

The effect of metals and soil pH on the growth of
Rhododendron and other alpine plants in limestone soil.

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Declaration

This thesis has not been submitted, in whole or in part, for any degree at this or any other university. The work is original and my own, carried out under the direction of Prof. D. W. H. Rankin and Prof S. Chapman. Where this is not so credit has been duly given.

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Abstract

Rhododendrons are economically important plants in horticulture, and many species are threatened in the wild by habitat degradation. It is therefore doubly important that their nutritional needs should be understood.

It is well-known that rhododendrons and most other members of the family *Ericaceae* are in general calcifuge plants, i.e. they do not grow well, or at all, on limestone-rich soils. However, there have been many observations of the fact that in the wild there are many *Rhododendron* and other *Ericaceae* species that grow healthily on, over or near limestone formations, and other alkaline soils.

In the present research the behaviour of *Rhododendron* in the wild has been studied, investigating the factors that determine the ability of rhododendrons to survive on alkaline soils, to promote the preservation of threatened plants. Discovering conditions for cultivating these lime-tolerant rhododendron species increases the opportunities for conservation of these plants in cultivation.

Dry leaf and soil material for *Rhododendron* species collected in the wild, as well as from species studied in growing experiments, were analysed for concentrations of calcium, iron, magnesium and manganese. Both leaf and soil samples were first treated with extraction solutions in order to extract the desired nutrients in ionic form in solution. The resulted extracts were then analysed spectroscopically, using inductively coupled plasma optical emission spectroscopy. The data obtained from the analyses were interpreted statistically. The pH and organic matter content of the soil samples were analysed, and their correlation with the levels of the nutrients in the soil and the leaves was also interpreted statistically. In order to provide information on whether the soil-plant relationship for other mountain plants, growing in acid and alkaline conditions, were the same as those for rhododendrons, data on the behaviour of other plant species growing in the same habitat as *Rhododendron* were also collected. Leaf and soil material for *Gentiana*, *Primula* and other genera collected in the wild, and from species which were studied in growing experiments, was treated and analysed for the same information as for the *Rhododendron* samples. In addition to laboratory analyses, visual observations on the factors determining the growth of the subjected plant species in their natural habitats were correlated to the

results obtained practically in the laboratory. This was achieved through undertaking a scientific expedition to Yunnan, China, regarded as the region containing the richest number and highest density of *Rhododendron* species, as well as *Gentiana*, *Primula* and other genera.

The studies demonstrated that, at least for *Ericaceae*, it is deficiencies of manganese, and to a lesser extent iron, which are crucial for the survival of these plants in alkaline soils. It was also shown that rhododendrons growing in situations where manganese is readily available accumulate large amounts in their leaves. It was shown that the (small) organic component of the limestone soils in which rhododendrons flourish contains an unusually high level of manganese. It can be therefore suggested that recycling of this nutrient is the key to the unusual behaviour in the wild.

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Chapter 1 Introduction

General introduction

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The Genus *Rhododendron*

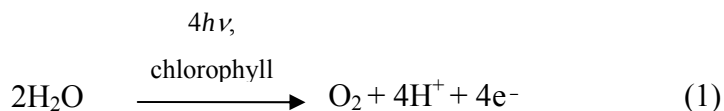
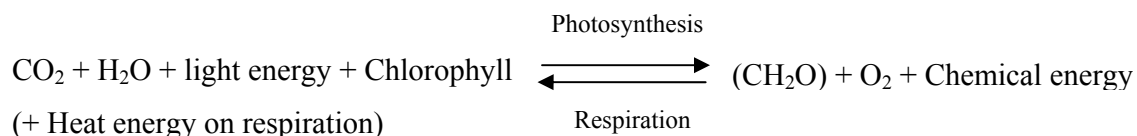
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General introduction

1.1. Plant nutrient requirements

1.1.1. Photosynthesis

Photosynthesis in all chlorophyll-containing organisms (except for photosynthetic bacteria), is the light-induced reaction in which CO_2 is reduced by water to produce carbohydrates, while CO_2 oxidises two molecules of water to molecular oxygen as an end product (reaction (1))^{1, 2}. There are several types of pigments that accumulate light. In higher plants the primary pigment is chlorophyll *a* (chl *a*), with a magnesium atom as metal centre.



Between the removal of electrons from water and the CO_2 accepting them there are many steps of chains of redox reactions, usually catalysed by enzymes. The least understood part of the process for a long time has been the splitting of the water molecule, which is carried out in the enzyme complex Photosystem II (PS II) in the thylakoid membranes of chloroplasts with the water-oxidising complex (WOC) as an active site of the oxidation reaction^{3, 4}. The WOC consists of four manganese atoms, the cofactors Ca^{2+} and Cl^- , and a closely interacting, redox-active tyrosine side chain, TyrZ (D1-Y161)^{3, 4}. Upon light absorption the reaction centre in PS II, the chlorophyll dimer P680, which is the primary electron donor, is excited and subsequently reduced by the WOC. The active Mn centre in the WOC undergoes one-electron oxidation from the P680 through five redox intermediates, S_0 , S_1 , S_2 , S_3 , and S_4 , to the four-step oxidation

of the water molecule^{3, 5}. At the present time, electron paramagnetic resonance (EPR) and x-ray absorption spectroscopy (XAS) studies have provided evidence on the Mn-cluster transformations that occur in the steps between S_0 , S_1 , S_2 and S_3 , and the transitions $S_3 \rightarrow S_4$ and $S_4 \rightarrow S_0$ are still not well understood³. It is generally agreed that the transitions $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_2$ involve oxidation of the Mn cluster, while in $S_2 \rightarrow S_3$ changes in the coordination environment rather than in oxidation state occur^{3, 5}.

1.1.2. Nutrients and their role in plant life

This discussion is concerned mainly with the nutrients for which analyses were performed as part of this research programme.

The nutrients crucial for healthy plant growth are divided into two main groups, macronutrients and micronutrients (Table 1).

Table 1 Nutrients essential* for healthy plant growth.

Essential elements		Beneficial elements
Macronutrients	Micronutrients	
Carbon	Iron	Selenium
Nitrogen	Manganese	Cobalt
Oxygen	Boron	Sodium
Hydrogen	Zinc	Silicon
Phosphorus	Copper	Fluorine
Potassium	Molybdenum	
Sulfur	Nickel	
Calcium	Chlorine	
Magnesium		

* Essential nutrients – the elements the absence of which will cause the plant to fail to complete its life cycle; that the element cannot be replaced by the presence of another element; and that the element is directly involved in plant metabolism (Arnon and Stout, 1939)

Nutrient requirements for healthy growth for *Rhododendron* and for other trees and shrubs for comparison have been experimentally shown to be within the ranges listed in Table 2^{6,7,8}.

Table 2 Concentration ranges of some nutrients in the leaves of healthy and deficient shrubs and trees in comparison to *Rhododendron* [ppm].

Plant	Deficient/ Healthy	Ca	Fe	Mg	Mn
<i>Rhododendron</i> ⁷	Healthy	7200 – 25000	53 – 363	1300 – 4600	63 – 2720
Woody plants ⁷	Healthy	6400 – 36000	65 – 200 ⁶	1700 – 8000	20 – 300
Fruits ⁸	Deficient	7000 – 20000	< 35 – 60	1300 – 2000	< 15 – 25
	Healthy	10000 – 60000	50 – 250	2500 – 8000	25 – 160
Sugar beet plants ⁸	Deficient	1000 – 4000	20 – 55	250 – 500	4 – 20
	Healthy	4000 – 15000	60 – 140	1000 – 25000	25 – 360

1.1.2.1 Macronutrients: chemistry, functions and deficiency in plants

Macronutrients are the nutrients that plants require in concentration above 1 ppm to support healthy growth.

1.1.2.1.1. Chemical compounds of macronutrients in plants and their transport and functions in plant metabolism

i. Calcium

Calcium compounds and transport inside the plant

Ca is absorbed by plant roots as Ca(II). It is believed that the young roots closer to the top soil absorb Ca more than the older roots⁹. Inside plants it is mainly present in compounds with oxalate (Ca oxalate occurs in crystalline form in leaf cell vacuoles of some plants) and salts of other carboxylic acids, such as γ -carboxyglutamate. Calcium transport and binding may depend on a specific Ca-binding amino acid in proteins produced by secondary reactions, such as γ -carboxyglutamate⁸. One of the most important, specifically binding Ca proteins, is calmodulin, which is thought to have four Ca-binding sites with dissociation constants around 10 to 17 μ M. Calmodulin is localised in nuclear material and it first binds to Ca and then reacts with calcium-dependant enzymes and proteins (ATP-ase, phosphorylases, phospholipases, kinases, etc.).

Calcium biological functions in plant metabolism

Calcium has two roles in plant metabolism: it has a structural function and it is an activator for a number of enzymes.

At the level of cell structure calcium is very important for formation of membranes, in cell adhesion, cell wall stability, cell division, and also in nuclei and chromatin behaviour. Calcium controls the germination and the growth of pollen cell tubes. Severe Ca deficiency causes chromosomes to fail to separate completely and the cell plate is not formed. Organelles such as mitochondria, chloroplasts, vacuole tonoplasts and plasmalemma become defective or morphologically abnormal; their membranes may become fragmented and irregularly detached from the cell walls⁸. Nuclear membranes may perforate or form hollow fragments.

In cell wall composition, calcium forms salts with pectic acid (a polymeric galacturonic acid), forming cross-link between the non-methylated carboxyls in different fibrils by calcium bridges.

The affinity of phospholipids in membranes for Ca^{2+} ions stabilises the membranes and controls their permeability for other ions. This role of Ca is particularly important for promoting potassium transport across the membrane, and for K absorption. The mechanism may involve Ca-dependent ATP-ase functioning as an ion pump to transport K across the plasmalemma or tonoplast membranes. It was shown that some ATP-ase systems are inhibited by Ca^{8} .

Calcium activates enzymes such as phospholipase D, lecithinase (which hydrolyses lecithin to choline and phosphatidic acid), ATP-ase systems, and α amylase (starch hydrolyses in seed germination). Ca may specifically activate one or two steps in the electron transport sequence in photosystem, between photosystem II (PSII) for the oxygen evolution and photosystem I (PSI) for the ferredoxin reduction⁸.

ii. Magnesium

Magnesium compounds inside the plant

Magnesium is mainly contained in the molecules of chlorophylls *a* and *b*.

Magnesium biological functions in plant metabolism

The best known role of magnesium is as a constituent of chlorophyll *a* and *b* molecules. Therefore, lack of Mg is first seen in decreased chlorophyll concentration due to its breakdown in the older leaves, and in decreased formation in the younger leaves when deficiency is more severe⁸. The largest part of the essential Mg concentration (about 75% of the minimal leaf Mg concentration in healthy plants) is associated with ribosomal structure and functions. Magnesium is important in the stability of ribosomal particles (especially the polysomes), and required in critical concentrations for the isolation of functional RNA-protein particles that perform *in vitro* the sequential

reactions of protein synthesis from amino acids. In addition, magnesium may also bind the transfer (tRNA) acylamino acid complex ribosome⁸.

Magnesium-dependent enzymes are very important in photosynthesis, e.g. in enolase, ribulose-bisphosphate carboxylase, etc. Many enzymes are activated by Mg, in some cases partially or adequately substituted by Mn (in decarboxylating systems) or by Zn. There are several magnesium-dependent enzymes in chloroplasts and mitochondria, which undergo structural degeneration under Mg deficiency conditions. Magnesium (in the form of an Mg-ATP complex) is also important in dinitrogen fixation by the root nodule bacteria in legume and non-legume plants⁸.

iii. Nitrogen

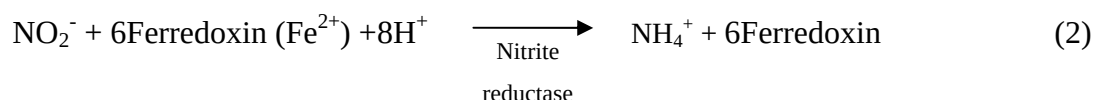
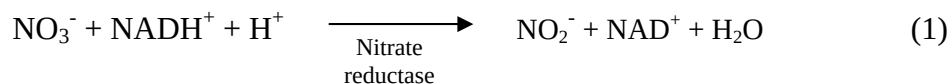
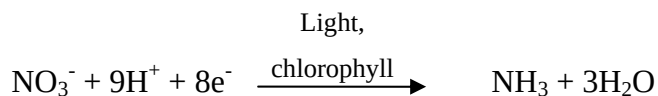
Nitrogen compounds inside the plant

The principal compounds of nitrogen in plants are the large number of proteins which represent the most important end-products of plant growth, and the nucleic acids DNA and RNA. Nitrogen is a constituent of amino acids, which are precursors of the polypeptide chains that compose all proteins, and glycine or glutamate are additionally precursors of the bases in nucleic acids. Nitrogen is also part of a number of haem proteins such as some cytochromes, legume haemoglobin, and also catalase and peroxidase. Other nitrogen-containing compounds include adenosine triphosphate (ATP), involved in energy transfer, guanosine triphosphate (GTP), involved in translation of mRNA during protein synthesis, and the nicotinamide adenine dinucleotide co-enzymes NAD and NADP, involved in enzymic electron and hydrogen transport⁸.

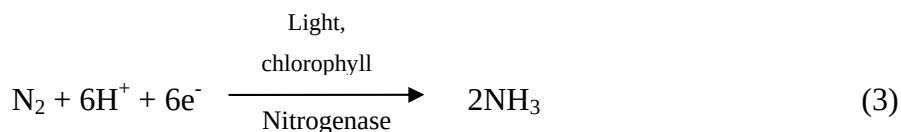
Nitrogen biological functions in plant metabolism

The functions of nitrogen in plant metabolism and growth are defined by the functions of the principal compounds, which are end-products of N-assimilation (proteins, etc.).

Plants absorb nitrogen mainly and preferentially from nitrate (NO_3^-), reducing it to ammonia, and to a lesser extent from ammonia directly. Nitrate ions are reduced to ammonia in several stages (reactions (1) and (2)), as the ammonia is subsequently consumed for the synthesis of amino acids, and etc⁸.



Nitrogen may also be utilised directly from the atmosphere after fixation by reduction of dinitrogen (N_2) to NH_4^+ in photosynthetic bacteria, blue-green algae and in the root nodules of legume and non-legume plants, by bacteria that exist in a symbiotic relationship with the plant (reaction (3))⁸.



The ammonia is further converted to glutamate and used for protein synthesis.

Rhododendron and other plants in the family *Ericaceae* have shown preference for NH_4^+ to NO_3^- ^{10, 11}. This may be due to the fact that in some plants in the family *Ericaceae* nitrate reductase is either absent or inactive⁸.

iv. Phosphorus

Phosphorus compounds and biological functions in plant metabolism

Phosphorus is absorbed by plants in the form of phosphates. In plant metabolism phosphorus is a constituent element of the nucleic acids, phospholipids and many coenzymes, including the pyridine nucleotides and nucleoside phosphates. It is required for energy storage - ATP synthesis, cell division, and root development, and also for nitrogen fixation. Phosphorus is taken up by the plant as phosphate and it is not reduced in the plant. It is further transported in the xylem either as inorganic phosphates or as sugar phosphates.

Phosphorus is particularly important for fruit and seed ripening and germination.

1.1.2.1.2. Macronutrient geochemistry. Deficiency disorders and their treatments

i. Calcium

Calcium mineral forms in the soil

The most abundant sources of calcium are calcite (CaCO_3), aragonite - a metastable polymorph of calcite, ankerite ($(\text{Ca,Fe})\text{CO}_3$), gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), and dolomite ($(\text{Ca,Mg})\text{CO}_3$).

Calcium deficiency in plants

According to their tolerance to calcareous soils, plants are divided into two groups: calcifuges, which cannot grow in calcareous soils but grow in acidic soils, and calcicoles, which have a preference for alkaline soils (e.g. limestone).

Calcium deficiency occurs mostly in plants growing in acidic soils, such as peat (*Sphagnum* or moss peat), and in soils originating from rocks that have low Ca content, such as many granite rocks and silica sandstones. Calcium is relatively immobile in plant tissues, not moving readily from old to young leaves, and so it is usually in its highest concentration in the older leaves and less in young leaves and fruits. As a result, deficiency of Ca appears first on the young leaves and shoot tips, which may become curled inwards or ragged, scorched and followed by necrosis¹². The most common way to correct Ca deficiency in acid soils is by applying lime to the soil until it reaches a pH of around 6.5. Any higher pH may inhibit the availability of other nutrients to plants. To avoid further increase of pH, application of CaSO_4 , CaCl_2 , or $\text{Ca}(\text{NO}_3)_2$ is preferable to the use of CaCO_3 .

ii. Magnesium

Magnesium mineral forms in the soil

In most soils the main source of magnesium is weathered secondary minerals such as dolomite $(\text{Ca,Mg})\text{CO}_3$, serpentine or primary minerals such as hornblende (occurring in metamorphic rocks), providing exchangeable Mg on the clay-humus colloid sites¹³.

Magnesium deficiency in plants

The most characteristic symptoms of magnesium deficiency are yellowing between the veins, while the veins remain green¹², or reddish-purple coloration between the veins of the older leaves, but without marginal scorch (shown in black currants)⁸. The purpling symptom of Mg deficiency may often be mistaken for phosphorus deficiency.

Because of its high mobility in plant tissues Mg deficiency symptoms appear first in the older leaves as during periods of shortage it is transported into the young leaves¹². Mg deficiency can be treated with magnesium-limestone or Epsom salts (MgSO_4).

iii. Nitrogen

Nitrogen mineral forms in the soil

Nitrogen in soils occurs as organic compounds (proteins, aminoacids), NH_4^+ , NO_3^- , NO_2^- (nitrite), nitrous oxide, and N_2 in the soil air space. The largest concentration of nitrogen in the surface soil is in the organic form.

Nitrogen deficiency in plants

Nitrogen can be deficient in almost all types of soils, but those having low organic matter content are more susceptible. Some peat soils containing insufficiently decomposed organic matter can become N-deficient¹². Symptoms of N-deficiency begin in the older leaves (N-compounds are highly mobile and readily transported from old to young leaves) and include weak growth, decreased leaf-size, and pale colour of the leaves, which in some plants may also have red, purple or yellow tints¹². Under conditions of deficiency of nitrogen supply, nitrate reduction failure, or inefficient nitrogen fixation, the result will be impaired growth due to reduced protein synthesis, and breakdown of reserve forms of chlorophyll and proteins in the older leaves⁸.

Nitrogen is commonly applied to soil as $(\text{NH}_4)_2\text{SO}_4$, KNO_3 , NH_4NO_3 , and in forms such as animal manure.

It has been shown that *Rhododendron* plants growing in high pH soils take up nitrogen as ammonium in preference to nitrate¹⁰.

In a growing experiment including *Rhododendron* seedlings and mature plants, planted in two media pH, 4.6 and 7.6, both seedlings and mature plants developed chlorotic symptoms when grown in soil where the source of nitrogen was nitrate and remained healthy when the source was ammonium salts^{10, 14}. The seedlings given nitrate were either severely chlorotic or dead 20 weeks after they were potted. With the nitrate treatment at pH 4.6, growth, although very weak and unhealthy, did occur, whereas at

pH 7.6 growth did not develop at all, and most seedlings died¹⁰. Meanwhile, the seedlings given the ammonia treatment were healthy at both pH levels, although the seedlings in the pH 4.6 medium had stronger growth than those in pH 7.6¹⁰. The symptoms reported were pale yellowish-green colour of leaves of the plants grown in the nitrate medium, while those grown in the ammonium medium remained green. In the study of the mature *Rhododendron* plants the plants grown in the nitrate medium became chlorotic at both pH 7.6 and 4.6, but more severely at pH 7.6. The plants in the ammonium medium remained healthy, but towards the end of the trial the plants at pH 7.6 were showing a tendency to develop chlorotic symptoms¹⁴.

An explanation for the preference of *Rhododendron* for ammonium nitrogen is perhaps that the conversion of ammonium to nitrate increases with an increase in pH. It has also been suggested that the activity of nitrate reductase is reduced by high levels of manganese, so the uptake rates of Mn and its concentration in the shoots increase significantly if, under high levels of Mn in the soil, N is supplied as NO_3^{-15} .

iv. Phosphorus

Phosphorus mineral forms in the soil

Phosphorus in soils occurs mainly in inorganic form, bound to other metals such as Ca, Fe or Al in water-insoluble complexes, such as minerals in the apatite group ($\text{Ca}_5(\text{PO}_4)_3(\text{F}, \text{Cl}, \text{OH})$), thus being slowly released into the soil¹⁶. Generally, the mechanism by which plants dissolve the P locked in Ca minerals is by exuding di- and tricarboxylic acids and oxalic acid in particular, which is due to the low solubility of Ca oxalate. It has been shown that calcicole plants exude much more oxalic acid or oxalate than calcifuge plants do if exposed to calcareous soils, thus solubilising and making P available for uptake. Two important observations were made. 1) The calcicole plants had significantly higher levels of inorganic phosphate in their foliage than the calcifuge plants on both calcareous and acidic soils. 2) The calcifuge species were also less

capable of controlling the excessive take-up of calcium when growing on calcareous soil, as they had higher concentrations of Ca in their leaves than the calcicole plants¹⁶. It has also been suggested that high HCO_3^- concentrations in the soil may increase the solubility of phosphorus complexes, and thus the availability of HPO_4^{2-} ¹⁶.

Phosphorus deficiency in plants

The symptoms of phosphorus deficiency in plants could be similar to those of nitrogen deficiency. All growth is restricted and weak, and flowering and fruiting are reduced and delayed. Leaves are smaller in size and, beginning from the oldest, may drop. Phosphorus deficiency rarely induces yellow or red colours in leaves. The leaves will more likely become dull blue-green with purple tints¹².

1.1.2.2 Micronutrients: functions and deficiency in plants

1.1.2.2.1. Chemical compounds of micronutrients in plants and their transport and functions in plant metabolism

i. Manganese

Manganese compounds in the plant and biological functions in plant metabolism

Plants roots absorb Mn from the soil as Mn(II), which is the stable soluble state of manganese in acidic aqueous solution. In alkaline solution and in the presence of oxygen, the insoluble MnO_2 is the stable form¹⁷.

There is evidence that manganese in plants is distributed unevenly, being more concentrated in the cell vacuoles and chloroplasts¹⁷.

The biological functions of manganese are determined by the chemistry of Mn(II), and the powerful oxidation properties of Mn(IV), both having a role in PS II.

The most important function of manganese is as a part of the Mn cluster in PS II, which oxidises the water molecule to produce molecular oxygen, discussed earlier in this chapter. However, Mn also plays a role in activating several enzymes, such as the auxin oxidase system and the NAD^+ - malate enzyme (in C_4 photosynthetic plants)⁸.

ii. Iron

Iron transport from the soil into the plant and inside the plant

Plants have developed two strategies to secure iron uptake by roots under different soil conditions, proposed first by Römheld and Marschner in 1986¹⁸. All higher plants, except for grasses, carry out Strategy I to absorb iron. This consists of three main reactions: a) acidifying the soil solution surrounding the roots, by release of H^+ , and thus increasing Fe^{3+} solubility; b) reduction of Fe^{3+} by Fe^{3+} -chelate reductase to Fe^{2+} ; and c) transport of Fe^{2+} in the plasmalemma by iron transporters - the major iron uptake transporter from the soil is established to be the IRT1 transporter (iron-regulating transporter)¹⁸. Under conditions of iron deficiency all three processes have increased activity, along with other adaptive measures that may also occur, such as increase of citrate concentrations in the phloem, change in root morphology, etc.

Inside the plant iron is transported after it is bound to organic acids and certain proteins, before it can be inserted into the targeted molecules for biological functions¹⁸. In higher plants (Strategy I) iron is transported in Fe^{2+} form by nicotianamine (NA). In contrast, in grasses (Strategy II) iron is taken up from the soil in the form of Fe^{3+} and transported into the xylem, where it is bound by citrate, which is a main carrier for Fe^{3+} in plants¹⁸. Further on, ferrochelatase is regarded as a transporter of Fe^{2+} ions into the porphyrin ring to form ferrous protoporphyrin, although the affinity of the enzyme is low and an intermediate reaction may take place first⁸.

Non-physiological forms of iron are FeEDTA and other Fe chelates, which, despite their large stability constants, are readily available sources of Fe to plants. Conversely,

phosphate and bicarbonate in the root medium readily immobilise iron and cause chlorosis⁸.

Iron biological functions in plant metabolism

The functions of iron are mostly based on the redox reactions of Fe^{2+} (ferrous) and Fe^{3+} (ferric) ions, its ability to form octahedral complexes with many ligands and to vary its redox potential in response to different ligand environments. These properties make iron excellent for electron-transfer reactions and, indeed, the essential electron-transport chains of life in respiration and photosynthesis are based on non-haem iron-sulfur proteins of 2Fe-2S type or 4Fe-4S type, as found in photosynthetic reaction centres and ferredoxins^{8, 18}. In plants the best known iron-sulfur protein is chloroplast ferredoxin, which contains 2Fe-2S, and transfers electrons from PS I to specific chloroplast enzymes (e.g. nitrite reductase).

Several enzymes and electron carriers contain haem prosthetic groups, which in plants are of four types: cytochromes *a*, cytochromes *b*, cytochromes *c*, and sirohaem proteins. Cytochrome *b* is contained in the nitrate reductase enzyme in the reduction of nitrate to ammonia, and sirohaem protein is contained in nitrite reductase. It contains both haem and non-haem iron⁸. Iron is also part of the two proteins involved in the fixation of dinitrogen to ammonia by nodule bacteria, found in the root nodules of plants (e.g. clover, legumes). Also in the root nodules, the symbiosis between the roots and the bacteria produces haemoglobin (related to mammalian haemoglobin), containing ferrous iron-porphyrin protein (cytochrome *b* type), on which the dinitrogen fixation is very dependent⁸. Another iron-containing protein is phytoferritin, which is an iron-storage protein, and it holds about 2000 iron atoms per mole⁸.

1.1.2.2.2. Micronutrient geochemistry. Deficiency disorders and their treatments

i. Manganese

Manganese mineral forms in the soil

The majority of the Mn present in rocks occurs as a minor constituent of the main minerals¹⁹. It is mostly present in primary silicate minerals, such as olivine, biotite, serpentine, muscovite, magnetite, quartz, etc., in most of which it is in the Mn(II) state, or as secondary minerals, oxides and hydroxides. In oxidising weathering environments and under alkaline conditions Mn(II) is rapidly oxidised to Mn(III) and Mn(IV) in the form of MnO₂, which has an extremely low solubility, about 10⁻²⁸ M, at pH 7.0¹⁹. In contrast, in hydrated reducing soils Mn(II) will form. This has a solubility about of 10⁻¹ M. The secondary minerals of manganese are oxides such as δ-MnO₂ (vernadite) and (Na_{0.7}Ca_{0.3})Mn₇O₁₄·2.8H₂O (birnessite, also known as manganous manganate), which are the most commonly found ones, manganite (γ-Mn³⁺O OH) and hausmannite (Mn²⁺Mn₂³⁺O₄), which are rare, and other higher oxides of Mn¹⁹.

Manganese deficiency and toxicity in plants

Manganese deficiency is very common in the UK⁸. The soil conditions that favour occurrence of Mn deficiency are: high organic matter (fen peat overlying calcareous soils, or marshy land soils, and sandy soils), high pH, and not very good drainage (calcareous soils). Unlike Fe deficiency, Mn deficiency is not a serious problem on naturally calcareous soils, but is much more likely to occur in neutral or acid soils that are overlimed⁸.

Manganese is generally a relatively immobile nutrient and so deficiency symptoms appear first in the young leaves. The symptoms can be variable but most often, as with Mg deficiency, the symptoms are expressed in interveinal chlorosis, which may also be pale green rather than yellow⁸. Grey specks or streaks and lesions may appear at a more developed stage, and severely affected tissues eventually turn brown.

The most commonly used treatment for Mn deficiency is a foliar spray with MnSO_4 hydrate, although it does not have a long-release effect and it is utilised very quickly by the plant, so the treatment should be repeated.

In addition to Mn deficiency, another common problem is also Mn toxicity, which may occur in very acidic or waterlogged soils. For most plants that do not tolerate high Mn levels in their tissues the typical limit of concentrations toxic to them is 300 ppm. Manganese is readily taken up and transported from the roots to the shoots, and so Mn toxicity appears first in the shoots. Generally symptoms of high Mn may differ in distribution between the plant organs, depending on the tolerance of the different tissues. It has been shown that within the old tissues the distribution of Mn is uneven and it accumulates preferentially in the cell walls, mostly as insoluble MnO_2 , as this is believed to be a mechanism of detoxification²⁰. Therefore, in old leaves, stems and petioles symptoms appear as dark brown speckles due to accumulated MnO_2 . In young plant tissues, the Mn toxicity appearance of “crinkle leaf” is caused by high-Mn-induced Ca deficiency in leaves²⁰. Since both Ca and Mn are preferentially and readily transported to fast-growing tissues, as a result high Mn levels in the soil inhibits both Ca uptake from the soil and Ca transport to the young expanding leaves. Therefore, even if Ca is applied to the soil to counteract the high soil Mn, this may still not rectify and elevate the transport of Ca to the young leaves, which will still be impeded by a prioritised Mn transport²⁰. High Mn levels may also result in Fe deficiency, particularly in the young leaves.

Some plant species have tolerance to high Mn supply, which differs among different genotypes, and climate conditions, and which depends mainly on the abilities of the shoots and other plant tissues to tolerate high Mn. Mn tolerance has been shown to be dependent on temperature and light intensity, as well as on leaf age. In some species physiologically older leaves are more sensitive to Mn than younger leaves, as was demonstrated by applying Mn via the petioles²⁰. It is suggested that in some species silicon decreases the sensitivity to high Mn supply²⁰.

Overall, for some plants it was demonstrated that Mn oxides were absent in Mn-tolerant tissues; this therefore suggests that Mn oxidation is increased and occurs more in the Mn-sensitive plant tissues²⁰.

ii. Iron

Iron mineral forms in the soil

Iron in the soil exists in the Fe(II) (ferrous) state in primary minerals, and in the Fe(III) (ferric) state in secondary minerals, in the forms of inorganic and organic complexes and chelates (Table 3).

Table 3 Iron-containing minerals.

Type of mineral		Mineral		Colour
Primary		olivine	(Mg,Fe ²⁺) ₂ [SiO ₄]	olive green
Secondary	Oxides	hematite	α -Fe ₂ O ₃	dull to bright rust-red in soil
		maghemite	Fe ₂ O ₃	brown-brick red
		magnetite	Fe ²⁺ Fe ³⁺ ₂ O ₄	greyish black or iron black
	Oxyhydroxides	goethite	α -Fe ³⁺ O(OH)	
		lepidocrocite	γ -Fe ³⁺ O(OH)	deep red-brown
		akaganeite	β -Fe ³⁺ O(OH,Cl)	yellowish brown, rusty brown
		ferrihydrite	Fe ₅ O ₃ (OH) ₉	dark brown, yellow- brown
	Other	pyrite	FeS ₂	pale brass-yellow
		siderite	FeCO ₃	

Although iron is abundant in primary and secondary minerals in soils, in alkaline soils it is often unavailable to plants as its availability is hindered by the chemistry of the iron oxidation states. This is mainly expressed in low solubility of iron secondary minerals (Fe(III)), especially in alkaline and calcareous soils, which limits plant growth. Fe(II) is soluble but is readily oxidised by atmospheric oxygen (in oxidising conditions - drought), precipitating as secondary minerals. At lower pH and in a more reducing environment Fe(III) is soluble enough (10^{-6} M at pH = 3.3), but its solubility decreases dramatically with increasing pH, due to hydrolysis as Fe(OH)₃, polymerisation or precipitation with inorganic anions; at pH = 7.0 the Fe(III) concentration is only 10^{-17} M. However, plants require between 10^{-4} M and 10^{-8} M Fe(III), so in well-aerated soils with pH above 7.0 the solubility of iron is lower than required for optimal plant growth¹⁸.

At the same time, in low pH soils and/or reducing conditions soluble forms of Fe can be in sufficient concentrations to be toxic to plants.

Iron deficiency in plants

Essentially, true iron deficiency in soils is uncommon. However, in alkaline (limestone) soils iron precipitation as insoluble oxides and hydroxides causes iron deficiency, with symptoms known as 'lime-induced chlorosis'^{8, 12}. The symptoms associated with this are bleaching (pale green colour) or yellowing of the new-growth foliage, while the veins remain unaffected, dark green, or the young leaves become uniformly yellow^{8, 12}. The pale coloration of the leaves is caused by the lack of chlorophyll in the leaves, since iron takes part in its formation. If the Fe deficiency becomes acute, its appearance may progress to yellow leaves, brown leaf margins and tips (an advanced stage), failure to fruit and death¹². Regardless of the factor that caused iron deficiency, it usually develops and appears first in the youngest leaves, as Fe is a relatively immobile nutrient^{8, 18}.

On the other hand, iron deficiency can also appear in acid soils, as a secondary effect of another nutritional disorder, such as high levels of Mn or P, or too low Ca, or in acid and anaerobic (waterlogged) soils, with pH < 4.0, due to Fe being leached out of the soil

because of its high solubility. In these situations liming may alleviate the iron deficiency. Metal-induced iron chlorosis can be caused by interactions of other metals (Zn, Cu, Co, Mn, Ni) with iron proteins, by replacing iron⁸.

To treat iron deficiency in alkaline soil, mere application of inorganic iron compounds to the soil rarely improves the deficiency since the added iron will again become unavailable. The most reliable treatment will, therefore, be to apply iron in the form of iron chelates (Fe-EDTA, Fe-EDDHA) or sequestrenes*. In these compounds iron is bound to organic molecules, which make it less likely to react with other chemicals in the soil, thus preventing it from becoming unavailable to plants¹². However, in acidic soils foliage sprays of iron sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), and iron citrate can also be efficient¹⁸. Iron chelates, although more expensive than iron sulfate, persist longer.

Summary of plant - nutrient relationships

In order to relate some of the important theoretical points to the results and conclusions of this thesis, which will be discussed later, it will be helpful to give a brief summary of some factors relevant to the research.

Mineral disorders can be triggered by fungal or viral infections, as plants are more susceptible under such infections and/or more prone to mineral deficiency when infected. On the other hand, symptoms induced by non-nutritional factors, such as seasonal changes (some leaves become yellow or red towards the beginning of autumn), growth regulators, herbicides, pests and diseases (insects, fungi, viruses), and air pollutants, often result in appearances that can be mistaken for nutrient disorders⁸. Furthermore, when more than one nutrient is deficient in plants, the symptoms may appear very different from those typically caused by deficiency of either of the nutrients alone. Often mere analyses of leaves can be misleading, especially if the wrong type of

* Sequestrene 330 (Fe-DTPA) contains 10% iron; manufacturer, Becker-Underwood.
Sequestrene 138 (Fe-EDDHA) contains 6% iron; manufacturer, Becker-Underwood.

plant material is analysed (roots, stems, bark, fruits, petioles, leaf tip or leaf margins, shoots, etc.). Generally, with immobile nutrients (Ca, Fe, Mn) deficiency and adequate concentrations may exist simultaneously in different parts of the same plant. If supply of calcium, for instance, is interrupted, it will result in Ca deficiency in the new leaves only. Therefore, analyses of mature leaves to establish deficiency of an immobile nutrient will be unsatisfactory for diagnosing the cause of the disorder⁸.

It is found that if plants are supplied with excess of some nutrients an excess proportion of these nutrients will be usually present in ionic or soluble form somewhere within the plant. For example, for some plants, if the supply of nitrates exceeds the capacity of the plant to reduce nitrates they accumulate it in the conducting tissues (xylem) and leaves⁸.

1.2. Soil-plant interactions

1.2.1. Nutrient uptake by plants

The majority of plant nutrients are taken up by the plant in ionic form in the soil water solution. The contact of the plant with the film of water that surrounds the soil particles is through the main root, the root hairs and mycorrhiza, as the latter two greatly increase the contact surface area. There are three ways that nutrients in the soil become available to the root surface: interception – growth of roots into new, nutrient-rich area, mass flow – water-borne movement of ions into the root driven by transpiration, and diffusion – movement of ions into areas depleted in nutrients.

Further transport of nutrient ions across membranes is carried out by protein transporters that are specific for each nutrient²¹.

1.2.2. Microbial activity in soil: roots exudates and root clusters as mechanisms for counteracting mineral deficiency in plants.

As a response to growing in a soil environment poor in certain nutrients, plants are known to develop a few different mechanisms of adaptation in such soils.

1.2.2.1. Release of exudates

It is well known that calcareous (limestone) soils have low nutritional content as some of the nutrients that are very important for plant growth, such as P, Fe, Mn, and Zn, are present in insoluble forms in the minerals. In such high-pH, nutrient-poor soils a general mechanism solubilising 'locked' minerals in is the exudation of organic acids from the roots of plants, and consequently acidification of the immediate rhizosphere, making the nutrients available for uptake^{16, 22}. Such exudates are usually di- and tricarboxylic organic acids such as oxalic, citric and malonic acids, which under deficiency conditions are exuded at higher rates than under normal conditions and mobilise the deficient nutrients from less soluble formations in the soil^{22, 23}.

There is evidence for soluble organic complexes exuded by plants that are important in solubilising ferric iron⁸.

1.2.2.2. Root clusters

It has been found that in some extremely infertile soils, formation of cluster roots is an adaptation for nutrient acquisition²³. To date, such cluster roots have been observed and studied only for plants growing in soils in SW Australia, S Africa, and the Mediterranean. Clusters develop on root systems of a range of species. Some of those adapted to the most extreme nutrient-poor soils belong to the families *Proteaceae*, *Casuarinaceae*, *Fabaceae*, *Betulaceae*, *Eleagnaceae*, *Moraceae* and *Myricaceae*. A single cluster is composed of shorter and finer rootlets on the parental root forming a bottlebrush-like shape, and cluster roots consist of many groups of clusters creating a "beaded" shape along the parental roots²³. As with non-cluster roots, the function of the cluster roots is expressed in the release under condition of mineral deficiency of organic acids that mobilise the deficient nutrients. However, cluster roots exude organic acids at rates higher than those of the non-cluster roots²³. Reduction of MnO₂ has been

demonstrated in the rhizosphere of cluster-rooted species. It has been suggested that this allows hyper-accumulation of Mn by plants tolerant to very high Mn concentrations to occur, as observed for members of the *Proteaceae* family²⁴. The cluster roots have been found to be very efficient particularly in the acquisition of phosphorus, which has generally low availability²³. Plants that form cluster roots acquire more phosphorus from soils of low soluble P than plants with non-cluster roots. So far, the plant species identified to form cluster roots are species growing in low-pH soils²³. There are observations of a few cluster-rooted plant species that form only weak associations with mycorrhiza, although generally they are not associated with mycorrhiza²³.

1.2.2.3. Mycorrhiza-plant relationships

Mycorrhizal fungi are living in symbiosis with the roots of most vascular plants, working for mutual benefit. Mycorrhiza are highly efficient at absorbing nutrients from the soil²¹. The most common form of mycorrhiza is the vascular-arbuscular mycorrhiza (VAM = endomycorrhiza) but another widespread type is ectomycorrhiza (EM), which is characteristic of many trees of temperate and boreal environments and some tropical trees, particularly those growing in nutrient-poor acidic soils²¹. Plants associated with the VAM fungi can also live without them, especially if in nutrient-rich soils, but for the plants associated with the EM, the symbiosis is usually obligatory at least at some stage of their life cycle²¹. Compared to non-mycorrhizal plants VAM-colonised plants may be able to increase nutrient uptake. VAM fungi have been observed to increase the phosphatase activity in the soil²⁵. There are also reports of effects of VAM on the pH of the media, and, *vice versa*, VAM colonisation is also influenced by the pH and nutrient status of the soil. The growth of some VAM may be stimulated by an increase or a decrease in soil pH, while others are not affected by pH²⁵. Overall, each VAM community is adapted to its native soil.

1.2.3. Soil taxonomy, mineral composition and structure

Depending on the way in which they formed, rock types are divided into the three groups listed in Table 4.

Table 4 Classification and origin of rock formations.

Rock type	Formation process
Igneous	Solidification on cooling of molten rock material - magma. Mineral crystals grow in the cooling magma until solidification is complete. – granite, basalt (Fe-rich)
Sedimentary	Transport and deposition of material such as sand, mud and gravel, produced by the breaking-up of rock and marine animal skeletons and shells at the Earth's surface under the action of wind and water.
Metamorphic	Re-crystallisation of rocks under heat and/or pressure (squeezing) inside the Earth – schist (>50% layered rock containing mica – muscovite, biotite; hornblende), gneiss (<50% layered); granite; marble, slate

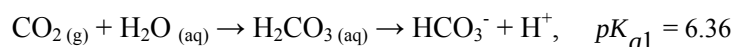
The target of this research is to study plant growth on alkaline soils and particularly on soils derived from limestone rock formations. However, another source of alkaline soil, in which a few groups of plants are found to thrive, is serpentinite rock formations. Serpentinite can be very high in nickel, chromium, cobalt, or magnesium. The Serpentine group is the phyllosilicate class (minerals with sheet structures) of minerals with the general formula $M_3[Si_2O_5](OH)_4$, which were formed as a result of metamorphic changes or weathering of igneous rock. When ‘serpentine’ is used alone it typically refers to either antigorite $((Mg, Fe^{2+})_3[(OH)_4Si_2O_5])$ or amesite $(Mg_2Al[(OH)_4AlSiO_5])$, but may also refer to either lizardite $(Mg_3Si_2O_5(OH)_4)$ or any of the chrysotile species^{26, 27}. Generally, limestone and serpentine-derived soils as well as

sand soils are low in organic content, which is usually richer in nutrients, and this is often a reason why many plants are unable to grow in these soils. At the other extreme, too acidic soils are also considered as nutrient-poor soils.

1.2.3.1 Soil solution - acidity and alkalinity

Soil pH is a very important criterion when deciding on plant culture or which ornamental plant species can be grown. The two main factors that affect soil pH are the balance between H^+ and OH^- ions in the soil solution and the balance between acid and nonacid cations on colloid surfaces¹³.

The main factor controlling the balance between H^+ and OH^- ions in the soil solution is precipitation. Therefore, soil acidity reaches its highest levels in humid and waterlogged areas, creating reducing conditions in the soil. Precipitation (rain, snow, fog, and dust) may contain various acids that contribute H^+ ions to the soil. Precipitating through unpolluted air the rain water dissolves atmospheric CO_2 , forming the weak carbonic acid (H_2CO_3), and giving the aqueous solution a pH of around 5.6.



Simultaneously with the formation of H_2CO_3 in the atmosphere, this process occurs in the soil air from the root respiration and decomposition of soil organic matter by microorganisms, which produce high levels of CO_2 .

In addition to the precipitation factor, the accumulation of organic matter in the soil also increases the soil acidity, firstly because organic matter contains a large number of acid functional groups from which H^+ dissociate, and secondly because organic matter forms soluble complexes with nonacid cations such as Ca^{2+} and Mg^{2+} ¹³.

The second important pH balance for soil, between acid and nonacid cations, is also influenced by precipitation, which in humid areas contributes to soil acidity as rainfall or soil erosion leaches the nonacid cations out the soil.

1.2.3.2 Limestone rock composition and formation

Limestone is a sedimentary, fragmental rock, which consists mainly of the secondary minerals calcite and aragonite but may also contain some amount of dolomite (dolomitic limestone). Calcite is a calcium carbonate mineral that is relatively soft (hardness 3 on Mohs' scale). Calcite is usually white or colourless but occasionally it may have tints of some other colour. Dolomite ($(\text{Ca,Mg})\text{CO}_3$, hardness 3.5) is calcium and magnesium carbonate and is very similar to calcite but it may be honey coloured²⁸. Often, limestone is referred to as a *calcareous* rock, which means that it is a rock rich in calcium. There are several types of limestone rock, depending on the nature of the activity that led to its formation. Sediments maybe the result of the activities of plants and animals, for example, but they can also be the result of chemical processes. As the sea water evaporates, calcite separates from the sea water and deposits as rocks (the least soluble one, calcite, deposits first, then dolomite, gypsum and halite). Wave action causes the calcite to form round grains called ooliths, and the limestone made up of those is an oolitic limestone. Chalk is a white and fine-grained limestone which is entirely made of fossils of microscopic shells, remains of plankton and skeletons of microscopic plants called *coccoliths*. Shelly and Coral limestone are made up of shell fragments and coral fragments respectively, which are the deposits of skeletons or shells of marine animals, such as coral, made from calcium carbonate accumulated from the sea water. The types of sedimentary rocks and their mineral constitutions are classified according to particle size are shown in Table 5.

Table 5 Classes of sedimentary rocks

Type of particle	Diameter of particle / mm	Name and mineral composition of sedimentary rock	
boulder	> 256	Conglomerates - round particles: pebbles Breccias - sharp and pointy particles	Mixture minerals
cobble	64 – 256		
pebble	4 – 64		
gravel	2 – 4		
sand	0.16 – 2	sandstones	Grit Greywacke – mixed sediment Arkose – quartz and > 25% feldspar (K-silicate or Na, Ca-silicate). Sandstone – mainly composed of quartz, and also feldspar or mica. The particles are held together with <i>cement</i> that consists of silica, calcite or Fe-oxides.
silt	0.0039 – 0.16	siltstones	Siltstone – compacted, silt-sized particles Limestone – containing mainly calcite
clay or mud	< 0.0039	mudstones	Clay – contains clay minerals - water-containing silicates (Kaolinite), occurring as tiny crystals, formed from the chemical weathering of feldspars, caused by carbonic acid allowing it to combine with water. Mudstone – compacted, cemented mud Shale - flaky mudstone Marl –calcareous (calcite) mudstone

The genus *Rhododendron*

1.3. Distribution and characteristics

The genus *Rhododendron* includes around 1000 species, 90% of which are native to SE Asia (80° - 125° E and 0° - around 35° N), from the NW Himalaya through Nepal, NE India, northern Myanmar, and western and central China (including eastern Tibet - now called Xizang) and south through Thailand, Vietnam, Malaysia, Indonesia and the Philippine Islands²⁹. Of the 90% of *Rhododendron* species native to Asia, the highest density is located in W China and particularly the province of Yunnan, which is the eastern extension of the Himalayan range (Fig. 1).



Figure 1 Map of China³⁰

A small number of *Rhododendron* species are native to Europe, Canada and the USA. Generally, rhododendrons in the temperate parts of Asia grow in the region from 1700 m, reaching elevations as high as 6000 m (*R. nivale*)^{29, 31}. Rhododendrons are deciduous or evergreen shrubs or trees varying from a few centimeters in the alpine region (e.g. *R. pumilum*) to trees of 25 m or more (*R. arboreum*)²⁹. The bigger bushes and trees usually inhabit the open forests and meadows of subtropical (between 150 m and 1000 m - *R. arboreum*) and temperate zones (1800 m - 4200 m), whereas the smaller shrubs and dwarf species are found in the subalpine and alpine meadows, open rocky slopes and mountain screes between 3500 m and 6000 m^{29, 32}. The leaves of the larger species can reach 30 cm long²⁹, whereas those of some dwarf species are only a few millimeters long. Although they can reach great heights, the genus *Rhododendron* is known for developing shallow root systems^{15, 33}. Rhododendrons have variable growth rates, which are often faster in young plants, although later they may slow down considerably^{29, 34}. *R. roxieanum* was found in Yunnan, 8 m tall but growing only at 5 mm per year. At constant growth rate this would correspond to an age of 1600 years, although growth was probably much faster when it was young³⁴.

1.4. Classification of *Rhododendron*

The genus *Rhododendron* belongs to the family *Ericaceae* and was described for the first time by Carl Linnaeus in 1753³⁵. Linnaeus created two genera, *Rhododendron* and *Azalea*. The latter contained 9 species and since then it has been included in *Rhododendron*. Over the next 250 years many botanists have developed the taxonomy of rhododendron further. From 1916 until 1980's, the classification of the genus has been mostly carried out by botanists working in the herbarium of the Royal Botanic Garden, Edinburgh³⁵ (RBGE). In 1922 Prof. Sir Isaac Balfour developed a temporary system for classification in which the level below genus he named “series”, where he grouped related or seemingly related species. Since he died before revising that system, it was

used by gardeners until 1949 when H. Sleumer suggested that Balfour's system was scientifically inaccurate³⁵. Sleumer proposed a new hierarchical structure in the classification of the genus by inclusion of the levels of subgenus and section and subsection in the place of series (Table 6). Later, from 1972, following the work of Sleumer with some modifications, and using morphological, cytological, anatomical and chemical research, botanists in the RBGE also began revision of the Balfour's system. J. Cullen summarised the major inadequacies of Balfour's system: the importance of geographical distribution of the species was ignored; many species were named using garden material of unknown origin and hybrids; there was lack of hierarchy in the structure showing the true relationship between groups of species; the concept of a 'species' was too limited - during the first half of the 20th century every time a slight variation in a specimen was found, a new species name was given. As a result of the latter, Cullen gave clear and precise definition of a species as applied to rhododendrons in the RBGE revision³⁵:

“Species should differ from each other in at least two independent but correlatedly varying characters, and have geographical or ecological distributions different from those of their closest allies.”

Table 6 Divisions in Balfour, Sleumer and RBGE systems for classification of the genus *Rhododendron*³⁵.

Balfour system	Sleumer and RBGE revision
Genus	Genus
	Subgenus
	Section
Series	Subsection
Sub-series	(alliance - not official)
Species	Species

At present, the RBGE revision is considered to be the most comprehensive and satisfactory classification of the genus *Rhododendron*. Current taxonomy is concerned with the evolution of a genus and the relationship between different subgenus, sections and subsections (Fig. 2). Nowadays, except for herbarium-based and horticultural-based taxonomy, botanists use many more strategies and methods in their work, such as fieldwork on populations in the wild, DNA analyses - research undertaken in University of Seattle, USA and the RBGE³⁵. In particular, DNA analyses have resulted in the inclusion of plants from other genera in *Rhododendron* (e.g. *Ledum*)³⁶, leading to revision of the classification within the genus.

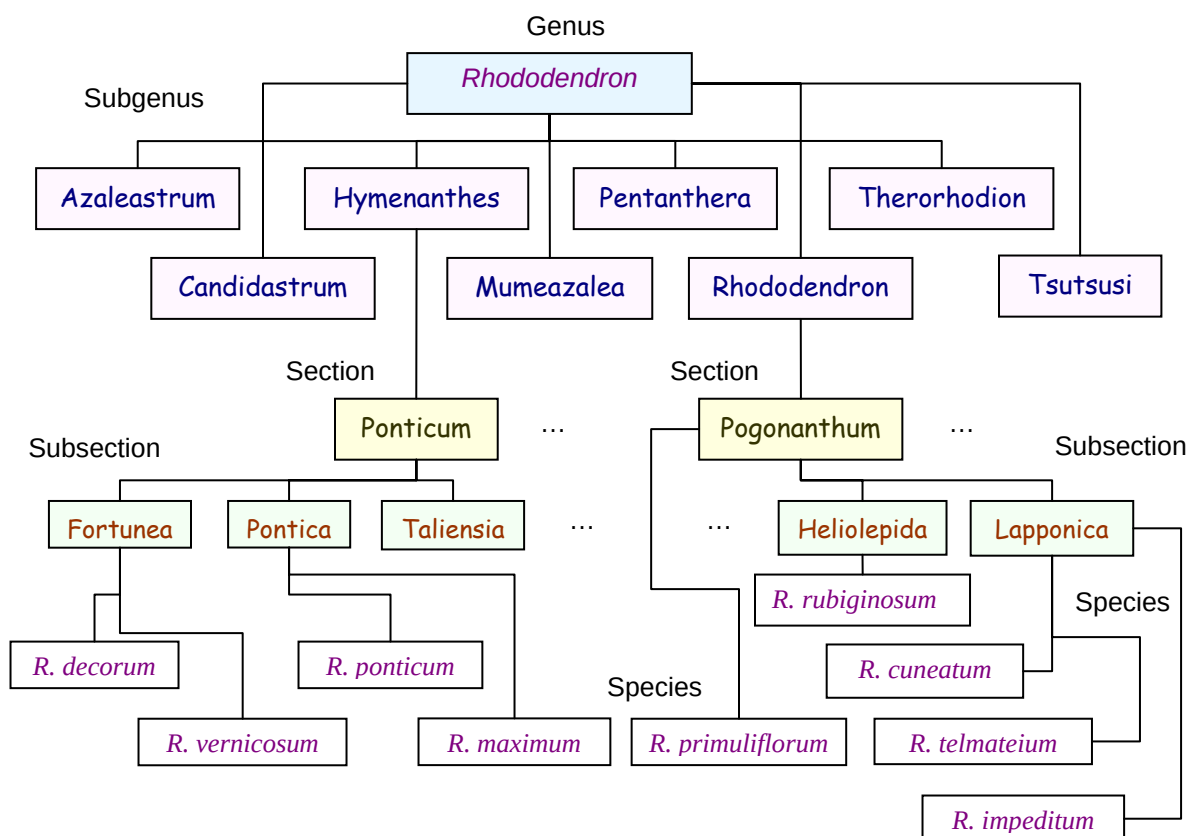


Figure 2 Present classification of the genus *Rhododendron*. Only representative examples of section, sub-section and species are given³⁵.

1.5. Introduction of *Rhododendron* to cultivation from SE Asia

A large number of *Rhododendron* species originate from W China. For a long time China was almost completely closed to Europeans and to foreign travel, until the middle of the 19th century. No foreigners were allowed to go more than a few miles outside Canton and Macao, as these were the only ports open to Europeans³⁷. Thus it was impossible for plant collectors to explore the country. However, it would appear that most of the most important plants growing in and around Canton, Macao and Peking were introduced to western cultivation. During this period, between 1840 and 1860, one of the greatest collectors in the Far East was Robert Fortune. Soon after his third expedition, war broke out between China and the allied powers of Britain, France, Germany and the United States. After the treaty of 1860, China was fully open to foreign travel and a new period in the history of plant hunting in China began³⁷.

During this period, 1860-1900, many plants were introduced into cultivation, but most of the plant collecting was done by amateurs, missionaries, merchants and diplomats³⁷. With the beginning of the 20th century a new era of professional botanical and horticultural collectors began with E. H. Wilson and George Forrest³⁷, followed by F. Kingdon Ward, Joseph Rock, Reginald Farrer and Euan Cox, all working in China. The *Rhododendron* species native to Nepal, NE India, northern Burma, and south through Thailand, Vietnam, Malaysia, Indonesia and the Philippine Islands were explored at the same time. Botanical expeditions and research in NE India were undertaken in the 1850's by Joseph D. Hooker. Parts of NE India, notably Arunachal Pradesh in particular, have only been open to foreign plant explorers since the end of 1990s, revealing that it is the second place after Yunnan in the number of temperate *Rhododendron* species³⁸.

1.6. Significance and values of *Rhododendron* species

From an ecological point of view, the genus *Rhododendron* is very important for the maintenance of the ecological balance in surrounding ecosystems. It forms a wide variety of forest types in cool temperate and sub-alpine regions, supporting a large range

of biodiversity. This provides food and shelter for birds and animals, and stabilises the mountain's soil profile, preventing erosions.

Some species of *Rhododendron* are known to have medicinal use. *R. arboreum* and *R. campanulatum* are used in traditional medicine to treat diarrhea, dysentery, rheumatism and sciatica. *Rhododendron* wood is used in some Asian regions for carving traditional wooden bowls and containers and the vegetative parts of *R. thomsonii* are used as a natural insecticide³². Flowers of *R. decorum* are used as a vegetable in soups³⁴.

A very important use of *Rhododendron* is in their role as ornamental plants, for which they are valuable in gardening. They provide a vast range of flower shapes, colours and fragrance, as well as great diversity of foliage shape and size. The different heights of the species are very useful in the design of gardens having various levels of plant lines, and evergreen plants with attractive foliage have an important structural role in garden

1.7. A century of research on the genus *Rhododendron*

1.7.1. Cultivation of *Rhododendron*

Rhododendrons have always been considered to grow healthily only in acidic soils, having pH between 4.0 and 6.0, and not being able to survive at all growing in soils with pH of 7.0 and above. Ever since their introduction from Asia to Europe and North America, gardeners have been cultivating rhododendrons mostly in acidic soils, since for many years attempts to grow them in alkaline soils were unsuccessful.

Early in the twentieth century, the observations by plant explorers like George Forrest (described in *Journeys and Plant Introductions of George Forrest* by Dr. Cowan³³), who reported rhododendrons growing in limestone on a number of occasions, provoked English growers to try to grow rhododendrons on English chalk^{31, 39, 40}. The experiments resulted in what they had already known - that rhododendrons would not grow in such conditions.

Despite the observations of Forrest and others, in the first half of the 20th century, gardeners worldwide were convinced that rhododendrons cannot possibly be grown healthily on limestone under any circumstances. The three main widely-accepted reasons for the failure of rhododendrons to grow in limestone soils were as follows:

A. “The calcium in the limestone has a toxic effect on the plants.”

In the early 1950’s gardeners were convinced that Ca is harmful to rhododendrons, and disagreed that rhododendrons cultivated in peat should receive any doses of limestone (or any lime-supplements) to provide the so-important nutrient calcium.

This belief was based on examples such as failure of rhododendrons to grow when planted in soils containing alkaline rubble from buildings. To support that theory, in 1956, Tod carried out a study of plants grown in acidic wood soil to which MgCO_3 was added to raise the pH to around 7.0 and 8.0⁴¹. The plants grown at the high pH did not show any deficiency symptoms, and so Tod attributed this to the fact that MgCO_3 was used to raise the soil pH and not CaCO_3 . Furthermore, since the chlorosis effect occurred more rapidly than the change in soil pH could occur Tod interpreted his observations on the effect of limestone on plant’s growth as an effect caused by Ca^{2+} and not by pH⁴². Additionally, Tod suggested that the available in the soil excessive amount of Ca^{2+} , which *Rhododendron* plants absorb, causes mineral imbalance, leading to impaired take-up and deficiencies of other nutrients^{7, 42}. Overall, based on his past experience as well as on that reported by others, Tod attributed the failure of *Rhododendron* plants to grow in calcareous soil to a complex harmful effect of Ca^{2+} ions on the plants.

Nevertheless, some authors observed that peat can not supply sufficient amounts of calcium for *Rhododendron* healthy growth, and they did not agree that Ca from the limestone was toxic to plants^{43, 44, 45}. It was critical to demonstrate to gardeners not only that calcium is not toxic to rhododendrons, but also that it is essential for their healthy growth. To provide evidence for that, A. Cook carried out a series of experiments on rhododendrons that had obvious symptoms of Ca deficiency such as short new growth

and dark green leaves. The recognised Ca deficiency was treated simply by spreading agricultural lime over the top soil around the sick plants⁴³. It gave fast and striking results to all those gardeners who were strongly against any addition of lime to their rhododendrons. The treated plants improved over a few weeks and throughout the year, and this was confirmed by the better leaf colour, new growth length and more buds in the next season⁴³. In those years Cook was one of the few authors to rectify calcium deficiency with limestone and to propose that rhododendrons grown in acidic soil can only benefit from addition of calcium in the form of limestone and that no plant is going to suffer as long as it is within the healthy range of concentrations. Studies by Leiser carried out between 1959 and 1969 also demonstrated that if free carbonate was not present high levels of Ca did not inhibit the growth of rhododendrons. Conversely, *Rhododendron* showed surprisingly good rooting over the whole range of Ca concentrations that the plants were subjected to⁴⁴.

Later, with the development of science and practical experience, scientists began to question more strongly if calcium is actually toxic to plants, or whether it is the high pH that causes the failure of rhododendrons to survive in limestone. After claiming in 1956 that Ca^{2+} is what is responsible for the failure of rhododendrons to survive in limestone soil rather than pH, Tod directed his research towards providing scientific evidence for it rather than just assuming it. After a series of trials, in 1971 he recognised that leaf chlorosis in plants growing in alkaline soils was actually most likely caused by manganese or iron deficiencies induced by the high soil pH⁷. Additionally, Tod, and other authors, independently, also concluded that *Rhododendron* plants quite evidently tolerated high levels of Ca^{7, 42, 44}. Following these findings, another experiment showed that rhododendrons grown for six years in pots to which calcium was applied using three different sources failed to show signs of chlorosis⁴⁶. The calcium was supplied in the form of CaCO_3 , CaSO_4 and Ca(OH)_2 , which were mixed with the top 3 cm of the soil. The levels of calcium were increased on four occasions, in the 1st, 2nd, 3rd and 5th seasons. The experiment resulted in no symptoms of disorder in the analysed plants, even though the pH in the top soil was high enough to induce manganese and iron

deficiency. The absence of chlorosis in the plants proved that rhododendrons can tolerate calcium and that it is not the Ca in the limestone that is harmful to them but it is the fact that limestone raises the pH. Another experiment in which CaCl_2 and $\text{Ca}(\text{NO}_3)_2$ were used as the sources of calcium also proved that a high concentration of Ca does not suppress the growth of rhododendrons⁴⁷. Moreover, the emphasis of study was on the fact that Ca alone has no harmful effect on rhododendrons, so as long as it does not come from CaCO_3 , as the failure of rhododendrons to grow in limestone was attributed to toxicity of the HCO_3^- ions⁴⁷.

Experiments confirmed that adding lime to rhododendrons as a source of Ca is often essential^{43, 44, 45}. Calcium is especially required in areas where the soil is originally very acidic and there is very little or no lime to provide calcium present in it. Without sufficient calcium, plants develop an unhealthy appearance and symptoms of calcium deficiency. In addition to being poor in Ca, too acidic soil (naturally acidic or from continuous use of peat to maintain low pH) may cause iron to be leached from the soil, as below a critical pH value (around 5.0) its solubility increases so much that it could be washed away from the soil, resulting in deficiency of these nutrients in rhododendron plants. In such situations, what will prevent this, or change such conditions, and make the element available to rhododendrons, will be addition of lime, which will slightly increase the pH to an optimal, slightly acidic level.

The significant point that needed to be well established amongst gardeners was that limestone increases pH to levels at which some essential nutrients are unavailable to plants to take up. Unfortunately, this concept of the actual effect of limestone on the soil chemistry was not considered and therefore barely studied at all until the late 1970's. Nowadays, it is a well-known fact that limestone raises pH, and therefore, in recent years horticulturists have used other sources of calcium supplements to counteract calcium deficiency in plants, such as calcium salts, some of which are CaSO_4 , CaCl_2 , or $\text{Ca}(\text{NO}_3)_2$.

B. “Bicarbonate ions (HCO_3^-) are toxic to plants.”

In 1993 a study carried out by Mordhorst *et al.* proved that calcium is not a marker for lime tolerance of *Rhododendron* plants and that it does not play a role in their adaptation to calcareous soils⁴⁷. The team carried out experiments on *Rhododendron* clones pre-screened *in vitro* for Ca tolerance and lime tolerance together at the Institute for Ornamental Plant Breeding in Ahrensburg, Germany⁴⁸. The experiment involved two groups of *Rhododendron* plants, the Ca and lime-tolerant *Rhododendron* clones and a control group of lime-sensitive *Rhododendron* hybrids. The effect of Ca on the growth of the *Rhododendron* clones was studied by addition of $\text{Ca}(\text{NO}_3)_2$ and CaCl_2 as a source of Ca. The results showed that in both groups the plants grew healthily in Ca-rich soils with increasing supply of Ca and that even at the highest levels of Ca none of the *Rhododendron* plants showed symptoms of disease that could be correlated with the levels of Ca. On the contrary, the control plants, which were lime-sensitive, even showed the best growth at high Ca content in the leaves. The authors concluded that since the high amounts of Ca supplied as $\text{Ca}(\text{NO}_3)_2$ and CaCl_2 did not inhibit the growth of *Rhododendron*, the Ca present in CaCO_3 is therefore not what causes failure of rhododendrons to survive in limestone soil. Consequently, although the direct effect of addition of CaCO_3 to the rhododendron plants was not studied, the authors assumed that if not due to the high Ca levels in CaCO_3 rhododendrons must then fail to grow in limestone because of the high levels of HCO_3^- ions in the CaCO_3 soil equilibrium solution. So rhododendrons will remain healthy under high Ca levels provided HCO_3^- is absent, as they regarded the harmful effect of high levels of bicarbonate ions as inducing Fe deficiency⁴⁷. Overall, the study made it clear that high Ca content does not affect the health of *Rhododendron* plants, regardless of whether they are calcifuge or calcicole plants.

The same Ca and lime-tolerant *Rhododendron* clones, selected as rootstocks for hybrid cultivars at the Institute for Ornamental Plant Breeding in Ahrensburg under the trade mark of INKHARO®, were later used in further experiments^{48, 49}. The following

experiments were set up to establish the bicarbonate concentrations that the clones can tolerate, and the effect of the increase of HCO_3^- ions supplied with CaCO_3 on the availability of iron⁴⁹. The selected lime-tolerant rootstocks tolerated HCO_3^- concentrations in the soil of up to 400 mg kg^{-1} , which were achieved with addition of 5 g L^{-1} CaCO_3 to the soil substrate ($\text{pH} = 5.8$). However, growth was inhibited in the medium containing 10 g L^{-1} CaCO_3 ($\text{pH} = 6.8$), which showed HCO_3^- ions concentrations of 684 mg kg^{-1} outdoors and 582 mg kg^{-1} indoors. The inhibited growth was accompanied by signs of Fe chlorosis and a significant decrease in iron concentrations in young leaves with increasing pH was observed⁴⁹. However, a significant fluctuation of the bicarbonate concentration with the weather was also observed: wet and cold weather showed higher levels of bicarbonate in the same medium.

A similar study with seeds of different *Rhododendron* species and hybrids germinated in a peat substrate containing 1, 5, and 10 g L^{-1} CaCO_3 , resulting in pH levels of 4.2, 6.4 and 7.1 respectively, showed the same results⁴⁸. At the highest level of CaCO_3 (10 g L^{-1} , $\text{pH} = 7.1$) most plants died, with the exception of *R. micranthum* and a few seedlings of *R. schlippenbachii* and *R. occidentale*. (The latter is also found to grow on serpentine.) *R. micranthum* had a healthy appearance and iron chlorosis in the seedlings did not occur. In a further trial *R. micranthum* plants were grown in media with 1, 5, 10 and 20 g L^{-1} CaCO_3 , as the last showed an HCO_3^- concentration of around 3000 mg L^{-1} ⁴⁸. After eight months even the plants at the highest CaCO_3 concentration were healthy and there was no difference in condition between the plants in the lowest and the highest concentrations of CaCO_3 ⁴⁸. The seedlings of *R. micranthum* reached lime-tolerance of $3000 \text{ mg HCO}_3^- \text{ L}^{-1}$ ⁴⁸.

Experiments on other plant genera have shown that in the absence of HCO_3^- ions in soil media of pH 6.0 and 8.0, the absorption of cations, including Fe(III), was lower than in the presence of HCO_3^- ions⁵⁰. This suggests that the decreased absorption of Fe(III) at high pH, as an effect of the pH alone, may actually be improved by the presence of bicarbonate ions in the soil solution.

Overall, experiments with addition of bicarbonate ions to soil showed that it does not necessarily increase the concentration of HCO_3^- in the soil. The reason for this is that since HCO_3^- is in equilibrium with the CO_2 in the atmosphere it will always be present in the soil in a certain pH range⁴⁰ (pH $\sim$ 6.0 - 7.0) (Table 7).

Table 7 Theoretical concentrations of the carbonic acid equilibrium species as a function of the partial pressure of CO_2 ⁵¹.

$p\text{CO}_2$ atm	pH	$[\text{CO}_2]$ mol L ⁻¹	$[\text{H}_2\text{CO}_3]$ mol L ⁻¹	$[\text{HCO}_3^-]$ mol L ⁻¹	$[\text{CO}_3^{2-}]$ mol L ⁻¹
10^{-8}	7.00	3.36×10^{-10}	5.71×10^{-13}	1.42×10^{-9}	7.90×10^{-13}
10^{-6}	6.81	3.36×10^{-8}	5.71×10^{-11}	9.16×10^{-8}	3.30×10^{-11}
10^{-4}	5.92	3.36×10^{-6}	5.71×10^{-9}	1.19×10^{-6}	5.57×10^{-11}
3.5×10^{-4} *	5.65	1.18×10^{-5}	2.00×10^{-8}	2.23×10^{-6}	5.60×10^{-11}
10^{-3}	5.42	3.36×10^{-5}	5.71×10^{-8}	3.78×10^{-6}	5.61×10^{-11}
10^{-2}	4.92	3.36×10^{-4}	5.71×10^{-7}	1.19×10^{-5}	5.61×10^{-11}
10^{-1}	4.42	3.36×10^{-3}	5.71×10^{-6}	3.78×10^{-5}	5.61×10^{-11}
1	3.92	3.36×10^{-2}	5.71×10^{-5}	1.20×10^{-4}	5.61×10^{-11}
2.5	3.72	8.40×10^{-2}	1.43×10^{-4}	1.89×10^{-4}	5.61×10^{-11}
10	3.42	0.336	5.71×10^{-4}	3.78×10^{-4}	5.61×10^{-11}

* Highlighted is the partial pressure of CO_2 at standard conditions (1 atm, 298 K)

In aqueous solution, carbonate, bicarbonate, carbon dioxide, and carbonic acid exist together in a dynamic equilibrium. The raised pH in limestone soil is a consequence of the increased concentrations of HCO_3^- and CO_3^{2-} with respect to the CO_2 concentrations, which are a consequence of reducing the partial pressure of CO_2 .

Carbon dioxide in the air dissolves in water under pressure. The change in the partial pressure of CO_2 reflects the equilibrium of each of the forms of carbonate and determines the pH of the soil. In soil in which the respiration of soil fauna and flora is higher the CO_2 partial pressure is also higher, and so the pH can decrease down to 4.4. As the partial pressure of CO_2 increases, the hydrated $\text{CO}_{2(\text{aq})}$ combines more and more with water to give carbonic acid. Hence, at higher partial pressure the carbonic acid

becomes the dominant species, with respect to the bicarbonate ions, and dissociates less and less to bicarbonate, making the pH of the solution lower. As the partial pressure of CO_2 decreases, $\text{CO}_{2(\text{g})}$ dissolves less and less in water and the dissolved $\text{CO}_{2(\text{aq})}$, converted into carbonic acid, dissociates more and more to HCO_3^- and CO_3^{2-} so the concentrations of the HCO_3^- and CO_3^{2-} are higher, and the pH simultaneously increases.

$$\text{CO}_{2(\text{g})} \longleftrightarrow \text{CO}_{2(\text{aq})}, \quad \frac{[\text{CO}_2]}{p\text{CO}_2} = \frac{1}{k_c} \quad (1)$$

k_c' is Henry's constant, $k_c' = 29.76, \text{ atm mol L}^{-1}$, at 25°C

$$\text{H}_2\text{O}_{(\text{rain})} + \text{CO}_{2(\text{aq})} \rightleftharpoons \text{H}_2\text{CO}_3, \quad K_h = \frac{[\text{H}_2\text{CO}_3]}{[\text{CO}_2]} = 1.70 \times 10^{-3} \text{ mol L}^{-1}, \text{ at } 25^\circ\text{C} \quad (2)$$

$$\text{H}_2\text{CO}_{3(\text{aq})} \rightleftharpoons \text{H}^+ + \text{HCO}_3^-, \quad K_{a1} = \frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} = 4.3 \times 10^{-7} \text{ mol L}^{-1}, \text{ at } 25^\circ\text{C}, \quad pK_{a1} = 6.36 \quad (3)$$

$$\text{HCO}_3^-_{(\text{aq})} \rightleftharpoons \text{H}^+ + \text{CO}_3^{2-}, \quad K_{a2} = \frac{[\text{H}^+][\text{CO}_3^{2-}]}{[\text{HCO}_3^-]} = 4.84 \times 10^{-11} \text{ mol L}^{-1}, \text{ at } 25^\circ\text{C}, \quad pK_{a2} = 10.32 \quad (4)$$

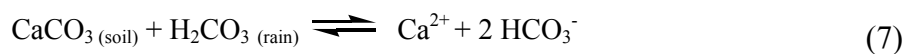
Overall, at standard atmospheric conditions the partial pressure of CO_2 is $p\text{CO}_2 = 3.5 \times 10^{-4} \text{ atm}$, so the process in equation (2) gives rain water a $\text{pH} = 5.7$.

The solubility of CaCO_3 in pure water is very low and a suspension in pure water has a $\text{pH} = 9.6$:

$$\text{CaCO}_{3(\text{s})} + \text{H}_2\text{O} \rightleftharpoons \text{Ca}^{2+} + \text{CO}_3^{2-}, \quad K_{sp} = [\text{Ca}^{2+}][\text{CO}_3^{2-}] = 4.47 \times 10^{-9} \text{ mol L}^{-1}, \text{ at } 25^\circ\text{C} \quad (5)$$

$$\text{CO}_3^{2-} + \text{H}_2\text{O} \rightleftharpoons \text{HCO}_3^- + \text{OH}^-, \quad K_b = \frac{[\text{HCO}_3^-][\text{OH}^-]}{[\text{CO}_3^{2-}]} = 2.1 \times 10^{-4} \text{ mol L}^{-1} \quad (6)$$

However, the system is always in equilibrium with CO_2 , so when acidic rain water contacts the limestone soil the pH for the equilibrium with atmospheric CO_2 drops down to 8.35 for saturated carbonate (7):



Equation (7) represents the process of chemical weathering of limestone, which usually neutralises the rain (soil) water from pH ~ 5.7 to pH ~ 7.0. Reactions (5), (6) and (7) are the reactions determining the pH of calcareous soil.

The main conclusion is that with or without addition of bicarbonate to the soil in the forms of carbonates or bicarbonate compounds the HCO_3^- content in the soil will not necessarily increase, as up to the pH maximum of limestone soils HCO_3^- is in equilibrium with the soil-air CO_2 . Therefore, within the pH range occurring in limestone-derived soils it will always be present in the soil within a certain concentration range, which will be determined by the pH.

Presence of bicarbonate ions is required for the normal function of PS II⁵².

C. “There is a shortage of magnesium in limestone soils, and as a result plants suffer from Mg deficiency.”

This suggestion was based on the assumption that Ca competes with Mg for uptake by plants. It seems to be based on the fact that they are in the same group (IIA). However, there does not seem to be any experimental evidence to support the assertion about a competition between the two ions, which is also quite unlikely as Ca^{2+} and Mg^{2+} cations have very different sizes. In support of the argument that calcium does not cause shortage of magnesium, in 1981 Hanger showed that the addition of calcium compounds to rhododendrons did not decrease the take-up of magnesium⁴⁶. Magnesium concentrations increased normally, at a steady rate, through the seasons and with the age of the leaves. The range of concentrations of Mg in the leaves also showed consistency regardless of the different amounts of Ca given to the plants. The addition of Ca compounds did not affect the concentrations of Mg, and so did not prevent the healthy growth of the studied rhododendrons.

Today, research has shown that plants grow well and meet their Ca and Mg requirements in soils with Mg/Ca ratios between 1/1 and 1/15¹³ and plant's health and growth is not affected by alterations of the levels of either of these two nutrients in the soil.

Summary of *Rhododendron* in cultivation

Despite George Forrest's efforts to convince the public in Britain that in the wild rhododendrons cover the limestone hillsides, the suggestion that some rhododendrons like limestone in cultivation was still not accepted in Britain. However, there were still some people who were more open to the idea and even skeptics considered *Rhododendron* species that tolerate limestone as an interesting occurrence and worthwhile to investigate⁵³. In 1927, supporting the evidence that some rhododendrons tolerate limestone, Grove grew a number of cuttings and mature plants of *Rhododendron* species native to western China on the loams overlying the chalk soil of southern England. Expectedly, a group of plants died shortly after planted. A few years from the start of the experiment the rhododendrons that were growing undisturbed or even flourishing had their roots extended well into the chalk soil which was all around the plant. After growing healthily in that soil for seven years, that group of rhododendrons gave clear evidence that some rhododendrons are tolerant to the chalky soil, and the author concluded that none of the tested rhododendrons native to China were dramatically susceptible to limestone, although some were clearly more calcifuge than others, which were sufficiently tolerant to limestone⁵³.

Later, in Switzerland *R. ferrugineum* and *R. hirsutum* were cultivated in simulated natural conditions in a rock garden made of limestone⁴³. This experience may be seen as slightly controversial as *R. ferrugineum* is usually found naturally in acidic conditions, whereas, *R. hirsutum* normally prefers limestone.

Within the last ten years, with the increased scientific interest and research in the subject, the knowledge and experience of cultivation of *Rhododendron* has significantly developed. Trials on breeding lime-tolerant species with the trade mark INKHARO®

have already been carried out at the Institute for Ornamental Plant Breeding in Ahrensburg, Germany. *R. micranthum* showed high tolerance to limestone but this valuable quality of the species was also accompanied with the disadvantage as a breeding parent of not hybridising well with other rhododendron species⁴⁸.

1.7.2. *Rhododendron* species that tolerate limestone and other alkaline soil in the wild

Some rhododendrons have been observed growing healthily in limestone in the wild^{31, 39, 40, 43}. Some such tolerant species are listed in Table 8, but altogether nearly 80 species showing tolerance have been recorded by botanists and plant hunters. A tendency for lime-tolerance has been observed amongst most members of the *Laponica* and *Taliensia* sub-sections⁵⁴.

Table 8 Some *Rhododendron* species, which have been established to grow in extreme lime-tolerant conditions.

<i>Rhododendron</i> species	Pure limestone, no organic layer	Thin organic layer over limestone rock or soil, with pH > 7.0 at root zone
<i>R. cuneatum</i> ³⁴	*	*
<i>R. telmateium</i> ^{34, 55}	*	*
<i>R. decorum</i> ^{34, 56}		*
<i>R. vernicosum</i> ^{34, 57}		*
<i>R. primuliflorum</i> ^{55, 58}	*	*
<i>R. rupicola</i> ³⁴		*
<i>R. rubiginosum</i> ³⁴		*
<i>R. yunnanense</i> ^{34, 56}		*
<i>R. impeditum</i> ⁵⁹		*
<i>R. racemosum</i> ^{34, 55}		*
<i>R. fastigiatum</i> ⁵⁵		*
<i>R. yungningense</i> ⁵⁵		*
<i>R. phaeochrysum</i> ³⁴		*
<i>R. saluenense</i> ⁵⁵		*
<i>R. hirsutum</i> ^{34, 55, 56}		*

Some *Rhododendron* species have also been observed to thrive in other alkaline soils such as serpentine-derived soils. Examples are *R. neoglandulosum* and *R. occidentale*⁵⁴. The latter is native to W USA and S California and occurs at elevations from sea-level to 2700 m³⁵. In California it is found growing on serpentine soil with pH over 7.0 and low fertility³³.

Without having any clear understanding of what causes *Rhododendron* plants to fail to grow in limestone soil in cultivation, horticulturists and gardeners looked for answers to a second question:

"What maintains the healthy growth of rhododendrons in limestone soil in the wild?"

In fact, nobody really attempted to answer this question by doing an experiment. Instead, it was assumed that the rhododendrons could not possibly be growing in contact with calcium carbonate in the soil, and without any evidence people came up with reasons why the apparent observations of Forrest and others must be wrong. The main suggestions for such reasons were as follows:

A. “The limestone in China is insoluble and it does not affect the associated soil. The soil remains acidic and the CaCO_3 has no effect on the plants.”

One of the theories why *Rhododendron* plants grow healthily in limestone soil in the wild was that the limestone rock formation is ancient and, therefore, it is very hard and very resistant to weathering. Therefore, in such areas of old limestone, due to its low solubility calcium ‘poisoning’ of the rhododendrons is not so likely to occur, and also the high pH of the rock has much less effect on the soil⁷.

However, studies of the limestone rock formations in Yunnan proved this hypothesis wrong. Theoretically, the highest possible pH of water saturated with CaCO_3 is 8.4. Water samples from springs and small rivers collected in two different limestone regions in Yunnan showed $\text{pH} = 8.2 - 8.4$ ⁵⁸, which clearly shows that the limestone is soluble enough to affect the chemistry of the local soil.

Geological analyses of limestone rock specimens collected in Yunnan proved that the limestone rock in Yunnan is soft and soluble enough to affect the associated soil^{39, 40}. This was also confirmed by the high calcium concentrations present in samples of water, collected in the same region, and by analyses of the available calcium in the soil samples themselves^{39, 40}.

The limestone rock in NW Yunnan is breaking laterally along the mountains slopes, where the water from the melting glaciers, heavy rainfall during the monsoon season, and the melt of the winter snow form rivers, carrying limestone rocks down to the valleys and breaking it further to give very fine limestone silt. This has been deposited on the ground for thousands of years, forming a soil profile at least 3 m thick (Fig. 3,

Photo 1 and 2). Several species of *Rhododendron*, notably *R. telmateium* and *R. cuneatum*, grow in these most extreme conditions (Photo 3).



Figure 3 Gang He Ba valley, Yunnan, China, Satellite image⁶⁰



Photo 1 Soil profile in Gang He Ba valley



Photo 2 Gang He Ba valley, Yunnan, China

B. “There is always a layer of organic soil covering the limestone and preventing the roots from any contact with the CaCO_3 coming from the underlying rock.”

In 1915 George Forrest reported that the same species that he saw growing in the decaying humus of forests and meadows, he also often found growing in direct contact with limestone and in rock crevices^{39, 40, 53}.

In 1956, quoting Hartge, Tod too claimed that *Rhododendron* plants are healthy in limestone soil in the wild due to the presence of a humus layer overlying the limestone soil⁴², or that they grow in crevices and isolated pockets of soil in rocks, where the pH remains lower⁶¹.

In an attempt to explain how rhododendrons survive growing over limestone in China and why they refuse to grow in it in cultivation, an expedition to Yunnan, China, in 1996 set off to investigate the climate in Yunnan, so it could be correlated to this phenomenon. Based on field measurements of soil pH and climatic data provided for both Yunnan and the UK, it was concluded that the occurrence of rhododendrons growing over limestone is entirely due to the presence of a thin layer of organic matter over the limestone formation⁵⁸. Indeed, in some cases, there is an organic layer covering the limestone rock thus allowing the less limestone-tolerant species to thrive growing over limestone rock or limestone-derived soil. Such an organic layer can vary in thickness up to 20 cm or more and may consist of moss or pine needles on the top surface and rhododendron mulch underneath, or the whole organic layer could be from decaying leaves of rhododendrons and plants. However, this is not the only case; there are some *Rhododendron* species that are lime-lovers, and they grow in pure, white limestone and do not need an organic layer to survive the alkalinity of the soil^{39, 40, 62}.

