate-citrate extractable Fe and Al concentrations. Similarly, the amorphous Fe and Al oxy-hydroxides concentrations were determined by subtracting the exchangeable Fe and Al concentrations from the acid oxalate Fe and Al concentrations.

Cation exchange capacity (CEC) was determined as described by Blakemore et al. (1987), by mixing 1 g air-dry soil (< 2 mm) with 2 g acid-washed silica sand into leaching tube. The tubes were leached with 50 ml  $\text{NH}_4\text{OAC}$  (1 M; pH 7), rinsed with ethanol, and then re-leached with water, 45 ml of  $\text{NaCl}$  (1 M). The  $\text{NaCl}$  leachate was collected, diluted to 50 ml with water, and the concentration of cations ( $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Na}^+$ ) were determined by atomic adsorption concentration were expressed as meq/100 g soil and summed to quantify CEC. Organic C was determined by LECO CR-412 carbon analyser

### **5.3.2 Particle size distribution**

The particle size distribution of each soil was determined using the ultrasonic dispersion method and the pipette method as described by Claydon (1989).

### **5.3.3 Boron adsorption batch study**

Adsorption studies were conducted by way of batch systems. Soil samples (1g) in 100-ml polypropylene centrifuge tubes were equilibrated with 20 ml 0.01 M  $\text{CaCl}_2$  solution containing varying concentrations of B (i.e. 0.5, 2, 3, 5, 10 and 15 mg B/L) as  $\text{B}(\text{OH})_3$ . The centrifuge tubes were first equilibrated on an end-over-end shaker for 48 hours at room temperature, followed by centrifugation (4000 rpm for 20 minutes), and filtration through Whatman-42 filter paper. The supernatants were analysed for B using the modified Azomethine-H method (Gaines and Mitchell, 1979) where aliquots of the filtrate were reacted with Azomethine-H at pH 5.1 buffered with glacial acetic acid and ammonium acetate, and the absorbance read at 420 nm using a PU 8625 UV/VIS spectrophotometer. Boron adsorbed to the soil was calculated by the difference between the amount of initially added B and the amount of B left in supernatant solution after subtracting the native B concentration in the soil.

The B adsorption isotherm was described using Langmuir and Freundlich adsorption isotherm models. The Langmuir isotherm model was originally formulated to study gas adsorption onto a planar solid surface (Langmuir, 1918) and assumes that sorbate forms only a single layer. However, in the current study the Langmuir isotherm equation (Equation 5.2) was adjusted to determine solute sorption onto soil by replacing the  $p$  (the gas pressure term) with  $C$  (solution concentration). Such replacement helps obtain an isotherm for solution in equilibrium with a known amount of solid (Campbell and Davies, 1995). The Langmuir adsorption model was converted to a linear form with Equation 5.2.

$$C/(x/m) = 1/Kb + C/b \text{ (Bohn et al., 1985)} \quad \text{Equation (5.2)}$$

where  $C$  (mg/L) is the equilibrium concentration of adsorbate (B),  $x/m$  (mg/kg) is the amount of adsorbate (B) adsorbed per unit mass of soil (adsorbent) at equilibrium while  $b$  (mg/kg) and  $K$  (L/mg) are the Langmuir constants related to the adsorption capacity (maximum adsorption) and energy of adsorption, respectively. The Langmuir isotherm was constructed by plotting  $C/(x/m)$  against  $C$ . The adsorption maximum ‘ $b$ ’ was calculated as the reciprocal of the slope of the line obtained by plotting  $C/(x/m)$  against  $C$ . The bonding energy coefficient ( $K$ ) was calculated as the slope divided by the intercept.

Though widely used to describe adsorption in soil, Sparks (1995) reported that the Langmuir adsorption isotherm cannot deliver mechanistic information of the system under study. Therefore, another widely used adsorption model, the Freundlich isotherm, was also used to study B adsorption onto soil. The linearized form of the Freundlich isotherm is shown (Equation 5.3).

$$\log X = n \log C + \log K \quad \text{Equation (5.3)}$$

where the slope and intercept of the mathematical model,  $n$  and  $K$  respectively, are empirical constants correlated with intensity and capacity. Huang (1980) reported ‘ $n$ ’ to be a bonding energy-related term in the Freundlich isotherm. One of the major disadvantages of the Freundlich isotherm is that it cannot predict an adsorption maximum. Therefore, Equation 5.3 does not allow for the calculation of adsorption maximum for the data set in the current research. However a high correlation ( $r = 0.984$ ) between  $K$  and soil

maximum adsorption capacity shows that Freundlich adsorption isotherm can give a relative measure of soil adsorption capacity (Mead, 1981).

All adsorption determinations on soils for the current study were conducted in triplicate. Mean values were used for adsorption analysis.

#### **5.3.4 Boron adsorption at varying pH**

Boron adsorption in response to different suspension pH was determined in 6 soils (Recent Soil, Gley Soil, Pallic Soil, Allophanic Soil, Pumice and Ultic Red Soil) of this study at pH 2.0, 4.0, 7.8, 8.5, 9.6 and 11.5. The pH of an extracting solution containing 5 mg B/L was adjusted using dilute HCl and NaOH and equilibrated with 1 g of soil on an end-over-end shaker for 48 hours at room temperature ( $25 \pm 2^\circ \text{C}$ ). Following centrifugation (4000 rpm for 20 min.), and filtration of the suspension through Whatman-42, the concentration of B in solution was analysed using the Azomethine-H method. The amount of B adsorbed at the different pHs was determined by the difference between the amount of B added to the soils and the amount left in solution at the end of the adsorption period.

#### **5.4 Data analysis**

Data analysis was carried out using Microsoft Excel 2007. Simple linear correlation and Pearson's correlation matrix was conducted among the soil properties. Statistical analysis of the B adsorption data set was conducted using SAS<sup>®</sup> (SAS Institute Inc. 2004) to determine the soil properties that influence B adsorption on soils. pH-B adsorption curves were drawn using SigmaPlot<sup>®</sup> (Stat software, Inc. 2008).

## **5.5 Results and Discussion**

### **5.5.1 Soil chemical and physical properties**

Selected soil chemical and physical properties including pH, CEC, organic C, amorphous Al and Fe, crystalline Fe, Al oxy-hydroxides and soil particle size distribution are listed in Table 5.3. Soil pH ranged from 4.9 to 5.9, with the Ultic Red Soil from Tangahoe in Northland having the lowest pH. The CEC ranged from 15.9 to 22 meq/100 g soil. The highest value of 22 meq/100 g soil was obtained for the Pallic Soil sampled from Massey University. Organic C ranged from 0.92% in the Ultic red Soil to 11.2% in the Allophanic Soil collected from Taranaki.

Amorphous Al and Fe oxides (Al-Am, Fe-Am) ranged from 0.15 to 2.79% and 0.37 to 3.02%, respectively, while crystalline Fe, Al oxy-hydroxides (Al-Cr and Fe-Cr) ranged 0.04-4.19 % and 0.03-5.81 %, respectively. The pale colour of the Pallic Soil and the red colour in Ultic Red Soil are attributed to low and high content of Fe oxide, respectively.

**Table 5.3 Physical and Chemical properties of soils from Jeyakumar (2010)**

Soil Site	Soil Classification*	pH	CEC (meq/100g)	Organic C (%)	Amorphous (%)		Crystalline (%)		Particle Size distribution (%)		
					Al	Fe	Al	Fe	Sand	Silt	Clay
1. Manawatu silt loam, Palmerston North	Recent Soil	5.9	11.6	1.22	0.15	0.37	0.04	0.15	74.0	15.5	10.5
2. Tangahoe, Northland Tokomaru silt loam, No. 4	Ultic Red Soil	4.9	15.0	0.92	0.55	1.21	4.17	5.81	3.5	44.0	52.5
3. dairy farm Massey University Palmerston North	Pallic Soil	5.9	22.1	3.56	0.20	0.39	0.15	0.22	59.5	28.0	12.5
4. Carnarvon black sandy loam	Gley Soil	5.6	19.0	4.33	0.20	0.51	0.34	0.32	57.0	31.0	12.0
5. Tuapaka farm, Palmerston North	Brown Soil	5.3	20.5	4.79	0.43	0.76	1.03	0.40	5.0	72.5	22.5
6. Egmont silt loam, Taranaki	Allophanic Soil	5.3	21.6	11.20	2.79	3.02	2.38	0.03	12.0	56.5	31.5
7. Taupo	Pumice Soil	5.7	13.6	2.79	2.13	0.99	0.37	0.25	34.0	45.5	20.5

\*(Hewitt, 1998)

## 5.5.2 Correlation between soil properties

Simple linear correlations between each pair of the soil properties are presented in Table 5.4. It is evident that soil pH had a significant correlation with AlCr, FeCr, silt and clay content and that the correlation coefficients were negative. Organic C was positively and significantly related only with FeAm content. FeAm was positively related with Al-Am content, and FeCr was positively correlated with AlCr and clay content.

**Table 5.4 Simple linear correlation coefficients between soil properties**

	pH	CEC	Org C	Alam	Feam	AlCr	FeCr	Silt
<b>CEC</b>	-0.06							
<b>Org C</b>	-0.149	0.680*						
<b>ALAm</b>	-0.274	0.56	0.64					
<b>FeAm</b>	-0.544	0.271	0.785*	0.852**				
<b>AlCr</b>	-0.935***	-0.009	0.105	0.286	0.585			
<b>FeCr</b>	-0.744*	-0.287	-0.432	-0.145	0.703	0.837**		
<b>Sand</b>	0.90*	-0.163	-0.294	-0.486	-0.615	-0.768*	-0.487	
<b>Silt</b>	-0.681*	0.322	0.433	0.490	0.530	0.441	0.111	
<b>Clay</b>	-0.936***	-0.099	0.018	0.335	0.545	0.978***	0.848**	0.51

\* $p < 0.05$       \*\*  $p < 0.01$       \*\*\*  $p < 0.001$

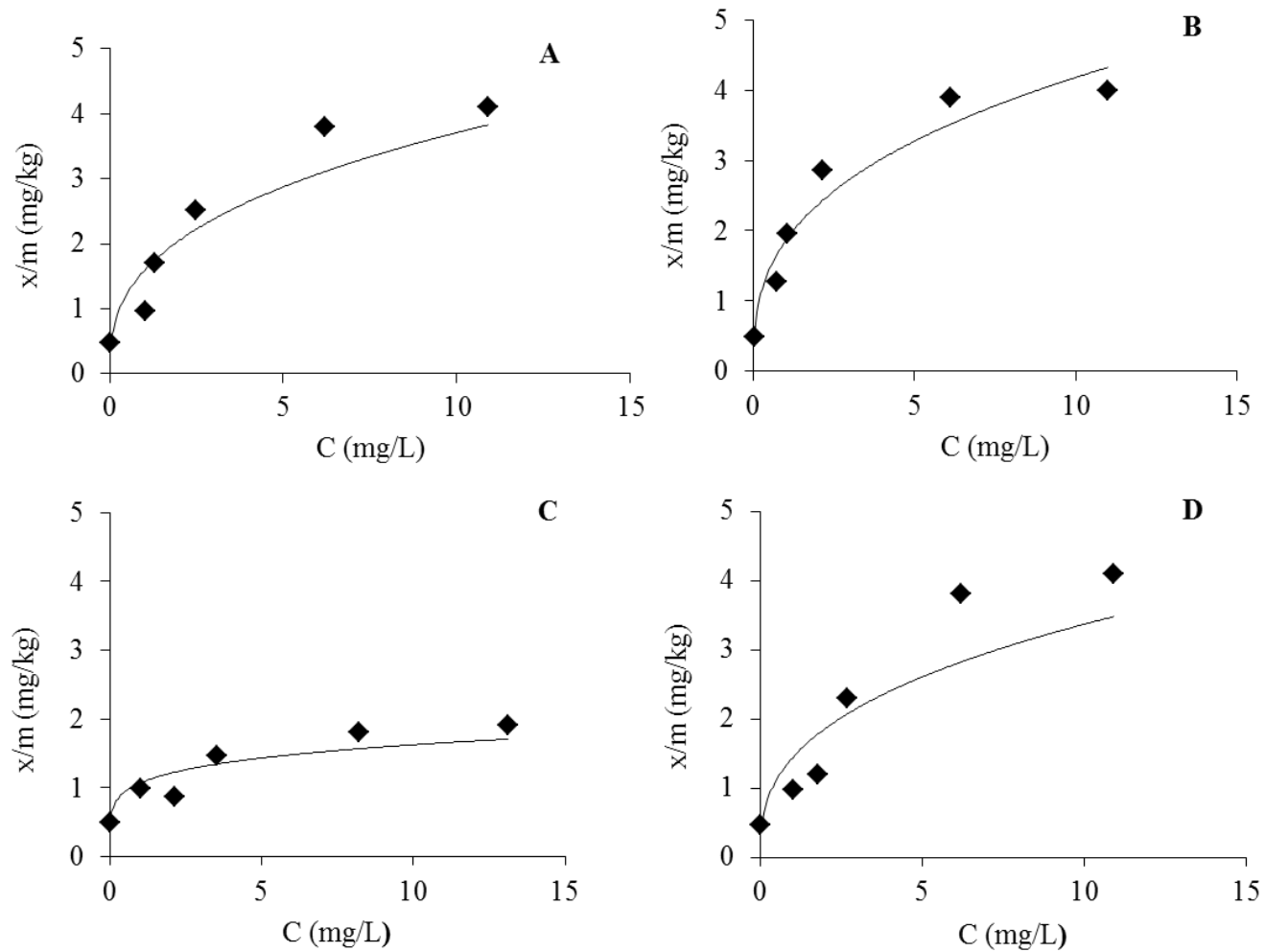
CEC	Cation exchange capacity	b	Langmuir maximum adsorption
OrgC	Organic Carbon	AlCr	Crystalline-Al
Alam	Amorphous-Al	FeCr	Cystalline-Fe
Feam	Amorphous-Fe		
AlCr	Crystalline-Al		
FeCr	Cystalline-Fe		



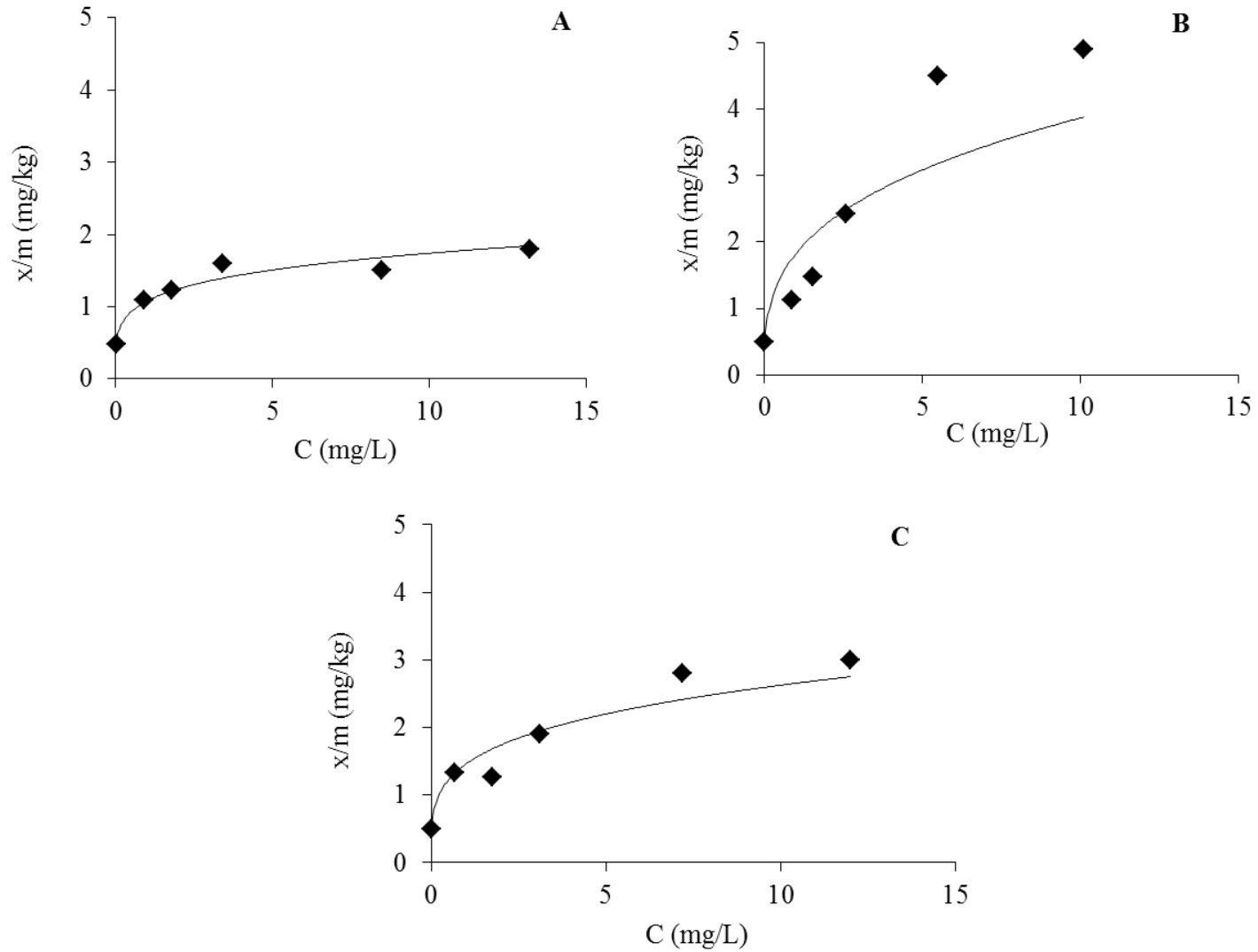
### 5.5.3 Adsorption isotherms

Boron adsorption (expressed as  $x/m$ ) as a function of equilibrium concentration ( $C$ ) for the 7 soils is presented in Figures 5.1-5.2. At low B concentration, B adsorption increased linearly as the B concentration in equilibrium solution increased, demonstrating that all soils tested in this study have a strong affinity for B adsorption. Interpretation of the results shows that the adsorption isotherms can be divided into a high affinity zone where B adsorption increased at a faster rate with increasing B concentration in equilibrium solution, and a low affinity zone where there is an apparent low rate of B adsorption with further increase of B concentration in the equilibrium solution (Figure 5.3). The faster rate of adsorption at low B concentration is due to the availability of a greater number of vacant adsorption sites in the soils relative to those that exist when the B concentration in solution is high.

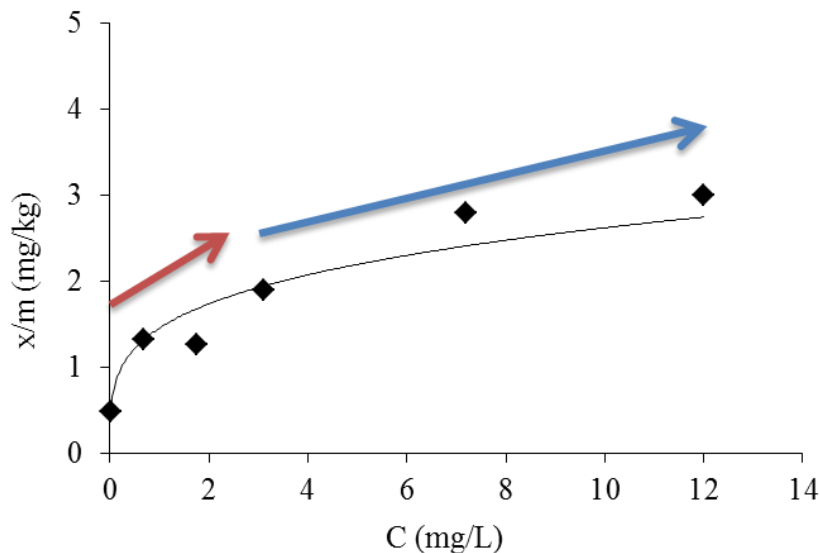
The schematic Figure 5.3 shows an initial steep slope that decreases steadily towards a zero slope, representing the zone of adsorption maximum. As discussed earlier adsorption isotherms showing such behaviour are classified as L-type (non-linear) or Langmuir adsorption isotherms.



**Figure 5.1 Relationship between B in equilibrium solution and B adsorbed on (A) Recent Soil (B) Ultic Red Soil (C) Pallic Soil and (D) Gley Soil**



**Figure 5.2** Relationship between B in equilibrium solution and B adsorbed on (A) Brown Soil (B) Allophanic Soil (C) Pumice Soil



**Figure 5.3 Schematic transformation of an adsorption isotherm from a non-linear (red) portion to a linear (blue) portion as the equilibrium B concentration increases**

#### 5.5.4 Adsorption isotherms model: Langumir versus Freundlich

##### 5.5.4.1 The Langmuir adsorption model

The coefficient of determination ( $r^2$ ) for the Langmuir isotherm ranges from 0.81 to 0.98 (Figures 5.4-5.5; Table 5.5). The Langmuir isotherm gave a good fit for all 7 soils. The data fit was found to be very highly significant ( $p < 0.001$ ) for the Ultic red, Brown, Pallic and Pumice Soils, highly significant ( $p < 0.01$ ) for the Recent Soil, and significant for the Gley Soil ( $p < 0.05$ ). Statistical interpretation of the data showed that the Langmuir isotherm modelled B adsorption well in all soils.

The values of calculated bonding energy 'K' and calculated adsorption maxima 'b' for the soils presented in Table 5.5, show that 'K' varied from 0.29 to 1.78 with a mean value of 0.82 L/mg. These values are higher than those reported by Singh et al. (1987), Mondal et al. (1993) and Biggar and Fireman (1960), who reported mean values of 0.410, 0.113 and 0.720, respectively for B on a range of soils. The high values in this study indicate capacity of each soil to retain B against leaching in the case of high annual rainfall. The value of adsorption maxima 'b' for the soils used in this study (Table 5.5) ranged from 1.78 to 6.26

mg B/kg soil with a mean value of 4.8 mg/kg. The highest value was recorded for the Allophanic Soil while the lowest was recorded for the Brown Soil. The values of 'b' reported in this study are much lower than those reported by Evans (1987), Mondal et al. (1993), Ryan (1989) and Elrashidi and O'Connor (1982) who reported values ranging between 6.4 to 78.7, 4.8 to 95.59, and 13.6 to 52.9, and 2.27 to 33.90 mg/kg, respectively and having mean values of 25.8, 21.5, 40.1 and 12.30 mg/kg respectively. The low B adsorptive capacity of the New Zealand soils of the current study is ascribed to B deficiency in these soils. The maximum value for the Allophanic soil can be related to the high amorphous alumina-silicate mineral content of the soil derived from volcanic ash in the Taranaki district. Schalscha et al. (1973) also reported that a high amount of B is adsorbed by volcanic ash derived Allophanic soils in southern Chile and that is contributed by a high B affinity for amorphous aluminum-silicate.

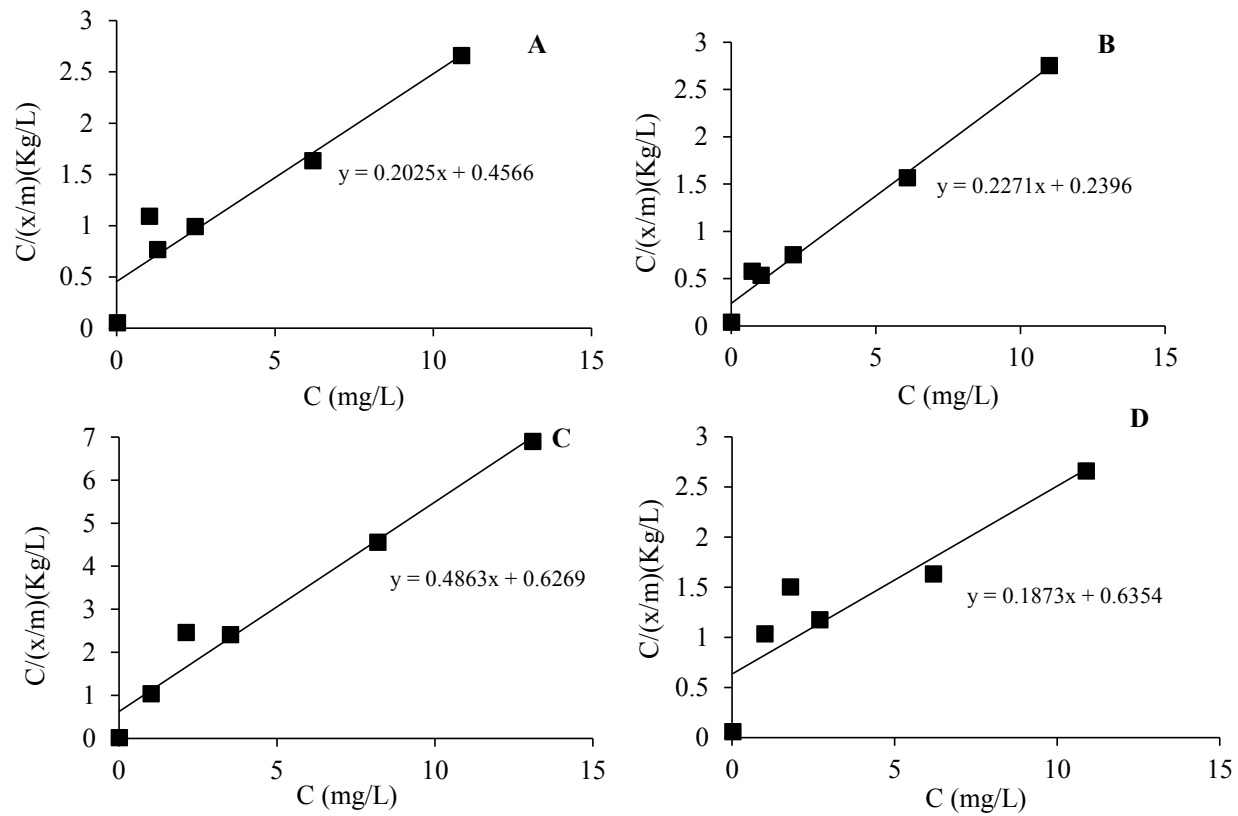
Several studies on B adsorption have been conducted around the world with an aim to investigate the effect of soils with diverse mineralogy on B adsorption (Table 5.6). The maximum B adsorption recorded for the current study is similar to the values reported by Mezuman and Keren (1981), who conducted B adsorption studies on soils having different textural classes (loamy sand, loam, clay and clay), with values of 0.43, 0.65, 3.22 and 4.53 mg B/kg, respectively, but less than the maximum adsorption value reported by Datta and Bhadoria (1999) for soils with textures varying from clay to sandy loam. Mean 'b' values reported by Alleoni and De Carmargo (2000) were 14.81, 5.59, 2.62, 10.33 and 3.57, greater than the 'b' values reported by Mezuman and Keren (1981).

**Table 5.5 Boron adsorption parameters of the fitted Langmuir isotherms**

Soil Orders	Langmuir isotherm parameters		
	K (L/mg)	b (mg/kg)	R <sup>2</sup>
Recent Soil	0.44	4.93	0.91**
Ultic red Soil	0.94	4.40	0.98***
Pallic Soil	0.77	2.05	0.96***
Gley Soil	0.29	5.33	0.81*
Brown Soil	1.78	1.78	0.98***
Allophanic Soil	0.33	6.26	0.83*
Pumice Soil	0.71	3.28	0.96***

\* $p < 0.05$  \*\*  $p < 0.01$  \*\*\*  $p < 0.001$

b Langmuir maximum adsorption  
K Langmuir bonding energy coefficient



**Figure 5.4** Langmuir isotherms for B sorption onto (A) Recent Soil (B) Ultic Red Soil (C) Pallic Soil and (D) Gley Soil

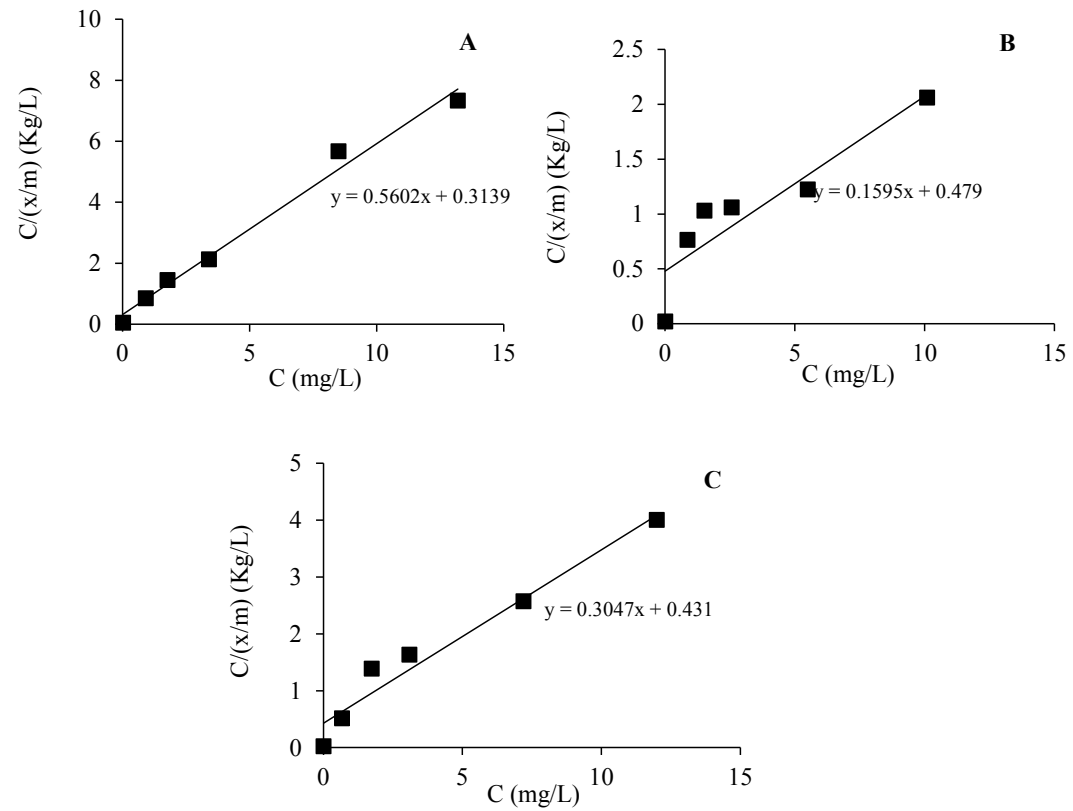


Figure 5.5 Langmuir isotherms for B sorption onto (A) Brown Soil (B) Allophanic Soil (C) Pumic Soil



**Table 5.6 Boron adsorption reported in literature from studies around the world compared with that for the current study**

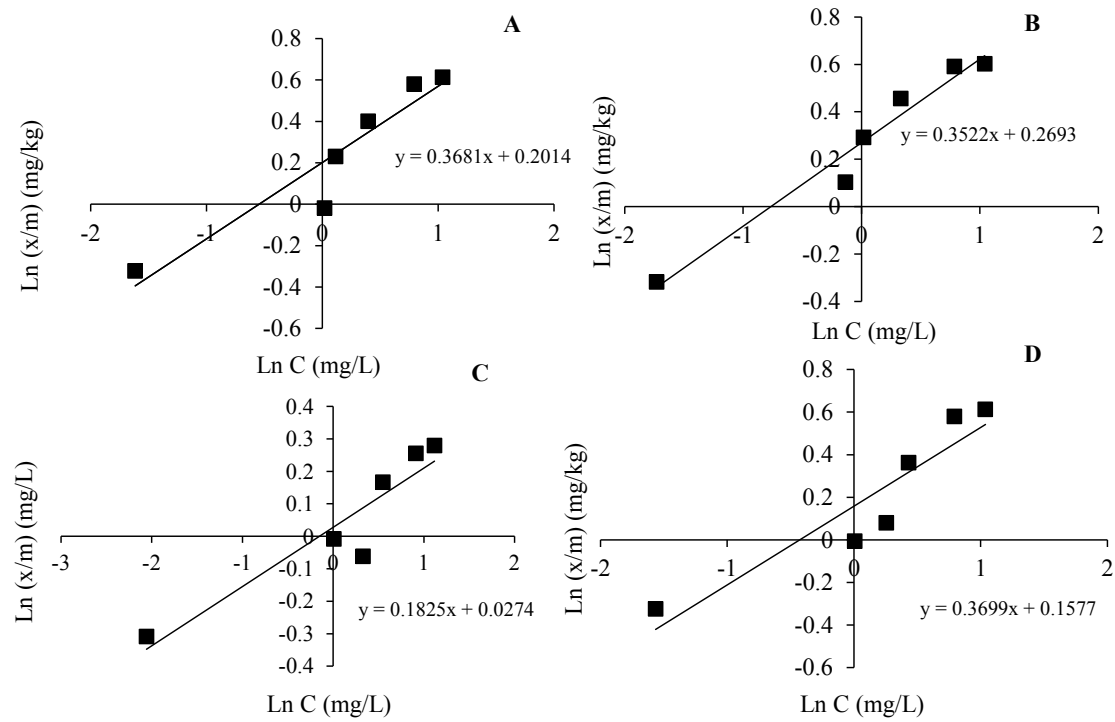
Soils (Location)	Description (Sample, depth etc.)	Langmuir 'b'(mg B/kg)	Reference
Soils collected from different locations of North Island, across New Zealand	7 soil samples collected at 0-10 cm	1.78-6.26	This thesis
Collected from arid, semi-arid zones of Punjab, India	4 soils collected 0-10 cm	4.54-7.96 (b1) 36.11-46.08 (b2)	Arora and Chahal (2010)
Soils collected from Sao Paulo state, Brazil	5 soils collected at 0-10 cm	0.43- 4.53	Alleoni and De Camargo (2000)
Soil collected from West Bengal, India	25 soil samples collected from 0-10 cm,	6.35-45.50 0.81-9.23 (b1)) 6.56-70.49 (b2)	Datta and Bhadoria (1999)
Soil collected from USA	3 soil series Imperial, Holtville and Bonsall collected at different depth; 61-76 cm 0-25 cm	0.103-0.108 0.119-6.64	Goldberg and Forster (1991)
Soil collected from New Mexico state, USA	10 soil samples ( Carjo silt loam, puye sandy loam, R-30 sandy, Glendale clay, Reagan clay loam, Lea sandy loam, Harvey sandy loam)	2.27-33.9, highest in Glendale clay soil	Elrashidi and O'Connor (1982)
Soil collected from Tel Yosef, Israel	4 soils (Loamy sand, loam, clay, clay) collected at 0-10 cm	0.4-4.52	Mezuman and Keren (1981)

b1; lower part of the curve b2; upper part of the curve

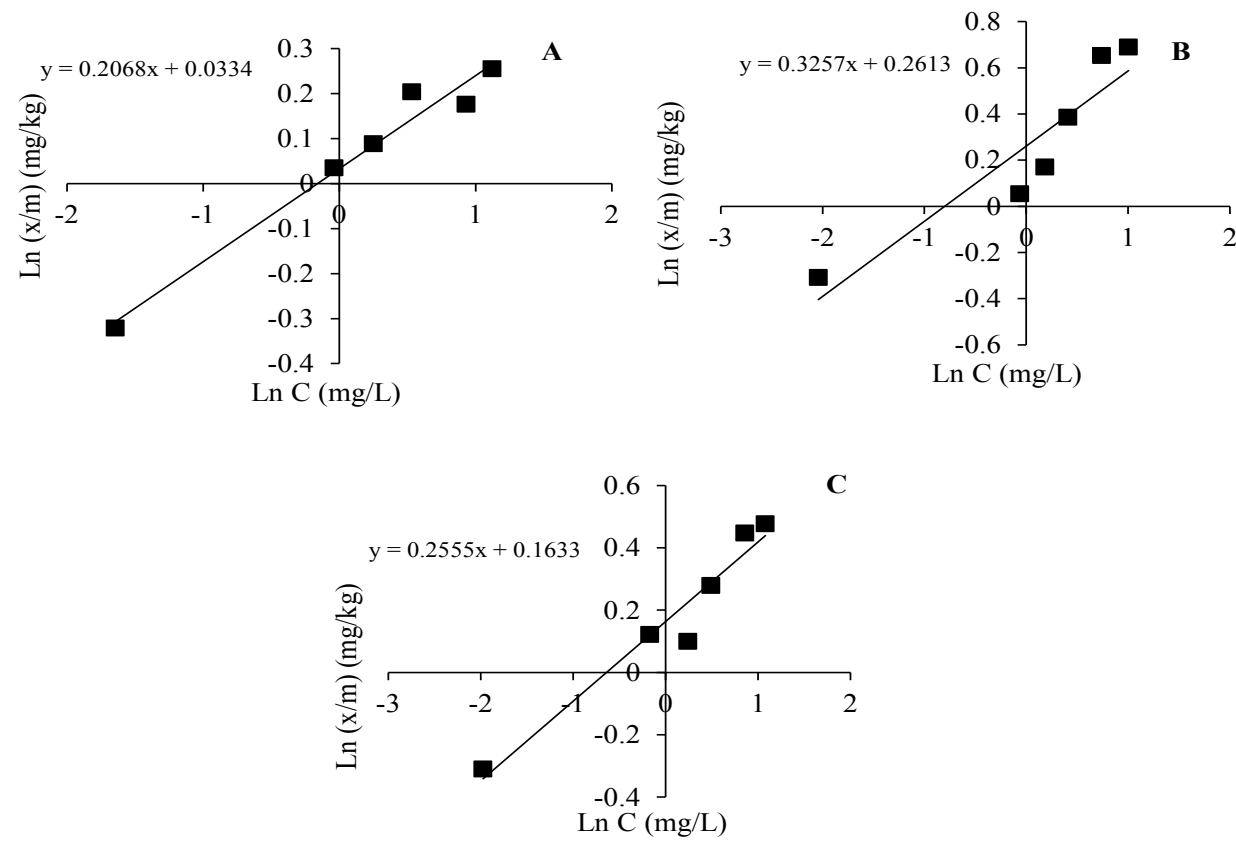
#### 5.5.4.2 The Freundlich adsorption model

Plots of B adsorption against B concentration in equilibrium solution on a log-log scale (Figure 5.6-5.7) show a linear relationship for all soils especially at higher concentration confirming good fit of data to the Freundlich isotherm. The suitability of the Freundlich isotherm to describe the adsorption data was assessed by the coefficient of determination values ( $R^2$ ) for each plot (Table 5.7). The high  $R^2$  values indicate that the adsorption isotherm satisfactorily describes soil B adsorption for the soils used in this study. The  $R^2$  values for the Langmuir adsorption model and the Freundlich model are not significantly different showing that both of the models can be used to describe B adsorption on the studied soils.