The claim that there is always an organic layer covering the limestone, as a reason why rhododendrons survive in limestone, can easily be refuted. First of all, many explorers have observed that in some places in China rhododendrons grow directly over limestone-derived soil. Secondly, in this part of China, in many cases where a top

organic layer is present, the *Rhododendron* roots still penetrate through the organic layer and are directly in contact with the subsoil. (Elsewhere the organic layer can certainly be too thick so the roots do not reach the limestone subsoil.) As a result, the plants are subjected to the alkaline soil. The subsoil is usually limestone-derived, or the roots could be growing in crevices and pockets of limestone rocks. Thirdly, the research states that the definite presence of an organic layer counteracts the alkalinity of the limestone subsoil and, therefore, the plants would grow healthily. However, soil analyses show that a layer of organic material does not always counter the effects of limestone soil (unavailability of iron and manganese), when the roots of the plants penetrate the latter.

To confirm that the presence of an organic layer is not always the reason why rhododendrons survive on limestone, experiments on the amount of organic matter in limestone soils were carried out^{39, 40}. The results showed that most of the soils, from very acidic (pH < 4.0) to very alkaline (pH close to 8.4), had less than 50% organic content. Therefore, the theory that an organic layer covering the limestone aids rhododendrons to survive on limestone by eliminating any contact with it is shown to be wrong. It is clear that, even when a substantial amount of organic material is present, there is mixing of soil layers, and the limestone is present throughout the rooting zone of the plants⁶². Some rhododendrons clearly have their roots in white, powdered limestone, with very little organic material (Photos 3 and 4). The same observations have been made in California, where *R. occidentale* was found growing in serpentine soil with its roots penetrating through the whole profile, reaching into cracks in the serpentine rock, and in none of the explored sites was there an appreciable amount of organic matter³³.

Of course, many species grow in a thick organic layer over limestone, but that does not mean that such a layer is always essential for healthy plant growth.

In blueberries (*Vaccinium* - a genus in *Ericaceae*), for instance, the organic matter requirements can be satisfied with as little as 2 to 4% organic matter¹¹.



Photo 3 *R. cuneatum* roots growing in limestone soil



Photo 4 Soil profile of plants growing in limestone in Gang He Ba valley

C. “During the persistent heavy rain in the monsoon summer in China, the soil water flows only downwards through the soil profile.”

In 1959 Tod supported an idea of Hartge (1956) that *Rhododendron* plants grew in a humus layer overlying calcareous soil, and that because of high precipitation the water flow was entirely downwards, leaving the top soil layer, in which the plants grew, acidic and lime-free⁴².

Using climatic data, Kinsman (1997) pointed out that between April and September in most of the UK the process of evapotranspiration* exceeds the process of precipitation, leading to the occurrence of net water deficit in the soil until excess precipitation makes

* The sum of evaporation and plant transpiration: in evaporation, water is evaporated from soil. Transpiration takes water out of the watershed by evaporation of water through the pores in leaves.

up the deficit⁵⁸. With a focus on the chalky areas in the UK such water deficit would draw the water within the soil profile upwards in order to satisfy plant water requirement, thus increasing the pH of the top soil layer. Therefore, rhododendrons cultivated in soil over limestone formations in the UK could not survive the induced higher alkalinity of the soil. In contrast, the authors stated that there was high rainfall throughout the year in Yunnan. They suggested that in Yunnan, there would never be an upwards flux of underground Ca-rich water, compensating a net water deficit. In Yunnan the period from May to September, the season of high evapotranspiration, is also the season of very high precipitation - the monsoon - and the water would flow only downwards and there would not be subsoil water flux driven upwards. Therefore, the chemistry of the limestone-derived subsoil would not affect the organic top soil and it would remain acidic and wet enough over the whole year. This way rhododendrons growing in an organic layer in Yunnan, having their roots situated in the upper 10-20 cm of the soil profile, would not fail to grow, even if the subsoil was limestone⁵⁸.

However, such a downwards flow of water should not make much difference for the healthy growth of rhododendrons as in many cases their roots are located either in or very close to the alkaline subsoil (Photos 3 and 4). It should also be taken into consideration that the process of diffusion of solution from places of higher concentration towards places with lower concentration is a conventional process in soils. In the situation of limestone-derived soil, this would be diffusion of water solution with higher concentration of dissolved CaCO_3 from the limestone subsoil upwards towards the top soil layers, thus affecting the topsoil pH. Additionally, in Gang He Ba valley for example, the water from the monsoon rain, the melting glacier and the river formed from the melting snow, flowing down in the valley, dissolves the limestone in the soil resulting in white limy water flooding the entire valley in the summer (Photo 4). Moreover, the assumption that Yunnan has high rainfall all year is not correct. There are dry periods in spring and autumn, when some upward movement of water should occur. In addition, in California, *R. occidentale* grows in serpentine soil with its roots deeply penetrating through the soil profile, with pH between 7.2 at top-soil level and 8.5 at sub-

soil, and almost no organic matter present. The summers in that area are dry and it is believed that it is for this reason that the roots are penetrating more deeply into the soil, ‘fighting their way against the dangerously high pH’ to actually provide water from the sub-soil layers. Evidently, under those conditions, *R. occidentale* does flourish in alkaline soil, without enough organic matter or rain to acidify the immediate rhizosphere³³. This species often occurs in wet places where water seepage would ensure that alkaline conditions near the surface would affect the roots.

This theory was supported by analyses of a few samples of soils in which *Rhododendron* plants were growing, collected in Yunnan⁵⁸. The conclusion that the underlying limestone does not affect the plants was based on the pH that most of the soils showed and which was in the range pH = 4.5 - 5.8. However, the plants studied in the experiments were always plants growing in an organic layer of at least 10 cm and the pH measurements carried out were also mostly measured for that topsoil organic layer, which being organic would be expected to have a pH between 4.5 and 6.0. Only for two *R. primuliflorum* plants was the pH of the limestone subsoil at 20 cm depth, high, between 7.4 and 7.8⁵⁸. It will be a normal observation that for a plant growing over limestone rock pH measured at the soil level close to the underlying limestone will be high, perhaps 7.0 or above, and conversely, if it is taken in the acidic organic layer near the top soil, it will most probably have its pH below 6.5.

D. “The limestone rock in which rhododendrons grow in the wild is dolomitic limestone, the high magnesium content of which protects the plants from calcium toxicity.”

Based on observations of *Rhododendron* plants growing healthily in dolomitic limestone as well as serpentine, which is also an Mg-rich rock formation, these two types of soil, although having high pH, became widely accepted as favourable for rhododendrons conditions, simply because they have lower Ca content. That was further ‘evidence’ that “it is not the high pH, but the high Ca” that is poisoning the rhododendrons³³.

Essentially, pure limestone is CaCO_3 , while dolomite contains equal molar amounts of CaCO_3 and MgCO_3 , but as Ca is heavier than Mg, the proportion by weight is 45% MgCO_3 and 55% CaCO_3 . So-called dolomitic limestone contains from 2% up to 45% MgCO_3 and the rest is CaCO_3 .

The general hypothesis was that the Mg coming from the dolomite counteracts the harmful effect of Ca on plants^{39, 40}. In 1906 rhododendrons and azaleas were successfully grown in limed soils, provided MgSO_4 was also added⁷. In 1956 Tod reported that rhododendrons were occasionally observed growing healthily in alkaline soils, characterised by low calcium level with magnesium as the replacement cation⁴¹.

The series of ideas that developed this theory began with the suggestion that for plants to grow healthily in, near or over limestone rocks, the rock formations underlying the top layer where plants grow is dolomitic-limestone. As a result, in the process of weathering the higher precipitation in some areas leaches the calcium away first, resulting in a higher level of Mg that depresses the uptake of the remaining calcium by the plants growing in this soil⁴². Tod however excluded this idea as a possible solution for the drier areas, where this event would not occur and the plants would still suffer the consequences of high Ca⁴².

The next argument supporting the theory was largely based on the properties of the CaCO_3 formation called tufa*. It was proposed that if tufa was exposed to water from a Mg-rich rock formation the magnesium can replace the calcium in it, and vice versa, until reaching the theoretical limits of dolomite, 45% MgCO_3 and 55% CaCO_3 ⁶³. Originating from this idea, it was suggested that tufa can consist of either pure CaCO_3 or of various proportions of both CaCO_3 and MgCO_3 , up to the theoretical limits⁶³. This was suggested as an explanation for the observations of lime-hating alpine plants growing happily in tufa but not in chalk or limestone, presumably because of high MgCO_3 content⁶³.

* Calcium carbonate deposit which forms by the precipitation from water with a high content of dissolved calcium.

Tod rejected the idea of high rainfall washing away the calcium as valid for all areas, but at the same time agreed with the initial idea that the dolomitic origin of the rocks could be an explanation of why plants survive in limestone soil, and so he developed his views further on how the dolomitic limestone might be affecting the plants positively. Consequently, to show that the soil was actually dolomitic limestone, in 1962 Tod started work on the theory of the high Mg content in tufa⁶³. Based on the proposed dolomitic limestone content of tufa and on his experiments in 1956, where he showed that rhododendrons grew healthily at high pH provided the pH was raised using MgCO_3 but not with CaCO_3 , Tod analysed a number of soils collected in the Dolomites, UK and USA. From his results he concluded that most soils with high pH in which calcifuge plants were growing also had high Mg content⁶¹. Although this provided insufficient information, the widely accepted idea remained that where calcifuge plants were found growing in, near or over limestone, chalk or tufa, these soils must be high in Mg, which probably limited the Ca uptake of the plants to 'safe' levels⁶¹. Regardless of the rate of rainfall, even if the calcium could not be washed away and it was still in the soil, the Mg was in high enough amounts to inhibit the Ca take up by the plants. Additionally, those conclusions were made without having explicit evidence on which plant species were definitely calcifuge, or if possibly they could also tolerate calcareous soils, meaning these plants that Tod found growing in high pH could be healthy also because they were lime-tolerant.

Overall, it is an expected result that soil samples from the Dolomites will show high levels of Mg. However, it would be reasonable to say that although the soil in the Dolomites is high in Mg, it does not mean that the plants growing there survive just because of the high Mg level; they could simply be tolerant to limestone and dolomitic limestone, regardless of the levels of magnesium and the Mg/Ca. Moreover, Tod himself observed in 1959⁴² that rhododendrons can absorb large amounts of Ca. Secondly, because the dolomite sites contain high levels of Mg does not indicate that high Mg levels are aiding plant growth in the sites of more pure CaCO_3 limestone or chalk. Clearly, the species growing healthily in both cases, in dolomite or dolomitic limestone

and in limestone, are simply lime-tolerant. On the other hand, lime-hating plants will probably fail to grow at high pH anyway, regardless of the level of magnesium and the Mg/Ca ratio. In 1971 Tod confirmed his own theory and showed that rhododendrons grown in sand culture became chlorotic at high calcium levels and that the Mg/Ca ratio between the healthy and the chlorotic leaves varied⁷.

However, around 25 years later studies carried out on analyses of soils demonstrated that the above is not true. Measurements of the amounts of calcium and magnesium available to plants in soil samples collected in China showed that the Mg levels were about 15% of those of Ca^{39, 40}. This suggests that Mg dissolves more readily and/or comes from other sources (i.e. organic).

Furthermore, X-ray fluorescence analyses of rock samples, also collected in NW Yunnan, proved that the rocks in the area are almost pure calcium carbonate, as the amounts of MgCO₃ in the rocks were as little as 0.2 to 0.4 %³⁹. These results clearly proved that the limestone in China is not dolomitic, so the survival of plants in limestone soils can not be attributed to the high concentrations of Mg, from the "dolomite" rock.

Generally, regardless the amounts of Mg/Ca in the soil, the amounts of Mg in the leaves vary very little^{39, 40}. This indicated that rhododendrons take up magnesium from the soil only up to the concentrations they need for their healthy growth requirements, and control its levels in the leaves very well, and they would not take it up excessively to battle a possible Ca toxicity threat.

Overall, the presumed relative rates of dissolution of CaCO₃ and MgCO₃ were wrong way round, the antagonism between Ca and Mg uptake by plants is falsely, and in any case the rocks were simply assumed to be dolomitic. Nobody attempted to make measurements.

1.8. General growth characteristics of *Ericaceae* family members

The family *Ericaceae* is one of the largest plant families. Most of its members, *Vaccinium* (blueberries), *Calluna*, *Erica*, *Cassiope*, *Kalmia*, *Ledum*, etc., are famous for

their preference to grow in acidic soil. Additionally, these plants flourish more under high organic matter conditions on well-drained soils¹¹.

Low soil pH is a typical condition in places where ericaceous plants are dominant, as in much of the north and the west of Scotland, and in the monsoon-affected Sino-Himalayan region.

Very high levels of manganese, 6000 – 13 000 ppm, in the leaves of *Vaccinium* plants, without any symptoms of Mn toxicity, have been reported¹¹. Blueberries can accumulate Mn very efficiently even when it is very low in the soil, and plants in the family *Ericaceae* generally have the tendency to accumulate high levels of manganese¹¹.

1.9. Other plant species that grow in association with *Rhododendron* and tolerate extreme conditions in the wild, such as *Gentiana*, *Primula*

During the early times of plant hunting in Asia many *Primula* species proved to be difficult to cultivate and survived only a short time in western gardens⁷⁸. *Primula dryadifolia*, for example, was observed growing in crevices of limestone cliffs, sometimes in moss under *Rhododendron* plants³¹. Some lime-tolerant *Primula* species, often growing in association with *Rhododendron*, are *Primula dryadifolia* and *Primula forestii*. Other well-known alpine calcicole and also *Rhododendron* companion species are *Daphne calcicola* and some *Androsace* species⁵⁴.

The genus *Gentiana* is widely distributed throughout the world. It is believed that *Gentiana* plants have evolved in the tropics (Africa-South America), as the oldest ones are found in tropical Latin America, where they grow at the high altitudes. Later the genus spread to the North temperate region⁷⁹.

Their main distribution is in the mountains of the northern temperate zone and also New Zealand and the Andes in South America. *Gentiana* species have also been found on Mount Everest, as high as 5500 m. Relatively few species are found in the lowlands and the Arctic. Most gentians are perennial plants, having beautiful flowers, often with a

trumpet shape, most often coloured blue, and occasionally yellow, white or purple. In the early 20th century, the discovery of the new Chinese autumn-flowering species by western plant hunters introduced some completely new shades of blue such as ultramarine blue and turquoise blue - almost unknown in any other flower. The latter discovery was of great interest also for the fact that it gave gardeners the opportunity to have flowering gentians from early spring till late autumn⁸⁰.

Some species have medicinal values. For example, the dry roots of *G. lutea* are used in gentian schnapps, and in higher concentration the compounds in the roots are used in medicines designed to stimulate the appetite and gastric secretion. Another well-known use of gentian is as a remedy for lack of energy and anaemia. Gentians have also been used as a febrifuge and as a remedy for malaria. In larger doses their extract may have a depressant effect but in smaller doses it stimulates the central nervous system. These useful applications made *Gentiana* plants heavily exploited by man and in the present day they are protected species in most countries⁸⁰. However, the flowers are still collected in large quantities in China, and this could threaten populations of some species.

Some alpine *Gentiana* species often tolerate limestone-derived soil (Table 9), but others are strictly calcifuge. *Gentiana* are also often found growing in the same limestone areas in the wild, next to *Rhododendron* plants.

Table 9 pH requirements for some *Gentiana* species from Asia and Europe^{80, 34}.

Asia			Europe		
Lime-tolerant	Lime-intolerant	Not sensitive to pH	Lime-tolerant	Lime-intolerant	Not sensitive to pH
<i>G. prolata</i>	<i>G. sino-ornata</i>	<i>G. sikkimensis</i>	<i>G. verna</i>	<i>G. asclepiadea</i>	<i>G. lutea</i>
<i>G. veitchiorum</i>	<i>G. trichotoma</i>	<i>G. hexaphylla</i>	<i>G. augustifolia</i>		<i>G. purpurea</i>
<i>G. stipitata</i>		<i>G. szechenyii</i>	<i>G. lutea</i>		<i>G. acaulis</i>
					<i>G. bavarica</i>
					<i>G. verna</i>

Furthermore, in both cases most *Gentiana* species require moist and well-drained soil.

1.10. Modern interpretation of *Rhododendron* nutrient requirements and behaviour in the wild

All of these ‘explanations’ attempted to say that rhododendrons were not actually growing on limestone, i.e. to avoid the issue. All of them were at best hypotheses, without sufficient experimental data or observations, and as a result, they all failed.

If it is not the limestone that is harmful to rhododendrons, then what is the reason for their failure to grow in limestone in cultivation? What actually causes harm to rhododendrons in cultivation is either addition of high amounts of lime, which will raise the pH too much, or dehydration of the soil, which will change the redox conditions and will cause oxidising conditions in the soil (aerobic soil). Either of these two situations will result in unavailability of iron and manganese, as they become oxidised to higher oxidation states, Fe(III) and Mn(III) or Mn(IV), in the forms of water-insoluble oxides and hydroxides, unavailable for plant uptake. In the opposite situation, under low pH or reducing conditions (waterlogged - anaerobic soil) both Fe and Mn are in their second

oxidation state Fe(II) and Mn(II), in the form of water-soluble compounds. In extreme low-pH conditions, solubility may be so high that leaching causes deficiency.

Considering the low availability of Fe and Mn in limestone, there is still no clear explanation of how rhododendrons survive in limestone in the wild. What is clear is that there is, certainly, a way out for rhododendrons in the wild to prevent iron and manganese deficiency as a consequence of the limestone, by somehow providing these essential nutrients for their healthy growth. The next question is:

"What can be a possible mechanism for supplying rhododendrons with sufficient amounts of iron and manganese in limestone soil in the wild?"

Past analyses of soil and leaf samples showed that, as expected, the available iron content in the acidic soils is generally higher than that observed in alkaline or slightly acidic soils^{39, 40}. Even then, in the leaves of some samples in all the pH ranges, the level of iron was below the level normal for rhododendrons. This seemed to suggest a potential risk of iron deficiency, but there was never visible evidence of chlorosis in any of those specimens. However, the minimum level of Fe in all the pH range varied little. So, it appeared that *Rhododendron* iron requirements are satisfied even in the most alkaline conditions. Thus, iron deficiency does not appear to be a problem.

Unfortunately, this is not the case with manganese. In general, the lowest level of Mn required by plants for their healthy growth is 20 ppm and the highest is 300 ppm, as levels of Mn above that are usually toxic for plants. However, rhododendrons seem to behave in exactly the opposite way, as they seem to tolerate extremely high levels of Mn. Analysis of rhododendron leaves showed that the amounts of Mn in the leaves were generally extremely high, up to 4000 ppm^{39, 40}. The evidence is that these plants absorb huge amounts of manganese and there are no indications that they suffer from Mn toxicity. If the Mn is present in the soil, they just absorb it, steadily, during the entire growing period⁶⁴. This excess of Mn appeared to be stored in the leaves, and not to be subsequently redistributed throughout the plant, which indicates that rhododendrons are

very effective manganese accumulators. *Ericaceae* plants in general are found to take up high amounts of manganese¹¹. There have been suggestions that the ability of *Ericaceae* plants to deposit and overcome Mn toxicity may be related to presence of silicon in cell walls¹⁵. It has been observed that calcifuge species have greater capacity for Mn accumulation than the calcicole species¹⁵. It is not known why some plants accumulate manganese, but it has been suggested that uncontrolled uptake may be a mechanism for survival under extreme acidic, anaerobic conditions, where manganese levels are low as a result of leaching^{39, 40}.

Evidently, the uptake of Mn by *Rhododendron* is a process that is very complex and is apparently not controlled at all. No general correlation between its levels in leaves and soil pH was previously shown. Manganese availability and levels in soils can vary over a wide range, depending on dryness, temperature etc. It was found that even in the most alkaline soils there can be sufficient concentrations of Mn present. However, manganese in the above-mentioned high concentrations may not always come from the original soil. As analysis showed, the soil samples from China consisted mainly of fragments of rock, which was shown to contain very little manganese. Clearly, the high overall Mn level must arise from a very high level within the small organic component of the soil. Such a very high level can only come from manganese-rich ericaceous plants. So, it seems that in limestone soils it is rhododendrons themselves that provide Mn for their healthy growth, as a regular source of Mn is the falling and decaying rhododendron leaves. These do not necessarily all come from plants that are themselves growing on the limestone.

This proposed mechanism of supplying Mn, which *Rhododendron* plants may have developed, ensuring sufficient amounts of Mn for their own usage, is not typical for other woody plants. Failure to provide Mn from decaying leaves may be one of the main factors responsible for the unsuccessful attempts to grow rhododendrons on limestone in cultivation, whereas recycling Mn in this way provides a mechanism for surviving in such extreme conditions in the wild.

Certainly, depending on the soil environment, there could be other cases in which chlorosis may be caused by neither manganese nor iron deficiencies. Heavy application of CaSO_4 as a source of calcium for instance, caused interveinal chlorosis in the old leaves of rhododendrons, which was believed to have been induced by very high levels of total soluble salts, measured by soil conductivity⁴⁶.

Another possible reason why in some cases rhododendrons cultivated in chalk soil in the UK may fail to survive could be phosphorus availability. It is a well-known fact that in calcareous soils one of the factors causing deficiencies in plants is the lack of available phosphorus in such soils²².

To fully characterise the issue and establish a complete growing pattern that is adapted by these plants, some other factors and components that represent the rhododendron's environment in Yunnan should also be considered. What role do these components have on the ability of rhododendrons to develop tolerance to limestone as an extreme growing condition?

1.11. Geography and environment of Yunnan as factors related to the adaptation and growth of *Rhododendron* on limestone in Yunnan

1.11.1. Geographical features

Yunnan is reputed to have the richest flora diversity as well as cultural diversity, and is probably the most mysterious and relatively unexplored place in south Asia. Some authors refer to Yunnan as “The kingdom of plants and animals”^{65, 66}. The main factors responsible for the fortunate diversity in Yunnan are its location in terms of latitude and longitude, and topography: the wide range of altitudes within the province borders (from 6740 m to 760 m). To look further at these two important factors, it is useful to discuss the geography of the province in more details.

The climate of NW Yunnan, which is determined by its location, makes it an exceptional region, first because it is within the area that is mainly affected by the southwest summer

monsoon, and secondly, because it is close to the equator, and the lower altitudes (typically around 1800 m) are dominated by an arid climate in northern, or a tropical climate in southern Yunnan (Fig. 4).

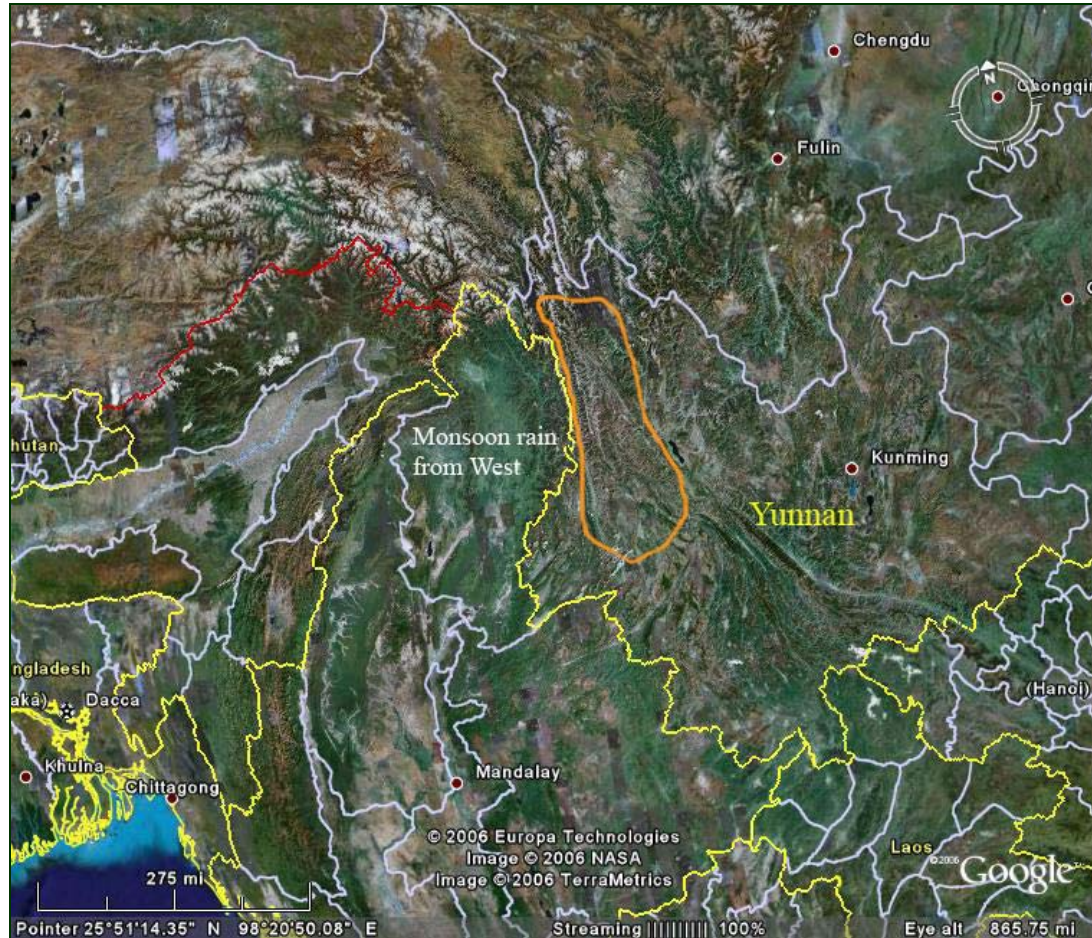


Figure 4 Satellite image of Yunnan⁶⁰

This change of the landscape and climate from the northern cold zone (Tibetan Plateau), to the tropical zone of southern China allows the adaptation of vast numbers of plant species between these zones. The unique geographic profile provides a wide range of climatic zones in the province (Fig. 5)^{32, 62}:

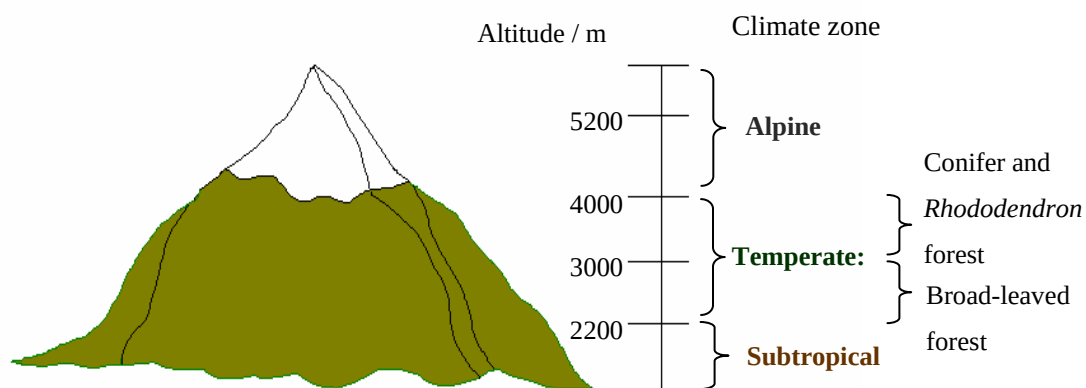


Figure 5 An approximate division of climate zones in NW Yunnan⁶²

The alpine and sub-alpine zones of Yunnan are located in the north-west, in the mountain range Hengduan Shan, which forms a barrier separating the Tibetan plateau and the peaks of the central Himalaya from the flat lands of eastern China. A unique characteristic of NW Yunnan is provided by the three parallel rivers flowing through the province: the Salween, Mekong and Yangtze Rivers (Fig. 4 and 6). The first two flow all the way from north to south, while the Yangtze follows their course from north to south down to the prefecture of Lijiang, where the river turns its course back to the north, flowing below the Yulong Mountain⁶⁵. At this point the Yangtze cuts a defile that isolates the Yulong Mountain from the Tibetan plateau. As a result of this, the plants found in the Yulong Mountain have evolved to species that are not present in other parts of the Hengduan Shan mountain range. Yunnan province has around 16000 plant species, which represent about 50% of the flora in China. (In comparison, Britain has around 1500 known plant species⁶⁷.) Of these, around 3200 species are recorded on the Yulong Mountain^{65, 66}.

The geography of Yunnan gives the area a mild climate, with few frosts at the lower altitudes, and a dry season between October and April and a very wet one from May to September⁶⁶. There are some places in the mountains where the snow cover is lost in the

summer to unusually high altitudes, above around 5200 m, but generally most of these heights are covered by glaciers (Photo 4)³⁴.

The second factor that contributes to the botanical wealth of Yunnan is the variable altitude range, composed of high mountains in NW Yunnan, up to 6740 m (Meili Xue Shan, Photo 4), going down to 760 m⁶⁸.



Photo 4 Meili Xue Shan, 6740 m, NW Yunnan

Thirdly, the fact that the mountain ranges run North-South has enabled plants to migrate, and thus survive the effects of repeated glaciations, while separation between ranges has led to extensive speciation³⁴.

1.11.2. The effect of low temperatures at high altitude

Tiwari investigated the Indian *Rhododendron* species in the Sikkim Himalaya and observed the variation of their distribution with altitude, starting from 1500 m to up to 6000 m, with the densest occurrence of rhododendrons between 3000 m - 3500 m²⁹. This is consistent with our observations in China, although at higher latitudes the altitude range becomes lower. The distribution and growth are, therefore, probably affected and

dependent on temperatures rather than altitude in itself^{29, 34}. In Bhutan as well as in NW Yunnan, the *Rhododendron* species growing between 3000 m and 5200 m are considered as alpine zone species. These high-altitude alpine species are dwarf or shrubby plants, often growing in large colonies, with the size of their leaves from 5 mm - 2 cm long and 3 mm - 2 cm wide³².

Winter injuries are generally common for trees growing in poorly drained soils or in areas exposed to wind⁶⁹. Injuries to *Rhododendron* and *Azalea* are rarely caused by excessive cold during the winter. If damage occurs, it is caused by excessive and rapid fluctuations in temperature or by late spring freezes after the plants have resumed activity. Plants growing in sunny, protected areas are frequently subjected to this type of injury. When strong cold March winds follow warm days, many rhododendrons may be killed. The leaves become brown, especially at the edges and near the tips, which is due to loss of water by the leaves at a time when the water in the soil is still frozen or unavailable for other reasons⁶⁹.

Generally, Yunnan has a mild climate, with a dry season from October to April and a very humid climate between May and September at very high altitudes⁶⁶. However, during the winter months it is drier and colder, especially in the high mountain ranges, above 3500 m. Many *Rhododendron* species have evolved to grow in very cold areas and using special mechanisms have adapted to tolerate very low temperatures, around – 30 °C^{70, 71}. In the USA there have been experiments aiming to induce adaptation to low temperatures artificially in *Rhododendron* hybrids, and to increase genetically their freezing tolerance in cultivation. A hybrid genetically derived from a *Rhododendron* cultivar that was tolerant to freezing temperatures was designed to tolerate even lower temperatures than its parents. However, the approach showed that after two winters of temperatures around –20°C the new hybrid, which was expected to withstand the cold winter, suffered frost injuries, while the cultivar with the naturally adaptive genes (unaltered) was unaffected by the low temperatures⁷¹. This experiment indicates very clearly how well evolved and established the mechanisms that *Rhododendron* plants in the wild use for living in extreme conditions are, and that this ability for quick

acclimatisation can be preserved with migration of these plants over large distances, and performed also in cultivation. Studies on acclimatisation of *Rhododendron* species have shown that some species grown in Benmore Botanic Garden, UK, were able to perceive environmental changes and react to them and that such reactions may occur rapidly⁷².

1.11.3. Effects of precipitation

Temperature, rainfall and evaporation are the main factors that condition the growth of plants. Wind has less direct effect on the growth. Where the cycle of wet and dry seasons is combined with a great range of temperature, the cool winter results in greater rain efficiency, so that the trees are predominantly evergreen and broad-leaved; examples are rhododendrons, camellias and magnolias⁷³.

It is characteristic for rhododendrons in NW Yunnan to occupy the west slopes of the mountains to a higher extent than the east slopes, which indicates that they definitely like more moist slopes, where they are exposed to half a day of sun radiation (the sunshine from the west in the second half of the day)⁶².

The precipitation in NW Yunnan varies very much from season to season. Despite some speculations about the high summer monsoon rainfall leaching the limestone away from the soil, in NW Yunnan precipitation is not permanently high and neither the limestone in the top soil layers nor that in the subsoil is leached away from it⁵⁴. In fact, some areas are surrounded by sheer limestone cliffs from which rocks break off by weathering, thus continually providing more limestone in the soil, and even if there was continuous rainfall over the whole year, it would be very unlikely for the limestone ever to be completely leached out of the soil. From such processes of weathering and regular deposition in the lower lands, as in areas as Gang He Ba (Fig. 4, Photo 1, 2, and 3), the soil is composed of a very thick profile of compacted pure limestone silt. This type of soil generally has poor drainage and during the summer monsoon season the area becomes waterlogged providing an anaerobic, reducing environment. However, since the water is saturated with limestone, the pH is often at its maximum of 8.4, and so the

environment is still not reducing enough for the species Fe^{2+} and Mn^{2+} to be dominant in the soil. Despite the seasonal waterlogging the plants in this area, including the *Rhododendron* species, do not seem to suffer poor-drainage damage and grow very healthily.

1.11.4. Mycorrhiza - *Rhododendron* relationship

Generally the most widely spread, geographically and in terms of variety of host plant species, is the vascular-arbuscular mycorrhiza (VAM).

The present knowledge on mycorrhizal association of rhododendrons and the members of the *Ericaceae* family generally indicates that these plants have a few types of mycorrhiza partners. The main one is the Ericoid mycorrhiza, which is a specific for the *Ericaceae* family. However, VAM was also found in association with *Vaccinium* plants in Hawaii⁷⁴, and Dighton and Coleman (1992) reported VAM in *R. maximum*, and other authors reported its occurrence in *R. ponticum* and *R. simsii*⁷⁵. A study of the VAM occurrence in five *Rhododendron* species growing at a range of altitudes between 1500 m and 4500 m, native to the temperate, sub-alpine and alpine zones of the Pindari Glacier region of the Kumaun Himalaya, Indian Central Himalaya, was conducted⁷⁵. The species studied were *R. arboreum* (appearing between 1500 m and 2800 m elevation together with oak), *R. barbatum* (beyond 2800 m together with birch), *R. campanulatum*, *R. anthopogon* and *R. lepidotum*, growing in heaths and alpine meadows (above 3400 m). The pH of the soil in which the plants studied were growing was in the range 4.6 – 5.3. The results of the experiments showed that the richness of VAM species, the percent of colonisation of the roots (the volume of the root system that was colonised), and the dominance of the colonisation in the *Rhododendron* species (the number of plants that were colonised) decreases with increasing altitude, with maximum colonisation in roots of *R. arboreum* (42%) and minimum in the roots of *R. lepidotum* (28%)⁷⁵. In accordance with this finding, in the species *R. campanulatum* and *R. anthopogon* only, growing at the highest alpine altitudes, another structure of fungi, dark septate endophytes (DSE)

was also detected along with the VAM. These fungi were not found in any of the species of the lower altitudes⁷⁵. This is an interesting result as it may be one of the keys to growth of some *Rhododendron* species in extreme, highly stressed environments of low temperature, low nutrient status, and low decomposition rates, such as alpine and arctic habitats. Occurrences of DSE fungi were also reported from antarctic and sub-arctic sites⁷⁵. The occurrence of these fungi in such habitats suggests that there could be stronger mutual relationship between DSE and *Rhododendron* plants than that of VAM species and *Rhododendron* plants. However, the role of the DSE fungi in rhododendrons is still under investigation.

1.11.5. Acid exudation and formation of root clusters in *Rhododendron* as mechanisms for nutrient uptake from limestone soils

At present, no information or experimental data have been reported on the occurrence of root clusters on roots of *Rhododendron* and *Ericaceae* in general⁷⁶. Data on the release of exudates specifically by *Rhododendron* is also not available.

It has been found that the roots of calcicole plants generally exude organic acids, particularly oxalate and citrate, at higher rates than those of calcifuge plants²². Studies of the extraction of insoluble forms of P, Fe, Mn, Zn, and Cu from calcareous soils in Sweden showed that citrate, followed by malate, was generally more efficient in extracting cations than oxalate, but their efficiency also depended strongly on the pH of the extractant solution²².

Overall, in order to relate these factors, which are generally associated with other plant species, to the present study of *Rhododendron* growing on limestone-derived soil in China, more studies and information are needed.

1.12. Comparison between the environment in the British Isles and NW Yunnan

In contrast to the geologically young landscape and history of formation of the Himalaya, in geological terms the British Isles are much older and weathered lands. The general theory is that the altitudes present are therefore lower²⁸, and a lot lower than those in the Himalayas, which together with the coastal nature of the Isles makes the area a lot more open and windy, especially in the north.

Due to the higher latitudes and the lower altitudes of the British Isles the intensity of the sun radiation is lower than that in the Himalayas. Because of its open coastal landscape and lower sun radiation the weather in spring and summer in the British Isles is generally cooler and drier than the summer in NW Yunnan. However, being more an open landscape, plants may be exposed to more sun, having less shelter during the day, whereas in the mountainous ranges of NW Yunnan the plants may be exposed only to a half-day radiation.

As the summer monsoon rain in Yunnan and the Tibetan Plateau comes from southwest, from the Bay of Bengal, it affects mostly the west slopes of the mountains, as further east the precipitation decreases significantly with distance. Therefore, due to the differences in the moisture levels the vegetation on the west slopes is different to the vegetation on the east slopes, which are drier. Similarly, in the British Isles the rainfall is also higher on the west side. Generally, the precipitation in the British Isles is at lower rates but it is more continuous, through most of the year, and especially between September and April.

The coastal nature of the British Isles provides the land with a mild winter climate and a very low snow level. As a result, the vegetation in the British Isles is less hardy and the acclimatisation for cold weather is slower. Therefore, plants in areas where frost is likely to occur in the spring are more susceptible to it, and suffer frost damage. Conversely, plants in NW Yunnan are very hardy, often exposed in the alpine regions to very low temperatures, around -30°C , to which their acclimatisation is rapid and very efficient⁷⁰. Plants growing in the alpine region during the winter months are even often covered by

snow. This difference in the acclimatisation between the two regions is possibly also affected by different drought/rainfall regimes during the year. In Oregon for example, rhododendrons in dry soils were observed to freeze at higher temperatures than the same varieties of rhododendrons growing in moist soils⁷⁷.

The rivers in the British Isles also present an environment different to that of NW Yunnan. Because of the elevations, which are close to sea level, and because of the absence or low activity of tectonic plates, there is not much topographical diversity of features such as deep gorges and large valleys formed by rivers. In contrast, in NW Yunnan the three rivers cut very deep gorges, providing habitats for many different plant species (Photo 5, Fig. 6). In the Ice Ages plants could migrate from north to south and then return, which is important to maintain the diversity of species. Where there is rich variety of landscapes the diversity of vegetation is also richer.



Photo 5 Tiger Leaping Gorge, Yangtze river, Yulong Shan, NW Yunnan



Figure 6 Map of NW Yunnan, Salween, Mekong and the Yangtze river gorges (Map of China, MairDumont/RV Verlag)

Summary of the differences in the climates and geography of NW Yunnan and the British Isles is presented in Table 10.

Table 10 Contrasts between growing conditions in the British Isles and NW Yunnan.

Factor	British Isles, maritime climate	NW Yunnan
Limestone formations	Chalk in south. Plenty of limestone in north and west	Old, weathered limestone ³¹
Precipitation	All-year-round rainfall, highest in the west and north UK, drier in south and east.	Monsoon summer rain, and relatively dry between October and April.
Altitudes	Low altitudes, thus not much shelter from wind and sun, lower capacity for moist atmosphere in the open areas, smaller variety of species.	High altitudes, providing different aspects, controlled doses of moisture, sunshine and wind ³¹ .
Rain shadow	Low altitudes. Rain shadow occurs to much lesser extent.	West sides of mountains are much wetter than east sides. East of the highest mountains are very dry areas, particularly in the river valleys.
Frost snow cold	Temperate, little snow. As it is a coastal area the high heat capacity of the sea, slowly warming up the land, gives mild winters, with temperatures above freezing. Frost may occur in early spring, damaging new growth.	Depends on the altitude, aspect, etc. - very variable. Acclimatisation of plants is quicker and more efficient ^{70, 72} . Plants are more frost-tolerant.
Rivers and gorges	On average, there are not many deep gorges and large glaciated valleys	Long rivers run in deep gorges from north to south.
Sun radiation	Lower in terms of both latitude and altitude.	Higher sun radiation due to lower latitudes and higher altitudes.

Summary of Introduction

Some *Rhododendron* species and many other alpine species grow in perfect health in very extreme environments, tolerating high pH, seasonal waterlogging, and high altitudes, where the temperatures are generally lower and freezing in winter, and the lower temperatures result in a low microbial activity, and hence low rates of decomposition and organic matter production. The mechanisms that these plants use to flourish in such areas are still not well understood and are highly intriguing phenomena.

Attempts to cultivate rhododendrons on limestone fail because of low availability of Fe and Mn, causing chlorosis. However, Fe deficiency as a consequence of limestone in soil does not seem to be the most widespread problem and, even if it occurs, there is fortunately a relatively easy way to treat iron deficiency, by adding iron in its chelate form - as its EDTA complex. So from the point of view of iron availability there should not be a problem to grow rhododendrons in limestone soils. Unfortunately, it is more difficult to treat Mn deficiency using the Mn-EDTA complex since the manganese is in its reduced form (Mn^{2+}) and it is oxidised very rapidly into the insoluble manganese dioxide MnO_2 . Additionally, it should be noted that at lower pH, 4.5 to about 6.0, the Fe-EDTA to supply Fe is stable and has no effect of providing iron. In contrast, Fe-EDTA added at high rates to soil of pH above 6.0 is less stable, and so Fe^{3+} is released in the soil, but then since above that pH Mn-EDTA is more stable, the free EDTA binds to the available Mn^{2+} ions in the soil, and actually decreases its availability to plants.

Generally, as a slow-release Mn fertiliser the best is shredded leaves from healthy Mn accumulators: rhododendrons or tea leaves – *Camellia*, species that also show Mn-accumulating properties⁵⁴.

With regards to the past studies that have been presented, intriguing science and the questions that have remained unanswered, the objectives of the research of this thesis are clearly established.

1.13. Challenges of studying *Rhododendron* genus

A long term goal of the research is conservation of endangered plant species. The world's list of extinctions of species increases continually. The International Union for Conservation of Nature and Natural Resources (IUCN)⁸¹. Over the past 20 years, 27 documented extinctions or extinctions in the wild have occurred, although these numbers underestimate the real number of extinctions as many described species have not been comprehensively assessed, and proving that a species has become extinct can take years to decades. While the vast majority of extinctions since 1500 AD have occurred on oceanic islands, recently extinctions have become increasingly common on continents too. A recent assessment of indicates that around 50% of the extinctions over the past 20 years occurred on continents. Table 11 represents the trends in threatened plant species between 1998 and 2006, and Table 12 gives up-to-date information about the numbers of threatened species in the families *Ericaceae*, *Gentianaceae* and *Primulaceae*. Table 13 shows the total number of threatened species by country up to 2006⁸¹.

Table 11 Changes in numbers of species in the threatened categories* (CR, EN, VU) from 1996 to 2006⁸¹

Group	Critically Endangered (CR)						Endangered (EN)						Vulnerable (VU)					
Year	1996/98	2000	2002	2003	2004	2006	1996/98	2000	2002	2003	2004	2006	1996/98	2000	2002	2003	2004	2006
Plants	909	1,014	1,046	1,276	1,490	1,541	1,197	1,266	1,291	1,634	2,239	2,258	3,222	3,331	3,377	3,864	4,592	4,591

* IUCN Red List Categories abbreviations:

Threatened species - **CR** - Critically Endangered; **EN** – Endangered; **VU** – Vulnerable

Table 12 Numbers of extinct and threatened plant species* included in the IUCN Red List for plant class *Magnoliopsida* and in some families belonging to this class⁸¹.

Class *Magnoliopsida*

Family	EX	EW	Subtotal	CR	EN	VU	Subtotal	LR/cd	NT	DD	LC	Total
<i>Ericaceae</i>	0	1	1	1	3	14	18	3	3	0	2	27
<i>Gentianaceae</i>	0	0	0	1	10	12	23	0	2	0	8	33
<i>Primulaceae</i>	0	1	1	0	0	0	0	0	0	0	0	1
Subtotal (<i>Magnoliopsida</i>)	77	21	98	1,277	1,836	3,973	7,086	196	807	441	910	9,538

* IUCN Red List Categories abbreviations:
 Extinct species - **EX** – Extinct; **EW** - Extinct in the Wild
 Species at lower risk of threat - **NT** - Near Threatened (includes LR/nt - Lower Risk/near threatened);
LR/cd - Lower Risk/conservation dependent
DD - Data Deficient
LC - Least Concern (includes LR/lc - Lower Risk, least concern)

Table 13 Numbers of extinct and threatened plant species* included in the IUCN Red List, according to countries of origin⁸¹.

	EX	EW	Subtotal	CR	EN	VU	Subtotal	LR/cd	NT	DD	LC	Total
East Asia												
China	3	1	4	73	172	197	442	5	47	19	111	628
South & Southeast Asia												
Bhutan	0	0	0	1	2	4	7	0	3	2	17	29
Myanmar	0	0	0	13	12	13	38	0	10	8	54	110
Nepal	0	0	0	0	2	5	7	0	2	1	24	34
India	7	2	9	45	113	89	247	1	22	18	68	365
Europe												
France	0	0	0	5	0	2	7	0	0	0	16	23
Italy	1	0	1	17	2	1	20	0	0	0	16	37
Spain	1	1	2	21	17	10	48	6	7	0	19	82
United Kingdom	0	0	0	4	2	7	13	0	1	0	7	21
Portugal	0	0	0	2	6	7	15	3	4	0	16	38
North America												
United States	23	7	30	104	64	75	243	4	23	3	83	386

* Refer to footnote to Table 12

The IUCN Red List reports that the main causes of threat of extinction to plants with which this work is concerned are:

(1) Social and economic factors - people and threatened species are often concentrated in the same areas. This is especially true in much of Asia, in particular southeast China and the Western Ghats of India. The number of threatened species is likely to increase rapidly in regions where human population growth rates are high⁸¹.

(2) Habitat destruction - deforestation disturbances and unsustainable extraction of rhododendrons due to construction work and tourism influx has led to severe pressure on the availability of these plants (Photos 6, 7, 8 and 9)^{29, 82}. In Sikkim *R. leptocarpum*, *R. maddenii* and *R. niveum* are under serious threat of extinction as they are widely used as fuel. Such disturbances trigger further troubles for the habitat such as avalanches, floods and soil erosion⁸².



Photo 6 Building of a chairlift, Zhongdian Plateau



Photo 7 Soil erosion from horse-riding tourism



Photo 8 Dead forests, Tianchi Lake, Zhongdian Plateau



Photo 9 Deforestation, Tianchi Lake, Zhongdian Plateau

(3) Climate change – cold or ice-age periods in the long term, or global warming and drier regimes in the short term, may enforce migration of species, or invading by species that can resist the new climate conditions, thus increasing the competition between species.

(4) Over-harvesting of rare plants – many rare and medicinally important species are continuously harvested for illegal trade. As a result of the natural disturbances referred to above, some species in the wild grow in isolated populations. The regeneration of seedlings in such places is particularly inhibited and poor⁸².

Factors (1) and (2), the social factors and the habitat destruction, result in a habitat fragmentation in the way of separation of blocks of vegetation, so that migration in response to factor (3), the climate change, becomes impossible, and also so that extinction within a block due to factor (4) can occur³⁴. Additionally, any potential threat to *Rhododendron* forests in the wild would also result in a potential threat to the large number of terrestrial animal species which *Rhododendron* forests harbour. The patterns evident for mammals, birds and amphibians are therefore likely to be representative of plant taxonomic groups⁸¹.

For these and other commercial reasons, methods for propagation of *Rhododendron* are widely studied, and there is a demand for lime-tolerant idiotypes suitable for use as

rootstocks in commercial gardening and growing^{47, 48, 82}. In addition to generally advancing the knowledge and techniques of cultivating *Rhododendron* species, and of rescuing potentially threatened species in the wild, the information gained from research on *Rhododendron* may also potentially provide a solution for management and control of invasive species, such as *R. ponticum* in the British Isles. *R. ponticum* and its hybrids pose a major problem for local endemic fauna and flora where they have been introduced, often on large estates, while in their original habitats they are rare species. The poor understanding of its invasive character also presents difficulties in fighting the very damaging virus *Phytophthora ramorum*, which causes ‘Sudden Oak’ death to trees, and of which *R. ponticum* is a transmitter in the UK.

Chapter 2 Materials and methods

2.1. Sample collections selection criteria

The collections of plant and soil material were carefully planned and designed to include material from plants specially selected according the following criteria.

1) Plant genus of main interest:

- a. *Rhododendron*
- b. *Primula*
- c. *Gentiana*
- d. other typical alpine plant genus: *Androsace*, *Daphne*, *Saxifraga*, etc.

2) Healthy plants:

- e. growing on or near limestone-derived soil
- f. growing on or near dolomitic limestone-derived soil
- g. growing on organic or other non-limestone-derived soil*

3) Unhealthy plants showing symptoms of chlorosis (yellow veins or green veins and yellow between the veins):

- h. growing on or near limestone-derived soil
- i. growing on or near dolomitic limestone-derived soil
- j. growing on organic or other non-limestone-derived soil

4) Unhealthy plants showing unhealthy symptoms other than chlorosis (fungal infections, red or brown marks)

- k. growing on or near limestone-derived soil
- l. growing on or near dolomitic limestone-derived soil
- m. growing on organic or other non-limestone-derived soil

The plants' health condition was assessed at the time of collection on the basis of visually observable symptoms of chlorosis, necrotic marking, reddish-purple coloration,

*1) thick organic layer covering limestone rocks: loams, forest soils

2) soil derived from metamorphic or igneous rock formations: forests, screes

3) peat: bogs, moors, meadows, woods

scorched and bleached leaves, and etc. The results obtained from the analyses for nutrient concentrations were later related to those symptoms.

5) Age of leaf

- n. leaves from current season growth (denoted as '0')
- o. leaves from previous years of growth, sampled for each age of leaf present on the plant (denoted from '0' to '5', for five-years-old leaves).

The age of the leaves was determined using the marks on plants' stems that remain after a leaf bud develops into a leaf.

2.2. Location of plants subjected to sample collection

The locations where samples were collected depended on the natural distribution of plant species targeted to be studied in the selection criteria, and therefore were carefully pre-selected. The areas of natural distribution of the plants were chosen so that their local geology comprised of rocks of the types included in the selection criteria, i.e. limestone, dolomite and dolomitic limestone, where the soil is also mainly derived from those rock types. Within the selection criteria, the non-limestone/dolomitic-derived soils were present, easily accessible and rich in plant species in all selected areas. Therefore, areas having such soils were not specially pre-selected according to local geology, but samples from plants growing on those soils were collected simply when the plants of interest were observed.

According to these factors for pre-selecting the locations of samples collections, the main areas visited were the limestone mountains of SW China, the Dolomites in the Alps, and non-limestone/dolomitic areas in the USA.

Samples of soil and leaf material for 20 species of *Rhododendron* and around 150 species of alpine plants were collected by Prof. David Rankin and his team in Yunnan and Sichuan provinces in SW China, N Carolina (USA), N Italy and Switzerland, between 1999 and 2005. During the last expedition to NW Yunnan, China in 2005,

samples for around 20 species of *Rhododendron* and 20 species of alpine plants were collected by Prof. Rankin and Maria Kaisheva.

2.3. Summary of plant genus/species from which samples were collected, soil type, and numbers of samples collected

The numbers of plant species collected in the wild, and the numbers of soil and leaf samples analysed for each species are listed in groups according to genus, type of soil and soil pH in Table 14.

Table 14 Soil and leaf samples analysed experimentally and interpreted statistically.

Genus	Total number of plants/cases*	Soil	Leaves	Plants on limestone soil	Plants on dolomitic limestone formations	Plants on non-limestone soil	at pH 4.0-6.7	at pH 6.7-8.0
<i>Rhododendron</i>	216	63	209	39	9	66	40	55
<i>Androsace</i>	8	7	8	4	0	4	3	4
<i>Daphne</i>	7	7	6	4	1	2	1	6
other <i>Ericaceae</i>	14	14	13	3	4	7	7	6
<i>Gentiana</i>	47	40	37	2	3	33	25	11
<i>Primula</i>	48	40	48	18	1	28	18	26
<i>Rheum</i>	5	1	5	2	0	3	1	4
<i>Other</i>	49	26	44	3	13	28	18	26
TOTAL analysed	396	168	370	75	31	171	113	136

* Number of plants for which a leaf and/or a soil sample was analysed in each species or group of species.

As in some cases one soil sample was collected for several plants growing in the same location, the total number of analysed soils (168) is less than the sum of the numbers in the 'Soil' column. Similarly, some plants in the 'Genus' groups do not have

representative of both a leaf and a soil sample, but each plant has at least one leaf and/or soil sample. Some plants have more than one leaf and/or soil sample, others have only leaf samples, and others have only soil samples but not leaf samples. Therefore, the total number of plants in a genus group (2nd column) can be different from the sum of the number of ‘Soil’ and ‘Leaves’ samples (3rd and 4th columns respectively).

Additionally, the statistical analyses of the variation in concentrations of the studied nutrients with leaf age were performed on groups of plants that have leaves representatives of each year of age to. For instance, when variation of [Mn] in the leaves was plotted against leaf age including current-year-old, one-year-old and two-years-old leaves, only plants that have representatives of all three ages were included in the plot. In this respect, for plants for which only current-year-old leaves (1st row) or only current-year-old and one-year-old leaves (2nd row) were collected statistical analyses involving the concentrations of metals and leaf age were not carried out (Table 15). Such analyses were only performed for the plants for which current-year-old, one-year-old and two-years-old leaves (3rd row), and current-year-old, one-year-old, two-years-old, three-years-old, four-years-old, and five-years-old leaves (4th row) were collected.

Table 15 Number of *Rhododendron* plants in each group of leaf samples of consecutive years of age collected.

Leaf age / y per plant	Total number of plants	Plants on limestone soil	Plants on dolomitic limestone formations	Plants on non- limestone soil
0	31	13	5	13
0, 1	69	27	3	39
0, 1, 2	10	4	0	6
0 - 5	1	0	0	1

As the genus *Rhododendron* was of priority interest, analyses were carried out on the *Rhododendron* species listed in Table 16.

Table 16 Taxonomy and numbers of analysed leaf and soil samples per species.

Subgenus	Section	Subsection	Species	Number of plants	Leaf sample	Soil sample
Hymenanthus	Ponticum	Campylocarpa	<i>R. wardii</i>	1	1	1
		Fortunea	<i>R. vernicosum</i>	9	14	8
			<i>R. decorum</i>	5	9	3
		Irrorata	<i>R. anthosphaerum</i>	1	1	1
		Pontica	<i>R. maximum</i>	2	8	2
			<i>R. catawbiense</i>	1	1	1
		Taliensia	<i>R. phaeochrysum</i>	7	17	3
			<i>R. balfourianum</i>	1	1	1
			<i>R. aganniphum</i>	1	2	1
Rhododendron	Rhododendron	Heliolepidia	<i>R. rubiginosum</i>	12	22	11
		Lapponica	<i>R. cuneatum</i>	12	23	12
			<i>R. telmateium</i>	7	13	7
			<i>R. nitidulum</i>	1	1	1
			<i>R. nivale</i> ssp. <i>boreale</i>	2	2	3
			<i>R. setosum</i>	1	2	0
			<i>R. hippophaeoides</i>	1	2	1
			<i>R. rupicola</i>	1	1	1
			<i>R. yungkingense</i>	1	2	1
			<i>R. impeditum</i>	1	2	1
			<i>R. fastigiatum</i>	3	6	2
		Lepidota	<i>R. lepidotum</i>	1	2	0
		Rhododendron	<i>R. hirsutum</i>	7	9	7
			<i>R. ferrugineum</i>	4	9	4
		Saluenensia	<i>R. saluenense</i>	2	3	2
		Scabrifolia	<i>R. racemosum</i>	2	4	1
		Trichoclada	<i>R. trichocladum</i>	2	4	2
		Triflora	<i>R. yunnanense</i>	3	4	2
	Pogonanthum		<i>R. anthopogon</i>	2	2	0
			<i>R. primuliflorum</i>	9	17	8
			<i>R. trichostomum</i>	1	2	1

2.4. Collection and handling of leaf and soil material

Leaf sample collection was carried out by tearing a sufficient number of leaves, together with the petiole, off the plants' branches. The number of leaves collected per plant was dependent on the surface area and thickness of the leaves, as well as on the total number of leaves that were present on the plant, i.e. on the size of the whole plant. The amount of leaf material was considered to be sufficient when the total surface area collected was at least 4 cm² for thin leaves and 2 cm² for thicker leaves, which is the least required for leaf extraction amount. However, to ensure enough leaf material for repetitions of analyses greater than 4 cm² is preferable. Collected leaves were placed and stored in paper bags and left to dry in air.

Leaf samples for leaves of different age were collected depending on the availability of material: very few *Rhododendron* plants in the wild have leaves older than two-years-old, and so above that age, representatives of older leaves were not always found on rhododendrons on each of the types of soil studied. As a result, the absence of plants having representative leaves of more than three consecutive years of age, in some groups of soil type, may affect the overall correlations between 'Age of leaf' variable and nutrients concentrations variables.

Most of the soil samples were collected from the top 10 cm of the soil profile, around the plants' root system, and where easy access to a deeper soil profile was allowed, from the zone below the plants' root system, at depth depending on the size of the plant. The soils were kept in plastic bags and dried in air.

2.5. Materials and chemicals used in experimental methods

The materials used in the laboratory for the sample treatments are listed in Table 17 (ref. also Table 20).

Table 17 Chemicals used in the analytical laboratory treatment of the leaf and soil samples.

Chemical	Source
Deionised water	Milli-Ro 15 water purification system, Millipore
HNO ₃	Fisher Scientific, 69%, d 1.42
CH ₃ COONH ₄	Fisher Scientific, 500 g, Ca < 0.001%, Fe < 1ppm, Mg < 5 ppm, 5% solution – pH = 6.6 – 7.5
Calibration standard solutions	Fisher Scientific: 1000 ppm Ca, Mg, Fe, Mn, K, P solutions in <i>ca.</i> 1M HNO ₃
CH ₃ COOH	Fisher Scientific
MnSO ₄ ·4H ₂ O	Fisher Scientific

2.6. Soil pH measurement

To minimise the effect of water in the soil samples on the pH readings dry soil was used. Soil pH was measured in suspension of 1: 2.5 v/v soil to water⁴⁸, after 24 hours of equilibration of the solutions, using a Jenway 370 pH/mV meter with accuracy ± 0.02 pH and resolution 0.01 pH. To ensure that pH was measured in a homogenous environment the soil suspension was thoroughly shaken before inserting the electrode in it. To account for reproducibility three readings of pH for each soil sample were taken: the variation of the measurements was ~ 0.03 .

2.7. Extraction of nutrients from leaf material

Since the spatial distribution of the nutrients being analysed in the leaves is still unclear, to take the average reading of the nutrient concentrations the leaves that were thick and bigger than 2 cm in length and 1 cm in width were ground to powder where possible,

whereas the leaves that were thinner and weighed less than 0.125 g were weighed as whole leaves.

Approximately 0.125 g dried leaf material was placed in a pre-weighed glass vial, washed with distilled water and then placed in a muffle furnace at 70 °C to dry for 2 hours⁸³. The vials were allowed to cool and were weighed again. 22.5 ml of 69% HNO₃ was then added to the dry leaf material using a measuring cylinder and shaken overnight, using an orbital electric shaker. The resulting extract was filtered through Whatman hardened ashless filter paper and the filtrate was diluted ten times with deionised water (1 ml of filtrate plus 9 ml water). The diluted solution was then analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES) for the concentrations of nutrients in the leaves. It is recommended that the diluted sample solution is analysed within seven days of the preparation.

2.8. Extraction of nutrients from soil

For extracting the metal ions from the soil a standard extraction procedure used widely in agricultural soil testing for characterising soils of different types was used⁸³. The test uses ammonium acetate as the extracting medium, assessing the exchangeable cations, which is the fraction of the metals' concentrations available to plants in the soil, so it does not show the total amount of the metals in the soils.

20 ml of 0.5 M CH₃COONH₄ solution, buffered to pH = 4.0 with CH₃COOH, was added to a weighed sample of 0.25 g of dried soil. The mixture was then shaken for at least 6 hours and filtered through Nalgene filter syringe units, SFCA (surfactant-free cellulose acetate) membrane, 0.2 µm pore size. The extract was then analysed by ICP-OES for the concentrations of nutrients in the soil.

2.9. Measuring the organic content in the soil

0.25 g of fine soil powder was weighed in a crucible with known mass. The crucibles were placed in a graphite furnace and heated to 200°C for 2 hours. After cooling their

weight was recorded and they were placed back in the furnace and heated at 650 °C for 8 hours⁸³. The temperature is high enough for the organic components to decompose, but not high enough to cause decomposition of CaCO₃, which occurs at 825 °C. During the course of the experiment it was observed that the soils with high CaCO₃ content were losing a greater percentage of weight than expected, perhaps due to loss of CO₂. It has been shown that the threshold temperature for decomposition of CaCO₃ is 710 °C, with a peak rate at 890° C⁸⁴. The measurement of soil organic matter in the CaCO₃ soils was therefore established to give more accurate results at a lower temperature, between 550 °C and 570 °C. For the rest of the soils, containing minerals other than carbonates, the soil organic content was measured at 650 °C.

The organic content was calculated as the percentage soil mass lost on ignition.

2.10. Growing experiments

2.10.1. Experiment set up

The growing experiments were carried outdoors, between April and October 2004, at the nursery of Kevoek Garden Plants, Lasswade, near Edinburgh.

The experiment was targeted to focus on studying *Rhododendron* and *Gentiana* species growing under controlled conditions in cultivation. The plant species studied in the growing experiment were provided according to availability of species offered by suppliers (Table 18). Some of the species of interest, although generally cultivated in the UK, is hard to provide in a sufficient for an experiment number, and others are unlikely to be found in commercial plant nurseries, or at all.

Table 18 Plants studied in the growing experiments.

Name of plant	Volume of pots / L	Number of plants	Natural distribution	pH tolerance	Provider
<i>R. hirsutum</i>	2	64	Europe	Lime-tolerant	PN*
<i>R. impeditum</i>	2	64	Himalaya	Lime-tolerant	PN*
<i>R. coeloreuron</i>	5	64	Himalaya	Lime-tolerant	Glendoick
<i>Gentiana sino-ornata</i>	0.75	24	Himalaya	Lime-intolerant	RBGE**

* PN - Plant nurseries in Scotland

** RBGE - Royal Botanic Garden Edinburgh

The plants were removed from the pots where they were previously growing and had their roots thoroughly washed from any remains of soil. The plants were then planted into the pre-mixed growing media. The current season's shoots of each plant were cut off so the effect of their new growing environment during the experiment could be detected in the shoots of the next season's growth.

The pots were kept outdoors, placed in a randomly selected area in the nursery site where the experiment took place according to the availability of space. The pots were arranged in lines in order of pH of the soil media. The plants were provided with water by natural rainfall and, when necessary, by watering with water from the neighbouring River North Esk (South of Edinburgh). The pots were sheltered from wind and sun-light under semi-permeable cover. Because of their arrangement in a block design, covering relatively small square area, all pots were exposed to the same climate conditions, and therefore, to approximately equal amounts of wind, sunshine and rain water.

In addition, to the rain water the pots were watered once every two days, if the precipitation was high, and once per day on dry days. The watering was coordinated with the addition of the low dose manganese solution over six months: the fertiliser was

poured into relatively dry to slightly damp soil so that it would not drain out from the pot, which would be likely to happen if the plants had been recently watered.

The plants were grown in the experimental environment for 6 months, from the beginning of May until the end of October. The average temperature from May to September varied between 15 and 18 °C, whereas in October it dropped down to 10-12 °C.

2.10.2. Growing media preparation

The plants were divided into four groups, all four of which were planted in soil media of pH 4.0, 5.3, 6.7 and 8.0. The pH value of each medium was achieved by the combinational effect of the main substrate used, followed by the addition of different quantities of garden limestone and magnesium limestone where applicable⁷⁷. For the media with pH 4.0, pH 5.3 and pH 6.7 peat was used as substrate. The main agents used for raising the pH were limestone and magnesium limestone, as to set up pH 6.7 and pH 8.0 both were added in equal amounts (Table 19). To further raise the alkalinity and so to achieve pH 8.0, coir was used as a main substrate. The pH of coir is higher than that of peat. Coir is composed of coarse fibre obtained from the tissues surrounding the coconut seed and it has recently been introduced in horticultural growing media, functioning as the organic component of composts. It is believed to be relatively environmentally friendly and therefore is used in horticulture preferentially to peat or other soil types, which have to be extracted from the ground, and are non-renewable resources.

Table 19 Quantities of ingredients used in growing experiment media.

Media ingredients	Media pH			
	pH = 4.0	pH = 5.3	pH = 6.7	pH = 8.0
Substrate	Peat 20 L	Peat 20 L	Peat 20 L	Coir 20 L
Mg-limestone	-	50 g	50 g	50 g
Limestone	-	50 g	250 g	250 g
KNO ₃	15 g	15 g	15 g	15 g
Superphosphate	15 g	15 g	15 g	15 g
FTE 255 WM	6 g	6 g	6 g	6 g
NH ₄ NO ₃	-	-	-	5 g

The materials used in the experiment were obtained from the sources listed in Table 20.

Table 20 Chemicals and materials used in growing experiment media.

Chemical	Source
Substrate	Keith Singleton, peat compost and coir
Mg-limestone	Chempak
Limestone	J. Arthur Bower, garden lime
KNO ₃	Fisher Scientific
Superphosphate	J. Arthur Bower, superphosphate plant food
FTE 255 WM	Fritted Trace Elements mixture containing ⁸⁶ : 1% B, 4.3% Cu, 13.8% Fe, 5.4% Mn, 1% Mo, 4.3% Zn. Clydeside Trading Society LTD, Horticultural Suppliers
NH ₄ NO ₃	Fisher Scientific

Furthermore, each of the four pH media was subdivided into another four groups, targeted to follow the effect of applying manganese fertiliser to the plants (Table 21 and 22). The first group, 'control', contained the original growing medium for each pH as shown in Table 19 without any other additives. The second group was labelled 'low dose Mn' and it was made of the original medium with the addition of manganese solution, poured into the soil once a week for the period of six months. The third group, 'high dose Mn', consisted of the initial medium with the addition of manganese solution equal to the total quantity of that added with the low dose over six months. The 'high dose Mn' solution was applied as a single dose at the start of the experiments by watering the solution into the soil. The last subgroup, 'mulch', included fallen and decaying *Rhododendron* leaves, mixed up with the initial medium soil, acting as a slow-release source of manganese. The purpose of the mulch experiment was to simulate the natural living conditions and to see whether the rhododendron leaves would release manganese, which they had been accumulating.

Table 21 Characteristics of subgroups within each pH medium.

Subgroup	Subgroups media content
Control	Each initial pH medium (Table 19).
Low dose	Each initial pH medium + 4.5×10^{-5} M solution of $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ applied to the soil once a week for 6 months (Table 22).
High dose	Each initial pH medium + 4.5×10^{-4} M solution of $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ applied to the soil at once, in the beginning of the experiment * (Table 22)
Mulch	Fallen and decaying <i>Rhododendron</i> leaves collected under <i>Rhododendron</i> plants in the wild in Scotland, mixed with the soil of the initial medium (Table 19).

* Since the quantity of the solution was too much to be added at once, it was added in two applications.

Table 22 Quantities of Mn fertiliser applied to number of studied plant species.

Subgroups	Plant species				
		<i>R. impeditum</i>	<i>R. hirsutum</i>	<i>R. coeloreuron</i>	<i>G. sino-ornata</i>
Control	No of plants	4 plants x 4 pH media	4 plants x 4 pH media	4 plants x 4 pH media	3 plants x 4 pH media
	Quantity applied	-	-	-	-
Low dose	No of plants	4 plants x 4 pH media	4 plants x 4 pH media	4 plants x 4 pH media	3 plants x 4 pH media
	Quantity applied	40 ml *pp, pw**	40 ml pp, pw	100 ml pp, pw	15 ml pp, pw
High dose	No of plants	4 plants x 4 pH media	4 plants x 4 pH media	4 plants x 4 pH media	Not applied
	Quantity applied	640 ml pp	640 ml pp	1600 ml pp	
Mulch	No of plants	4 plants x 4 pH media	4 plants x 4 pH media	4 plants x 4 pH media	Not applied
	Quantity applied	1/4 of total volume	1/4 of total volume	1/4 of total volume	

* the applied volume of solution varied with the volume of the pots but the standard dose was 20 ml/L added in the low dose and 320 ml/L in the high dose.

** pp = per plant, pw = per week

For all *Rhododendron* species four replicas per species, per subgroup in each pH were planted. As for the experiment the *G. sino-ornata* plants were available in fewer numbers, only three replicas per subgroup in each pH were planted.

The pH of the soils was measured three times: at the beginning, in the middle and at the end of the experiment.

On completion of the experiment leaf material of each plant and soil material for groups of plants were collected for further treatment and ICP-OES analyses. The river water used for irrigation was analysed for content of metal ions by ICP-OES. The mulch leaves used in the experiment were analysed.

2.11. Inductively-coupled plasma optical-emission spectroscopy (ICP-OES)

2.11.1. Instrument background

ICP-OES is a simultaneous, multi-elemental analytical method, where the plasma dissociates the analysed sample into its constituent atoms and ions. The same source is used to atomise the sample and to excite, and possibly ionise, the atoms of the sample⁸⁷. The resulting atoms and ions are excited to high energy levels and consequently emit light at characteristic wavelengths⁸⁸. The excitation is performed by the 'inductively coupled plasma', which has a very high temperature, starting from 6000 K and reaching 10 000 K at the tip of the flame (Fig. 7). The plasma source is a stream of argon. The high-temperature plasma is produced by energising the argon stream with a rapidly changing radio frequency radiation (RF). The sample is introduced into the plasma in the form of aerosol, which is carried into the plasma by a nebuliser gas flow. A monochromator disperses and can separate specific wavelengths of interest, and a detector measures the intensity of the emitted light. The information can then be presented as concentration units.

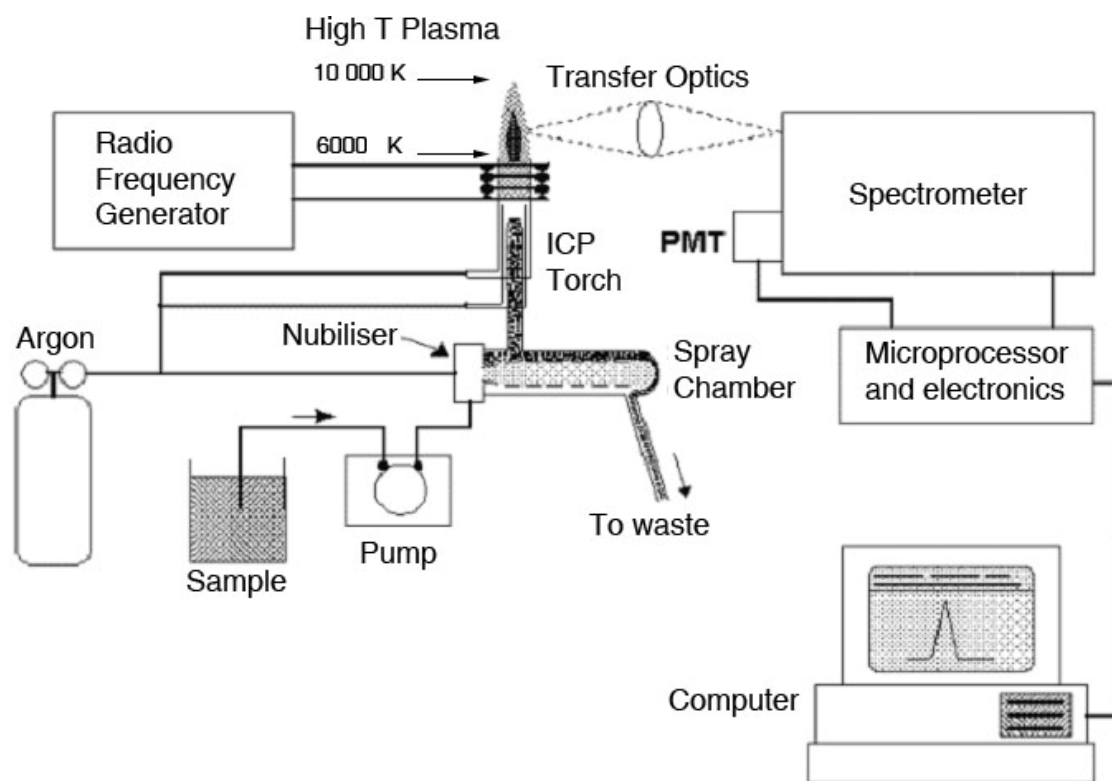


Figure 7 Components of ICP-OES instrument.

Figure used with the permission of Perkin-Elmer Corp.

The ICP method is generally superior in accuracy, precision, detection limit, low matrix interferences and dynamic range to Atomic Absorption Spectroscopy (AAS) or ICP-Mass Spectroscopy. Additionally, the costs of instrumentation and maintenance for ICP-OES are generally lower than those for ICP-MS. However, ICP-MS is better for determination of elemental isotope abundance, which is not needed for the purposes of this research⁸⁹.

ICP-OES is very useful for trace analysis in geological, environmental, and biological materials. Up to 60 elements can be simultaneously analysed at a time, as the analysis of one sample generally takes little time. Large volumes of data can therefore be generated very fast.

ICP-OES analysis is the most commonly used technology for chemical analyses of soils. The soil samples must first be digested for exchangeable ions (the ions available to

plants for uptake), using a multi-element solution for simultaneous extraction, as described in sections 2 and 3 of this chapter.

2.11.2. Method development for ICP-OES leaf and soil sample analyses

Between February 2003 and May 2004 the analyses were performed on a *Thermo Jarrel Ash IRIS* ICP – OES. From September 2004 until completion of project the analyses were performed on a *Perkin-Elmer Optima 5300 DV* spectrometer.

The analytical method was developed to present the wavelengths suitable for analysing the elements of interest and testing them over a wide range of concentrations. The standard solutions used for calibrating the machine are listed in Table 23.

Table 23 Concentrations in ppm of standard solutions used for calibration of the spectrometer for soil and leaf samples analyses.

Standard solution	Soil standard solutions / ppm	Leaf standard solutions / ppm	Element
Single	300	300	Ca Mg
	500	500	
	700	700	
Mixed	-	0.01	Fe Mn Ca Mg
	0.1	0.1	
	1	1	
	10	10	
	100	100	

The lines of emission were chosen according to their best representation of the analyte concentrations and least interference with neighbouring wavelengths of other elements. The wavelengths taken into consideration are listed in Table 24. The sensitivity of the spectrometer is presented by the detection limits for the analysed elements (Table 24).

Table 24 Wavelengths and detection limits for the analysed elements⁸⁹.

<i>Element</i>	<i>Wavelength /nm</i>			<i>Detection limits / mg L⁻¹</i>
Fe (II)	238	239	259	0.01
Mn (II)	260	293.3	-	0.01
Ca (II)	184	315	317	0.05
Mg (II)	279.5	280	383	0.04

The ICP-OES was calibrated using standard solutions, made up from single-element standards of 1000 ppm for Ca, Mg, Fe and Mn for ICP-OES analysis. The standards for the soil analysis were prepared in 0.5 M ammonium acetate, and these for the leaf analysis in 0.1 M nitric acid. Reference samples of ammonium acetate and nitric acid solutions were pre-treated and analysed as blanks for the standard solutions, using the same procedure as those used in the soil and leaf extractions. The standard solutions were diluted to concentrations comparable with those of the elements expected to be present in the samples, or aiming to cover wide range of concentrations in which the elements might be present. The analyses were tested for reproducibility by using reference samples of a certain soil and leaf material, which were tested regularly.

Sample material with SDR number (sample reference number, refer to Appendix 1) from 42xx to 51xx (see Appendix 1 Results tables), collected during the expedition to Yunnan in 2005, was chemically treated and analysed with ICP-OES by the project students Lesley Oliphant, MChem, and Rosie Hunter, BSc, under the supervision of the author of the thesis. The interpretation of the results obtained from the spectroscopic analyses was done by Maria Kaisheva.

The results of all experimental analyses and visual observations were stored, interpreted and assessed using Microsoft Access Database and Minitab and Statistica 6.0 statistical software.

Chapter 3 Statistical interpretation of results

Discussions of results for *Rhododendron* species in the wild

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Discussions of results for *Gentiana* and other alpine plant species
in the wild

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Discussions of results for growing experiments with
Rhododendron and *Gentiana* species

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Legend and abbreviations to figures and text:

- Current-year leaves – current-year leaf (newest growth), also may be referred to as young leaves.
- Old leaves - one-year-old leaf (growth from one year before collection) to five-year-old leaf samples.
- [M] (L) and [M] (S) – concentrations of the analysed metal in (L) leaves and (S) soil.
- SP – Scatter plot, representing linear correlation between variables.
- BP – Box plot, representing the discriminant analysis.
- LR – Linear regression plot.
- ANOVA – Analysis of variance. Vertical bars denote 0.95 confidence intervals
- CP – Column plot, presented in the Appendix.
- MP – Mean plot.
- Min – max – The full range of observed values for the variable on the y axis, from the lowest to the highest value for the given variable, represented by the min – max range.
- ‘25 % - 75 %’ – Represented by the box in the plot corresponding to the middle half (50 %) of the data, i.e. with the top 25 % and bottom 25 % of the y values removed.
- Median – The small box shape in a Box plot represents the middle of a distribution: half the observations are above the median and half are below the median.
- CM - Correlation matrix.
- r – correlation coefficient
- HC – Health condition
- SOM – Soil organic matter
- Case – Age of leaf sample for every plant (current, one-year-old, and etc.). Each

plant has at least one corresponding case (current year leaf) and may have maximum of six cases – up to six-year-old leaf samples. Each case (year of age) is represented by ten variables: soil pH, SOM, Ca (L), Fe (L), Mg (L), Mn (L), Ca (S), Fe (S), Mg (S), and Mn (S).

- Number of plant of each species in Column plots - (1), (2), etc. – corresponds to the count of plants from one species, from which either leaf and/or soil samples were collected. For some plants only soil or leaf samples were collected. Where sample from more than one plant was collected for a given species, they have been numbered sequentially.
- SD – Standard deviation
- SE – Standard error of the mean
- CI – confidence interval, $\pm 95\%$
- N – number of leaf samples in each age group involved in the analyses of “Leaf age” variable. In these analyses only those plants having representative leaves of all years of age were included.

3.1. Method development for the statistical interpretations

- Statistical analyses

The results for *Rhododendron* and alpine plants collected in the wild were interpreted using Statistica 6.0 software and KaleidaGraph. The performed analyses are: linear correlations presented as scatter plots (SP) and correlation matrices, box plots, mean plots, regression, and analysis of variance (ANOVA). Tables of summary of statistical analyses are presented in appendix 2.

For a summary representation of the results and variations between plant species Access database was used for creating column plots (Appendix 3).

- Significance of correlations

All correlation matrices presented in the text of this chapter and in Appendix 2 contain the same data. However, for easier reviewing of the coefficients during the discussions of results, the correlation coefficients are presented in three types of tables: (a) tables

containing only the significant and strong correlations (at the beginning of each nutrient discussion), (b) tables of all correlations of the discussed nutrient, i.e. weak and medium insignificant correlations as some of them will also be discussed in relation to the significant correlations, and (c) tables of all, correlations for all variables (in Appendix 2). In the correlation matrices (and regression summaries) in accordance with the number of degrees of freedom, values highlighted in red are the strongly significant (at $p < 0.05$) correlation coefficients, although in some cases correlations of medium significance are taken into account due to absence of strong ones. Those highlighted in blue (in appendix 2 only) are all, weak and strong, significant correlation coefficients (at $p < 0.05$). The strong insignificant correlations are not highlighted, and not considered as meaningful.

Due to the overall observation of generally medium correlations, below 0.50, in this study, correlations are regarded as strong when higher than 0.70 and those between 0.30 and 0.70 are considered as medium correlations.

Outliers were included in the correlation matrices, i.e. correlation coefficients in the matrices may contain values which could be different from the values for the same correlations on some of the graphs in the text, as outliers were selected out from the graphs.

- Samples grouping

For analysing the relation between a soil variable (soil pH, [M], and soil organic matter) and a leaf variable ([M], health condition, age) the samples taken into account were only those for plants having both soil and leaf samples. For some plants soil samples were not collected, only leaf samples, so these leaf samples do not have soil pH and soil organic matter variables to contribute to the total representation of those plants in the statistical interpretation. Such samples were included in the analyses of leaf variables but excluded where correlations of soil only variables were analysed. Conversely, samples for plants from which leaf sample was not collected but only soil sample, were included only in the soil variables interpretations and excluded from mixed (soil and leaf) interpretations (e.g. soil pH vs [Mn] in the leaves).

- Correlations between “Leaf age” and “[Me] (L)” variables

When correlations between Leaf age and concentrations of nutrients in the leaves are presented in the correlation matrices for each type of soil, these are calculated for all ages of leaves available for all plants per group. More significant correlation coefficients, only for the plants for which there are representatives of each leaf age (0, 1 and 2), are presented in the sections discussing the variation of each nutrient in leaves with time.

- Criteria for outliers exclusion

Outlier samples, defined as those having analysis values outside the bulk/general distribution of all samples, were generally included in the statistical analyses, but where specified these were excluded.

- Error estimation

The error of the experiments was expressed in terms of confidence intervals, with 95 % confidence concentration, which represents the random error of the experimental method. The random error of the method was calculated from the errors generated by the equipment used in the experiments: ICP-OES, in terms of the relative standard deviation (RSD %), balance, glass-ware, etc. The RSD from the ICP-OES includes the error of metal-ion concentration in the prepared standard solutions, the matrix interference/background correction, and spectral interferences. For both soil and leaf samples the random error was within the range 4.7 – 6.7 %. The nature of the samples is such that it is subjective to estimate a single number error for all plant material, as it is living material and the variables and their parameters vary from plant to plant, and therefore for all samples the systematic error is presented as the average, i.e. 5.7 %.

Discussion of results for *Rhododendron* species in the wild

3.2. General correlations between soil pH, soil organic matter and health for *Rhododendron*

The correlations observed for soil pH, soil organic matter (SOM), health condition (HC) and leaf age are listed in correlation matrix 1. Correlations between HC and leaf age are not shown in the following matrix and Table 25 as these correlations are not meaningful in this study, but are shown in the overall matrices in appendix 2.

Correlation matrix 1 Correlations for soil pH, SOM, HC and leaf age for *Rhododendron* plants.

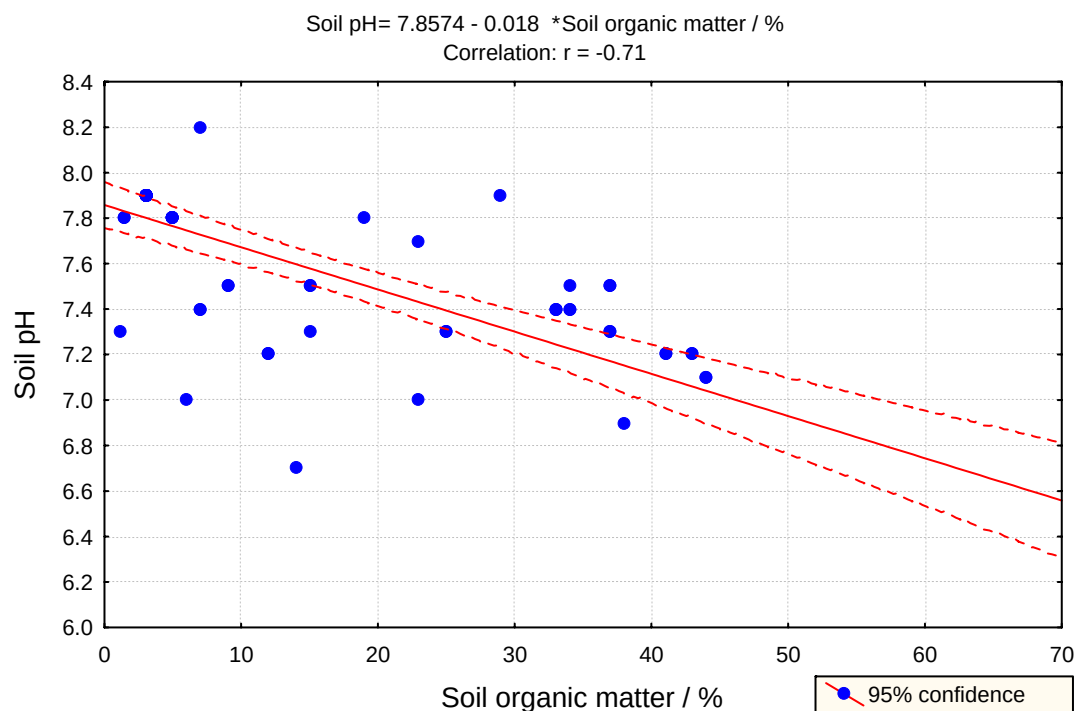
Variable	soil pH				SOM %			
	<i>all</i>	<i>limestone</i>	<i>dolomitic</i>	<i>non-limestone</i>	<i>all</i>	<i>limestone</i>	<i>dolomitic</i>	<i>non-limestone</i>
SOM %	-0.39	-0.68	-0.91	0.24	1.00	1.00	1.00	1.00
Leaf age	-0.15	0.16	-0.43	-0.08	-0.15	-0.17	0.35	-0.31
HC	0.19	-0.35	0	0.04	-0.09	0.27	0	0.01

As expected, the correlation between soil pH and SOM is significant in all types of soil but the strongest correlations are observed for the limestone and dolomitic limestone soils and are negative. The second significant but weak correlation is between soil pH and SOM and health condition: the health condition of rhododendrons is mostly affected in limestone soil (Table 25).

Table 25 Significant correlations for soil pH and SOM for *Rhododendron* plants

soil pH				SOM %
	<i>limestone</i>	<i>dolomitic</i>	<i>non-limestone</i>	<i>limestone</i>
SOM %	-0.68	-0.91	0.24	
HC	-0.35			0.27

For limestone and dolomitic limestone soils the correlation between soil pH and SOM is negative, i.e. soil pH decreases with increase in soil organic matter (LR 1). This clearly demonstrates that the pH of calcareous soils strongly depends on the presence of organic matter in it.



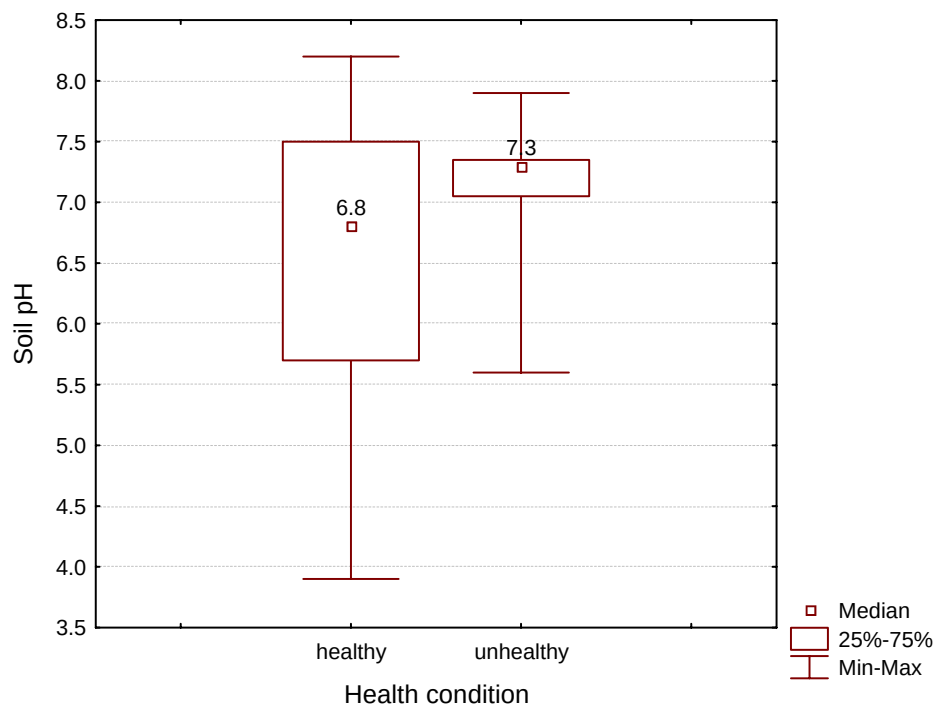
LR 1 Variation of soil pH with soil organic matter for *Rhododendron* plants on limestone soil.

From a chemical point of view, soil pH is a function of soil organic matter, and so the pH values of soils depend on the amount of organic matter in it, and are presented as the

dependent variable.

The *Rhododendron* plants studied were generally healthy over the entire range of most variables: pH, soil organic matter, calcium and manganese concentrations in leaves, and etc. As the above correlation coefficients show (Table 25) the HC in limestone soils correlates negatively with soil pH and positively with SOM, i.e. the higher the soil pH is, the more the health condition of rhododendrons declines, and the higher the SOM, the healthier the plants are. Box Plot 1 (BP 1) shows that the range of pH at which the unhealthy leaf samples were observed is narrower, between pH 5.6 – 7.9, than that for the healthy leaves. Half of the unhealthy plants were showing the symptoms at pH between 7.1 and 7.4. Clearly, the *Rhododendron* plants are developing unhealthy symptoms mostly at high pH, and there are no unhealthy leaves below pH 5.6. However, healthy plants at the higher pH, above 7.0 were also observed, which is generally accepted for *Rhododendron* as a not very common observation. Overall, although there are some plants that may suffer the consequences of high pH in the wild, there is still a large group of plants which clearly tolerate growth at high pH.

The above result fits the visual observations in the wild of *Rhododendron* plants growing healthily on soils of high pH.



BP 1 Effect of pH on the health condition of *Rhododendron* plants.

3.3. Requirements for calcium and magnesium observed for *Rhododendron* plants in the wild

3.3.1. Calcium

The significant, strong correlations for [Ca] in leaves and soils for *Rhododendron* plants are listed in Table 26. Clearly, for Ca the most consistently strong correlations over the whole range of types of soils are those observed for [Ca] (S) and soil pH and soil organic matter. Very strong positive correlations are also observed between [Ca] and [Mg] in dolomitic limestone soils:

Table 26 Significant strong correlations for [Ca] in leaves and soils for *Rhododendron* plants

[Ca] (L)				[Ca] (S)			
	<i>limestone</i>	<i>dolomitic</i>	<i>non-limestone</i>		<i>limestone</i>	<i>dolomitic</i>	<i>non-limestone</i>
Leaf age	0.47		0.34	soil pH	0.63	0.74	0.56
[Fe] (L)			0.41	SOM %	-0.64	-0.94	0.68
[Mg] (L)	0.50	0.86	0.40	[Mg] (S)		0.99	0.51
				[Fe] (S)			-0.52

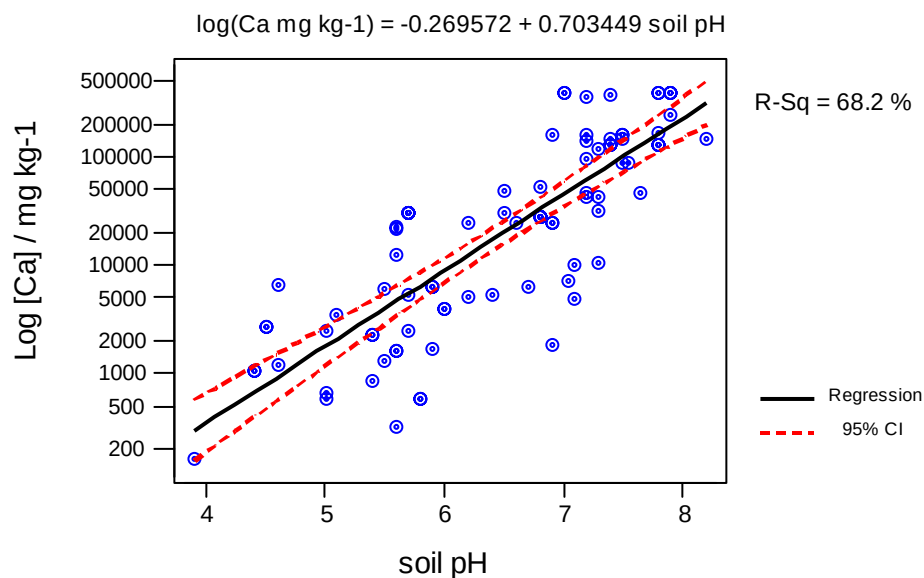
Correlation matrix 2 Correlations for [Ca] in leaves and soil for *Rhododendron* plants.

Variable	[Ca] (L)				[Ca] (S)			
	<i>all</i>	<i>limestone</i>	<i>dolomitic</i>	<i>non-limestone</i>	<i>all</i>	<i>limestone</i>	<i>dolomitic</i>	<i>non-limestone</i>
soil pH	0.20	0.05	0.26	0.02	0.71	0.63	0.74	0.56
SOM %	-0.15	-0.09	-0.33	0.09	-0.53	-0.64	-0.94	0.68
Leaf age	0.32	0.47	0.50	0.34	-0.06	0.18	-0.28	-0.15
HC	0.07	-0.04	0*	0.06	0.02	-0.35	0*	-0.03
[Ca] (L)	1.00	1.00	1.00	1.00	0.30	0.25	0.34	0.05
[Fe] (L)	0.13	0.29	-0.08	0.41	-0.03	-0.14	0.27	0.02
[Mg] (L)	0.32	0.50	0.86	0.40	-0.05	-0.04	0.45	-0.27
[Mn] (L)	0.04	0.08	0.07	0.18	-0.30	-0.30	-0.45	-0.36
[Ca] (S)	0.30	0.25	0.34	0.05	1.00	1.00	1.00	1.00
[Fe] (S)	-0.02	0.03	0.25	0.09	-0.39	-0.28	0.06	-0.52
[Mg] (S)	0.06	0.15	0.36	0.06	0.26	0.22	0.99	0.51
[Mn] (S)	0.02	0.00	0.04	0.07	-0.16	-0.26	0.10	0.23

* There is no variable "Health condition" for the samples collected on the dolomitic sites

Calcium in soil and leaves for *Rhododendron* plants

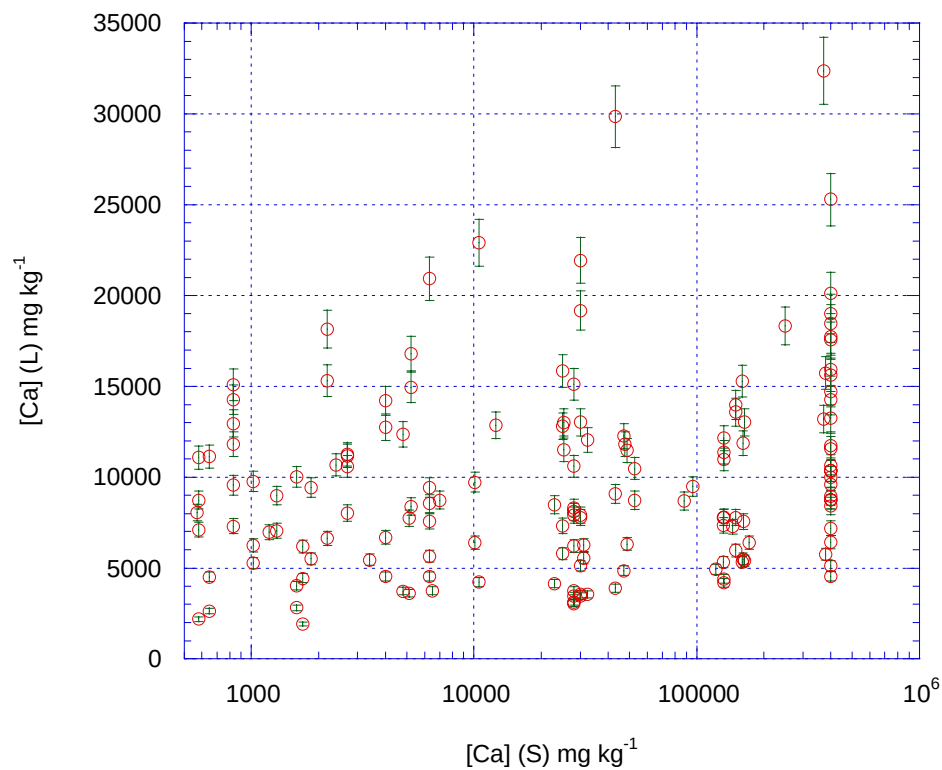
As expected, there is a strong positive correlation between calcium in the soil and soil pH for all soils (LR 2), $r_{Ca(S)/pH} = 0.71$ (CM 2).



LR 2 Variation of [Ca] (S) with soil pH for all *Rhododendron* plants.

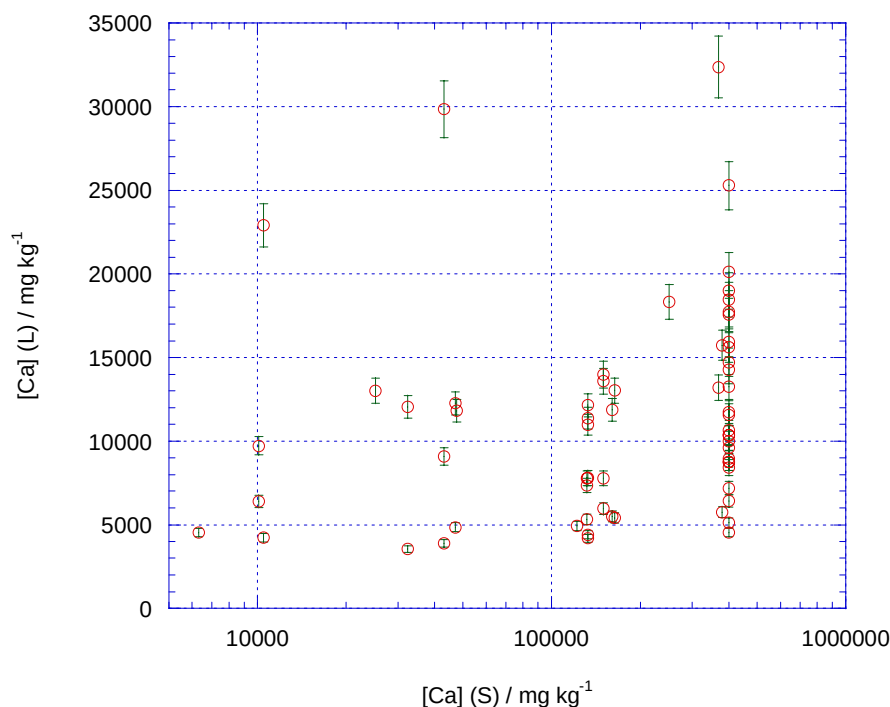
The status of calcium concentrations in the leaves, however, is different. The general correlation between [Ca] (L) and soil pH of all soils is much weaker ($r_{Ca(L)/pH} = 0.20$) than that between [Ca] (S) and soil pH. Similar correlations are observed for [Ca] (L) and pH in limestone soil ($r_{Ca(L)/pH} = 0.05$ $r_{Ca(S)/pH} = 0.63$).

Additionally, the correlation between [Ca] in soil and [Ca] in leaves (SP 1 and SP 2) shows that as [Ca] in the soil increases [Ca] in the leaves remains in consistent range of concentrations, 3000 - 15000 mg kg⁻¹ and does not change significantly.



SP 1 Variation of [Ca] (L) with [Ca] (S) for all *Rhododendron* plants.

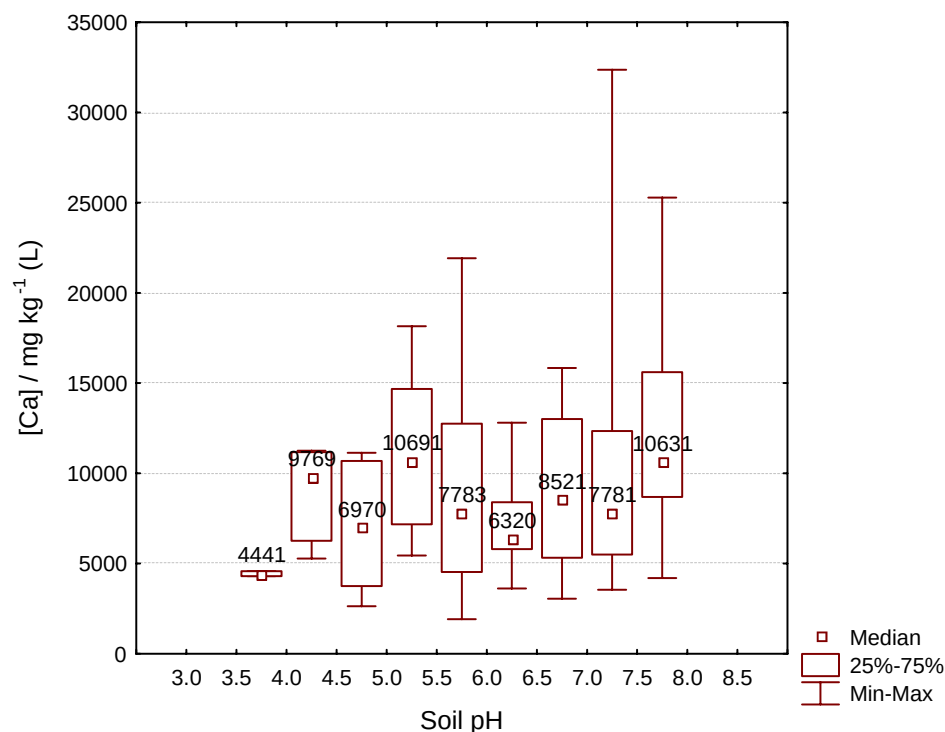
Note: Maximum possible [Ca] in the soil is 400 000 mg kg⁻¹



SP 2 Variation of [Ca] (L) with [Ca] (S) for *Rhododendron* plants growing on limestone.

Following the results in LR 2 and SP 1 and 2, it is clear that regardless of how high the soil pH or the Ca concentration in the soil is, the concentration of calcium in the leaves correlate only weakly with both of these two factors ($r_{\text{Ca(L)}/\text{Ca(S)}} = 0.30$, $r_{\text{Ca(L)}/\text{pH}} = 0.20$). This result shows that *Rhododendron* plants have developed an ability to regulate very well the concentrations of calcium they take up from the soil, and especially from high-pH calcareous soils. In this manner, even when growing on extreme, calcareous soils, rhododendrons take up concentrations of calcium from the soil within certain limits and not indefinitely, and this is also shown by the weak correlation between [Ca] (L) and [Ca] (S) for rhododendrons in limestone soils, $r_{\text{Ca(L)}/\text{Ca(S)}} = 0.25$.

The controlled Ca concentrations in the leaves are clearly manifested above pH 6.0 (BP 2) as its variation from low to high pH shows. The ranges of the middle 50 % distribution of in Ca the leaves (the boxes in the box plot) are very similar over the whole pH range, between 3000 and 15000 mg kg⁻¹, and there is no significant increase as the pH increases.



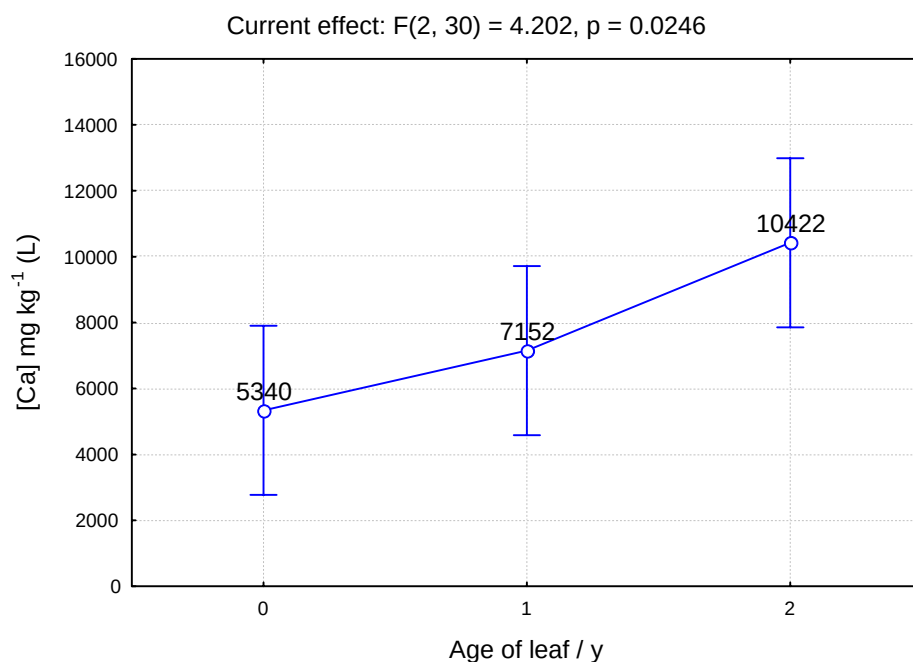
BP 2 Variation of [Ca] (L) with soil pH for all *Rhododendron* plants.

As a function of soil organic matter, in limestone soil the concentration of Ca (S) decreases with increasing the organic matter, as expected. The general trend in soils is a decrease in soil Ca with increasing of soil organic matter, as organic matter contains low concentrations of this nutrient.

Calcium variation in leaves with time

The correlation between Leaf age and [Ca] (L) for plants having representatives of all three years of age: current-year-old, one-year-old, and two-years-old, is $r_{Ca(L)/AGE(0, 1, 2)} = 0.67$ (this correlation coefficient is calculated including all leaves: those having just current-year-old leaves, and those having current-year-old and one-year-old, or more, leaves. Therefore r in the correlation matrix is different than R in the ANOVA summary shown in Table 27). Calcium in *Rhododendron* leaves of consecutive years of age show

a gradual increase with time over the whole range of soil pH (3.9 – 8.2). This is an indication that there is well-controlled accumulation of calcium in leaves older than the current year growth (ANOVA 1, Table 27).



ANOVA 1 Variation of [Ca] (L) in *Rhododendron* leaves with age of leaves.

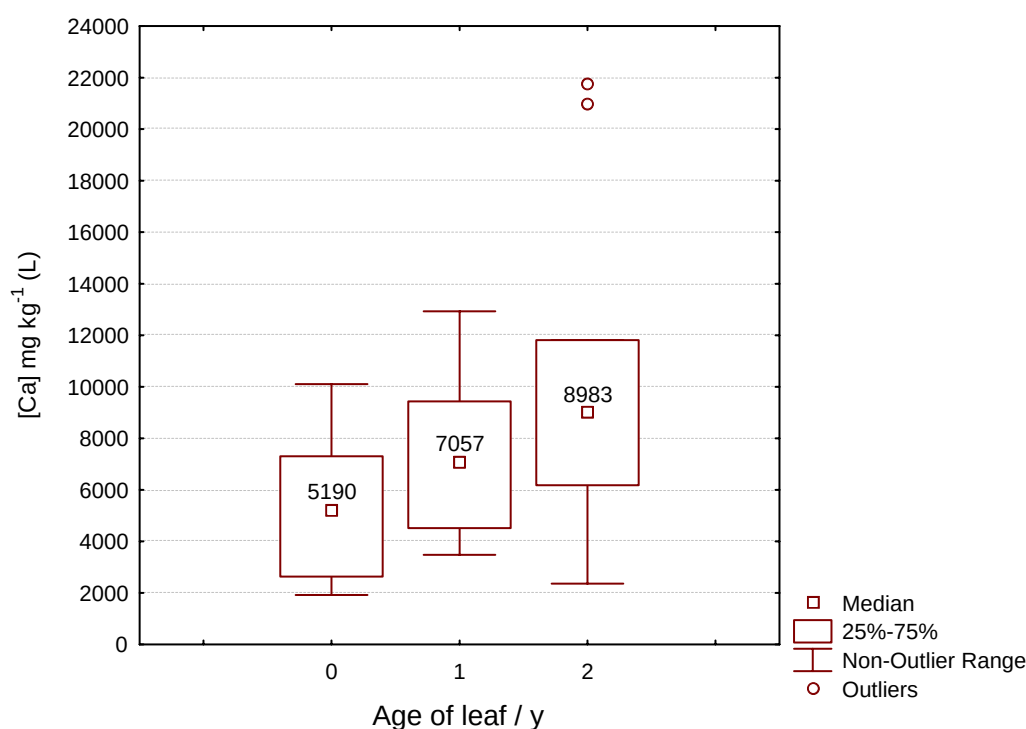
Table 27 ANOVA summary for [Ca] in *Rhododendron* leaves of age 0, 1 and 2 years old.

Effect	N	[Ca] (L)				
		$R = 0.4677$		$R^2 = 0.2188$		$p = 0.0246$
		Mean	SD	SE	CI, -95%	CI, +95%
Total	33	7638	4565	794	6019	9257
0 (Age of leaf / y)	11	5339	2817	850	3447	7232
1 (Age of leaf / y)	11	7152	2885	870	5214	9090
2 (Age of leaf / y)	11	1042	5987	1805	6400	14444

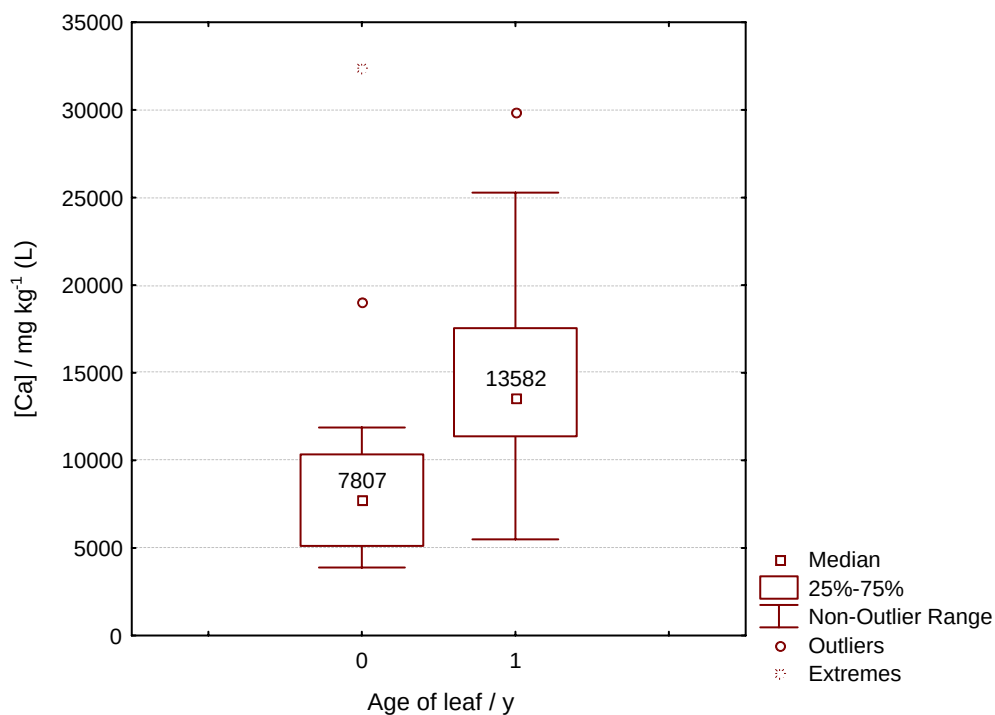
The concentrations of Ca (L) in one-year-old leaves of plants growing on limestone soil

is greater by 43 % ($r_{Ca(L)/AGE} = 0.47$, BP 4) compared to that in one-year-old leaves of the plants growing on non-limestone soil (23 %, $r_{Ca(L)/AGE} = 0.34$, BP 3). However, as leaf age increases the maximum limits become lower, demonstrating that the take-up of Ca from the soil by *Rhododendron* plants tends to certain limits, above which it is not further absorbed. Part of the absorbed Ca is utilised in the physiological functions, and the excess is deposited in the older leaves.

Once again controlled uptake of Ca is demonstrated, which was already suggested earlier by the medium correlation between [Ca] (S) and [Ca] (L) ($r_{Ca(L)/Ca(S)} = 0.30$).



BP 3 Distribution of [Ca] in current-year, one-year-old and two-years-old leaves for *Rhododendron* plants on non-limestone soil.

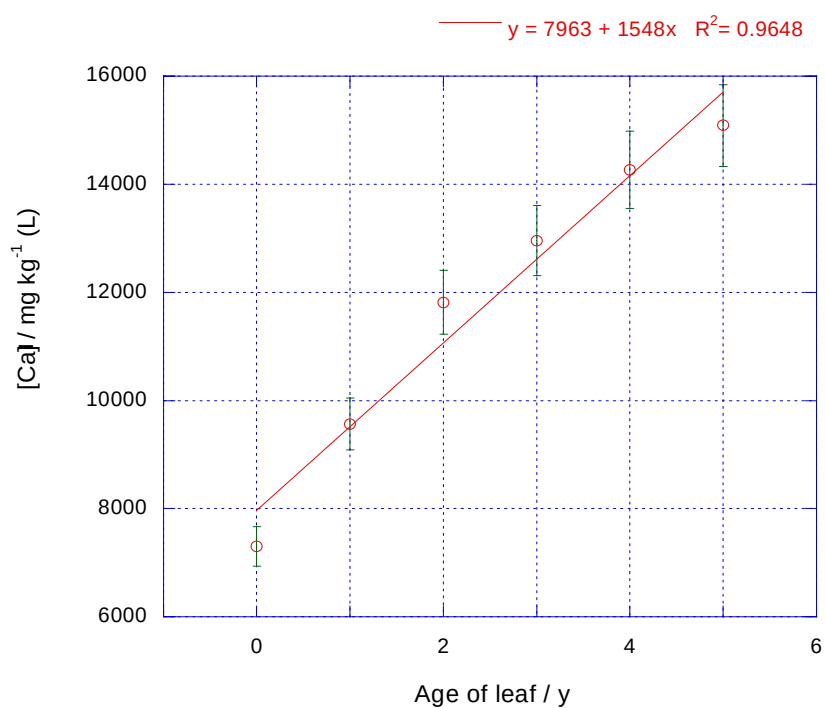


BP 4 Distribution of [Ca] in current-year and one-year-old leaves for *Rhododendron* plants on limestone soil.

Overall, there is an indication that calcium in the older leaves is present in concentrations greater than those in the young leaves. One explanation to this can be that most of the absorbed Ca is transported in the young leaves while decreasing concentrations of it continue to reach the leaves as they become older. This is particularly expressed in the [Ca] in the leaves of *R. maximum*, which is the only plant from which leaves of six consecutive years were collected (Table 28, SP 3).

Table 28 Increase in [Ca] (L) with leaf age for *R. maximum*

Age of leaf	[Ca] / mg kg ⁻¹
0	10000
1	12000
2	14500
3	15500
4	17000
5	17400

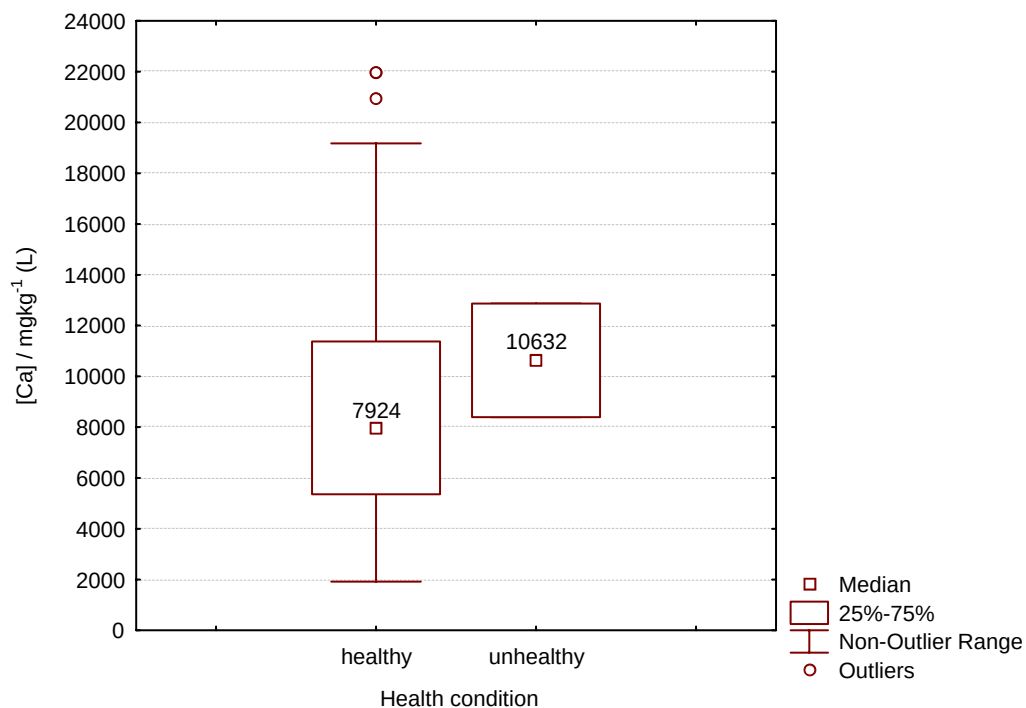


SP 3 Variation of [Ca] (L) in *R. maximum* leaves with age of leaves.

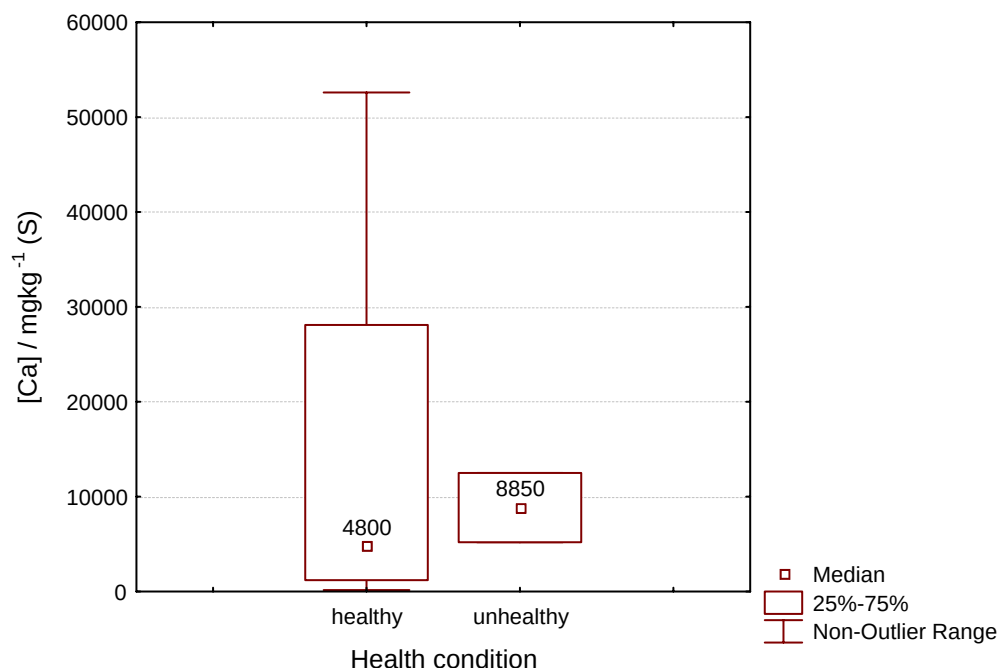
Calcium and Rhododendron health

With respect to the health condition, box plots 5 and 6 show that Ca concentrations in leaves and soils for both healthy and unhealthy leaves for rhododendrons on non-limestone soils are in close ranges. This indicates that healthy and unhealthy plants may

both be found growing within the same ranges of [Ca] in their leaves and in the soil (BP 5 and 6).

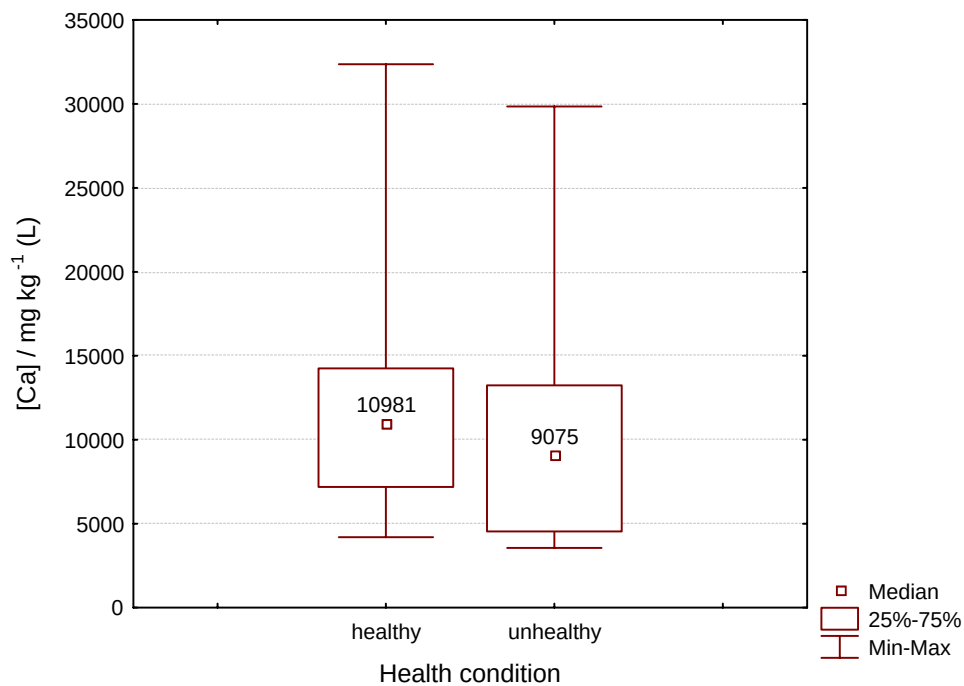


BP 5 Range of [Ca] in healthy and unhealthy leaves for *Rhododendron* plants on non-limestone soil.

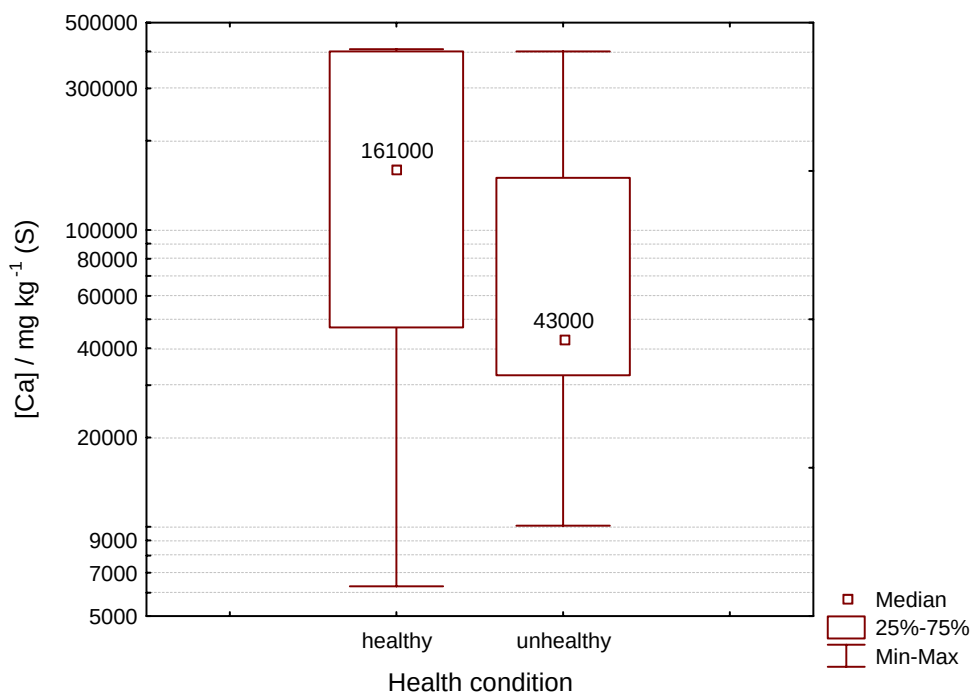


BP 6 Range of [Ca] (S) in the soils of healthy and unhealthy leaves for *Rhododendron* plants on non-limestone soil.

The observations of [Ca] on the health condition of rhododendrons growing on limestone soils is the same as that observed for plants on non-limestone soil: the ranges for both, healthy and unhealthy, overlap ($r_{Ca(L)/HC} = -0.04$, on limestone) (BP 7). Regarding the effect of [Ca] (S) in the limestone soil on health, healthy plants are evidently present at even higher Ca concentrations in the soil than the unhealthy plants (BP 8). This clearly invalidates the long-standing dispute that in limestone soils the very high Ca concentrations inhibited *Rhododendron* growth and that it was calcium concentrations that accounted for the difficulties encountered in cultivation of rhododendrons in limestone-derived soil. This is another indication that the health condition of rhododendrons in all environments is not affected either by high concentrations of calcium in the leaves or in the soil. This conclusion is confirmed by the correlation coefficient for the general [Ca] (L)-Health condition relationship, $r_{Ca(L)/HC} = 0.07$.



BP 7 Range of [Ca] (L) in healthy and unhealthy leaves for *Rhododendron* plants growing on limestone soil.



BP 8 Range of [Ca] (S) in the soils for healthy and unhealthy leaves for *Rhododendron* plants growing on limestone soil.

3.3.2. Magnesium

The significant, strong correlations for [Mg] in leaves and soils for *Rhododendron* plants are listed in Table 29. The significant correlations are mostly observed for dolomitic limestone soil: there is a strong positive correlation of [Mg] with [Ca], [Mg] (S) and soil pH, and strong negative correlation between [Mg] (S) and SOM. Additionally, in limestone soils there are significant medium correlations between [Mg] (L) and [Ca] (L) and [Fe] (L).

Table 29 Significant strong correlations for [Mg] in leaves and soils for *Rhododendron* plants

[Mg] (L)				[Mg] (S)		
	<i>limestone</i>	<i>dolomitic</i>	<i>non-limestone</i>		<i>dolomitic</i>	<i>non-limestone</i>
[Ca] (L)	0.50	0.86	0.40	soil pH	0.68	
[Fe] (L)	0.46			SOM %	-0.92	
				[Ca](S)	0.99	0.51

Correlation matrix 3 Correlations for [Mg] in leaves and soil for *Rhododendron* plants.

Variable	[Mg] (L)				[Mg] (S)			
	<i>all</i>	<i>limestone</i>	<i>dolomitic</i>	<i>non-limestone</i>	<i>all</i>	<i>limestone</i>	<i>dolomitic</i>	<i>non-limestone</i>
soil pH	-0.02	-0.19	0.14	-0.02	0.30	0.34	0.68	0.26
SOM %	0.04	0.11	-0.34	-0.39	0.03	-0.08	-0.92	0.12
Leaf age	0.11	0.04	0.41	0.23	-0.13	-0.08	-0.25	-0.06
HC	-0.06	-0.01	0*	0.03	-0.08	-0.21	0*	-0.03
[Ca] (L)	0.32	0.50	0.86	0.40	0.06	0.15	0.36	0.06
[Fe] (L)	0.31	0.46	-0.19	0.37	0.41	0.36	0.31	0.14
[Mg] (L)	1.00	1.00	1.00	1.00	0.52	0.37	0.49	0.00
[Mn] (L)	0.12	0.31	-0.09	0.27	-0.17	-0.10	-0.40	-0.14
[Ca] (S)	-0.05	-0.04	0.45	-0.27	0.26	0.22	0.99	0.51
[Fe] (S)	-0.00	0.04	-0.06	0.17	-0.26	-0.10	0.03	-0.19
[Mg] (S)	0.52	0.37	0.49	0.00	1.00	1.00	1.00	1.00
[Mn] (S)	-0.00	-0.03	-0.27	0.05	-0.04	-0.07	0.06	0.14

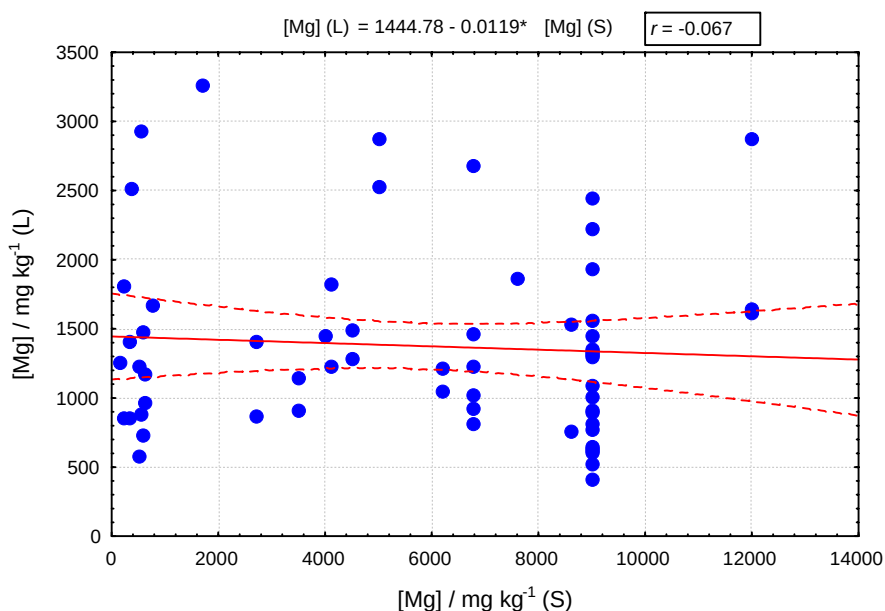
* There is no variable "Health condition" for the samples collected on the dolomitic sites.

Magnesium in soil and leaves for Rhododendron plants

The situation with magnesium is different from that with calcium. Compared to [Ca], [Mg] in the soil correlates significantly with soil pH ($r_{\text{Mg(S)/pH}} = 0.68$) and SOM ($r_{\text{Mg(S)/pH}} = -0.92$) only in dolomitic limestone soils, where the [Mg] in the soil are greater than in any other type of soil.

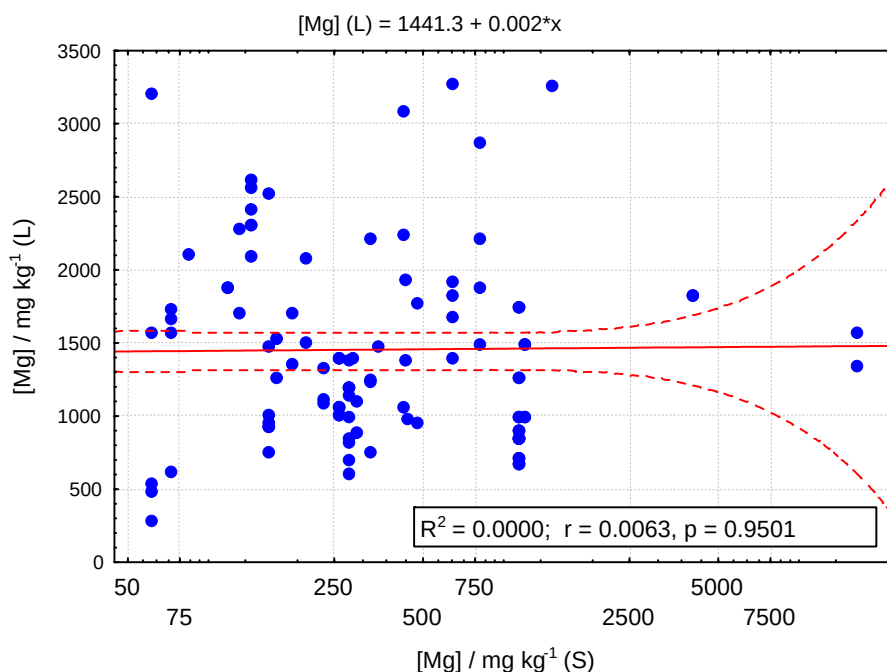
The concentrations of Mg in leaves of rhododendrons in all types of soils correlate significantly only with [Ca] (L), and in limestone soils with [Fe] (L), as all these are positive correlations (Table 29). This means that increased [Ca] in the leaves of rhododendrons is related to increased [Mg] in the leaves, and not to a shortage of Mg. This clearly disproves the postulate that in limestone soils the high concentrations of Ca in the soil reduce the magnesium uptake by the plants.

For limestone soils, although the correlation coefficient in CM 3 is medium ($r_{\text{Mg(L)/Mg(S)}} = 0.37$), after excluding the outliers from the analysis, shown on SP 4, the correlation coefficient becomes very low ($r_{\text{Mg(L)/Mg(S)}} = -0.067$), which clearly shows that [Mg] in leaves of rhododendrons in limestone soils do not depend on the [Mg] in the soil (SP 4).



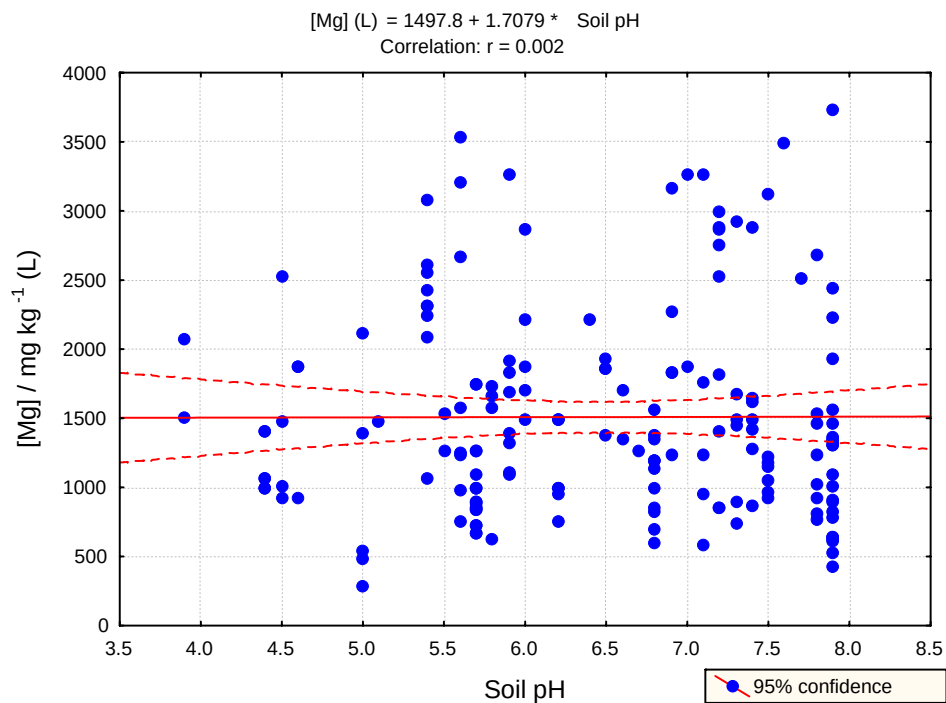
SP 4 Variation of [Mg] (L) with [Mg] (S) for *Rhododendron* plants on limestone soil.

Furthermore, it is also evident from the results that the concentrations of Mg in the leaves of rhododendrons growing on limestone soil are within the same range as those observed in the leaves of rhododendrons growing on non-limestone soil, 500 -3500 mg kg⁻¹ (SP 5), and in dolomitic limestone soil (1800 – 3500 mg kg⁻¹). Clearly, in all types of soil rhododendrons ensure the same concentrations of Mg required for their healthy growth, i.e. there is no shortage of Mg in the leaves of rhododendrons growing in limestone soil.

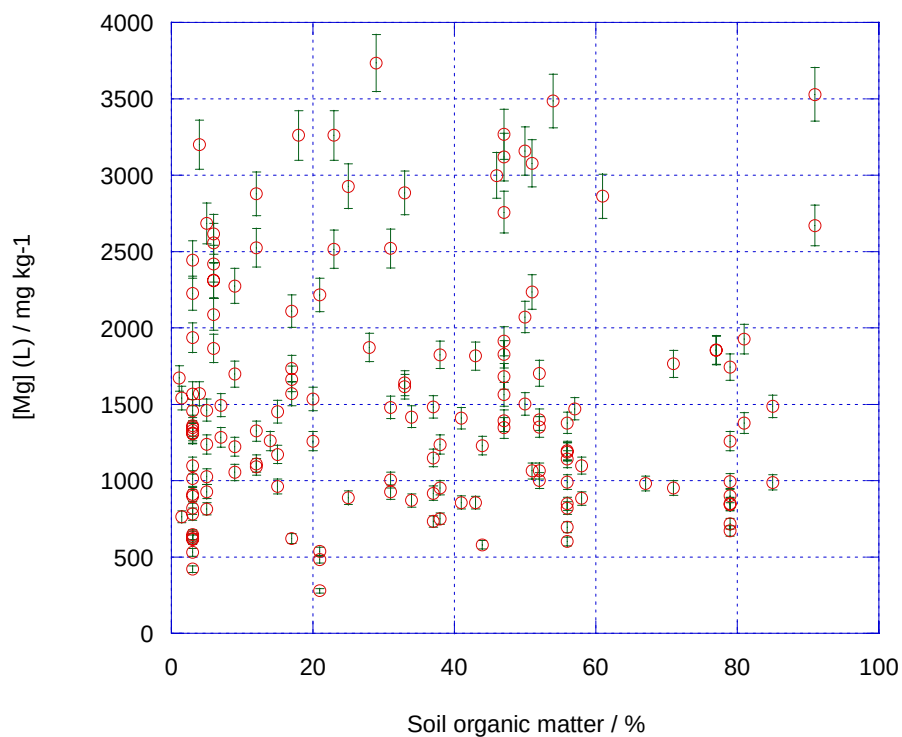


SP 5 Variation of [Mg] (L) with [Mg] (S) for *Rhododendron* plants on non-limestone soil.

Further reviewing of the weaker and insignificant correlation coefficients, shown in CM 3, shows that [Mg] in leaves of rhododendrons in all soils do not correlate with soil pH and SOM as no correlation is observed ($r_{\text{Mg (L)/pH}} = -0.02$, $r_{\text{Mg (L)/SOM}} = 0.04$) (SP 6 and 7). Both graphs show that Mg in the leaves of *Rhododendron* plants at all pH and SOM content is present in a narrow range of concentrations. With increasing soil pH the average concentrations of Mg in the leaves remain within the same close range.



SP 6 Variation of $[Mg] (L)$ with soil pH for all *Rhododendron* plants.



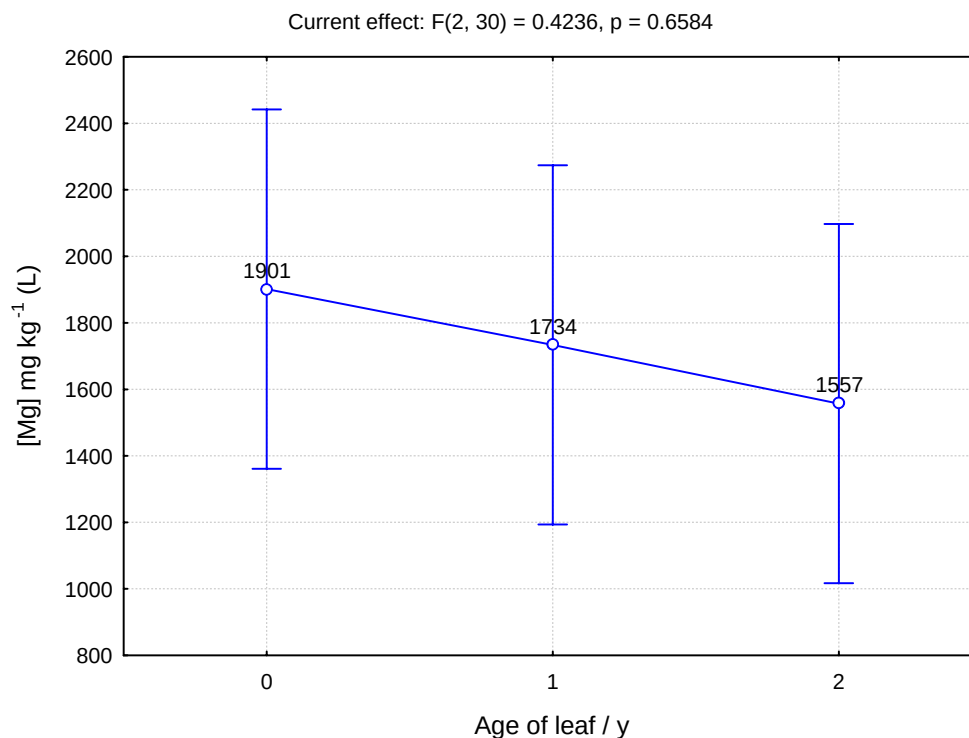
SP 7 Variation of $[Mg] (L)$ with soil organic matter for all *Rhododendron* plants.

A conclusion that can be drawn is that regardless of the type of soil (dolomitic or non-dolomitic), and how high the soil pH or the concentration of Mg in the soil is, Mg in the leaves is present within a consistent and narrow range of concentrations, on average between 1200 and 3500 mg kg⁻¹. In contrast, calcium in the leaves for rhododendrons growing on all types of soil, also being present within certain limits, is in much wider range, 2000 – 23 000 mg kg⁻¹.

Overall, as Mg and Ca concentrations in soil increase, both Mg and Ca in leaves also increase, but Mg increases to a lower maximum limit compared to Ca, which is, Ca (L)/Ca (S) = 0.5, whereas Mg (L)/Mg (S) = 0.3 (CM 3).

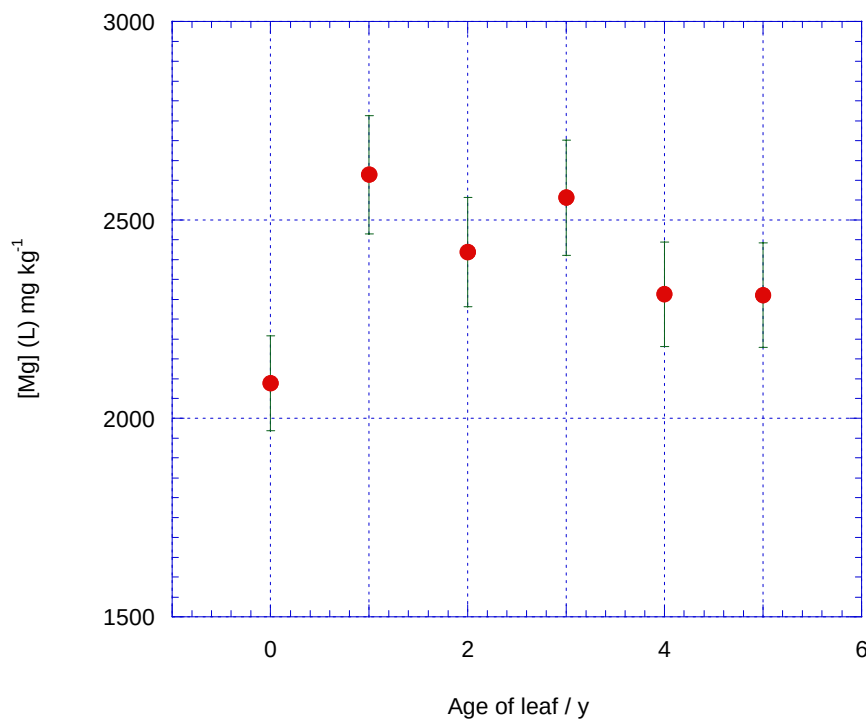
Magnesium variation in leaves with time

The variation of magnesium with the age of leaves shows that there is no increase of [Mg] in leaves with leaf age. The correlation of Mg in current-year-old and one-year-old leaves with the leaf age is very weak positive, $r_{\text{Mg(L)/AGE (0, 1)}} = 0.13$, and that for current-year-old, one-year-old and two-year-old leaves with leaf age is even negative, $r_{\text{Mg(L)/AGE (0, 1, 2)}} = -0.17$. As a result, the overall correlation is weak positive, $r_{\text{Mg(L)/AGE (all)}} = 0.11$. The variation of [Mg] in current-year-old, one-year-old and two-year-old leaves, although showing a decline over time within a close range of concentrations, is statistically insignificant correlation ($p = 0.6584$, see ANOVA summary in Appendix 2) (ANOVA 2).



ANOVA 2 Variation of [Mg] in *Rhododendron* leaves with age of leaves.

Furthermore, in many cases [Mg] (L) was lower in the older leaves than in the current-year leaves. Similar trend is also well demonstrated in the case of *R. maximum* where [Mg] in the leaves of six consecutive years decreases with leaf age (SP 8). This is consistent with Mg being mobilised from the old leaves into the young leaves if it becomes in excess in the old leaves.

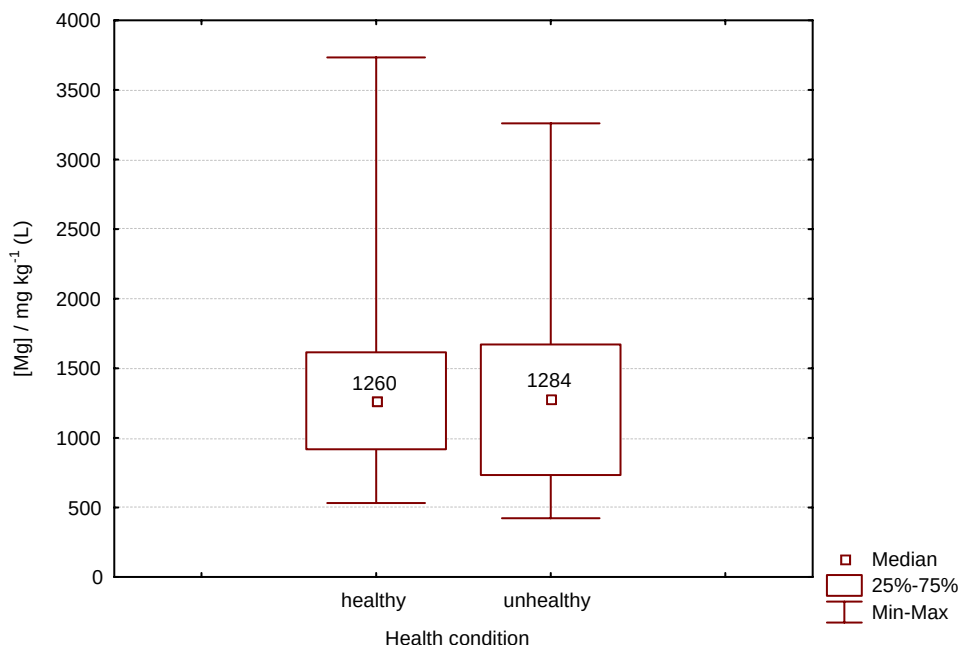


SP 8 Variation of [Mg] (L) in *R. maximum* leaves with age of leaves.

Evidently, after it is taken up from the soil Mg is not present in excess in the leaves, and therefore there is no accumulation in the older leaves. This demonstrates that Mg uptake is controlled. This may either be by limiting absorption from the soil (which may be by simply controlling the rate of absorption so that it cannot be in excess or less likely by stopping uptake once the leaves have sufficient concentrations of Mg), or by mobilisation of excess Mg from leaves. The latter mechanism would readily account for the fact that Mg concentrations do not increase greatly after the first year of leaf growth.

Magnesium and Rhododendron health

For *Rhododendron* growing on limestone soil the health condition of the plants does not correlate with [Mg] (L), and it correlates insignificantly with [Mg] (S), and in this respect it is like calcium ($r_{\text{Mg(L)}/\text{HC}} = -0.01$) (BP 9).



BP 9 Range of [Mg] in healthy and unhealthy leaves for *Rhododendron* plants on limestone soil.

3.4. Relationship between Ca and Mg in soil and leaves. Effect of Mg/Ca ratio in limestone and dolomitic limestone soils

As already mentioned earlier for Ca, in dolomitic limestone soil, as expected, there is a strong positive correlation of [Mg] with [Ca]. In non-limestone soils medium correlation between the latter two variables is observed.

The mean ratio Mg/Ca in soils varies significantly between limestone soil, Mg/Ca = 21/1, and dolomitic limestone soil, Mg/Ca = 1.8/1. The mean Mg/Ca ratios in the leaves are 8/1 for plants growing on limestone and 2.4/1 on dolomitic limestone soil.

Considering the discussion of the concentrations of Mg and Ca in soils earlier in this section, there are two clear points that need to be made.

First, in limestone soil Mg concentrations in leaves are very similar to those in non-limestone soils, i.e. concentrations of Mg in the leaves do not change or decrease with the increase of Ca in the soil ($r_{\text{Mg(L)}/\text{Ca(S)}} = -0.04$). Additionally, in limestone soils Mg in

the leaves correlates strongly with Ca in the leaves ($r_{\text{Mg(L)}/\text{Ca(L)}} = 0.50$) (CM 3), indicating that in the leaves Mg not only does not decrease but it actually increases with increasing Ca. This, in addition with the absence of correlation between [Mg] (L) and [Ca] (S) indicates that Mg uptake does not become restricted by an increased Ca concentrations in the leaves and higher Ca concentration in the soil, therefore, the idea that calcium and magnesium compete, resulting in magnesium shortage, is clearly incorrect.

The second point is in regard to the past postulates that the limestone soils in which rhododendrons were found to grow healthily was dolomitic limestone, and the high concentrations of Mg (S) in it counteracted the uptake of high concentrations of Ca by rhododendrons. Therefore, as a result of the reduced concentrations of Ca in their leaves the rhododendrons could survive in such soil. However, in agreement with past experiments^{39, 40}, in the current research the soil samples from the limestone areas that showed maximum concentrations of Ca, suggesting that the limestone rock in those areas is pure CaCO_3 , had a mean of 0.8 % Mg in the total soil. This is below the concentrations expected for dolomitic limestone and it indicates that the soil is not derived from dolomitic limestone rock. Additionally, the average concentrations of Ca in the leaves of rhododendrons growing in limestone and dolomitic limestone are very similar (ratio Ca (L) limestone / Ca (L) dolomitic limestone = 1.1/1).

Evidently, even if the limestone rock is a dolomitic limestone the absorption of Ca by rhododendrons is not inhibited by higher concentration of Mg and they absorb Ca at the same rates as in limestone soil. *Rhododendron* plants in dolomitic limestone soil take up similar concentrations of Ca as they do in limestone soil. Or in other words, [Ca] (L) in rhododendrons in limestone soil are not greater, thus do not cause a supposed “poisoning of rhododendrons with Ca in the limestone soil”, which in contrast “would not occur in the dolomitic limestone”. Additionally, as pointed out earlier, in dolomitic limestone soil [Mg] in the leaves of rhododendrons are also not elevated compared to [Mg] (L) in limestone soils, and are within the same range of concentrations. Hence, increased take up of Mg in dolomitic limestone soils which inhibits the take up of “toxic” Ca, also does

not occur. Regardless of how high the [Mg] (S) in the soils are, the plants absorb the same concentrations of Mg in their leaves.

Furthermore, as previously discussed *Rhododendron* plants are very tolerant to excessive concentrations of Ca in their leaves, so with or without elevated concentrations of Mg in the soil, the high Ca concentration in limestone soil does not harm the rhododendron plants growing in it.

Additional column plots showing the variation of Mg/Ca ratio between *Rhododendron* species can be seen in the appendix 3 (CP 1a, 1b and 1c).

3.5. Requirements for iron observed for *Rhododendron* plants in the wild

Results for iron requirements of *Rhododendron* plants in the wild show that [Fe] (S) correlates significantly with [Ca] (S) and SOM in non-limestone soil, and with [Mn] (S) in limestone and dolomitic limestone soils. In the leaves of rhododendrons [Fe] (L) correlates significantly with [Ca] (L) in non-limestone soil, and with [Mg] (L) in limestone soils (Table 30).

Table 30 Significant strong correlations for [Fe] in leaves and soils for *Rhododendron* plants

[Fe] (L)			[Fe] (S)			
	<i>limestone</i>	<i>non-limestone</i>		<i>limestone</i>	<i>dolomitic</i>	<i>non-limestone</i>
[Ca] (L)		0.41	SOM %			-0.40
[Mg] (L)	0.46		[Ca] (S)			-0.52
			[Mn] (S)	0.47	0.65	

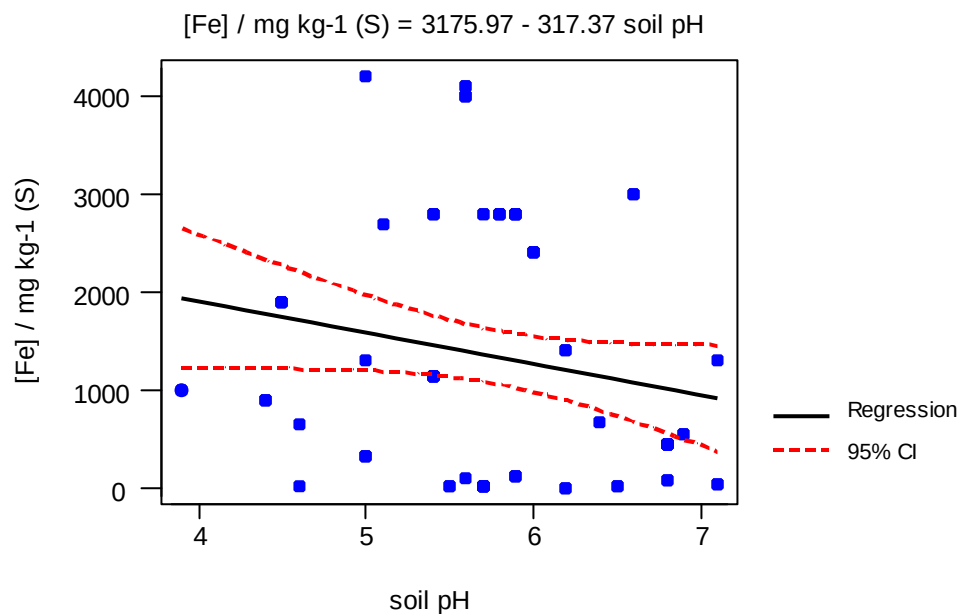
Correlation matrix 4 Correlations for [Fe] in leaves and soil for *Rhododendron* plants.

Variable	[Fe] (L)				[Fe] (S)			
	<i>all</i>	<i>limestone</i>	<i>dolomitic</i>	<i>non-limestone</i>	<i>all</i>	<i>limestone</i>	<i>dolomitic</i>	<i>non-limestone</i>
soil pH	0.02	-0.20	0.15	0.04	-0.48	-0.10	0.40	-0.25
SOM %	0.13	0.25	-0.24	0.02	-0.07	0.18	-0.19	-0.40
Leaf age	-0.01	0.15	-0.25	0.07	0.08	-0.11	-0.29	0.02
HC	-0.02	0.13	0	-0.02	-0.13	0.10	0	-0.12
[Ca] (L)	0.13	0.29	-0.08	0.41	-0.02	0.03	0.25	0.09
[Fe] (L)	1.00	1.00	1.00	1.00	0.03	0.05	-0.01	0.21
[Mg] (L)	0.31	0.46	-0.19	0.37	-0.00	0.04	-0.06	0.17
[Mn] (L)	0.02	0.25	-0.08	0.09	0.37	0.08	-0.10	0.25
[Ca] (S)	-0.03	-0.14	0.27	0.02	-0.39	-0.28	0.06	-0.52
[Fe] (S)	0.03	0.05	-0.01	0.21	1.00	1.00	1.00	1.00
[Mg] (S)	0.41	0.36	0.31	0.14	-0.26	-0.10	0.03	-0.19
[Mn] (S)	0.01	0.09	-0.02	-0.00	0.14	0.47	0.65	0.02

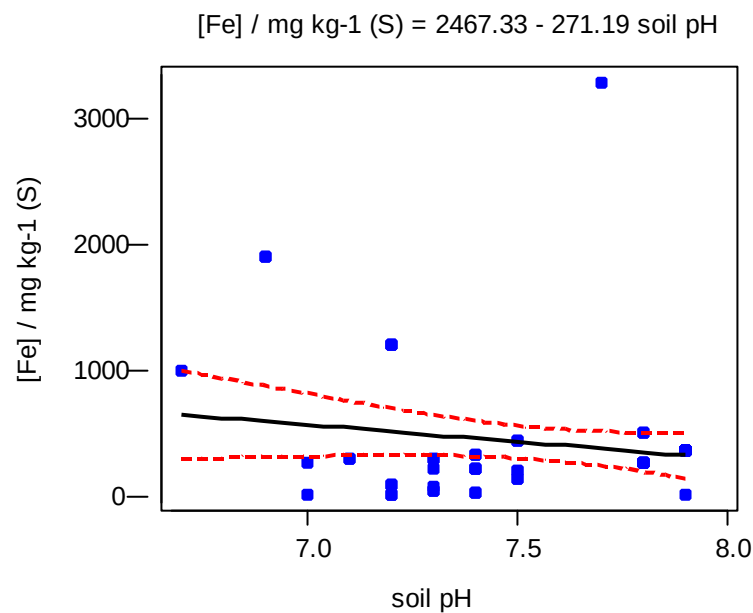
Iron in soil and leaves for Rhododendron plants

Correlations of [Fe] (S) with soil pH in all three types of soils, limestone, dolomitic limestone and non-limestone, are insignificant. However, from CM 4, the overall correlation of [Fe] (S) with soil pH for all analysed rhododendrons in all soils is significant, and is in agreement with the usually observed trend: soil iron decreases with increasing soil pH ($r_{\text{Fe(S)/pH}} = -0.48$).

Furthermore, it is also evident that the concentrations of Fe in limestone soil are lower, in the range 45 – 1900 mg kg⁻¹ (the maximum and minimum excluded), than those in non-limestone soil, 110 – 4200 mg kg⁻¹ (LR 3 and 4).

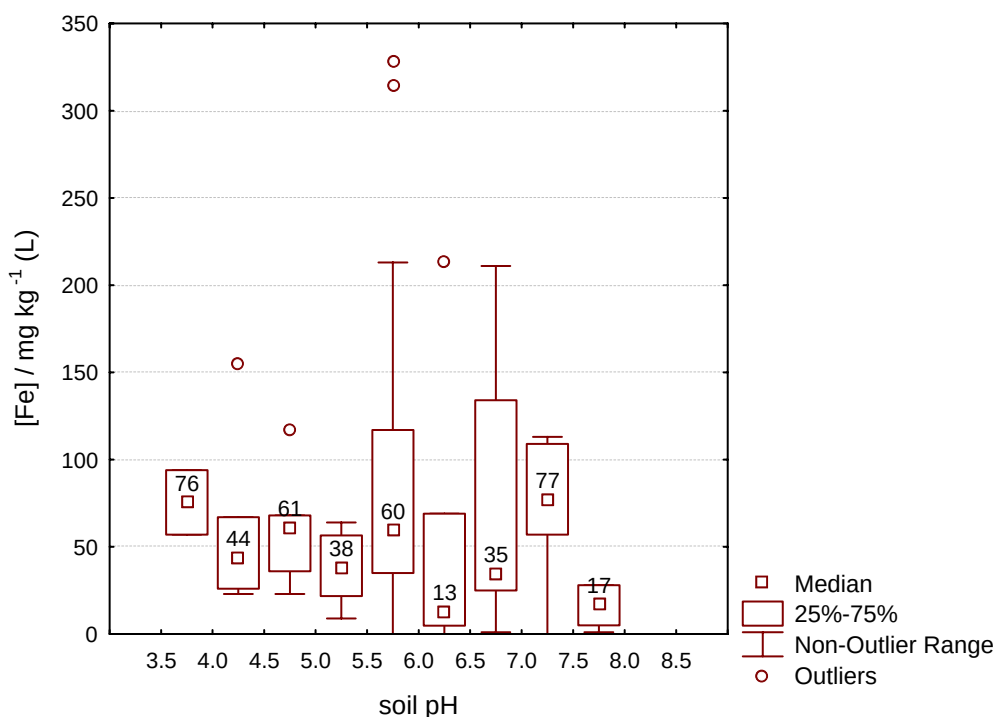


LR 3 Variation of $[\text{Fe}] (\text{S})$ in the soil with soil pH for *Rhododendron* plants on non-limestone soil.



LR 4 Variation of $[\text{Fe}] (\text{S})$ in the soil with soil pH for *Rhododendron* plants on limestone soil.

Although, the correlation between Fe (S) and soil pH is negative, as expected, correlation between Fe (L) and soil pH is absent, $r_{\text{Fe(L)/pH}} = 0.04$, meaning that in non-limestone soils [Fe] in the leaves is not affected by an increase in soil pH. For *Rhododendron* plants growing on non-limestone soils the average concentration of Fe in the leaves is steady, within the range of 20 – 120 mg kg⁻¹ over the whole pH range (BP 10).

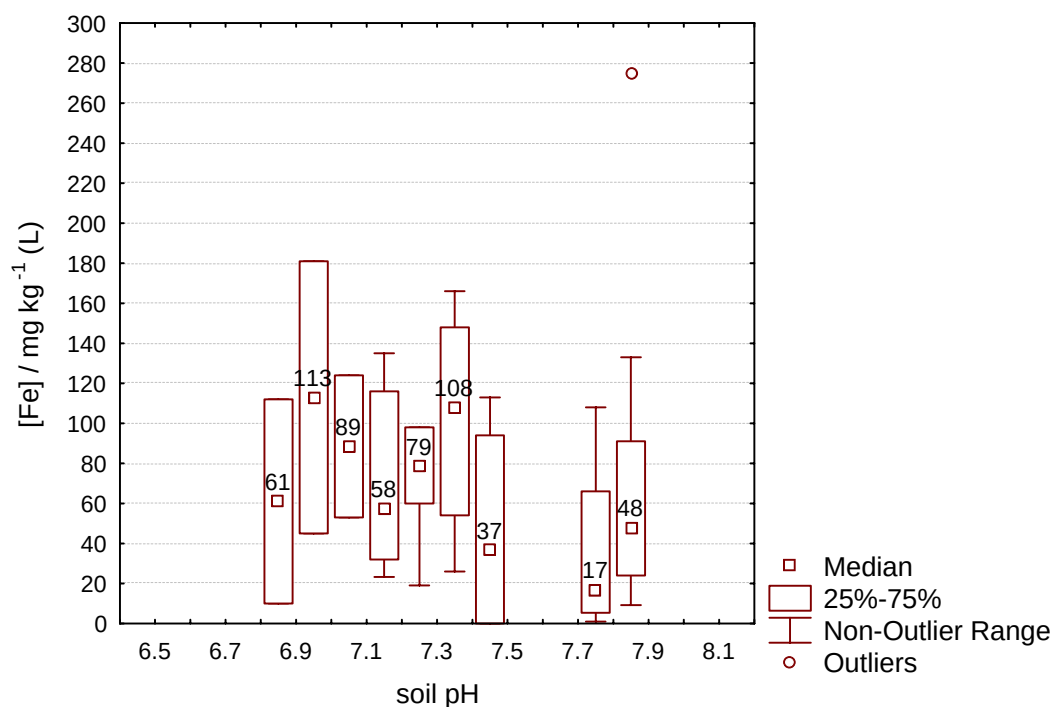


BP 10 Variation of [Fe] in leaves with soil pH for *Rhododendron* plants on non-limestone soil.

In some leaves at pH between 6.0 and 6.8, concentrations of Fe as low as 3 mg kg⁻¹ are found, demonstrating that low concentrations of Fe may generally also be present in leaves of rhododendrons growing in non-limestone, generally low-pH soils. Some leaves have extremely low Fe concentrations, close to 1 mg kg⁻¹, and except for one *R. decorum* which had [Fe] in its leaves between 1 and 17 mg kg⁻¹, they were all healthy. Clearly, such low [Fe] are more prevalent among the plants growing at pH above 7.0.

Despite that, the top limits of [Fe] (L) in those leaves are at concentrations as high as the top concentrations found in leaves at the lower pH, showing that although Fe in the leaves generally decreases with pH, at high pH there are still many plants that contain more than enough Fe in their leaves.

In limestone soil, the correlation between [Fe] (L) and soil pH is again weak and insignificant ($r_{\text{Fe(L)}/\text{pH}} = -0.20$), indicating that for rhododendrons growing on the studied limestone soil sites [Fe] in the leaves do not show dependence on the soil pH. The same is observed in dolomitic limestone soils. In support of this observation, the concentrations of Fe between pH 6.5 and 7.9 in the majority of the leaves appear to be present between 20 and 100 mg kg^{-1} , and a few leaf samples, at pH 6.8 and 7.8 only, have Fe in their leaves below 5 mg kg^{-1} (BP 11).