A difference in the data fit to the Langmuir and Freundlich models appears at low B concentration, where the Freundlich model appears to be less accurate. A Freundlich isotherm assumes multilayer adsorption on heterogeneous sites against monolayer adsorption on homogeneous sites in the Langmuir isotherm. The Freundlich isotherm has shown an inability to model monolayer adsorption (Arora and Chahal, 2010). However, as B concentrations increase the data appears to fit the model very well, probably as a result of multilayer adsorption or adsorption at heterogeneous binding sites with different energies of adsorption (Saha and Singh, 1997; Spark, 1995). The Freundlich K value range was 0.02-0.26 L/kg with the highest K value recorded for the Ultic red and the Allophanic soil, and the lowest K value recorded for the Pallic soil (Table 5.7). This range of values is smaller than the K value reported by Elrashidi and O'Connor (1982) for the soils from New Mexico, USA named Carjo (1.93); Puye (0.409), R-28 (0.421), Glendale (3.99), Reagan (3.33), Lea (2.35), but larger than the K value reported for Tuff (0.087) and Chem-B (0.125) (Table 5.6).



**Figure 5.6** Freundlich isotherms for B sorption onto (A) Recent Soil (B) Ultic red Soil (C) Pallic Soil and (D) Gley Soil



**Figure 5.7** Freundlich isotherms for B sorption onto (A) Brown Soil (B) Allophanic Soil and (C) Pumic Soil

**Table 5.7 Boron adsorption parameters of Freundlich isotherms<sup>Δ</sup>**

Soil Orders	Freundlich isotherm parameters		
	n	K (L/mg)	R <sup>2</sup>
Recent Soil	0.37	0.21	0.89**
Ultic Red Soil	0.35	0.26	0.96***
Pallic Soil	0.18	0.02	0.87**
Gley Soil	0.37	0.15	0.86*
Brown Soil	0.20	0.03	0.96***
Allophanic Soil	0.32	0.26	0.86*
Pumice Soil	0.25	0.16	0.95***

\* $p < 0.05$     \*\*  $p < 0.01$     \*\*\*  $p < 0.001$     Δ See Fig 5.6 and 5.7 for fitted relationship

N            Freundlich intensity factor

K            Freundlich capacity factor

### 5.5.5 Correlation study

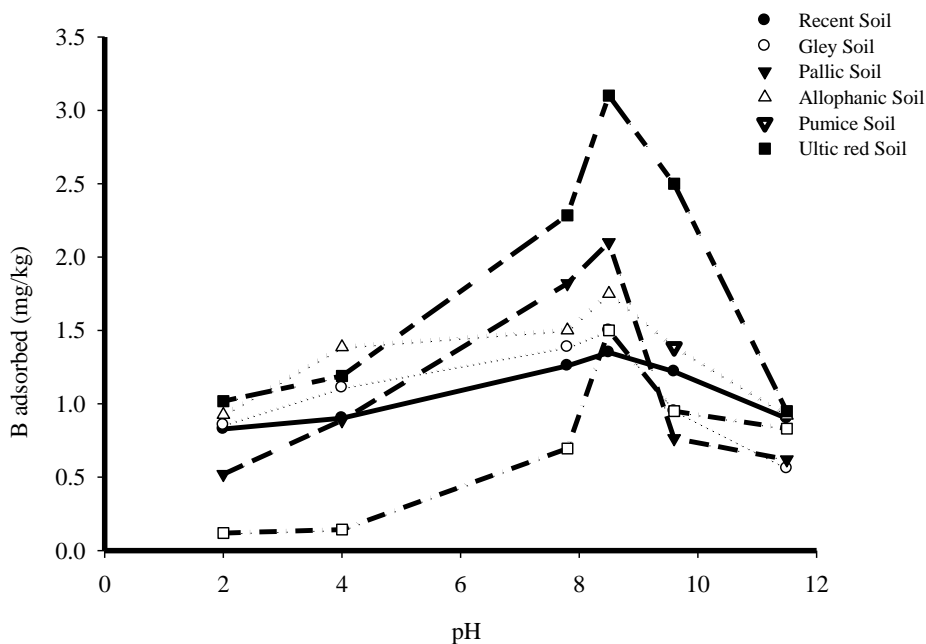
Pearson's correlation coefficients between the modelled Langmuir maximum adsorption (b), and modelled Freundlich capacity k, and the experimentally determined soil properties are listed in Table 5.8. Simple correlation analysis showed that none of the soil properties were statistically significantly correlated with the adsorption parameters. The lack of correlation in the current study could be associated to the low number of soils analysed (< 10), and the widely different mineral makeup of the soils tested.

**Table 5.8 Correlation coefficient (r) for comparison of each adsorption isotherm parameter and soil properties**

	pH	OrgC	Alam	Feam	AlCr	FeCr	Sand	Silt	Clay
<b>Langmuir</b>									
<b>B</b>	-0.039	0.36	-0.19	0.44	0.21	0.02	0.19	-0.35	0.21
<b>Freundlich</b>									
<b>K</b>	-0.326	0.12	-0.01	0.47	0.49	0.37	-0.03	-0.25	0.41
OrgC	Organic Carbon			AlCr	Crystalline-Al				
Alam	Amorphous-Al			FeCr	Cystalline-Fe				
Feam	Amorphous-Fe			b	Langmuir maximum adsorption				
				K	Freundlich capacity factor				

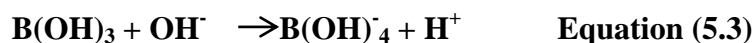
### 5.4.6 The influence of pH on B adsorption

Boron adsorption as a function of solution pH (Figure 5.8) showed that for all 6 soils tested, B adsorption increased as solution pH increased up to a pH of around 9 and beyond this pH adsorption decreased with increasing pH. This is in agreement with the findings of Goldberg et al. (2005) who also reported increased B adsorption with increasing pH, reaching an adsorption maximum around pH 9, with a subsequent decrease of adsorption with any further increase in pH.



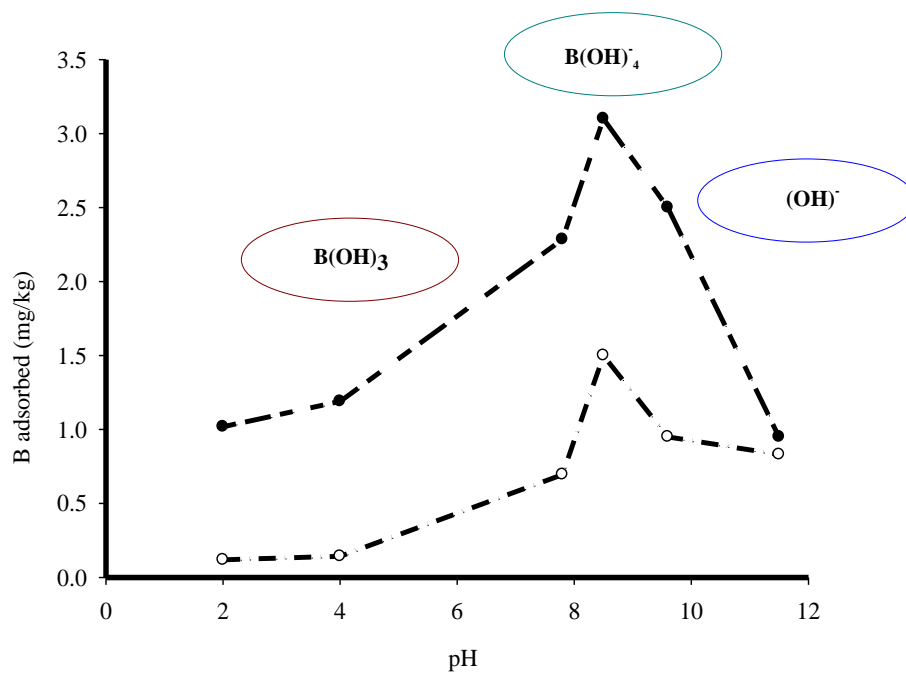
**Figure 5.8 Boron adsorption as a function of solution pH**

The sharp increase in adsorption upon raising solution pH from 6.5 to 9 is ascribed to a shift in B speciation from  $B(OH)_3$  to the  $B(OH)_4^-$  ion (Equation 5.3) which has the ability to exchange with the  $OH^-$  groups on soil particle surfaces by ligand exchange. As the pH increases the concentration of  $B(OH)_4^-$  increases and therefore B adsorption increases. However, any further increase in pH beyond the pH of adsorption maximum decreases B adsorption because of competition with an increasing concentration of  $OH^-$  ions in solution. Also the soil particle surface becomes more negatively charged at very high pH (variable charge) and this reduces the potential for adsorption of negatively charged ions like  $B(OH)_4^-$  (Equation 5.3).



Overall, the relative dynamics of B speciation in solution ( $B(OH)_3$  and  $B(OH)_4^-$ ) is strongly linked to pH (Figure 5.9), with an increased proportion of the species  $B(OH)_3$  apparent from pH 2 to pH 6.5, changing to  $B(OH)_4^-$  as the pH increases past 6.5. This phenomenon is of practical significance in agriculture, as liming, when used to raise soil pH and to help maintain the level of Ca base saturation in soil, could lead to increased B

adsorption and ultimately B unavailability to the plant as the soil pH increases to above 6.5. Hatcher et al. (1967) reported a 2-5 fold increase in B adsorption after liming of acidic soils of North America. Similarly, Alleoni and De Camargo (2000) reported a 33 % increase in B adsorption in sandy, medium-textured soils, and an increase of less than 20 % in clayey soil through the application of lime in some Brazilian soils. In soils where lime-induced B deficiency occurs, primarily caused by increased B adsorption, a high dose of B fertiliser is required to maintain optimum plant growth.



**Figure 5.9** Relative distributions of B species with changing pH



### 5.5.7 Conclusions

Boron adsorption over an initial concentration range of 0.5-15 mg B/L on 7 soils of variable physical and chemical composition showed that the proportion of B adsorbed to all soils increased as the concentration of B in the equilibrium solution increased. This phenomenon indicates that all soils will respond to B fertilisation and reflects the importance of proper understanding of B fertiliser dynamics to manage B deficiency for plant growth. This is particularly true for *P. radiata* forestry as this is one of the most wide-spread forestry plantation crops on a range of major soils found in different locations of New Zealand. As adsorption increases one needs to apply B at higher rates to overcome the amount of B temporarily unavailable through adsorption. Allophanic Soil and Ultic Soil had very high B adsorption and this suggests that these soils require a higher rate of B fertiliser provided they do not already have high B concentrations.

Modelling adsorption data using Langmuir and Freundlich isotherms showed that both models satisfactorily explain B adsorption on the soils of the current study. Langmuir maximum adsorption capacities obtained in this study (1.78 mg/kg to 6.26 mg/kg) were found to be in the range generally reported for other soils in the literature. A satisfactory fit of experimental data to the Langmuir isotherm suggests that there was monolayer adsorption with sites of adsorption having similar energies.

Simple correlation analysis showed that none of the soil properties have a significant influence on maximum B adsorption. This lack of relationship may be a function of the low number of soil tested with widely different soil mineralogy. To further investigate this issue, the study should be repeated with a larger number of soils. The use of multiple regression analysis may also better define potential relationships between adsorption and soil properties.

Investigation of the effect of solution pH on B adsorption in 7 soils showed that adsorption capacity generally increased as pH increased from 2 to 9, followed by an abrupt decline when pH was increased further. These results suggest that liming of acid soils, as normally practiced in modern agriculture may reduce B availability to plants.

## Chapter Six

# Overall conclusions and recommendations for future research work

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### 6.1 Review of the current study

*Pinus radiata* is an important forest plantation species for New Zealand. Every year the *P. radiata* forestry sector makes a significant contribution to New Zealand's GDP through the export of good quality wood and wood-related products to different destinations across the globe.

Boron is an important micronutrient that is deficient in the soil of *P. radiata* growing regions in New Zealand. As a result of low levels of soil B, *P. radiata* plantations in these regions develop B deficiency symptoms such as leader tip dieback, emergence of multi-leaders and needle tip yellowing. These symptoms negatively affect overall tree geometry, growth and wood quality. As discussed in Chapter 2 (review of literature) environmental and weather conditions such as low rainfall and droughts, especially on the East Coast in New Zealand, further aggravate this situation particularly on marginally deficient soils.

Published literature (Chapter 2) supports the use of B fertiliser to address the problems of B deficiency in *P. radiata* forestry. However, with the exception of a small number of studies, most information pertains to the use of highly soluble B fertilisers such as Na-borate and borax. Moreover, reported research findings (Chapter 2) show that the use of highly soluble and fast-release fertiliser provides only a short-term solution and the problems of B deficiency can re-emerge 2-3 years after fertiliser application.

No previous study has investigated the response of soil microbes to slow-release B fertilisers. Similarly, the B fertiliser effect on plant physiology in terms of plant photosynthesis and stomata conductance is poorly described in literature. There is also scarce information on the contribution of applied B fertiliser to pools or fractions of B in soil.

The research work described in this thesis presents the results of studies conducted to investigate the response of *P. radiata* clones to the slow-release fertiliser ulexite. This research was conducted to investigate the dynamics of B in the *P. radiata* forest system.

## **6.2 Objectives of the study**

The main objective of this study was to investigate the influence of different rates of slow-release B fertiliser on plant-available soil B using  $\text{CaCl}_2$  as an extractant to model the plant-available concentration of the nutrient. To achieve this objective, ulexite, a slow-release B fertiliser, was used on different clones of *P. radiata* grown on Pumice soil collected from the Taupo district of Central North Island, New Zealand. The effect of B treatments was quantified after *P. radiata* harvest by assessing plant B uptake, plant growth and physiology (photosynthesis and stomata conductance), and the distribution of B into different functionally defined geochemical fractions in the soil.

Glasshouse studies were designed and run to appraise the response of soil microbial properties to B fertiliser through measurement of soil dehydrogenase activity and the extent of mycorrhizal colonisation in *P. radiata* roots after harvest. Soil B dynamics, and the fractionation of B into different pools (geochemical fractions) were analysed in the soil after plant harvest. Laboratory studies were conducted on 7 benchmark soils collected from different locations of the North Island of New Zealand to investigate the role of soil chemistry in controlling B dynamics. Boron adsorption isotherms were constructed for this range of soils and the influence of variable pH on soil B adsorption was assessed in six of these seven soils.

### 6.3 General discussion and conclusions

Results from both glasshouse studies (Chapter 3 and Chapter 4) showed that B application had a significant influence on the concentration of plant-available soil B, plant B uptake and subsequently plant growth. Plant-available soil B increased as a function of increasing the rate of B fertilisation.

Review of the available literature (as described in Chapter 2) showed that a  $\text{CaCl}_2$ -extractable plant-available B concentration less than 0.5 mg/kg can be defined as critically deficient, and will therefore detrimentally affect plant growth. However, soil B greater than 14 mg/kg can be considered toxic. Results from the growth trials showed that B toxicity was apparent at a B application fertilisation rate of 32 kg/ha. All treatments above 16.7 mg B/kg soil (equivalent to a field application rate of 2 kg/ha) overcame B deficiency that was apparent for the control soil in glasshouse trial 1. But only a rate somewhat between 4 and 16 kg/ha overcame deficiency in trial 2.

Boron application increased the magnitude of plant growth in both glasshouse studies (Chapter 3 and Chapter 4). Boron application in the equivalent range 4-8 kg/ha positively increased the plant growth parameters height and fresh and dry weight, however with a further increase in soil B level the performance of these plant growth parameters declined.

The results of Chapter 4 on B adsorption onto a range (seven) of soils collected from around the North Island of New Zealand showed that both Langmuir and Freundlich isotherms can be successfully used to model B adsorption in all soils tested.

Although B adsorption to soil will reduce the potential for loss of nutrient out of the soil through leaching, particularly in high rainfall areas, adsorbed B is not linearly related with plant B uptake. Therefore the addition of B in the form of B fertiliser is considered as an important and realistic approach to maintain a sufficient B concentration in soil solution for adequate plant uptake. The results from Chapter 3 and Chapter 4 showed that B fertiliser application increased the concentration of B in the readily-available soil fraction

with a corresponding relative decrease in that associated with the specifically adsorbed fraction, which can be considered as plant unavailable.

Boron adsorption was a function of pH across the range of 2-9, with adsorption increasing with pH. Maximum adsorption occurring at pH 9.0 implies that liming of acidic soil will increase B retention in such soils.

### **6.3.1 The availability of B to clones and the subsequent distribution of B within the clones**

The B concentration throughout plant organs of all clones tested in this work was significantly increased with B fertilisation during both greenhouse trials, from a level of deficiency in the control plants to toxicity at the highest application rate (32 kg/ha) (both Chapter 3 and 4). Boron distribution between plant parts was in the order, needle > stem > root. Needles alone accounted for 52-85% of total plant B and can be classified as the main B sink. Among needle ages of year-old to current-year needle class, one-year-old needles were the primary sink for plant B.

Boron translocation from roots to needles behaved differently under different B fertilisation rates. Boron was confined to source organs (roots) under both the control and the higher fertilisation rate (32 kg/ha), but was distributed to sink organs (needles) under optimum B application rates (4-16 kg/ha) presumably in response to demand for new plant growth. These results suggest that B translocation from roots to shoots is restricted under conditions of both low and excess B supply. Such an uneven distribution between plant parts may result from B toxicity-or deficiency-induced impairment of vesicular tissues in *P. radiata*.

Needle tip yellowing was observed at the highest B application rate used in the current study (32 kg/ha). Boron in excess of 32 kg/ha will likely promote toxicity symptoms, and hence impair plant growth. The apparent effect on plant growth at both low levels of soil B (deficiency) and high levels (toxicity), confirms the narrow range of optimal B concentration reported in literature. The relationship between soil B (Plant available CaCl<sub>2</sub>-

extractable B) and the deficiency/toxicity status of *P. radiata* under different B application rates is summarised in Table 6.1.

**Table 6.1 Effect of soil B on plant deficiency and toxicity symptoms**

<b>Plant health indicator (deficiency and toxicity)</b>		
<b>B rate (kg/ha)</b>	<b>Concentration is the CaCl<sub>2</sub>-extractable plant available B concentration in soil (mg/kg)</b>	
	<b>Glasshouse trial 1</b>	<b>Glasshouse trial 2</b>
0	0.30 (deficient)	0.09-0.10 (deficient)
2	[a]	0.20-0.24 (deficient)
4	3.04 (sufficient)	0.42-0.45 (deficient)
8	5.39 (sufficient) [c]	0.84-0.85 (deficient) 1.45-1.60
16	5.95 (sufficient)	(sufficient)
32	14.05 (toxic)	[b]

Notes: [a] the rate of 2 kg/ha was not used in trial 1, [b] the rate of 32 kg/ha was not used in trial 2.  
[c] toxic for one-year-old needles]

The major difference in the plant-available B concentration in soil for the same fertilisation rate between greenhouse trial 1 and 2 is ascribed to the difference in B absolute fertiliser application due to surface area difference, difference in depth, distribution of fertiliser between the two experiments and relatively more plant growth during glasshouse trial 2.

Table 6.1 implies that fertilisation with ulexite at rates between 2-16 kg/ha will maintain plant-available B in pumice soils at a rate sufficient to maintain the optimal plant nutrition of *P. radiata* plantations under glasshouse studies.

The concentration of B in soil responded to B fertilisation, and increased with the B application rate (Chapter 3). The observed B distribution between the surface soil (0-10 cm) and sub-surface soil (10-20 cm) showed that B is more plant available in the surface soil relative to the deeper soil (Chapter 4).

Both clones used in trial 2 (Clone 37 and Clone 18) were sensitive to a low soil B concentration and responded to B application. Needles represent the dominant site of B accumulation regardless of clones. Although both clones showed no difference in stem and root B concentrations across B treatments, the needle B concentration in Clone 37 was greater than in Clone 18. As Clone 37 showed a greater response to B applied at higher application rate and was more susceptible to lower B application, these results imply that Clone 37 is a faster growing clone having a relatively higher demand for B than Clone 18.

### **6.3.2 The effect of variable B fertiliser rates on soil microbes with particular focus on ectomycorrhizae**

Soil dehydrogenase activity and mycorrhizal colonization were used in this work as indices of soil microbiology and responded to B application rates in both glasshouse trials of the study (Chapter 3 and Chapter 4).

Boron application has an effect on soil dehydrogenase activity (DHA). Activity increased up to a fertilisation rate of 8 kg/ha, but decreased beyond. The inference from this result is that B is toxic to microbiological activity at a lower rate of application than is apparent for *P. radiata* itself. The level of DHA activity was elevated in the rhizosphere soil when compared to the bulk soil (Chapter 4) suggesting that B plays a more influential role on the functioning of soil microbes in the rhizosphere than in the bulk soil. This study showed that instead of having a direct effect on DHA, B had an indirect effect on soil microbes. Low photosynthate partitioning manifest for a plant either deficient in B or subject to toxic levels of soil B triggered competition between plant and soil microbes for soil nutrients, leading to a decline in the soil microbe population measured in terms of DHA.

Both inadequate and excess B concentration may therefore be deleterious to mycorrhizal colonization in *P. radiata*. Boron application at the rate of 8 kg/ha resulted in maximum colonization of *P. radiata* roots in greenhouse trial 1. However, the maximum colonization in trial 2 occurred for a B rate of 2-4 kg/ha. The reason for this variable result is unclear, but may be a function of clone-specific mycorrhizae relationships and/or differences in the experimental conditions such as plant age, soil volume, and method of fertiliser application.

### **6.3.3 Response of plant photosynthesis to variable B fertiliser rates**

A significant relationship between plant B concentration and photosynthesis was recorded throughout the current study. Chapter 3 showed that B application in excess of 8 kg/ha reduced photosynthesis in *P. radiata*. However, Chapter 4 showed that B-effected reduction in photosynthetic activity will vary with the clone used. Clone 18 recorded the same level of photosynthesis across a B application range of 2-16 kg/ha, but this reduced for a B rate greater than 16 kg/ha (significantly greater than in the control treatment). For



Clone 37, maximum photosynthesis was recorded at a B application rate of 2 kg/ha, and although further increases in B reduced photosynthesis, activity was greater even at 32 kg/ha than the control treatment. This result may imply that B is only indirectly involved with photosynthesis. The shape of the photosynthesis light response curves showed an interrelation between the gradient of light adsorption and plant photosynthesis capacity. The light photosynthesis response curve reaches a peak for Clone 18 at a B rate of 8 kg/ha, but at 2 kg/ha for Clone 37. These results imply that photosynthesis in both clones does not equally respond to a variation in light at different B levels.

A reduction in photosynthetic activity as a function of increasing levels of soil B may be responsible for chlorosis and necrosis of plant tissues recorded for the high rates of B fertilisation.

#### **6.3.4 Summary for the optimal level of B fertilisation for both plant and soil microbes species**

Table 6.2 summarises the optimum B fertilisation rate derived from this study for both plant and microbial growth under glasshouse conditions. In this study the effect of application of low, medium and excess B fertiliser rates was assessed on *P. radiata* under glasshouse conditions using a Pumice soil collected from the Central North Island of New Zealand (Taupo soil). The results demonstrated the effectiveness of B fertiliser to alleviate B deficiency in soil and to subsequently improve plant B uptake and consequently improve plant growth, physiology, and soil microbial indexes.

Considering plant growth, it was found, in this study, that application of ulexite at a fertiliser rate at 4-8 kg/ha was most effective in improving plant growth and overcoming native soil B concentration-induced deficiency, but B application at a rate greater than 16 kg/ha appeared to be possibly toxic, not only for plant growth and physiology, but to soil microbes as well. Toxicity is proposed to be a function of high B application. Different soils show a variable rate of B adsorption maximum (Table 6.3), with the highest apparent for Allophanic Soil followed by Gley Soil, and lowest for Brown Soil. Variable adsorption suggests that for Allophanic Soil with a high potential to adsorb B, fertiliser application at the higher end of fertiliser recommendation is warranted. In contrast, for the Brown soil

with the lowest adsorption maximum, application at the low-end of the recommended range is warranted.

**Table 6.2 Optimum B level for plant and microbes under glasshouse conditions**

<b>Parameters</b>	<b>Optimal B rate (kg/ha)</b>
<b>Plant index</b> Plant height, fresh and dry weight, dry weight	8-16
<b>Plant physiology</b> Photosynthesis, stomata conductance	2-8
<b>Soil B index</b> CaCl <sub>2</sub> extractable-B	2-16
<b>Microbial and microbiological index</b> Dehydrogenase activities, Mycorrhizal colonization	4-8

**Table 6.3 Adsorption maximum observed in range of soils**

<b>Soil</b>	<b>Adsorption maximum (Langmuir b mg B / kg soil)</b>
Allophanic Soil	6.26
Gley Soil	5.33
Recent Soil	4.93
Ultic red Soil	4.40
Pumice	3.28
Pallic Soil	2.05
Brown Soil	1.78

#### 6.4 Boron cycling in the *Pinus radiata* - soil system

The findings of the thesis can now be used to propose a general model for B cycling under *Pinus radiata* forestry (Figure 6.1). Anthropogenic input of 'new' B into the biogeochemical cycle occurs through the application of slow-release B fertiliser, ulexite. Boron applied in slow-release fertiliser will first dissolve into soil solution, and can then be partially immobilized through complexation with soil organic matter (SOM), and adsorption to clay minerals and oxides and hydrous oxides, as reviewed earlier in this chapter. Such forms of B are removed from the soluble pool and become unavailable for plant uptake. Some fraction of the applied B will move down the soil profile with water and ultimately disappear from the plant-soil system due to leaching. Significant amounts of B leached downward in heavy rainfall areas results in B deficiency in coarse textured soils where there is limited potential for immobilisation. Recycling of B through the biogeochemical system can occur through wet and dry precipitation. Boron added to soil by this mechanism will undergo the same processes as new B.

A major flux in the B cycle is the biological fixation of B within a forest ecosystem. This process is initiated through B movement via mass flow to a plant's root system across the soil-plant interface. From roots, B is translocated to above-ground biomass. Boron will be released back into the soil as a result of the decomposition of plant litter. Although this is a long-term process, organic matter decomposition ultimately adds to the magnitude of the soluble B pool in the soil.

Mycorrhizal colonisation of *Pinus radiata* roots plays an important role in this B cycling. Where there is sufficient B available for optimal plant growth, the production of indole acetic acid increases, which subsequently enhances carbohydrate allocation to roots. This causes an increase in mycorrhizal colonization. Therefore, B fertiliser application not only promotes B cycling in the forest ecosystem, but also stimulates biological activity in soil, driving other nutrient cycles, creating a win-win situation for both plant and microorganisms living at the soil-plant interface.

The major mechanisms for net removal of B from the biogeochemical cycle are leaching and plant harvest. Although B adsorption onto soil particles reduces the potential for B leaching, sorbed B is not available for plant uptake until converted into a soluble B form.

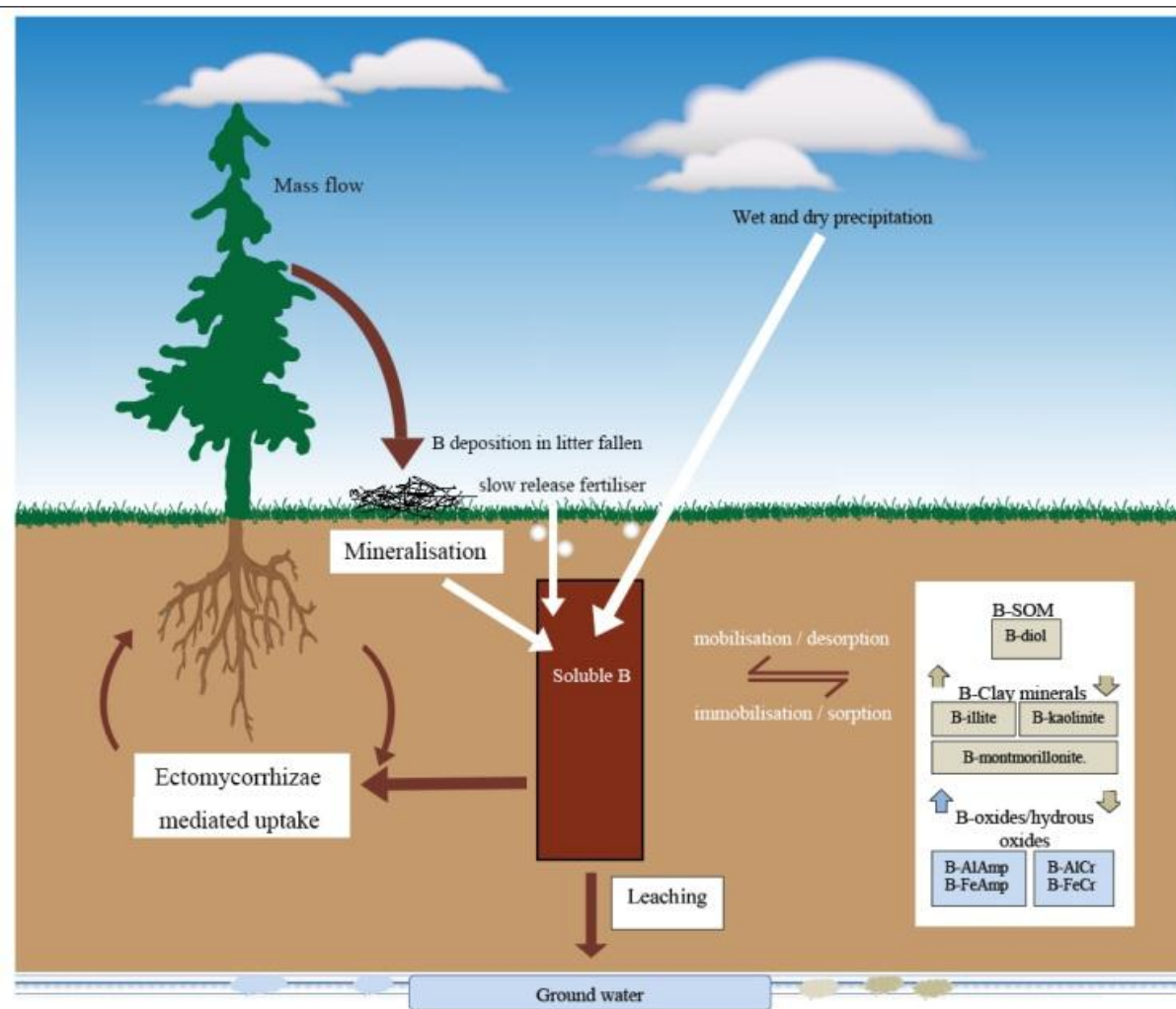


Figure 6.1 Schematic model for B cycling in *Pinus radiata* forestry

## 6.5 Recommendations for future research work

The results reported in this thesis demonstrate a significant effect of B application on ECM fungi development. However, in order to distinguish which fungi species dominate plant roots at each of deficient, optimum and toxic B levels, there is need for better knowledge of the fungal species biodiversity on *P. radiata* root. Furthermore, there is need for ultra-structural study of different fungi species using electron microscopy. As ECM colonization decreased at high B level, it is plausible that ECM control B toxicity, to some extent. However, at a critical level ECM become vulnerable to further increases and toxicity symptoms subsequently develop in plant parts.

The soil B fractionation data reported in this thesis show that residual-B is the major fraction of soil B in the Taupo soil used. There is need for further study to investigate B fractionation in a variety of soils and to better establish the relationship between B fractionation and soil properties.

Future B adsorption studies must include soils of diverse mineralogy, pH and organic matter content. In the current study simple correlation based on seven soils exhibiting a limited range of chemistry could not provide significant explanation of B adsorption.

The modified sequential extraction technique used in the B fractionation study needs further evaluation through the use of a series of soil materials along with a range of standard reference materials.

The findings of the glasshouse trials reported in this thesis on slow-release B fertiliser rate needed to be confirm and there is need of further investigation on conducting long term field trial by including adult *P. radiata* grown under diverse soil and climate conditions. It is also realized to assess further plant physiological and soil microbial response to B fertiliser and see how the findings here in these trials hold true under field conditions.

## References

- Adams J., Hamzah Z., Swift R. (1991) Availability and uptake of boron in a group of pedogenetically related Canterbury, New Zealand soils. *Soil Research* 29:415-423. DOI: <http://dx.doi.org/10.1071/SR9910415>.
- Adriano D.C. (1986) Trace elements in the terrestrial environment. Trace elements in the terrestrial environment.
- Adriano D.C. (2001) Boron In: Trace elements in terrestrial environments biogeochemistry, bioavailability and risks of metals' pp 459-489. Springer New York.
- Aitken R., Jeffrey A., Compton B. (1987) Evaluation of selected extractants for boron in some Queensland soils. *Soil Research* 25:263-273. DOI: <http://dx.doi.org/10.1071/SR9870263>.
- Aitken R.L., Bell L.C. (1985) Plant uptake and phytotoxicity of boron in Australian fly ashes. *Plant and Soil* 84:245-257.
- Aitken R.L., McCallum L.E. (1988) Boron toxicity in soil solution. *Australian Journal of Soil Research* 26:605-610.
- Alleoni L.R.F., De Camargo O.A. (2000) Boron adsorption in soils from the state of São Paulo, Brazil. *Pesquisa Agropecuaria Brasileira* 35:413-421.
- Alvarez-Tinaut M.C., Leal A., Agui I., Recalde L. (1979) physiological effects of boron manganese interaction in tomato *lycopersicon-esculentum* cultivar marglobe plants 2. absorption and distribution of macro elements. *Anales de Edafologia y Agrobiologia* 38:991-1012.
- Aphalo P.J., Schoettle A.W., Lehto T. (2002) Leaf life span and the mobility of "non-mobile" mineral nutrients - The case of boron in conifers. *Silva Fennica* 36:671-680.
- Apostol K.G., Zwiazek J.J. (2004) Boron and water uptake in jack pine (*Pinus banksiana*) seedlings. *Environmental and Experimental Botany* 51:145-153. DOI: 10.1016/j.envexpbot.2003.09.002.
- Archer F.C. (1980) Trace elements in soil in England and Wales. Inorganic pollution and agriculture. U K Ministry of Agriculture, Fisheries and Food Ref Book 326, HMSO, London.