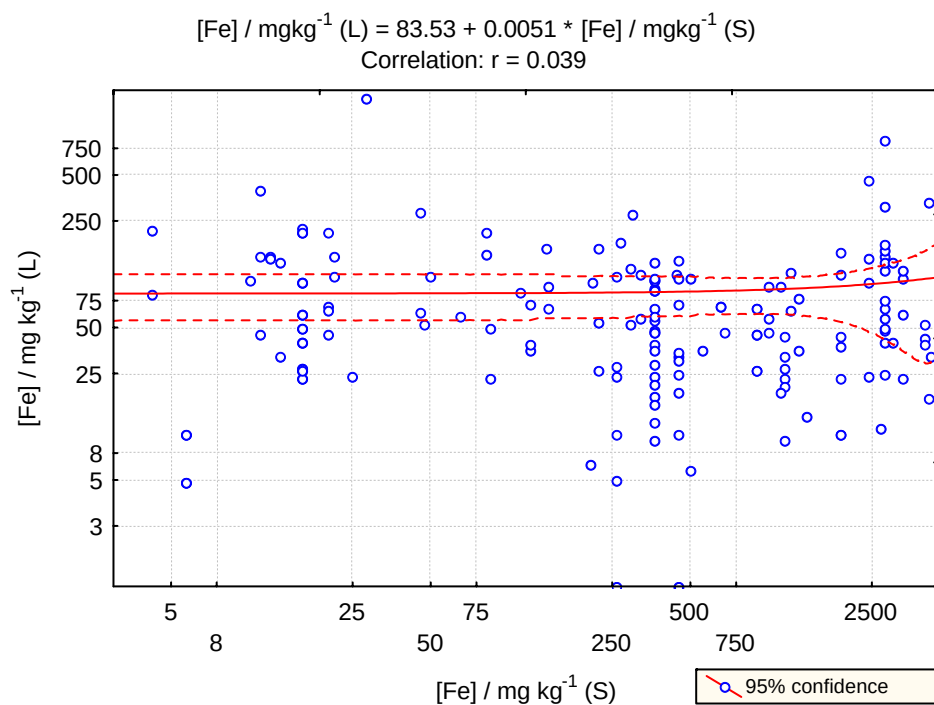
BP 11

Variation of [Fe] in leaves with soil pH for *Rhododendron* plants growing on limestone soils.

It appears that in limestone the overall concentrations of Fe in leaves cover the same range of concentrations as in non-limestone soil. Clearly, above pH 7.5 there are leaves with generally lower concentration of Fe, i.e. in more leaves the concentrations fall below 20 mg kg⁻¹. This drop appears only at pH 7.7 – 7.8, while the top limit is still around 60 mg kg⁻¹. However, at pH 7.8 – 7.9, most leaves sampled have Fe concentration between 25 and 85 mg kg⁻¹, which is within the range generally required for a healthy growth of plants.

Evidently, there are large variations in the concentration of Fe in *Rhododendron* leaves above pH 6.0, in both limestone and non-limestone soils. It is also important to note that compared to the total number of analysed leaves, those with extremely low concentrations of iron, below 10 mg kg⁻¹, are very few: 13 leaf samples for plants growing on non-limestone soils and 14 on limestone soils. Such an arbitrary pattern of the variation of [Fe] in leaves is also observed for the plants growing on dolomitic limestone soils, with [Fe] (L) higher, between 100 and 250 mg kg⁻¹, than those in limestone and non-limestone soils, which is a normal concentration to support healthy growth.

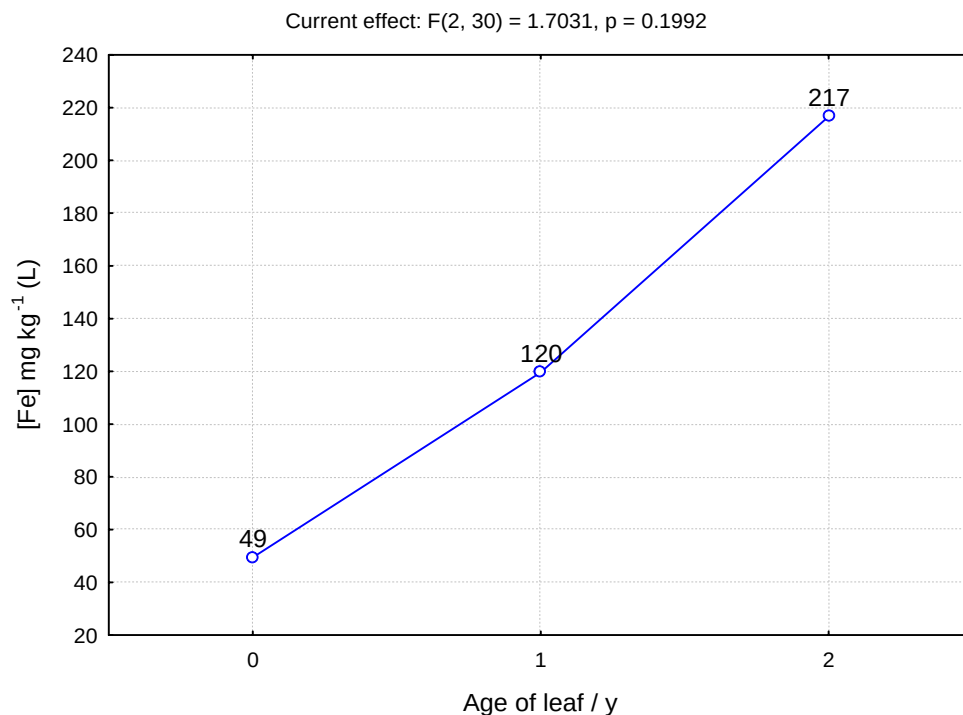
Generally, *Rhododendron* plants growing on all types of soils seem to absorb Fe from the soil in limited concentrations, normally between 20 and 300 mg kg⁻¹, which again demonstrates a well controlled uptake of Fe, as in this respect uptake of Fe seems to be similar to that of Ca ($r_{\text{Fe(L)}/\text{Fe(S)}} = 0.03$, SP 9). Alternatively, uptake may continue, but excess Fe is mobilised from leaves.



SP 9 Variation of [Fe] in leaves with soil pH for all *Rhododendron* plants.

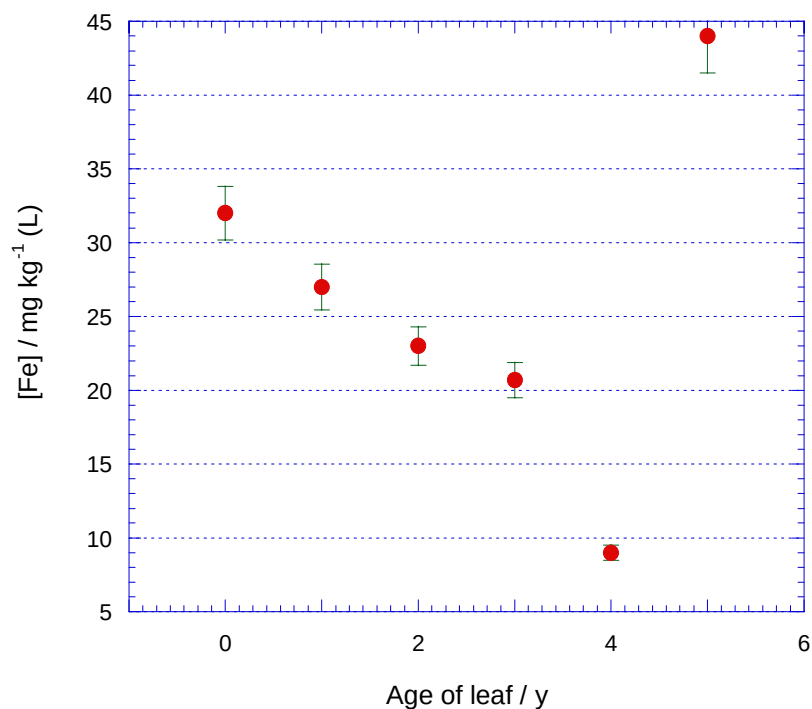
Iron variation in leaves with time

Although the concentrations of iron in leaves of consecutive years of age appear to increase with time, $r_{\text{Fe(L)/AGE}(0, 1, 2)} = 0.40$, this correlation is statistically insignificant ($p = 0.1992$) because the number of observations is low, from only few plants for all of which the [Fe] (L) increases dramatically from age to age (ANOVA 3, see ANOVA summary in Appendix 2).



ANOVA 3 Variation of [Fe] (L) in *Rhododendron* leaves with age of leaves.

However, the majority of leaf sets of three leaf ages have [Fe] fluctuating significantly between current-year-old, one-year-old and two-years-old. For instance, for the majority of plants the [Fe] in one-year-old are higher than in two-years-old. An overall trend can be, however, suggested by the variation of [Fe] in the leaves of *R. maximum* from current-year-old to five-years-old leaves (SP 10). There is a clear decrease in [Fe] in the leaves of this species with leaf age.



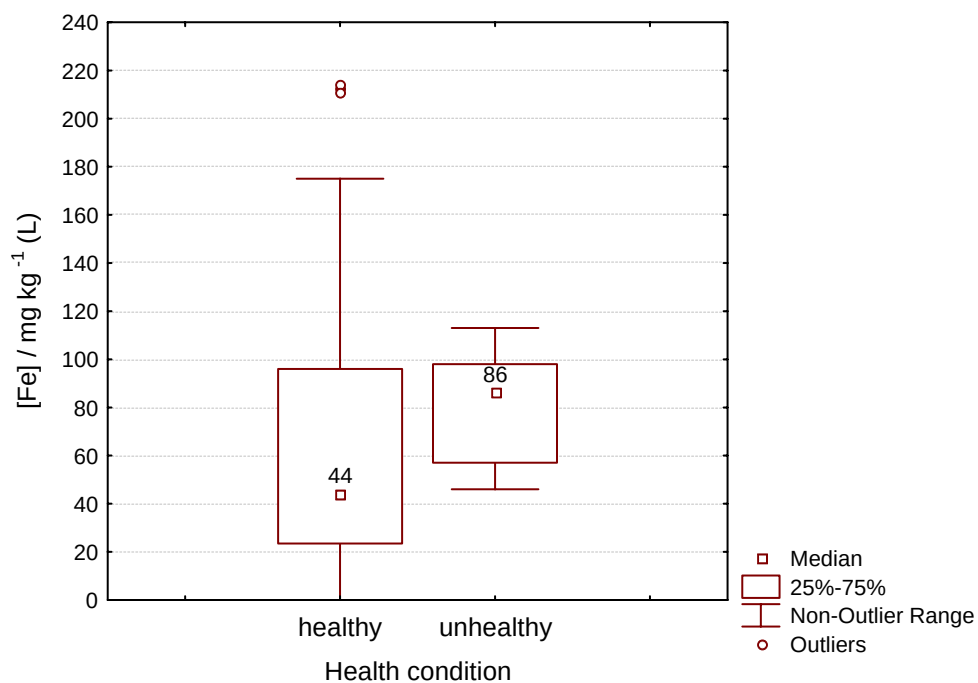
SP 10 Variation of [Fe] (L) in *R. maximum* leaves with age of leaves.

As with magnesium, it seems that rhododendrons either control their Fe take-up, and they do not absorb it into mature leaves above a certain concentration, or Fe in *Rhododendron* plants is more mobile than it is generally and it moves in both directions, in and out of the leaves. In the event of excess of Fe, it may be mobilised to other leaves.

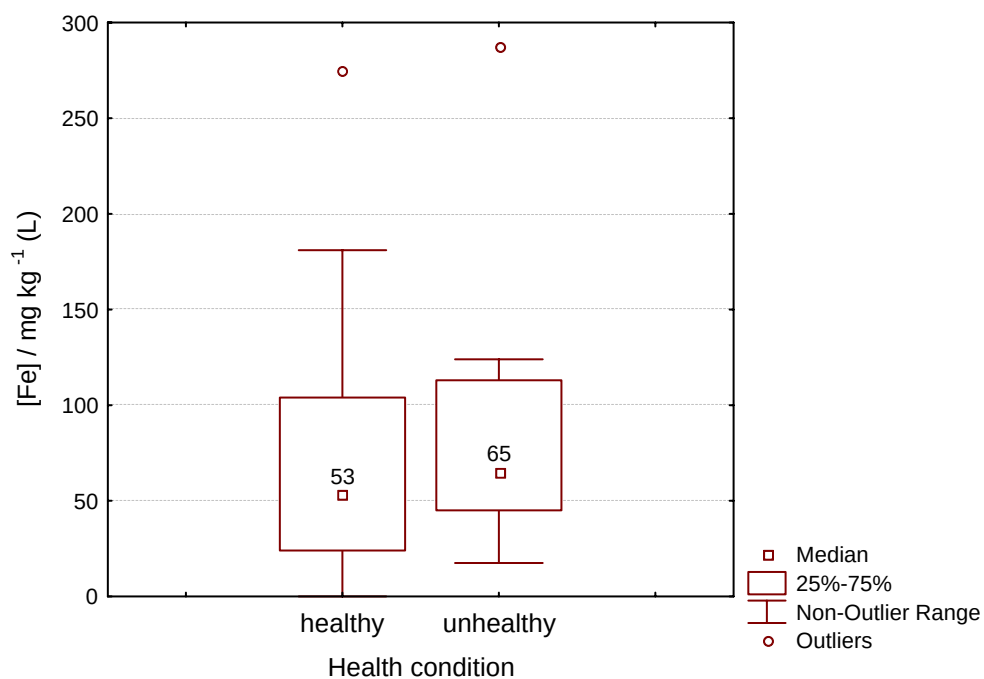
Iron and Rhododendron health

There is no correlation between the concentrations of iron in the leaves and health condition for rhododendrons in all soils ($r_{\text{Fe(L)/HC}} = -0.02$).

For *Rhododendron* plants growing on non-limestone soil the iron concentrations in both healthy and unhealthy leaves are within a similar range (BP 12).

**BP 12**

Range of [Fe] in healthy and unhealthy leaves for *Rhododendron* plants on non-limestone soils.

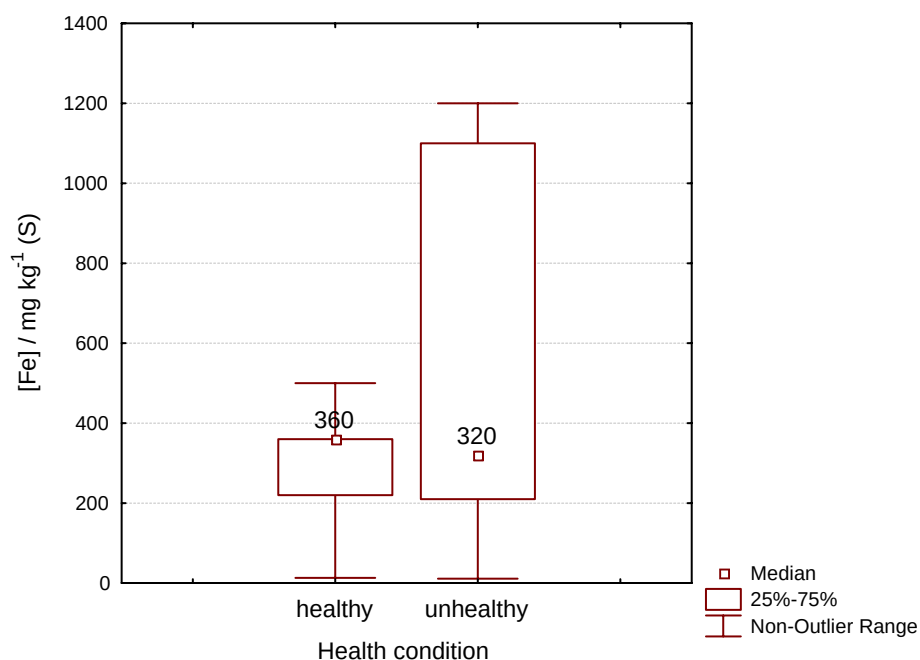
**BP 13**

Range of [Fe] in healthy and unhealthy leaves of *Rhododendron* plants on limestone soils.

In comparison, [Fe] in the leaves of rhododendrons growing on limestone soil are generally in a similar range as those in the leaves of rhododendrons on non-limestone soil, and are in some cases even present in higher concentrations (BP 13).

A few leaf samples, with unusually high [Fe] (L), are regarded as outliers and therefore are excluded from the total number of healthy leaves.

As already observed, the concentrations of Fe in the leaves of rhododendrons growing on limestone soil are sufficiently high. It is also evident that in unhealthy leaves iron is present at concentrations even higher than these in the healthy leaves, between 19 and 124 mg kg⁻¹, which is generally a satisfactory range of concentrations for healthy growth (BP 13). Additionally, the concentrations of Fe (S) present in the limestone soil for the unhealthy rhododendrons indicate that the iron available in the soil is also in sufficient range of concentrations. Low [Fe] (S) concentrations, such as those observed for some of the unhealthy rhododendrons, were also observed for some of the healthy plants (13 – 21 mg kg⁻¹) (BP 14).



BP 14 Range of [Fe] (S) in the soil for healthy and unhealthy leaves of *Rhododendron* plants on limestone soil.

One *R. decorum* plant in particular, which was growing in a boggy meadow had chlorotic symptoms and showed low concentrations of Fe in the leaves but very high concentrations of Mn. In this case it is probable that the poor health of the plant was not due to simple nutrient (Fe or Mn) deficiency (fungal infection can result in low [Fe]).

Overall no evidence that insufficient concentration of iron is the cause of the poor health in limestone soil was observed in the present study. There is no evidence that the rhododendrons studied in the wild were suffering from iron deficiency, even at the most extreme high-limestone, high-pH conditions. Therefore, the unhealthy symptoms shown by the analysed plants are not attributed to Fe deficiency.

3.6. Requirements for manganese observed for *Rhododendron* plants in the wild

The significant, strong correlations for [Mn] in leaves and soils for *Rhododendron* plants are listed in Table 31. As the correlations for Mn are generally not strong, the listed significant correlations include medium rather than only strong coefficients.

In limestone soils there is significance in the otherwise weak correlations between [Mn] (S) and soil pH, SOM, and [Ca] (S). In dolomitic limestone soils [Mn] (S) correlates significantly only with [Fe] (S), and in non-limestone soils, with soil pH and [Ca] (S).

Table 31 Significant correlations for [Mn] in leaves and soils for *Rhododendron* plants

[Mn] (L)				[Mn] (S)			
	<i>all</i>	<i>limestone</i>	<i>non-limestone</i>		<i>limestone</i>	<i>dolomitic</i>	<i>non-limestone</i>
soil pH	-0.46		-0.35	soil pH	-0.33		0.33
SOM %			-0.34	SOM %	0.29		
Leaf age	0.57		0.35	[Ca] (S)	-0.26		0.23
[Fe] (L)		0.25		[Fe] (S)	0.47	0.65	
[Mg] (L)		0.31					

Manganese in the leaves of rhododendrons growing in limestone soils correlates significantly only with [Fe] (L) and [Mg] (L), and in non-limestone soils with soil pH, SOM and Leaf age.

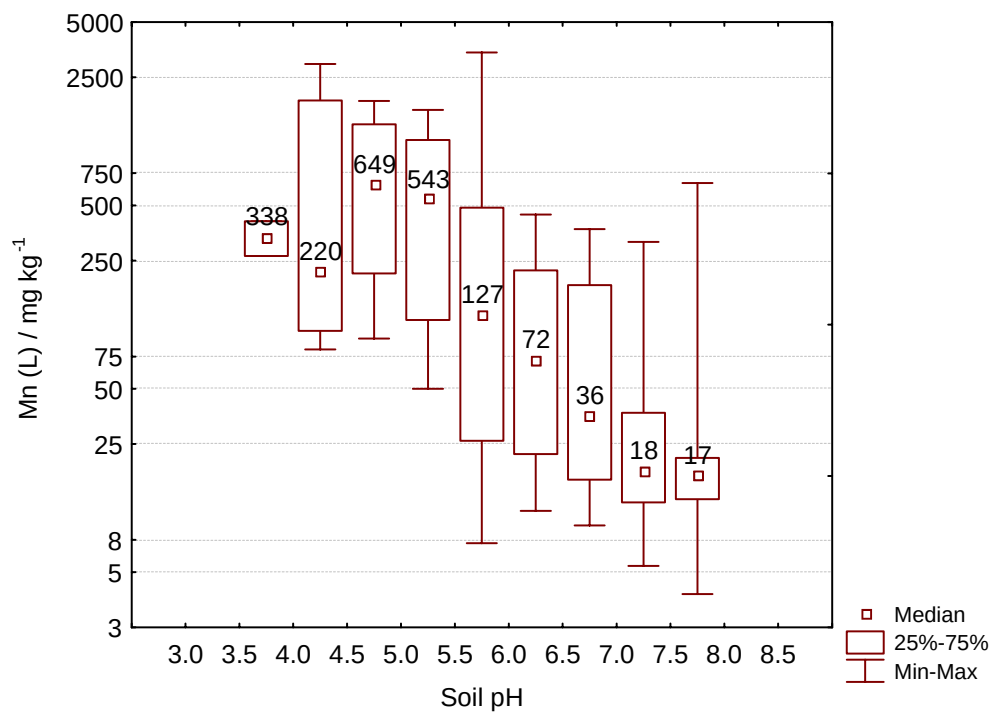
Correlation matrix 5 Correlations for [Mn] in leaves and soil for *Rhododendron* plants.

Variable	[Mn] (L)				[Mn] (S)			
	<i>all</i>	<i>limestone</i>	<i>dolomitic</i>	<i>non-limestone</i>	<i>all</i>	<i>limestone</i>	<i>dolomitic</i>	<i>non-limestone</i>
soil pH	-0.46	-0.22	-0.42	-0.35	0.03	-0.33	0.57	0.33
SOM %	-0.07	0.04	0.43	-0.34	0.15	0.29	-0.35	0.09
Leaf age	0.57	0.13	0.56	0.35	-0.09	-0.12	-0.41	-0.10
HC	-0.11	0.04	0	-0.05	-0.04	-0.02	0	-0.05
[Ca] (L)	0.04	0.08	0.07	0.18	0.02	0.00	0.04	0.07
[Fe] (L)	0.02	0.25	-0.08	0.09	0.01	0.09	-0.02	-0.00
[Mg] (L)	0.12	0.31	-0.09	0.27	-0.00	-0.03	-0.27	0.05
[Mn] (L)	1.00	1.00	1.00	1.00	-0.02	0.19	0.01	-0.09
[Ca] (S)	-0.30	-0.30	-0.45	-0.36	-0.16	-0.26	0.10	0.23
[Fe] (S)	0.37	0.08	-0.10	0.25	0.14	0.47	0.65	0.02
[Mg] (S)	-0.17	-0.10	-0.40	-0.14	-0.04	-0.07	0.06	0.14
[Mn] (S)	-0.02	0.19	0.01	-0.09	1.00	1.00	1.00	1.00

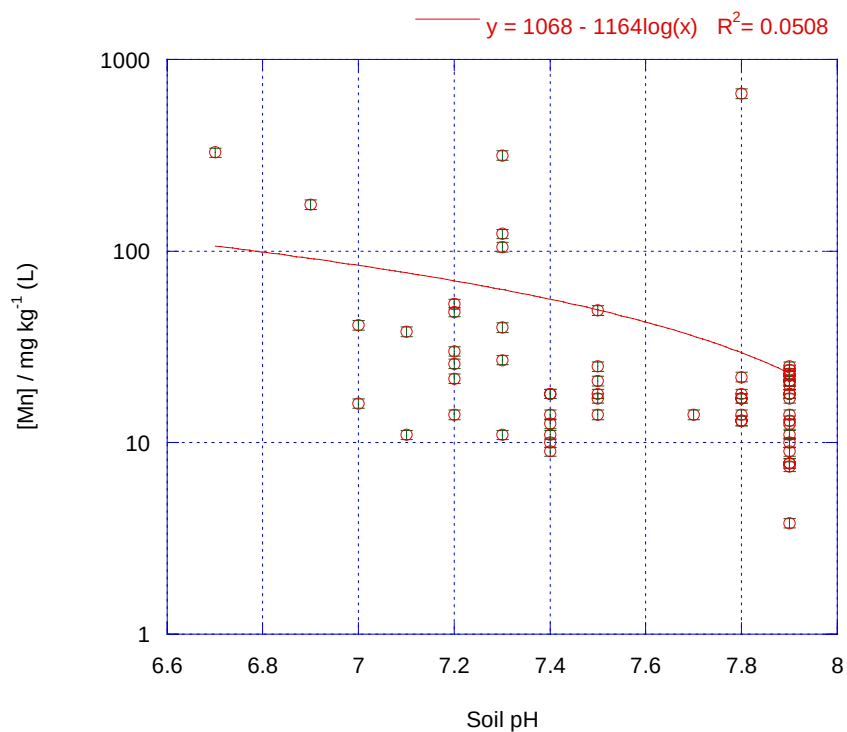
Manganese in soil and leaves for Rhododendron plants

In contrast to the status of iron in the leaves, the situation with manganese, in all types of soil, is very different.

First of all, while [Fe] (L) decreases more steadily and to higher bottom limits, [Mn] in the leaves of all rhododendrons clearly decreases more dramatically with increase in soil pH, with a threshold of around pH 5.5 (BP 15, SP 11). This is demonstrated by the significant correlation of [Mn] (L) with soil pH ($p = 0.00001$ and $R^2 = 0.2121$) (CM 5).

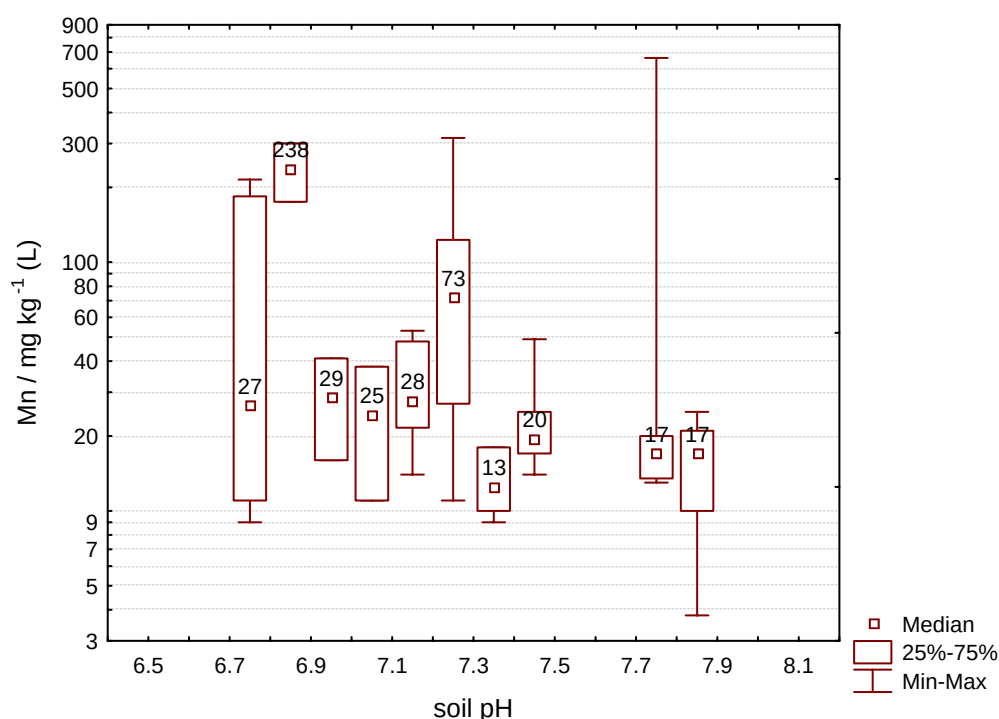


BP 15 Variation of [Mn] in leaves with soil pH for all *Rhododendron* plants.



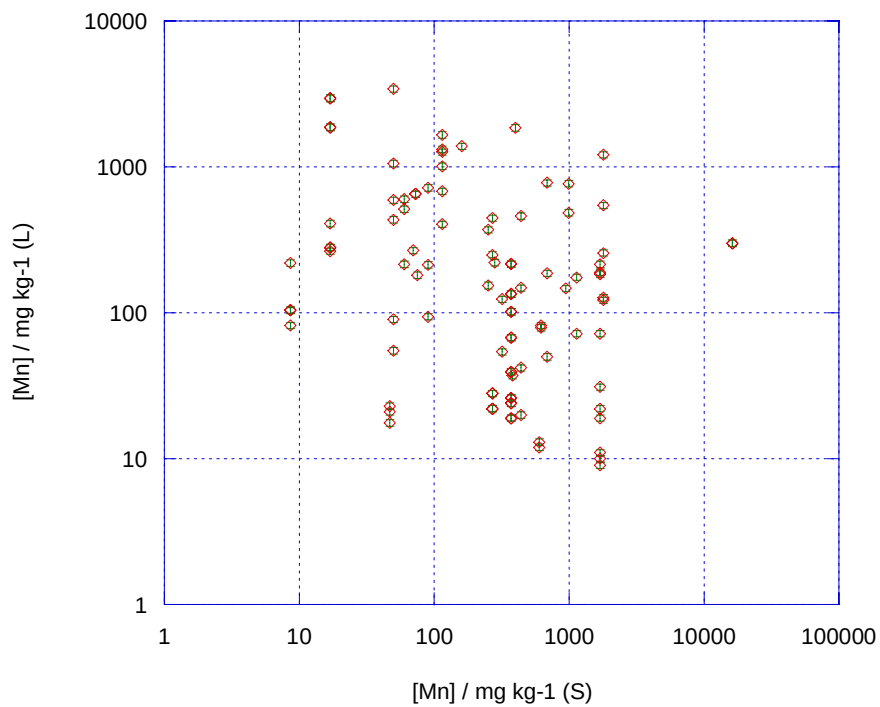
SP 11 Variation of [Mn] in leaves with soil pH for *Rhododendron* plants on limestone soils.

Secondly, in all soils in the high pH range, between 6.9 and 7.9, in contrast to [Fe] (L), the majority of rhododendrons have much lower [Mn] (L), 10 - 30 mg kg⁻¹, and for only a few leaf specimens the are concentrations between 30 and 300 mg kg⁻¹ (min = 3.8 mg kg⁻¹ and max = 663 mg kg⁻¹, excluded from these ranges) (BP 15 and 16). In limestone soil [Mn] in the leaves of rhododendrons growing at pH between 6.5 and 7.9 is very low, in the range 5 - 53 mg kg⁻¹, with minimum of 3.8 mg kg⁻¹ and maximum of 633 mg kg⁻¹. There were only three samples of leaves with concentrations near the top limits, at 300, 316 and 663 mg kg⁻¹ (SP 11, BP 16).

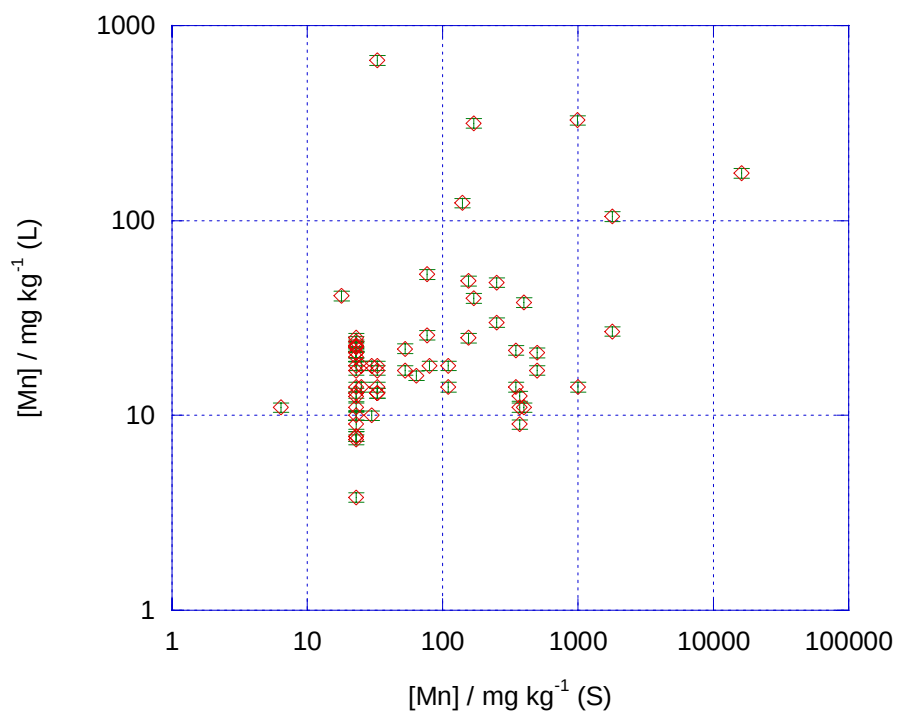


BP 16 Variation of [Mn] in leaves with soil pH for *Rhododendron* plants on limestone soil.

A third major characteristic of the behaviour *Rhododendron* species towards manganese, observed in previous experiments, is that *Rhododendron* plants generally absorb very high concentrations of Mn in their leaves, up to 3000 mg kg⁻¹. Such high Mn absorption appears particularly in the leaves of rhododendrons growing in soils of pH below 7.2 (non-limestone soils) (SP 12), and to a lesser extent above that pH (SP 13).



SP 12 Variation of [Mn] in the leaves with [Mn] in the soil for *Rhododendron* plants on non-limestone soil.



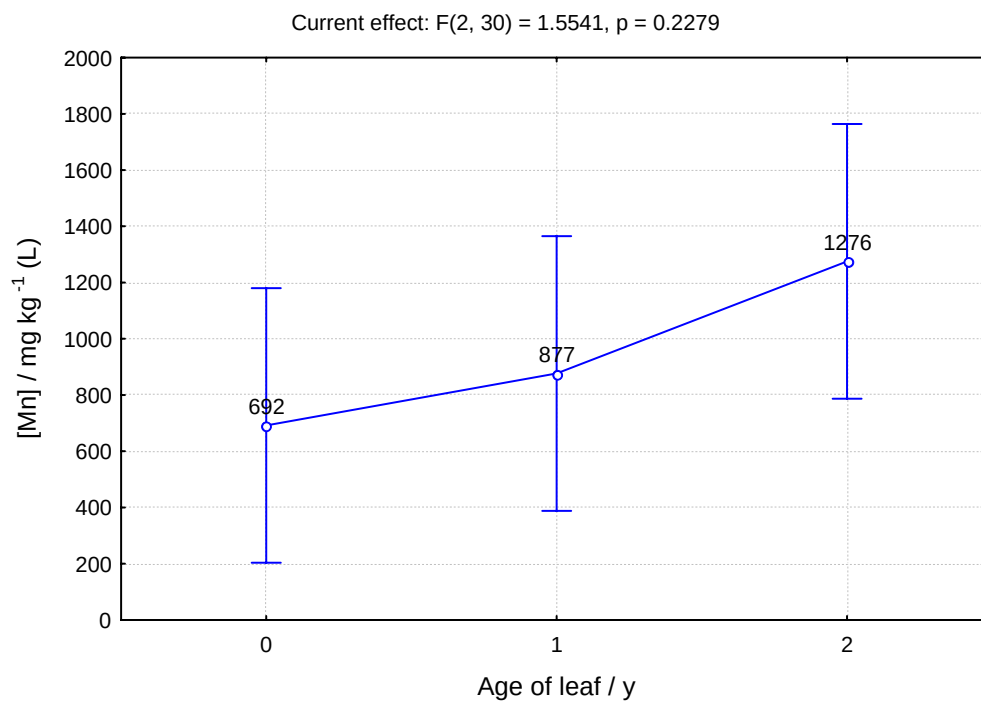
SP 13 Variation of [Mn] (L) with [Mn] (S) for *Rhododendron* plants on limestone soil.

With regards to the observations of high Mn absorption by *Rhododendron* plants, such difference in Mn take-up between plants in non-limestone and limestone soils may be due to the fact that Mn in low-pH, non-limestone soils is more sufficiently available and therefore, as long as it is present in the soil, it is efficiently and readily absorbed by *Rhododendron* plants. In contrast, in limestone soils there is generally low available Mn present in the soil and as a result the plants are simply exposed to lower concentrations for absorption and consequent accumulation in their leaves (SP 13).

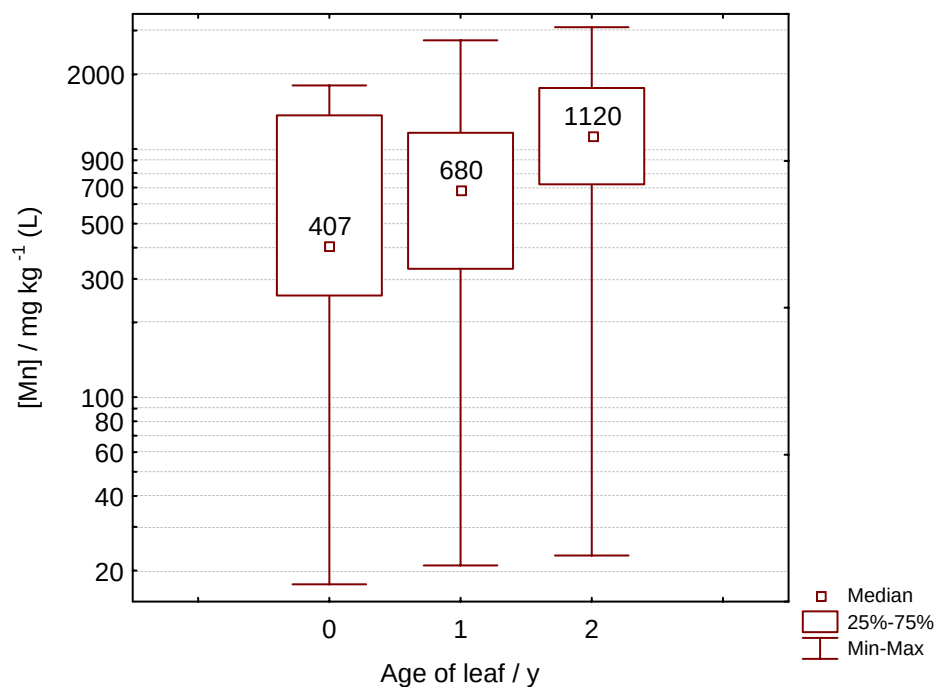
In these extreme soils there are many plants that do not display high leaf [Mn], and also have low soil [Mn]. It appears that high Mn concentrations in high pH soils occur to a lesser extent, and there are fewer plants absorbing and accumulating [Mn] in their leaves in high concentrations, compared to non-limestone soils.

Manganese in non-limestone soils

Since the generally shown by plants normal requirements for [Mn] for use in physiological functions are within the range of 20 – 300 mg kg⁻¹, it appears that rhododendrons absorb much more Mn than they need in their metabolism. Following past observations of high [Mn] in *Rhododendron* leaves, analyses of the concentrations of Mn in leaves of successive years of age, indicated by the significant at $p = 0.049$, positive correlation, $r_{\text{Mn(L)}/\text{AGE}} = 0.57$, showed that [Mn] in the leaves increases with the age of leaves. (ANOVA 4, BP 17, SP 14).



ANOVA 4 Variation of [Mn] (L) in *Rhododendron* leaves with age of leaves.



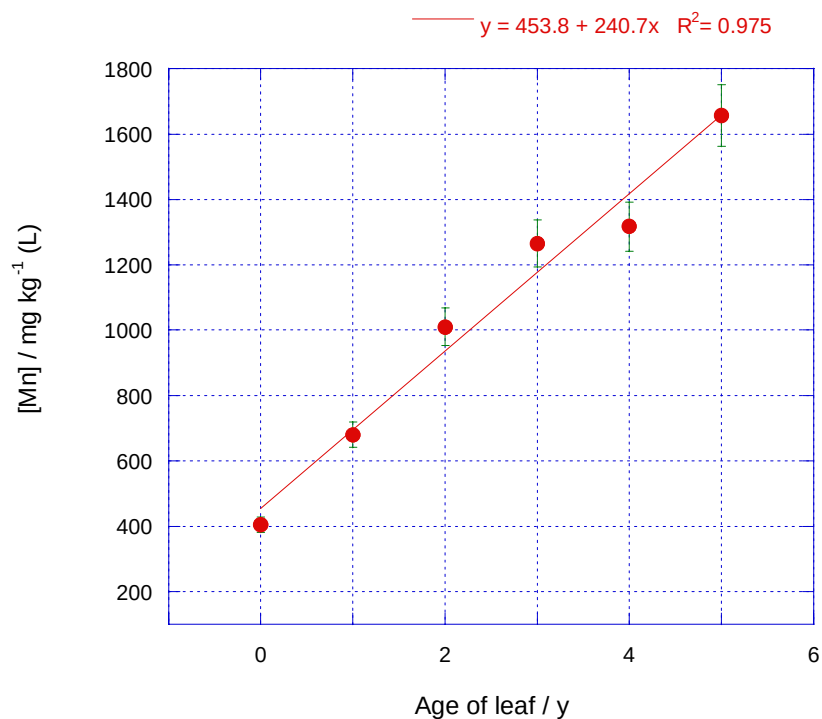
BP 17

Variation of [Mn] in current-year, one-year-old and two-year-old leaves for all *Rhododendron* plants.

The significance of the accumulation of [Mn] (L) with leaf age is confirmed even more strongly with the data from the analyses of six consecutive years of age collected from *R. maximum* plant growing at pH 5.4. More Mn is taken up by the leaves each season (SP 14). The concentrations of Mn in the leaves show a remarkably consistent and unlimited increase with the age of leaves (Table 32).

Table 32 Increase in [Mn] (L) with leaf age for *R. maximum*

Age of leaf / y	[Mn] / mg kg ⁻¹
0	405
1	680
2	1010
3	1270
4	1320
5	1660



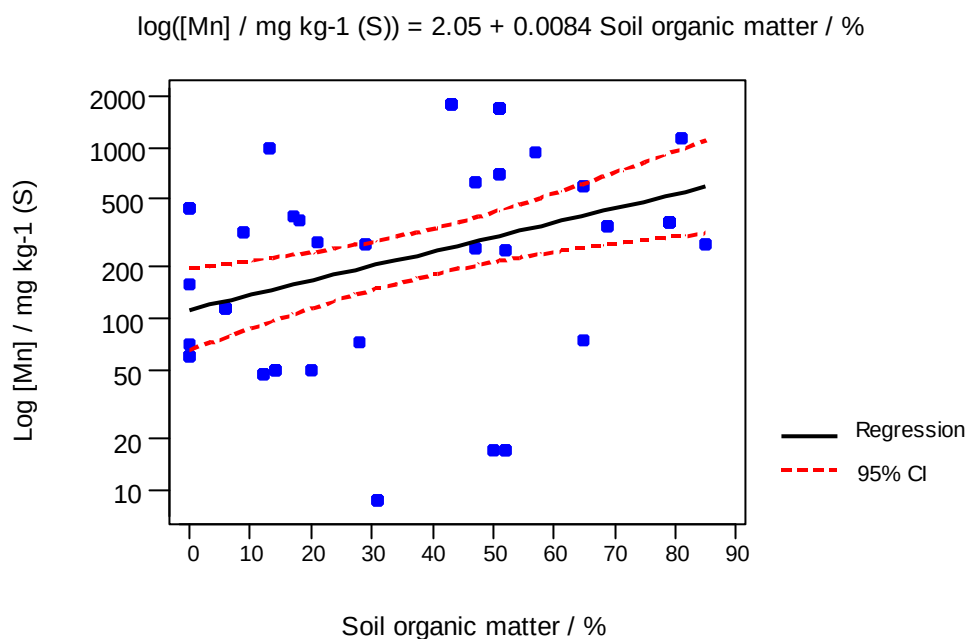
SP 14 Variation of [Mn] in *R. maximum* leaves with age of leaves.

Because few plants had leaves older than two-years-old, most collected leaves are current, one and two-years-old.

The increase in [Mn] in the leaves of consecutive years of age for each plant can be viewed in column plots CP 2a, 2b and 2c in appendix 3.

Manganese and soil organic matter

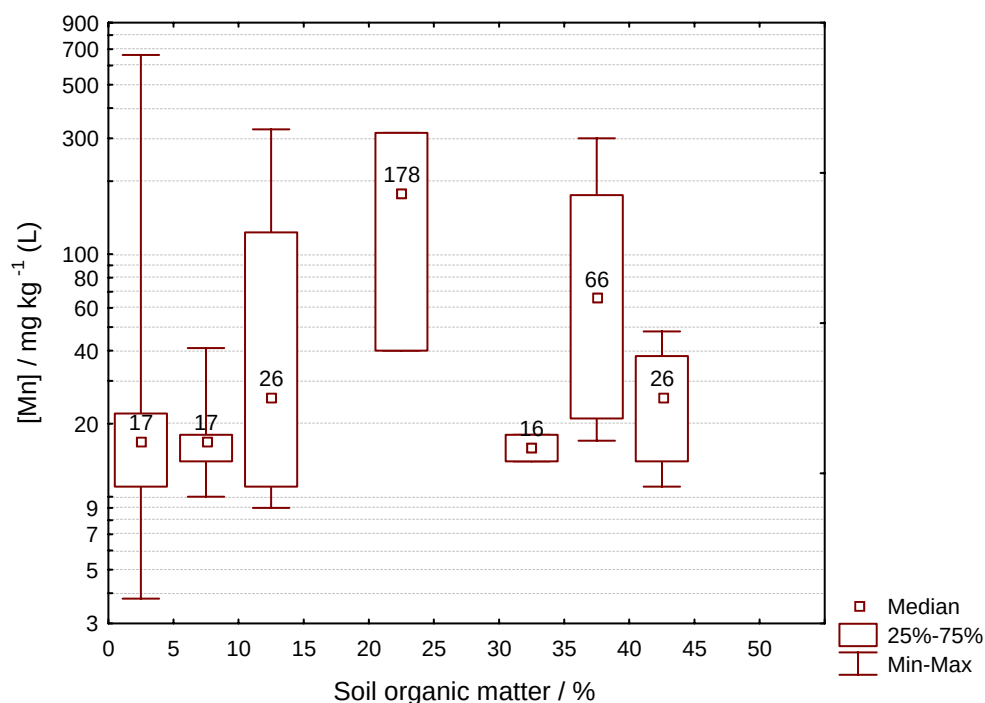
Furthermore, the observation of higher concentrations of leaf Mn in soils with lower pH (non-limestone soils) is closely related to the higher content of soil organic matter in those soils. As expected for soils with high content of soil organic matter, the [Mn] in the soil increases with increasing the soil organic matter (LR 5).



LR 5 Variation of [Mn] (S) with SOM for *Rhododendron* plants on non-limestone soil.

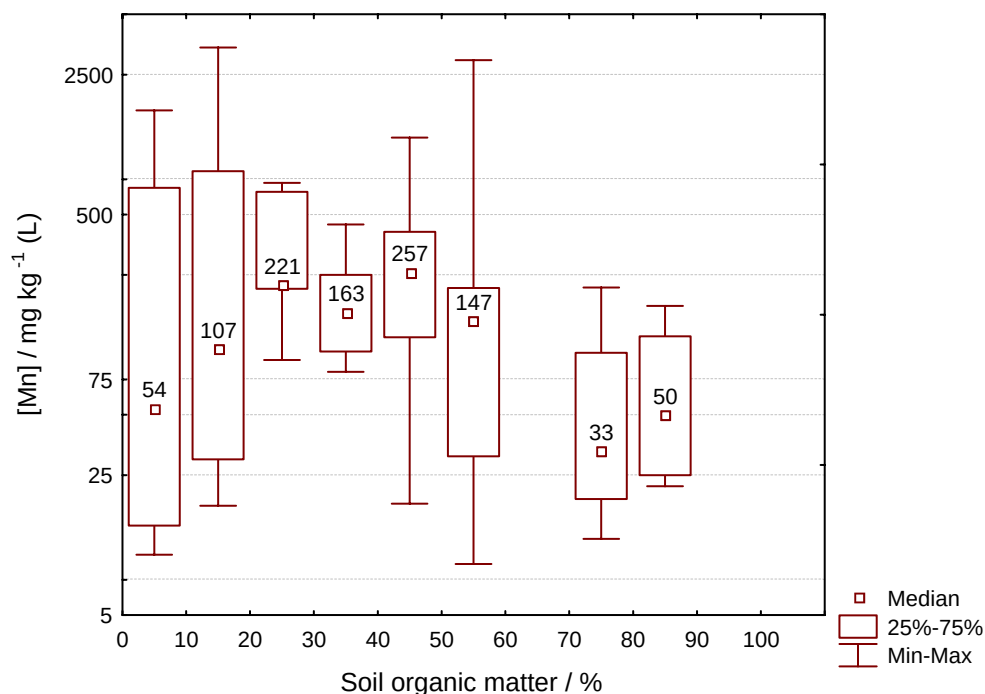
However, despite the increasing concentrations of Mn with increasing the organic matter, it is generally known that soil organic matter usually contains Mn in

concentrations lower than those that it was already shown could be present in *Rhododendron* leaves³⁴. Clearly, the concentrations of Mn observed in both soils and leaves for rhododendrons growing on non-limestone soils, reach much higher maxima, around 2000 mg kg⁻¹ in the soils and around 3000 mg kg⁻¹ in the leaves, than those observed in limestone soils (around 1000 mg kg⁻¹ in the soils and around 600 mg kg⁻¹ in the leaves, BP 18).



BP 18 Variation of [Mn] (L) with soil pH for *Rhododendron* plants on limestone soils.

In addition, interestingly, these high [Mn] in the non-limestone soils are found in the soil samples having lower soil organic matter, between 5 and 55 % (BP 19), and again, soil organic matter generally provides lower concentrations of Mn. So where do these high concentrations of Mn in the soil come from?



BP 19 Variation of [Mn] (L) with SOM for *Rhododendron* plants on non-limestone soils.

If the [Mn] in the organic component of the soil is high (and particularly if it is $> 300 \text{ mg kg}^{-1}$) it is likely that the organic part of the soil is provided by Mn-hyperaccumulating plants, such as rhododendrons. A possible mechanism of supplying the soil organic fraction with large concentrations of manganese involves the following steps. 1) The excess of Mn deposited in the older leaves most probably becomes oxidised in the leaves to the water-insoluble MnO_2 , thereby countering possible Mn toxicity. 2) After falling on the ground at the end of their life cycle, in the process of their decay in the soil the MnO_2 becomes reduced by bacteria to a water-soluble form of Mn^{2+} . The reduced Mn^{2+} is then available for re-absorption and readily taken up by rhododendrons (Fig. 8).

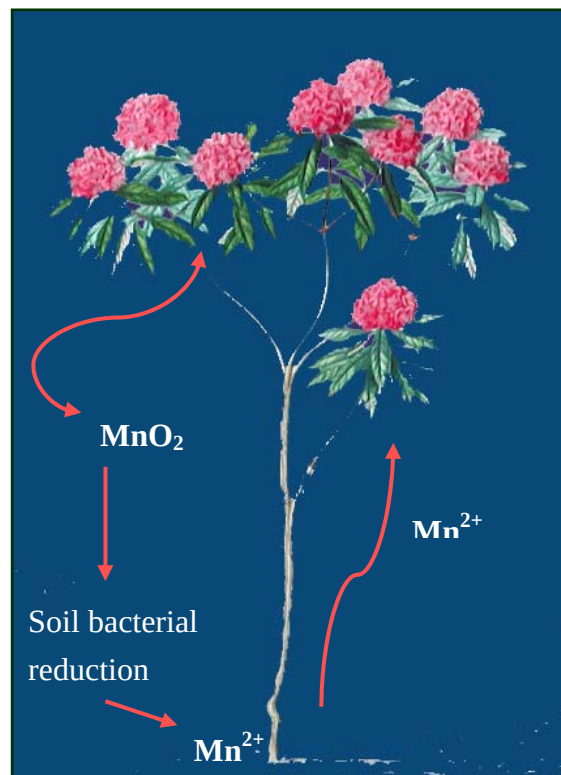
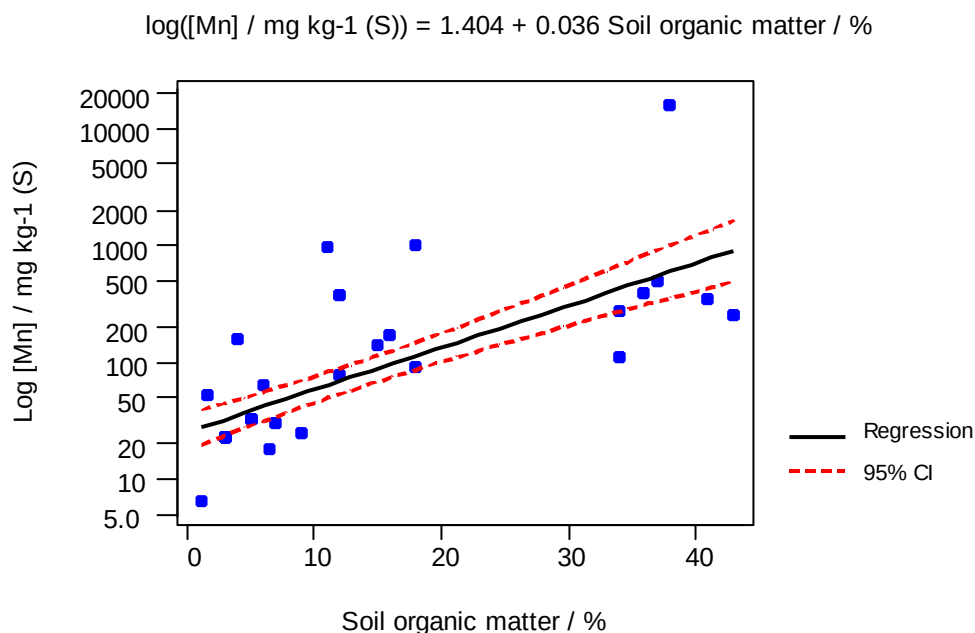


Figure 8 Scheme of the mechanism of Mn-recycling in *Rhododendron* plants.

One way of confirming the above hypothesis explaining the Mn-rich non-limestone soils is by analysing what the correlations are for limestone soils between the same variables, [Mn] in soil, SOM, and [Mn] in leaves, and whether the organic component in limestone soils is Mn-rich.

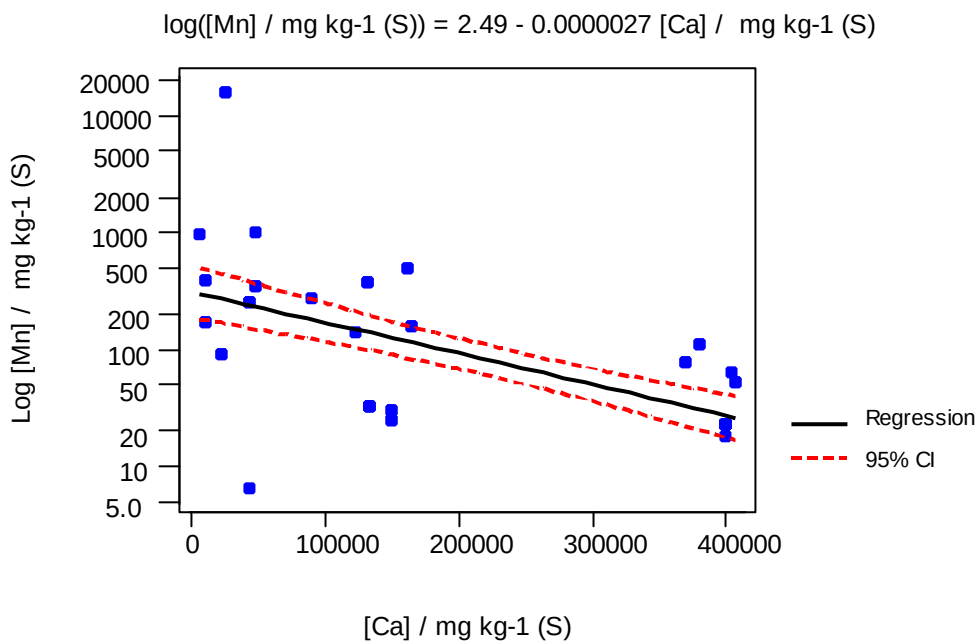
Manganese in limestone soils

In agreement with the already discussed lower concentrations of Mn in high-pH soils, the concentrations of Mn in limestone soil (and consequently [Mn] (L)) decrease also with decreasing the soil organic matter ($r_{\text{Mn(S)}/\text{SOM}} = 0.29$, $r_{\text{Mn(L)}/\text{SOM}} = 0.04$) (LR 6)



LR 6 Variation of [Mn] (S) with soil organic matter for *Rhododendron* plants on limestone soil.

On the other hand, as a consequence of the higher pH, in limestone soil [Mn] (S) decreases with the increase in [Ca] (S) ($r_{Mn(S)/Ca(S)} = -0.26$) (LR 7). Past analyses of limestone rock collected in the Lijiang and Zhongdian areas showed that it contains only traces of manganese, 20 - 70 mg kg⁻¹³⁹. In the present study the concentrations [Mn] (S) in the extreme limestone soils, in which the concentrations of Ca are consistently close to, even reaching, the maximum of 400000 mg kg⁻¹, vary from 18 to 110 mg kg⁻¹, which fits closely to the past observations that pure limestone soil contains only a little Mn.



LR 7 Variation of [Mn] in the soil with [Ca] in the soil for *Rhododendron* plants on limestone soil.

Such large [Ca] (S) and low [Mn] (S) are consistently observed in one of the most extreme limestone sites, Gang He Ba valley, where the soil is composed of pure white, finely powdered CaCO_3 with almost no organic matter present. The *Rhododendron* species populating this area are *R. cuneatum* and *R. telmateium* (members of the Lapponica subsection), growing directly in the limestone soil, and *R. vernicosum* and *R. yunnanense*, growing at the bottom of the side moraines of the valley, in a thin layer of humus.

However, in contrast to the generally observed [Mn] that soil organic matter and limestone soil provide, some of the analysed limestone soil samples contained more Mn than could normally come from the limestone and organic matter together: at SOM 11 - 19 % the [Mn] (S) is between 380 and 1800 mg kg^{-1} . Clearly, such high manganese concentrations in soils of low organic matter and high limestone content can be present

in the soil only via organic matter soil fraction. As in the case of non-limestone soils, this strongly suggests that since organic matter generally contains a lot less Mn, such high [Mn] must come from a particular Mn-rich organic material. A major source of organic matter under woody plants is their own fallen and decaying foliage. Indeed, some of the plants growing on the limestone soils for which the soil samples contained high concentrations of Mn have also high Mn in their leaves, 100 – 330 mg kg⁻¹ (SP 13). This is a strong indication that where there is high [Mn] in the little organic matter, in a soil composed of minerals containing low [Mn], Mn comes from the Mn-rich *Rhododendron* leaves upon their decay in the soil, or from leaves in Mn-rich plants in neighbouring areas.

Amongst the plants growing in the limestone areas, those few plants showing higher [Mn] in both soil and leaves than is usually observed were members of the larger-leaved species: *R. rubiginosum*, *R. yunnanense* and *R. vernicosum*. Generally, the [Mn] (L) in the leaves of *R. vernicosum* and *R. yunnanense* is significantly higher (123 – 300 mg kg⁻¹) than those in the leaves of the small-leaved *Lapponica* species, growing directly in limestone soil (10 – 25 mg kg⁻¹ on average). From the above results on the species growing in Gang He Ba and those growing in other limestone areas, two conclusions may be drawn. First, the limits required by *R. vernicosum* and *R. yunnanense* for their healthy growth are higher than those required by the two *Lapponica* species, which is why they are found mostly growing on the banks of the valley but not on the floor. Secondly, the soil samples for the *R. vernicosum* and *R. yunnanense* plants growing on the side moraines of Gang He Ba valley, and the soil samples for the species growing in the other limestone areas, on average all have higher concentrations of [Mn] (S).

It seems that in extreme conditions (high pH and high limestone content) there may be a correlation between the absorption of high [Mn] and the size of leaves. The species with small leaves that were found in such soils, the *Lapponica* species, have consistently low concentrations of Mn in their leaves (11 – 25 mg kg⁻¹) and soils, whereas the *Lapponica* species found in more favourable conditions, limestone soil with high organic matter content and lower pH, have higher [Mn] in their leaves: 60 – 1100 mg kg⁻¹, and soils

(CP 4a). Additionally, it seems that in any type of soil some species generally absorb less Mn in their leaves than others. This was observed for the species *R. primuliflorum*, also a small leaved-species, and the Lapponica species, which although growing in higher in [Mn] non-limestone soils, still had low [Mn] in their leaves. Also, in many of the cases when those species had high soil [Mn], the plants were growing in association with other rhododendrons such as *R. vernicosum* and *R. phaeochrysum* (large-leaved species), so there is a possibility that soil where a mixture of different species are growing together could be Mn-rich as a result of a supply of Mn accumulated in the leaves of the large-leaved species.

The concentrations of Mn in the leaf and soil samples for each plant can be followed in CP 3a, 3b, and 3c, and the ratio Mn (L)/Mn (S) for each plant, in CP 4a, 4b, and 4c in appendix 3.

Manganese variation in leaves with time

Overall, the earlier observed high concentrations in *Rhododendron* leaves of successive years of age suggest two points (ANOVA 4, SP 14).

First, it appears that rhododendrons do not show maximum limits of Mn concentrations; they absorb this nutrient to indefinite concentrations, and so long as it is in the soil they take it up very efficiently and in an uncontrolled manner.

Secondly, [Mn] (L) in the leaves of *Rhododendron* plants is observed at much higher concentrations than the top limit for healthy growth for normal plants. The maximum observed concentration of Mn in *Rhododendron* leaves is 3400 mg kg^{-1} , whereas the upper limit for most other plants is 300 mg kg^{-1} , above which evidence of Mn toxicity usually appears. Clearly, rhododendrons do tolerate very high concentrations of manganese.

Due to the generally lower absorption of Mn in the high pH range, already discussed, the accumulation of Mn in the older leaves is also pH dependent. Within the pH range 6.9 - 7.9 (for all types of soil), out of 77 leaf samples the only leaf concentrations of Mn

higher than the usual were for six plants which had [Mn] (L) 120 – 330 mg kg⁻¹ in the current year leaves, and three plants with [Mn] (L) 100 – 660 mg kg⁻¹ in the one-year-old leaves. The majority of the plants in the soils of high pH have [Mn] in their leaves between 10 and 50 mg kg⁻¹. In contrast, between pH 6.8 and 5.8, out of 37 leaves (current and old), half of the plants have leaf [Mn] between 120 and 1370 mg kg⁻¹, and the other half have [Mn] between 10 and 50 mg kg⁻¹. Below pH 5.9 leaf [Mn] is much higher in both current leaves and older leaves, and is mostly between 500 and 3400 mg kg⁻¹. It seems that since it is more available in the soil, and as rhododendrons take Mn up readily as long as it is in the soil, at lower pH rhododendrons absorb Mn at higher rates, and as a result at the lower pH they also accumulate it in their leaves to a greater extent. *Rhododendron* plants show a tendency to accumulate Mn in their leaves at all pH. This tendency appears to be weaker at high pH and stronger at low pH, presumably reflecting the generally lower availability of Mn in the soil at high pH.

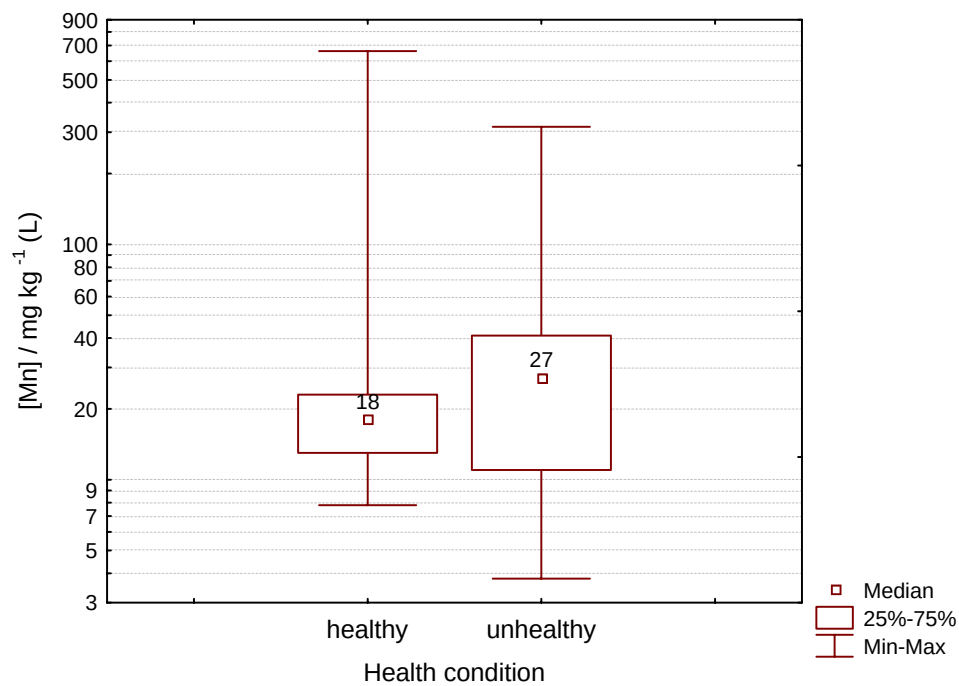
It can be therefore speculated that hyper-accumulation of Mn evolved as a response to growing in soils of very low pH, where manganese would have been leached away due to its very high solubility. However, as a result of the unregulated Mn absorption by rhododendrons, the manganese available in the low-pH soils was readily and efficiently absorbed and accumulated by *Rhododendron* plants. Fallen leaves from such plants could be transported to neighbouring areas with soils of higher pH. This way of carrying manganese within their own foliage, while moving from soils of low pH and high [Mn] to soils of high pH and low [Mn], could thus have provided rhododendrons with a self-sustaining mechanism of supplying manganese for physiological functions in regions where otherwise the plants would have been unable to grow.

Generally, another environment which it is important to study as a comparison to limestone soils is that of soils of high pH, 6.9 to 7.9, in which the source of alkalinity is other than limestone. In this study only two high-pH soil samples were derived from non-limestone minerals and organic components. Therefore, in order to study the accumulation of Mn by rhododendrons more soils from sites with minerals other than limestone as the source of alkalinity should be analysed, which is a point for future

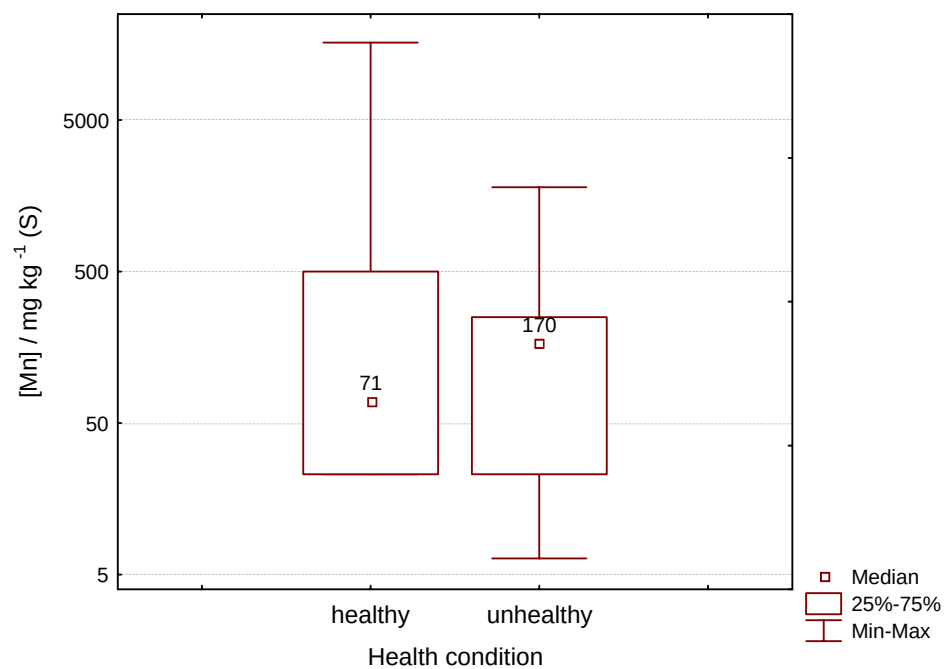
work.

Manganese and Rhododendron health

Most plants in both extremes, having very low concentrations of [Mn] in their leaves and those with very high concentrations of [Mn], were in perfect health (BP 20 and 21). Among the plants growing directly in the limestone soil only two unhealthy *R. cuneatum* plants were found, and the concentrations of [Mn] (L) in their leaves were 3.8 mg kg⁻¹ in current-year leaves and 7.5 mg kg⁻¹ in one-year-old leaves of one of the plants, and 11 mg kg⁻¹ in the second plant. However, there were still a few other plants that had [Mn] (L) in their leaves between 7.5 mg kg⁻¹ and 9 mg kg⁻¹, and which were also healthy. It appears that in this very extreme area, the *Rhododendron* species growing directly in limestone soil on the valley floor, *R. cuneatum* and *R. telmateium*, have adapted to living healthily under extremely low concentrations of manganese in both soil (23 – 77 mg kg⁻¹) and leaves (10 – 25 mg kg⁻¹). The other two species, found on the valley lateral moraines, *R. vernicosum* and *R. yunnanense*, which are slightly more protected from the white limestone soil with a thin organic layer, have higher concentrations of Mn in both soil (140 mg kg⁻¹) and leaves (123 – 300 mg kg⁻¹). There was one *R. vernicosum* plant that suffered from a fungal disease, and the concentrations of [Mn] in its leaves and soil were very low, 10 and 18 mg kg⁻¹ in the leaves and 30 mg kg⁻¹ in the soil. It is not possible to say whether the low manganese concentrations is a consequence of fungal disease, or the low manganese concentrations made the plant more susceptible to fungal attack.



BP 20 Range of [Mn] (L) in healthy and unhealthy leaves of *Rhododendron* plants on limestone soil.



BP 21 Range of [Mn] (S) in the soil for healthy and unhealthy leaves for *Rhododendron* plants on limestone soil.

The large-leaved species *R. rubiginosum*, which was also found in limestone areas, was the species that had the highest number of plants suffering from chlorosis in limestone soils. The [Mn] in the leaves of those plants showed both sufficient Fe concentrations in the leaves, 60 – 200 mg kg⁻¹, and concentrations of Mn between 27 and 40 mg kg⁻¹. As already observed this species, together with other large-leaved species, are not adapted to extremely low [Mn] as observed for the Lapponica species, and therefore are not generally found growing directly in the limestone soil, but in a thin organic layer over limestone soil. As a result, their minimum concentrations of Mn required for healthy growth seem also to be higher, between 20 and 50 mg kg⁻¹, than those for the small-leaved species, between 10 and 30 mg kg⁻¹, and which were mostly healthy. Only for one of the unhealthy plants growing on limestone soil the [Fe] in leaves was as low as 19 mg kg⁻¹, and [Mn] was 27, while for healthy plants growing at low pH [Fe] was between 22 and 24 mg kg⁻¹ and [Mn] was 26 - 160 mg kg⁻¹. Since the same concentrations of Fe that were found among the unhealthy plants were also found in healthy plants, but the concentrations of Mn in healthy plants were mostly much higher than those in unhealthy plants, the chlorotic symptoms in *R. rubiginosum* displayed in limestone soils were attributed to Mn deficiency rather than to Fe deficiency.

In summary, there is a strong relationship between *Rhododendron* plants and manganese in both extremes: when leaf [Mn] concentrations are critically low and when they could be potentially toxically high. In the first situation, rhododendrons growing in soils where manganese is insufficiently present in the soil, such as limestone soil, seem to have adapted to tolerate and grow healthily with extremely low concentrations of Mn. Almost half of the leaves of plants growing in limestone (29 leaves out of 65, as this includes all current year and old leaves) showed perfect health with [Mn] between 8 and 18 mg kg⁻¹. Of course, there are unhealthy leaves, but surprisingly only six of them (2 pairs of current and old leaves for two plants, and two plants having only current-year leaves, four plants in total) have [Mn] 4 – 11 mg kg⁻¹, and the remaining seven unhealthy leaves have sufficient [Mn], 30 – 320 mg kg⁻¹. For the latter unhealthy leaves, the [Mn] at the higher end correspond to older leaves and those at the lower end correspond to the

current-year leaves, in four plants in total. It appears that unhealthy symptoms are evenly distributed among plants with both low and relatively high concentrations of Mn. In total, between the observed high and low [Mn] in the limestone soil, there are five unhealthy plants (out of 44 in total in the limestone soils), in which the symptoms of chlorosis persist over the period of two years. The rest of the plants were growing in very good health.

Discussions of results for alpine plant species in the wild

All leaf samples collected from alpine plant species in the wild were current-year-old leaves, and all observed plants were healthy. Therefore, grouping by health condition and age of leaf sample is not performed in the statistical interpretation for alpine plant species.

As expected for high pH soils, the correlation between the soil pH and soil organic matter in limestone and dolomitic limestone soil is significant negative and strong (Table 33).

Table 33 Significant strong correlations of soil pH with SOM for alpine plants

	soil pH	
	<i>dolomitic</i>	<i>limestone</i>
SOM %	-0.86	-0.80

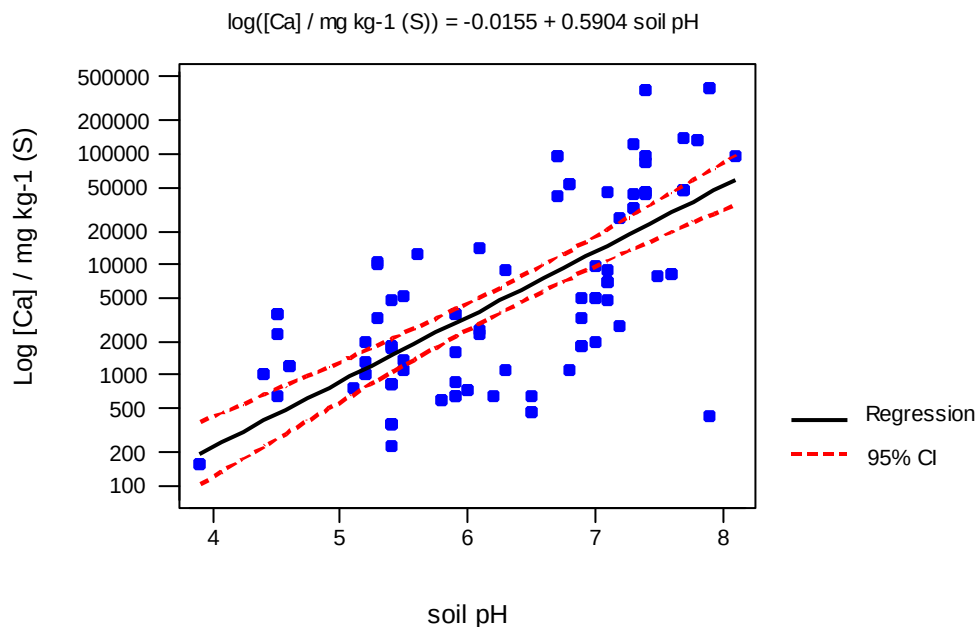
3.7. Requirements for calcium and magnesium observed for alpine plants in the wild

Calcium in soil and leaves for alpine plants

Table 34 Significant strong correlations for [Ca] in leaves and soils for alpine plants

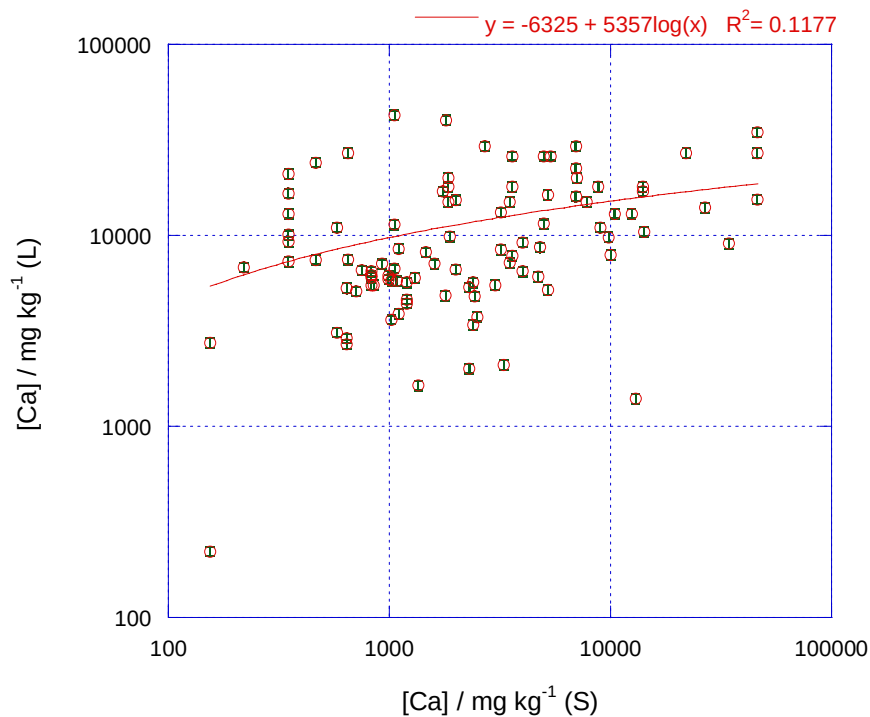
[Ca] (L)			[Ca] (S)		
	<i>dolomitic</i>	<i>non-limestone</i>		<i>dolomitic</i>	<i>non-limestone</i>
soil pH		0.44	soil pH	0.66	0.45
[Ca] (S)		0.40	SOM %	-0.53	0.42
[Mg] (S)		0.43	[Mg] (S)	0.67	0.89
[Mg] (L)	0.65	0.61	[Mn] (S)	0.48	0.73
[Mn] (S)		0.45	[Fe] (S)	-0.44	

The correlations between [Ca] (S) and [Ca] (L) and soil pH for alpine plants are almost the same as those observed for *Rhododendron* plants: [Ca] (S) in all soils increases with increasing soil pH (LR 8), and on limestone soil [Ca] (L) correlates only weakly with soil pH ($r_{\text{Ca(L)}/\text{pH}} = 0.02$ for limestone).

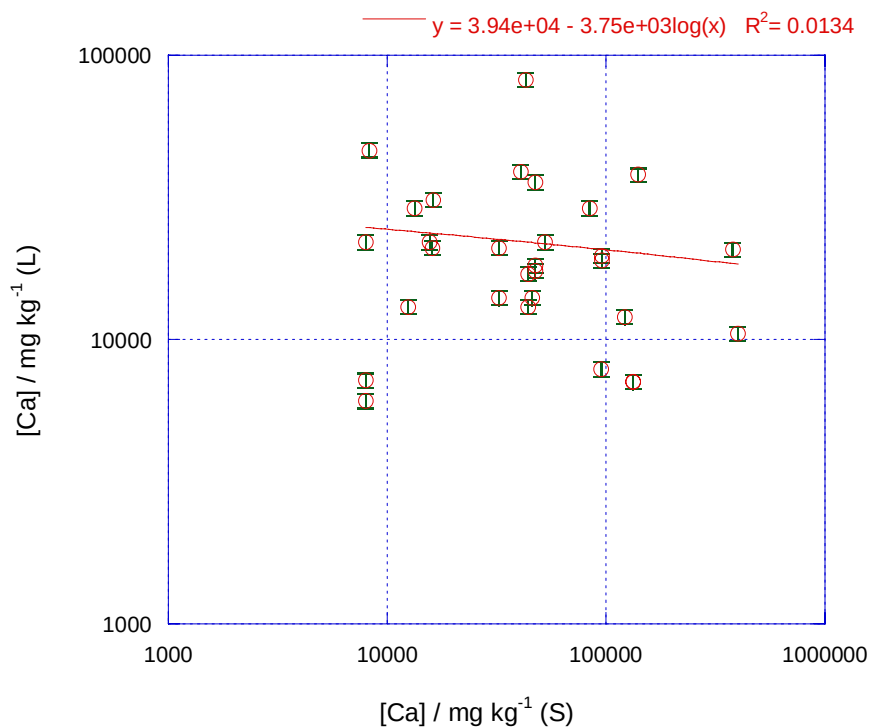


LR 8 Variation of [Ca] (S) with soil pH for all alpine plants.

The correlations between [Ca] in leaves and [Ca] in soils for alpine plants growing on all types of soils demonstrate that alpine plants generally absorb Ca in their leaves up to very high concentrations, with similar top limits in both limestone and non-limestone soils, around 30000 mg kg^{-1} (SP 15 and 16). The same is observed for *Primula* plants alone (SP 17 and 18). The minimum limits of [Ca] in non-limestone and limestone soils, however, are different: those in non-limestone soil being much lower. This wide range of [Ca] (L) resembles that observed in the leaves of *Rhododendron* plants.



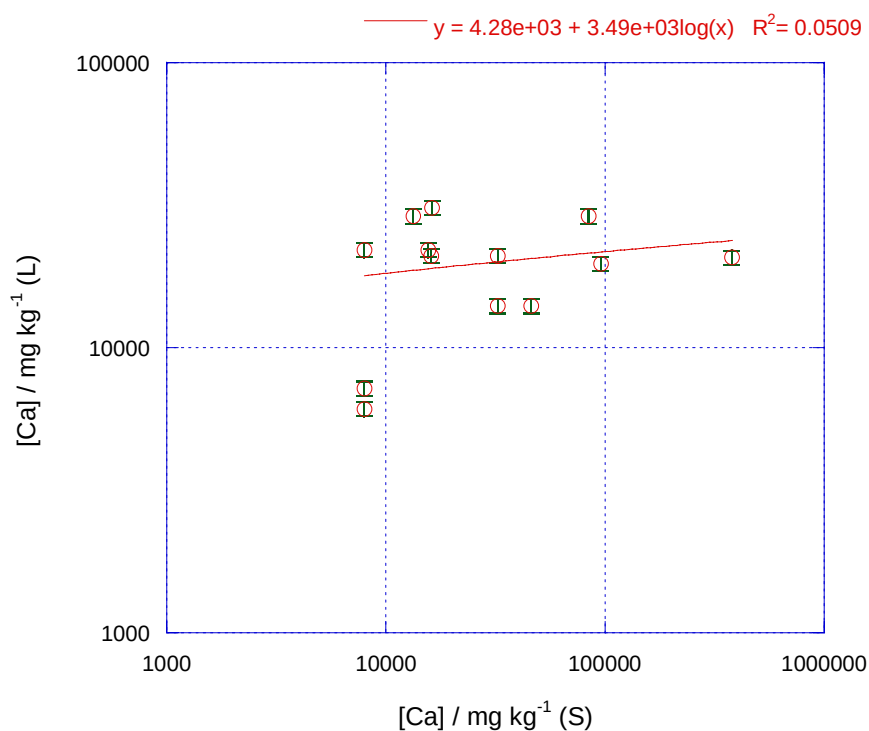
SP 15 Variation of [Ca] (L) with [Ca] (S) for alpine plants on non-limestone.