- Argust P. (1998) Distribution of boron in the environment. *Biological Trace Element Research* 66:131-143. DOI: 10.1007/bf02783133.
- Aronsson A. (1983) Growth disturbance caused by B deficiency in some fertilised pine and spruce stands on mineral soils. *Communicationes Instituti Forestalis Fenniae* 116:116-122.
- Arora S., Chahal D.S. (2007) Suitability of adsorption isotherms for behaviour of boron in soils varying in clay and organic matter content. *Agrochimica* 51:182-193.
- Arora S., Chahal D.S. (2010) Effect of Soil Properties on Boron Adsorption and Release in Arid and Semi-Arid Benchmark Soils. *Communications in Soil Science and Plant Analysis* 41:2532-2544. DOI: 10.1080/00103624.2010.514372.
- Artes O.C., Ruiz R.O.C. (1987) Effects of boron in tomato plant 1. Leaf Evolutions. *Agrochimica* 31:391-400.
- Arunachalam J., Emons H., Krasnodebska B., Mohl C. (1996) Sequential extraction studies on homogenized forest soil samples. *Science of The Total Environment* 181:147-159. DOI: 10.1016/0048-9697(95)05005-1.
- Asad A., Blamey F.P.C., Edwards D.G. (2002) Dry matter production and boron concentrations of vegetative and reproductive tissues of canola and sunflower plants grown in nutrient solution. *Plant and Soil* 243:243-252.
- Atalay A., Garrett H.E., Mawhinney T.P., Mitchell R.J. (1988) Boron fertilization and carbohydrate relations in mycorrhizal and nonmycorrhizal shortleaf pine. *Tree Physiology* 4:275-280. DOI: 10.1093/treephys/4.3.275.
- Aubert H., Pinta M. (1977) Boron In: Trace Elements in soils. pp 5-12, Amsterdam.
- Awad F., Mikhael M.I. (1980) A contribution to the boron content in some selected soils of Egypt. *Egypt Journal of Soil Science* 20:89-98.
- Ayars J.E., Hoffman G.J., Schrale G. (1990) Strategies to reduce salt load in drainage water: 331-338.
- Bääth E. (1989) Effect of heavy metals in soil on microbial process and populations (a review). *Water Air Soil Pollution* 47:335-379.
- Bais H.P., Weir T.L., Perry L.G., Gilroy S., Vivanco J.M. (2006) The role of root exudates in rhizosphere interaction with plants and other organisms *Annual Review of Plant Biology* 57:233-266. DOI: doi:10.1146/annurev.arplant.57.032905.105159.

- Barrow N.J. (1989) Testing a mechanistic model. X. The effect of pH and electrolyte concentration on borate sorption by a soil. *Journal of Soil Science* 40:427-435.
- Bell R.W. (1997) Diagnosis and prediction of boron deficiency for plant production. *Plant and Soil* 193:149-168. DOI: 10.1023/a:1004268110139.
- Bell R.W. (1999) Boron In: *Soil Analysis an Interpretation Manual*. (Eds. KI Peverill., LA Sparrow., DJ Reuter). pp 309-317
- Bell R.W., Rerkasem B., Keerati-Kasikom P., Phetchawee S., Hiranburana N., Ratanarat S., Pongsakul P., Loneragan J.F. (1990) *Mineral Nutrition of Food Legumes in Thailand with Particular Reference to Micronutrients*. ACIAR, Technical Reports.
- Bellaloui N., Brown P.H., Dandekar A.M. (1999) Manipulation of in Vivo Sorbitol Production Alters Boron Uptake and Transport in Tobacco. *Plant Physiology* 119:735-742. DOI: 10.1104/pp.119.2.735.
- Bennett W.F. (1993) *Nutrients deficiencies and toxicities in crop plants*. pages 202 APS Press, St Paul MN,.
- Berger K.C. (1949) Boron in Soils and Crops, in: A. G. Norman (Ed.), *Advances in Agronomy*, Academic Press. pp. 321-351.
- Berger K.C., Truog E. (1939) Boron Determination in Soils and Plants. *Industrial & Engineering Chemistry Analytical Edition* 11:540-545. DOI: 10.1021/ac50138a007.
- Berger K.C., Truog E. (1940) Boron deficiencies as revealed by plant and soil tests. *American Journal of Agronomy* 32:297-301.
- Berger K.C., Truog E. (1945) Boron availability in relation to soil reaction and organic matter content. *Soil Science Society of American Proceedings* 10:113-116.
- Bergmann W. (1992) *Nutritional disorders of plants-visual and analytical diagnosis*. Colour Atlas: Gustav Fischer, New York.:204-239.
- Beyer L., Wachendorf C., Balzen F.M., Balzer-Graf U.R. (1992) The effect of soil texture and soil management on micorbial biomass and soil enzyme activites in arable soils of northwest Germany. *Agrobiolology Research* 45:276-283.



- Bhumbla D.R., Ckhabra R. (1982) Chemistry of sodic soil in review of soil research in India. In: Trans. 12<sup>th</sup> Inter. Congress Soil Science. New Delhi, India 169 pp.
- Biggar J.W., Fireman M. (1960) Boron Adsorption and Release by Soils. Soil Science Society of American Journal 24:115-120. DOI: 10.2136/sssaj1960.03615995002400020015x.
- Bingham F.T. (1982) Boron. In: Method of soil analysis. (Eds. A.L Page). pp 431-447. American Society of Agronomy.
- Bingham F.T., Page A.L., Coleman N.T., Flach K. (1971) Boron adsorption characteristics of selected soils from Mexico and Hawaii. Soil Science Society of American Journal 35:546-550.
- Bingham F.T., Strong J.E., Rhoades J.D., Keren R. (1985) An application of the Maas-Hoffman salinity response model for boron toxicity. Soil Science Society of American Journal 49:672-674. DOI: 10.2136/sssaj1985.03615995004900030029x.
- Blakemore L.C. (1953) The chloride content of rain-water collected in the Wellington province. New Zealand Journal of Sciences and Technology 35B:193-197.
- Blakemore L.C., Searle P.L., Daly B.K. (1987) New Zealand Bureau Scientific Report 80. New Zealand.
- Blevins D.G. (1987) Future developments in plant nutrition research In: Future developments in soil science research. (Eds LL Boersma). pp 445-458. Soil Science Society of American Journal.
- Bloesch P., Bell L., Hughes J. (1987) Adsorption and desorption of boron by goethite. Soil Research 25:377-390. DOI: doi:10.1071/SR9870377.
- Bohn H., McNeal B., O'Connor G. (1985) Soil chemistry 2<sup>nd</sup> edition. Pages 341
- Bohnsack C.W., Albert L.S. (1977) Early Effects of Boron Deficiency on Indoleacetic Acid Oxidase Levels of Squash Root Tips. Plant Physiology 59:1047-1050. DOI: 10.1104/pp.59.6.1047.
- Bolaños L., Lukaszewski K., Bonilla I., Blevins D. (2004) Why boron? Plant Physiology and Biochemistry 42:907-912.
- Bonilla I., Garcia-González M., Mateo P. (1990) Boron requirement in cyanobacteria: Its possible role in the early evolution of photosynthetic organisms. Plant Physiology 94:1554-1560.

- Bowden J.W., Nagarajah S., Barrow N.J., Posner A.M., Quirk J.P. (1980) Describing the adsorption of phosphate, citrate, and selenite on a variable charge mineral surface. *Australian Journal of Soil Research* 18:49-60.
- Bowen J.E. (1972) Effect of environmental factors on water utilisation and boron accumulation and translocation in sugarcane. *Plant and Cell Physiology* 13:703-714.
- Braekke F.H. (1983) Occurance of growth disturbance problems in Norwegian and Swidish forestry. *Commun. Inst. for Fenn.*, 116:17-25.
- Bray R.H., Kurtz L.T. (1945) Determination of total, organic, and available forms of phosphorus in soils. *Soil Science* 59:39-45.
- Brennan R.F., Adcock K.G. (2004) Incidence of boron toxicity in spring barely in south western Australia. *Journal of Plant Nutrition* 27:411-425.
- Brinton W.F., Evans E., Blewett C. (2008) Extractability, Plant Yield and Toxicity Thresholds for Boron in Compost. *Compost Science & Utilization* 16:114-118.
- Brouwer R. (1962) Nutritive influences on the distribution of dry matter in the plant. *Netherland Journal of Agriculture Science* 10:399-408.
- Brown P.H., Bellaloui N., Wimmer M.A., Bassil E.S., Ruiz J., Hu H., Pfeffer H., Dannel F., Römheld V. (2002) Boron in Plant Biology. *Plant Biology* 4:205-223. DOI: 10.1055/s-2002-25740.
- Brown P.H., Hu H. (1996) Phloem mobility of boron is species dependent: Evidence for phloem mobility in sorbitol-rich species. *Annals of Botany* 77:497-505.
- Brown P.H., Shelp B.J. (1997) Boron mobility in plants. *Plant and Soil* 193:85-101. DOI: 10.1023/a:1004211925160.
- Brundrett M.C., Abbott L.K. (1994) Mycorrhizal fungus propagules in the jarrah forest. *New Phytologist* 127:539-546. DOI: 10.1111/j.1469-8137.1994.tb03972.x.
- Cakmak I., Kurz H., Marschner H. (1995) Short-term effects of boron, germanium and high light intensity on membrane permeability in boron deficient leaves of sunflower. *Physiologia Plantarum* 95:11-18. DOI: 10.1111/j.1399-3054.1995.tb00801.x.
- Cakmak I., Römheld V. (1997) Boron deficiency-induced impairments of cellular functions in plants. *Plant and Soil* 193:71-83. DOI: 10.1023/a:1004259808322.

- Camacho-cristóbal J.J., Rexach J., González-Fontes A. (2008) Boron in plants: Deficiency and toxicity. *Journal of Integrative Plant Biology* 50:1247-1255.
- Campbell L.S., Davies B.E. (1995) Soil sorption of caesium modelled by the Langmuir and Freundlich isotherm equations. *Applied Geochemistry* 10:715-723. DOI: 10.1016/0883-2927(95)00056-9.
- Carlson C.L., Adriano D.C. (1993) Environmental impacts of coal combustion residues. *Journal of Environmental Quality* 22:227-247.
- Cartwright B., Zarcinas B.A., Spouncer L.R. (1986) Boron toxicity in South Australian barley crops. *Australian Journal of Agricultural Research* 37:351-359.
- Chander K., Brookes P.C. (1991) Is the dehydrogenase assay invalid as a method to estimate microbial activity in copper-contaminated soils? *Soil Biology and Biochemistry* 23:909-915.
- Chao T.T. (1972) Selective dissolution of manganese oxides from soils and sediments with acidified hydroxylamine hydrochloride. *Soil Science Society of American Journal* 36:764-768. DOI: 10.2136/sssaj1972.03615995003600050024x.
- Chao T.T., Sanzolone R.F. (1984) Fractionation of soil selenium by sequential extraction partial dissolution. *Soil Sci. Soc. Am. J.* 53:385-392.
- Chao T.T., Sanzolone R.F. (1989) Fractionation of soil selenium by sequential partial dissolution. *Soil Science Society of America Journal* 53:385-392.
- Chauhan R., Powar S. (1978) Tolerance of wheat and pea to boron in irrigation water. *Plant and Soil* 50:145-149. DOI: 10.1007/bf02107164.
- Chesworth W. (1991) Geochemistry of micronutrients In: *Micronutrients in Agriculture* (Eds. JJ Mortvedt., FR Cox, LM Shuman., RM Welch). Soil Science Society of American Journal. pp1-30.
- Claydon J.J. (1989) Determination of particle size distribution of fine grained soils-pipette method: DSIR Division of Land & Soil Science technical record LH5. Division of Scientific and Industrial Research, Wellington, New Zealand.
- Comerford N.B., Skinner M.F. (1989) Residual phosphorus solubility for an acid, clayey, forested soil in the presence of oxalate and citrate. *Canadian Journal of Soil Science* 69:111-117. DOI: doi:10.4141/cjss89-010.

- Communar G., Keren R. (2008) Boron adsorption by soils as affected by dissolved organic matter from treated sewage effluent. *Soil Science Society of American Journal* 72:492-499. DOI: 10.2136/sssaj2007.0086.
- Conant T.T., Ryan M.G., Goñan i.a.G., Birge H.E., Davidson E. (2011) Temperature and soil organic matter decomposition rates– synthesis of current knowledge and a way forward. *Global Change Biology* 17:3392-3404.
- Cox R.R., Kamprath E.J. (1977) Essential micronutrients soil test. In: *Micronutrients in agriculture*. (Eds. JJ Morrvedt., P.M Giordano., WL Lindsay). pp 289-317. *Soil Science Society of American Journal*.
- Craw D., Rufaut C.G., Haffert L., Todd A. (2006) Mobilisation and attenuation of boron during coal mine rehabilitation, Wangaloa, New Zealand. *Science of The Total Environment* 368:444-455. DOI: <http://dx.doi.org/10.1016/j.scitotenv.2006.04.020>.
- Dannel F., Pfeffer H., Romheld V. (2000) Characterization of root boron pools, boron uptake and boron translocation in sunflower using the stable isotopes B. *Functional Plant Biology* 27:397-405. DOI <http://dx.doi.org/10.1071/PP99086>.
- Datta S.P., Bhadoria P.B.S. (1999) Boron adsorption and desorption in some acid soils of West Bengal, India. *Journal of Plant Nutrition and Soil Science* 162:183-191. DOI: 10.1002/(sici)1522-2624(199903)162:2<183::aid-jpln183>3.0.co;2-a.
- Datta S.P., Rattan R.K., Suribabu K., Datta S.C. (2002) Fractionation and colorimetric determination of boron in soils. *Journal of Plant Nutrition and Soil Science* 165:179-184. DOI: 10.1002/1522-2624(200204).
- Davies B.E., White H.M. (1981) Trace elements in vegetables grown on soils contaminated by base metal mining. *Journal of Plant Nutrition* 3:387-396.
- Day T.A., Heckathorn S.A., DeLucia E.H. (1991) Limitations of Photosynthesis in *Pinus taeda* L. (Loblolly Pine) at Low Soil Temperatures. *Plant Physiology* 96:1246-1254. DOI: 10.1104/pp.96.4.1246.
- DeEndredy A.S. (1963) Estimation of free iron oxides in soils and clays by a photolytic method. *Clay Minor Bulletin* 9:209-217.
- Dell B., Huang L. (1997) Physiological response of plants to low boron. *Plant and Soil* 193:103-120.

- Dell B., Malajczuk N. (1994) Boron deficiency in eucalypt plantation in China. *Canadian Journal of Forest Research* 24:2409-2416.
- Diana G., Beni C., Marconi S. (2010) Comparison of adsorption isotherm equations to describe boron behavior in soils affected by organic and mineral fertilization. *Communications in Soil Science and Plant Analysis* 41:1112-1128.
- Dible W.T., Truog E., Berger K.C. (1954) Boron Determination in Soils and Plants. *Analytical Chemistry* 26:418-421. DOI: 10.1021/ac60086a047.
- Dieffenbach A., Matzner E. (2000) Soil solution chemistry in the rhizosphere of mature Norway spruce (*Picea abies Karst.*) trees. *Plant and Soil* 222:149-161. DOI: 10.1023/a:1004755404412.
- Dixon R.K., Garrett H.E., Cox G.S. (1989) Boron fertilization, vesicular-arbuscular mycorrhizal colonization and growth of citrus *Jambhiri lush*. *Journal of Plant Nutrition* 12:687 - 700.
- Eaton F.M. (1944) Deficiency, toxicity, and accumulation of boron in plants. *Journal of Agricultural Research* 69:237-277.
- El-Mogazi D., Lisk D.J., Weinstein L.H. (1988) A review of physical, chemical, and biological properties of fly ash and effects on agricultural ecosystems. *The Science of The Total Environment* 74:1-37. DOI: 10.1016/0048-9697(88)90127-1.
- El-seewi A.A., Elmalky A.E. (1979) Boron distribution in soils and water of Egypt. *Soil Science Society of American Journal* 43:297-300.
- El-Shintinawy F. (1999) Structural and functional damage caused by boron deficiency in sunflower leaves. *Photosynthetica* 36:565-573.
- Elrashidi M.A., O'Connor G.A. (1982) Boron sorption and desorption in soils. *Soil Science Society of American Journal* 46:27-31. DOI: 10.2136/sssaj1982.03615995004600010005x.
- Entry J.A., Rygiewicz P.T., Watrud L.S., Donnelly P.K. (2002) Influence of adverse soil conditions on the formation and function of Arbuscular mycorrhizas. *Advances in Environmental Research* 7:123-138. DOI: 10.1016/s1093-0191(01)00109-5.
- Evans C.M., Sparks D.L. (1983) On the chemistry and mineralogy of boron in pure and in mixed systems: a review. *Communications in Soil Science and Plant Analysis* 14:827-846.

- Evans L.J. (1987) Retention of boron by agricultural soil from Ontario. *Canadian Journal of Soil Science* 67:33-42. DOI: doi:10.4141/cjss87-003.
- FAO/UNESCO. (1978) Soil maps of the world 1:5 000 000. UNESCO, Paris 1–10.
- Ferreira R.E., Aljaro A.U., Sch. Ruiz R., Rojas L.P., Oster J.D. (1997) Behavior of 42 crop species grown in saline soils with high boron concentrations. *Agricultural Water Management* 34:111-124. DOI: 10.1016/s0378-3774(97)00014-0.
- Fleming G.A. (1980) Essential, micronutrients In: Boron and molybdenum (Eds. Davies) pp 155-197 *Applied soil trace elements*, Wiley New York.
- Fogg T.R. (1983) Source and sinks of atmospheric boron. PhD dissertation, university of Rhode Island, Kingston. 319 pp.
- Fogg T.R., Duce R.A. (1985) American Geophysical Union Paper no.4D1385. AGU Washington, DC.
- Gaines T.P., Mitchell G.A. (1979) Boron determination in plant tissue by the Azomethine H method. *Communications in Soil Science and Plant Analysis* 10:1099 - 1108.
- Gestring W.D., Soltanpour P.N. (1984) Evaluation of the ammonium bicarbonated-DTPA soil test for assessing boron availability to alfalfa. *Soil Science Society of American Journal* 48:96-100.
- Gestring W.D., Soltanpour P.N. (1987) Comparison of soil tests for assessing boron toxicity to alfalfa. *Soil Science Society American Journal* 51:1214-1219. DOI: 10.2136/sssaj1987.03615995005100050022x.
- Giovannetti M., Mosse B. (1980) Evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist* 84:489-500. DOI: 10.1111/j.1469-8137.1980.tb04556.x.
- Goldbach H.E., Amberger A. (1986) Influence of boron nutrition on cell wall polysaccharides in cell culture of *Daucus carota* L. *Journal of Plant Physiology* 123.
- Goldbach H.E., Blaser-Grill J., Lindemann N., Porzelt M.H.o., Lupp C., Gessner B. (1991) Influence of boron on net proton release and its relation to other metabolic process. *Current topics Plant Biochemistry. Physiology* 10:195–220.
- Goldberg S. (1997) Reactions of boron with soils. *Plant and Soil* 193:35-48. DOI: 10.1023/a:1004203723343.

- Goldberg S., Corwin D.L., Shouse P.J., Suarez D.L. (2005) Prediction of boron adsorption by field samples of diverse textures. *Soil Science Society of America Journal* 69:1379-1388.
- Goldberg S., Forster H.S. (1991) Boron Sorption on Calcareous Soils and Reference Calcites. *Soil Science* 152:304-310.
- Goldberg S., Forster H.S., Heick E.L. (1993) Temperature Effects on Boron Adsorption By Reference Minerals and Soils. *Soil Science* 156:316-321.
- Goldberg S., Forster H.S., Heick E.L. (1993b) Boron adsorption mechanisms on oxides, clay minerals, and soils inferred from ionic strength effects. *Soil Science Society of American Journal* 57:704-708. DOI: 10.2136/sssaj1993.03615995005700030013x.
- Goldberg S., Forster H.S., Lesch S.M., Heick E.L. (1996) Influence of Anion Competition on Boron Adsorption By Clays and Soils. *Soil Science* 161:99-103.
- Goldberg S., Glaubig R.A. (1985) Boron adsorption on aluminum and iron oxide minerals. *Soil Science Society of American Journal* 49:1374-1379.
- Goldberg S., Glaubig R.A. (1986) Boron adsorption on California soils. *Soil Science Society of America Journal* 50:1173-1176.
- Goldberg S., Suarez D.L., Shouse P.J. (2008) Influence of soil solution salinity on boron adsorption by soils. *Soil Science* 173:368-374.
- Graham R.D., Welch R.M., Grunes D.L., Cary E.E., Norvell W.A. (1987) Effect of Zinc deficiency on the accumulation of boron and other mineral nutrients in barley. *Soil Science Society of American Journal* 51:652-657. DOI: 10.2136/sssaj1987.03615995005100030018x.
- Greenwood H.N. (1973) Boron *In: Comprehensive inorganic chemistry*. I Oxford; Pergamon Press:665-991.
- Gregory P.J. (2006) Roots, rhizosphere and soil: the route to a better understanding of soil science? *European Journal of Soil Science* 57:2-12. DOI: 10.1111/j.1365-2389.2005.00778.x.
- Grieve C.M., Poss J.A. (2000) Wheat response to interactive effects of boron and salinity. *Journal of Plant Nutrition* 23:1217-1226. DOI: 10.1080/01904160009382095.
- Gu B., Lowe L.E. (1990) Studies on the adsorption of boron on humic acids. *Canadian Journal of Soil Science* 70:305-311.

- Gunes A., Alpaslan M., Cikili Y., Ozcan H. (2000) The effect of zinc on alleviation of boron toxicity in tomato plants ( *Lycopersicon esculentum* L.). Turkish Journal of Agriculture & Forestry 24:505-509.
- Güneş A., Alpaslan M., Çikili Y., Özcan H. (1999) Effect of zinc on the alleviation of boron toxicity in tomato. Journal of Plant Nutrition 22:1061-1068.
- Gupta U.C. (1967) A simplified method for determination of hot-water soluble boron on podzol soil. Soil Science 103:424-428.
- Gupta U.C. (1968a) Relationship of Total and Hot-Water Soluble Boron, and Fixation of Added Boron, to Properties of Podzol Soils. Soil Sci. Soc. Am. J. 32:45-48. DOI: 10.2136/sssaj1968.03615995003200010011x.
- Gupta U.C. (1968b) Relationship of total and hot-water soluble boron, and fixation of added boron, to properties of podzol soils. Soil Science Society of American Journal 32:45-48. DOI: 10.2136/sssaj1968.03615995003200010011x.
- Gupta U.C. (1979a) Boron nutrition of crops. Advance Agronomy 31:273-307.
- Gupta U.C. (1979b) Some factors affecting the determination of hot-water soluble B from podzol soil using Azomethine-H. Canadian Journal of Soil Science 9:241-247.
- Gupta U.C. (1993a) Boron and its role in crop production. CRC Pres, Cleveland, Ohio.
- Gupta U.C. (1993b) Deficiency, sufficiency, and toxicity levels of boron in crops. In Boron and its role in crop production pp 137-145. CRC Press Boca Raton, FL USA
- Gupta U.C., Cutcliffe J.A. (1973) Boron nutrition of broccoli, brussel sprout, and cauliflower grown on Prince Edward Island soils. Communication Soil Science and Plant Analysis 53:275-279.
- Gupta U.C., Cutcliffe J.A. (1978) Effects of methods of boron application on leaf tissue concentration of boron and control of brown-heart in rutabaga. Canadian Journal of Plant Science 58:63-68. DOI: 10.4141/cjps78-012.
- Gupta U.C., Jame Y.W., Campbell C.A., Leyshon A.J., Nicholaichuk W. (1985) Boron toxicity and deficiency: a review. Canadian Journal of Soil Science 65:381-409.
- Haddad K.S., Kaldor C.J. (1982) Effect of parent material, natural available soil boron, and applied boron and lime on the growth and chemical composition



- of lucerne on some acidic soils of the Central Tablelands of New South Wales. *Australian Journal of Experimental Agriculture and Animal Husbandry* 22:317-323.
- Han S., Chen L.-S., Jiang H.-X., Smith B.R., Yang L.-T., Xie C.-Y. (2008) Boron deficiency decreases growth and photosynthesis, and increases starch and hexoses in leaves of citrus seedlings. *Journal of Plant Physiology* 165:1331-1341. DOI: 10.1016/j.jplph.2007.11.002.
- Han S., Tang N., Jiang HX, L.T Y., Y L., Chen L.S. (2009) CO<sub>2</sub> assimilation, photosystem II photochemistry, carbohydrate metabolism and antioxidant system of citrus leaves in response to boron stress. *Plant Science* 176:143-135.
- Handreck K.A. (1990) Method of assessing boron availability in potting media with special reference to toxicity. *Communication Soil Science and Plant Analysis* 21:2265-2280.
- Harmesen K., Vlek P.L.G. (1985) 1. The chemistry of micronutrients in soil. *Nutrient Cycling in Agroecosystems* 7:1-42. DOI: 10.1007/bf01048994.
- Hartmann A., Rothballer M., Schmid M. (2008) Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. *Plant and Soil* 312:7-14. DOI: 10.1007/s11104-007-9514-z.
- Hatcher J.T., Bower C.A., Clark M. (1967) Adsorption of Boron By Soils As Influenced By Hydroxy Aluminum and Surface Area. *Soil Science* 104:422-426.
- Hedley M.J., Nye P.H., White R.E. (1982) Plant-Induced Changes in the Rhizosphere of Rape (*Brassica napus* Var. Emerald) Seedlings. II. Origin of the pH Change. *New Phytologist* 91:31-44.
- Hegan C. (1993) Radiata, the prince of pines. *New Zealand Geographic* 20:88-114.
- Hewitt A.E. (1993) New Zealand soil classification. Landcare Research Science Series No. 1 Lincoln, New Zealand, Manaaki-Whenua Press.
- Hewitt A.E. (1998) The New Zealand soil classification series 2. Manaaki whenua press: Lincoln, 133P.
- Hill J., Lambert M.J. (1981) Physiology and management of micronutrients in forest trees in Australia. In: Productivity in perpetuity Proc. Australian Forest Nutrition Workshop, 10-14 August 1981 Canberra. CISRO , Melbourne:pp 93-103.

- Hingston F. (1964) Reactions between boron and clays. *Soil Research* 2:83-95. DOI: <http://dx.doi.org/10.1071/SR9640083>.
- Hobson K., Armstrong R., Nicolas M., Connor D., Materne M. (2006) Response of lentil (*Lens culinaris*) germplasm to high concentrations of soil boron. *Euphytica* 151:371-382. DOI: 10.1007/s10681-006-9159-7.
- Holloway R., Alston A. (1992) The effects of salt and boron on growth of wheat. *Australian Journal of Agricultural Research* 43:987-1001. DOI: <http://dx.doi.org/10.1071/AR9920987>.
- Hopmans P., Clerehan S. (1991) Growth and uptake of N, P, K and B by *Pinus radiata* D. Don in response to application of borax. *Plant Soil* 131:115-127.
- Hopmans P., Flinn D.W. (1984) Boron deficiency in *Pinus radiata* D. Don and the effect for applied boron on height growth and nutrient uptake. *Plant Soil*:295-298.
- Hou J., Evans L.J., Spiers G.A. (1994) Boron Fractionation in Soils. *Communications in Soil Science and Plant Analysis* 25:1841-1853. DOI: 10.1080/00103629409369157.
- Hou J., Evans L.J., Spiers G.A. (1996) Chemical fractionation of soil boron .1. Method development. *Canadian Journal of Soil Science* 76:485-491.
- Houba V.J.G., Lexmond T.M., Novozamsky I., Van der Lee J.J. (1996) State of the art and future developments in soil analysis for bioavailability assessment. *Soil Total Environment* 178:21-28.
- Houba V.J.G., Temminghoff E.J.M., Gaikhorst G.A., Van Vark W. (2000) Soil analysis procedure using 0.01 M Calcium chlorid as extraction agent. *Communications in Soil Science & Plant Analysis* 31:1299-1396.
- Hu H., Brown P.H. (1997) Absorption of boron by plant roots. *Plant and Soil* 193:49-58. DOI: 10.1023/a:1004255707413.
- Huang P.M. (1980) *The handbook of environmental chemistry, Vol. 2, Part A*, ed. O. Hutzinger, 47. Amsterdam, The Netherlands: Springer Verlag.
- Hübel F., Beck E. (1993) In-situ determination of the P-relations around the primary root of maize with respect to inorganic and phytate-P. *Plant and Soil* 157:1-9. DOI: 10.1007/bf02390221.
- Hue N.V., Hirunburana N., Fox R.L. (1988) Boron status of Hawaiian soils as measured by B sorption and plant uptake 1. *Communications in Soil Science and Plant Analysis* 19:517-528. DOI: 10.1080/00103628809367956.

- Huettl P.J.V. (1976) The pH dependent sorption of boron by soil organic matter. MSc dissertation., University of Wisconsin, Madison, Wisconsin, USA.
- Hung L.-L.L., Trappe J.M. (1987) Ectomycorrhizal inoculation of Douglas-fir transplanted container seedlings with commercially produced inoculum. *New Forests* 1:141-152. DOI: 10.1007/bf00030058.
- Hunter I.R., Will G.M., Skinner M.F. (1990a) A strategy for the correction of boron deficiency in radiata pine plantations in New Zealand. *Forest Ecology and Management* 37:77-82. DOI: 10.1016/0378-1127(90)90047-f.
- Hunter I.R., Will G.M., Skinnner M.F. (1990b) A strategy for the correction of boron defeciciency in radiata pine plantation in New Zealand. *Forest Ecology and Management* 37::77-82.
- Ibekwe A.M., Poss J.A., Grattan S.R., Grieve C.M., Suarez D. (2010) Bacterial diversity in cucumber (*Cucumis sativus*) rhizosphere in response to salinity, soil pH, and boron. *Soil Biology and Biochemistry* 42:567-575. DOI: 10.1016/j.soilbio.2009.11.033.
- Ilin V.B., Anikina A.P. (1974) Boron salinization of soils. *Soviet Soil Science Society* 6:68-74.
- Jackson M.L. (1958) *Soil chemical analysis*. Elsevier, Englewood Cliff, JJ.
- Jakobsen I., Abbott L.K., Robson A.D. (1992) External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *trifolium subterraneum* L. 1. spread of hyphae and phosphorus inflow into roots. *New Phytologist* 120:371-379.
- James W.D., Graham C.C., Glascock M.D., Hanna A.S.G. (1982) Water-leachable boron from coal ashes. *Environmental Science and Technology* 16:195-197.
- Jasmund K., Lindner B. (1973) Experiments on the fixation of boron by clay minerals:399-413.
- Jeyakumar P. (2010) Copper and zinc dynamics and bioavailability in soils amended with biosolids PhD thesis Soil and Earth science Group, INR, Massey University, Palmerston North.
- Jeyakumar P., Loganathan P., Sivakumaran S., Anderson C.W.N., McLaren R.G. (2008) Copper and zinc spiking of biosolids: Effect of incubation period on metal fractionation and speciation and microbial activity. *Environmental Chemistry* 5:347-354.

- Jeyakumar P., Loganathan P., Sivakumaran S., Anderson C.W.N., McLaren R.G. (2010) Bioavailability of copper and zinc to poplar and microorganisms in a biosolids-amended soil. *Australian Journal of Soil Research* 48:459-469.
- Jin J.Y., Martens D.C., Zelazny L.W. (1987) Distribution and plant availability of soil boron fractions. *Soil Science Society of America Journal* 51:1228-1231.
- José J.F.B.S., da Silva I.R., de Barros N.F., Novais R.F., Silva E.F., Smyth T.J., Leite F.P., Nunes F.N., Gebrim F.O. (2009) Boron mobility in eucalyptus clones. *Revista Brasileira de Ciencia do Solo* 33:1733-1744.
- Kabata-Pendia A., Pendias H. (1992) *Trace Elements in Soils and Plants* 2<sup>nd</sup> edition. CRC Press, Boca Raton, Florida,
- .
- Kabata-Pendias A., Pendias H. (2001) *Trace elements in soils and plants*. 413 pages CRC Press, Boca Raton, Florida.
- Kang H., Freeman C. (2007) Interactions of marsh orchid (*dactylorhiza spp.*) and soil microorganisms in relation to extracellular enzyme activities in a peat soil. *Pedosphere* 17:681-687. DOI: 10.1016/s1002-0160(07)60082-4.
- Kastori R., Petrović N. (1989) Effect of boron on nitrate reductase activity in young sunflower plants. *Journal of Plant Nutrition* 12:621-632. DOI: 10.1080/01904168909363978.
- Kastori R., Plesničar M., Panković D., Sakač Z. (1995) Photosynthesis, chlorophyll fluorescence and soluble carbohydrates in sunflower leaves as affected by boron deficiency. *Journal of Plant Nutrition* 18:1751 - 1763.
- Keren R., Bingham F.T. (1985) Boron in water, soils, and plants. *Advances in Soil Science* 1:229-276.
- Keren R., Bingham F.T., Rhoades J.D. (1984) Plant uptake of boron as affected by boron distribution between liquid and solid phases. *Soil Science Society of American Proceedings* 48:297-302.
- Keren R., Bingham F.T., Rhoades J.D. (1985) Plant uptake of boron as affected by boron distribution between liquid and solid phases in soil. *Soil Science Society of America Journal* 49:297-302.
- Keren R., Gast R.G. (1981) Effects of wetting and drying, and of exchangeable cations, on boron adsorption and release by montmorillonite. *Soil Science Society of American Journal* 45:478-482. DOI: 10.2136/sssaj1981.03615995004500030007x.

- Keren R., Gast R.G. (1983) pH-Dependent Boron Adsorption by Montmorillonite Hydroxy-Aluminum Complexes. *Soil Science Society of American Journal* 47:1116-1121. DOI: 10.2136/sssaj1983.03615995004700060011x.
- Keren R., Gast R.G., Baryosef B. (1981) pH dependent boron adsorption by Namontmorillonite. *Soil Science Society of America Journal* 45:45-48.
- Keren R., Mezuman U. (1981) Boron adsorption by clay minerals using a phenomenological equation. *Clays & Clay Minerals* 29:198-204.
- Keren R., Talpaz H. (1984) Boron adsorption by montmorillonite as affected by particle size. *Soil Science Society of American Journal* 48:555-559. DOI: 10.2136/sssaj1984.03615995004800030017x.
- Kluge R. (1971) Contribution to the problem of drought-induced B deficiency in agricultural crops. *Acker-Acker-Pflanzenbau Bodenkd* 15:749-754.
- Knight P.J., Jacks H., Fitzgerald R.E. (1983) Longevity of response in *Pinus radiata* foliar concentrations to nitrogen, phosphorus, and boron fertilisers. *New Zealand Journal of Forestry Science* 13:305-324.
- Kochian L.V. (1995) Cellular Mechanisms of Aluminum Toxicity and Resistance in Plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 46:237-260. DOI: doi:10.1146/annurev.pp.46.060195.001321.
- Kot F. (2009) Boron sources, speciation and its potential impact on health. *Reviews in Environmental Science and Biotechnology* 8:3-28. DOI: 10.1007/s11157-008-9140-0.
- Kovada V.A. (1973) *Fundamental of soil science*, Vol 2 Nauka, Moscow (in Russian).
- Kral R. (1993) *Pinus Linnaeus*. In: *Flora of North America north of Mexico*, volume 2: Pteridophytes and gymnosperms, *Flora of North America Editorial Committee*. Oxford University Press, New York:373-398.
- Krauskopf K.B. (1972) Geochemistry of micronutrients. *Micronutrients in agriculture*:7-40.
- Krishnamurti G.S.R., Huang P.M., Vanrees K.C.J., Kozak L.M., Rostad L.M. (1995) Speciation of particulate-bound cadmium of soils and its bioavailability. *Analyst* 120:659-665.
- Kubota J., Berger J.C., Truogh E. (1948) Boron movement in soils. *Soil Science Society of American Proceedings*. 13:130-134.

- Kulkarni M., Phalke S. (2009) Evaluating variability of root size system and its constitutive traits in hot pepper (*Capsicum annum* L.) under water stress. *Scientia Horticulturae* 120:159-166.
- Lambert D., Cole H., Baker D. (1980) The role of boron in plant response to mycorrhizal infection. *Plant and Soil* 57:431-438. DOI: 10.1007/bf02211700.
- Lambert M.J., Ryan P.J. (1990) Boron nutrition of *Pinus radiata* in relation to soil development and management. *Forest Ecology and Management* 30:45-53.
- Lambert M.J., Turner J. (1977) Dieback in high site quality *Pinus radiata* stands-the role of sulphur and boron deficiency. *New Zealand Journal of Forest Research* 7:333-348.
- Langmuir I. (1918) The adsorption of gases on plane surface of glass, mica and platinum. *Journal of the American Chemical Society* 40:1361-1403. DOI: 10.1021/ja02242a004.
- Lehto T. (1994) Effects of liming and boron fertilization on mycorrhizas of *Picea abies*. *Plant and Soil* 163:65-68.
- Lehto T. (1995) Boron retention in limed forest mor. *Forest Ecology and Management* 78:11-20.
- Lehto T., Lavola A., Kallio E., Aphalo P.J. (2004) Boron uptake by ectomycorrhizas of silver birch. *Mycorrhiza* 14:209-212.
- Lehto T., Mälkönen E. (1994) Effects of liming and boron fertilization on boron uptake of *Picea abies*. *Plant and Soil* 163:55-64. DOI: 10.1007/bf00033940.
- Lehto T., Ruuhola T., Dell B. (2010) Boron in forest trees and forest ecosystems. *Forest Ecology and Management* 260:2053-2069.
- Lemarchand D., Gaillardet J., Lewin E., Allegre C.J. (2000) The influence of rivers on marine boron isotopes and implications for reconstructing past ocean pH. *Nature* 408:951-954.
- Lemarchand E., Schott J., Gaillardet J. (2005) Boron isotopic fractionation related to boron sorption on humic acid and the structure of surface complexes formed. *Geochimica et Cosmochimica Acta* 69:3519-3533. DOI: 10.1016/j.gca.2005.02.024.
- Lenoble M.E., Blevins D.G., Miles R.J. (1996a) Prevention of aluminium toxicity with supplemental boron. II. Stimulation of root growth in an acidic, high-aluminium subsoil. *Plant, Cell & Environment* 19:1143-1148. DOI: 10.1111/j.1365-3040.1996.tb00429.x.