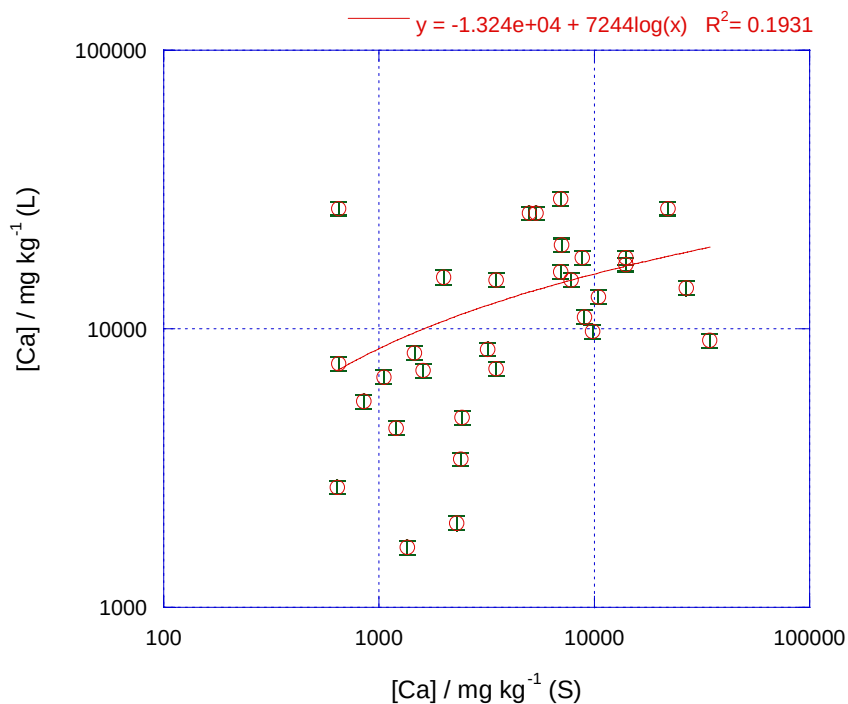
SP 16 Variation of [Ca] (L) with [Ca] (S) for alpine plants on limestone.

As with *Rhododendron* plants, for alpine plants the range of [Ca] in leaves is wider for plants in the non-limestone soils than for those in limestone soils. The lower bottom limit in non-limestone soil and the very high top limit in limestone soil suggest that if there is high [Ca] in the soil there is also high [Ca] in the leaves, i.e. in non-limestone soil the bottom limit is lower simply because there is less Ca in the soil. On the other hand, in limestone soil, where the [Ca] (S) is higher of course the bottom limit is also higher. This suggests that when it is available in the soil alpine plants tolerate and take up more Ca in their leaves, as with *Rhododendron*. Meanwhile, this take up is also well controlled and does not proceed beyond certain top limits, as observed on SP 16.

However, there is a very large scatter in the data, particularly for limestone soils (SP 16). The observations for the plants from the genus *Primula* growing on limestone and non-limestone soils are very similar to the above (SP 17 and 18).

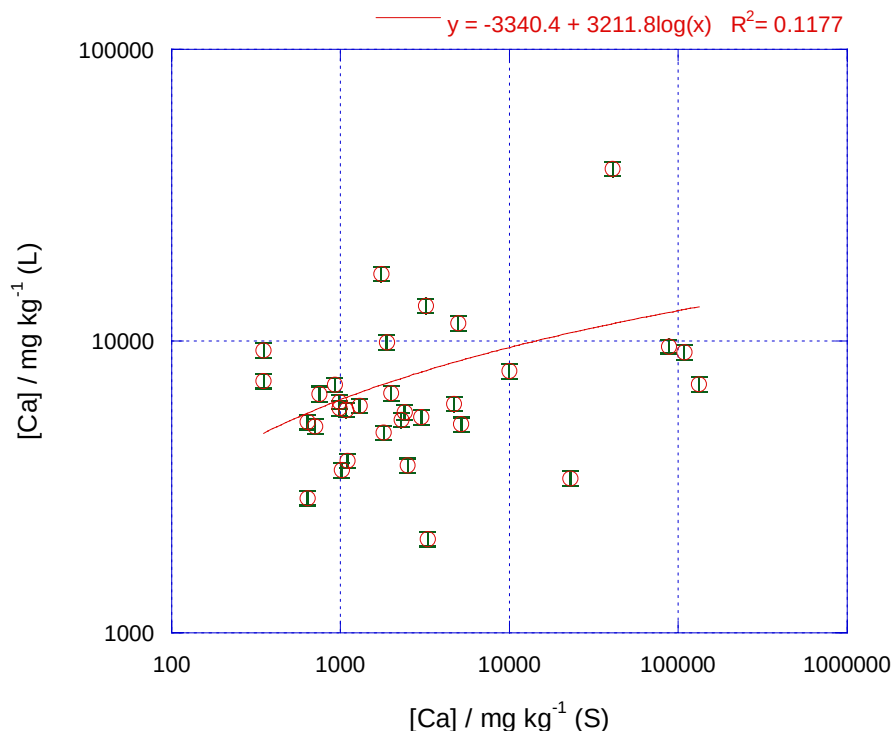


SP 17 Variation of [Ca] (L) with [Ca] (S) for *Primula* plants on limestone.



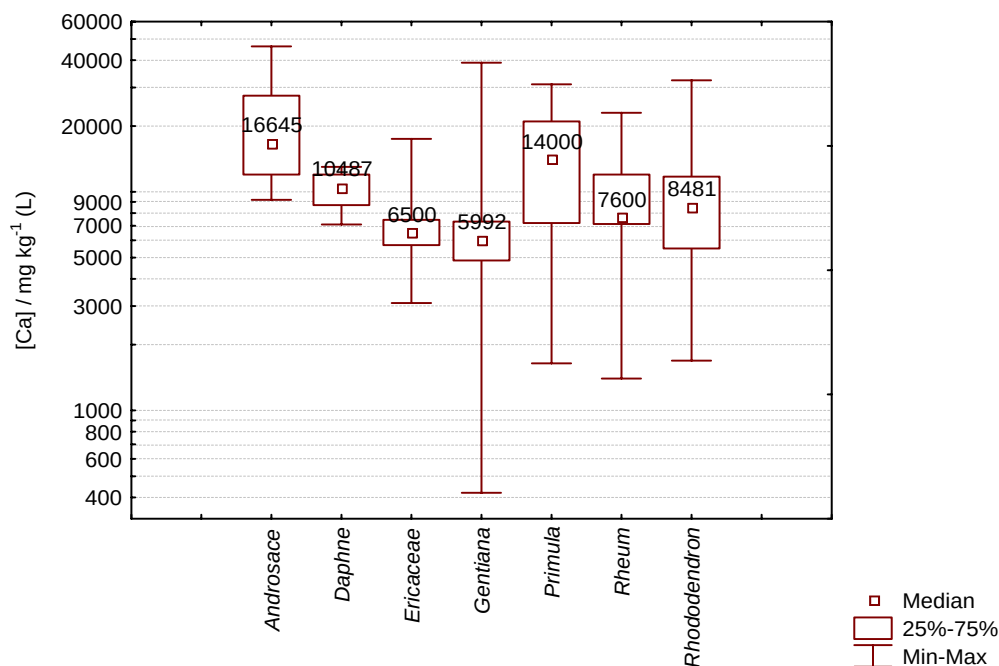
SP 18 Variation of [Ca] (L) with [Ca] (S) for *Primula* plants on non-limestone.

For *Gentiana* plants the observed correlation is that [Ca] in leaves increases with an increase in [Ca] in the soil (SP 19). However, the majority of the samples for *Gentiana* plants showed low soil [Ca], up to around 10000 mg kg⁻¹. As a result, [Ca] in the leaves was also generally lower than the concentrations of Ca observed in the leaves of the plants in other studied genera. This result fits well with the observation that fewer *Gentiana* plants were found growing on Ca-rich soils.



SP 19 Variation of [Ca] (L) with [Ca] (S) for *Gentiana* plants on all types of soil.

Overall, the results for [Ca] in the leaves of plants in all studied genera show that the highest concentrations of Ca in leaves are displayed by the genus *Androsace*, 9000 – 50000 mg kg⁻¹, followed by *Primula*, *Daphne*, *Rhododendron* and *Rheum*, for the latter two the ranges of Ca concentrations are very similar (BP 22). Additionally, in comparison to *Rhododendron* plants, more *Primula* plants had leaves with [Ca] above 10000 mg kg⁻¹ (the middle 50% of [Ca], BP 22). This suggests that *Primula*, together with *Androsace*, tolerate the highest concentrations of Ca in their leaves of all the studied genera.

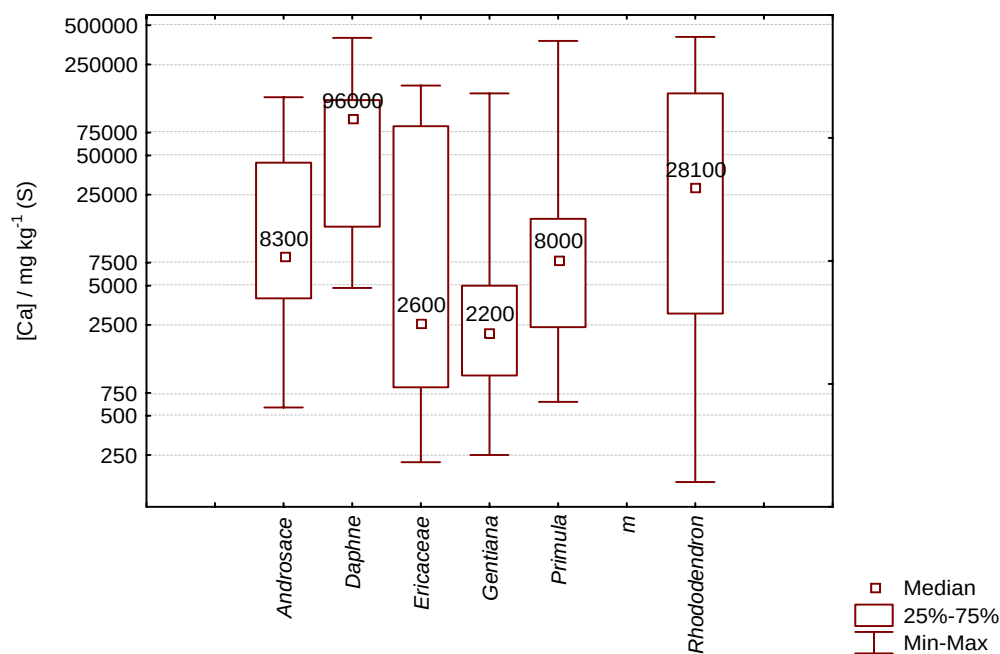


BP 22 Variation of [Ca] in leaves of alpine and *Rhododendron* genera.

On the other hand, the two groups that clearly show the lowest concentrations of Ca in their leaves are *Gentiana*, which also displays a very wide range of [Ca] (L), 400 – 40000 mg kg⁻¹, and the family *Ericaceae* (excluding *Rhododendron*), which includes the genera *Vaccinium*, *Cassiope*, *Erica*, *Kalmia*, *Rhodothamnus*, and *Arctostaphylos*. Despite the evidently lower and narrower range of [Ca] (L) for the group of plants in the family *Ericaceae*, it is still close to the range displayed by *Rhododendron*. This suggests that if a larger number of plants and a wider range of species from the family *Ericaceae* were studied, the range for the group *Ericaceae* may potentially widen and overlap with that for *Rhododendron*.

With respect to Ca concentrations in the soil, the highest observed concentrations are displayed by *Daphne* and *Rhododendron*, reaching the maximum of 400000 mg kg⁻¹ (BP 23). The concentrations of Ca in the soil for *Primula* plants also reach the maximum but more plants (50% of the observations) are observed at lower concentrations, between around 2500 and 18000 mg kg⁻¹. *Androsace*, and some species in the family *Ericaceae*

group also tolerate and grow at high [Ca] in the soil, but not so high as those for *Daphne* and *Rhododendron*. As for *Rhododendron*, the plants in the family *Ericaceae* group appear to grow in a very wide range of [Ca] in soils, which considering the much lower concentrations and much narrower range of [Ca] in the leaves, suggests that the studied species in the family *Ericaceae* group also control the Ca take-up in their leaves very efficiently. The same can be concluded for *Daphne*.



BP 23 Variation of [Ca] in soil of alpine and *Rhododendron* genera.

On the other hand, the observations for *Primula* and *Androsace* show that some of the plants in these genera may absorb concentrations of Ca in their leaves as high as the concentrations at which Ca is present in the soils in which they grow (BP 23, CP 6a, 6b). Although *Gentiana* shows a wide range of [Ca] in the soil, most of the plants were found growing towards the lower limits, between 300 and 3000 mg kg⁻¹. On the other hand, [Ca] in the leaves of *Gentiana* plants is generally higher than [Ca] in the soil, suggesting that although these plants may grow in soils of low [Ca], they still ensure a take-up of

sufficient concentrations in their leaves (BP 23, CP 7a, 7b). Correlations for the genus *Gentiana* are presented in appendix 2.

Overall, the data are consistent with the genus *Gentiana* and the family *Ericaceae* as both include high proportions of lime-hating species. Sampling was not intended to be statistically representative, but nevertheless reflects the natural distribution of plants.

Calcium variation in leaves with time in alpine plants

Being members of the same family *Ericaceae*, as for *Rhododendron* species the species *Kalmia latifolia* showed only a little increase in [Ca] in the leaves with leaf age, from 5500 mg kg⁻¹ in current-year, to 6000 mg kg⁻¹ in one-year-old, and 6500 mg kg⁻¹ in two-year-old leaves. In contrast, *Primula forestii* showed a large increase in Ca concentrations in leaves with age, 14000 mg kg⁻¹ in current-year and 21000 mg kg⁻¹ in one-year-old leaves. This can only suggest that this species is very tolerant to high concentrations of Ca in its leaves, and readily absorbs it. It characteristically grows on limestone-rich soils, and so would usually have the potential to absorb a lot of Ca.

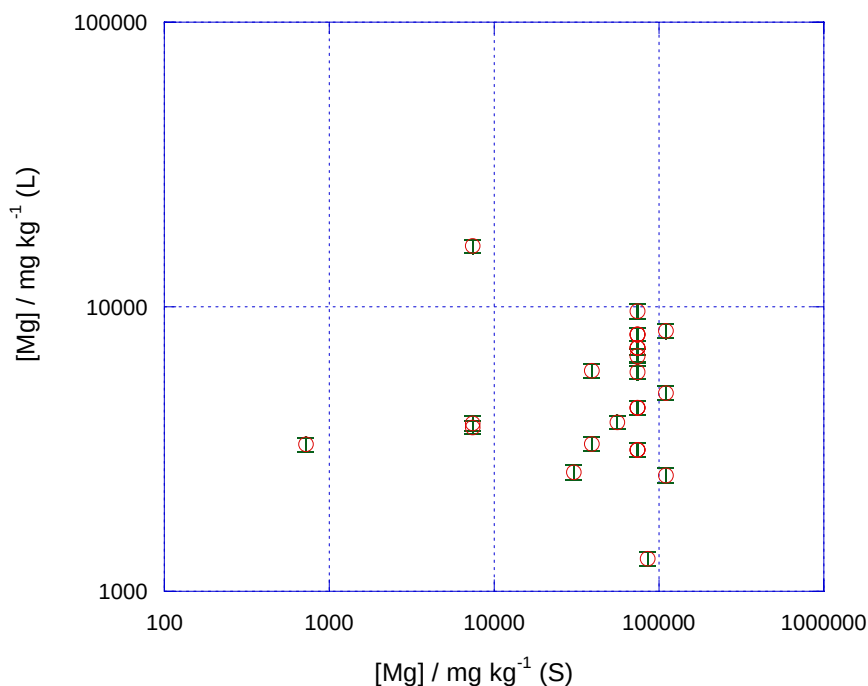
Magnesium soil and leaves for alpine plants

The significant correlations for alpine plants are listed in Table 35.

Table 35 Significant strong correlations for [Mg] in leaves and soils for alpine plants

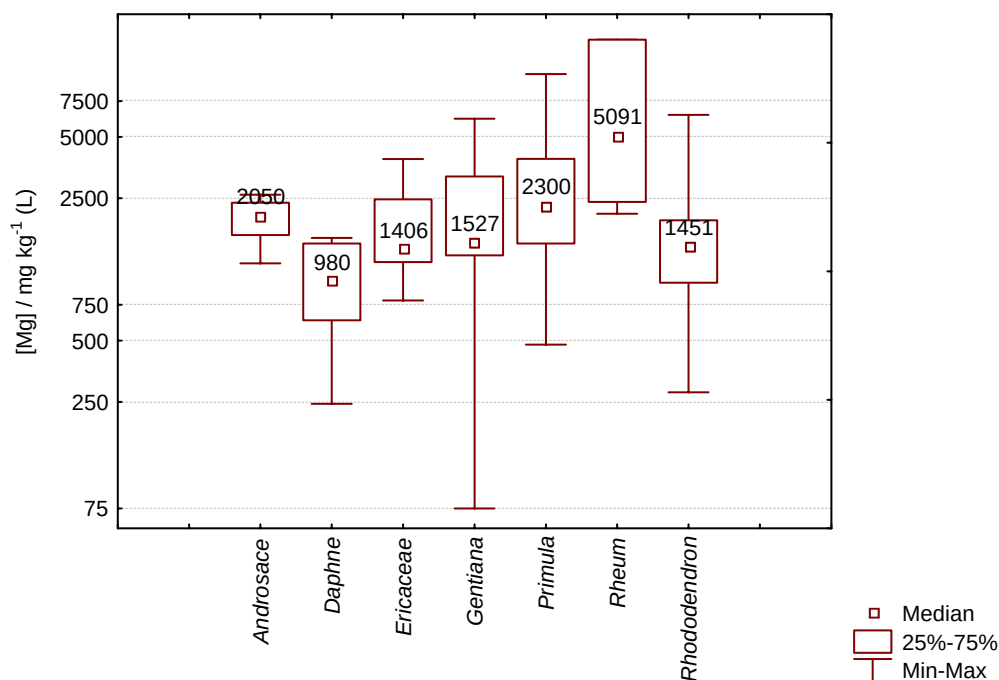
[Mg] (L)			[Mg] (S)		
	dolomitic	non-limestone		dolomitic	non-limestone
soil pH		0.41	soil pH	0.72	0.55
[Ca] (L)	0.65		SOM	-0.64	0.31
[Mg] (S)		0.38	[Ca] (L)		0.43
			[Ca] (S)	0.67	0.89
			[Mn] (S)		0.72

The situation with [Mg] in both soil and leaves for alpine plants is the same as that for *Rhododendron* plants. Although it is present in higher concentrations, on dolomitic limestone soils alpine plants appear to absorb Mg in a controlled way, and not indefinitely, ($r_{\text{Mg(L)}/\text{Mg(S)}} = -0.08$, SP 20).



SP 20 Variation of [Mg] (L) with [Mg] (S) for alpine plants on dolomitic limestone.

It appears that concentrations of Mg in the leaves of most plants are generally within a similar range, 1000 - 5000 mg kg⁻¹. An exception seems to be the genus *Rheum*, which shows much higher [Mg] in the leaves, and especially two plants that were growing on limestone scree, and with concentrations of 15000 mg kg⁻¹ in the leaves (BP 24). However, although this result is interesting it cannot be conclusive, because only five samples from *Rheum* plants were collected.



BP 24 Variation of [Mg] in leaves of alpine plants genera and *Rhododendron*

3.8. Requirements for iron observed for alpine plants in the wild

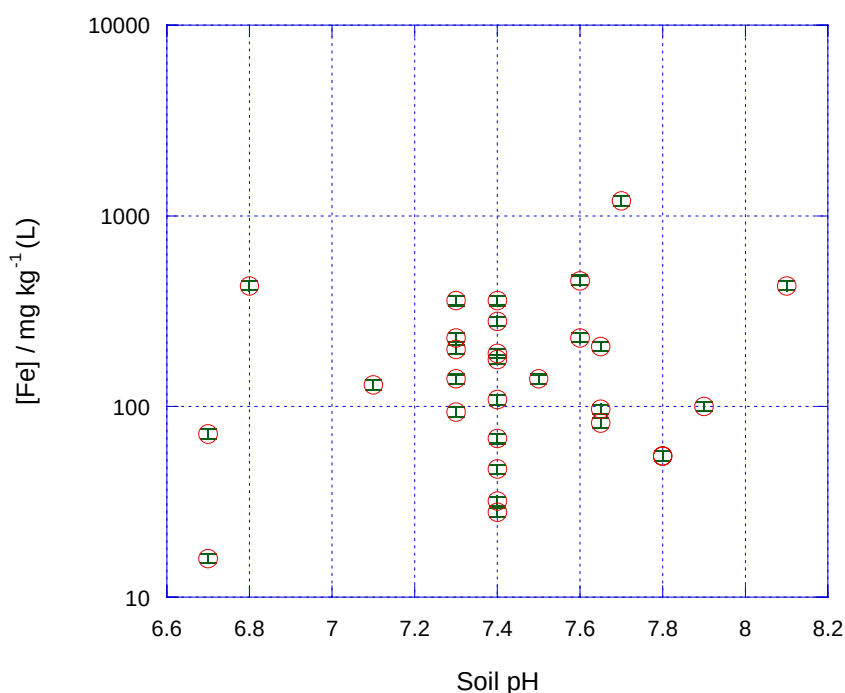
For alpine plants the only significant correlations appear to be between Fe and [Mn] (S): [Fe] (L) correlates negatively with [Mn] (S) and only in dolomitic soil, suggesting that greater [Mn] in the dolomitic soil inhibit the take-up of iron. The concentrations of Fe in the soil on the other hand correlate positively with [Mn] (S), which is a common observation in soil (Table 36).

Table 36 Significant strong correlations for [Fe] in leaves and soils for alpine plants

[Fe] (L)		[Fe] (S)		
	<i>dolomitic</i>		<i>limestone</i>	<i>non-limestone</i>
[Mn] (S)	-0.53	[Mn] (S)	0.53	0.26

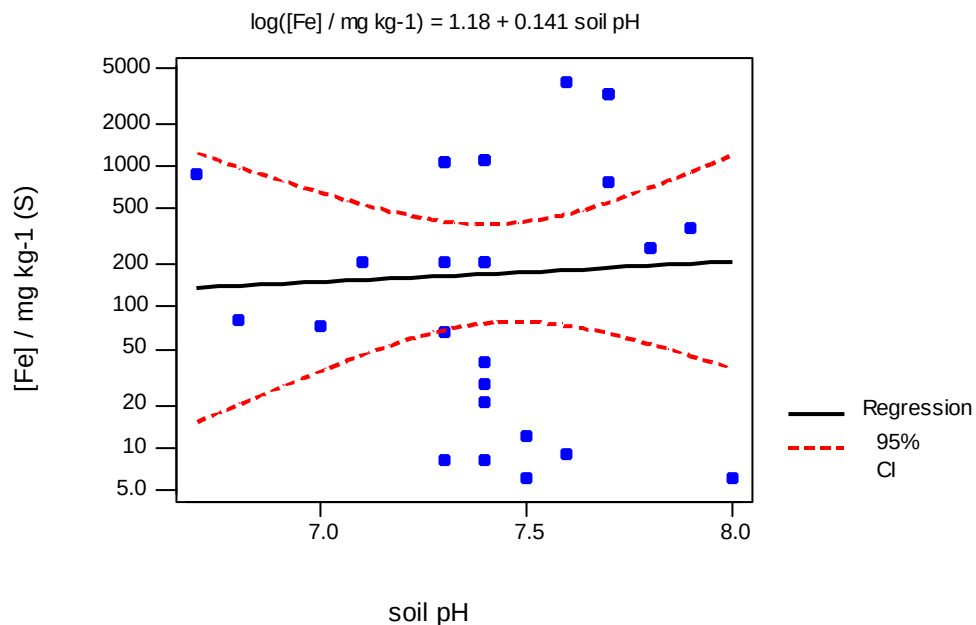
Generally, the analyses for alpine plants growing in any type of soil showed very weak or no correlations at all between leaf and soil [Fe] and soil pH (see correlation matrices E to J in appendix 2).

For alpine plants growing on limestone there is weak insignificant correlation of [Fe] in leaves with soil pH ($r_{\text{Fe(L)/pH}} = 0.24$, SP 21). The minimum concentration of Fe in leaves, around 15 mg kg^{-1} , is observed for one plant only, growing at $\text{pH} < 7.0$. Almost all plants growing on limestone soils have [Fe] in their leaves in sufficient concentrations, above 30 mg kg^{-1} .



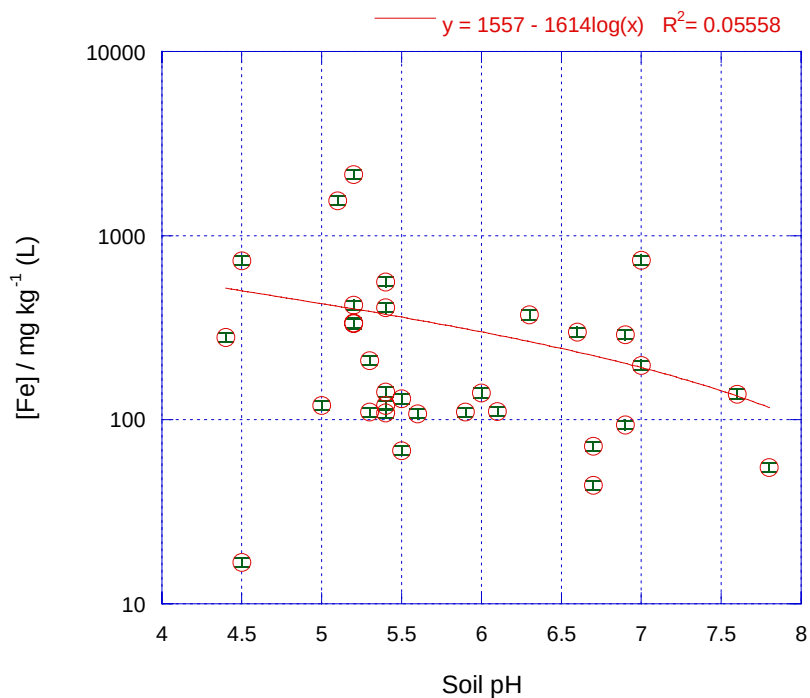
SP 21 Variation of [Fe] in leaves with soil pH for alpine plants on limestone soils.

The concentrations of Fe in limestone soils vary randomly with soil pH. For some soils [Fe] (S) are as low as $5 - 10 \text{ mg kg}^{-1}$, while for others concentrations reach 1000 mg kg^{-1} (LR 9).



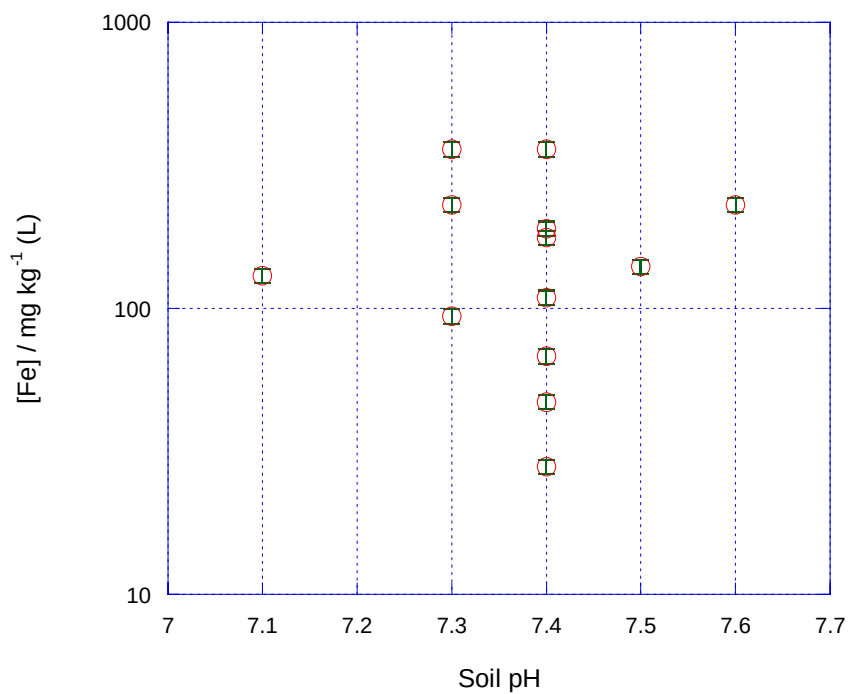
LR 9 Variation of [Fe] in soil with soil pH for alpine plants on limestone soils.

For *Gentiana* plants the [Fe] in the leaves decrease with increasing soil pH, which generally fits the observations made earlier in the discussions that a smaller number of *Gentiana* species are commonly found growing on high-pH soils (SP 22). Despite that, for the *Gentiana* species tolerating high-pH soils, the concentrations of Fe in their leaves are in concentrations sufficient to maintain perfect health, between 50 and 500 mg kg⁻¹ (CP 8b).

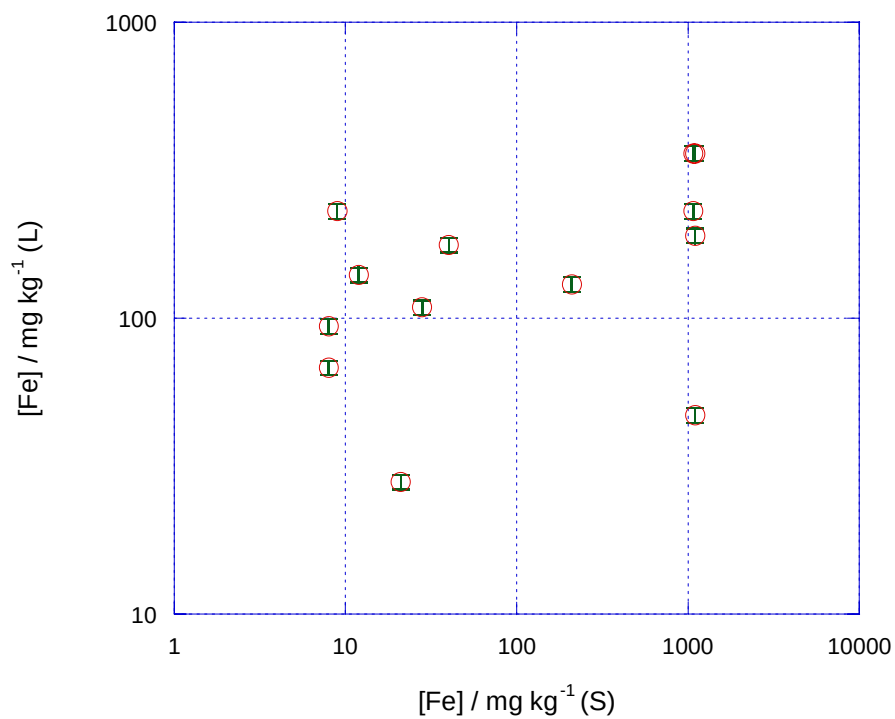


SP 22 Variation of [Fe] in leaves with soil pH for all *Gentiana* plants.

The observations for *Primula* plants are similar. The [Fe] in the leaves growing on limestone soils does not change significantly with increasing the soil pH, and there are sufficient concentrations of Fe in the leaves to support healthy growth, between 20 and 400 mg kg⁻¹ (SP 23 and 24, CP 9).

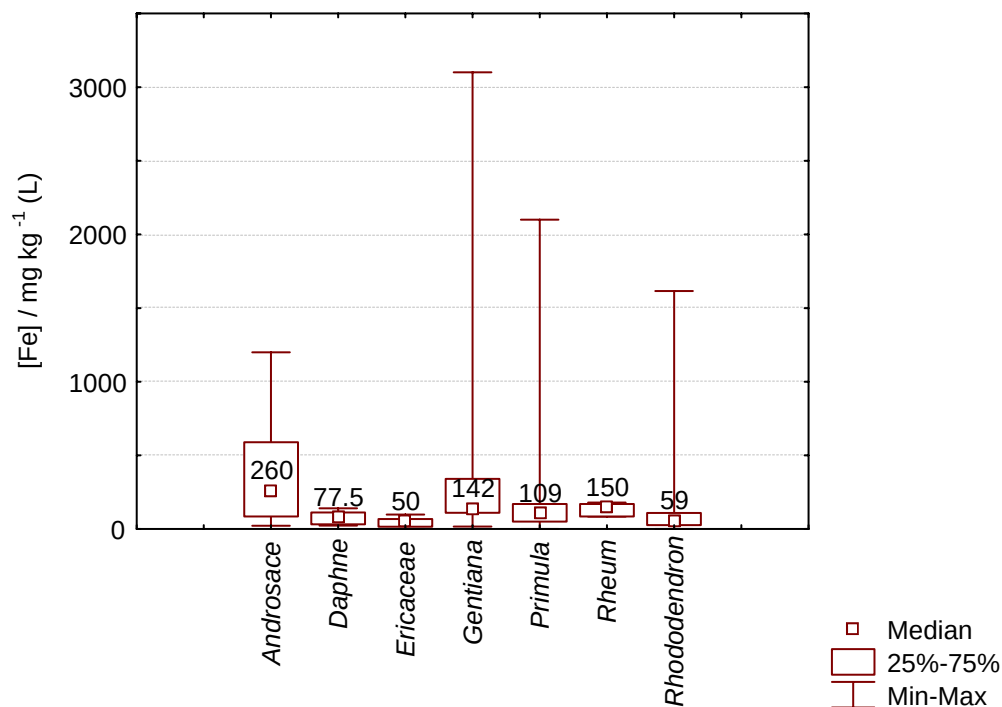


SP 23 Variation of [Fe] (L) with soil pH for *Primula* plants on limestone soils.



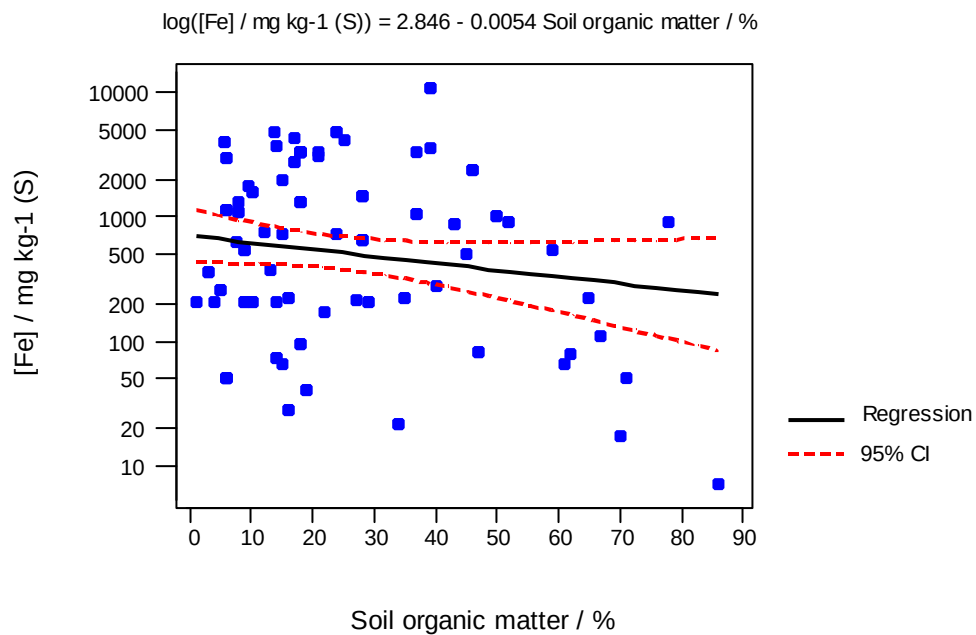
SP 24 Variation of [Fe] (L) with [Fe] (S) for *Primula* plants on limestone soils.

Generally, concentrations of Fe in soils and leaves for all studied alpine plants, growing in all soil conditions, are within the ranges of concentrations required for healthy growth (BP 25). However, there are a very few plants, in several different genera, with exceptionally high concentrations. It is not known what causes these, but it must be noted that there is no apparent negative effect on the health of the plants.

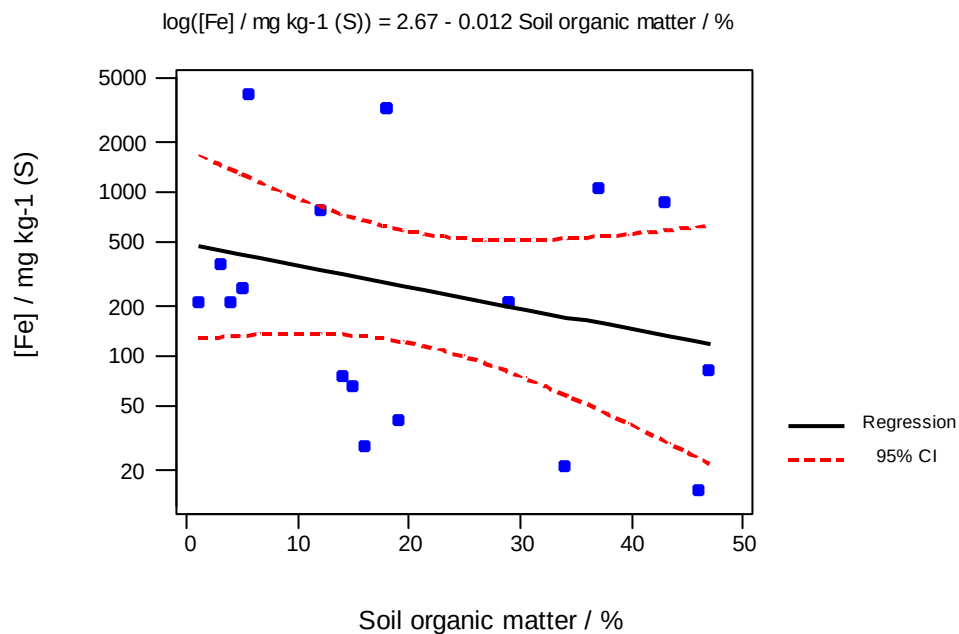


BP 25 Variation of [Fe] in leaves of alpine genera and *Rhododendron*.

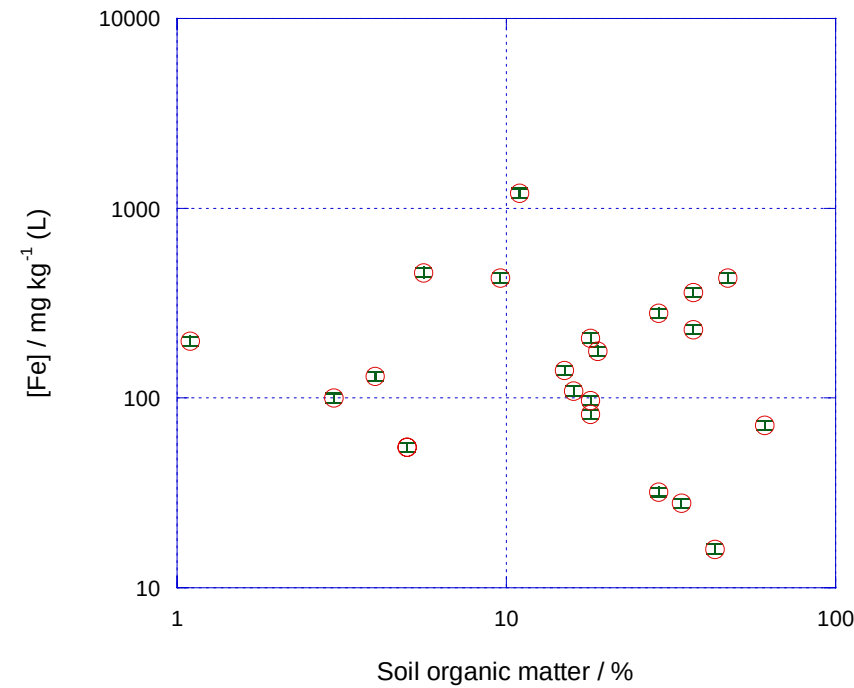
The concentrations of Fe in soils for alpine plants growing on all soils, and on limestone soils in particular seem greater as the soil organic matter decreases (LR 10 and 11). This trend is also observed in the leaves as in limestone soils there is a slight decrease in the absorption of Fe in leaves as the soil organic matter increases (SP 25). This suggests that the available iron in the soil originates from the inorganic (rock) component of the soil.



LR 10 Variation of [Fe] (S) with soil organic matter for all alpine plants.



LR 11 Variation of [Fe] (S) with soil organic matter for alpine plants on limestone soil.



SP 25 Variation of [Fe] (L) with soil organic matter for alpine plants on limestone soil.

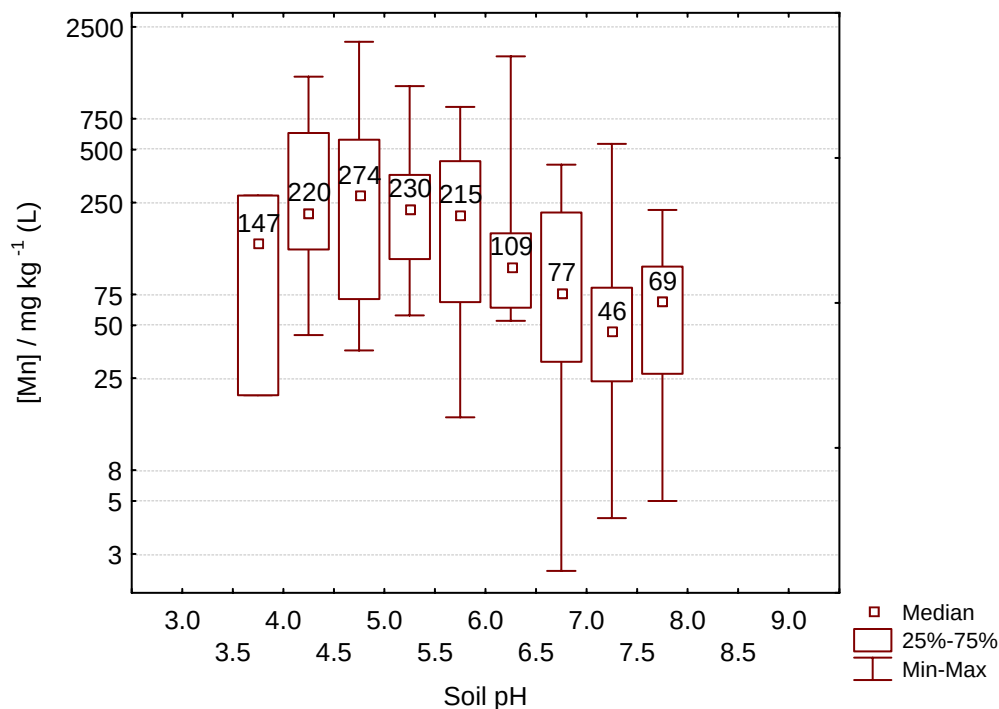
3.9. Requirements for manganese observed for alpine plants in the wild

In agreement with the general trend in the availability of Mn in plants as a response to pH changes, the only significant and strong correlation of [Mn] in the leaves is with the soil pH (Table 37).

Table 37 Significant strong correlations for [Mn] in leaves and soils for alpine plants

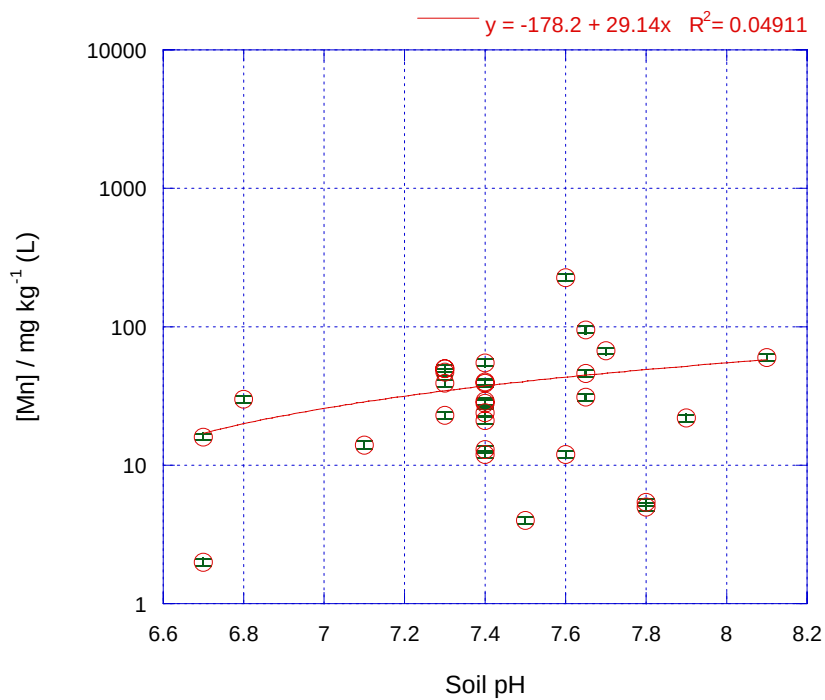
[Mn] (L)			[Mn] (S)		
	<i>limestone</i>	<i>non-limestone</i>		<i>dolomitic</i>	<i>non-limestone</i>
soil pH	-0.61	-0.34	soil pH		0.37
			SOM %	-0.45	

Generally, the concentrations of Mn in the leaves of all alpine plants decrease with increasing the soil pH, $r_{\text{Mn(L)}/\text{pH}} = -0.61$ (BP 26).



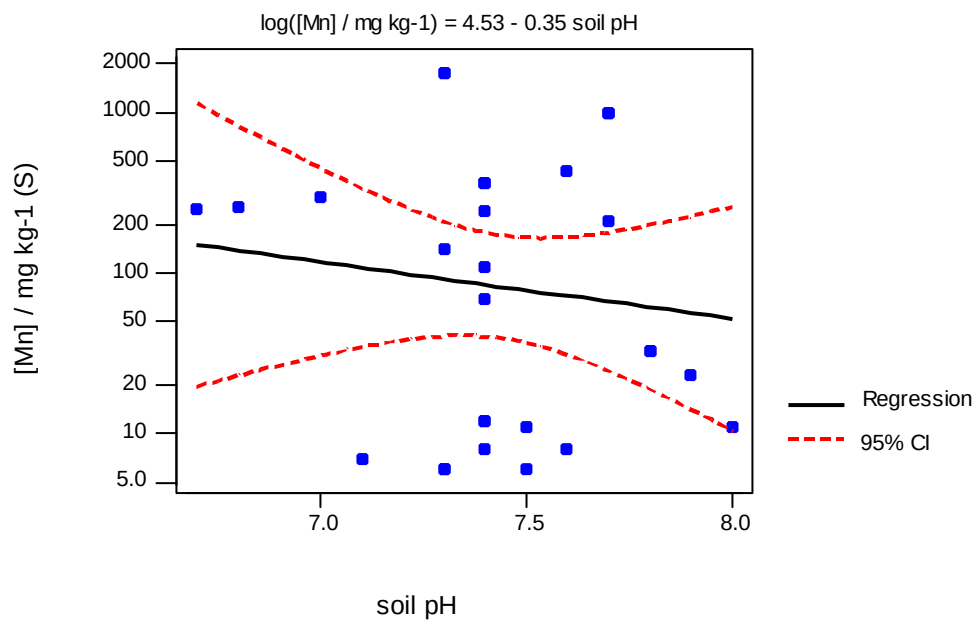
BP 26 Variation of [Mn] (L) with soil pH for alpine plants in all soils.

However, even at high pH 50% of the Mn concentrations are between 22 and 95 mg kg⁻¹. In limestone soils the [Mn] in the leaves of alpine plants varies very broadly with soil pH (SP 26). Despite that [Mn] in some leaves were as low as 2 - 10 mg kg⁻¹, in many other leaves [Mn] was in the range 20 – 200 mg kg⁻¹, which is within the range required for healthy growth. Additionally, the visual observations on the plants that had the low [Mn] showed no evidence of poor health condition.

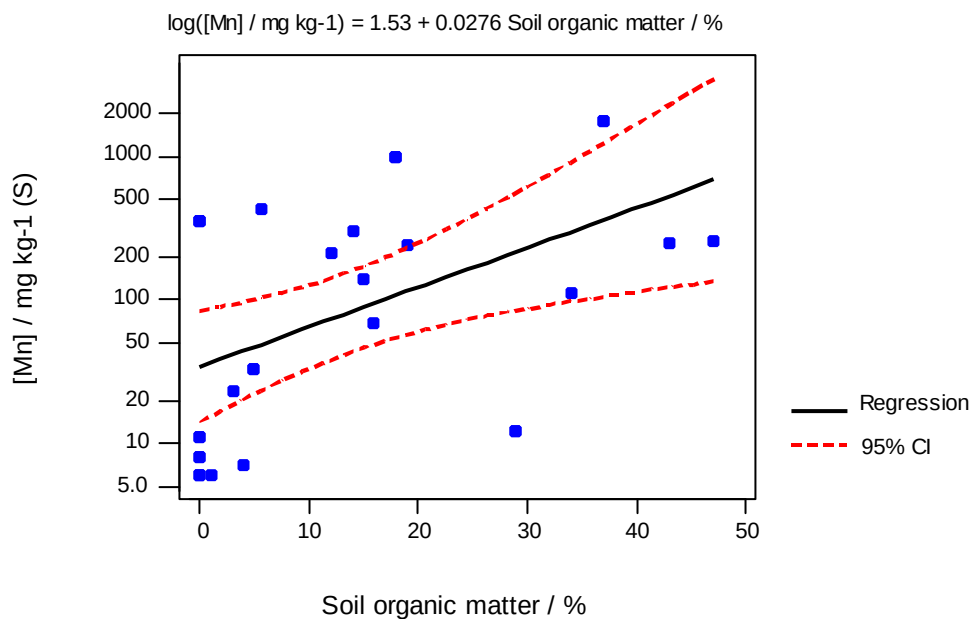


SP 26 Variation of [Mn] (L) with soil pH for alpine plants on limestone soils.

In agreement with the general trend that high pH soils are associated with low content of soil organic matter, the limestone soils here also show low soil organic matter content, below 15%. As a result of the low organic matter present, the high pH limestone soil is also associated with very low soil [Mn], 5 – 30 mg kg⁻¹ (LR 12 and 13).

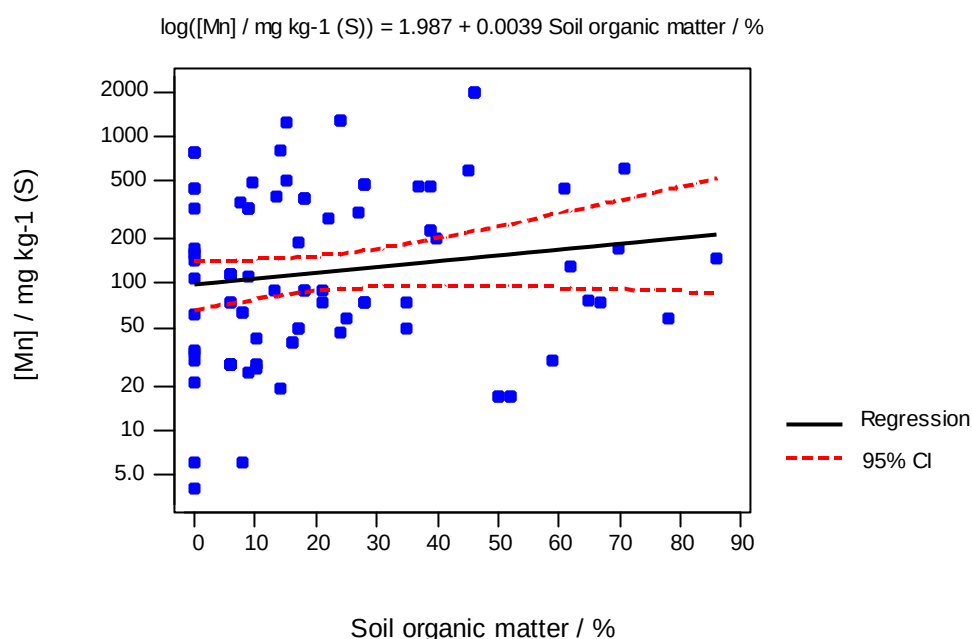


LR 12 Variation of [Mn] (S) with soil pH for alpine plants on limestone soils.



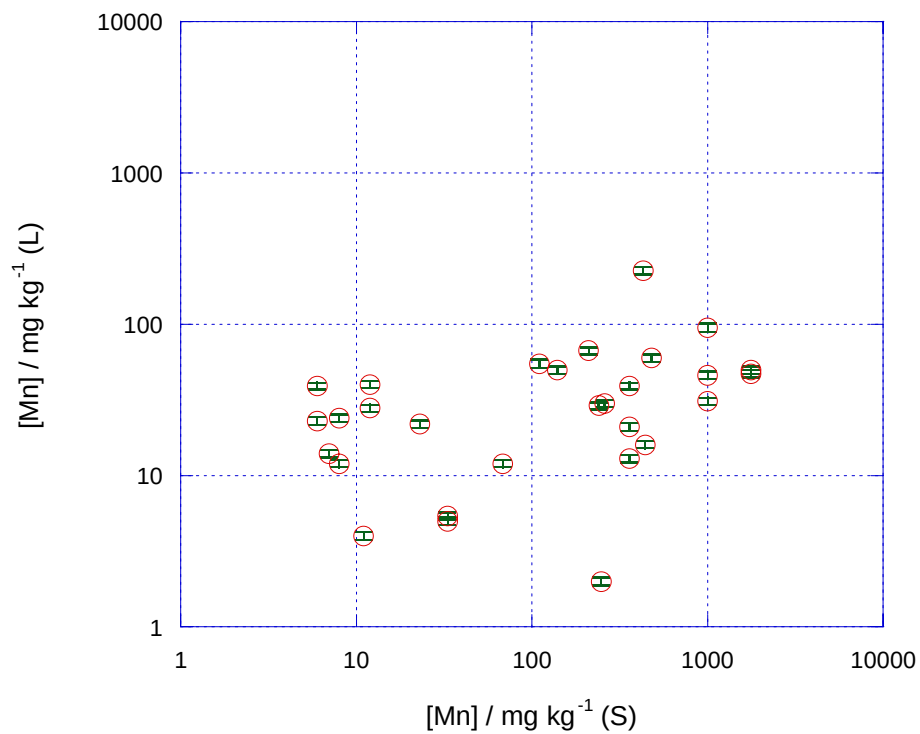
LR 13 Variation of [Mn] (S) with SOM for alpine plants on limestone soils.

High manganese concentrations in the soils in which alpine plants grow are more commonly observed in limestone soils with higher soil organic matter (e.g. places where a thin organic layer covers the limestone) and also in non-limestone soils with low or high organic matter (LR 13 and 14). This matches well with the observation that generally more rhododendrons are found growing in non-limestone soils in which the [Mn] is greater (which include limestone subsoil covered by a thicker organic layer), than in the Mn-poor limestone soil in which there is almost no organic matter present.

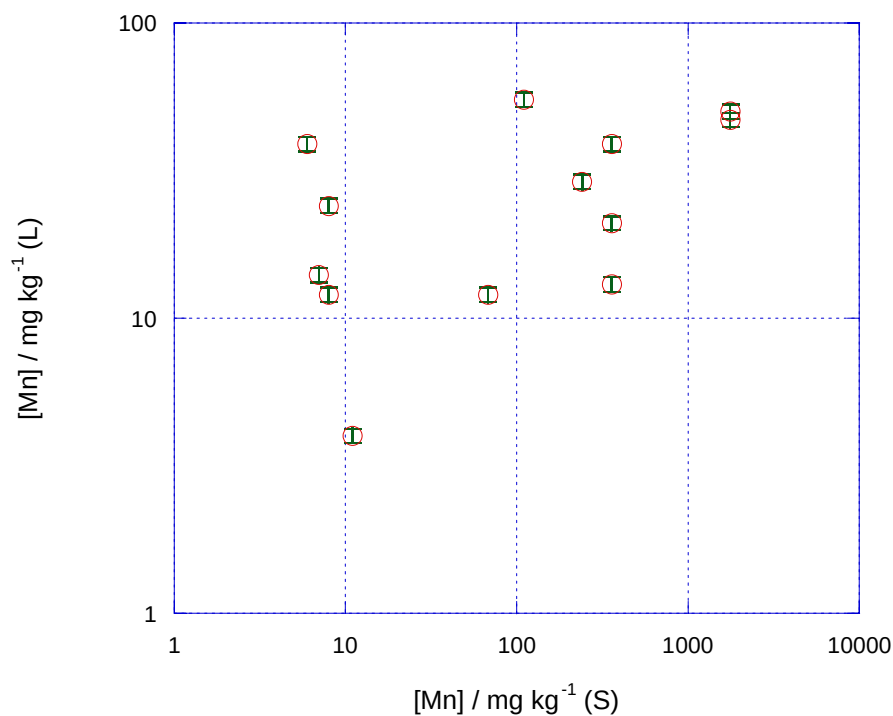


LR 14 Variation of [Mn] (S) with SOM for alpine plants on non-limestone soils.

Another observation is that in limestone soils, even when high [Mn] are present in the soil, the concentrations of Mn observed in the leaves of alpine plants collected from those soils, and in the leaves of *Primula* plants in particular, are only up to 200 mg kg^{-1} , and do not reach concentrations above toxic limits of 300 mg kg^{-1} (SP 27 and 28).

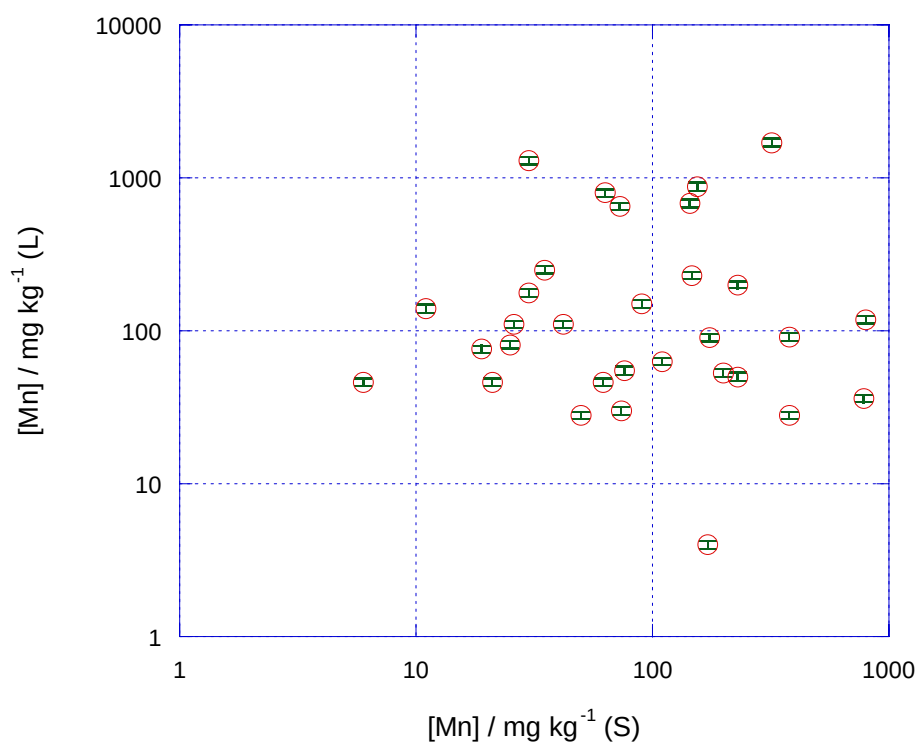


SP 27 Variation of [Mn] in leaves with [Mn] in soil for alpine plants on limestone soils.



SP 28 Variation of [Mn] (L) with [Mn] (S) for *Primula* plants on limestone soils.

On the other hand, *Primula* plants growing on non-limestone soils absorb Mn in the leaves up to concentrations higher than the normal toxic limit, up to 1700 mg kg^{-1} , without any symptoms of toxicity (SP 29, CP 10). Additionally, in some instances the high [Mn] detected in leaves of *Primula* plants were for plants growing in *Rhododendron* forests with low pH soils. It appears that some *Primula* species, and most notably *Primula hongshanensis*, *Primula boreiocaliantha*, *Primula calliantha*, *Primula dryadifolia*, *Primula sonchifolia*, and *Primula muscariodes*, all growing at pH below 6.5, also absorb very high concentrations of Mn in their leaves.



SP 29 Variation of [Mn] (L) with [Mn] (S) for *Primula* plants on non-limestone soils.

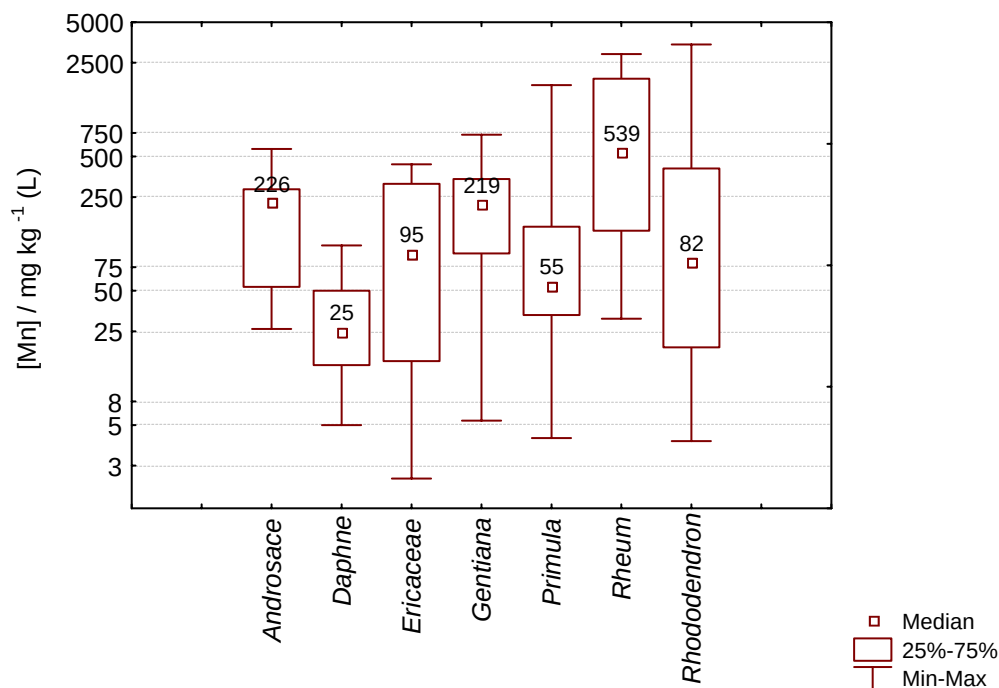
As the species *Primula boreiocaliantha*, *Primula calliantha* and *Primula hongshanensis* are closely related, the above is an indication that for this group of *Primula* ‘relatives’ there is a pattern showing tolerance to [Mn] beyond the toxic limits.

For *Primula dryadifolia* and *Gentiana wardii* [Mn] in their leaves was between 400 and

800 mg kg⁻¹, and the plants were growing on low-pH metamorphic scree with 8% soil organic matter, and [Mn] in the soil of only 63 mg kg⁻¹. This strongly suggests that these two species cope with growing in such poor in nutrients but low-pH soils simply by the adaptation of absorbing high [Mn] in their leaves. Other *Primula dryadifolia*, which were growing in other low-pH soils, also had higher [Mn] in their leaves. In contrast, a *Primula dryadifolia* plant found on a limestone rocky slope, at pH 7.0, with 37% organic matter, and far away from any rhododendrons, had 28 mg kg⁻¹ of Mn in its leaves.

The above suggests that as long as the pH is not too high, this species absorbs high concentrations of Mn in its leaves as a way of adapting to nutrient-poor soils. Conversely, at high pH, due to the lower [Mn] in the soil, and no rhododendrons around, there is also low [Mn] in the leaves of *Primula dryadifolia*. It would be interesting to observe in the future if *Primula dryadifolia* plants, growing in high-pH nutrient-poor soils (such as limestone) and in *Rhododendron*-rich environments, would benefit from the Mn-rich decaying leaves of the surrounding rhododendrons, and also absorb Mn at the high concentrations they attain in low-pH nutrient-poor soils. Decaying *Rhododendron* leaves might also help cultivation of the other high-[Mn] *Primula* species in soils with high pH.

High concentrations of Mn in leaves were also observed for some *Rheum*, *Androsace*, *Galax*, *Pulsatilla*, *Gentiana* and *Ericaceae* plants (BP 27, CP 11, 12, 13 and 14). Most plants with high [Mn] in the leaves were growing at low pH, usually in the vicinity of rhododendrons. The *Rheum* plants, however, were growing on high-pH limestone and metamorphic screes, again away from *Rhododendron* plants.



BP 27 Variation of [Mn] in leaves of alpine and *Rhododendron* genera.

Manganese distribution with time in alpine plants

The variable ‘Age of leaf’ here is not studied in depth, since most alpine plants are herbaceous and only occasionally leaves older than current year can be observed. Despite this, leaf samples of the current-year, one-year-old and two-years-old were collected for a shrub from the *Ericaceae* family *Kalmia latifolia*, and current-year and one-year-old leaves were collected for one *Primula forestii* plant.

In agreement with the previously shown ability of other plants in the *Ericaceae* family¹¹ and *Rhododendron* plants to accumulate Mn in their leaves, the [Mn] concentrations in the leaves of *Kalmia latifolia* also reaches limits higher than the healthy limits for normal plants, and it also increases with the age of the leaf, from 313 mg kg⁻¹ in current-year-old, to 366 mg kg⁻¹ in one-year-old, and 437 mg kg⁻¹ in two-year-old leaves (CP 15).

This indicates that as for *Rhododendron*, *Kalmia* species potentially also absorb excess

Mn and store it in their leaves. However, to confirm this, more leaf samples will be needed for further analyses.

The situation with *Primula forestii*, however, is different: first, Mn is not absorbed to high concentrations, only 50 mg kg⁻¹ in current-year and 47 mg kg⁻¹ in one-year-old leaves, and secondly, there is clear evidence that in this particular plant it is not accumulated in the leaves with age. This again is not a conclusive result, as more plants need to be studied, but it still provides some direction for Mn requirements of *Primula* plants. In contrast, as already observed that *Primula* plants absorb high concentrations of Fe in their leaves, which increase with increasing the soil pH, the *Primula forestii* plant showed very high concentrations of Fe in the leaves. However, [Fe] was not observed to be increasing with the age of leaves (CP 15).

Discussions of results for growing experiments with *Rhododendron* and *Gentiana* species

The *R. coeleureuron* plants were supplied in originally not very good health. Most leaves had brown, dry tips. Some plants had suffered frost or fungal damage, and others had yellow leaves. All plants from *R. impeditum*, *R. hirsutum* and *G. sino-ornata* were in perfect health from the start of the experiment.

For all four studied species, calcium and magnesium concentrations were within the normal ranges of concentrations required for healthy growth, as the results from the *Rhododendron* species in the wild also showed. Therefore, Ca and Mg were not discussed in relation to the growing experiments.

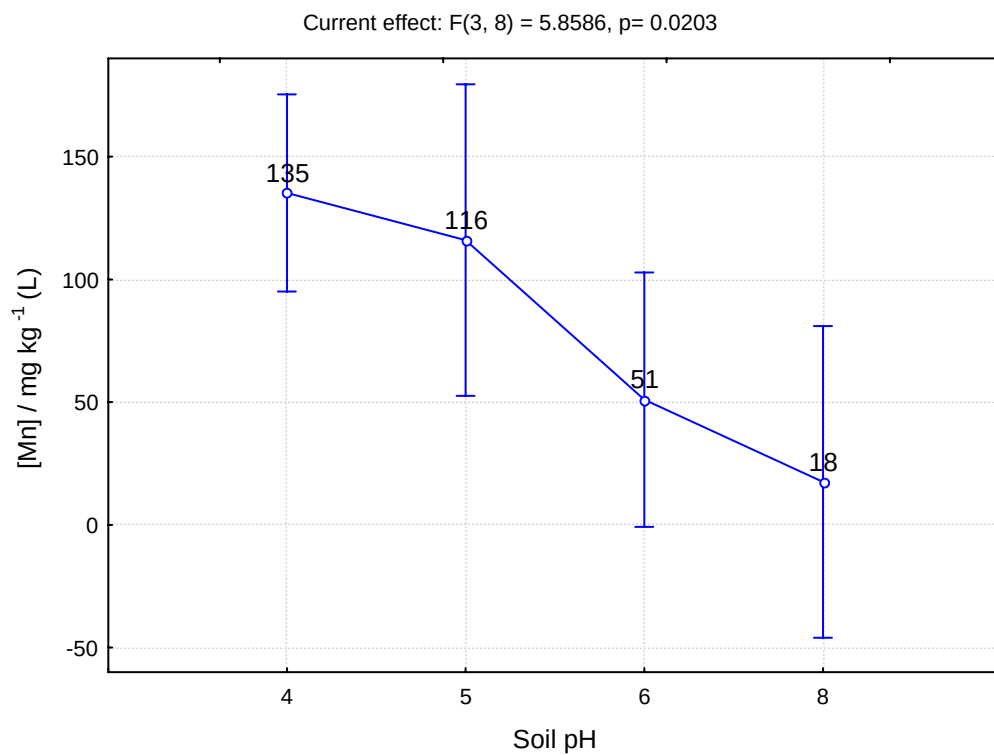
3.10. Discussions of results for *Gentiana sino-ornata*

The only significant correlations observed for the *Gentiana sino-ornata* plants are of [Ca] (L) and [Mn] (L) with soil pH (CM 6).

Correlation matrix 6 Correlations *Gentiana sino-ornata* plants.

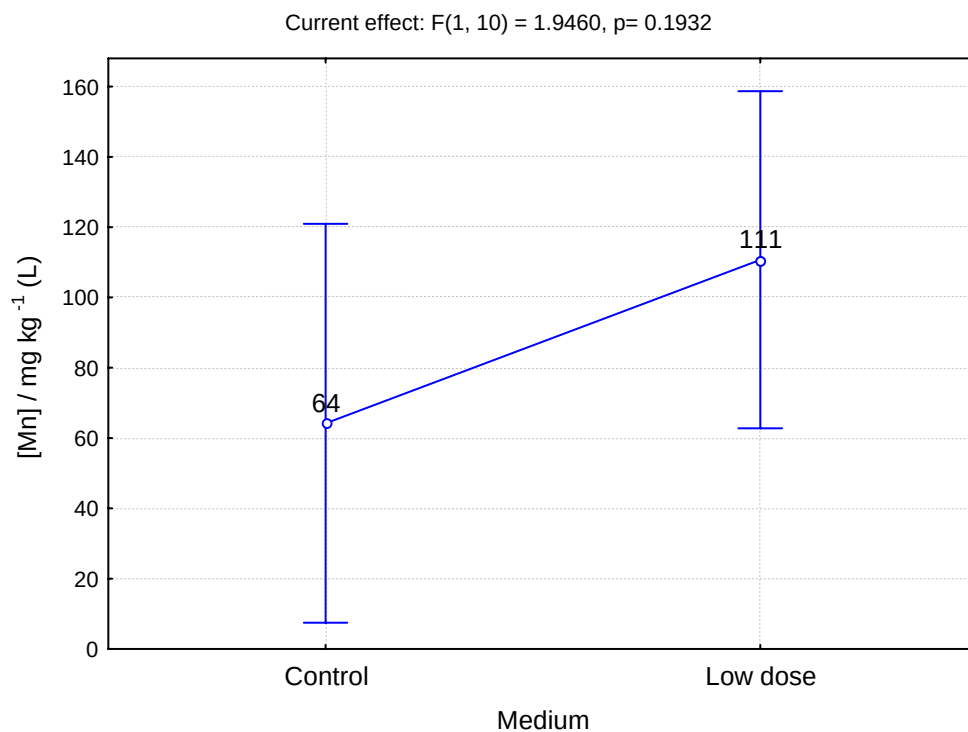
	Medium	Soil pH	[Ca] (L)	[Fe] (L)	[Mg] (L)	[Mn] (L)
Medium	1.00					
Soil pH	-0.35 p = 0.285	1.00				
[Ca] (L)	-0.39 p = 0.233	0.72 p = 0.012	1.00			
[Fe] (L)	0.02 p = 0.947	0.38 p = 0.249	0.48 p = 0.132	1.00		
[Mg] (L)	0.13 p = 0.70	0.15 p = 0.660	0.40 p = 0.218	-0.02 p = 0.936	1.00	
[Mn] (L)	0.54 p = 0.083	-0.79 p = 0.004	-0.57 p = 0.065	-0.10 p = 0.769	0.19 p = 0.573	1.00

G. sino-ornata is a species generally intolerant to alkaline soil and the results from the growing experiment confirmed that it cannot grow at high pH. Three weeks after being planted the *G. sino-ornata* plants in the media of pH 6.7 and 8.0 began developing the typical symptoms of Fe or Mn deficiency, i.e. chlorosis, yellowing of the leaves. After Week 3 most plants grown at pH = 8.0 in both media control and low dose manganese application died, and those that were still alive were in a bad health. In support to the significance of the correlation between [Mn] (L) and soil pH (p = 0.004), ANOVA analysis demonstrates the clear and consistent decrease of [Mn] in the leaves as the pH increases (ANOVA 5, CP 16).



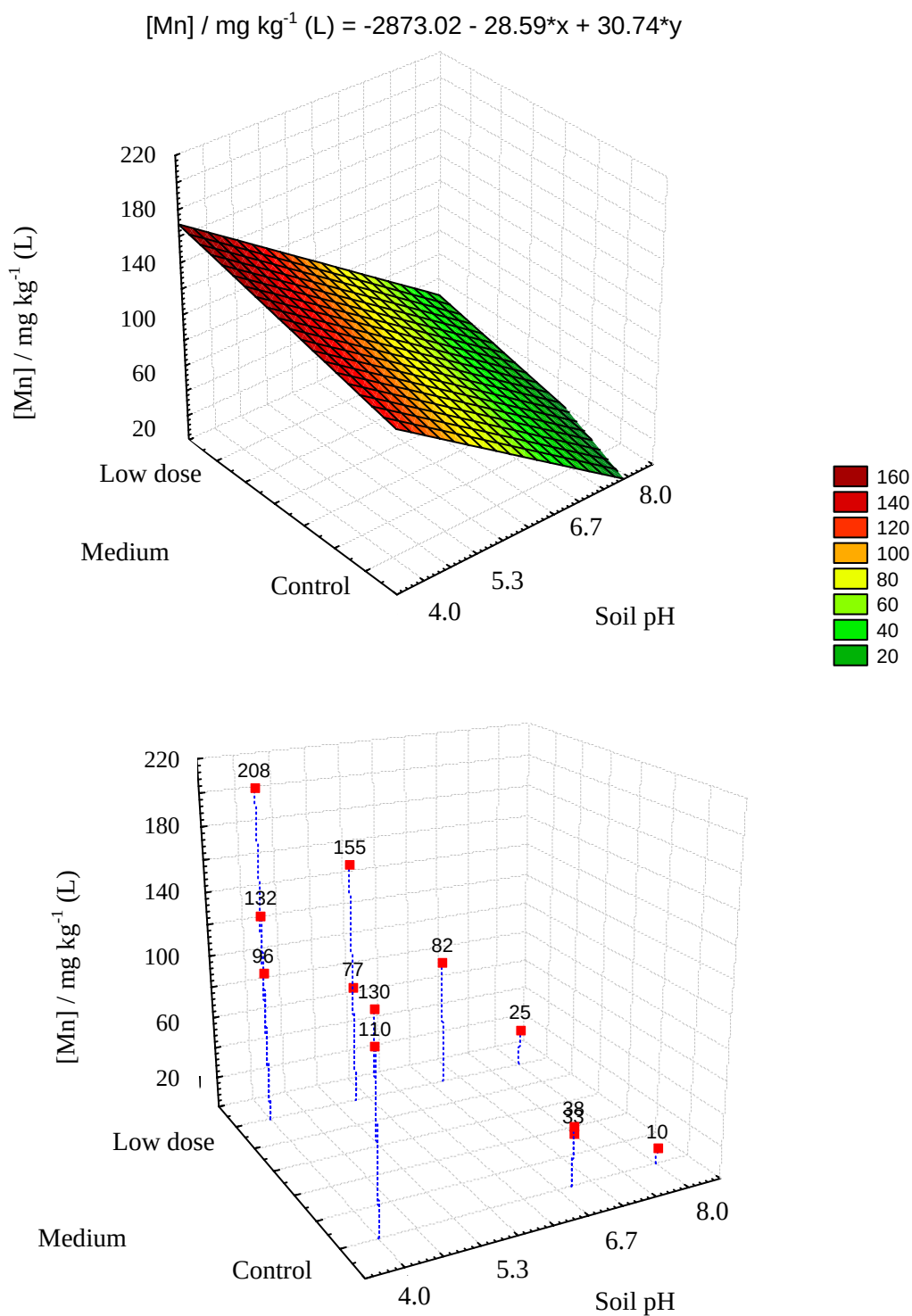
ANOVA 5 Variation of [Mn] (L) in *G. sino-ornata* leaves with soil pH.

The low dose medium supplement of Mn increased noticeably the [Mn] in the leaves from the control medium (ANOVA 6).



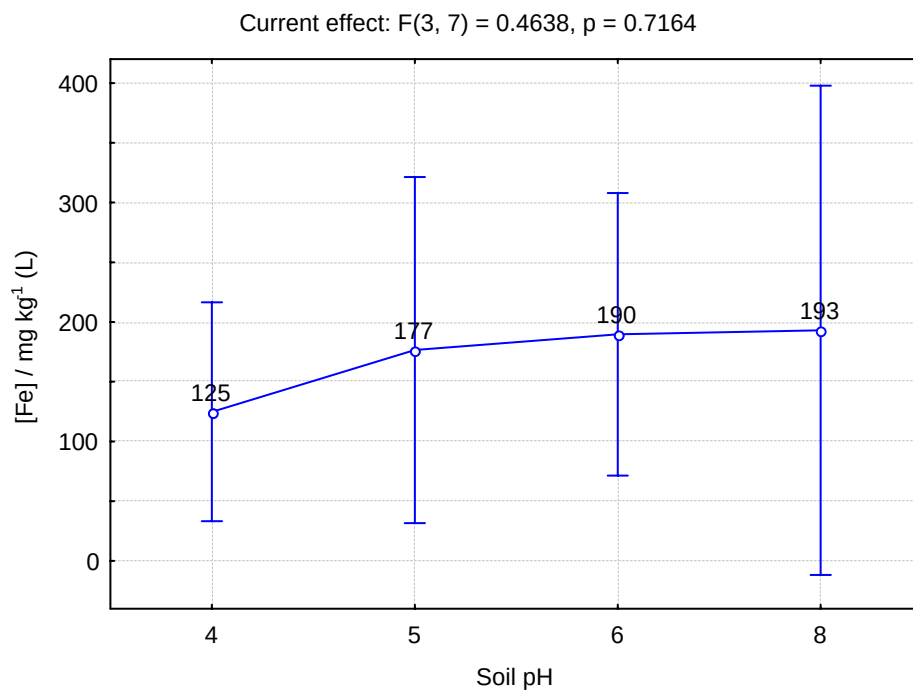
ANOVA 6 Variation of [Mn] (L) in *G. sino-ornata* leaves with soil pH.

However, the deteriorating condition of the plants in both media, resulting in death of most plants at pH 8.0, indicates that although higher the concentrations of Mn supplied via the low dose medium were perhaps still too low to maintain healthy growth of *G. sino-ornata* at pH above 6.7 (SP 30).

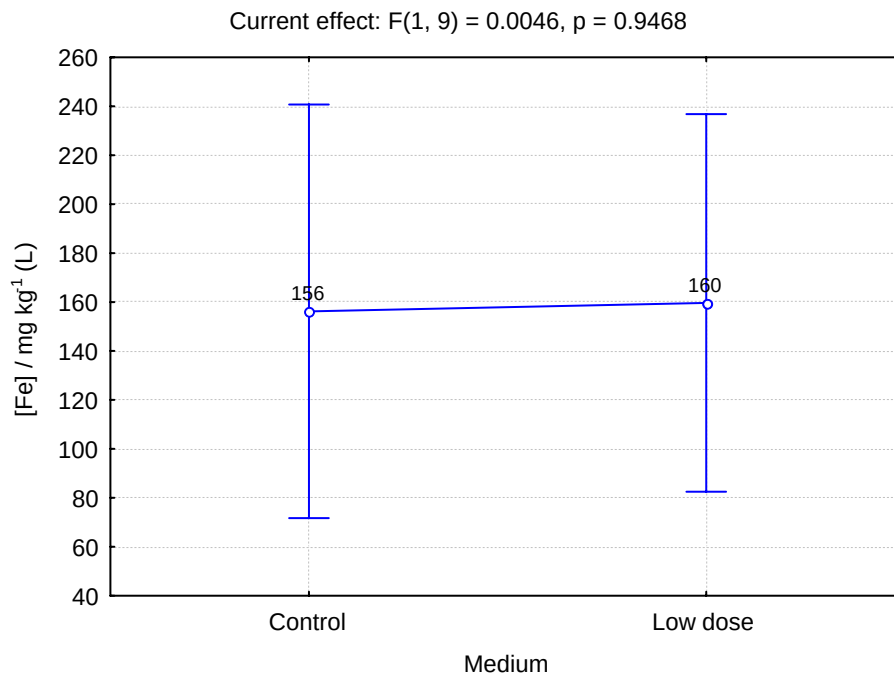


SP 30 Variation of [Mn] in leaves with soil pH for *G. sino-ornata* in all media.

Although there is no change in [Fe] (L) of *G. sino-ornata* plants between the two media, the concentrations of Fe in the leaves in both media appear to increase with increasing soil pH (ANOVA 7 and 8, CP 16 see Appendix 3).

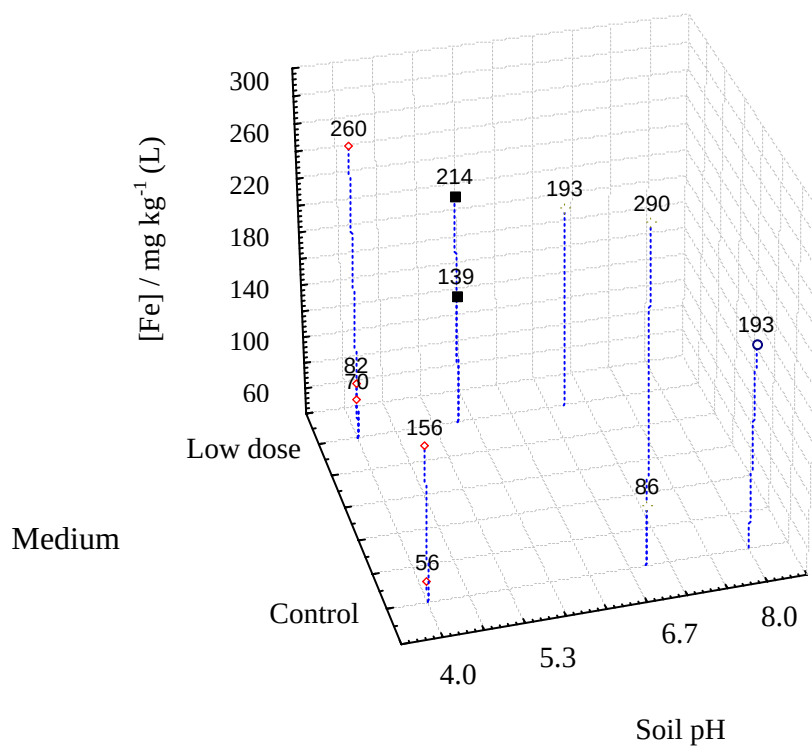
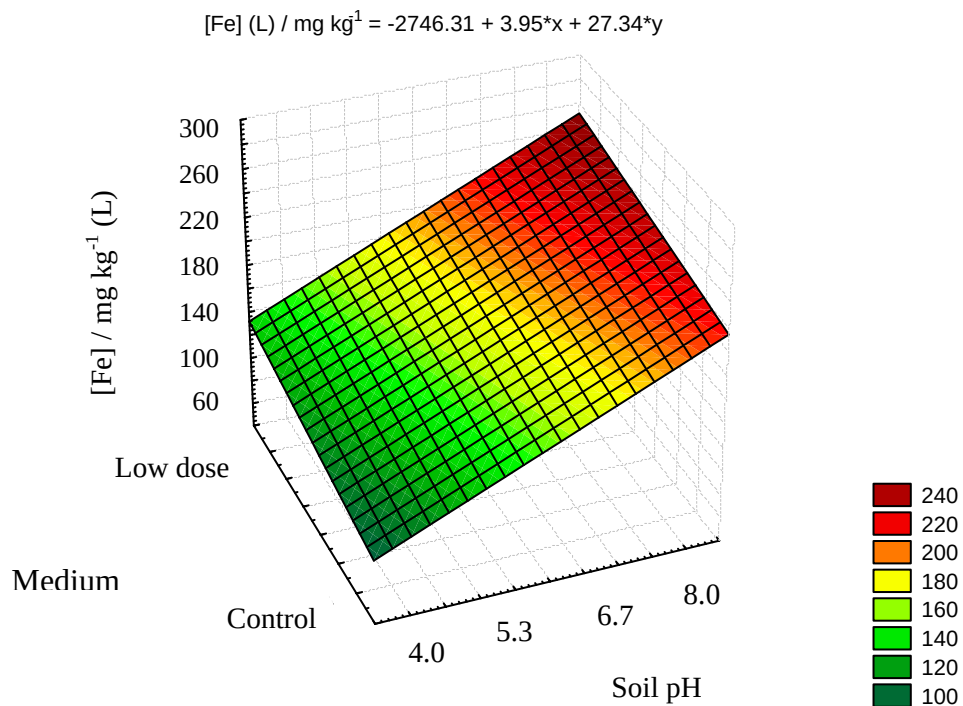


ANOVA 7 Variation of [Fe] (L) in *G. sino-ornata* leaves with soil pH.



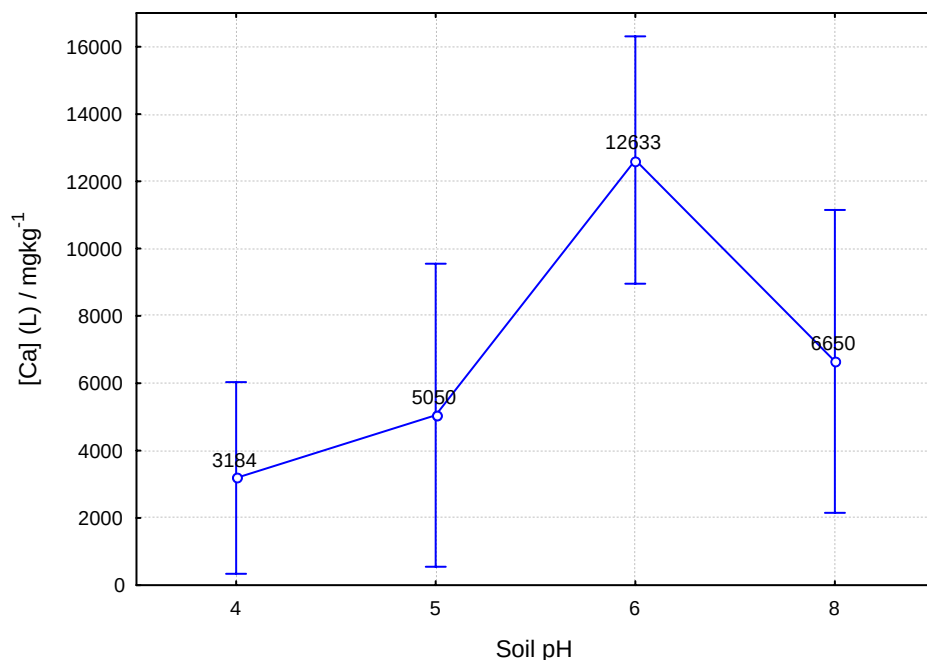
ANOVA 8 Variation of [Fe] (L) in *G. sino-ornata* leaves with soil medium.

Generally, in the leaves of *G. sino-ornata*, in all pH the [Fe] are high and sufficient to support healthy growth, between 60 and 300 mg kg⁻¹ (SP 31). Considering the very low concentrations of Mn found in the leaves of *G. sino-ornata*, there is clear evidence that the ‘lime-hating’ character of this species is a consequence of potential manganese deficiency, not iron deficiency.



SP 31 Variation of [Fe] in leaves with soil pH for *G. sino-ornata* in all media.

As mentioned above, the correlation between [Ca] (L) and soil pH is also significant, with $r_{[\text{Ca}] (\text{L})/\text{pH}} = 0.72$ (ANOVA 9). However, the concentrations of Ca in the leaves are still in the range required for a healthy growth.



ANOVA 9 Variation of [Ca] (L) in *G. sino-ornata* leaves with soil pH.

Overall, it appears that Fe does not cause a threat in the cultivation of *G. sino-ornata* in moderately alkaline soil. Provided enough Mn is supplied in the soil *G. sino-ornata* may successfully be cultivated in high-pH or at least moderately alkaline soils in the future.

3.1. Discussions of results for *Rhododendron coeloreuron*

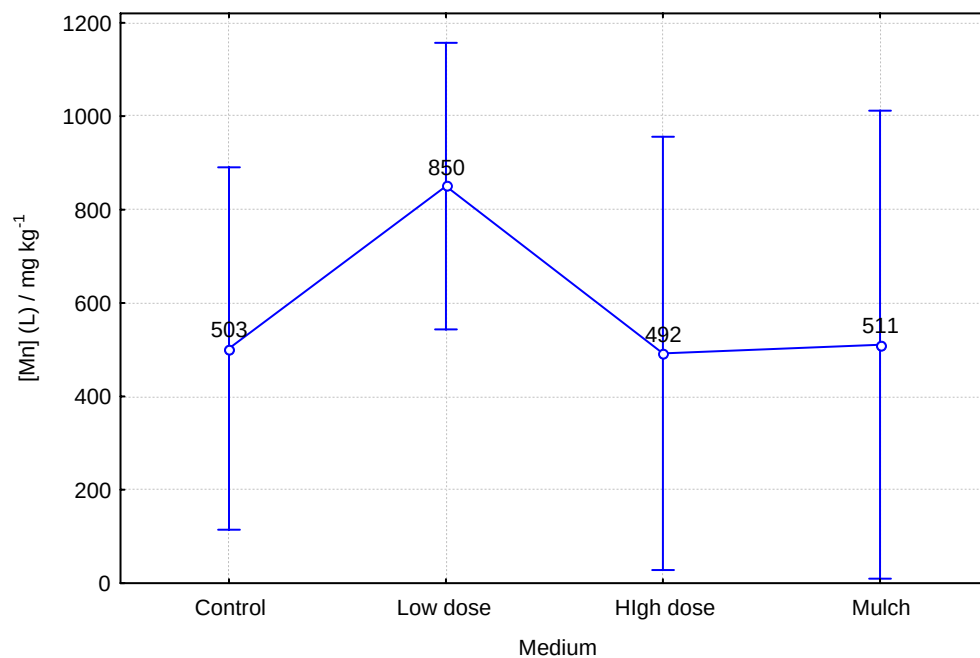
For *R. coeloreuron* significant correlations are observed for [Ca] (L) and [Mn] (L) and leaf age (CM 7), as this result was already established for the rhododendrons growing in the wild. There is also a strong significant correlation between [Ca] (L) and [Mn] (L) and not so strong one with [Mg] (L), indicating that Mn and Mg concentrations increased with increasing Ca concentrations in the leaves of this species.

Correlation matrix 7 Correlations for *R. coeloreuron* plants.

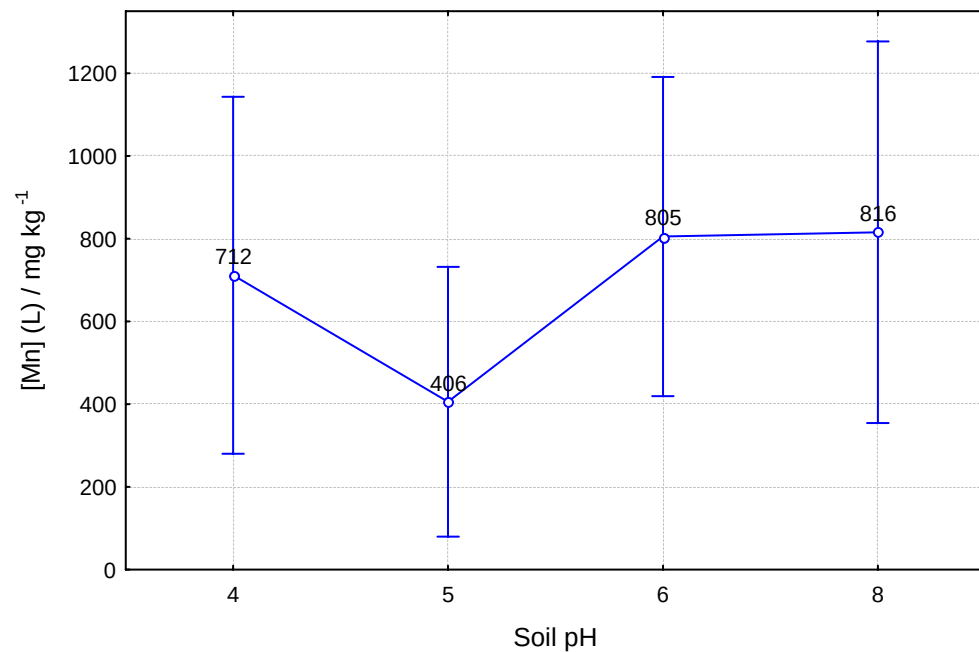
	Medium	Soil pH	Leaf age	[Ca] (L)	[Fe] (L)	[Mg] (L)	[Mn] (L)
Medium	1.00						
Soil pH	-0.14 p = 0.381	1.00					
Leaf age	-0.16 p = 0.314	0.30 p = 0.062	1.00				
[Ca] (L)	-0.20 p = 0.200	-0.16 p = 0.308	0.55 p = 0.000	1.00			
[Fe] (L)	0.05 p = 0.747	0.18 p = 0.262	0.10 p = 0.540	0.07 p = 0.666	1.00		
[Mg] (L)	-0.02 p = 0.873	-0.22 p = 0.169	-0.17 p = 0.294	0.34 p = 0.030	0.07 p = 0.667	1.00	
[Mn] (L)	-0.05 p = 0.745	0.15 p = 0.356	0.72 p = 0.000	0.71 p = 0.000	0.21 p = 0.181	0.06 p = 0.681	1.00

The plants grown in the low dose media show higher [Mn] in their leaves than the plants grown in the control media, as expected. The [Mn] in some leaves in the control media, however, were higher than those in the high dose media (ANOVA 10, CP 17). This difference may be caused as a result of the application of the Mn-solution to the high dose media: the solution was added in three portions over 5 days, which sometimes coincided with rainfall. As a result, the solution applied within such a short period of time may have partially drained out of the pots. In the same way, the [Mn] (L) in the mulch media do not differ from those in the control media. It is difficult to predict a definite and strong increase in [Mn] (L) as a result of absorption from Mn-rich decaying mulch leaves, so it is difficult to know whether the plants in the mulch media showed low or high concentrations of Mn compared to any of the other media.

There appears to be no correlation between the [Mn] and soil pH between the media, as the concentrations of Mn in pH 5.3 media, for instance, are much lower and in a narrower range than those in the pH 4.0, 6.7 and 8.0 media (ANOVA 11).



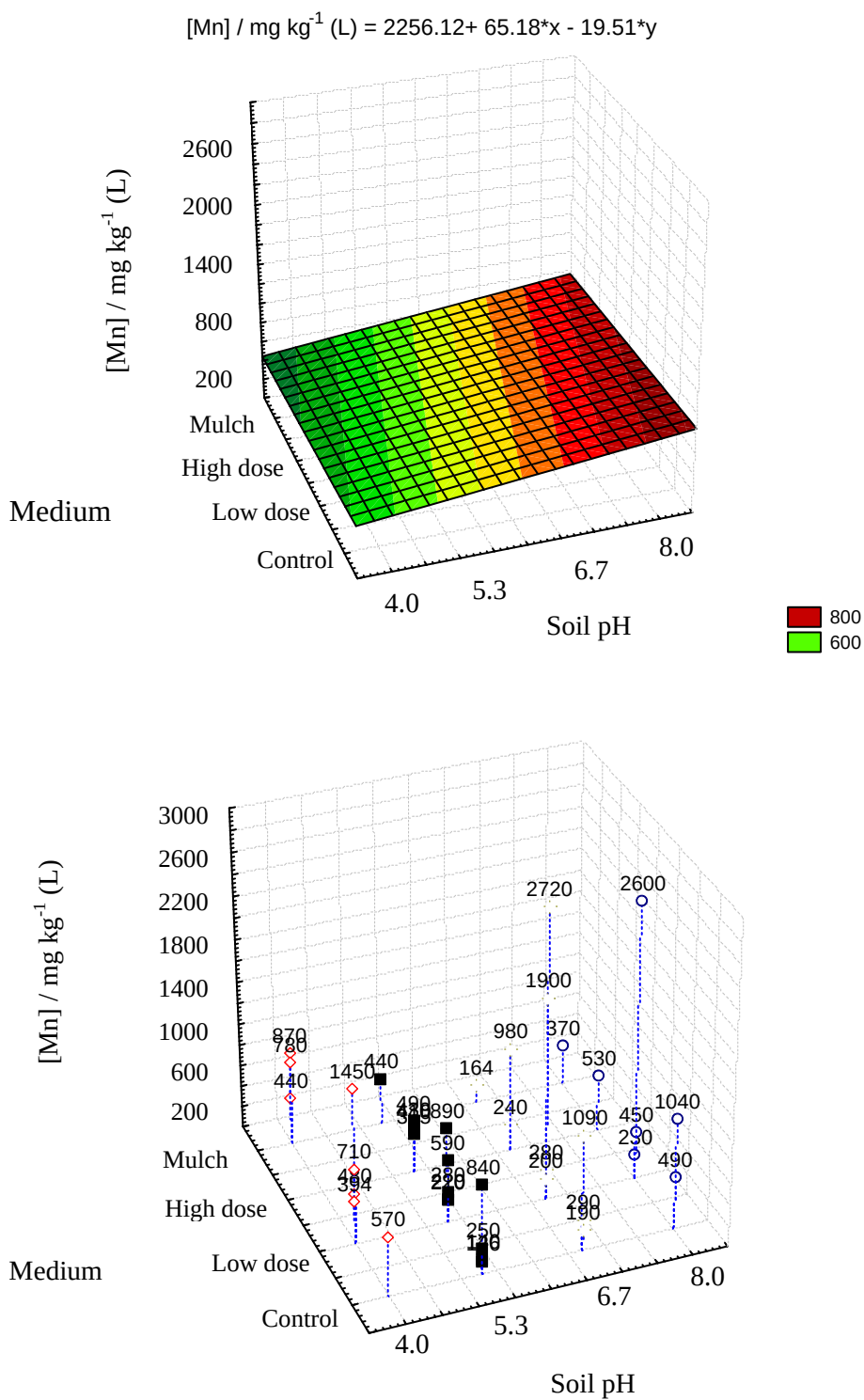
ANOVA 10 Variation of [Mn] in leaves of *R. coeloreuron* with soil media.



ANOVA 11 Variation of [Mn] in leaves of *R. coeloreuron* with soil pH.

Generally, Mn in the leaves of *R. coeloreuron* in all media is in very high concentrations, 120 – 2700 mg kg⁻¹, sufficient for healthy growth (SP 32). Although Mn was not added to the control medium, there are high Mn concentrations observed in the plants growing in it at all pH (490 – 100 mg kg⁻¹ at pH 8.0). This is again a sign that some rhododendrons absorb Mn into the leaves in concentrations sufficient to maintain healthy growth in alkaline soils. Additionally, the significant and strong positive correlation between [Mn] (L) and leaf age ($r_{[\text{Mn}] (\text{L})/\text{AGE}} = 0.72$, CM 7) again clearly confirms that Mn is accumulated in *Rhododendron* leaves over time. These high [Mn] (L) in the control media, and the unchanged health condition of plants after six months in all high-pH soil mixtures, indicate that this species can be cultivated in soils of high pH, regardless of whether Mn was specially added to the growing medium or not. However, the Mn concentrations in the plants were very variable, and it is clear that even plants within a single batch may differ widely in their responses to nutritional limitations or excesses. Overall, the results do not provide sufficient information on the [Mn] supplied to *R. coeloreuron* via the low dose and high dose media, hence on the effect the two media had on the Mn concentrations in the soil and its absorption by the plants.

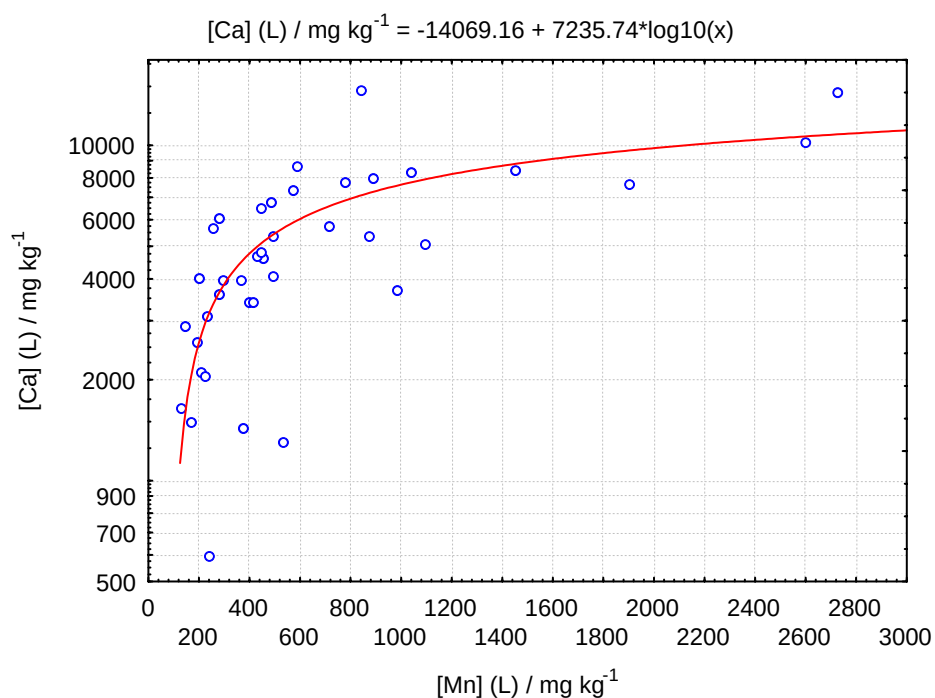
It is also difficult to estimate exactly how much Mn was supplied with the mulch media, since in order to do so the exact concentration of Mn has to be measured from the same one single leaf before the start of the experiment, and after the end of it, which was not performed in this study. However, the high concentrations of Mn, 510 mg kg⁻¹, observed in the decaying *R. ponticum* leaves used for the mulch media, suggest that Mn could have been released from it and supplied to the plants grown in those media.



SP 32 Variation of [Mn] in leaves of *R. coeloreuron* with soil pH and soil media.

The concentrations of Fe in all media in which *R. coeloreuron* plants were growing were generally sufficient to support healthy growth. The [Fe] in the leaves of the plants grown in the high pH media, 6.7 and 8.0, in particular, were even higher than those in pH 4.0 and 5.3 media. Some leaves showed very low [Fe], 8 – 20 mg kg⁻¹, which could be due to uneven distribution of the added mixture of trace elements in the experimental soil media. Overall, the correlation analyses show that correlations between [Fe] and soil pH in this experiment are insignificant.

Additionally, it was observed that for *R. coeloreuron* the correlation of [Ca] (L) with [Mn] (L) is significant (CM 7). In this growing experiment the concentrations of Ca in the leaves of *R. coeloreuron* increase with an increase in [Mn] in the leaves, demonstrated by a significant and strong positive correlation ($r_{[Ca](L)/[Mn](L)} = 0.71$, SP 33).



SP 33 Variation of [Ca] with [Mn] in leaves of *R. coeloreuron*.

Furthermore, as the results for the rhododendrons growing in the wild have already shown, there is a positive correlation of Ca and age of leaves, $r_{[Ca](L)/AGE} = 0.55$,

confirming once again that Ca accumulates in older leaves of rhododendrons.

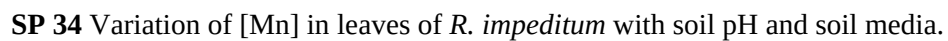
3.2. Discussions of results for *Rhododendron impeditum*

The results for *R. impeditum* plants show the significant correlations are mainly regarding [Fe] in the leaves, and are positive with [Ca] (L) and [Mg] (L) but negative with [Mn] (L). There is also significant correlation between [Mn] (L) and soil pH (CM 8).

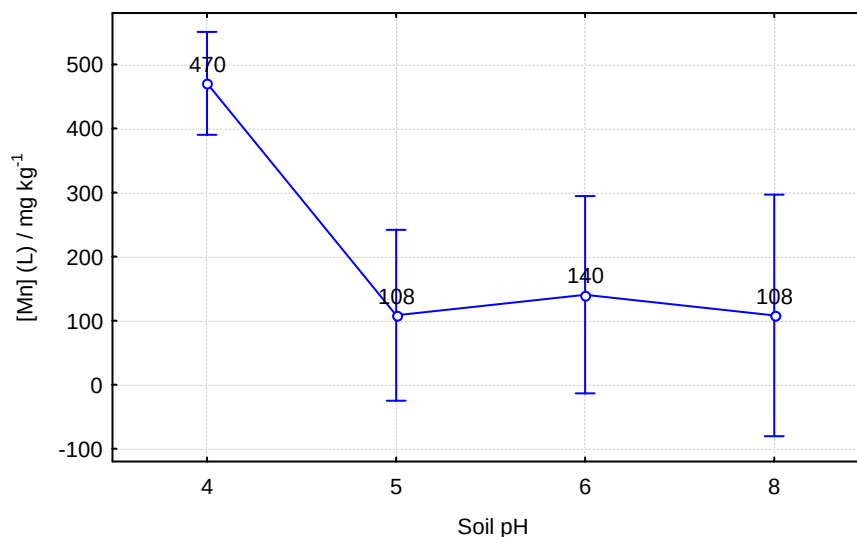
Correlation matrix 8 Correlations for *R. impeditum* plants.

	Medium	Soil pH	Leaf age	[Ca] (L)	[Fe] (L)	[Mg] (L)	[Mn] (L)
Medium	1.00						
Soil pH	-0.16 p = 0.479	1.00					
Leaf age	-0.04 p = 0.836	0.22 p = 0.337	1.00				
[Ca] (L)	0.001 p = 0.997	0.009 p = 0.969	-0.29 p = 0.210	1.00			
[Fe] (L)	-0.13 p = 0.570	0.37 p = 0.100	0.09 p = 0.682	0.54 p = 0.013	1.00		
[Mg] (L)	0.03 p = 0.894	0.12 p = 0.611	-0.11 p = 0.619	0.92 p = 0.000	0.76 p = 0.000	1.00	
[Mn] (L)	0.009 p = 0.970	-0.71 p = 0.000	-0.15 p = 0.521	-0.14 p = 0.549	-0.44 p = 0.047	-0.30 p = 0.198	1.00

In the case of *R. impeditum* the addition of Mn solution to the soil in two different doses seem to have affected the Mn uptake by *R. impeditum* plants accordingly: the [Mn] in the leaves of the plants growing in the low dose media are lower than those in the high dose media (SP 34).

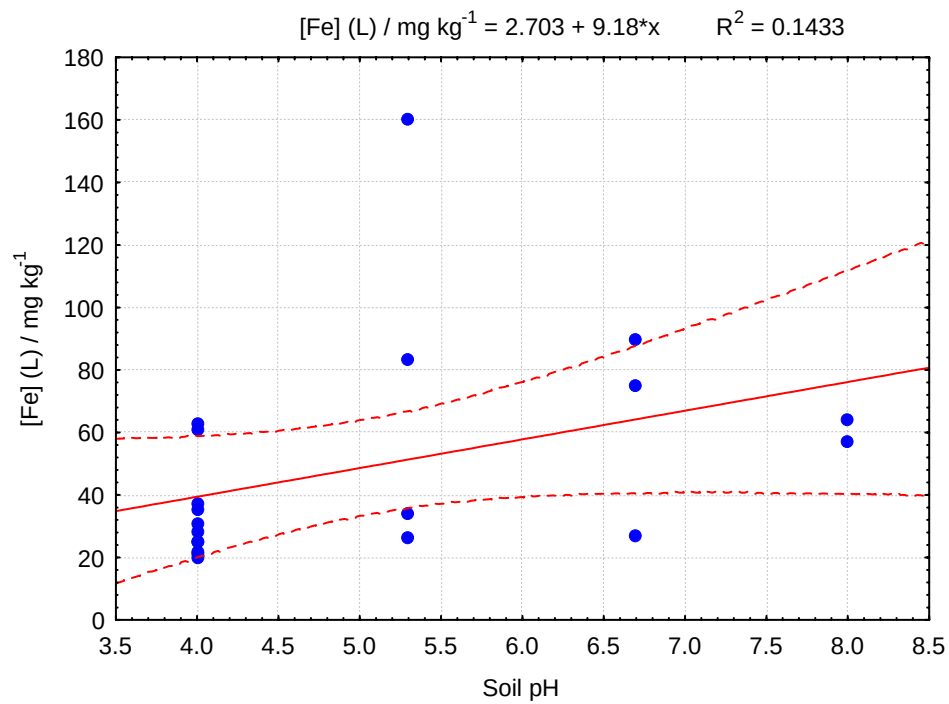


The concentrations of Mn in the leaves varied with pH as expected, and decreased with increase in soil pH (ANOVA 12). Despite that, the concentrations of Mn in the leaves for all *R. impeditum* plants are sufficient, and this was also confirmed by the perfect health of all plants, through the entire period of time of the experiment.



ANOVA 12 Variation of [Mn] in leaves of *R. impeditum* with soil pH.

The concentrations of Fe in the leaves are also within the ranges of normal for healthy growth concentrations, in all pH media (SP 35).



SP 35 Variation of [Fe] in leaves of *R. impeditum* with soil pH.

3.3. Discussions of results for *Rhododendron hirsutum*

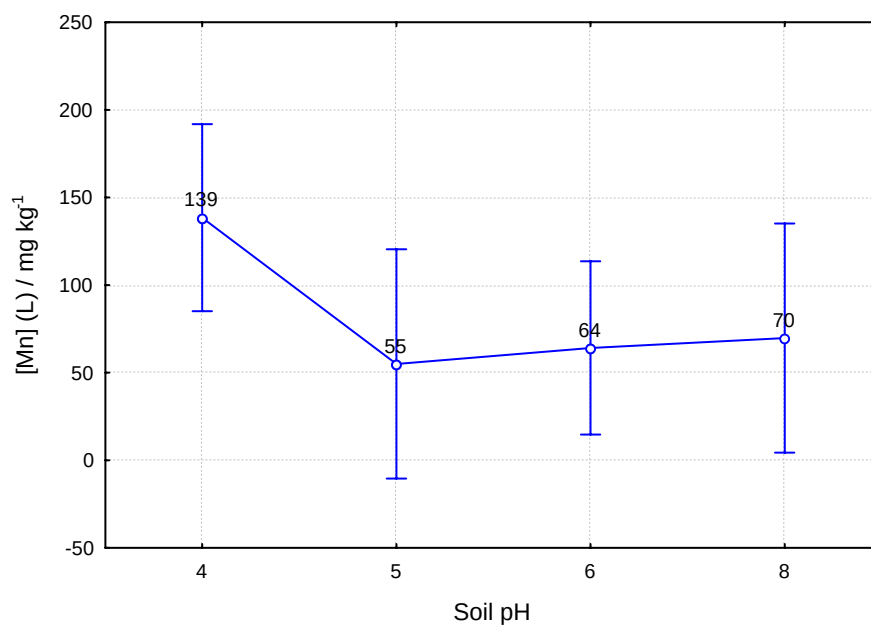
The only significant correlations observed for the *R. hirsutum* plants are of [Ca] (L) with [Fe] (L) and [Mn] (L) with leaf age (CM 9).

Correlation matrix 9 Correlations for *R. hirsutum* plants.

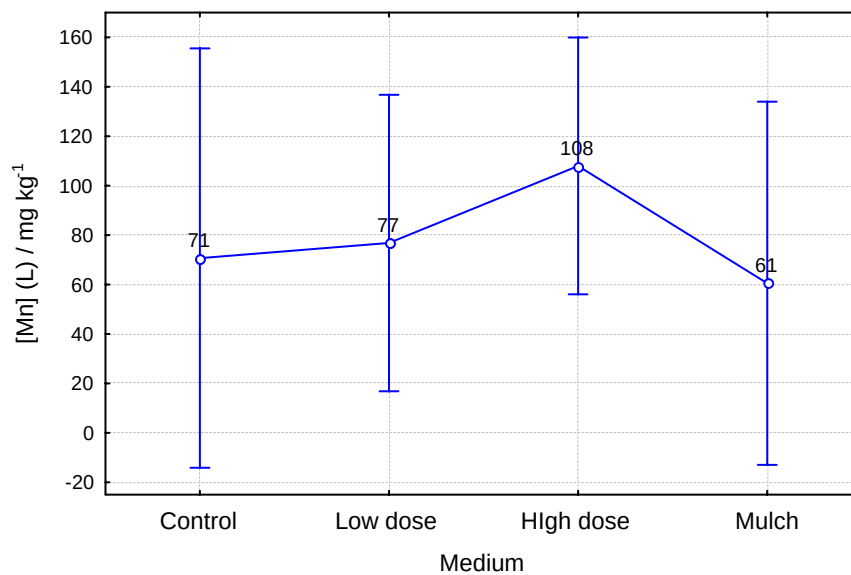
	Medium	Soil pH	Leaf age	[Ca] (L)	[Fe] (L)	[Mg] (L)	[Mn] (L)
Medium	1.00						
Soil pH	-0.06 p = 0.766	1.00					
Leaf age	-0.04 p = 0.861	0.02 p = 0.931	1.00				
[Ca] (L)	0.16 p = 0.464	0.24 p = 0.280	-0.18 p = 0.419	1.00			
[Fe] (L)	-0.31 p = 0.171	0.08 p = 0.698	0.30 p = 0.184	-0.61 p = 0.003	1.00		
[Mg] (L)	0.09 p = 0.692	-0.10 p = 0.663	0.03 p = 0.893	0.001 p = 0.995	0.36 p = 0.108	1.00	
[Mn] (L)	0.02 p = 0.909	-0.38 p = 0.082	0.56 p = 0.007	-0.05 p = 0.824	0.30 p = 0.176	0.30 p = 0.179	1.00

The results for *R. hirsutum* are very similar to those for *R. impeditum*. The concentrations of both Mn and Fe in the leaves are generally sufficient. The concentrations of Mn in the leaves are mainly above 30 mg kg⁻¹, and only two leaf samples, for plants growing at pH 5.3, had low [Mn] (ANOVA 13 and 14).

As observed for *R. coeloreuron* and *R. impeditum*, the [Mn] in the leaves of *R. hirsutum* in the mulch media are generally lower than the [Mn] (L) in the rest of the media (ANOVA 14). This again demonstrates that the supplement of Mn by Mn-rich mulch is unclear, and requires further studies to establish more clearly the mechanisms of Mn release and its uptake by rhododendrons from their own decaying leaves.



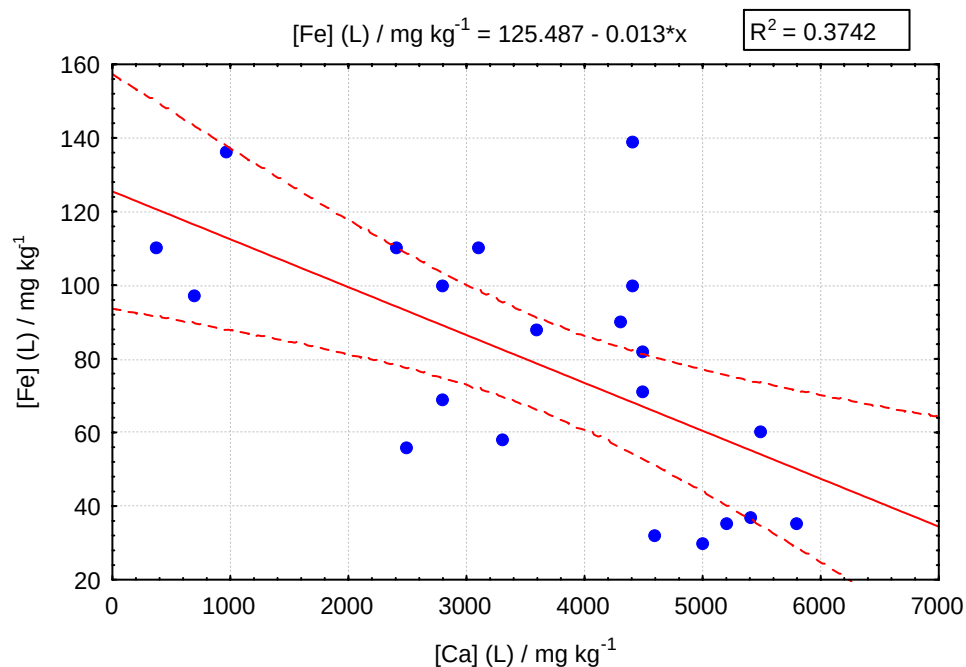
ANOVA 13 Variation of [Mn] in leaves of *R. hirsutum* with soil pH.



ANOVA 14 Variation of [Mn] in leaves of *R. hirsutum* with soil media.

As with *R. coeloreuron*, in *R. hirsutum* there is a significant positive correlation between [Mn] (L) and leaf age ($r_{[\text{Mn}] (\text{L})/\text{AGE}} = 0.56$).

Additionally, as also observed for *R. impeditum* there is also significant negative correlation of [Fe] (L) with [Ca] (L) (SP 36).



SP 36 Variation of [Fe] with [Ca] in leaves for *R. hirsutum* in all media.

The concentrations of Fe in the leaves of *R. hirsutum* were also sufficient, above 30 mg kg⁻¹, to maintain healthy growth. Few plants growing in the pH 6.7 media had [Fe] between 30 and 40 mg kg⁻¹, and the rest, including the leaves at pH 8.0 had [Fe] above 50 mg kg⁻¹.

However, the *R. hirsutum* plants in all tested soil media developed red colouring, followed by a change in the colour to brown, and in some cases to drying of the leaves and death of the plant. Those symptoms could not be related to either the nutrients concentration analysed in this study, or to the soil pH. Therefore, other factors in the experiment must have had an effect on the plants, such as availability of other nutrients

Chapter 4 Conclusions on the statistical interpretation

4.1. Conclusions for *Rhododendron* plants growing in the wild

The majority of *Rhododendron* plants, from which material was collected for analyses, were growing healthily in their environment. Among the plants that were growing on, near, or over limestone soil only very few were showing chlorotic symptoms. Around half of these were growing in a thick top layer of organic soil, covering limestone subsoil, and the rest were growing with very little organic material present in the soil and almost entirely in limestone soil.

4.1.1. Observations for Ca requirements by *Rhododendron* in the wild

Disproving past theories

The carried out research provided clear and undisputable proof that the theories claimed in the past are wrong.

“The calcium in the limestone has a toxic effect on the plants.”

Rhododendron species studied in the wild and in cultivation showed clear tolerance of very high concentrations of Ca in the soil in which they grow. The observations were that for very high concentrations of Ca in the soil, as in limestone soil, where concentrations of Ca are higher by several orders of magnitude in comparison to non-calcareous soils, the Ca concentrations in the leaves increases only a little, by less than one order of magnitude. It appears that in the wild *Rhododendron* plants have very similar requirements for Ca concentrations in their leaves in both calcareous and non-calcareous soils: rhododendrons in limestone areas do not take up Ca in their leaves to any greater extent than when growing on any other soils. This indicates that the roots of rhododendrons absorb Ca from the soil in a very well controlled manner, within certain limits of maximum concentrations, regardless of the maximum concentrations of Ca present in the soil.

Each season, in all types of soil, *Rhododendron* plants absorb calcium in new-growth leaves, on average between 3500 and 10000 mg kg⁻¹, and with a very well controlled mechanism. The results suggest that *Rhododendron* plants absorb calcium in the leaves in each year of their growth, but the gradient of absorption becomes less – the concentration absorbed is less each year.

The above is sufficient evidence that *Rhododendron* plants are not sensitive to high concentrations of Ca in the soil.

Regardless of whether the species grow in calcareous or non-calcareous soil, they do not suffer from an excess of Ca either in the soil or in the leaves. On the contrary, almost all rhododendrons in this study that were exposed to high Ca in soil were growing in very good health.

“The limestone in China is insoluble and it does not affect the associated soil. The soil remains acidic and the CaCO₃ has no effect on the plants.”

The pH of 7.9 to 8.2 recorded for the limestone soil samples collected from the most extreme limestone areas, Gang He Ba, which was close to the maximum possible pH for CaCO₃, confirms that the limestone in this area is soluble and affects the soil pH.

Further findings

The results demonstrated that *Rhododendron* species are able to accumulate Ca in their older leaves. This ability was indicated by the results to be significant particularly for the rhododendrons growing in limestone soils. However, it is not clear if this is storage of absorbed excess of Ca, or it is simply further absorption of Ca by older leaves.

4.1.2. Observations for Mg requirements by *Rhododendron* in the wild

Disproving past theories

Once again, the results of this study provided proof that explanations claimed in the past were wrong:

“There is a shortage of magnesium in limestone soils, and as a result plants suffer from Mg deficiency.”

First of all, magnesium in the limestone soils studied is available in sufficient concentrations, within the normal range required for healthy growth. Secondly, despite the lower [Mg] (S) in the limestone soil, magnesium concentrations in the leaves of rhododendrons growing in it are not lower than those for rhododendrons growing on dolomitic limestone soil, but are within the same ranges of concentrations. *Rhododendron* plants growing in limestone soil in the wild have sufficient [Mg] in their leaves and do not suffer from Mg shortage.

Further findings

In contrast to calcium, magnesium in leaves of rhododendrons of successive years does not increase after the first year of growth. *Rhododendron* plants do not store excess Mg in their leaves. Either very little is taken up by the older leaves after their first season, or any excess is mobilised.

Overall, the absorption of magnesium in *Rhododendron* plants is also very well controlled. Magnesium availability does not pose a threat to the healthy growth of *Rhododendron*, and it is sufficiently available in all types of soil studied.

4.1.3. Observations for Mg/Ca ratio effect in the growth of *Rhododendron* in the wild

Disproving past theories

“The limestone rock in which rhododendrons grow in the wild is dolomitic limestone, the high magnesium content of which protects the plants from calcium toxicity.”

The past analyses of rock samples from the limestone formations in Yunnan^{40, 54} were confirmed with the results of this study. The average Mg content as percentage of the total soil for the samples collected from the limestone areas is 0.8 %. In contrast, the average Mg content as percentage of the total soil for the samples collected from the dolomitic limestone areas is 6 %. Once again, the clear evidence is that the limestone formations in Yunnan are composed mainly of CaCO_3 and it does not contain dolomitic limestone.

Furthermore, the results for rhododendrons growing on dolomitic limestone showed that greater Mg concentrations in the soil do not lead to a reduced uptake of Ca by rhododendrons. The Ca concentrations in leaves of rhododendrons growing on dolomitic limestone soil are within the same range as those on limestone soil.

Therefore, the healthy growth of *Rhododendron* in the limestone areas in Yunnan cannot be attributed to a suppressed calcium uptake, induced by higher concentrations of magnesium.

Overall, the ratio Mg/Ca is not a criterion for the healthy growth of *Rhododendron* species in any type of soil.

4.1.4. Observations for Fe requirements by *Rhododendron* in the wild

As expected, the concentrations of iron in limestone soil are lower than those in non-limestone soil. Despite that, in limestone soil there are sufficient concentrations of available Fe, 45 – 1900 mg kg⁻¹. Although there were sites of limestone soil having Fe as low as 11 – 21 mg kg⁻¹, the plants growing there were in a perfect health.

In limestone soil in the wild concentrations of iron in the leaves of *Rhododendron* plants are also generally sufficient (23 – 275 mg kg⁻¹) to support healthy growth, although a few plants in this range of [Fe] were unhealthy with chlorotic symptoms. Only a small number of rhododendrons had very low [Fe], < 15 mg kg⁻¹, and they were all healthy. Moreover, the same low concentrations of Fe were also observed in *Rhododendron* plants growing on non-limestone soil, which in contrast to the plants in the limestone soil, were often unhealthy.

Therefore, in limestone soil the chlorotic symptoms observed in some of the plants must have been caused to a lesser extent, if at all, by Fe deficiency.

Additionally, on limestone soils, amongst the rhododendrons with sufficient [Fe] in the leaves, only plants from a certain range of species were found to be unhealthy. Therefore, it seems that these are less lime-tolerant rhododendron species, which possibly fail to become established in limestone soil at all and they will only thrive in more acidic soils. It can be therefore concluded that in limestone soils iron is generally sufficient to maintain healthy growth of the lime-tolerant rhododendron species. This applies only to the species studied.

It is also established that iron is not stored in the older leaves of *Rhododendron* plants.

4.1.5. Observations for Mn requirements by *Rhododendron* in the wild

Based on the results, the following main conclusions can be drawn:

- 1) *In low pH soils Rhododendron plants absorb Mn in their leaves in large concentrations.*

In contrast to the concentrations of 300 mg kg⁻¹ of manganese, above which toxicity in most plants will be induced, *Rhododendron* plants with much higher concentrations, up to at least 3000 mg kg⁻¹, do not show any signs of susceptibility to Mn.

- 2) *Rhododendron plants accumulate the absorbed Mn in their leaves over time.*

Manganese is accumulated every year in the leaves gradually and at consistent rates.

- 3) *No maximum limits of Mn concentrations in the leaves were observed, i.e. Rhododendron appear to absorb unlimited concentrations of Mn, for as many years as they hold their leaves.*

The observation that Mn is absorbed each year in similar concentrations indicates that Mn is absorbed in unlimited concentrations, and as long as it is available it will be absorbed by *Rhododendron* plants.

- 4) *Some Rhododendron species grow healthily in high pH soils, between 7.0 and 8.0, with minimal concentrations of Mn, 10 - 30 mg kg⁻¹, in the leaves.*

Based on the results it can be deduced that the “functional nutrient requirement”, defined by Loneragan and Snowball (1969) as the minimum concentration of nutrients within the plant that can sustain its metabolic functions at rates that do not limit growth⁸, for Mn in *Rhododendron* species growing on Mn-poor limestone soil is in the range 10 - 30 mg kg⁻¹, which is usually a deficiency range for most other plants.

- 5) *The range of manganese concentrations in the leaves within which the healthy requirements of Rhododendron plants are met is very wide, 10 – 3000 mg kg⁻¹, between the limits inducing chlorosis or potential toxicity.*

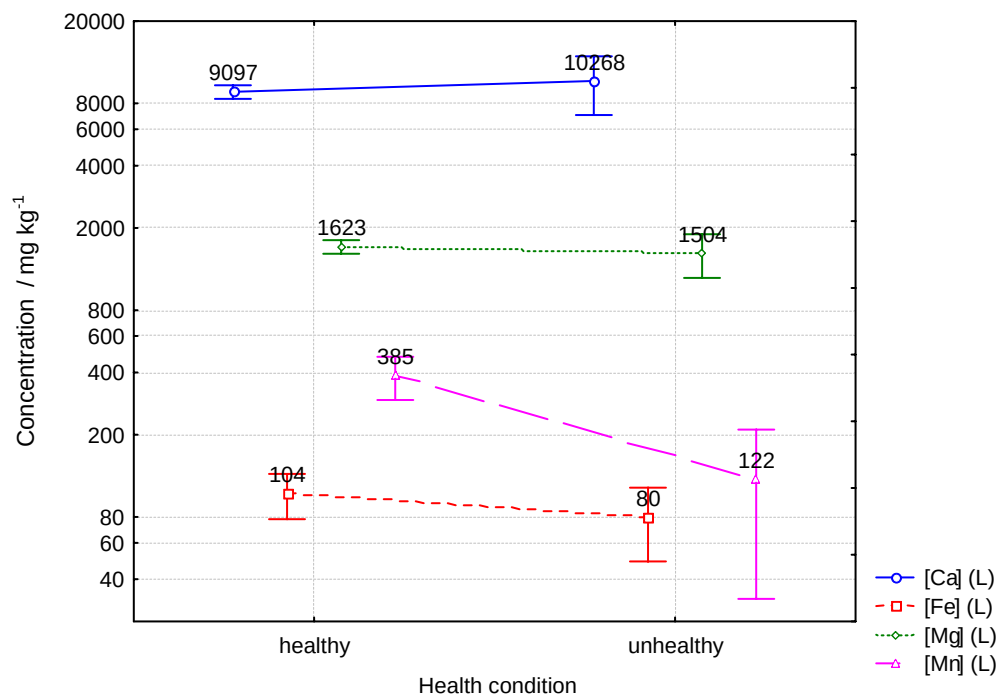
4.1.6. Origins of Mn for *Rhododendron* species on limestone soils in the wild

Although Mn requirement for rhododendrons is low (10 – 30 mg kg⁻¹ in leaves) the achievement of even these low concentrations is surprising. At pH 7.0 - 8.0, availability of Mn is normally very low. Therefore, a continually renewed source of Mn is required and we believe this comes by the decay of Mn-rich leaves. Some of these will come from the local rhododendrons themselves but others may blow or wash in from neighbouring regions with higher pH soils.

It should be noted that the analyses carried out in this project measure the concentrations of available Mn in soils. The samples were air-dried, a requirement for import from China. The measured concentrations of Mn therefore may be lower than those in the wild, where a steady state may be maintained by decay of Mn-rich leaves.

Summary for nutrient requirements by *Rhododendron* species in the wild

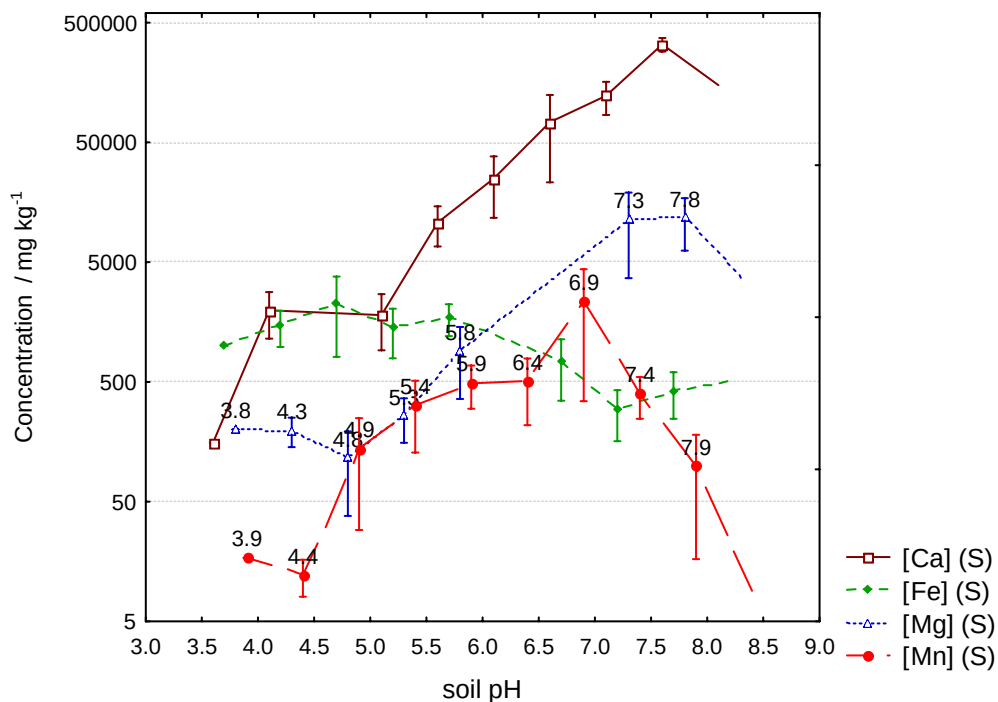
With respect to the health condition of the *Rhododendron* plants, mean plot 1 shows a gradual drop for Mn, a very small drop for Fe, and none for Ca and Mg. This suggests that [Mn] is the primary factor in poor health, with [Fe] either a secondary cause or simply correlated with [Mn] (MP 1).



MP 1 Means and min-max ranges of [Ca], [Fe], [Mg] and [Mn] in healthy and unhealthy *Rhododendron* plants.

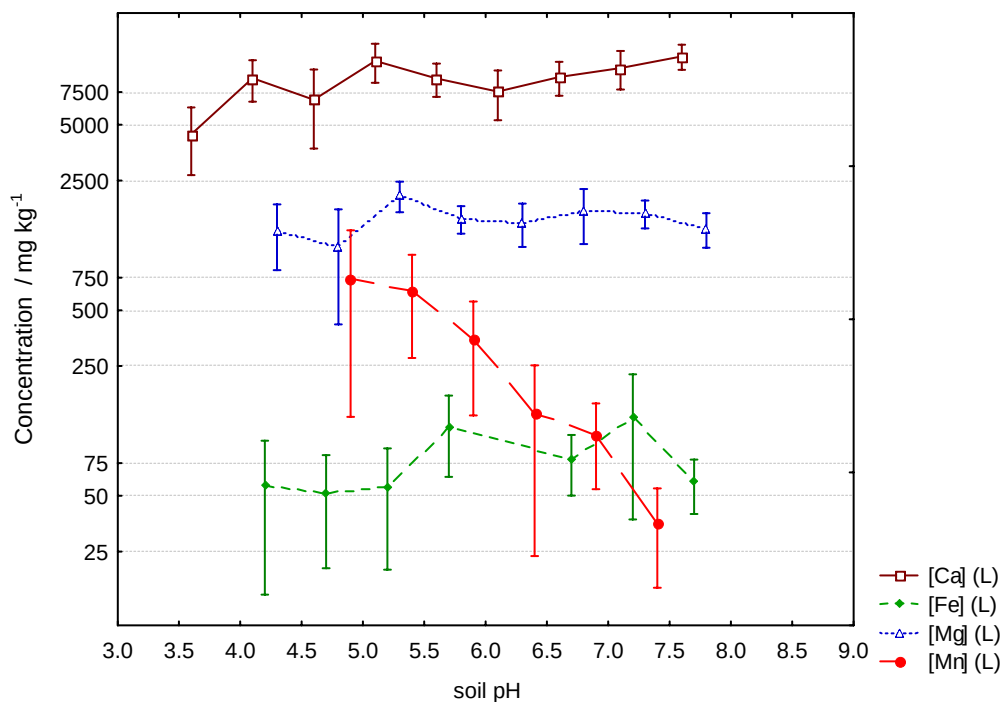
However, there were only few samples collected from unhealthy plants (13 plants), compared to the number of samples from healthy plants. Therefore, this drop in [Mn] in leaves for unhealthy plants cannot be regarded as a general rule until a comprehensive study, involving focus on the inclusion of a greater number of unhealthy samples, is carried out. Such a collection of unhealthy samples may, however, be difficult as in the wild unhealthy plants are only occasionally observed.

As [Ca] in the soil increases the soil pH also increases, which is common in limestone soils, and it is paralleled by [Mg] in the soil (MP 2). On the other hand, [Fe] decreases with soil pH, but not very much, while [Mn] is low at both low pH, because of being too soluble and lost from the soil, and high pH, due to reduced availability.



MP 2 Means and min-max ranges of [Ca], [Fe], [Mg] and [Mn] in the soil in the observed pH range for *Rhododendron* plants.

In the leaves, the [Ca] and [Mg] show little variation, indicating a controlled uptake, while the [Fe] are remarkably steady, indicating that there is no evidence for shortage of Fe at high pH (MP 3). Conversely, [Mn] in leaves show a drop with an increase in soil pH, as expected, but the studied plants do not appear to suffer from deficiency due to this decrease (MP 3).



MP 3 Means for [Ca], [Fe], [Mg] and [Mn] in leaves in the observed pH range for *Rhododendron* plants.

So after all, it seems that in limestone soils lack of Fe is not a threat for the healthy growth of rhododendrons, as it is in sufficient concentrations in both soil and leaves. On the other hand, the concentrations of Mn clearly decrease, particularly above pH 7.0, but the *Rhododendron* species growing and tolerating those conditions seem to have adapted to grow healthily at low concentrations. This indicates that for these *Rhododendron* species and in these sites Mn also does not generally seem to be a problem at high pH. However, the few species that showed chlorotic symptoms also showed very low [Mn], while the [Fe] were normal. So, although plants were generally healthy at the bottom limits required for healthy growth of both Mn and Fe, lack of Mn is a greater threat to rhododendrons than lack of Fe.

Overall, from the results for both Mn and Fe it seems that as long as Mn deficiency does not occur, even concentrations of Fe that are normally accepted as low are satisfactory for rhododendrons to grow healthily on limestone soil.

4.1.7. Variations between *Rhododendron* species

Mn and Ca in soil and leaves have been shown to have more effect on rhododendrons than Fe and Mg, for which very steady concentrations were observed. Therefore, with respect to variations in [Ca] and [Mn] in the leaves between different studied *Rhododendron* species, the average [Ca] and [Mn] in the leaves of plants from each species are summarised in Table 38 (CP 1 and 3, Appendix 3).

Table 38 Maximum soil pH, average concentrations of Ca and Mn [mg kg⁻¹] in leaves, and health condition observed for each *Rhododendron* species.

Subgenus	Section	Subsection	Species	Max pH	Average of Ca	Average of Mn	Health*
Hymenanthus	Ponticum	Campylocarpa	<i>R. wardii</i>	5.7	3500	24	H
		Fortunea	<i>R. vernicosum</i>	7.4	11340	220	3 UnH
			<i>R. decorum</i>	7.0	9300	770	5 UnH
		Irrorata	<i>R. anthosphaerum</i>	5.0	10700	1400	H
		Pontica	<i>R. maximum</i>	5.4	10000	880	H
			<i>R. catawbiense</i>	5.0	8000	1860	H
		Taliensia	<i>R. phaeochrysum</i>	6.8	5800	1020	H
			<i>R. balfourianum</i>	7.4	7300	13	H
			<i>R. aganniphum</i>	5.8	5500	2000	H
Rhododendron	Rhododendron	Heliolepidia	<i>R. rubiginosum</i>	7.3	9300	147	9 UnH
		Lapponica	<i>R. cuneatum</i>	7.9	11650	15	3 UnH
			<i>R. telmateium</i>	7.9	12500	70	H
			<i>R. nitidulum</i>	7.7	11800	14	H
			<i>R. nivale ssp. boreale</i>	7.9	13000	13	H
			<i>R. setosum</i>	-	8700	260	H
			<i>R. hippophaeoides</i>	5.4	12400	120	H
			<i>R. rupicola</i>	5.4	15300	780	H
			<i>R. yungningense</i>	6.8	5800	130	H
			<i>R. impeditum</i>	5.6	8700	630	H
			<i>R. fastigiatum</i>	6.0	7000	480	H
		Lepidota	<i>R. lepidotum</i>	-	6600	700	H
		Rhododendron	<i>R. hirsutum</i>	7.6	8000	12	H
			<i>R. ferrugineum</i>	6.8	6900	41	H
		Saluenensia	<i>R. saluenense</i>	5.6	6400	370	H
		Scabrifolia	<i>R. racemosum</i>	5.9	5200	94	H
		Trichoclada	<i>R. trichocladum</i>	6.8	5800	180	H
		Triflora	<i>R. yunnanense</i>	7.3	6900	100	H
	Pogonanthum		<i>R. anthopogon</i>	-	7000	176	H
			<i>R. primuliflorum</i>	7.5	10000	41	H
			<i>R. trichostomum</i>	6.8	8400	26	H

* Where health condition is noted as “H” the leaves of all samples were healthy, and “UnH” refers to the number of unhealthy specimens. The total number of specimens is given in Table 14 Chapter 2.

It appears that the species that accumulate the highest concentrations of Mn in their leaves are members of subsections Taliensia and Pontica. This finding may provide useful information in future studies on the invasive nature of *R. ponticum* and its hybrids on the west coast of the British Isles, as it is a species from the Pontica subsection.

It should also be noted that Taliensia rhododendrons are almost ubiquitous in Yunnan at around 3700 to 4000 m, i.e. above the main regions where rhododendrons on particularly limestone-rich soils have been found. They are, therefore, a potential source of manganese, as their leaves can be washed and/or blown to lower elevations. In particular, Gang He Ba is a flood plain, so there is an easy mechanism for this transport. For the species *R. decorum*, *R. vernicosum* and *R. rubiginosum* there is a higher tendency to develop unhealthy symptoms. *R. decorum* and *R. vernicosum* do grow in limestone soil, although it seems that this environment is at the extreme limits for their normal growth, as a higher number of plants are found to be unhealthy in these soils, compared to *R. cuneatum* and *R. telmateium*. It seems that even though *R. decorum* and *R. vernicosum* seem generally to accumulate more Mn in their leaves (not as a mean of [Mn], but when the Mn concentrations in each leaf sample of every plant are compared), as already discussed for large-leaved species in general, they are still more prone to suffer from the alkalinity, expressed in leaf Mn deficiency and fungal diseases, which are more likely to occur under Mn deficiency in the soil. In contrast, none of the dwarf, small-leaved species suffered from any diseases and none of the Lapponica species was found to be showing symptoms other than chlorosis (e.g. fungal). Moreover, in Gang He Ba Valley the Lapponica species *R. cuneatum* and *R. telmateium* were observed growing in the lowest, most flooded and limestone saturated floor of the valley, whereas the other *Rhododendron* species were mostly growing a little to the sides of the valley, at the higher levels. This suggests that Lapponica species, Pogonanthum section species, and dwarf species in general, perform better than the large-leaved species in limestone soils. With respect to variations between *Rhododendron* species, studied in this research, and soil pH Table 39 shows the pH ranges within which different species were mostly found growing.

Table 39 Distribution of *Rhododendron* species with soil pH in the wild.

Subgenus	Section	Subsection	Species	pH		
				7.2 – 7.9	6.0 – 7.2	4.0 – 6.0
Hymenanthus	Ponticum	Campylocarpa	<i>R. wardii</i>			*
		Fortunea	<i>R. vernicosum</i>	*~	*	*
			<i>R. decorum</i>		*	*
		Irrorata	<i>R. anthosphaerum</i>			*
		Pontica	<i>R. maximum</i>			*
			<i>R. catawbiense</i>			*
		Taliensia	<i>R. phaeochrysum</i>		*	*
			<i>R. balfourianum</i>	*		
			<i>R. aganniphum</i>			*
Rhododendron	Rhododendron	Heliolepidia	<i>R. rubiginosum</i>	*~	*	*
		Lapponica	<i>R. cuneatum</i>	*		
			<i>R. telmateium</i>	*		
			<i>R. nitidulum</i>	*		
			<i>R. nivale ssp. boreale</i>	*		
			<i>R. hippophaeoides</i>			*
			<i>R. rupicola</i>			*
			<i>R. yungningense</i>		*	
			<i>R. impeditum</i>			*
			<i>R. fastigiatum</i>			*
		Rhododendron	<i>R. hirsutum</i>	*	*	*
			<i>R. ferrugineum</i>		*	*
		Saluenensia	<i>R. saluenense</i>			*
		Scabrifolia	<i>R. racemosum</i>			*
		Trichoclada	<i>R. trichocladum</i>		*	
		Triflora	<i>R. yunnanense</i>	*		*
	Pogonanthum		<i>R. primuliflorum</i>	*	*	*
			<i>R. trichostomum</i>		*	

*Plants found growing in good health.

*~ Plants that were not coping well and are generally in unstable health within this pH range.

Evidently, *Rhododendron* species that tolerate high pH are also often found growing in soils of lower pH, whereas the species that are generally growing in low-pH soils are rarely observed at high pH. The species that tolerate limestone soil the most are mainly dwarf species with small leaves.

However, for many of the species, samples of only one plant were collected, which is not enough to conclude the pH range within which the species are mostly found growing. For example, *R. impeditum* is a lime-tolerant species, but samples from this species were collected from only one plant growing at pH = 5.6, but it is also frequently found growing in soils with pH above 7.0.

All conclusions have more significant meaning for the species and subsections from which enough number of samples was analysed (see Chapter 2). Of the subsections represented by only one member species and by only one or two plants, more specimens need to be analysed to contribute to the summary for their behaviour.

All summary data are averages for the samples collected. These samples are not random, but selected specifically for the present studies. In particular, most samples are for limestone areas, but very few for other alkaline soils, and the plants from which samples were collected were of course those that were growing in the area. Nothing can be said about species that were not present in the collecting sites.

All evidence presented in this study is yet not enough for conclusions to be made about whether the observed differences in the tolerance of the studied species to high soil pH, [Mn] and [Ca] are related to the origin of the species or root selectivity. Adaptivity of species native to Europe and Asia cannot be compared since the species native to Europe are not native to Asia and *vice versa*. The available results can only suggest that although different, in both Europe and Asia there are species that tolerate conditions of high soil pH. Therefore, it is unlikely that extreme soil pH is tolerated by some species as a result of the area/continent of their natural habitat.

Summary for variations observed between *Rhododendron* species in the wild

There seems to be a special correlation between the subsection to which rhododendrons growing in extreme limestone soils belong and their absorption of Mn and generally thriving in such extreme conditions.

The above results lead to the conclusion that following their general ability to absorb high concentrations of Mn and store it in their old leaves, especially in low-pH soils, *Rhododendron* species have evolved to be able to grow in less favourable, Mn-poor conditions. These are particularly the species members of the Lapponica subsection and Pogonanthum section, and they have adapted very efficiently to grow healthily with minimum soil and leaf manganese (10 – 30 mg kg⁻¹). Meanwhile, the same species are also found growing in more favourable, lower pH and richer in Mn conditions (organic top layer overlying limestone subsoil) and consequently having higher [Mn] in their leaves. Based on these results from the present study, the following hypothesis can be proposed:

A long time ago, when rhododendrons were mainly growing and populating areas of low-pH soils, they had developed ability to absorb large concentrations of Mn from the soil. Consequently, having to accommodate such high concentrations of Mn, they began depositing the excess of Mn in their leaves. The decaying leaves of these plants, if transported to neighbouring high-pH soils, could provide the manganese needed for rhododendrons to grow there. Their leaves could then, in turn, widen the region in where rhododendrons could grow. Slowly, this enabled those species most tolerant of low [Mn] to migrate into the limestone regions. The pressure to migrate could be increased by the coming and going of ice ages, and species with the ability to migrate over limestone areas would have an evolutionary advantage. Lime-tolerant species or variety would therefore gradually evolve.

However, the current work cannot explain why different species demonstrate adaptive capability. Investigating this matter would require studying factors such as root microbiology and genetics of species, which are outside the scope of this project.

4.2. Conclusions for alpine plants growing in the wild

4.2.1. Observations for Ca requirements by alpine plant species in the wild

The genera for which the highest concentrations of Ca in the soils were observed are *Daphne* and *Rhododendron*, i.e. some species that are members of those two genera are definite calcicole species.

From the genus *Gentiana* the species observed growing at both high pH, above 6.7, and high [Ca] in the soil were *G. veitchiorum*, *G. szechenyii*, *G. asclepiadea*, *G. angustifolia* and *G. lutea* (CP 7a and 7b). Those species also showed higher [Ca] in their leaves, at concentrations similar to the concentrations of Ca observed in *Rhododendron* (CP 8a).

However, the observations of some genera growing at lower [Ca] in the soil cannot be conclusively attributed to a general preference of these genera for low [Ca] in the soil and consequently in the leaves, and it may be a result of a preference for high soil organic matter and low pH, which are variables correlated with lower Ca concentrations.

4.2.2. Observations for Mg requirements by alpine plant species in the wild

The concentrations of magnesium in the leaves of alpine plants are observed to be well controlled and within a close range of concentrations regardless of the type of soil in which the plants were growing.

4.2.3. Observations for Fe requirements by alpine plant species in the wild

As for *Rhododendron*, the concentrations of Fe in the leaves of *Gentiana*, *Primula* and the rest of the alpine plants, are within a range of concentrations satisfactory for healthy growth, between 50 and 500 mg kg⁻¹, even at high pH (CP 8b and CP 9).

4.2.4. Observations for Mn requirements by alpine plant species in the wild

It appears that as with *Rhododendron* plants tolerating limestone soils, the studied alpine plants observed growing on high-pH soils may show extremely low concentrations of Mn in their leaves. Even so, all alpine plants studied were growing very healthily. This indicates that, as with *Rhododendron* plants, alpine plants generally have adapted to growing at the extreme minimum of Mn concentrations in soils with high pH, where Mn is generally present in low concentrations in the soil.

From the soil samples for alpine plants collected from sites where rhododendrons were growing in the limestone areas of low soil organic matter, below 10%, none showed high [Mn] in the soil. Manganese could be supplied by the Mn-rich organic matter present in the soil, supplied by the decaying leaves of the surrounding rhododendrons. However, those alpine plants were not growing directly under *Rhododendron* plants, but some small distance away from them. Generally, in the extreme limestone areas, with open scree and sparse vegetation, fewer rhododendrons are observed growing, and they grow less densely. The soil samples for these *Rhododendron* plants, which are often also the soil samples corresponding to alpine plants collected on the same sites, contain low soil organic matter, below 10%. In those soils with a low amount of organic matter, the [Mn] are lower, between 7 and 70 mg kg⁻¹, than could be obtained if the small amount of organic material was rich in Mn, which might be expected if it was provided by the decaying leaves of the surrounding rhododendrons. This suggests that in those very extreme areas the organic matter probably contains to a lesser extent, if at all, the high concentrations of Mn generally observed in soils in which *Rhododendron* plants grow.

These high concentrations may be more localised, closer to the stems under the plant for example. In addition to the fact that fewer *Rhododendron* species occupy these extreme limestone areas, another possible reason for the lower [Mn] in these soils may be a landscape factor. *Rhododendron* populations in scree and open areas seem to grow in patterns where the plants do not form thickets or woods, growing close to each other, but are distributed more openly with larger spaces between the plants. Consequently, such patterns cannot create a continuous area of *Rhododendron* plants, in which fallen leaves would form an interrupted 'carpet' of Mn-rich decaying leaves, which would provide and maintain high concentrations of Mn in the soil. Therefore, because of the rarer *Rhododendron* vegetation in those places the soil concentrations of Mn are also lower than those in *Rhododendron* woods, and so other plant species growing under or near rhododendrons would also grow in soils of [Mn] lower than those contained in decaying *Rhododendron* leaves. Even in the cases in which rhododendrons in the open limestone areas provide higher concentrations of Mn within their immediate rhizosphere from their own foliage, alpine plants growing exactly under such rhododendrons were not observed and therefore not collected. For most cases of alpine plants observed growing under rhododendrons, those were usually found in areas where the limestone is covered by an organic layer (Napa Hai, foot of East Bai Ma Shan, Shika Shan, etc.). This was the case with *Androsace delavayi* growing at the foot of a scree slope, where the habitat consists of dwarf *Rhododendron* and *Cassiope* (another genus in the family *Ericaceae*) plants covering the ground. The soil sample for this species showed [Mn] in the soil higher than would be provided by a normal organic matter source or mineral soil. In contrast, two other *Androsace* species as well as the all other alpine species, collected just 500 m above, on the open scree, showed [Mn] in the soil between 26 and 70 mg kg⁻¹.

With respect to the abilities of alpine plants to absorb high concentrations of Mn in their leaves, most species that had high [Mn] in the leaves, including the above *Androsace delavayi* plant growing next to rhododendrons, were growing in low-pH soils. As with the *Rhododendron* species found growing on screes and in Gang He Ba, the alpine

species growing on high-pH soils appeared to absorb Mn in the leaves within the normal ranges of concentrations (CP 11, 12, 13 and 14). Some alpine plants species observed to have unusually high concentrations of Mn in their leaves are listed in Table 40.

Table 40 *Primula* species and other alpine species having high concentrations of Mn in their leaves in the wild.

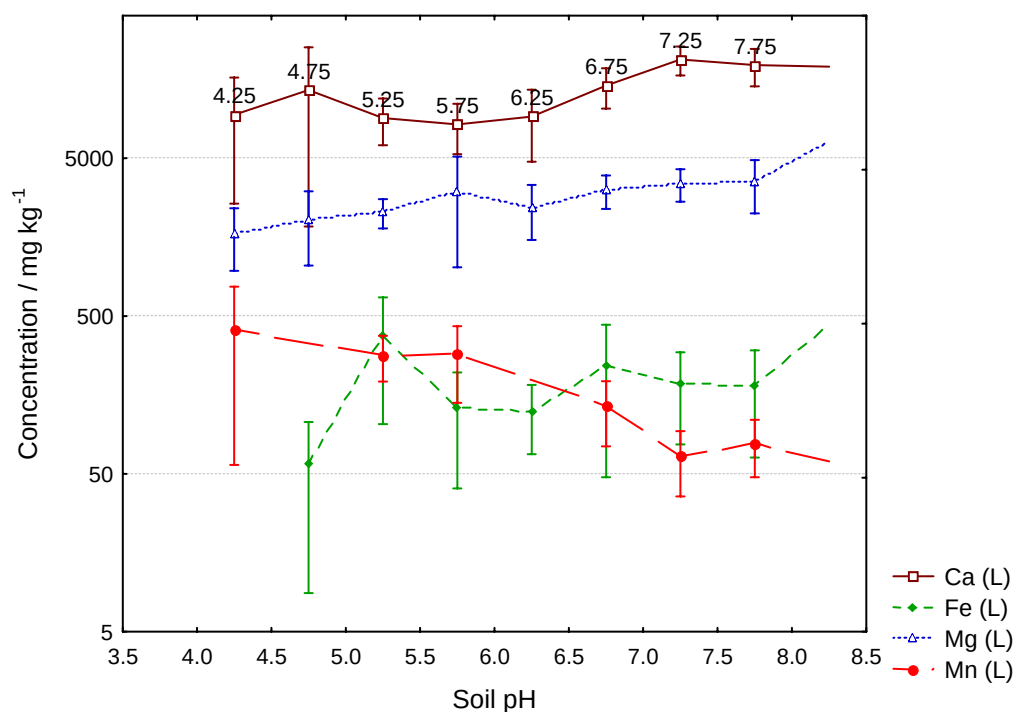
[Mn] / mg kg ⁻¹	Name of species	[Mn] / mg kg ⁻¹	Name of species
1300	<i>Primula boreiocalliantha</i>	300 – 440	<i>Kalmia latifolia</i>
		540	<i>Rheum nobile</i>
1700	<i>Primula calliantha</i>	3000	<i>Rheum sp</i>
650 – 800	<i>Primula dryadifolia</i>	530	<i>Pulsatilla alpina</i>
700	<i>Primula sonchifolia</i>	1200	<i>Galax aphylla</i>
900	<i>Primula muscariodes</i>	500 – 730	<i>Gentiana atuntsiensis</i>
1500	<i>Primula hongshanensis</i>	560	<i>Gentiana purpurea</i>
2000	<i>Astragalus yunnanensis</i>	520	<i>Gentiana sikkimensis</i>
340	<i>Androsace delavayi</i>	350 – 500	<i>Gentiana sino-ornata</i>

Overall, the studies of other alpine plant species confirmed that Mn accumulation is not limited to the family *Ericaceae*.

Rhododendron, *Primula*, *Daphne* and *Androsace* appear to include the highest number of species observed tolerating alkaline soils, of the genera studied. In contrast, the genus *Gentiana* in general seems to prefer soils of pH below 7.0. Only a few species were observed growing on soils with pH above 7.0.

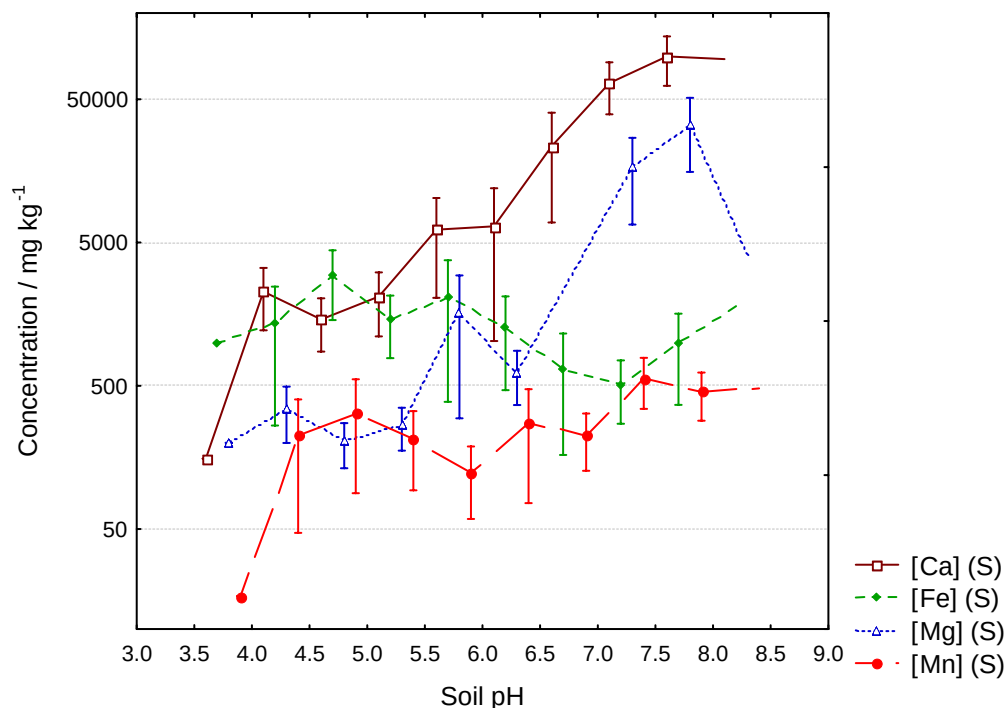
Summary for nutrient requirements by alpine species in the wild

Overall, for the studied alpine plants the concentrations of Fe and Mn in the soils in which they grow and in their leaves at high pH are within ranges of normal and sufficient concentrations, above 40 mg kg^{-1} , to satisfy healthy growth (MP 4 and 5). At pH above 6.5 the concentrations of these two nutrients in the leaves are generally higher for alpine plants than observed for *Rhododendron* plants.



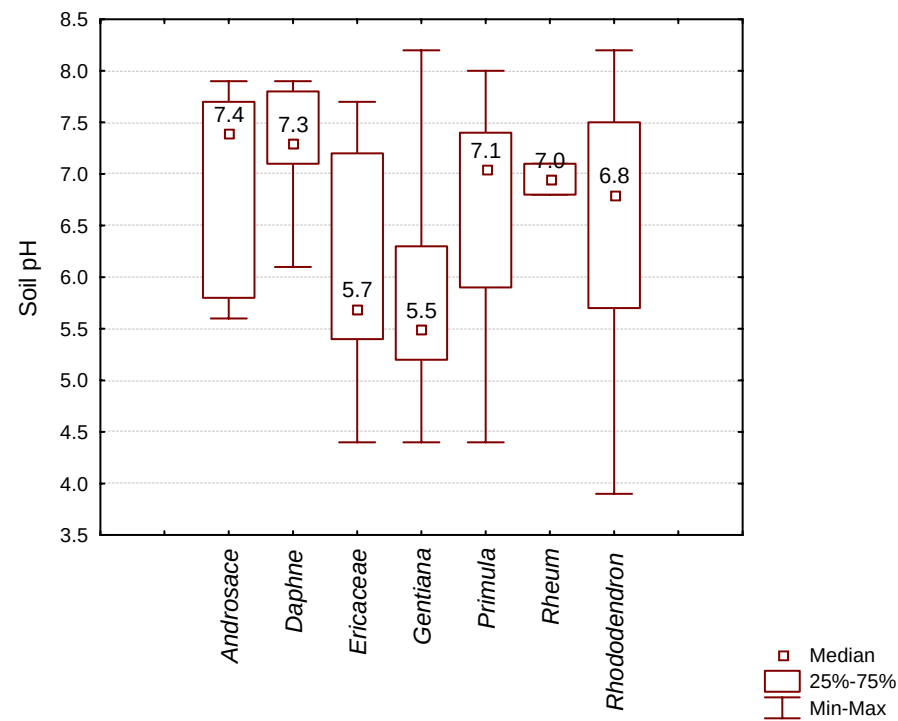
MP 4 Means of [Ca], [Fe], [Mg] and [Mn] in the leaves in the observed pH range for alpine plants.

Again, all the evidence points to decreasing concentrations of Mn in leaves for plants growing in high-pH soils as being the most likely cause of chlorosis. However, even in the most alkaline soils there are many plants with sufficient leaf Mn for healthy growth.



MP 5 Means of [Ca], [Fe], [Mg] and [Mn] in the soil in the observed pH range for alpine plants.

Once again, *Gentiana*, followed by the *Ericaceae* plants, seem to be the least tolerant to high-pH soils among the studied genera, and to have many species with a clear preference for acidic soils (BP 28). From the studied genera, those found growing mainly, or containing many species that grow healthily, on alkaline soils are *Androsace*, *Daphne*, *Primula* and *Rhododendron*. Of course, there were many other alpine plants species studied, but samples from a single plant representative of those species were analysed, so the conclusions of the results they showed are discussed in a collective group as ‘alpine plants’.



BP 28 Distribution of all studied genera with soil pH.

The *Primula* species observed growing at high pH are listed in Table 41.

Table 41 *Primula* species observed growing in soils of high pH in the wild.

Name of species	Soil pH		
	7.5 – 8.0	7.0 – 7.5	
	<i>P. malvacea</i>	<i>P. sikkimensis</i>	<i>P. pulchella</i>
		<i>P. minor</i>	<i>P. secundiflora</i>
		<i>P. sinopurpurea</i>	<i>P. forestii</i>
		<i>P. souliei</i>	<i>P. bracteata</i>
		<i>P. zambalensis</i>	<i>P. albenensis</i>
			<i>P. polyneura</i>

4.3. Conclusions for *Rhododendron* and *Gentiana* growing experiments

None of the *Rhododendron* species tested in the growing experiments for lime tolerance developed symptoms of typical Mn or Fe deficiency.

After six months, the *R. coeloreuron* plants in all media did not appear to have changed since they were planted in the experimental media.

R. hirsutum developed unhealthy symptoms of red-brown colouring on the leaves, which for some plants were followed by necrosis and plant death. The symptoms were evenly distributed among plants in all pH media, so correlation of the health deterioration with the soil conditions cannot be deduced.

R. impeditum in all pH media showed perfect health throughout the entire experiment at all pH.

G. sino-ornata in all media showed clear intolerance to high-pH soils as the plants gradually developed chlorosis and those at pH 6.7 and 8.0 died two months after being planted.

However, some of the *G. sino-ornata* plants in pH 4.0 and 5.3 media also died over the same period as the plants at the high pH media, suggesting that some other factors of the designed growing media were not good for growing *G. sino-ornata* plants. For instance, if the season was too wet for the freshly mixed soil media, the rain may have washed some of the nutrients away from the soil in the pots before the roots could even take them up, and as a result, some plants in all pH media developed certain symptoms of chlorosis.

Overall, the results from the growing experiments performed provided experience and knowledge on how future experiments involving cultivation of *Rhododendron* species can be improved and designed to give more accurate results. Some improvements include: using both acid-tolerant and lime-tolerant species; using young and healthy plant material; better drainage control in order to ensure maximum effects of added nutrient solutions; climate factors – shelter against heavy and continuous rainfall and strong wind; more comprehensive study of the ranges of concentrations of Mn that

would be supplied by *Rhododendron* leaf mulch; analyses of the concentrations of other nutrients in the leaves, such as phosphorus, potassium, nitrogen, etc., in order to establish if more complex nutrient disorders (e.g. mixed nutrient deficiencies) may occur during the cultivation of rhododendrons in the designed environment.

Summary

The results obtained in this study have certainly proved that the leaves of *Rhododendron* plants contain large concentrations of manganese and that they can be an efficient source in providing this nutrient in soils where there is a shortage of it.

Furthermore, the *Rhododendron* species observed to be tolerant to limestone soils in the wild can be cultivated in the UK in alkaline soils with pH above 7.0, and particularly when nutrient supplements are also added.

The association with rhododendrons and the nutrient requirements of various alpine plant species growing in limestone soils in the wild were studied.

The behaviour of rhododendrons and alpine plant species growing on dolomite and dolomitic limestone soil were also studied.

The accumulation of manganese in leaves of successive years of age was studied for first time.

Appendix 1 Tables of results

Results for *Rhododendron* species in the wild

Page 237

Results for alpine species in the wild

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Results for growing experiments on *Rhododendron* and *Gentiana*

Page 296

Legend to tables of results for *Rhododendron* and alpine plants collected in the wild:

- o SDR number: The code number of the collected sample.
- o Age of leaf/y: The year of growth of the leaf sample collected.
 - ‘0’ – current-year leaf (newest growth),
 - ‘1’ – one-year-old leaf (growth from one year before collection),
 - ‘2’ – two-year-old leaf (growth from two years ago), etc.
- o Top/Sub/Mulch: In the tables it appears respectively as ‘T’, ‘S’ or ‘M’. It describes the depth at which the soil was collected. ‘Top soil’ is that collected at the roots of the plant, whereas the ‘Sub soil’ is soil collected at the zone below the plants’ root system, at depth depending on the size of the plant. ‘M’ appears when mulch, decaying rhododendron leaves under the plant but above the levels of the roots, was collected.
- o pH: The pH of the soil, measured in the lab as described in Chapter 2, Materials and methods.
- o OC % = Organic Content: The percentage content of organic matter present in the collected soil samples.
- o Type of soil: The type of soil, regarding the particle size and mineral content (Table 22).
- o Ca, Fe, Mg and Mn: the concentrations of these elements presented in ppm.
- o Healthy/Unhealthy: Visual condition of the plant from which samples were collected.
- o Note: Notes were made of specific factors relating to the plant that was sampled. Where such a ‘Note’ is not present, there is no comment on the sample.
- o Altitude / m: Represents the altitude range in which the samples were collected, not the altitude range of the area/mountain.