- Lenoble M.E., Blevins D.G., Sharp R.E., Cumbie B.G. (1996b) Prevention of aluminium toxicity with supplemental boron. I. Maintenance of root elongation and cellular structure. *Plant, Cell & Environment* 19:1132-1142. DOI: 10.1111/j.1365-3040.1996.tb00428.x.
- LeRiche H.H., Weir A.H. (1963) A method for studying trace elements in soil fractions. *Journal of Soil Science China* 14:225-235.
- Lewis D.H. (1980) Boron, lignification and the origin of vascular plants-a unified hypothesis. *New Phytologist* 84:209-229.
- Leyval C., Turnau K., Haselwandter K. (1997) Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhiza* 7:139-153. DOI: 10.1007/s005720050174.
- Li M.D., Dai P.A., Luo C.X., Zheng S.X. (1989) Interactive effects of K and B on rape seed yield and the nutrient status in rape plants. *Journal of Soil Science China* 20:31-33.
- Lindsay W. (1972) Inorganic phase equilibria of micronutrients in soils. *Micronutrients in agriculture*:41-57.
- Little E.L.J. (1980) *The Audubon Society field guide to North American trees*. New York: Alfred A. Knopf.
- Liu Z., Zhu Q., Tong T. (1980) Boron deficient soils and their distribution in China. *Acta Ped. Sin.*:228-239.
- Liu Zheng, Qi-Qing Z., Li-Hua T. (1983) Boron deficient soil and their distribution in China. *Acta Ped Sin.* 17:228-239.
- Lloyd J., Farquhar G.D. (1996) The CO<sub>2</sub> dependence of photosynthesis, plant growth responses to elevated atmospheric CO<sub>2</sub> concentrations and their interaction with soil nutrient status. I. General principles and forest ecosystems. *Functional Ecology* 10:4-32.
- Loomis W.D., Durst R.W. (1992) Chemistry and biology of boron. *BioFactors* 3:229-239.
- Lopez-Bucio J., Cruz-Ramírez A., Herrera-Estrella L. (2003) The role of nutrient availability in regulating root architecture. *Current Opinion in Plant Biology* 6:280-287.
- Lovatt C.J. (1985) Evolution of xylem resulted in a requirement for boron in the apical meristems of vascular plants. *New Phytologist* 99:509-522. DOI: 10.1111/j.1469-8137.1985.tb03679.x.

- Lovatt C.J., Bates L.M. (1984) Early effects of excess boron on photosynthesis and growth of curcuma-pepo. *Journal of Experimental Botany* 35:297-305. DOI: 10.1093/jxb/35.3.297.
- Madgwick H.A.I. (1990) Boron, copper and zinc in plantation forestry. Proceedings of a workshop, Rotorua, New Zealand, October 1987. *Forest Ecology & Management* 37:1-221.
- Madgwick H.A.I., Sim A., Oliver G.R. (1988) Nutrients content and uptake of closed-spaced *Pinus radiata*. *New Zealand Journal of Forestry Science* 18:65-76.
- MAF. (2010/2011) Ministry of Agriculture and Forestry. Monitoring & Evaluation Invoation and Research Policy. MAF, Po box 2526, Wellington 6140.
- Manoharan V., Yunusa I.A.M., Loganathan P., Lawrie R., Murray B.R., Skilbeck C.G., Eamus D. (2010) Boron contents and solubility in Australian fly ashes and its uptake by canola (*Brassica napus* L.) from the ash-amended soils. *Soil Research* 48:480-487. DOI: <http://dx.doi.org/10.1071/SR10073>.
- Marschner H. (1986) *Mineral Nutrition of Higher Plants*, 1<sup>st</sup> ed. Academic Press, London.
- Marschner H. (1995) *Mineral Nutrition of Higher Plants*, 2<sup>nd</sup> ed. Academic press, New York. pp 379-396.
- Marschner H., Römheld V., Horst W.J., Martin P. (1986) Root-induced changes in the rhizosphere: Importance for the mineral nutrition of plants. *Zeitschrift für Pflanzenernährung und Bodenkunde* 149:441-456. DOI: 10.1002/jpln.19861490408.
- Marzadori C., Antisari L.V., Ciavatta C., Sequi P. (1991) Soil organic matter influence on adsorption and desorption of boron. *Soil Science Society of American Journal* 55:1582-1585. DOI: 10.2136/sssaj1991.03615995005500060013x.
- Mass E.V. (1987) Salt tolerance of plants in *Handbook of plant science in agriculture*. (Eds. I.B.R Christie) CRC, Press Boca Raton, Florida. Vol 2 p 57
- McGeehan S.L., Topper K., Naylor D.V. (1989) Sources of variation in hot water extraction and colorimetric determination of soil boron. *Communications in Soil Science & Plant Analysis* 20:1777-1786.



- McLaren R.G., McLenaghan R.D., Swift R.S. (1990) Boron applications to pastures: effect on herbage and soil boron concentration. *New Zealand Journal of Agricultural Research* 33:277-284.
- McLean E.O., Heddleson M.R., Bartlett R.J., Holowaychuk N. (1958) Aluminum in Soils: I. Extraction Methods and Magnitudes in Clays and Ohio Soils. *Soil Science Society of American Journal* 22:382-387. DOI: 10.2136/sssaj1958.03615995002200050005x.
- Mead J. (1981) A comparison of the Langmuir, Freundlich and Temkin equations to describe phosphate adsorption properties of soils. *Soil Research* 19:333-342. DOI: <http://dx.doi.org/10.1071/SR9810333>.
- Mehmet B., Sait G., Ali T., Bayram S., Huseyin D. (2004) *Gypsophila sphaerocephala* Fenzl ex Tchihat.: A Boron Hyperaccumulator Plant Species That May Phytoremediate Soils with Toxic B Levels. *Turk. Journal of Botany* 28:273-278.
- Mehmet B, 2009 Investigation of sodium borohydride production process; ulexite mineral as boron source. *International J, Hydrogen Energy* 34: 4773-4779.
- Mehra O.P., Jackson M.L. (1960) Iron oxide removal from soils and clays by a dithionite-citrate system buffered with sodium bicarbonate. *Clays Clay Miner* 7:317-327.
- Mehrotra N.K., Khan S.A., Agarwall S.C. (1989) High SAR (sodium adsorption ratio) irrigation and boron phytotoxicity in sugar beet. *Ann. Arid Zone* 28:69-78.
- Mei L., Sheng O., Peng S.-a., Zhou G.-f., Wei Q.-j., Li Q.-h. (2011) Growth, root morphology and boron uptake by citrus rootstock seedlings differing in boron-deficiency responses. *Scientia Horticulturae* 129:426-432. DOI: 10.1016/j.scienta.2011.04.012.
- Mengal K., Kirkby E.A. (1982) *Principles of plant nutrition*. 3<sup>rd</sup> eds. Potash Institute, Bern. 655 p.
- Mezuman U., Keren R. (1981) Boron Adsorption by Soils Using a Phenomenological Adsorption Equation. *Soil Science Society of American Journal* 45:722-726. DOI: 10.2136/sssaj1981.03615995004500040009x.
- Miljkovic N.S., Matthews B.C., Miller M.H. (1966) The availability of boron content of the genetic horizons of some Ontario soils: I. The relationship between

- water-soluble boron and other soil properties. *Canadian Journal of Soil Science* 46:133-138. DOI: doi:10.4141/cjss66-022.
- Milmile S.N., Pande J.V., Karmakar S., Bansibal A., Chakrabarti T., Biniwale R.B. (2011) Equilibrium isotherm and kinetic modeling of the adsorption of nitrates by anion exchange Indion NSSR resin. *Desalination* 276:38-44. DOI: 10.1016/j.desal.2011.03.015.
- Mitchell R.J., Garrett H.E., Cox G.S., Atalay A. (1986) Boron and ectomycorrhizal influences on indole-3-acetic acid levels and indole-3-acetic acid oxidase and peroxidase activities of *Pinus echinata* Mill. roots. *Tree Physiology* 1:1-8.
- Mitchell R.J., Garrett H.E., Cox G.S., Atalay A. (1990) Boron and ectomycorrhizal influences on mineral nutrition of container-grown *Pinus ehinta* mill. *Journal of Plant Nutrition* 13:1555-1574.
- Mitchell R.J., Garrett H.E., Cox G.S., Atalay A., Dixon R.K. (1987) Boron fertilization, ectomycorrhizal colonization, and growth of *Pinus echinata* seedlings. *Canadian Journal of Forest Research* 17:1153-1156.
- Miwa K., Fujiwara T. (2010) Boron transport in plants: co-ordinated regulation of transporters. *Annals of Botany* 105:1103-1108. DOI: 10.1093/aob/mcq044.
- Mondal A.K., Mandal B., Mandal L.N. (1993) Boron adsorption characteristics of some acidic alluvial soils in relation to soil properties. *Communications in Soil Science and Plant Analysis* 24:2553-2567. DOI: 10.1080/00103629309368977.
- Moody D.B.A., Rathjen B., Cartwright B., Paull J.G., Lewsi J. (1988) Genetic diversity and geographical distribution of tolerance to high levels of soil boron. (Eds. TE Miller and RMD Koebner). *Proceedings of 7<sup>th</sup> interanational Wheat Genetic symposium*. pp 859-966
- Mortvedt J.J., Woodruff J.R. (1993) Boron compounds, used as fertiliser : In: *Boron and Its Role in Crop Production*. pp 157-176. CRC press, Boca Raton, Florida.
- Möttönen M., Lehto T., Aphalo P.J. (2001) Growth dynamics and mycorrhizas of Norway spruce (*Picea abies*) seedlings in relation to boron supply. *Trees - Structure and Function* 15:319-326. DOI: 10.1007/s004680100106.
- Möttönen M., Lehto T., Aphalo P.J., Kukkola M., Mälkönen E. (2003) Response of mature stands of Norway spruce (*Picea abies*) to boron fertilization. *Forest Ecology and Management* 180:401-412.

- Nable R.O., Bañuelos G.S., Paull J.G. (1997) Boron toxicity. *Plant and Soil* 193:181-198. DOI: 10.1023/a:1004272227886.
- Nable R.O., Paull G. (1991) Mechanism and genetics of tolerance to boron toxicity in plants. In: *Current topics in plant biochemistry and physiology*. (Eds Randall, D.D., Blevins, D.G and Miles, C.D) Vol 10 , Columbia University press, Columbia pp 257-273.
- Nable R.O., Paull J.G., Cartwright B. (1990) Problems associated with the use of foliar analysis for diagnosing boron toxicity in barley. *Plant and Soil* 128:225-232.
- Nelson D.R., Mele P.M. (2007) Subtle changes in rhizosphere microbial community structure in response to increased boron and sodium chloride concentrations. *Soil Biology and Biochemistry* 39:340-351. DOI: 10.1016/j.soilbio.2006.08.004.
- Neumann G., Massonneau A., Langlade N., Dinkelaker B., Hengeler C., Römheld V., Martinoia E. (2000) Physiological Aspects of Cluster Root Function and Development in Phosphorus-deficient White Lupin (*Lupinus albus* L.). *Annals of Botany* 85:909-919. DOI: 10.1006/anbo.2000.1135.
- Noppakoonwong R.N., Bell R.W., Dell B., Loneragan J.F. (1993) An effect of shade on the boron requirement for leaf blade elongation in black gram (*Vigna mungo*). *Plant and Soil* 155-156:317-320. DOI: 10.1007/bf00025046.
- Novozamsky I., Barrera L.L., Houba V.J.G., van der Lee J.J., van Eck R. (1990) Comparison of a hot water and cold 0.01 M CaCl<sub>2</sub> extraction procedures for the determination of boron in soil. *Communications in Soil Science and Plant Analysis* 21:2189-2195. DOI: 10.1080/00103629009368370.
- Novozamsky I., Lexmond T.M., Houba V.J.G. (1989) A single extraction in studies on trace metals in soils, sewage sludges and sludge-treated soils. *International Journal Environ. Anal. Chemistry* 9:743-179.
- NZFOA. (2011) New Zealand Forest Owners Association. New Zealand Plantation Forest Industry, Facts & Figures, 85 The Terrace PO Box 1208, Wellington.
- NZWWA. (2003) Guidelines for the safe application of biosolids to land in New Zealand. pp 1-5 (New Zealand Water and Wastes Association: Wellington).
- O'Neill M.A., Eberhard S., Albersheim P., Darvill A.G. (2001) Requirement of borate cross-linking of cell wall rhamnogalacturonan II for *Arabidopsis* growth. *Science* 294:846-849.

- O'Neill M.A., Ishii T., Albersheim P., Darvill A.G. (2004) Rhamnogalacturonan II: Structure and function of a borate cross-linked cell wall pectic polysaccharide. *Annul Review of Plant Biology* 55:109-139.
- Oertli J.J. (1994) Non-homogeneity of boron distribution in plants and consequences for foliar diagnosis. *Communication in Soil Science and Plant Analysis* 25:1133-1147.
- Ogilvie D. (1998) National study of the composition of sewage sludge. . New Zealand, Water Wastes Association. Drainage Managers, Group Part B:1-5.
- Okazaki E., Chao T.T. (1968) Boron Adsorption and Desorption By Some Hawaiian Soils. *Soil Science* 105:255-259.
- Olykan S.T., Adam J.A., Nordmeyer A.H., McLaren R.G. (1995) Micronutrient and macronutrient uptake by *pinus radiata*, and soil boron fraction, as affected by added nitrogen and boron. *New Zealand Journal of Forest Research* 25:61-72.
- Olykan S.T., Xue J.M., Clinton P.W., Skinner M.F., Graham D.J., Leckie A.C. (2008) Effect of boron fertiliser, weed control and genotype on foliar nutrients and tree growth of juvenile *Pinus radiata* at two contrasting sites in New Zealand. *Forest Ecology and Management* 255:1196-1209. DOI: 10.1016/j.foreco.2007.10.025.
- Page N.R., Cooper H.P. (1955) Minor Plant Nutrients, Less-Soluble Boron Compounds for Correcting Boron Nutritional Deficiencies. *Journal of Agricultural and Food Chemistry* 3:222-225. DOI: 10.1021/jf60049a002.
- Paliwal K.V., Mehta K.K. (1973a) Boron status of some soils irrigated with saline in Kota and Bhilwara regions of Rajasthan *Indian Journal of Agriculture Science* 43:766-772.
- Paliwal K.V., Mehta K.K. (1973b) Interactive effect of salinity, SAR and boron on the germination and growth of seedlings of some paddy (*Oryza sativa*. L) varieties. *Plant and Soil* 39:603-609. DOI: 10.1007/bf00264177.
- Papadakis K., K.N D., Therios I.N. (2003) Response of two citrus genotypes to six boron concentrations: concentration and distribution of nutrients, total absorption, and nutrient use efficiency. *Australian Journal of Agricultural Research* 54:571-580.
- Parfitt R.L. (1979) Anion Adsorption by Soils and Soil Materials *Advances in Agronomy* 30:1-50.

- Park C.S., Park N.J. (1966) Studies on the available boron content of soils in the upland crop area in Korea. Annual Research Report, Office of Rural development 9:163-174.
- Park H., Schlesinger W.H. (2002) Global biogeochemical cycle of boron. *Global Biogeochemical Cycles* 16:20-1.
- Parker D.R., Gardner E.H. (1981) The determination of hot-water-soluble boron in some acid Oregon soils using a modified Azomethine-H procedure. *Communications in Soil Science and Plant Analysis* 12:1311-1322.
- Parks W.L., White J.L. (1952) Boron retention by clay and humus systems saturated with various cations. *Soil Science Society of American Journal* 16:298-300. DOI: 10.2136/sssaj1952.03615995001600030018x.
- Parr A.J., Loughman B.C. (1983) Boron and membrane function in plants. In: *Metals and Micronutrients: Uptake and utilization by plants*. (Eds D.A. Robb and W.S. Pierpoint): Academic Press pp 87-107. London, England.
- Payn T.W., Skinner M.F., Clinton P.W. (1998) Future nutrient requirements of New Zealand plantation forest. In: *Long-term Nutrient Needs for New Zealand's Primary Industries* (Eds L D Currie and P Loganathan) Occasional report No. 11 pp 97-110 (Fertiliser and Lime Research Centre, Massey University).
- Power P.P., Woods W.G. (1997) The chemistry of boron and its speciation in plants. *Plant and Soil* 193:1-13. DOI: 10.1023/a:1004231922434.
- Purves D., Mackenzie E.J. (1974) Phytotoxicity due to boron in municipal compost. *Plant and Soil* 40:231-235. DOI: 10.1007/bf00011427.
- Ramon A.M., Carpenaruiz R.O., Garate A. (1990) The effects of short-term deficiency of boron on potassium, calcium, and magnesium distribution in leaves and roots of tomato (*Lycopersicon esculentum*). In: *Proceedings of 11<sup>th</sup> international Plant Nutrition Colloquium, Wageningen, The Netherlands*. Plant nutrition-Physiology and applications. Kluwer Academic Publ, Dordrecht.
- Raven J.A. (1980) Short and long distance transport of Boric acid in plant. *New Phytologist* 84:231-249. DOI: 10.1111/j.1469-8137.1980.tb04424.x.
- Reisenauer H.M., Walsh L.M., Hoelt R.G. (1973) Testing soils for sulfur, boron, molybdenum and chlorine. In 'Soil testing and plant analysis' (Eds L.M. Walsh, J.D. Beaton) pp. 173-200. Soil Science Society of American Journal.