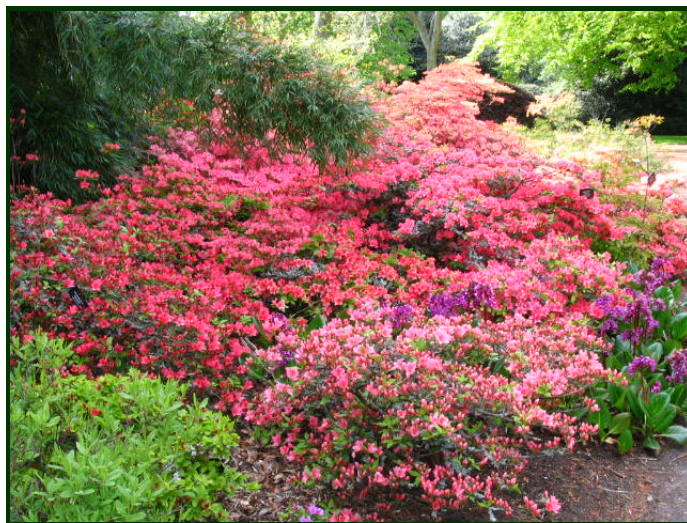
Abbreviations and descriptions used:

- o (P): A photo in support of the description of the plant condition is available on a CD attached to the back of the thesis. Where specified the photos were taken by Jane Armstrong (J. A), and the rest, by Maria Kaisheva (M.K.).
- o NM: ‘Not measured’, mostly used for the measurements of organic content in the soil samples.
- o H: Healthy
- o Unhealthy/Chlorotic: Where the health condition is described as ‘Unhealthy’ it is a condition with visual symptoms for disease other than chlorosis, such as fungal, viral, etc.
- o Mixed forest: Evergreen and deciduous, including *Rhododendron* species.
- o 0 ppm concentration of Fe or Mn: The concentration of Fe and/or Mn in the leaf is below the detection limits of the ICP-OES.

The descriptions of the type of soil of the analysed soil samples are listed in Table 42. The descriptions are based on the appearance of the soil. Additionally, for certain areas, information was available from past analyses of rock material. The purpose of describing the type of soil is to have a reference for the correlations between soil pH and soil organic matter.

Table 42 Soil classification used in Results tables.

Type of soil	Description
<u>White limestone silt</u>	Pure limestone, with almost no organic matter present
<u>Yellow- grey limestone (silt/sand)</u>	Limestone soil containing iron minerals
<u>Grey limestone (sand/silt)</u>	Limestone sand/silt mixed with a little organic matter (limestone : organic matter = <i>ca.</i> 2:1)
<u>Dark grey (silt)</u>	Impure limestone with higher content of other minerals.
<u>Dolomite/Dolomitic limestone</u>	Dolomite , 45% $MgCO_3$ and 55% $CaCO_3$, or Dolomitic limestone, 2.5% - 45% $MgCO_3$ and the rest $CaCO_3$
<u>Red-brown (limestone)</u>	Soil containing a large amount of iron, formed by the decay of iron contained in the parent limestone formation (could also have orange colour)
<u>Mineral limestone</u>	Higher proportion of mineral soil, containing a small amount of limestone (mineral soil: limestone = <i>ca.</i> 3:1)
<u>Brown limestone (sand)</u>	Limestone soil/silt mixed with higher amount of organic matter (limestone : organic matter = <i>ca.</i> 1:1)
<u>Organic limestone/dolomite</u>	Grey organic soil mixed with some limestone/dolomite soil (limestone : organic matter = <i>ca.</i> 1:3)
<u>Black/brown organic</u>	Humus or lighter organic soil, woodland or moor soil, bog
<u>Black/brown organic-mineral</u>	Organic soil containing little other than limestone minerals and/or limestone (organic soil : minerals = <i>ca.</i> 3:1)
<u>Brown mineral</u>	Mixture of mineral soil (igneous, metamorphic etc. minerals) and organic matter
<u>Grey metamorphic</u>	Dark grey slate/granite-derived soil, occasionally having organic matter
<u>Metamorphic mineral (sand)</u>	Yellowish-light brown/grey granite or slate-derived sandy soil, without organic matter

Results for *Rhododendron* species in the wild

- Table 43 ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil samples from *Rhododendron* species collected in China, in NW Yunnan and SW Sichuan. - page 238
- Table 44 ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil samples from *Rhododendron* species collected in Europe. – page 259
- Table 45 ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil samples from *Rhododendron* species collected in the USA and Canada. – page 263
- Table 46 ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil samples from *Rhododendron* species collected in Sikkim, India. – page 265

Table 43 ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil samples from *Rhododendron* species collected in China, in NW Yunnan and SW Sichuan.

Gan He Ba, Yunnan, 3200 m	A glaciated valley surrounded by pure limestone cliffs and moraines. The valley soil profile is composed of pure limestone silt, around 7 m deep in places.

SDR number	Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
1510	soil		T	7.5	37	Brown limestone	161000	204	3500	500
	leaf	0	H				11900	6	1200	21
		1					5500	0	920	17
		Note	From same site as the plants below, collected in later years.							
1513	soil		T	7.2	12	White limestone silt	391000	89	5200	82
	leaf	0	H				32400	50	2900	53
		1					13200	23	2500	26
		Note	From same site as the plants below, collected in later years.							
1516	soil		T	7.0	6	White limestone silt	400000	270	7700	64
	leaf	1	H				14700	180	1900	16
		Note	From same site as the plants below, collected in later years.							
1525	soil		T	7.8	1.5	White limestone silt	400000	470	9000	55
			M			Decaying leaves	1050	26	4700	91
	leaf	0	H				19000	6	1500	17
		1					8800	100	770	22
		Note	From same site as the plants below, collected in later years.							
1528	soil		T	6.9	38	Mineral limestone	25200	190	400	1600
	leaf	0	Unhealthy, fungal disease				13000	110	1200	175
		Note	Very slight rust.							

Gan He Ba, Yunnan, 3200 m

A glaciated valley surrounded by pure limestone cliffs and moraines. The valley soil profile is composed of pure limestone silt, around 7 m deep in places.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
1529	soil	<i>R. vernicosum</i>		T	6.9	38	Brown mineral	25200	190	400	1600
	leaf		0	H				11500	10	1800	300
			Note	Very slight rust.							
3314 A	soil	<i>R. cuneatum</i>		T	7.9	3	White limestone silt	400000	270	4500	54
	leaf		0	H				8400	29	600	13
			1					18500	90	640	17
			Note	On the same site as plants with SDR numbers 15xx, 4319 and 4320							
3314 B	soil	<i>R. cuneatum</i>		T	7.9	3	White limestone silt	400000	270	4500	54
	leaf		0	H				7200	24	820	8
			1					15600	56	1000	9
			Note	On the same site as plants with SDR numbers 15xx, 4319 and 4320							
3314 C	soil	<i>R. cuneatum</i>		T	7.9	3	White limestone silt	400000	270	4500	54
	leaf		0	H				11600	12	1100	11
			1					25300	48	1300	14
			Note	On the same site as plants with SDR numbers 15xx, 4319 and 4320							
3314 D	soil	<i>R. cuneatum</i>		T	7.9	3	White limestone silt	400000	270	4500	54
	leaf		0	H				10600	39	1300	13
			1					14200	46	620	8
			Note	On the same site as plants with SDR numbers 15xx, 4319 and 4320							
3314 E	soil	<i>R. cuneatum</i>		T	7.9	3	White limestone silt	400000	270	4500	54
	leaf		0	H				5100	36	800	10
			1					10000	46	530	10
			Note	On the same site as plants with SDR numbers 15xx, 4319 and 4320							

Gan He Ba, Yunnan, 3200 m

A glaciated valley surrounded by pure limestone cliffs and moraines. The valley soil profile is composed of pure limestone silt, around 7 m deep in places.

SDR number		Name of plant	Age of leaf / y	Top/Sub/ Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
3315	soil	R. cuneatum		T	7.9	3	White limestone silt	400000	270	4500	54
	leaf		0	Unhealthy plant				4500	21	420	3.8
			1					6400	18	650	7.5
			Note	On the same site as plants with SDR numbers 15xx, 4319 and 4320							
3317 A	soil	R. telmateium		T	7.9	3	White limestone silt	400000	270	4500	54
	leaf		0	H				8800	100	900	21
			1					11700	16	910	23
			Note	On the same site as plants with SDR numbers 15xx, 4319 and 4320							
3317 B	soil	R. telmateium		T	7.9	3	White limestone silt	400000	270	4500	54
	leaf		0	H				9000	66	1500	18
			1					15900	100	2200	20
			Note	On the same site as plants with SDR numbers 15xx, 4319 and 4320							
3317 C	soil	R. telmateium		T	7.9	3	White limestone silt	400000	270	4500	54
	leaf		0	H				9600	100	1400	20
			1					20100	88	2400	22
			Note	On the same site as plants with SDR numbers 15xx, 4319 and 4320							
3317 D	soil	R. telmateium		T	7.9	3	White limestone silt	400000	270	4500	54
	leaf		0	H				10300	60	1400	21
			1					17700	89	1600	24
			Note	On the same site as plants with SDR numbers 15xx, 4319 and 4320							
3317 E	soil	R. telmateium		T	7.9	3	White limestone silt	400000	270	4500	54
	leaf		0	H				10400	9	1300	23
			1					17600	130	1900	25
			Note	On the same site as plants with SDR numbers 15xx, 4319 and 4320							

Gan He Ba, Yunnan, 3200 m

A glaciated valley surrounded by pure limestone cliffs and moraines. The valley soil profile is composed of pure limestone silt, around 7 m deep in places.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4312	soil	R. telmateium		T	7.8	5	White limestone silt	133000	260	6800	34
	leaf		0	H				4200	1	810	14
			1					12100	110	2700	660
			Note	Growing on the river gravel, next to 4314, 4315 and 4316 (P1). On the same site as plants above.							
4313	soil	R. cuneatum		T	7.8	5	White limestone silt	133000	260	6800	34
	leaf		0	H				7800	24	1500	18
			1					11300	5	1200	13
			Note	Growing on the river gravel. On the same site as plants above.							
4319	soil	R. cuneatum		T	7.5	9	White limestone silt	150000	440	6200	25
	leaf		0	H				6000	110	1200	14
			1					13600	0	1100	18
			Note	1 cm top organic soil (conifer needles) (P2 a, b)							
4320	leaf	R. cuneatum	0	Unhealthy plant				9000	100	1800	11
			Note	Young leaves are pale green. The plant was growing 10 cm away from a healthy R. cuneatum plant (P3 a, b)							
4324	soil	R. yunnanense		T	7.3	15	Grey limestone silt	122000	65	4000	140
	leaf		0	H				4900	60	1450	123
			Note	20 cm mossy top layer. Growing at bottom side of moraine.							
4326	soil	R. vernicosum		T	7.4	7	White limestone silt	150000	320	4500	30
	leaf		0	Fungal disease symptoms				7800	57	1500	10
			1					14000	110	1300	18
			Note	Growing directly in limestone, at bottom of moraine.							

Gan He Ba, Yunnan, 3200 m

A glaciated valley surrounded by pure limestone cliffs and moraines. The valley soil profile is composed of pure limestone silt, around 7 m deep in places.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4335	soil	R. cuneatum		T	7.8	5	White limestone silt	133000	260	6800	34
	leaf		0	H				4400	10	930	17
			1					11000	28	1000	13
				Note							

Soil samples only:

285 soil	Soil under <i>R. cuneatum</i>	T	7.8	NM	White limestone silt	400000	330	7600	570
225 soil	The valley soil	T	8.2	7	White limestone silt	150000	500	3700	9
343 soil	The valley soil	T	7.9	16	White limestone silt	150000	540	4000	13
206 soil	River gravel (4312-4313)	T	7.3	1.1	White limestone silt	43000	210	750	6

Napa Hai, Yunnan, 3500 m

Limestone hills, where *Rhododendron* and evergreen forests dominate. The soil profile consists of limestone subsoil, which in the forest is covered with a top organic layer and needles and/or moss. There is no top layer in the more open places. Part of the area was deforested in the past. The present forests are young.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
1600	soil	<i>R. rubiginosum</i>		T	5.7	79	Black organic	30300	16	1050	370
	leaf		0	H				7900	24	1750	26
	Note		At the very edge of a limestone quarry.								
1601	soil	<i>R. vernicosum</i>		T	5.7	79	Black organic	30300	16	1050	370
	leaf		0	H				22000	40	1300	215
			1					5100	27	850	66
			Note	1 m away from the edge of the quarry .							
1602	soil	<i>R. rubiginosum</i>		T	5.7	79	Black organic	30300	16	1050	370
	leaf		0	H				13000	210	1000	100
			1					3500	46	840	19
			Note	5 m away from the edge of the quarry.							
1603	soil	<i>R. wardii</i>		T	5.7	79	Black organic	30300	16	1050	370
	leaf		0	H				3500	27	700	24
			Note	5 m away from the edge of the quarry.							
1604	soil	<i>R. vernicosum</i>		T	5.7	79	Black organic	30300	16	1050	370
	leaf		0	H				19200	62	670	135
			1					7800	98	900	40
			Note	5 m away from the edge of the quarry.							
2853	soil	<i>R. rubiginosum</i>		T	4.5	31	Brown organic-mineral	2700	1900	150	9
	leaf		0	H				8000	23	2500	104
			1					11250	160	1500	82
			Note	At the edge of the same limestone quarry as samples 1600 and 4633.							

Napa Hai, Yunnan, 3500 m

Limestone hills, where *Rhododendron* and evergreen forests dominate. The soil profile consists of limestone subsoil, which in the forest is covered with a top organic layer and needles and/or moss. There is no top layer in the more open places. Part of the area was deforested in the past. The present forests are young.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2854	soil	<i>R. vernicosum</i>		T	4.5	31	Brown organic-mineral	2700	1900	150	9
	leaf		0	H				10600	38	930	105
			1					11000	44	1000	220
			Note	Near the same limestone quarry.							
4633	soil	<i>R. rubiginosum</i>		S	7.6	18	White limestone silt	122000	500	920	40
	leaf			T	5.6	60	Brown organic	21700	290	220	90
			0	Not so healthy plant				3600	19	730	27
			1					12000	93	1500	100
			Note	On the very edge of the same limestone quarry as samples 1600 and 2853. (P4 a, b, c)							
4634	soil	<i>R. vernicosum</i>		T	5.6	65	Black organic	12500	110	440	75
	leaf		0	In better health than 4633				12900	86	980	180
			1					5100	87	1900	350
			Note	5 m behind the quarry and plant 4633. (P4 d)							
4637	soil	<i>R. rubiginosum</i>		T	4.6	NM	Brown organic	6500	25	150	70
	leaf		0	H				3800	24	920	270
			Note	In a young <i>R. rubiginosum</i> forest. Young plant.							
4639	soil	<i>R. rubiginosum</i>		T	7.1	36	Mineral limestone	10100	290	500	400
	leaf		0 A	Unhealthy branch				9700	124	580	11
			0 B	Healthy branch				6400	53	1200	38
			Note	Very poor, stony soil. SW face. (P5 a)							

Napa Hai, Yunnan, 3500 m

Limestone hills, where *Rhododendron* and evergreen forests dominate. The soil profile consists of limestone subsoil, which in the forest is covered with a top organic layer and needles and/or moss. There is no top layer in the more open places. Part of the area was deforested in the past. The present forests are young.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4641	soil	<i>R. vernicosum</i>		M	Pine needles mulch, top 5 cm			9400	270	290	5
	leaf		0	H			12400	93	2000	690	
			1				8600	20	940	570	
			Note	Growing in a pine and <i>R. vernicosum</i> forest, in a top layer of pine needles.							
4644	leaf	<i>R. rubiginosum</i>	0 A	Unhealthy branch – yellow				15000	180	1600	38
			0 B	Unhealthy branch – purple spots				14800	70	1900	27
			Note	Growing directly in limestone gravel, SW face. Some young leaves had dark purple, uneven marks of necrosis. (P5 b, c, d)							
4645	soil	<i>R. rubiginosum</i>		T	7.2	43	Sample lost	43000	1200	230	250
	leaf		0	Not so healthy plant				4000	120	900	31
			1					30000	65	1800	48
			Note	Growing near to a limestone quarry. Top soil had red-brown colour.							
4646	soil	<i>R. rubiginosum</i>		T	7.3	16	Yellow-grey	10500	45	530	170
	leaf		0	Unhealthy, yellow leaves				4200	63	900	40
			1					23000	290	2900	320
			Note	Growing 10 m away from plant 4633, near the limestone quarry. (P5 e)							
4649	soil	<i>R. rubiginosum</i>		T	7.2	43	Sample lost	43000	1200	230	250
	leaf		0	H				5800	22	1550	158
			Note	Growing next to 4645.							

Shika Shan, Yunnan,
3500 – 4200 m

Limestone hills, where rhododendron forests dominate. The area below the cliffs is wet; the vegetation is growing in thick, mossy organic layer. The summit is loam over limestone subsoil. (**P** Shika Shan 1, 2)

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
1548	soil	<i>R. primuliflorum</i>		T	6.2	85	Black organic	25000	6	1100	270
	leaf		0	H				12800	10	1500	28
			1					5800	5	990	22
4410	soil	<i>Rhododendron</i> Lapponica subsect.		T	7.1	65	Black organic	4800	50	480	600
	leaf		0	H				3700	0	1000	13
			1					12400	110	1800	12
			Note	The plant had its roots in a rock crevice, covered with thin mossy layer.							
4425	soil	<i>R. trichocladum</i>		T	6.2	29	Brown organic-mineral	5100	1400	150	270
	leaf		0	H				3600	0	800	250
			1					7700	13	1000	450
			Note	On a rocky W slope.							
4428	soil	<i>R. trichocladum</i>		T	6.8	51	Black organic-mineral	28100	450	280	1700
	leaf		0	H				3500	32	700	10
			1					8300	10	1000	11
			Note	At the summit. Open moor-land, covered with ‘cushions’ of dwarf rhododendrons, including 4428-4431. (P6 a)							
4429	soil	<i>R. yungningense</i>		T	6.8	51	Black organic-mineral	28100	450	280	1700
	leaf		0	H				3800	70	600	72
			1					7900	100	900	184
			Note	At the summit. Open moor-land, covered with ‘cushions’ of dwarf rhododendrons, including 4428-4431. (P6 a)							

Shika Shan, Yunnan,
3500 – 4200 m

Limestone hills, where rhododendron forests dominate. The area below the cliffs is wet; the vegetation is growing in thick, mossy organic layer. The summit is loam over limestone subsoil. **(P Shika Shan 1, 2)**

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4430	soil	<i>R. trichostomum</i>		T	6.8	51	Black organic-mineral	28100	450	280	1700
	leaf		0	H				6200	34	1200	31
			1					10600	19	1200	22
			Note	At the summit. Open moor land, covered with ‘cushions’ of dwarf rhododendrons, including 4428-4431. (P6 a, b)							
4431	soil	<i>R. primuliflorum</i>		T	6.8	51	Black organic-mineral	28100	450	280	1700
	leaf		0	H				8100	140	1200	19
			1					15000	25	1400	9
			Note	At the summit. Open moor land, covered with ‘cushions’ of dwarf rhododendrons, including 4428-4431. (P6 b)							
4432	soil	<i>R. phaeochrysum</i>		T	6.8	51	Black organic-mineral	28100	450	280	1700
	leaf		0	H				3100	31	1200	190
			1					3000	1	820	215
			Note	At the summit. Open moor land. (P6 c)							

East Bai Ma Shan,
Yunnan, 4300 – 4600 m

Limestone, well-drained, alpine scree slopes. The vegetation is alpine plant species growing on the scree, and dwarf *Rhododendron* species, mainly at the bottom of the hill, growing on scree with very little grassy top layer. (**P** East Bai Ma Shan 1, 2, 3, 4)

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4508	soil	<i>R. fastigiatum</i>		T	6.0	NM	Brown organic-mineral	4100	2400	780	440
	leaf		0	H				4500	24	1500	43
			1					6700	100	2200	150
			Note	At the foot of a scree slope. (P7), (P15 a)							
4509	soil	<i>Rhododendron</i> (dwarf)		T	6.0	NM	Brown organic-mineral	4100	2400	780	440
	leaf		0	H				14200	460	1900	20
			1					12800	140	2900	460
			Note	At the foot of a scree slope.							

Bai Shui Tai
Haba Shan, Yunnan, 2600 m

Travertine pool, lime river.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
3208	soil	R. decorum		T	7.0	6.4	Grey limestone	400000	7	1900	15
	leaf		0	Unhealthy				13200	45	3300	41
			Note	Growing over limestone.							

West Bai Ma Shan,
Yunnan, 4300 – 4800 m

Granite, well-drained, alpine scree slopes. The vegetation is alpine plant species growing on the scree, and dwarf *Rhododendron* species, mainly at the bottom of the hill, growing on scree with very little grassy top layer, or on moor. (**P** West Bai Ma Shan 1, 2 (M.K.), 3 (J.A.))

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
1706	soil	<i>Rhododendron</i> <i>Lapponica</i> subsect.		T	4.6	28	Brown mineral	1200	650	109	73
	leaf		0	H				7000	68	1900	650
1710	soil	<i>R. saluenense</i>		T	4.4	52	Brown organic-mineral	1020	900	260	17
	leaf		0	H				5300	67	1000	280
1712	soil	<i>R. phaeochrysum</i> <i>agglutinatum</i>		T	4.4	52	Brown organic-mineral	1020	900	260	17
	leaf		0	H				9800	26	1400	3000
			1					6300	45	1100	1870
4539	soil	<i>R. aganniphum</i>		T	5.8	14	Metamorphic mineral	580	2800	70	50
	leaf		0	H				2200	25	620	590
			1					8700	140	1600	3400
4540	soil	<i>R. fastigiatum</i>		T	5.8	14	Metamorphic mineral	580	2800	70	50
	leaf		0	H				7100	310	1700	440
			1					11000	75	1700	1100
			Note	Growing on moor.							
4554	leaf	<i>R. fastigiatum</i>	0	H				3200	15	760	260
			1					9400	71	1600	960
			Note	Growing on moor.							

Haba village (above),
Haba Shan, Yunnan, 2900 m

Open deciduous woods.

SDR number	Name of plant	Age of leaf / y	Top/Sub/Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
3218	soil		T	6.4	21	Grey metamorphic	5200	680	330	280
	leaf	0	Unhealthy				8400	45	2200	220
		Note	Growing in pine wood.							

Da Xue Shan, West side,
Yunnan 4200 – 4600 m

Mainly limestone area.

SDR number	Name of plant	Age of leaf / y	Top/Sub/Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2729	soil		T	7.5	4	Yellow- grey limestone	170600	150	640	162
	leaf	0	H				5400	67	1000	25
		1					13000	94	1200	50
		Note								
2739	soil		T	7.2	41	Mineral limestone	47000	13	310	350
	leaf	0	H				4900	32	860	14
		1					12300	135	1400	22
		Note	Growing on limestone and organic soil.							
2744	soil		T	7.4	34	Grey limestone	396000	21	2800	114
	leaf	0	H				5800	110	870	14
		1					16000	150	1400	18
		Note	Growing on lime scree.							

Yulong Xue Shan,
Yunnan, 3200 – 4300 m

Limestone mountain. Between 3200 m and 3800 m, and depending on the aspect and steepness, the area is dominated by *Rhododendron*, oak and conifer forests, boggy meadows, and is generally a wet area. The vegetation is growing in a thick, mossy organic layer. Ascending to the summits, the landscape changes to loam over limestone subsoil covered with dwarf *Rhododendron* species.

SDR number		Name of plant	Age of leaf / y	Top/Sub/Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4202	soil	<i>R. decorum</i>		T	5.9	43	Brown organic-mineral	6300	2800	630	1800
	leaf		0	H				5600	57	1800	260
			1					9400	50	1900	550
			2					21000	840	3300	1200
			Note	Growing in a mixed forest, near cement rubble.							
4203	soil	<i>R. racemosum</i>		T	5.9	43	Brown organic-mineral	6300	2800	630	1800
	leaf		0	H				8500	150	1700	130
			1					7600	66	1400	123
			Note	Growing in a mixed forest, next to plant 4202.							
4205	soil	<i>R. hippophaeoides</i>		T	5.4	41	Brown organic-mineral	2200	2800	430	690
	leaf		0	H				6600	50	1100	50
			1					18000	160	2200	185
			Note	Growing on an open rock face, in 15 cm mossy top layer.							
4206	soil	<i>R. rupicola</i>		T	5.4	41	Brown organic-mineral	2200	2800	430	690
	leaf		0	H				15300	175	3000	780
			Note	Growing on an open rock face, 10 m away from plant 4205. (P9)							
4212	leaf	<i>R. decorum</i>	0	Unhealthy, red leaves with purple marks of necrosis				3000	0	1200	390
			0	Unhealthy, yellow and purple spots				6800	17	2300	790
			1	Healthy, green				6200	61	2100	1400
			Note	Growing in a boggy meadow, pH taken 10 m away is pH = 6.0. (P10 a, b)							

Yulong Xue Shan,
Yunnan, 3200 – 4300 m

Limestone mountain. Between 3200 m and 3800 m, and depending on the aspect and steepness, the area is dominated by *Rhododendron*, oak and conifer forests, boggy meadows, and is generally a wet area. The vegetation is growing in a thick, mossy organic layer. Ascending to the summits, the landscape changes to loam over limestone subsoil covered with dwarf *Rhododendron* species.

SDR number		Name of plant	Age of leaf / y	Top/Sub/ Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4221	soil	<i>R. rubiginosum</i>		S	5.0	18	Yellow-grey	650	3300	60	90
			T	5.5	66	Black organic-mineral	5900	330	640	350	
	leaf		0	H				2600	61	540	94
			1					4500	22	480	210
			2					11200	120	280	720
			Note					Large <i>R. rubiginosum</i> forest. (P11 a, b, c)			
4233	soil	<i>R. anthosphaerum</i>		T	5.0	NM	Brown organic	2400	4200	290	160
	leaf		1					10700	32	1400	1400
	Note										
4245	soil	<i>R. phaeochrysum</i> var. <i>phaeochrysum</i>		T	5.6	NM	Brown organic-mineral	1600	4000	330	60
	leaf		0	H				2800	43	1250	510
			Note	Peaty soil from a very wet meadow/moor.							
4246	soil	<i>R. saluenense</i>		T	5.6	NM	Brown organic-mineral	1600	4000	330	60
	leaf		0	H				4000	40	800	215
			1					10000	53	1200	600
			Note	On a loam field on top of a hill.							
4255	soil	<i>R. impeditum</i>		T	5.6	13	Red-brown	320	4100	60	990
	leaf		0	H				6000	17	1600	500
			1					11300	330	3200	770
			Note	Growing in a mixed forest in an organic layer covering the limestone.							

Yulong Xue Shan,
Yunnan, 3200 – 4300 m

Limestone mountain. Between 3200 m and 3800 m, and depending on the aspect and steepness, the area is dominated by *Rhododendron*, oak and conifer forests, boggy meadows, and is generally a wet area. The vegetation is growing in a thick, mossy organic layer. Ascending to the summits, the landscape changes to loam over limestone subsoil covered with dwarf *Rhododendron* species.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4262	soil	<i>R. primuliflorum</i>		T	5.7	47	Brown organic	5200	2800	300	620
	leaf		0	H				15000	40	900	80
			1					16800	120	1100	83
			Note	Growing in a mixed forest in an organic layer covering the limestone.							
4265	soil	<i>R. yunnanense</i>		T	5.1	52	Brown organic	3400	2700	350	950
	leaf		0	H				5500	11	1500	150
			Note	Growing in a mixed forest in an organic layer covering the limestone.							
4270	leaf	<i>R. decorum</i>	0	Unhealthy				3400	73	1700	123
			Note	Growing next to steps, in rubble of cement, left from building work. Near sample 4202. (P13)							

Huluhai,
Yunnan, 4300 m

Open valley. **No** signs of limestone.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4756	leaf	<i>R. phaeochrysum</i>	0	H				3300	22	920	320
			1					3500	170	950	330
			2					2400	27	540	240
			Note								

Xiang Chen
Yunnan, 3800 m

Deciduous and mixed forest.

SDR number	Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4957	leaf	<i>R. yunnanense</i>	0			H	8600	34	3100	69
			1				8800	49	2600	62
			Note							

Ri Zhao Xue Shan,
Yunnan, 3600 - 4600 m

Low rainfall area, mainly limestone mountain. Poor in rhododendrons, only very few observed.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4811	soil	<i>R. primuliflorum</i>		T	5.5	16	Red-brown silt	1300	20	160	50
	leaf		0	H				7000	45	1500	55
			1					9000	64	1260	90
			Note								
4856	soil	<i>Rhododendron</i> Lapponica subsect.		T	6.6	52	Brown organic	25000	3000	180	250
	leaf		0	H				7300	40	1400	154
			1					15800	130	1700	370
			Note								

GaJin mountain,
Yunnan, 3800 – 4200 m

Dry area. Limestone mountain.

SDR number	Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
5001	leaf	<i>R. primuliflorum</i>	0		H		5800	130	1600	56
			1				11400	150	2200	110
			Note				In perfect health.			
5002	leaf	<i>R. phaeochrysum</i> <i>var. agglutinatum</i>	0		H		5200	20	1800	720
			1				7200	180	1700	1160
			2				9000	130	1700	1300
			Note				In perfect health.			
5021	leaf	<i>R. phaeochrysum</i> <i>var. agglutinatum</i>	0		H		4900	40	1400	1570
			1				5200	24	860	1160
			2				9200	65	760	2800
			Note				In perfect health.			
5023	leaf	<i>R. phaeochrysum</i> <i>var. agglutinatum</i>	0		H		5600	13	1900	410
			1				9300	80	2500	820
			2				8800	62	1800	750
			Note				In perfect health.			
5026	leaf	<i>Rhododendron</i> <i>aff. decorum</i>	0		H		10300	28	2700	1270
			1				14900	76	3000	2250
			Note				In perfect health.			

Weixi,
Yunnan, 2500 m

Wet area, **No** sign of limestone.

SDR number	Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
5108	leaf	<i>Rhododendron</i>	0			H	2300	55	2400	1370
			1				7000	220	3300	1520
			2				5900	62	1800	1760
			Note							
5109	leaf	<i>R. racemosum</i>	0			H	1700	70	1100	30
			1				2900	35	690	100
			Note							

Wu Min Shan,
East side, Yunnan, 3900 m

Moor land.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
1827	soil	<i>Rhododendron</i> Lapponica subsect.		T	7.1	18	Brown mineral	7000	1300	1370	380
	leaf		0	H				8700	77	3300	37
			Note								

Li Ti Ping,
Yunnan, 3250 m

Deciduous forest, open areas. **No** sign of limestone.

SDR number	Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
5116	leaf	<i>R. rubiginosum</i>	0			H	4300	410	2500	280
			1				7100	1100	3300	480
			Note							
5117	leaf	<i>Rhododendron</i>	0			H	10100	180	3100	1800
			1				12900	450	1800	2740
			2				21700	970	2200	3100
			Note							
5149	leaf	<i>Rhododendron</i>	0			H	9800	30	3700	660
			1				5500	60	1900	450
			2				7600	20	1300	1120
			Note							

East of Daochen
Yunnan, 3900 m

Open moor land. **No** limestone.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
1872	soil	<i>Rhododendron</i> Lapponica subsect.		T	6.9	9	Brown mineral	1850	550	120	320
	leaf		0	H				9400	36	2300	124
			1					5500	0	1700	54
			Note								

Road Daochen - Ya Ding
and south of Ya Ding
Sichuan, 3500 – 4000 m

Valley with mixed forest and moor land over limestone formations.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
3011	soil	<i>R. nitidulum</i>		T	7.7	18	Yellow- grey	47500	3300	370	1000
	leaf		0	H				12000	100	2500	14
			Note	Limestone soil.							
3020	soil	<i>R. vernicosum</i>		T	6.7	11	Red-brown	6300	1000	160	990
	leaf		0	H				4500	47	1300	330
			Note	Young plant, the soil is over limestone.							
3034	soil	<i>R. nivale</i> subsect. <i>boreale</i>		T	7.4	12	Grey limestone	136600	230	12700	380
	leaf		0	H				8000	54	2900	9
			Note	Limestone crevice soil.							
3035	soil	<i>R. primuliflorum</i>		T	7.4	12	Grey limestone silt	136600	230	12700	380
	leaf		0	H				5300	165	1600	11
			Note	Limestone crevice soil.							
3036	soil	<i>R. balfourianum</i>		T	7.4	12	Grey limestone silt	136600	230	12700	380
	leaf		0	H				7400	26	1600	13
			Note	Limestone crevice soil.							
3057	soil	<i>R. nivale</i> subsect. <i>boreale</i>		T	7.5	34	Brown limestone silt	91500	17	31200	270
				S	7.9	29	White limestone silt	250500	300	94000	80
	leaf		0	H				18300	270	3700	18
			Note	Around 5 cm top soil layer.							

Table 44 ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil samples from *Rhododendron* species collected in Europe.

Schachen, The Alps, Germany, 1150 m				Area dominated by dolomite.							
SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2101	soil	<i>R. hirsutum</i>		T	6.9	50	Brown dolomite	174100	13	90600	31
	leaf		0	H				5300	146	3200	11
			1					15300	140	6400	9
			Note								
2102	soil	<i>R. hirsutum</i>		T	5.6	91	Brown organic	23000	4	7400	46
	leaf		0	H				4100	220	2700	7.2
			1					8500	82	3500	25
			Note								

Pordoi, The Dolomites, Italy, 2300 m				Dolomite area.							
SDR number		Name of plant	Age of leaf / y	Top/Sub/ Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2105	soil	<i>R. ferrugineum</i>		T	6.5	77	Brown dolomite	31300	11	7000	430
	leaf		0	H				5500	390	1900	11
			1					6300	145	1850	16
			Note								

Sassolunga, The Dolomites,
Italy, 2100 m

Dolomite area.

SDR number		Name of plant	Age of leaf / y	Top/Sub/Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2115	soil	<i>R. hirsutum</i>		T	7.2	61	Brown organic	98500	150	44200	1250
	leaf		0	H				9500	165	2900	11
			Note								

Chignolo, The Alps,
Italy, 900 m

Dolomitic limestone area.

SDR number		Name of plant	Age of leaf / y	Top/Sub/Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2403	soil	<i>R. hirsutum</i>		T	7.2	46	Organic limestone	146300	16	75000	1200
	leaf		0	H				7300	230	3000	15
			Note								
2408	soil	<i>R. hirsutum</i>		T	7.2	47	Organic limestone	163000	28	85000	420
	leaf		0	H				7500	1600	2700	12
			Note								
2413	soil	<i>R. hirsutum</i>		T	7.5	47	Organic limestone	172300	11	86200	380
	leaf		0	H				6400	100	3100	5
			Note								
2418	soil	<i>R. hirsutum</i>		T	7.6	54	Brown organic	86000	48	39000	900
	leaf		0	H				8700	53	3500	13
			Note								

To Refuge d'Anterne,
SW Alps,
Switzerland, 1750 m

Non-limestone area. Open mountain.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
3528	soil	<i>R. ferrugineum</i>		T	6.5	81	Brown organic	48500	20	430	1140
	leaf		0	H				6300	210	1400	72
			1					11500	70	2000	175
				Note	Growing over limestone.						

Petit Mont Cenis, SW Alps,
Switzerland, 2100 m

Limestone area.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
3596	soil	<i>R. ferrugineum</i>		T	6.8	47	Organic limestone	52600	81	14700	260
	leaf		0	H				8700	210	1560	18
			1					10500	150	1400	20
				Note	On limestone.						

Arolla,
Switzerland, 2200 m

Non-limestone, very rocky area beside the glacier moraines.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4101	soil	<i>R. ferrugineum</i>		T	5.9	12	Grey metamorphic	1700	120	230	47
	leaf		0	H				1900	35	1300	18
			1					4400	40	1100	21
			2					6200	70	1100	23
			Note								

Table 45 ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil samples from *Rhododendron* species collected in the USA and Canada.

Crabtree Falls, Blue Ridge Parkway, North Carolina, 1000 m				Deciduous forest.							
SDR number		Name of plant	Age of leaf / y	Top/Sub/ Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2213	soil	<i>R. maximum</i>		T	5.4	6	Metamorphic mineral	830	1200	130	120
	leaf		0	H				7300	32	2000	405
			1					9600	27	2600	680
			2					11800	23	2400	1010
			3					13000	21	2550	1265
			4					14300	9	2300	1320
			5					15000	44	2300	1660
			Note								

Craggy Gardens,
Blue Ridge Parkway, North
Carolina, 1660 m

Deciduous forest.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2219	soil	<i>R. catawbiense</i>		T	5.0	17	Metamorphic mineral	570	1300	80	400
	leaf		0	H				8000	36	2100	1860
			Note								

Graveyard Fields,
Blue Ridge Parkway, North
Carolina, 1500 m

Deciduous forest.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2241	soil	R. maximum		T	3.9	50	Brown organic	160	1000	200	17
	leaf		0	H				4300	57	2100	265
			1					4600	94	1500	410
				Note							

Table 46 ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil samples from *Rhododendron* species collected in Sikkim, India.

Dzongri, Kanchenjunga, Sikkim, 4000 m			Non-limestone area, at transition from forest to grassy meadow area.								
SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
3942	leaf	<i>R. anthopogon</i>	0		H			4800	16	420	140
			1					9400	62	630	215
			Note								
3943	leaf	<i>R. setosum</i>	0		H			7500	81	740	210
			1					9800	140	1600	310
			Note								
3944	leaf	<i>R. lepidotum</i>	0		H			4000	500	780	280
			1					9200	80	1200	710
			Note								

Results for alpine species in the wild

- Table 47 ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil samples from *Gentiana* and other alpine species, collected in China, in NW Yunnan and SW Sichuan. – page 267
- Table 48 ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil samples from *Gentiana* and other alpine species, collected in Europe. – page 287
- Table 49 ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil samples from *Gentiana* and other alpine species, collected in the USA and Canada. – page 293
- Table 50 ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil samples from *Gentiana* and other alpine species, collected in Sikkim, India. – page 295

Table 47 ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil samples from *Gentiana* and other alpine species, collected in China, in NW Yunnan and SW Sichuan.

Gan He Ba, Yunnan, 3200 m	A glaciated valley surrounded by pure limestone cliffs and moraines. The valley soil profile is composed of pure limestone silt, around 7 m deep in places.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
3320	soil	<i>Daphne calcicola</i>		T	7.9	3	White limestone silt	400000	270	4500	54
	leaf		0				H	10500	100	760	22
			Note	Collected at the same site as the samples below in the table.							
4305	soil	<i>Primula forrestii</i>		T	7.3	37	Mineral limestone	32400	1100	580	1800
	leaf		0				H	14000	360	2200	50
			1					21000	230	2100	47
			Note	Growing in limestone, under half shadow (half day).							
4311	soil	<i>Primula pulchella</i>		T	7.1	4	White limestone silt	46000	210	750	7
	leaf		0				H	14000	130	2400	14
			Note								
4314	soil	<i>Daphne calcicola</i>		T	7.8	5	White limestone silt	133000	260	6800	34
	leaf		1				H	7100	55	630	5
			Note	Growing next to <i>R. telmateium</i> 4312. (P1)							
4315	soil	<i>Gentiana szechenyii</i>		T	7.8	5	White limestone silt	133000	260	6800	34
	leaf		0				H	11000	42	1200	5
			Note	Growing next to <i>R. telmateium</i> 4312, 4315 and 4316. (P1)							
4316	soil	<i>Saxifraga sp.</i>		T	7.3	1.1	White limestone silt	43000	210	750	6
	leaf		0				H	82000	200	3100	23
			Note	Growing next to <i>R. telmateium</i> 4312, 4314 and 4315. (P1 a)							
4325	soil	<i>Daphne tangutica</i>		T	7.3	15	Grey limestone silt	122000	65	4000	140
	leaf		0				H	12000	140	1600	50
			Note	Growing near plants 4312, 4314 and 4315.							

Gan He Ba, Yunnan, 3200 m

A glaciated valley surrounded by pure limestone cliffs and moraines. The valley soil profile is composed of pure limestone silt, around 7 m deep in places.

SDR number	Name of plant	Top/Sub/Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
014/02 soil	<i>Saxifraga sp.</i>	T	8.0	NM	White limestone silt	95600	225	6700	64
016/02 soil	<i>Allium beesianum</i>	T	8.2	NM	White limestone silt	95600	200	5400	40
019/02 soil	<i>Cyananthus sp.</i>	T	7.8	35	Grey limestone silt	95600	1900	2500	230
024 soil	<i>Primula malvacea</i>	T	8.0	NM	Red-brown	53400	90	11400	1200

Wu Min Shan, East side,
Yunnan, 3900 – 4700 m

Moor land.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
1823	soil	<i>Primula sp.</i>		T	7.1	18	Brown mineral	7000	1300	1370	380
	leaf		0				H	16000	130	9300	28
1825	soil	<i>Saussurea globosa</i>		T	7.1	18	Brown mineral	7000	1300	1370	380
	leaf		0				H	23000	120	540	83
1826	soil	<i>Primula sikkimensis</i>		T	7.1	18	Brown mineral	7000	1300	1370	380
	leaf		0				H	29300	170	10400	94
2906	soil	<i>Gentiana arethusae</i>		T	5.5	27	Brown organic-mineral	5200	220	400	300
	leaf		0				H	5200	68	1400	280
4906	soil	<i>Gentiana hexaphylla</i>		T	6.6	NM	Grey metamorphic	930	5	350	4
	leaf		0				H	7100	300	2000	330

Napa Hai, Yunnan, 3500 m

Limestone hills, where *Rhododendron* and evergreen forests dominate. The soil profile consists of limestone subsoil, which in the forest is covered with a top organic layer and needles and/or moss. There is no top layer in the more open places. Part of the area was deforested in the past. The present forests are young.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4636	soil	<i>Androsace mariae</i>		T	5.6	67	Black organic	12500	110	440	75
	leaf		0	H				13000	26	1600	570
			Note	Growing under a <i>Rhododendron</i> plant.							
247/02 soil		<i>Sedum sp.</i>			NM	NM		95600	530	730	260

N of Napa Hai, on way to
Bai Ma Shan
Yunnan, 3000 m

Terra rossa – red clay soil formed by decay of limestone.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4459	soil	<i>Daphne aurantiaca</i>		T	7.4	29	Red-brown	44000	210	920	12
	leaf		0	H				13000	32	1200	28
			Note	(P14 a) Photo by Jane Armstrong							
4460	soil	<i>Androsace bulleyana</i>		T	7.4	29	Red-brown	44000	210	920	12
	leaf		0	H				17000	280	1200	40
			Note	(P14 b and c)							

East Bai Ma Shan,
Yunnan, 4300 – 4800 m

Limestone, well-drained, alpine scree slopes. The vegetation is alpine plant species growing on the scree, and dwarf *Rhododendron* species, mainly at the bottom of the hill, growing on scree with very little grassy top layer. (**P** East Bai Ma Shan 1, 2, 3, 4)

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4506	soil	<i>Cassiope sp.</i>		T	6.0	NM	Brown organic-mineral	4100	2400	780	440
	leaf		0				H	6500	0	1400	250
			Note	At the foot of a scree slope.							
4507	soil	<i>Androsace delavayi</i>		T	6.0	NM	Brown organic-mineral	4100	2400	780	440
	leaf		0				H	9200	240	2200	340
			Note	At the foot of scree slope. (P15 b)							
4511	leaf	<i>Primula sp.</i>	0				H	6100	120	1400	130
			Note	On limestone scree slope.							
4515	leaf	<i>Rheum sp.</i>	0				H	12000	150	15000	1900
			Note	On limestone scree slope.							
4516	leaf	<i>Androsace zambalensis</i>	0				H	17000	22	2300	26
			Note	On limestone scree slope. (P15 c)							
4519	leaf	<i>Rheum sp.</i>	0				H	23000	170	15000	2900
			Note	On limestone scree slope.							
4527	soil	<i>Androsace yargongensis</i>		T	7.7	11	Dark grey silt	140000	770	900	210
	leaf		0				H	38000	1200	1900	67
			Note	On limestone scree slope.							
4531	soil	<i>Primula zambalensis</i>		T	7.4	NM	Dark grey silt	8000	1100	320	360
	leaf		0				H	22000	360	4400	39
			Note	On limestone scree slope.							
4532	soil	<i>Primula sinopurpurea chionantha subsp.</i>		T	7.4	NM	Dark grey silt	8000	1100	320	360
	leaf		0				H	6100	190	1800	13
			Note	On limestone scree slope.							

East Bai Ma Shan,
Yunnan, 4300 – 4800 m

Limestone, well-drained, alpine scree slopes. The vegetation is alpine plant species growing on the scree, and dwarf *Rhododendron* species, mainly at the bottom of the hill, growing on scree with very little grassy top layer. (P East Bai Ma Shan 1, 2, 3, 4)

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4533	soil	<i>Primula minor</i>		T	7.4	NM	Dark grey silt	8000	1100	320	360
	leaf		0	H				7200	47	990	21
			Note	On limestone scree slope.							

Ri Zhao Xue Shan,
Yunnan, 3600 - 4600 m

Limestone cliffs surrounding a valley.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4823	soil	<i>Gentiana veitchiorum</i>		T	6.9	NM	Brown mineral	1900	6	330	6
	leaf		0	H				9900	290	1100	180
			Note								
4833	leaf	<i>Primula bracteata</i>	1	H				28000	45	3100	33
			Note	Very old leaves (dry on the stem), Growing on W – facing limestone cliff.							
4835	soil	<i>Primula bracteata</i>		T	7.3	NM	Organic limestone	22000	11	1400	21
	leaf		0	H				27000	220	4800	46
			Note								
4837	soil	<i>Primula sp.</i>		T	7.3	NM	Yellow- grey	16300	8	420	6
	leaf		0	H				31000	94	2000	39

West Bai Ma Shan,
Yunnan, 4300 – 4800 m

Granite, well-drained, alpine scree slopes. The vegetation is alpine plant species growing on the scree, and dwarf *Rhododendron* species, mainly at the bottom of the hill, growing on scree with very little grassy top layer, or on moor. (**P** West Bai Ma Shan 1, 2, 3)

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
1704	soil	<i>Primula dryadifolia</i>		T	4.6	28	Brown mineral	1200	650	110	73
	leaf		0	H				4400	0	780	650
			Note								
1705	soil	<i>Cassiope selaginoides</i>		T	4.6	28	Brown mineral	1200	650	110	73
	leaf		0	H				5700	0	1400	83
			Note								
1707	soil	<i>Diapensia purpurea</i>		T	4.6	28	Brown mineral	1200	650	110	73
	leaf		0	H				4600	27	1700	400
			Note								
1709	soil	<i>Gentiana atunsiensis</i>		T	4.4	52	Brown organic-mineral	1000	900	260	17
	leaf		0	H				3600	280	1400	730
			Note								
1711	soil	<i>Cassiope selaginoides</i>		T	4.4	52	Metamorphic mineral	1000	900	260	17
	leaf		0	H				5800	100	1000	230
			Note								
4538	soil	<i>Cassiope wardii</i>		T	5.8	17	Metamorphic mineral	580	2800	70	50
	leaf		0	H				3100	8	1000	89
			Note	At the foot of the mountain and the scree slopes.							
4541	soil	<i>Androsace yargongensis</i>		T	5.8	17	Metamorphic mineral	580	2800	70	50
	leaf		0	H				11000	720	2600	230
			Note	On a moor.							

West Bai Ma Shan,
Yunnan, 4300 – 4800 m

Granite, well-drained, alpine scree slopes. The vegetation is alpine plant species growing on the scree, and dwarf *Rhododendron* species, mainly at the bottom of the hill, growing on scree with very little grassy top layer, or on moor. (**P** West Bai Ma Shan 1, 2 (M.K.), 3 (J.A.))

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4547	leaf	<i>Gentiana sp.</i>	0				H	2500	140	1200	530
			Note				(P16 a) Photo by Jane Armstrong				
4552	soil	<i>Gentiana wardii</i>		T	5.9	6.3	Grey metamorphic	640	1100	530	63
	leaf		0				H	2900	110	1000	340
			Note				Growing in colonies on scree on the flat parts of the hill. (P16 b)				
4553	soil	<i>Primula dryadifolia</i>		T	5.9	6.3	Grey metamorphic	640	1100	530	63
	leaf		0				H	2700	50	680	800
			Note				Growing on scree, next to the <i>Gentiana</i> 4552. (P17 a) Photo by Jane Armstrong, (P17 b)				
4563	soil	<i>Primula sino-plantaginea chionantha subsp.</i>		T	5.9	10	Grey metamorphic	1600	210	580	42
	leaf		0				H	7100	45	1000	110
			Note				Growing on flat area.				
4569	soil	<i>Primula sikkimensis</i>		T	6.9	14	Grey metamorphic	5000	210	570	19
	leaf		0				H	26000	110	4800	76
			Note				Growing in a crevice. (P17 c, d)				
078/00 soil		<i>Gentiana sp.</i>			5.2	21	Brown mineral	420	3100	75	74

Shika Shan, Yunnan,
3500 – 4200 m

Limestone hills, where *Rhododendron* forests dominate. The area below the cliffs is wet; the vegetation is growing in thick, mossy organic layer. The summit is loam over limestone subsoil. (**P** Shika Shan 1, 2)

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
1545	soil	<i>Corydalis sp.</i>		T	7.4	46	Black organic	46300	2400	2900	2000
	leaf		0	H				34700	140	7800	100
			Note								
1546	soil	<i>Cyananthus longituba</i>		T	7.4	46	Black organic	46300	2400	2900	2000
	leaf		0	H				15400	185	3500	110
			Note								
1547	soil	<i>Swertia sp.</i>		T	7.4	46	Black organic	46300	2400	2900	2000
	leaf		0	H				27000	260	2200	76
			Note								
4404	soil	<i>Primula polyneura</i>		T	7.2	62	Black organic	26800	17	1100	170
	leaf		0	H				14000	91	1400	4
			Note	Growing in wet, peaty soil.							
4411	soil	<i>Daphne aurantiaca</i>		T	7.1	71	Black organic	4800	50	480	600
	leaf		0	H				8700	23	1500	14
			Note	The plant had its roots in a rock crevice.							
4414	soil	<i>Primula sikkimensis</i>		T	7.9	NM	Brown organic	14000	220	840	62
	leaf		0	H				17000	60	2700	46
			Note	Growing in wet, peaty soil							
4415	soil	<i>Primula cf. deflexa</i>		T	6.3	65	Black organic	8800	220	1100	76
	leaf		0	H				18000	130	4700	55
			Note	Growing in wet, peaty soil.							

Shika Shan, Yunnan,
3500 – 4200 m

Limestone hills, where *Rhododendron* forests dominate. The area below the cliffs is wet; the vegetation is growing in thick, mossy organic layer. The summit is loam over limestone subsoil. (**P** Shika Shan 1, 2)

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4421	soil	<i>Primula sonchifolia</i>		T	6.9	NM	Black organic	14000	220	630	35
	leaf		0				H	18000	59	2700	250
			Note	Growing in wet, peaty soil, under shadow.							
4422	soil	<i>Primula dryadifolia</i>		T	7.0	35	Brown mineral	9800	220	670	50
	leaf		0				H	9800	260	1200	28
			Note	On a rocky W slope.							
4423	soil	<i>Primula minor</i>		T	7.1	35	Brown organic	9000	220	600	74
	leaf		0				H	11000	230	1200	30
			Note	On a rocky W slope.							
4434	leaf	<i>Gentiana trichotoma</i>	0				H	6400	75	1000	420
			Note	At the summit. Open moor land, together with dwarf rhododendrons, including 4428-4431. (P6 b)							

Xiao Xue Shan,
Yunnan, 4300 – 4800 m

Limestone cliffs.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2841	soil	<i>Primula bracteata</i>		T	7.4	19	Grey limestone	95600	40	730	240
	leaf		0				H	19700	180	990	29
			Note	On a shady face in crevice soil							
4722	soil	<i>Primula bracteata</i>		T	7.5	NM	Grey limestone	16100	13	330	11
	leaf		0					21000	140	1800	4
			Note	Growing on S –facing limestone slope							

Hong Shan,
Yunnan, 4100 – 4260 m

Wet, flat area of forests and meadows, but most samples were collected on non-limestone scree slope. (**P** Hong Shan Scree slope 1, 2)

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4435	soil	<i>Primula secundiflora</i>		T	7.5	10	Metamorphic mineral	7800	210	1500	26
	leaf		0	H				15000	2100	2500	110
			Note								
4438	leaf	<i>Primula hongshanensis</i>	0	H				12000	38	3000	1500
			Note	Growing in woodland in peaty soil and deep shade. (P18)							
4443	soil	<i>Primula amethystina</i>		T	6.5	9	Metamorphic mineral	650	210	530	110
	leaf		0	H				7500	90	4400	63
			Note	Growing on a slate slope.							
4449	soil	<i>Primula florida</i>		T	5.9	7.8	Metamorphic mineral	850	210	530	25
	leaf		0	H				5500	65	1500	81
			Note	Growing on a scree slope.							
4450	leaf	<i>Rheum delavayi</i>	0	H				7600	180	2100	140
			Note	Growing on a scree slope.							
4452	soil	<i>Primula brevicula</i>		T	5.3	83	Black organic	10500	7	580	147
	leaf		0	H				13000	26	2300	230
			Note	Growing on a scree slope. (P19) Photo by Jane Armstrong							

Yulong Xue Shan,
Yunnan, 3200 – 4300 m

Limestone mountain. Between 3200 m and 3800 m, and depending on the aspect and steepness, the area is dominated by *Rhododendron*, oak and conifer forests, boggy meadows, and is generally a wet area. The vegetation is growing in a thick, mossy organic layer. Ascending to the summits, the landscape changes to loam over limestone subsoil covered with dwarf *Rhododendron* species.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4210	soil	<i>Primula poissonii</i>		T	5.9	35	Brown organic	3700	11000	520	230
	leaf		0				H	15000	100	6700	200
			Note	Growing in a boggy meadow, pH taken 10 m away is pH = 6.0. (P20)							
4211	soil	<i>Primula beesiana bulleyana subsp.</i>		T	5.9	35	Brown organic	3700	11000	520	230
	leaf		0				H	7200	140	1600	50
			Note	Growing in a boggy meadow, pH taken 10 m away is pH = 6.0.							
4227	soil	<i>Gentiana sp.</i>		T	5.5	25	Brown mineral	1100	4100	340	57
	leaf		0				H	5800	130	1900	330
			Note	Growing in a mixed wood.							
4228	soil	<i>Primula cortusoides</i> sect.		T	5.0	21	Yellow-grey	650	3300	60	90
	leaf		0				H	27000	74	3500	150
			Note	Growing in a <i>R. rubiginosum</i> wood, in a wet, mossy layer, over limy soil.							
4230	soil	<i>Gentiana sp.</i>		T	5.4	37	Brown organic	4700	3300	840	450
	leaf		0				H	6100	120	1500	230
			Note								
4231	soil	<i>Gentiana trichotoma</i>		T	5.3	39	Brown organic	3300	3600	590	460
	leaf		0				H	2100	210	1100	59
			Note	(P21)							
4234	soil	<i>Gentiana sino-ornata</i>		T	5.0	NM	Brown organic	2400	4200	290	160
	leaf		0				H	5700	120	1500	490
			Note	Growing in a boggy meadow. (P22 a, b) Photo by Jane Armstrong							

Su La,
Yunnan, 4550 - 4710 m

High, non-limestone stony pass, on border of Yunnan and Tibet (Xizang).

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
1642	soil	<i>Androsace sp.</i>		T	7.9	7	Grey metamorphic	5200	1900	950	820
	leaf		0	H				16300	140	2500	230
			Note	Flaky, slate-like soil in sheets.							

To Lao Jun Shan,
Yunnan, 3870 m

Around a lake, conifer and *Rhododendron* forest.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2567	soil	<i>Primula sonchifolia</i>		T	5.2	NM	Brown organic	1500	700	300	150
	leaf		0	H				8200	8	3300	680
			Note								
2579	soil	<i>Gentiana atunsiensis</i>		T	4.5	62	Brown organic	2300	78	240	130
	leaf		0	H				5400	17	1500	520
			Note								
2596	soil	<i>Primula muscariodes</i>		T	5.8	NM	Brown organic-mineral	3200	170	500	160
	leaf		0	H				8500	130	1900	880
			Note								

Da Xue Shan, East side,
Yunnan 4100 – 4350 m

Non-limestone scree. Slate stony.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2773	soil	<i>Gentiana hexaphylla</i>		T	5.4	6	Grey metamorphic	350	50	31	28
	leaf		0	H				9300	410	3300	210
			Note	On slate soil.							
2774	soil	<i>Phlomis rotata</i>		T	5.4	6	Grey metamorphic	350	50	31	28
	leaf		0	H				21000	140	4800	57
			Note	On slate soil.							
2775	soil	<i>Euphorbia sp.</i>		T	5.4	6	Grey metamorphic	350	50	31	28
	leaf		0	H				16601	30	2995	112
			Note	On slate soil.							
2776	soil	<i>Gentiana szechenyii</i>		T	5.4	6	Grey metamorphic	350	50	31	28
	leaf		0	H				7300	560	1900	70
			Note	On slate soil.							
2777	soil	<i>Cremanthodium sp.</i>		T	5.4	6	Grey metamorphic	350	50	31	28
	leaf		0	H				13000	180	2700	60
			Note	On slate soil.							
2778	soil	<i>Saussurea sp.</i>		T	5.4	6	Grey metamorphic	350	50	31	28
	leaf		0	H				10100	90	3000	180
			Note	On slate soil.							
2785	soil	<i>Rheum nobile</i>	370	T	7.1	NM	Grey metamorphic	1700	400	260	220
	leaf		0	H				7100	84	5100	540
			Note	On slate scree.							
2786	soil	<i>Saussurea medusa</i>		T	7.1	NM	Grey metamorphic	1700	400	260	220
	leaf		0	H				38300	62	2600	120
			Note	On slate scree.							

Da Xue Shan, East side,
Yunnan 4100 – 4350 m

Non-limestone scree. Slate stony.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2787	soil	<i>Saussurea medusa</i>		T	7.1	NM	Grey metamorphic	1700	400	260	220
	leaf		0	H				22800	71	2200	100
			Note	On slate scree.							
2788	soil	<i>Corydalis calcicola</i>		T	7.1	NM	Grey metamorphic	1700	400	260	220
	leaf		0	H				32100	75	3800	70
			Note	On slate scree.							
2797	soil	<i>Primula dryadifolia</i>		T	6.1	40	Brown organic-mineral	2300	280	600	200
	leaf		0	H				2000	150	960	53
			Note	On slate scree.							
2798	soil	<i>Primula dryadifolia</i>		T	5.5	59	Brown organic	1350	540	420	30
	leaf		0	H				1600	25	480	180
			Note	Growing under <i>Rhododendron</i> .							
162/00 soil		<i>Rhododendron Taliensia</i> subsect.			5.7	10	Grey metamorphic	2400	1600	300	150

Da Xue Shan, West side,
Yunnan 4200 – 4600 m

Mainly limestone area.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
1749	soil	<i>Meconopsis pseudointegrifolia</i>		T	4.8	NM	Brown mineral	1100	5400	310	790
	leaf		0	H				42600	66	4300	58
1750	soil	<i>Primula brevicula</i>		T	4.8	NM	Brown mineral	1100	5400	310	790
	leaf		0	H				6700	15	1200	36
1755	soil	<i>Astragalus yunnanensis</i>		T	4.8	NM	Brown mineral	1100	5400	310	790
	leaf		0	H				11400	160	2200	2100
2716	soil	<i>Gentiana hexaphylla</i>		T	5.2	13	Brown mineral	1300	370	100	90
	leaf		0	H				6000	2200	2400	130
2735	soil	<i>Gentiana veitchiorum</i>		T	6.1	15	Red-brown	2500	2000	240	1250
	leaf		0	H				3800	110	1500	190
2743	soil	<i>Primula sikkimensis</i>		T	7.4	34	Grey limestone	396000	21	2800	110
	leaf		0	H				20800	28	2000	55
				Note	Growing on lime scree, on the same site as <i>Rhododendron</i> SDR 2744.						

Hai Zhi Shan,
Yunnan, 4250 m

Wet, rocky moor land.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2934	soil	<i>Meconopsis</i> sp.		T	7.2	15	Brown mineral	2700	730	310	500
	leaf		0	H				29300	190	3300	48
			Note	Soil collected at the roots.							

Tian Chi,
Yunnan, 3800 m

Flat and wet, peaty area of mixed forest. (**P** Tian Chi 1, 3 (J.A.), 2 (M.K.))

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4602	soil	<i>Rheum alexandrae</i>		T	6.8	NM	Brown organic	13000	220	890	34
	leaf		0	H				1400	85	2400	31
			Note	Growing in a wood bog. (P23 a, b)							
4605	soil	<i>Gentiana sino-ornata</i>		T	5.3	78	Brown organic	10000	920	680	58
	leaf		0	H				7900	110	4400	240
			Note	Growing in a boggy meadow near a lake. (P24 a, b, c)							
4611	soil	<i>Primula boreiocalliantha</i>		T	4.4	NM	Brown organic	2400	220	770	30
	leaf		0	H				3400	83	2900	1300
			Note	Growing in a wood, wet peaty soil. (P25)							

Between Dali and Lijiang,
Yunnan, around 2000 m

Open sunny hills, red-limy soil (Terra-rossa).

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4706	soil	<i>Primula malvacea</i>		T	7.5	NM	Dark red-brown	5400	6	740	6
	leaf		0	H				26000	21	7300	46
			Note	Growing on upper half of S –facing limestone slope.							
4714	soil	<i>Primula malvacea</i>		T	8.0	NM	Red-brown	7000	6	1400	11
	leaf		0	H				20000	170	7400	140

East of Daochen
Yunnan, 3900 - 4360 m

Open moor land. No limestone.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
1869	soil	<i>Lilium lophophorum</i>		T	6.9	9	Brown mineral	1850	550	120	320
	leaf		0	H				15000	0	3000	57
			Note								
1870	soil	<i>Stellera chamaejasme</i>		T	6.9	9	Brown mineral	1850	550	120	320
	leaf		0	H				18000	0	3000	140
			Note								
1871	soil	<i>Pedicularis sp.</i>		T	6.9	9	Brown mineral	1850	550	120	320
	leaf		0	H				20000	0	3800	410
			Note								
227/00 soil		<i>Gentiana stipitata</i>	T		5.0	8		250	1800	18	64

Near Daochen,
Yunnan, 3900 - 4360 m

On a plain, not very limy.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
3104	soil	<i>Gentiana stipitata</i>		T	5.2	19	Brown organic-mineral	2000	170	185	280
	leaf		0	H				6600	330	1500	120
			Note	Hard turf soil.							
3105	soil	<i>Gentiana veitchiorum</i>		T	5.2	18	Brown organic-mineral	990	93	100	90
	leaf		0	H				6200	420	1300	170
			Note	Hard turf soil.							
3106	soil	<i>Gentiana sino- ornata</i>			5.2	18	Brown organic-mineral	990	93	100	90
	leaf		0	H				6000	330	1700	350
			Note	Hard turf soil.							

Road Daochen - Ya Ding and
south of Ya Ding
Sichuan, 3500 – 4000 m

Valley with mixed forest and moor land, covering limestone formations.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
3012	soil	<i>Calanthe sp.</i>		T	7.7	18	Yellow- grey limestone silt	47500	3300	370	1000
	leaf		0				H	18200	210	1000	31
			Note				Limestone soil. On the same site as <i>Rhododendron</i> SDR 3011.				
3013	soil	<i>Orchidaceae</i>		T	7.7	18	Yellow- grey limestone silt	47500	3300	370	1000
	leaf		0				H	36000	100	860	46
			Note				Limestone soil. On the same site as <i>Rhododendron</i> SDR 3011.				
3014	soil	<i>Vaccinium sp.</i>		T	7.7	18	Yellow- grey limestone silt	47500	3300	370	1000
	leaf		0				H	17500	82	1600	95
			Note				Limestone soil. On the same site as <i>Rhododendron</i> SDR 3011.				
3031	soil	<i>Primula souliei</i>		T	7.4	16	Grey limestone silt	84000	28	8200	68
	leaf		0				H	29000	110	2500	12
			Note				Limestone soil				
3047	soil	<i>Primula bellidifolia</i>		T	6.1	NM	Brown organic	34000	8	1300	175
	leaf		0				H	9100	40	2200	90
			Note				Growing on moss over rocks. Woodland soil.				
3040	soil	<i>Gentiana veitchiorum</i>		T	7.0	14	Red-brown	5000	74	90	300
	leaf		0				H	11500	730	1500	220
			Note				Limestone soil.				
338 soil		<i>Gentiana sp.</i>		T	6.3	46	Brown organic-mineral	15500	15	1400	160

Haba village (above),
Haba Shan, Yunnan, 2900 m

Open deciduous woods.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
3225	soil	<i>Primula capitata</i> subsp. <i>sphaerocephala</i>		T	7.0	14	Brown mineral	2000	3700	700	800
	leaf		0	H				15300	1750	6100	120
			Note								

186 soil		<i>Gentiana sp.</i>		T	4.7	24	Brown mineral	1300	1900	240	260

Above Dali,
Yunnan, 2600 m

Mixed forest.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
3326	soil	<i>Gentiana melandriifolia</i>	027	T	5.1	24	Brown mineral	750	720	43	46
	leaf		0	H				6600	1600	3200	320
			Note								

On way to Geza,
Yunnan, 2800 m

On steep grassy bank.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2705	soil	<i>Cypripedium flavum</i>		T	8.1	10	Dark grey	95600	1800	3900	480
	leaf		0	H				19000	430	6400	60
			Note								

Bi Rong,
Yunnan, 2500 - 3500 m

Limestone gorge.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
4727	soil	<i>Primula cortusoides</i> sect.		T	7.6	NM	Dark grey	15600	9	390	8
	leaf		0				H	22000	230	2200	12
4728	soil	<i>Primula cortusoides</i> sect.		T	7.4	NM	Dark grey	13400	8	340	8
	leaf		0				H	29000	68	3000	24

Li Ti Ping,
Yunnan, 3100 m

Deciduous forest, open areas. **No** sign of limestone.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
5127	leaf	<i>Gentiana sino- ornata</i>	0				H	6500	150	3200	490
5131	soil	<i>Gentiana sino- ornata</i>		T	6.7	NM	Brown organic	3000	9	800	110
	leaf		0				H	5500	44	1500	340

Cang Shan,
Yunnan, 3800 m

Thick moss in deciduous forest, over acidic metamorphic rocks.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
5168	soil	<i>Primula calliantha</i>		T	6.5	NM	Black organic	2400	11	1100	320
	leaf		0				H	4800	86	2100	1700

Table 48 ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil samples from *Gentiana* and other alpine species, collected in Europe.

Schachen, The Alps, Italy, 1150 m				Area dominated by dolomite.							
SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2103	soil	<i>Gentiana asclepiadea</i>		T	5.6	91	Brown organic	23000	4	7400	46
	leaf		0	H				3400	110	3800	54
			Note								
2104	soil	<i>Erica herbacea</i>		T	5.6	91	Brown organic	23000	4	7400	46
	leaf		0	H				7500	84	3900	15
			Note	Ericaceous species.							
2110	soil	<i>Pinguicula vulgaris</i>		T	5.6	91	Brown organic	23000	4	7400	46
	leaf		0	H				23400	120	16300	54
Pordoi, The Dolomites, Italy, 2300 m				Dolomite area.							
SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2106	soil	<i>Pulsatilla alpina subsp. apiifolia</i>		T	6.5	44	Black organic	6400	3900	730	680
	leaf		0	H				10000	110	3300	120
2109	soil	<i>Erica herbacea</i>		T	7.7	23	Organic dolomite	84000	2800	30400	730
	leaf		0	H				10600	60	2600	5
			Note	Ericaceous species.							

Sassolunga, The Dolomites,
Italy, 2100 m

Dolomite area.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2111	soil	<i>Thlaspi rotundifolium</i>		T	7.7	38	Organic dolomite	84000	310	110000	310
	leaf		0	H				33500	100	8200	160
			Note								
2112	soil	<i>Salix reticulata</i>		T	7.7	38	Organic dolomite	84000	310	110000	310
	leaf		0	H				7500	40	5000	87
			Note								
2113	soil	<i>Dryas octapetala</i>		T	7.7	38	Organic dolomite	84000	310	110000	310
	leaf		0	H				1100	100	2600	48
			Note								

Chignolo, The Alps,
Italy, 900 – 1500 m

Dolomitic limestone area.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2401	soil	<i>Helleborus niger</i>		T	7.2	46	Organic limestone	146300	16	75000	1200
	leaf		0	H				16100	66	7200	42
			Note								
2402	soil	<i>Cyclamen purpurascens</i>		T	7.2	46	Organic limestone	146300	16	75000	1200
	leaf		0	H				18800	47	4400	56
			Note								
2404	soil	<i>Aquilegia vulgaris</i>		T	7.2	46	Organic limestone	146300	16	75000	1200
	leaf		0	H				24200	77	8000	87
			Note								

Chignolo, The Alps,
Italy, 900 – 1500 m

Dolomitic limestone area.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2405	soil	<i>Erica herbacea</i>		T	7.2	46	Organic limestone	146300	16	75000	1200
	leaf		0				H	4600	20	3100	9
			Note				A lime-tolerant, Ericaceous species.				
2411	soil	<i>Physoplexis comosa</i>		T	7.8	47	Organic limestone	172300	11	86200	380
	leaf		0				H	23200	77	6700	70
			Note								
2412	soil	<i>Potentilla caulescens</i>		T	7.8	47	Organic limestone	172300	11	86200	380
	leaf		0				H	18200	95	5900	100
			Note								
2414	soil	<i>Primula albenensis</i>		T	7.8	47	Organic limestone	172300	11	86200	380
	leaf		0				H	17500	140	9600	110
			Note								
2419	soil	<i>Veratrum album</i>		T	7.6	54	Brown organic	86000	48	39000	900
	leaf		0				H	19600	110	3300	49
			Note								
2421	soil	<i>Gentiana asclepiadea</i>		T	7.6	54	Brown organic	86000	48	39000	900
	leaf		0				H	9600	140	6000	70
			Note								
2424	soil	<i>Gentiana augustifolia</i>		T	7.0	46	Organic limestone	112000	37	57500	350
	leaf		0				H	9200	200	3900	53
			Note								

Gavia Pass, The Alps,
Italy, 2700 m

Non-limestone, rocky mountain pass.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2427	soil	<i>Gentiana bavarica</i>		T	6.8	10	Grey metamorphic	1100	1600	170	28
	leaf		0	H				3900	370	2300	95
2428	soil	<i>Pedicularis rostratocapitata</i>		T	6.8	10	Grey metamorphic	1100	1600	170	28
	leaf		0	H				8500	135	4000	140
2429	soil	<i>Ranunculus glacialis</i>		T	6.5	6	Grey metamorphic	470	3000	270	74
	leaf		0	H				24000	87	3500	130
			Note	The rock has layer structure, slate-like soil.							
2431	soil	<i>Pedicularis rostratocapitata</i>		T	6.5	6	Grey metamorphic	470	3000	270	74
	leaf		0	H				7500	84	1750	167
			Note	The rock has layer structure, slate-like soil.							

Petit Mont Cenis, SW Alps,
Switzerland, 2100 m

Limestone area.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
3597	soil	<i>Arctostaphylos uva-ursi</i>		T	6.8	47	Organic limestone	52600	81	14700	260
	leaf		0	H				22000	430	2800	30
			Note	On limestone, the same site as <i>Rhododendron</i> SDR 3596.							
3600	soil	<i>Rhodothamnus chamaecistus</i>		T	6.7	43	Organic limestone	95000	880	84800	250
	leaf		0	H				7900	16	1800	2
			Note	Ericaceous species.							
3609	soil	<i>Androsace cf. alpina</i>		T	7.6	6	Limestone	8300	4000	800	430
	leaf		0	H				46200	460	1700	230

Near Lac Vert, SW Alps,
Switzerland, 1750 m

High alpine stony area and open meadows.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
3517	soil	<i>Gentiana lutea</i>		T	6.9	8	Metamorphic mineral	3200	630	360	350
	leaf		0	H				13200	95	6200	77
3518	soil	<i>Gentiana purpurea</i>		T	5.4	14	Grey metamorphic	1750	4900	400	390
	leaf		0	H				16000	140	3500	550

To Samoens, SW Alps,
Switzerland, 1400 m

High alpine, open meadows.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
3521	soil	<i>Gentiana lutea</i>		T	6.7	61	Brown organic	40900	64	660	440
	leaf		0	H				40000	70	5000	16
			Note	On limestone.							

Above La Thuile, SW Alps,
Switzerland, 2350 m

High alpine, open meadows.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
3561	soil	<i>Gentiana acaulis</i>		T	4.5	17	Brown mineral	640	4300	250	190
	leaf		0	H				5300	730	1100	210

Above Val d'Isere, SW Alps,
Switzerland, 2250 m

High alpine, open meadows.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
3568	soil	<i>Gentiana acaulis</i>		T	5.4	24	Brown mineral	1800	4800	520	1280
	leaf		0	H				5700	43	2300	43
3569	soil	<i>Pulsatilla alpina</i>		T	5.4	24	Brown mineral	1800	4800	520	1280
	leaf		0	H				40000	88	5100	530

Below Refuge Femma,
SW Alps, Switzerland, 2300 m

Alpine meadows.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
3577	soil	<i>Daphne mezereum</i>		T	6.1	45	Organic limestone	14200	500	630	580
	leaf		0	H				10500	112	250	109
			Note	Usually grows on limestone.							
3579	soil	<i>Soldanella alpina</i>		T	4.5	28	Organic limestone	3600	1500	330	470
	leaf		0	H				7800	50	3200	44
3580	soil	<i>Alchemilla hoppeana</i>		T	4.5	28	Organic limestone	3600	1500	330	470
	leaf		0	H				18000	37	1200	130
3581	soil	<i>Dryas octapetala</i>		T	4.5	28	Organic limestone	3600	1500	330	470
	leaf		0	H				26000	200	1200	140
			Note	Always grows on limestone.							

Table 49 ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil samples from *Gentiana* and other alpine species, collected in the USA and Canada.

Crabtree Falls, Blue Ridge Parkway, North Carolina, 1000 m							Deciduous forest.				
SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2214	soil	<i>Kalmia latifolia</i>		T	5.4	6	Metamorphic mineral	830	1200	130	120
	leaf		0	H				5500	53	1400	310
			1					6000	67	1200	370
			2					6500	50	800	440
			Note					On the same site as <i>R. maximum</i> SDR 2213.			
2215	soil	<i>Galax aphylla</i>		T	5.4	6	Metamorphic mineral	830	1200	130	120
	leaf		0	H				6200	80	2200	1150
			Note	On the same site as <i>R. maximum</i> SDR 2213.							
Amicalola Falls, Georgia, 700 m							Deciduous forest.				
SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2304	soil	<i>Kalmia latifolia</i>		T	5.4	8	Red-brown	220	1300	170	6
	leaf		0	H				6800	50	2500	400
			Note								

Graveyard Fields,
Blue Ridge Parkway, North
Carolina, 1500 m

Deciduous forest.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
2243	soil	<i>Galax aphylla</i>		T	3.9	50	Brown organic	150	1000	200	17
	leaf		0	H				2800	65	3100	280
			Note	On the same site as <i>R. maximum</i> 2241.							
2244	soil	<i>Selaginella sp.</i>		T	3.9	50	Brown organic	150	1000	200	17
	leaf		0	H				220	16	620	20
			Note	On the same site as <i>R. maximum</i> 2241.							

Table 50 ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil samples from *Gentiana* and other alpine species, collected in Sikkim, India.

West Bengal, above Molloy
Sikkim, 3150 m

Shrubby half open place towards the summit.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
3827	soil	<i>Gentiana sikkimensis</i>		T	6.0	16	Sample lost	710	220	550	40
	leaf		0	H				5100	140	1900	520
			Note								
3828	soil	<i>Gentiana prolata</i>		T	6.0	16	Sample lost	710	220	550	40
	leaf		0	H				4400	340	1100	240
			Note								

Yoksam – Dzongri,
(Kanchenjunga),
Sikkim, 3150 m

Damp, evergreen forest.

SDR number		Name of plant	Age of leaf / y	Top/Sub /Mulch	pH	OC %	Type of soil	Ca	Fe	Mg	Mn
3908	leaf	<i>Primula scapigera</i>	0	H				5700	780	3900	460

Results for growing experiments on:

R. hirsutum

R. impeditum

R. coeloreuron

G. sino-ornata



- Table 52 Symptoms diagnosis and ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil from *R. coeloreuron* in the each medium. – page 300
- Table 53 Symptoms diagnosis and ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil from *G. sino-ornata* in the each medium. – page 302
- Table 54 Symptoms diagnosis and ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil from *R. impeditum* in the each medium. – page 303
- Table 55 Symptoms diagnosis and ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil from *R. hirsutum* in the each medium. – page 304

The experiment started on the 27th of March 2004. The plants were planted in the designed growing media, and their growth was observed periodically. Samples were taken according to the health condition of the plants (Table 51).

Table 51 Monitoring and sample collection of the *Rhododendron* and *Gentiana* species used in the experiment.

Date	Photo session	Sample collection	Condition of plants recorded
01 - 10 April 2004		Samples of some plants were collected for reference material.	Recorded
06 June 2004	P	1 st collection of <i>G. sino-ornata</i> plants only.	
10-15 October 2005	P	Collection of leaf samples from most plants.	Recorded
02 – 04 May 2005			Recorded

Strength of chlorotic symptoms increase in the order:

Dying > Yellow > Bright green with yellow veins > Bright green > Green new growth > Green

The conditions 'B, dry parts' and 'B, unwell' describe the inhibited healthy growth of the plants due to the manual handling of the plant material while planting in the experimental media. Therefore, this classification is not included in the above scale of chlorotic symptoms.

Tables of results legend:

- o Month of collection of the material: Where in the tables of results ‘April’ and ‘Oct’ are used it is in 2004, where ‘May’ is the month of collection, it is in May 2005
- o Age of leaf/y: The year of growth of the leaf sample collected.
 - ‘0’ – current-year leaf (newest growth),
 - ‘1’ – one-year-old leaf (growth from one year before collection),
 - ‘2’ – two-year-old leaf (growth from two years ago), etc.
- o pH: The pH established in the designed soil mixture.
- o Ca, Fe, Mg and Mn: the concentrations of these elements presented in ppm.
- o Condition of plant before experiment: Visual condition of the plant before the start of the experiment (available only for the plants that were initially having symptoms of a disorder).
- o Condition of plant at collection: Visual condition of the plant at sample collection.

Abbreviations and descriptions used:

- o 0 ppm concentration of Ca, Fe or Mn: The concentration of Ca, Fe and/or Mn in the leaf is below the detection limits of the ICP-OES.
- o Collection: Date of sample collection.
- o Age of leaf/y: The year of growth of the leaf sample collected.
 - ‘0’ – current-year leaf (newest growth),
 - ‘1’ – one-year-old leaf (growth from one year before collection).

Legend for column ‘Condition of plant before experiment’ in the tables of result:

G		leaves have dry, brown tips and/or edges.
Chl	1	leaves with subtle pale yellow / orange, randomly spread marks.
Chl	2	leaves with green veins and yellowing between the veins.
NA		Record on the condition of the plant before the start of the experiment was not taken.

Legend for column ‘Condition of plant at collection’ in the tables of result:

The condition of each plant is represented by a letter, corresponding to a description of the health condition of the plant:

A	<u>Healthy green</u> - usual for healthy plants green colour.
B	<u>Green</u> - Healthy green plant, but only a few yellow spots on some leaves or pale green to yellow colour of a few leaves.
C	<u>Green with a few red young/old leaves</u> – Plants generally have green leaves, but a few new leaves have dark red colour.
D	<u>Red spots</u> – The leaves of the plant are bright green with red spots at the tip and around the edges. This condition was the best that <i>R. hirsutum</i> plants were at when the experiment finished.
E	<u>Red-brown</u> - Most leaves are red with just a little bit green left around the veins, but there are a few leaves that are still green.
F	<u>Dying</u> – Whole plant is fully dark red-brown and reaching condition of becoming completely dead dry.

Table 52 Symptoms diagnosis and ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil from *R. coeloreuron* in each medium.

<i>R. coeloreuron</i>									
Medium	pH	N of Plant	Collection	Age of leaf / y	Ca	Fe	Mg	Mn	Condition of plant at collection
Control	4.0	1	Oct	0	7350	35	3000	570	B
	5.3	1	May	0	14700	8	3900	840	NA
		2	Oct	0	1640	39	2500	126	NA
		2	May	0	2900	80	1900	140	NA
		3	May	0	5700	9	2000	250	NA
	6.7	1	Oct	0	5100	140	2200	1090	NA
		1	May	0	4000	8.5	1900	290	A
		4	May	0	2600	11	1400	190	NA
	8.0	1	Oct	0	4100	90	1930	490	NA
		1	Oct	1	8300	20	1500	1040	NA

<i>R. coeloreuron</i>									
Medium	pH	N of Plant	Collection	Age of leaf / y	Ca	Fe	Mg	Mn	Condition of plant before experiment
Low dose	4.0	4	April	0	6800	150	2700	480	Chl 2 and G
		1	Oct	0	8500	90	2800	1450	NA
		3	May	0	5800	18	1650	710	NA
		4	May	0	3400	10	1300	394	NA
	5.3	2	May	0	8000	24	2200	890	Chl 1
		3	Oct	0	2100	13	1730	210	NA
		3	Oct	1	8700	29	1240	590	NA
		3	May	0	2050	7	1200	220	NA
		4	May	0	6100	9	1840	280	Chl 2 and G
	6.7	1	April	1	14400	140	1600	2720	Chl 2
		1	Oct	0	3600	13	1700	280	Chl 2
		1	Oct	1	7700	120	1900	1900	Chl 2
		1	May	0	4050	313	1600	200	Chl 2
	8.0	1	May	0	3100	33	1300	230	G
		3	Oct	0	4600	74	2400	450	NA
		3	Oct	1	10300	59	2000	2600	NA

R. coeloreuron

Medium	pH	N of Plant	Collection	Age of leaf / y	Ca	Fe	Mg	Mn	Condition of plant before experiment
High dose	5.3	1	May	0	3400	17	1700	410	Chl 1
		1	Oct	0	5400	86	3500	490	Chl 1
		3	May	0	4700	15	1000	430	NA
		4	May	0	4000	39	1800	365	NA
	6.7	1	Oct	0	600	74	1300	240	G
		4	April	0	3700	27	890	980	Chl 1
	8.0	2	Oct	0	1300	110	2200	530	NA

R. coeloreuron

Medium	pH	N of Plant	Collection	Age of leaf / y	Ca	Fe	Mg	Mn
Mulch	4.0	1	Oct	0	5400	51	3000	870
		2	May	0	4800	23	1640	440
		4	April	0	7800	100	1260	780
	5.3	2	Oct	0	6500	47	4100	440
	6.7	1	Oct	0	1500	80	1400	164
	8.0	1	Oct	0	1440	58	2050	370

Table 53 Symptoms diagnosis and ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil from *G. sino-ornata* in each medium.