- Rerkasem B., Netsangtip R., Bell R.W., Hiranburana N., Loneragan J.F. (1988) Comparative species responses to boron on a typic tropaqualf in Northern Thailand. *Plant and Soil* 106:15-21.
- Rerkasem B., Saunders D.A., Dell B. (1989) Grain set failure and boron deficiency in wheat in Thailand. *Journal of Agriculture (Chiang Mai University)* 5:1-10.
- Rhoades J.D., Ingvalson R.D., Hatcher J.T. (1970) Laboratory determination of leachable soil boron. *Soil Science Society of American Proceedings*. 34:871-875.
- Richardson D.M. (1998) *Ecology and Biogeography of Pinus*. Cambridge University Press. Pp 548. ISBN 0-521-55176-5.
- Ryan J., Singh M., Yau S.K. (1998) Spatial variability of soluble boron in Syrian soils *Soil Tillage* 45:407-417.
- Ryan P.J. (1989) Boron retention within a catena of rhyolitic soils and its effect on radiata pine growth and nutrition. *Australian Journal of Soil Research* 27:135-148.
- Ryle G.B. (1962) *Pinus radiata* by C. W. Scott Review and Synopsis. *Journal of Forestry* 35:183-186.
- Saarsalmi A., Tamminen P. (2005) Boron, phosphorous and nitrogen fertilization in Norway spruce stands suffering from growth disturbance. *Siva Fenn* 39:351-364.
- Saha J.K., Singh M.V. (1997) Boron adsorption-desorption characteristics of some major soil groups of India. *Journal of Indian Society of Soil Science* 45:271-274.
- Sarkar D., Das B. (1990) Effect of removal organic matter on boron adsorption by some soils of West Bengal. *Environmental Ecology* 8:280-282.
- Sarkar D., Mandal B., Kundu M.C., Bhat J.A. (2008) Soil properties influence distribution of extractable boron in soil profile. *Communications in Soil Science and Plant Analysis* 39:2319-2332. DOI: 10.1080/00103620802292418.
- Schalscha E.B., Bingham F.T., Galindo G.G., Galvan H.P. (1973) Boron Adsorption By Volcanic Ash Soils in Southern Chile. *Soil Science* 116:70-76.
- Schwertmann U. (1964) The differentiation of iron oxides in soils by extraction with NH<sub>4</sub>-oxalates solution. *Zeitschrift für Pflanzenernährung und Bodenkunde* 105:194-202.

- Scott H.D., Beasley S.D., Thompson L.F. (1975) Effect of lime on boron transport and uptake by cotton. *Soil Science Society of American Proceedings* 39:1116-1121.
- Serdar B., Murat B., B S. (2011) The effects of boron management on soil microbial population and enzyme activities. *African Journal of Biotechnology* 10:5311-5319.
- Sharma K.R., Srivastava P.C., Srivastava P., Singh V.P. (2006) Effect of farmyard manure application on boron adsorption-desorption characteristics of some soils. *Chemosphere* 65:769-777. DOI: 10.1016/j.chemosphere.2006.03.053.
- Sharma P.N., Kumar N., Bisht S.S. (1994) Effect of zinc-deficiency on chlorophyll content, photosynthesis and water relations of cauliflower plants. *Photosynthetica* 30:353-359.
- Sharma P.N., Ramchandra T. (1990) Water relation and photosynthesis in mustard plants subjected to boron deficiency. *Indian Journal of Plant Physiology* 33:150-154.
- Shelp B.J. (1988) Boron mobility and nutrition in Broccoli (*Brassica oleracea var. italica*). *Annals of Botany* 61:83-91.
- Shelp B.J. (1990) The influence of boron nutrition on nitrogen partitioning in broccoli plants. *Communications in Soil Science and Plant Analysis* 21:49-60. DOI: 10.1080/00103629009368214.
- Shelp B.J., Marentes E., Kitheka A.M., Vivekanandan P. (1995) Boron mobility in plants. *Physologia Plantarum* 94:356-361.
- Shelp B.J., Shattuck V.I. (1987) Boron nutrition and mobility, and its relation to the elemental composition of greenhouse grown root crops. 1. Rutabaga. *Communications in Soil Science and Plant Analysis* 18:187-201. DOI: 10.1080/00103628709367810.
- Sheng O., Song S., Chen Y., Peng S., Deng X. (2009) Effects of exogenous B supply on growth, B accumulation and distribution of two navel orange cultivars. *Trees - Structure and Function* 23:59-68. DOI: 10.1007/s00468-008-0254-3.
- Sheng O., Zhou G., Wei Q., Peng S., Deng X. (2010) Effects of excess boron on growth, gas exchange, and boron status of four orange scion-rootstock combinations. *Journal of Plant Nutrition and Soil Science* 173:469-476. DOI: 10.1002/jpln.200800273.

- Sherrell C.G. (1983) Comparison of materials of different solubility as sources of boron for plants. *New Zealand Journal of Experimental Agriculture* 11:325-329.
- Shorrocks V.M. (1964) Boron toxicity in *Hevea brasiliensis*. *Nature* 204:599-600.
- Shorrocks V.M. (1997) The occurrence and correction of boron deficiency. *Plant and Soil* 193:121-148. DOI: 10.1023/a:1004216126069.
- Shriver D., Atkins P. (1994) *Inorganic Chemistry*. Page 864 Oxford University Press, Oxford.
- Sillanpää M., Vlek P.L.G. (1985) Micronutrients and the agroecology of tropical and Mediterranean regions. *Nutrient Cycling in Agroecosystems* 7:151-167. DOI: 10.1007/bf01048999.
- Sims J.R., Johnson G.V. (1991) Micronutrients soil tests. In 'Micronutrients in Agriculture' (Eds. Mortvedt J.J) pp 427-476. PPSSSA Book series, Madison, WI.
- Singh R.N., Singh H., Roy N.H. (1987) Characteristics of adsorption equilibrium of added boron in red loam soils. *Annals of Agriculture Research* 8:252-257.
- Skinner M.F., Bowen G.D. (1974) Penetration of soil by mycelial strands of ectomycorrhizal fungi. *Soil Biology and Biochemistry* 6:57-61.
- Skujins J. (1973) Dehydrogenase: An Indicator of Biological Activities in Arid Soils. *Bulletins from the Ecological Research Committee* 17:235-241.
- Skujins J. (1978) History of abiotic soil enzymes research. In: soil enzymes (Eds.R.G Burns) pp 1-49. Academic press, New York.
- Smith G.S., Clark C.J. (1989) Effect of excess boron on yield and post-harvest storage of kiwifruit. *Scientia Horticulturae* 38:105-115. DOI: 10.1016/0304-4238(89)90024-1.
- Snowdon P. (1982) Diagnosis of boron deficiency in soils by pot experiments with *Pinus radiata*. *Australian Forest Research* 12:217-229.
- Snyder S.S., Hornsby A., Welch J., Gordon L., Franklin T. (1993) Effect of phosphate and poultry litter on cotton production on recently levelled lands in Lonoke county. *Research Services Arkansas Agriculture*. Experiment station, No. 425:64-66.
- Sohn S., Kim D. (2005) Modification of Langmuir isotherm in solution systems—definition and utilization of concentration dependent factor. *Chemosphere* 58:115-123. DOI: 10.1016/j.chemosphere.2004.08.091.



- Sotiropoulos T.E., Therios I.N., Dimassi K.N., Bosabalidis A., Kofidis G. (2002) Nutritional status, growth, co-assimilation, and leaf anatomical response in two kiwifruit species under boron toxicity. *Journal of Plant Nutrition* 25:1249 - 1261.
- Spark D.L. (1995) *Environmental soil chemistry*. Academic press Boston.
- Sposito G. (1984) *The surface chemistry of soils*. Oxford university press, New York, 234p.
- Spouncer L.R., Nable R.O., Cartwright B. (1992) A procedure for the determination of soluble boron in soils ranging widely in boron concentrations, sodicity, and pH. *Communications in Soil Science & Plant Analysis* 23:441-453.
- Stangoulis J., Tate M., Graham R., Bucknall M., Palmer L., Boughton B., Reid R. (2010) The mechanism of boron mobility in wheat and canola phloem. *Plant Physiology* 153:876-881.
- Stewart B.A. (1988) *Advances in Soil Science* Soil Science 145:257-271.
- Stone E.L. (1990) Boron deficiency and excess in forest trees: A review. *Forest Ecology and Management* 37:49-75.
- Stumm W. (1992) *Chemistry of the solid-water interface: Processes at the mineral-water and particle-water interface in natural systems* Wiley, New York.
- Su C., Suarez D.L. (1995) Coordination of Adsorbed Boron: A FTIR Spectroscopic Study. *Environmental Science & Technology* 29:302-311. DOI: 10.1021/es00002a005.
- Swaine D.L. (1955) *The trace elements content of soils*, Commonwealth Bureau of Soil Science Technical communication Pp. 48, Farnham Royal.
- Sword M.A., Garrett H.E. (1994) Boric acid–phenolic relationships within the *Pinus echinata*–*Pisolithus tinctorius* ectomycorrhizal association. *Tree Physiology* 14:1121-1130.
- Takano J., Noguchi K., Yasumori M., Kobayashi M., Gajdos Z., Miwa K., Hayashi H., Yoneyama T., Fujiwara T. (2002) *Arabidopsis* boron transporter for xylem loading. *Nature* 420:337-340. DOI: [http://www.nature.com/nature/journal/v420/n6913/supinfo/nature01139\\_S1.html](http://www.nature.com/nature/journal/v420/n6913/supinfo/nature01139_S1.html).
- Tamminen P., Saarsalm A. (2004) Needle boron concentration of young spruce stands on fertile sites in southern Finland. *Metsätieteiden aikakauskirja* 3:271-283.

- Tanaka H. (1967) Boron adsorption by plant roots. *Plant and Soil* 27:300-302. DOI: 10.1007/bf01373400.
- Tessier A., Campbell P.G.C., Bisson M. (1979) Sequential extraction procedure for the speciation of particulate trace-metals. *Analytical Chemistry* 51:844-851. DOI: 10.1021/ac50043a017.
- Theulier M., Duval Y., M D. (1979) Borate exchanges of *Lemna minor* L. as studied with the help of the enriched stable isotopes and of a (n, $\alpha$ ) nuclear reaction. *Plant Physiology* 63:283-288.
- Tollenaar H. (1969) Deficiency de boro en plantaciones de pino en la zona central de Chile. *Agric. Tech.*, 29:85-88.
- Toner C.V., Sparks D.L. (1995) Chemical relaxation and double layer model analysis of boron adsorption on alumina. *Soil Science Society of American Journal* 59:395-404.
- Tsadilas C.D., Dimoyiannis D., Samaras V. (1997) Methods of assessing boron availability to kiwifruit plants growing on high boron soils. *Communications in Soil Science and Plant Analysis* 28:973 - 987.
- Tsadilas C.D., Yassoglou N., Kosmas C.S., Kallianou C. (1994) The availability of soil boron fractions to olive trees and barley and their relationships to soil properties. *Plant and Soil* 162:211-217.
- Turan M.A., Taban N., Taban S. (2009) Effect of calcium on the alleviation of boron toxicity and localization of boron and calcium in cell wall of wheat. *Not. Bot. Agrobot* 37:99-103.
- Turner J., Lambert M.J. (1986a) Nutrition and nutritional relationships of *P. radiata*. *Annual Review Ecology System* 17:325-350.
- Turner J., Lambert M.J. (1986b) Nutrition and nutritional relationships of *Pinus radiata*. *Annual review of ecology and systematics*. Vol. 17:325-350.
- Van Duin M., Peters J.A., Kieboom A.P.G., Van Bekkum H. (1985) Studies on borate esters II: Structure and stability of borate esters of polyhydroxycarboxylates and related polyols in aqueous alkaline media as studied by  $^{11}\text{B}$  NMR. *Tetrahedron* 41:3411-3421. DOI: 10.1016/s0040-4020(01)96693-1.
- Vengosh A., Heumann K.G., Juraske S., Kasher R. (1994) Boron Isotope Application for Tracing Sources of Contamination in Groundwater.

Environmental Science & Technology 28:1968-1974. DOI: 10.1021/es00060a030.

- Venter H.A.V.D., Currier H.B. (1977) The Effect of Boron Deficiency on Callose Formation and  $^{14}\text{C}$  Translocation in Bean (*Phaseolus vulgaris L.*) and Cotton (*Gossypium hirsutum L.*). American Journal of Botany 64:861-865.
- Wallace T. (1961) Essential points in the nutrition of plants: The diagnosis in plants by visual symptoms, 3<sup>rd</sup> Eds. Her Majesty Stationary Office, London pp: 5-17.
- Wan X., Landhäusser S.M., Zwiazek J.J., Lieffers V.J. (1999) Root water flow and growth of aspen (*Populus tremuloides*) at low root temperatures. Tree Physiology 19:879-884.
- Wang K., Xu J.M., Wei Y.Z., Yang Y.A., Bell R.W. (1997) The influence of B fertiliser on distribution of soil extractable B in soil profile in rape-rice rotation in southeast China. In: Boron in Soils and Plants Proceedings of the international Symposium on Boron in Soil and Plant Chiang Mai, Thailand, 7-11 September 1997. Eds. R.W.Bell and B. Rerekasem. pp. 57-61. Developments in Plant and Soil Science, Vol 76 Kluwer Academics Press, Dordrecht.
- Wang K., Yang Y., Bell R.W., Xue J.M., Ye Z.Q., Wei Y.Z. (1999) Low risks of toxicity from boron fertiliser in oilseed rape–rice rotations in southeast China. Nutrient Cycling in Agroecosystems 54:189-197. DOI: 10.1023/a:1009714132135.
- Wear J.I. (1965) Boron In: Method of Soil Analysis-Part 3. Chemical and Biological Properties (Eds. C.A Black). pp 1059-1063.
- Whetstone R.R., Robinson W.O., Byers H.C. (1942) Boron distribution in the soil and related data. USDA Technical Bulletin 797.
- White J.B., Krause H.H. (2001) Short-term boron deficiency in a black spruce (*Picea mariana* [Mill] B.S.P.) plantation. Forest Ecology and Management 152:323-330.
- Wikner B. (1983) Distribution and mobility of boron in forest ecosystems. In : Kolari (eds.). Growth disturbance of forest trees. Communicationes Insituti Foresallis Fenniae 116:131-141.

- Wikner B. (1986) Pretreatment of plant and soil samples a problem in boron analysis. *Communications in Soil Science and Plant Analysis* 17:1-25. DOI: 10.1080/00103628609367693.
- Wilcox H.E. (1991) Root rhizosphere interaction Boron In: *Plant Roots: the hidden half* Pages 1670.:731-765.
- Will G.M. (1985) Nutrient deficiency and fertiliser use in New Zealand exotic forest. *FRI Bulletin No. 97*. Forest Research Institute, New Zealand Forest Service Private Bag, Rotorua.
- Wilson C.M., Lovvorn R.L., Woodhouse W.W. (1951) Movement and accumulation of water soluble boron within the soil profile. *Agronomy Journal* 43:363-367.
- Wilson J.D. (1988) A review of evidence on the control of shoot:root , in relation to models. *Annals of Botany* 61:433-449.
- Wimmer M.A., Lochnit G., Bassil E., Mühling K.H., Goldbach H.E. (2009) Membrane-Associated, Boron-Interacting Proteins Isolated by Boronate Affinity Chromatography. *Plant and Cell Physiology* 50:1292-1304. DOI: 10.1093/pcp/pcp073.
- Wójcik P. (2000) Behavior of soil boron and boron uptake by M.26 apple rootstock as affected by application of different forms and nitrogen rates. *Journal of Plant Nutrition* 23:1227-1239. DOI: 10.1080/01904160009382096.
- Wojcik P., Wojcik M., Klamkowski K. (2008) Response of apple trees to boron fertilization under conditions of low soil boron availability. *Scientia Horticulturae* 116:58-64. DOI: 10.1016/j.scienta.2007.10.032.
- Wolf B. (1974) Improvements in the azomethine-H method for the determination of boron. *Communications in Soil Science and Plant Analysis* 5:39-44.
- Xu J.M., Wang K., Bell R.W., Yang Y.A., Huang L.B. (2001) Division S-4 - Soil fertility & plant nutrition: Soil boron fractions and their relationship to soil properties. *Soil Science Society of America Journal* 65:133-138.
- Yadav H.D., Yadav O.P., Dhankar O.P., Oswal M.C. (1989) Effect of chloride salintiy and gernmination, growth and mineral composition of chickpea. *Ann. Arid Zone* 28:63-67.
- Yamauchi T., Hara T., Sonoda Y. (1986) Effects of boron deficiency and calcium supply on the calcium metabolism in tomato plant. *Plant and Soil* 93:223-230.
- Yermiyaho U., Keren R., Chen Y. (1988) Boron sorption on composted organic matter. *Soil Science Society of America Journal* 52:1309-1313.

- Yermiyahu U., Ben-Gal A., Keren R., Reid R. (2008) Combined effect of salinity and excess boron on plant growth and yield. *Plant and Soil* 304:73-87. DOI: 10.1007/s11104-007-9522-z.
- Yermiyahu U., Keren R., Chen Y. (1995) Boron sorption by soil in the presence of composted organic matter. *Soil Science Society of America Journal* 59:405-409.
- Yermiyahu U., Keren R., Chen Y. (2001) Effect of Composted Organic Matter on Boron Uptake by Plants. *Soil Science Society of American Journal*. 65:1436-1441. DOI: 10.2136/sssaj2001.6551436x.
- Youssef R.A., Chino M. (1987) Studies on the behaviour of nutrients in the rhizosphere. I. Establishment of a new rhizobox system to study nutrients status in the rhizosphere. *Journal of Plant Nutrition* 10:1185-1195.
- Yunusa I.A.M., Manoharan V., DeSilva D.L., Eamus D., Murray B.R., Nissanka S.P. (2008) Growth and elemental accumulation by canola on soil amended with coal fly ash. *Journal of Environmental Quality* 37:1263-1270.
- Zarcinas B.A. (1995) Suppression of iron interference in the determination of boron using the azomethine-H procedure. *Communications in Soil Science and Plant Analysis* 26:713-729.
- Zarcinas B.A., Cartwright B. (1987) Acid dissolution of soils and rocks for the determination of boron by inductively coupled plasma atomic emission spectrometry. *The Analyst* 112:1107-1112.
- Zhao D., D.M O. (2002) Cotton carbon exchange, non-structural carbohydrates, and boron distribution in tissues during development of boron deficiency. *Field Crop Research* 78:75-77.
- Zupancic J.M., Zupancic M. (2007) Boron in irrigation water and its interaction with soil and plants: an example of municipal landfill reuse. *Acra Slov* 89:289-300.