G. sino-ornata								
Medium	pH	N of Plant		Ca	Fe	Mg	Mn	
Control	4.0	2		2900	56	1400	130	
		3		3500	156	1970	110	
	6.7	1		18200	290	2700	33	
		2		10000	86	3800	38	
	8.0	2		7900	193	1700	10	
		3		Dead				
Low dose	4.0	1	June	3260	260	3070	208	
		1	May	4100	82	3900	132	
		2		2160	70	1300	96	
		3		Dead				
	5.3	1		2900	214	300	77	
		2		7200	139	3500	155	
	6.7	1		9700	193	3650	82	
	8.0	1		5400	900	1800	25	
		2		Dead				
		3						

Table 54 Symptoms diagnosis and ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil from *R. impeditum* in each medium.

<i>R. impeditum</i>									
Medium	pH	N of Plant	Collection	Age of leaf / y	Ca	Fe	Mg	Mn	Condition of plant at collection
Control	4.0	1	May	0	3800	35	950	575	A
		2	May	0	4000	22	1200	610	A
		3	May	0	4000	21	1200	472	A
		4	May	0	4000	20	1200	330	A
	6.7	3	Oct	0	3500	75	1600	180	A
		4	Oct	0	8500	27	1800	74	A

<i>R. impeditum</i>									
Medium	pH	N of Plant	Collection	Age of leaf / y	Ca	Fe	Mg	Mn	Condition of plant at collection
Low dose	4.0	1	May	0	3800	37	1000	340	A
		3	Oct	0	0	63	1050	300	A
		3	Oct	1	680	61	1200	360	A
	5.3	2	Oct	0	320	83	1500	133	C
		3	May	0	21600	160	10300	140	A
	6.7	3	Oct	0	5000	90	2100	167	C
	8.0	1	Oct	1	970	57	1150	74	B
		3	Oct	0	2000	64	1500	142	A

<i>R. impeditum</i>									
Medium	pH	N of Plant	Collection	Age of leaf / y	Ca	Fe	Mg	Mn	Condition of plant at collection
High dose	4.0	2	May	0	4800	25	1200	800	A
		3	May	0	4600	31	1250	580	A
	5.3	4	May	0	4500	26	2200	100	A

<i>R. impeditum</i>									
Medium	pH	N of Plant	Collection	Age of leaf / y	Ca	Fe	Mg	Mn	Condition of plant at collection
Mulch	4.0	2	May	0	3800	25	1400	438	A
		4	May	0	4700	28	1500	370	A
	5.3	2	May	0	5400	34	2200	60	A

Table 55 Symptoms diagnosis and ICP-OES results for concentrations of calcium, iron, magnesium and manganese in leaf and soil from *R. hirsutum* in the each media.

<i>R. hirsutum</i>									
Medium	pH	N of Plant	Collection	Age of leaf / y	Ca	Fe	Mg	Mn	Condition of plant at collection
Control	4.0	1	May	0	3300	58	1400	135	D
	5.3	1	May	0	2800	69	1400	24	F
	6.7	1	May	0	5500	60	1550	53	F

<i>R. hirsutum</i>									
Medium	pH	N of Plant	Collection	Age of leaf / y	Ca	Fe	Mg	Mn	Condition of plant at collection
Low dose	4.0	1	Oct	0	3600	88	1500	76	E
	5.3	1	Oct	0	2400	110	2300	90	E
	6.7	2	Oct	0	4400	139	2200	113	E
	8.0	3	Oct	0	4500	71	1300	53	F
		3	Oct	1	970	136	1400	58	F
		3	May	0	4400	100	1240	71	F

<i>R. hirsutum</i>									
Medium	pH	N of Plant	Collection	Age of leaf / y	Ca	Fe	Mg	Mn	Condition of plant at collection
High dose	4.0	1	Oct	0	370	110	1500	100	D
		1	Oct	1	4500	82	1800	340	D
		1	May	0	2500	56	1000	55	E
	5.3	1	Oct	0	700	97	1800	92	F
	6.7	1	May	0	5400	37	1500	32	F
		2	May	0	5200	35	1300	48	F
		4	Oct	0	3100	110	1800	100	NA
	8.0	3	Oct	0	2800	100	1400	97	F

<i>R. hirsutum</i>									
Medium	pH	N of Plant	Collection	Age of leaf / y	Ca	Fe	Mg	Mn	Condition of plant at collection
Mulch	4.0	2	Oct	0	4300	90	1640	125	D
	5.3	1	May	0	4600	32	1700	14	F
	6.7	1	May	0	5800	35	1670	65	E

Appendix 2

Summary of statistics

Results for *Rhododendron* species in the wild

In all tables, the concentration variables are presented in mg kg⁻¹.

Descriptive statistics summary 1 Group (1) All *Rhododendron* plants.

Variable	Mean	Minimum	Maximum	SD
soil pH	6.6	3.9	7.9	1.1
SOM %	32	1.1	91	26
[Ca] (L)	9800	1900	32400	5300
[Fe] (L)	91	0	1600	155
[Mg] (L)	1600	280	6400	860
[Mn] (L)	220	3.8	3400	490
[Ca] (S)	113000	155	400000	149600
[Fe] (S)	830	4.2	4100	1040
[Mg] (S)	6900	60	94000	16500
[Mn] (S)	610	6.4	16200	1900

Descriptive statistics summary 2 Group (2) *Rhododendron* plants growing on soils derived from limestone.

Variable	Mean	Minimum	Maximum	SD
soil pH	7.6	6.7	7.9	0.3
SOM %	14	1.1	44	14.8
[Ca] (L)	11300	3550	32400	6100
[Fe] (L)	70	0	287	59
[Mg] (L)	1400	422	3700	717
[Mn] (L)	49	3.8	663	103
[Ca] (S)	234400	6300	400000	158400
[Fe] (S)	430	11	3300	522
[Mg] (S)	7500	160	94000	11900
[Mn] (S)	690	6.4	16200	2800

Descriptive statistics summary 3 Group (3) *Rhododendron* plants growing on soils derived from dolomitic limestone.

Variable	Mean	Minimum	Maximum	SD
soil pH	6.7	5.6	7.6	0.68
SOM %	63	46	91	17.8
[Ca] (L)	7700	4100	15300	2300
[Fe] (L)	300	53	1600	446
[Mg] (L)	3200	1850	6400	1200
[Mn] (L)	12	5.4	25	5
[Ca] (S)	99300	23000	172000	63200
[Fe] (S)	27	4.2	139	39
[Mg] (S)	46300	7000	85000	34800
[Mn] (S)	454	29	1200	442

Descriptive statistics summary 4 Group (4) *Rhododendron* plants growing on soils derived from non-limestone.

Variable	Mean	Minimum	Maximum	SD
soil pH	6.03	3.9	7.8	0.97
SOM %	38	1.1	85	26
[Ca] (L)	8900	1900	21900	4300
[Fe] (L)	75	0	837	104
[Mg] (L)	1500	279	3300	653
[Mn] (L)	350	9	3400	600
[Ca] (S)	28300	155	150000	43940
[Fe] (S)	1150	5.7	4100	1200
[Mg] (S)	1400	60	14700	2800
[Mn] (S)	500	6.4	1800	617

ANOVA summary 1 for [Mg] in *Rhododendron* leaves of age 0, 1 and 2 years old.

Effect	N	[Mg] (L)				
		R = 0.1657	R ² = 0.0274		p = 0.6584	
		Mean	SD	SE	CI, -95%	CI, +95%
Total	33	1730	861	149	1425	2036
0 (Age of leaf / y)	11	1901	907	273	1291	2511
1 (Age of leaf / y)	11	1733	837	252	1171	2296
2 (Age of leaf / y)	11	1557	885	266	962	2151

ANOVA summary 2 for [Fe] in *Rhododendron* leaves of age 0, 1 and 2 years old.

Effect	N	[Fe] (L)				
		R = 0.3193	R ² = 0.1019		p = 0.1992	
		Mean	SD	SE	CI, -95%	CI, +95%
Total	33	128	217	37	51	205
0 (Age of leaf / y)	11	49	46	13	18	80
1 (Age of leaf / y)	11	119	129	38	32	206
2 (Age of leaf / y)	11	216	343	103	-13	447

ANOVA summary 3 for [Mn] in *Rhododendron* leaves of age 0, 1 and 2 years old.

Effect	N	[Mn] (L)				
		R = 0.3063	R ² = 0.0938		p = 0.2279	
		Mean	SD	SE	CI, -95%	CI, +95%
Total	33	948	806	140	662	1234
0 (Age of leaf / y)	11	692	613	184	280	1104
1 (Age of leaf / y)	11	876	765	230	362	1390
2 (Age of leaf / y)	11	1275	961	290	629	1921

Correlation matrix A Group (1) All *Rhododendron* plants.

Variable	soil pH	SOM %	Leaf age	HC	[Ca] (L)	[Fe] (L)	[Mg] (L)	[Mn] (L)	[Ca] (S)	[Fe] (S)	[Mg] (S)	[Mn] (S)
soil pH	1.00	-0.39	-0.15	0.19	0.20	0.02	-0.02	-0.46	0.71	-0.48	0.30	0.03
SOM %	-0.39	1.00	-0.15	-0.09	-0.15	0.13	0.04	-0.07	-0.53	-0.07	0.03	0.15
Leaf age	-0.15	-0.15	1.00	-0.08	0.32	-0.01	0.11	0.35	-0.06	0.08	-0.13	-0.09
HC	0.19	-0.09	-0.08	1.00	0.07	-0.02	-0.06	-0.11	0.02	-0.13	-0.08	-0.04
[Ca] (L)	0.20	-0.15	0.32	0.07	1.00	0.13	0.32	0.04	0.30	-0.02	0.06	0.02
[Fe] (L)	0.02	0.13	-0.01	-0.02	0.13	1.00	0.31	0.02	-0.03	0.03	0.41	0.01
[Mg] (L)	-0.02	0.04	0.11	-0.06	0.32	0.31	1.00	0.12	-0.05	-0.00	0.52	-0.00
[Mn] (L)	-0.46	-0.07	0.35	-0.11	0.04	0.02	0.12	1.00	-0.30	0.37	-0.17	-0.02
[Ca] (S)	0.71	-0.53	-0.06	0.02	0.30	-0.03	-0.05	-0.30	1.00	-0.39	0.26	-0.16
[Fe] (S)	-0.48	-0.07	0.08	-0.13	-0.02	0.03	-0.00	0.37	-0.39	1.00	-0.26	0.14
[Mg] (S)	0.30	0.03	-0.13	-0.08	0.06	0.41	0.52	-0.17	0.26	-0.26	1.00	-0.04
[Mn] (S)	0.03	0.15	-0.09	-0.04	0.02	0.01	-0.00	-0.02	-0.16	0.14	-0.04	1.00

Marked in blue correlations are significant at $p < 0.05$

Number of cases = 152

The significant correlations are highlighted in blue. Because the matrix is symmetric, only the correlation coefficients in the left section are marked.

Correlation matrix B Group (2) *Rhododendron* plants growing on soils derived from limestone.

Variable	soil pH	SOM %	Leaf age	HC	[Ca] (L)	[Fe] (L)	[Mg] (L)	[Mn] (L)	[Ca] (S)	[Fe] (S)	[Mg] (S)	[Mn] (S)
soil pH	1.00	-0.68	0.16	-0.35	0.05	-0.20	-0.19	-0.22	0.63	-0.10	0.34	-0.33
SOM %	-0.68	1.00	-0.17	0.27	-0.09	0.25	0.11	0.04	-0.64	0.18	-0.08	0.29
Leaf age	0.16	-0.17	1.00	-0.03	0.47	0.15	0.04	0.13	0.18	-0.11	-0.08	-0.12
HC	-0.35	0.27	-0.03	1.00	-0.04	0.13	-0.01	0.04	-0.35	0.10	-0.21	-0.02
[Ca] (L)	0.05	-0.09	0.47	-0.04	1.00	0.29	0.50	0.08	0.25	0.03	0.15	0.00
[Fe] (L)	-0.20	0.25	0.15	0.13	0.29	1.00	0.46	0.25	-0.14	0.05	0.36	0.09
[Mg] (L)	-0.19	0.11	0.04	-0.01	0.50	0.46	1.00	0.31	-0.04	0.04	0.37	-0.03
[Mn] (L)	-0.22	0.04	0.13	0.04	0.08	0.25	0.31	1.00	-0.30	0.08	-0.10	0.19
[Ca] (S)	0.63	-0.64	0.18	-0.35	0.25	-0.14	-0.04	-0.30	1.00	-0.28	0.22	-0.26
[Fe] (S)	-0.10	0.18	-0.11	0.10	0.03	0.05	0.04	0.08	-0.28	1.00	-0.10	0.47
[Mg] (S)	0.34	-0.08	-0.08	-0.21	0.15	0.36	0.37	-0.10	0.22	-0.10	1.00	-0.07
[Mn] (S)	-0.33	0.29	-0.12	-0.02	0.00	0.09	-0.03	0.19	-0.26	0.47	-0.07	1.00

Marked in blue correlations are significant at $p < 0.05$

Number of cases = 63

The significant correlations are highlighted in blue. Because the matrix is symmetric, only the correlation coefficients in the left section are marked.

Correlation matrix C Group (3) *Rhododendron* plants growing on soils derived from dolomitic limestone.

Variable	soil pH	SOM %	Leaf age	[Ca] (L)	[Fe] (L)	[Mg] (L)	[Mn] (L)	[Ca] (S)	[Fe] (S)	[Mg] (S)	[Mn] (S)
soil pH	1.00	-0.91	-0.43	0.26	0.15	0.14	-0.42	0.74	0.40	0.68	0.57
SOM %	-0.91	1.00	0.35	-0.33	-0.24	-0.34	0.43	-0.94	-0.19	-0.92	-0.35
Leaf age	-0.43	0.35	1.00	0.50	-0.25	0.41	0.56	-0.28	-0.29	-0.25	-0.41
[Ca] (L)	0.26	-0.33	0.50	1.00	-0.08	0.86	0.07	0.34	0.25	0.36	0.04
[Fe] (L)	0.15	-0.24	-0.25	-0.08	1.00	-0.19	-0.08	0.27	-0.01	0.31	-0.02
[Mg] (L)	0.14	-0.34	0.41	0.86	-0.19	1.00	-0.09	0.45	-0.06	0.49	-0.27
[Mn] (L)	-0.42	0.43	0.56	0.07	-0.08	-0.09	1.00	-0.45	-0.10	-0.40	0.01
[Ca] (S)	0.74	-0.94	-0.28	0.34	0.27	0.45	-0.45	1.00	0.06	0.99	0.10
[Fe] (S)	0.40	-0.19	-0.29	0.25	-0.01	-0.06	-0.10	0.06	1.00	0.03	0.65
[Mg] (S)	0.68	-0.92	-0.25	0.36	0.31	0.49	-0.40	0.99	0.03	1.00	0.06
[Mn] (S)	0.57	-0.35	-0.41	0.04	-0.02	-0.27	0.01	0.10	0.65	0.06	1.00

Marked in blue correlations are significant at $p < 0.05$

Number of cases = 11

Because the matrix is symmetric, only the correlation coefficients in the left section are marked.

Correlations for 'Health condition' are not presented since all samples for plants growing on dolomitic limestone derived soils were from healthy plants.

Correlation matrix D Group (4) *Rhododendron* plants growing on soils derived from non-limestone.

Variable	soil pH	SOM %	Leaf age	HC	[Ca] (L)	[Fe] (L)	[Mg] (L)	[Mn] (L)	[Ca] (S)	[Fe] (S)	[Mg] (S)	[Mn] (S)
soil pH	1.00	0.24	-0.08	0.04	0.02	0.04	-0.02	-0.35	0.56	-0.25	0.26	0.33
SOM %	0.24	1.00	-0.31	0.01	0.09	0.02	-0.39	-0.34	0.68	-0.40	0.12	0.09
Leaf age	-0.08	-0.31	1.00	-0.11	0.34	0.07	0.23	0.35	-0.15	0.02	-0.06	-0.10
HC	0.04	0.01	-0.11	1.00	0.06	-0.02	0.03	-0.05	-0.03	-0.12	-0.03	-0.05
[Ca] (L)	0.02	0.09	0.34	0.06	1.00	0.41	0.40	0.18	0.05	0.09	0.06	0.07
[Fe] (L)	0.04	0.02	0.07	-0.02	0.41	1.00	0.37	0.09	0.02	0.21	0.14	-0.00
[Mg] (L)	-0.02	-0.39	0.23	0.03	0.40	0.37	1.00	0.27	-0.27	0.17	0.00	0.05
[Mn] (L)	-0.35	-0.34	0.35	-0.05	0.18	0.09	0.27	1.00	-0.36	0.25	-0.14	-0.09
[Ca] (S)	0.56	0.68	-0.15	-0.03	0.05	0.02	-0.27	-0.36	1.00	-0.52	0.51	0.23
[Fe] (S)	-0.25	-0.40	0.02	-0.12	0.09	0.21	0.17	0.25	-0.52	1.00	-0.19	0.02
[Mg] (S)	0.26	0.12	-0.06	-0.03	0.06	0.14	0.00	-0.14	0.51	-0.19	1.00	0.14
[Mn] (S)	0.33	0.09	-0.10	-0.05	0.07	-0.00	0.05	-0.09	0.23	0.02	0.14	1.00

Marked in blue correlations are significant at $p < 0.05$

Number of cases = 89

The significant correlations are highlighted in blue. Because the matrix is symmetric, only the correlation coefficients in the left section are marked.

Results for alpine plant species in the wild**Descriptive statistics summary 5** Group (1) All *Gentiana* and alpine plants.

Variable	Mean	Minimum	Maximum	SD
soil pH	6.4	3.9	8.1	1.07
SOM %	29	1.1	91	21.7
[Ca] (L)	13900	222	82000	11000
[Fe] (L)	198	0	2150	333
[Mg] (L)	2900	245	16300	2300
[Mn] (L)	184	2	1700	249
[Ca] (S)	33000	155	400000	61800
[Fe] (S)	1100	4.2	11000	1700
[Mg] (S)	9300	31	110000	24400
[Mn] (S)	323	4.4	2000	438

Descriptive statistics summary 6 Group (2) *Gentiana* and alpine plants growing on soils derived from limestone.

Variable	Mean	Minimum	Maximum	SD
soil pH	7.4	6.7	7.9	0.3
SOM %	19	1.1	47	14
[Ca] (L)	22100	7100	82000	17200
[Fe] (L)	244	16	1200	281
[Mg] (L)	1640	630	3100	712
[Mn] (L)	52	2	227	61
[Ca] (S)	96800	5000	400000	105400
[Fe] (S)	938	21	4000	1306
[Mg] (S)	6900	90	84800	18300
[Mn] (S)	412	6	1766	557

Descriptive statistics summary 7 Group (3) *Gentiana* and alpine plants growing on soils derived from dolomitic limestone.

Variable	Mean	Minimum	Maximum	SD
soil pH	7.1	5.6	7.8	0.77
SOM %	51	23	91	18.7
[Ca] (L)	14300	3380	33440	8200
[Fe] (L)	95	20	198	40.8
[Mg] (L)	5500	1300	16340	3400
[Mn] (L)	71	5	160	44
[Ca] (S)	102900	6400	172000	55400
[Fe] (S)	415	4.2	3900	1050
[Mg] (S)	59000	725	110000	35800
[Mn] (S)	570	46	1200	417

Descriptive statistics summary 8 Group (4) *Gentiana* and alpine plants growing on soils derived from non-limestone.

Variable	Mean	Minimum	Maximum	SD
soil pH	5.87	3.9	8.1	0.98
SOM %	26	6	86	20.6
[Ca] (L)	11800	222	40000	8900
[Fe] (L)	230	0.0	2153	415
[Mg] (L)	2700	245	10178	1990
[Mn] (L)	210	4	1150	206
[Ca] (S)	6400	155	95600	14300
[Fe] (S)	1500	7	11000	2000
[Mg] (S)	540	31	3900	701
[Mn] (S)	300	6	2000	442

Correlation matrix E Group (1) All alpine plants.

Variable	soil pH	SOM %	[Ca] (L)	[Fe] (L)	[Mg] (L)	[Mn] (L)	[Ca] (S)	[Fe] (S)	[Mg] (S)	[Mn] (S)
soil pH	1.00	-0.09	0.42	0.02	0.24	-0.47	0.55	-0.12	0.40	0.36
SOM %	-0.09	1.00	-0.09	-0.20	0.25	-0.12	0.09	-0.11	0.27	0.16
[Ca] (L)	0.42	-0.09	1.00	0.03	0.35	-0.22	0.16	0.04	0.04	0.24
[Fe] (L)	0.02	-0.20	0.03	1.00	-0.00	-0.03	-0.10	0.02	-0.14	-0.05
[Mg] (L)	0.24	0.25	0.35	-0.00	1.00	-0.17	0.12	-0.01	0.33	0.17
[Mn] (L)	-0.47	-0.12	-0.22	-0.03	-0.17	1.00	-0.31	0.10	-0.19	-0.17
[Ca] (S)	0.55	0.09	0.16	-0.10	0.12	-0.31	1.00	-0.23	0.51	0.13
[Fe] (S)	-0.12	-0.11	0.04	0.02	-0.01	0.10	-0.23	1.00	-0.22	0.21
[Mg] (S)	0.40	0.27	0.04	-0.14	0.33	-0.19	0.51	-0.22	1.00	0.18
[Mn] (S)	0.36	0.16	0.24	-0.05	0.17	-0.17	0.13	0.21	0.18	1.00

Marked in blue correlations are significant at $p < 0.05$

Number of cases = 119

The significant correlations are highlighted in blue. Because the matrix is symmetric, only the correlation coefficients in the left section are marked.

Correlations for 'Health condition' and 'Leaf age' are not present since all samples were from healthy plants, and no leaf samples of successive years of age were collected.

Correlation matrix F Group (2) Alpine plants growing on soils derived from limestone.

Variable	soil pH	SOM %	[Ca] (L)	[Fe] (L)	[Mg] (L)	[Mn] (L)	[Ca] (S)	[Fe] (S)	[Mg] (S)	[Mn] (S)
soil pH	1.00	-0.80	0.02	0.24	-0.08	-0.61	0.33	0.35	-0.24	0.10
SOM %	-0.80	1.00	-0.18	-0.18	0.20	0.41	-0.24	-0.23	0.21	0.20
[Ca] (L)	0.02	-0.18	1.00	0.30	0.32	-0.00	-0.23	0.17	-0.24	-0.02
[Fe] (L)	0.24	-0.18	0.30	1.00	0.20	-0.00	-0.09	0.15	-0.17	0.11
[Mg] (L)	-0.08	0.20	0.32	0.20	1.00	-0.05	-0.18	-0.07	-0.03	0.06
[Mn] (L)	-0.61	0.41	-0.00	-0.00	-0.05	1.00	-0.23	0.12	-0.17	-0.04
[Ca] (S)	0.33	-0.24	-0.23	-0.09	-0.18	-0.23	1.00	-0.29	0.08	-0.32
[Fe] (S)	0.35	-0.23	0.17	0.15	-0.07	0.12	-0.29	1.00	-0.08	0.53
[Mg] (S)	-0.24	0.21	-0.24	-0.17	-0.03	-0.17	0.08	-0.08	1.00	-0.14
[Mn] (S)	0.10	0.20	-0.02	0.11	0.06	-0.04	-0.32	0.53	-0.14	1.00

Marked in blue correlations are significant at $p < 0.05$

Number of cases = 23

The significant correlations are highlighted in blue. Because the matrix is symmetric, only the correlation coefficients in the left section are marked.

Correlations for 'Health condition' and 'Leaf age' are not present since all samples were from healthy plants, and no leaf samples of successive years of age were collected.

Correlation matrix G Group (3) Alpine plants growing on soils derived from dolomitic limestone.

Variable	soil pH	SOM %	[Ca] (L)	[Fe] (L)	[Mg] (L)	[Mn] (L)	[Ca] (S)	[Fe] (S)	[Mg] (S)	[Mn] (S)
soil pH	1.00	-0.86	0.25	-0.12	-0.19	0.23	0.66	-0.03	0.72	0.31
SOM %	-0.86	1.00	-0.13	0.23	0.33	-0.18	-0.53	-0.32	-0.64	-0.45
[Ca] (L)	0.25	-0.13	1.00	0.09	0.65	0.35	0.21	-0.16	0.24	0.10
[Fe] (L)	-0.12	0.23	0.09	1.00	0.21	0.36	-0.28	0.02	-0.30	-0.53
[Mg] (L)	-0.19	0.33	0.65	0.21	1.00	0.17	-0.03	-0.26	-0.08	-0.15
[Mn] (L)	0.23	-0.18	0.35	0.36	0.17	1.00	0.13	0.05	0.30	-0.28
[Ca] (S)	0.66	-0.53	0.21	-0.28	-0.03	0.13	1.00	-0.44	0.67	0.48
[Fe] (S)	-0.03	-0.32	-0.16	0.02	-0.26	0.05	-0.44	1.00	-0.40	-0.01
[Mg] (S)	0.72	-0.64	0.24	-0.30	-0.08	0.30	0.67	-0.40	1.00	0.22
[Mn] (S)	0.31	-0.45	0.10	-0.53	-0.15	-0.28	0.48	-0.01	0.22	1.00

Marked in blue correlations are significant at $p < 0.05$

Number of cases = 23

The significant correlations are highlighted in blue. Because the matrix is symmetric, only the correlation coefficients in the left section are marked.

Correlations for 'Health condition' and 'Leaf age' are not present since all samples were from healthy plants, and no leaf samples of successive years of age were collected.

Correlation matrix H Group (4) Alpine plants growing on soils derived from non-limestone.

Variable	soil pH	SOM %	[Ca] (L)	[Fe] (L)	[Mg] (L)	[Mn] (L)	[Ca] (S)	[Fe] (S)	[Mg] (S)	[Mn] (S)
soil pH	1.00	-0.15	0.44	0.10	0.41	-0.34	0.45	-0.02	0.55	0.37
SOM %	-0.15	1.00	-0.06	-0.18	-0.11	-0.06	0.42	0.02	0.31	0.15
[Ca] (L)	0.44	-0.06	1.00	-0.03	0.61	-0.19	0.40	0.12	0.43	0.45
[Fe] (L)	0.10	-0.18	-0.03	1.00	0.07	-0.10	-0.03	-0.02	0.04	-0.03
[Mg] (L)	0.41	-0.11	0.61	0.07	1.00	-0.20	0.20	0.18	0.38	0.25
[Mn] (L)	-0.34	-0.06	-0.19	-0.10	-0.20	1.00	-0.20	-0.00	-0.22	-0.14
[Ca] (S)	0.45	0.42	0.40	-0.03	0.20	-0.20	1.00	0.02	0.89	0.73
[Fe] (S)	-0.02	0.02	0.12	-0.02	0.18	-0.00	0.02	1.00	0.11	0.26
[Mg] (S)	0.55	0.31	0.43	0.04	0.38	-0.22	0.89	0.11	1.00	0.72
[Mn] (S)	0.37	0.15	0.45	-0.03	0.25	-0.14	0.73	0.26	0.72	1.00

Marked in blue correlations are significant at $p < 0.05$

Number of cases = 78

The significant correlations are highlighted in blue. Because the matrix is symmetric, only the correlation coefficients in the left section are marked.

Correlations for 'Health condition' and 'Leaf age' are not present since all samples were from healthy plants, and no leaf samples of successive years of age were collected.

Correlation matrix I Group (5) *Gentiana* plants growing in the wild on all soils.

Variable	soil pH	SOM %	[Ca] (L)	[Fe] (L)	[Mg] (L)	[Mn] (L)	[Ca] (S)	[Fe] (S)	[Mg] (S)	[Mn] (S)
soil pH	1.00	-0.11	0.35	-0.24	0.41	-0.53	0.72	-0.29	0.51	0.25
SOM %	-0.11	1.00	0.14	-0.29	0.36	0.02	0.17	-0.11	0.28	0.03
[Ca] (L)	0.35	0.14	1.00	-0.10	0.54	-0.19	0.21	-0.12	0.05	0.10
[Fe] (L)	-0.24	-0.29	-0.10	1.00	-0.06	-0.05	-0.21	-0.12	-0.14	-0.25
[Mg] (L)	0.41	0.36	0.54	-0.06	1.00	-0.28	0.26	-0.22	0.42	0.16
[Mn] (L)	-0.53	0.02	-0.19	-0.05	-0.28	1.00	-0.43	0.14	-0.29	-0.26
[Ca] (S)	0.72	0.17	0.21	-0.21	0.26	-0.43	1.00	-0.28	0.74	0.10
[Fe] (S)	-0.29	-0.11	-0.12	-0.12	-0.22	0.14	-0.28	1.00	-0.22	0.38
[Mg] (S)	0.51	0.28	0.05	-0.14	0.42	-0.29	0.74	-0.22	1.00	0.21
[Mn] (S)	0.25	0.03	0.10	-0.25	0.16	-0.26	0.10	0.38	0.21	1.00

Marked in blue correlations are significant at $p < 0.05$

Number of cases = 28

The significant correlations are highlighted in blue. Because the matrix is symmetric, only the correlation coefficients in the left section are marked.

Correlation matrix J Group (6) *Primula* plants growing in the wild on all soils.

Variable	soil pH	SOM %	[Ca] (L)	[Fe] (L)	[Mg] (L)	[Mn] (L)	[Ca] (S)	[Fe] (S)	[Mg] (S)	[Mn] (S)
soil pH	1.00	-0.17	0.46	0.33	0.31	-0.57	0.43	-0.27	0.33	0.32
SOM %	-0.17	1.00	-0.10	-0.25	-0.09	-0.08	0.06	0.03	0.14	0.08
[Ca] (L)	0.46	-0.10	1.00	0.07	0.50	-0.39	0.31	-0.04	0.13	0.18
[Fe] (L)	0.33	-0.25	0.07	1.00	0.11	-0.10	-0.12	0.04	-0.05	0.16
[Mg] (L)	0.31	-0.09	0.50	0.11	1.00	-0.18	0.04	0.18	0.45	0.11
[Mn] (L)	-0.57	-0.08	-0.39	-0.10	-0.18	1.00	-0.17	0.04	-0.04	-0.16
[Ca] (S)	0.43	0.06	0.31	-0.12	0.04	-0.17	1.00	-0.19	0.38	-0.01
[Fe] (S)	-0.27	0.03	-0.04	0.04	0.18	0.04	-0.19	1.00	-0.11	0.08
[Mg] (S)	0.33	0.14	0.13	-0.05	0.45	-0.04	0.38	-0.11	1.00	0.01
[Mn] (S)	0.32	0.08	0.18	0.16	0.11	-0.16	-0.01	0.08	0.01	1.00

Marked in blue correlations are significant at $p < 0.05$

Number of cases = 27

The significant correlations are highlighted in blue. Because the matrix is symmetric, only the correlation coefficients in the left section are marked.

Results for growing experiments

ANOVA summary 4 for [Mn] in *Rhododendron* leaves vs soil pH.

Effect	N	[Mn] (L)				
		R = 0.8289	R ² = 0.6872		p = 0.0203	
		Mean	SD	SE	CI, -95%	CI, +95%
Total	12	91	59	17	53	129
4.0	5	135	43.	19	81	189
5.3	2	116	55	39	-379	611
6.7	3	51	27	15	-16	118
8.0	2	17	10	7	-77	112

ANOVA summary 5 for [Mn] in *Rhododendron* leaves vs soil medium.

Effect	N	[Mn] (L)				
		R = 0.4036	R ² = 0.1628		p = 0.1932	
		Mean	SD	SE	CI, -95%	CI, +95%
Total	11	91	59	17	53	129
Control	5	64	52	23	-0.986	129
Low dose	6	110	59	22	55	166

ANOVA summary 6 for [Fe] in *Rhododendron* leaves vs soil pH.

Effect	N	[Fe] (L)				
		R = 0.4072	R ² = 0.1658		p = 0.7164	
		Mean	SD	SE	CI, -95%	CI, +95%
Total	11	158	79	23	104	211
4.0	5	124	84	37	19	230
5.3	2	176	53	37	-299	653
6.7	3	189	102	58	-63	443
8.0	1	193				

ANOVA summary 7 for [Fe] in *Rhododendron* leaves vs soil medium.

Effect	N	[Fe] (L)				
		R = 0.0228	R ² = 0.0005	p = 0.9468		
		Mean	SD	SE	CI, -95%	CI, +95%
Total	11	158	79	24	104	211
Control	5	156	92	41	41	271
Low dose	6	159	75	30	80	239

Appendix 3

Column plots

Results for *Rhododendron* species in the wild

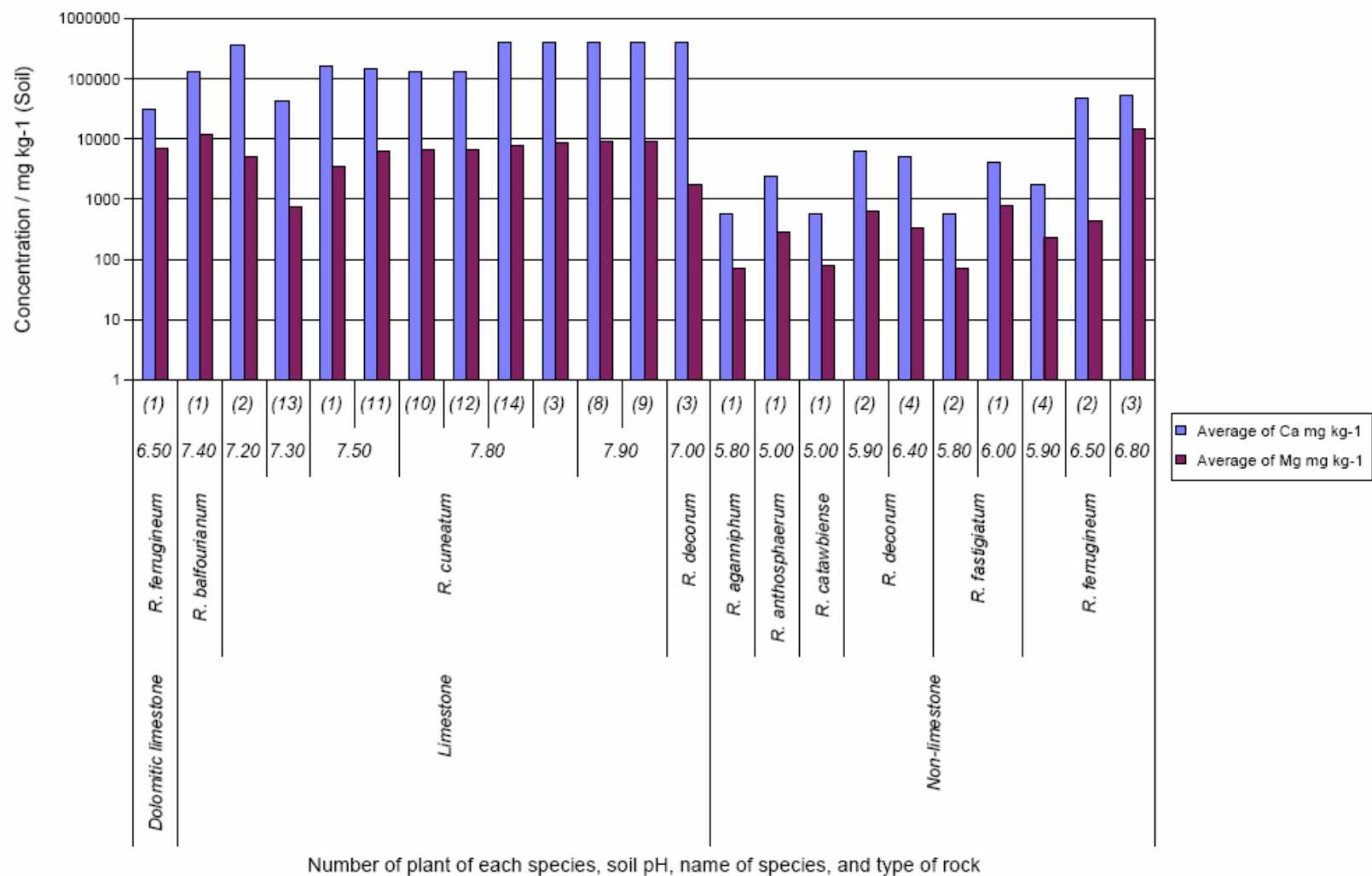
Page 324

Results for alpine plant species in the wild

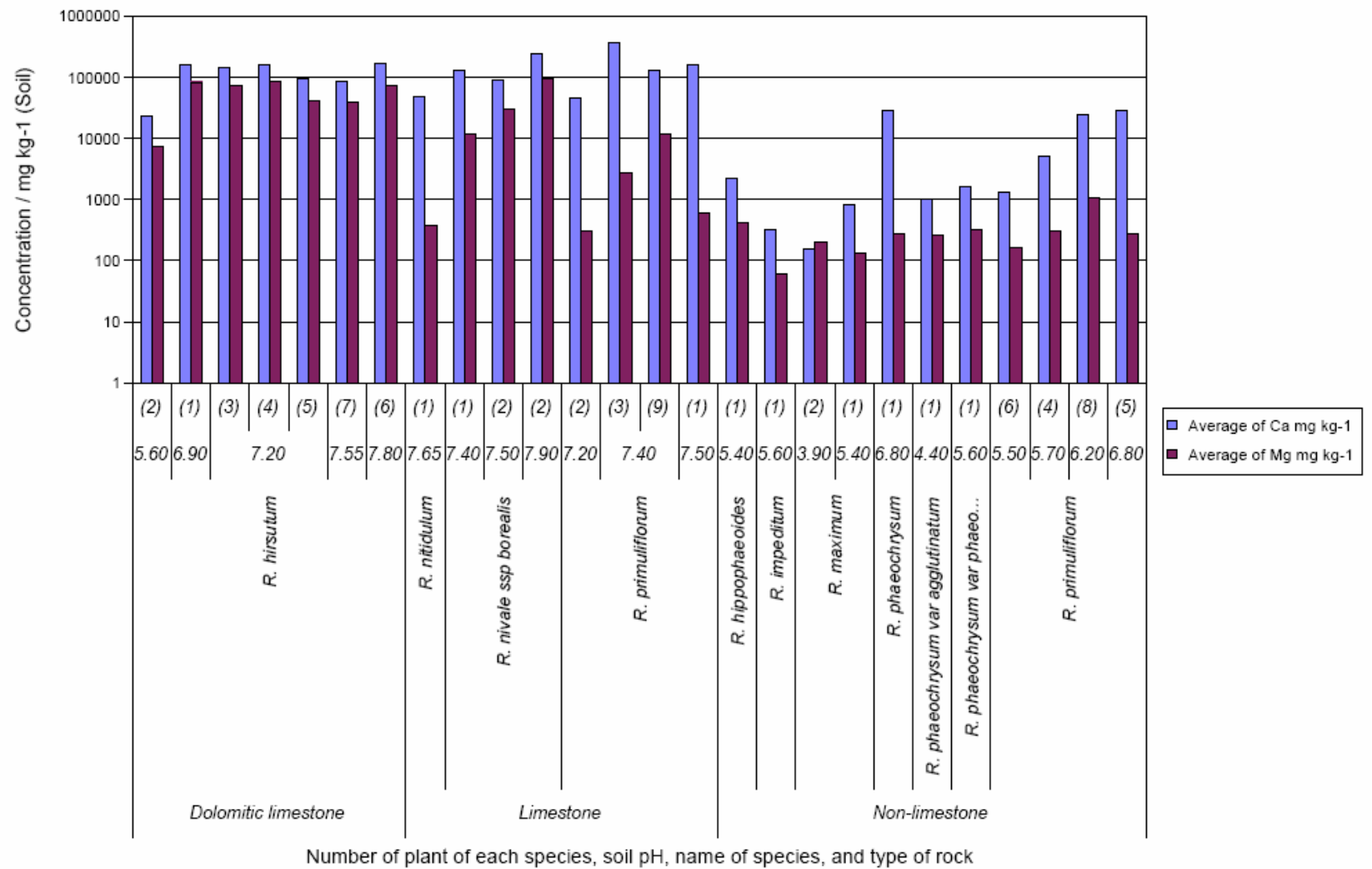
Page 339

Results for growing experiments on *Rhododendron* and *Gentiana*

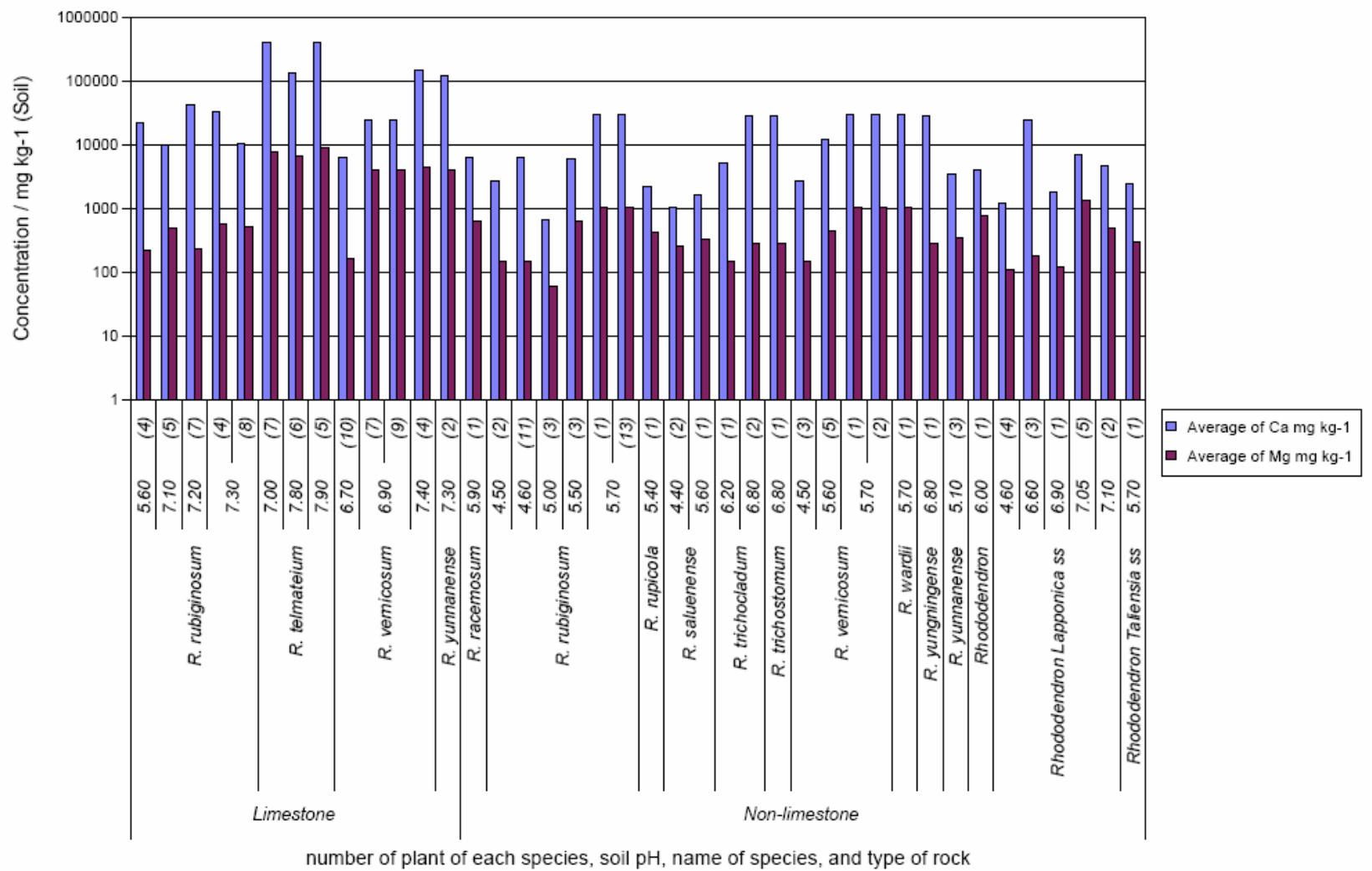
Page 352



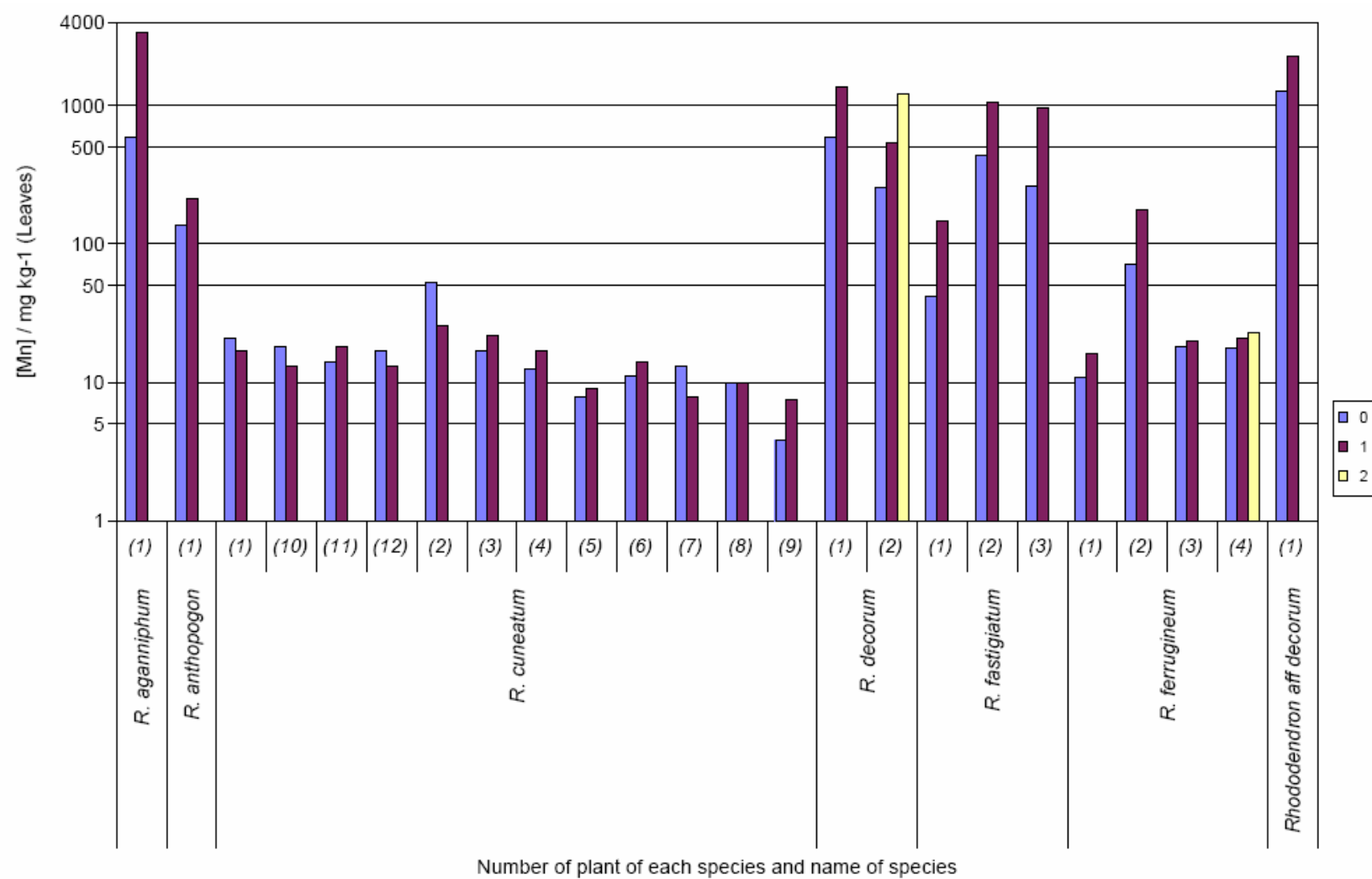
CP 1a Mg/Ca ratio in soils for *Rhododendron* species with names from A-F.



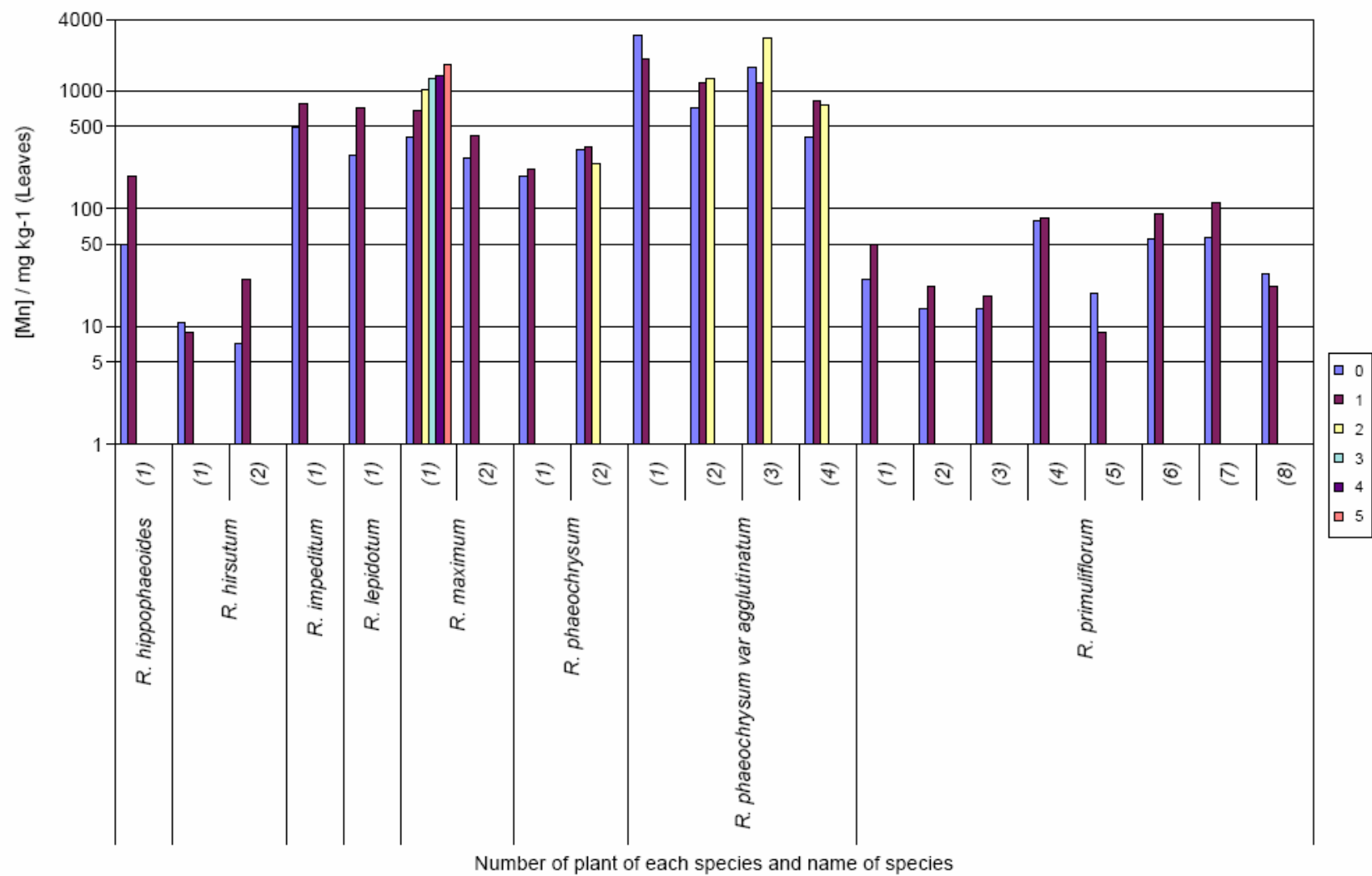
CP 1b Mg/Ca ratio in soils for *Rhododendron* species with names from G-P.



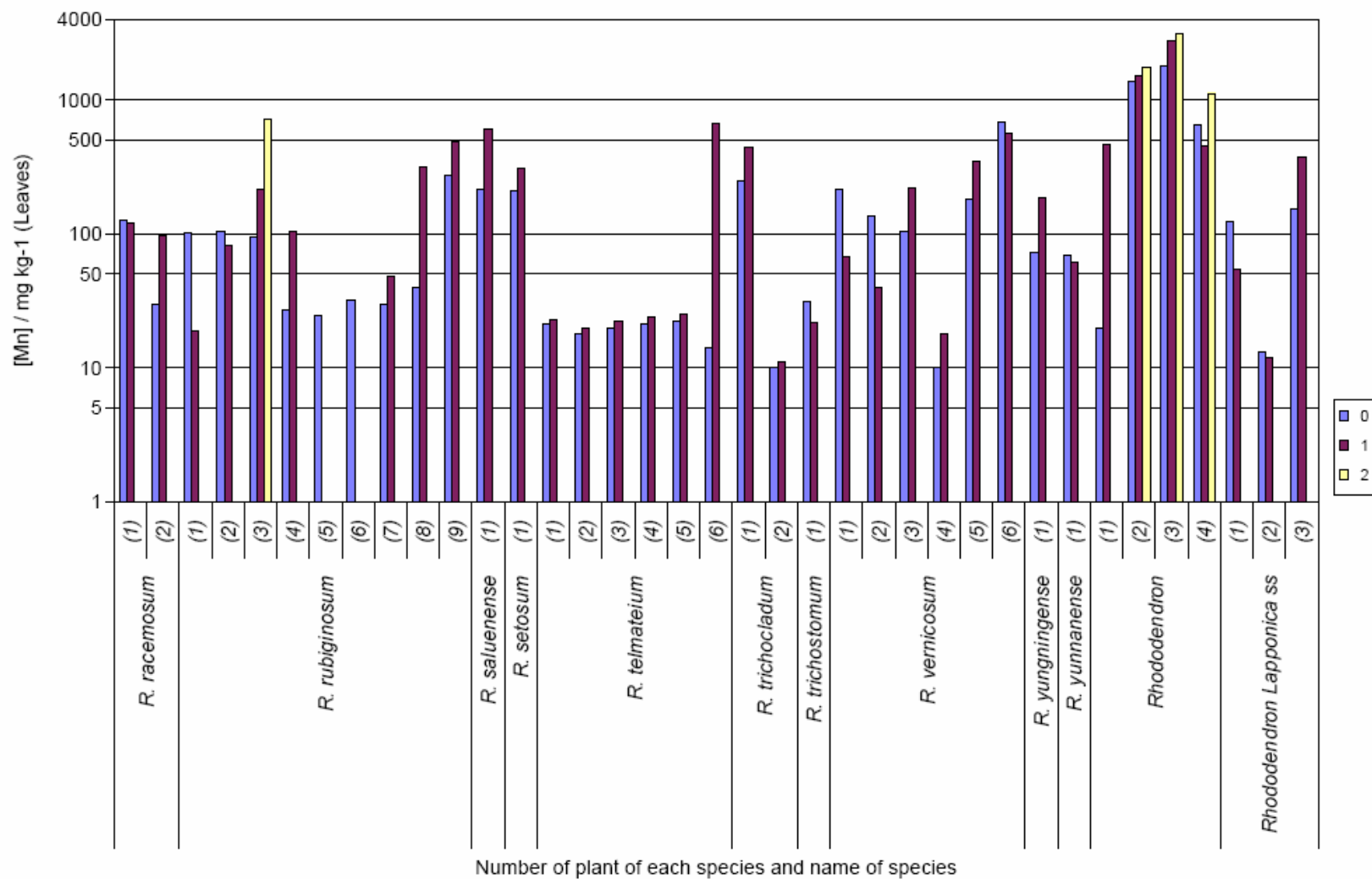
CP 1c Mg/Ca ratio in soils for *Rhododendron* species with names from Q-Z.



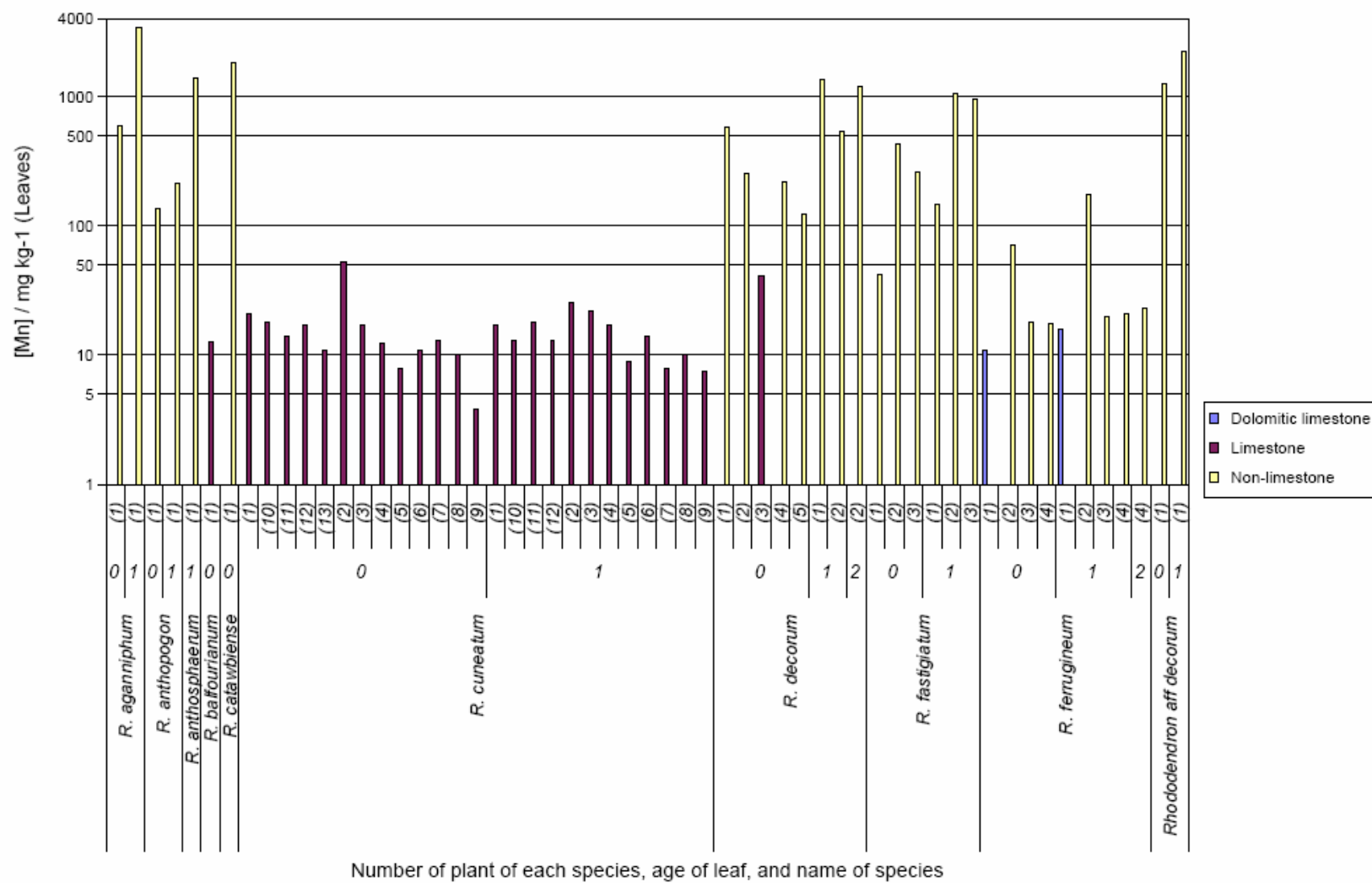
CP 2a [Mn] in leaves of consecutive years of age in each plant for *Rhododendron* species with names from A-F.



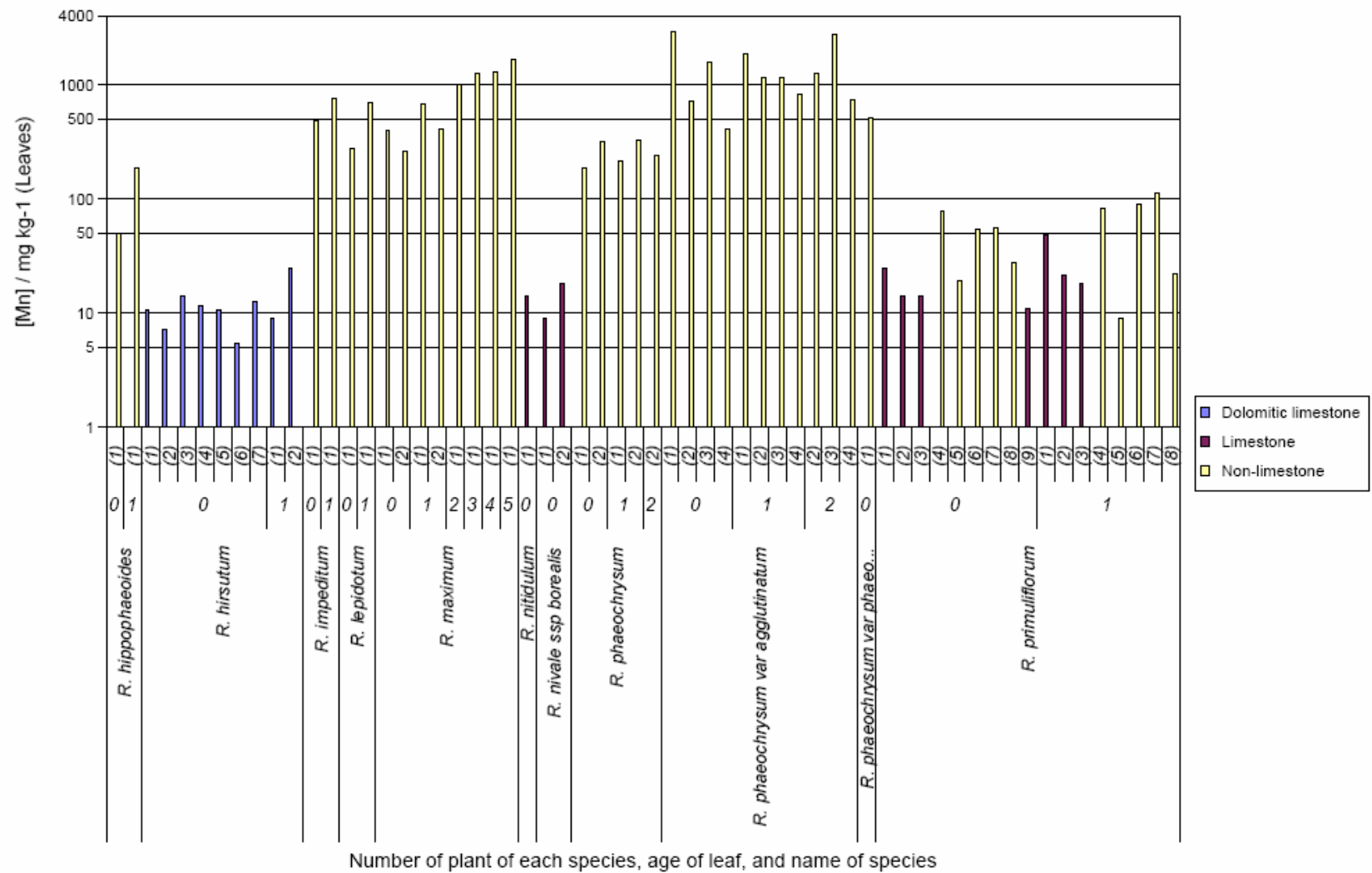
CP 2b [Mn] in leaves of consecutive years of age in each plant for *Rhododendron* species with names from G-P.



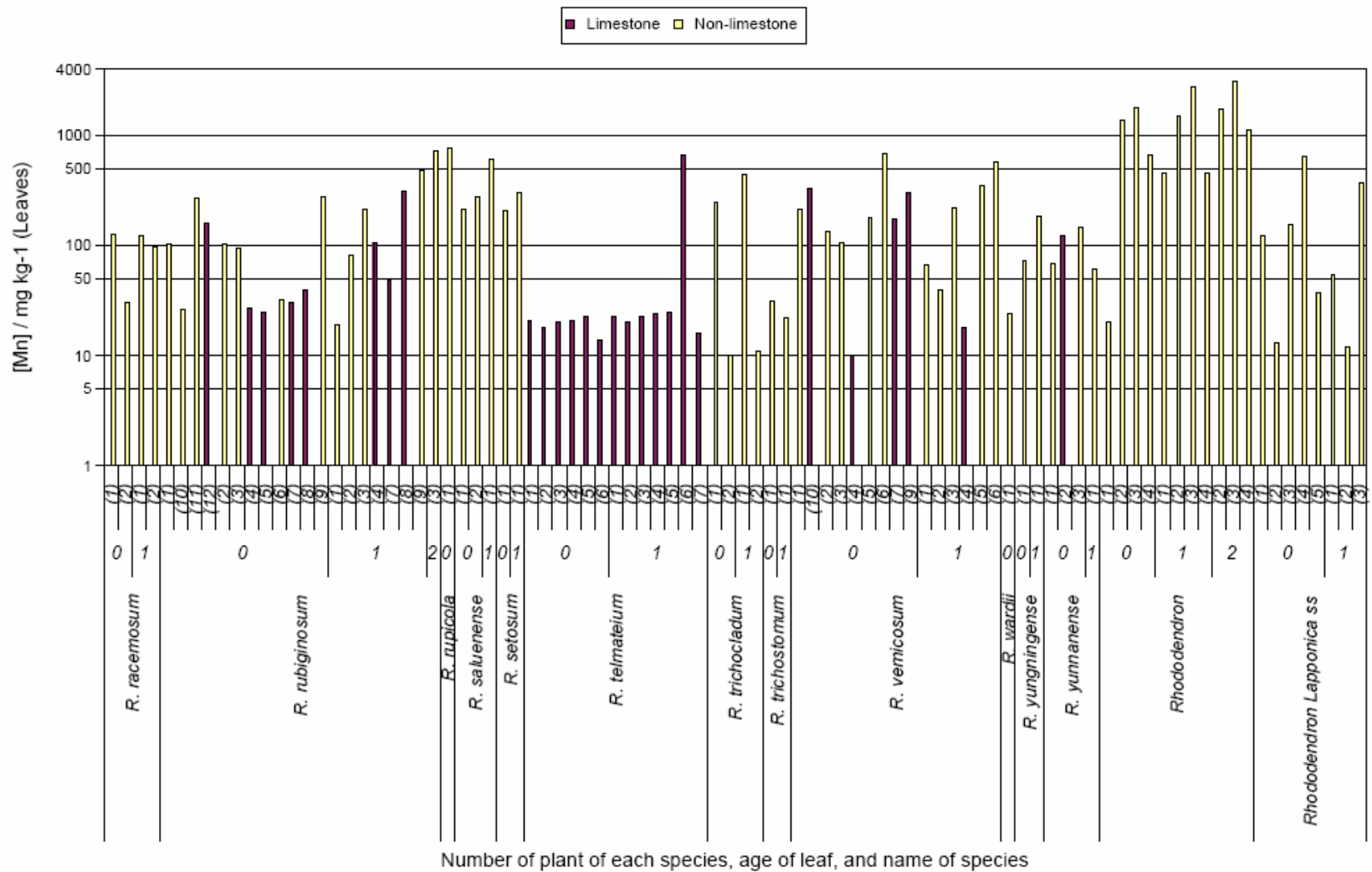
CP 2c [Mn] in leaves of consecutive years of age in each plant for *Rhododendron* species with names from Q-Z.



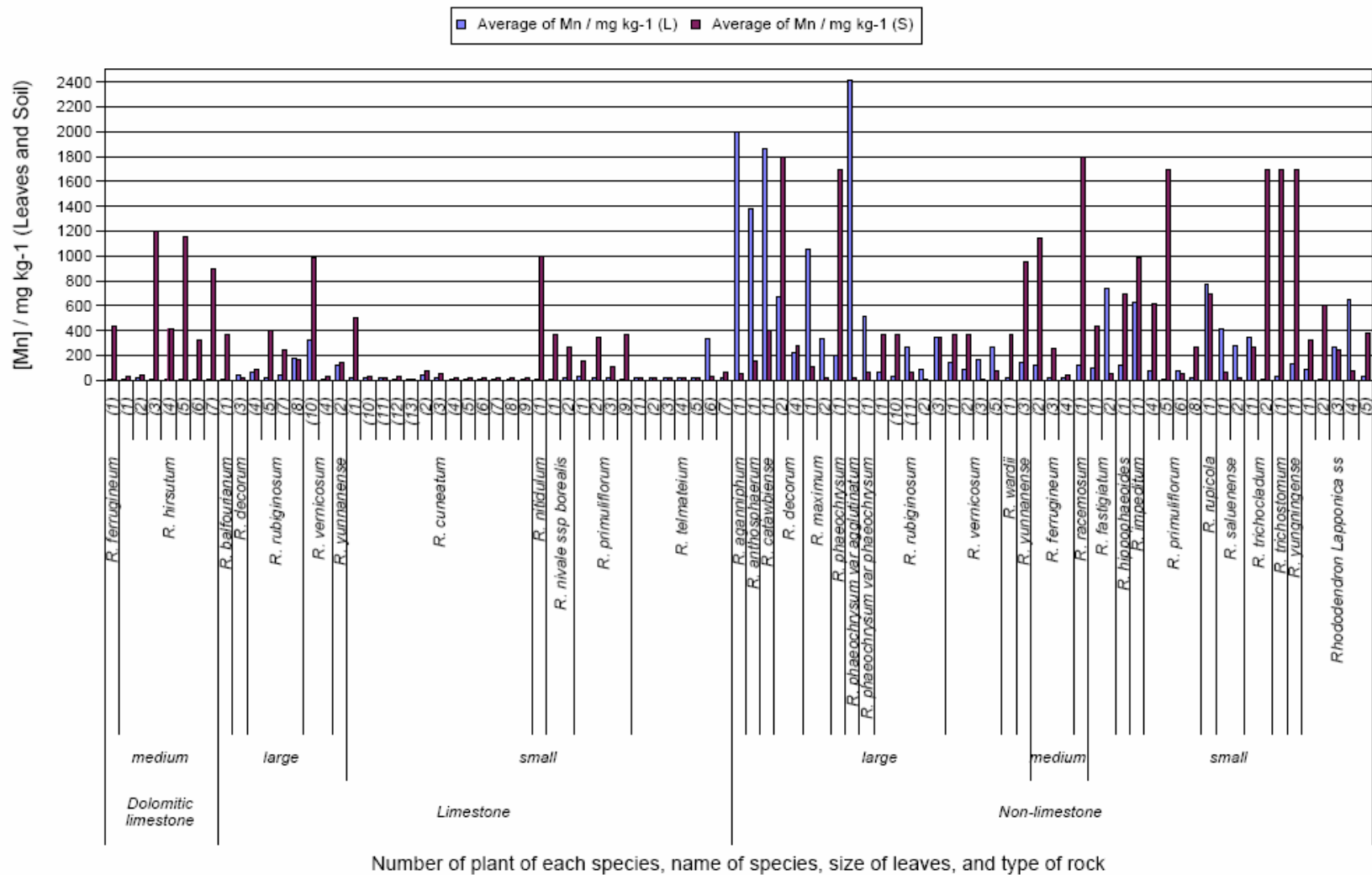
CP 3a [Mn] in leaves for each plant for *Rhododendron* species with names from A-F.



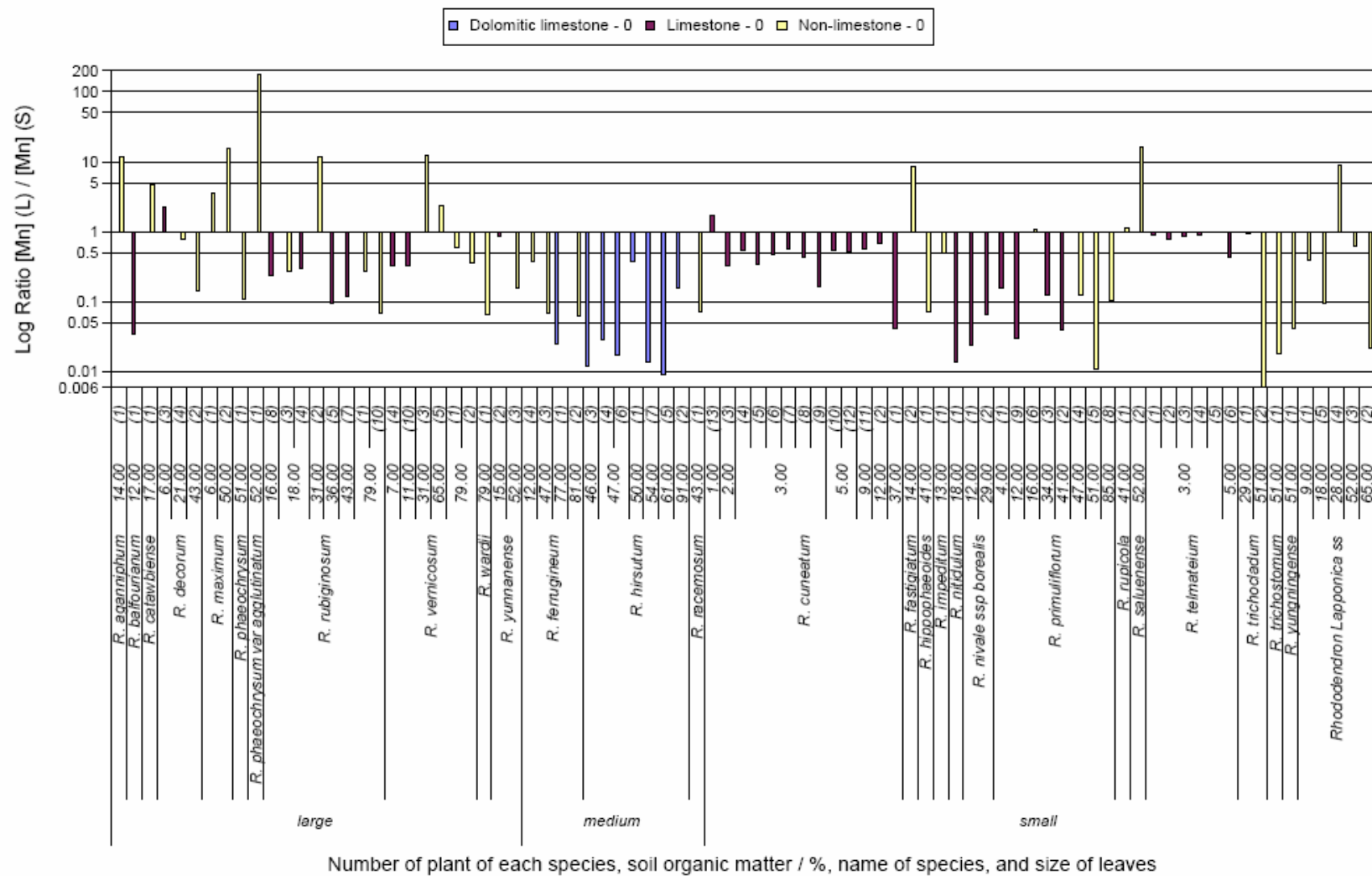
CP 3b [Mn] in leaves for each plant for *Rhododendron* species with names from G-P.



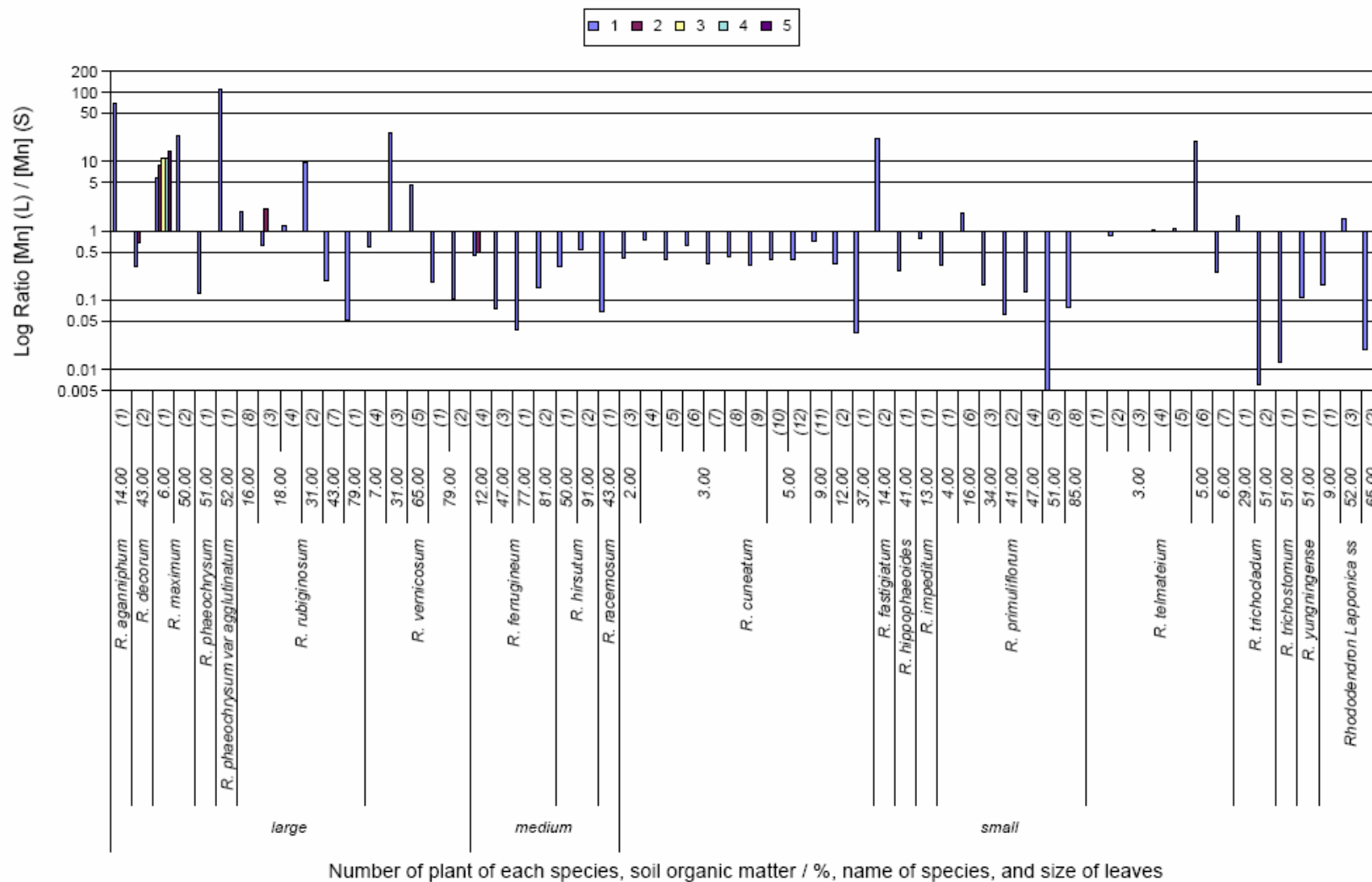
CP 3c [Mn] in leaves for each plant for *Rhododendron* species with names from Q-Z.



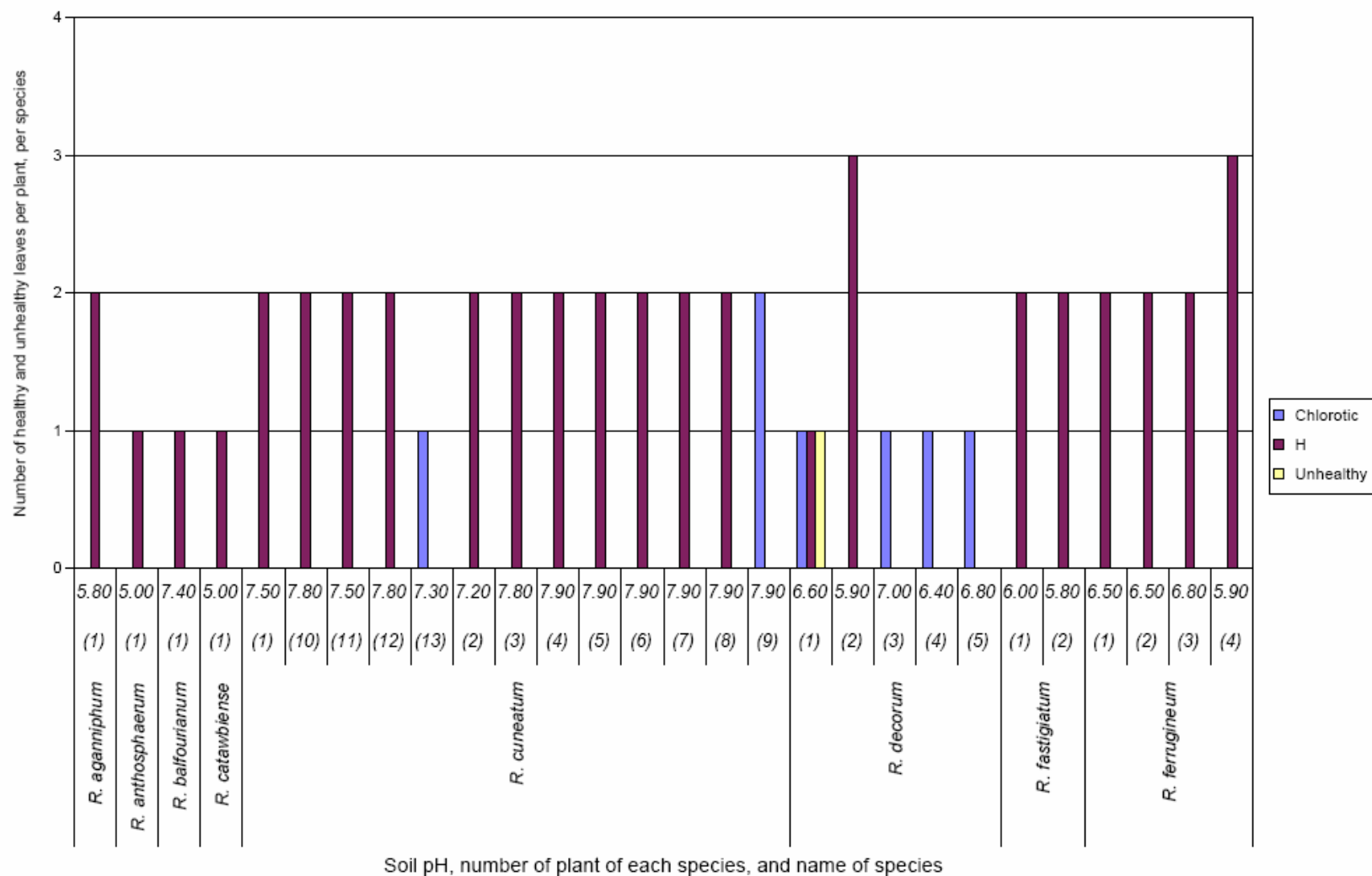
CP 4a Variation of [Mn] in leaves and [Mn] in the soil for each *Rhododendron* plant.



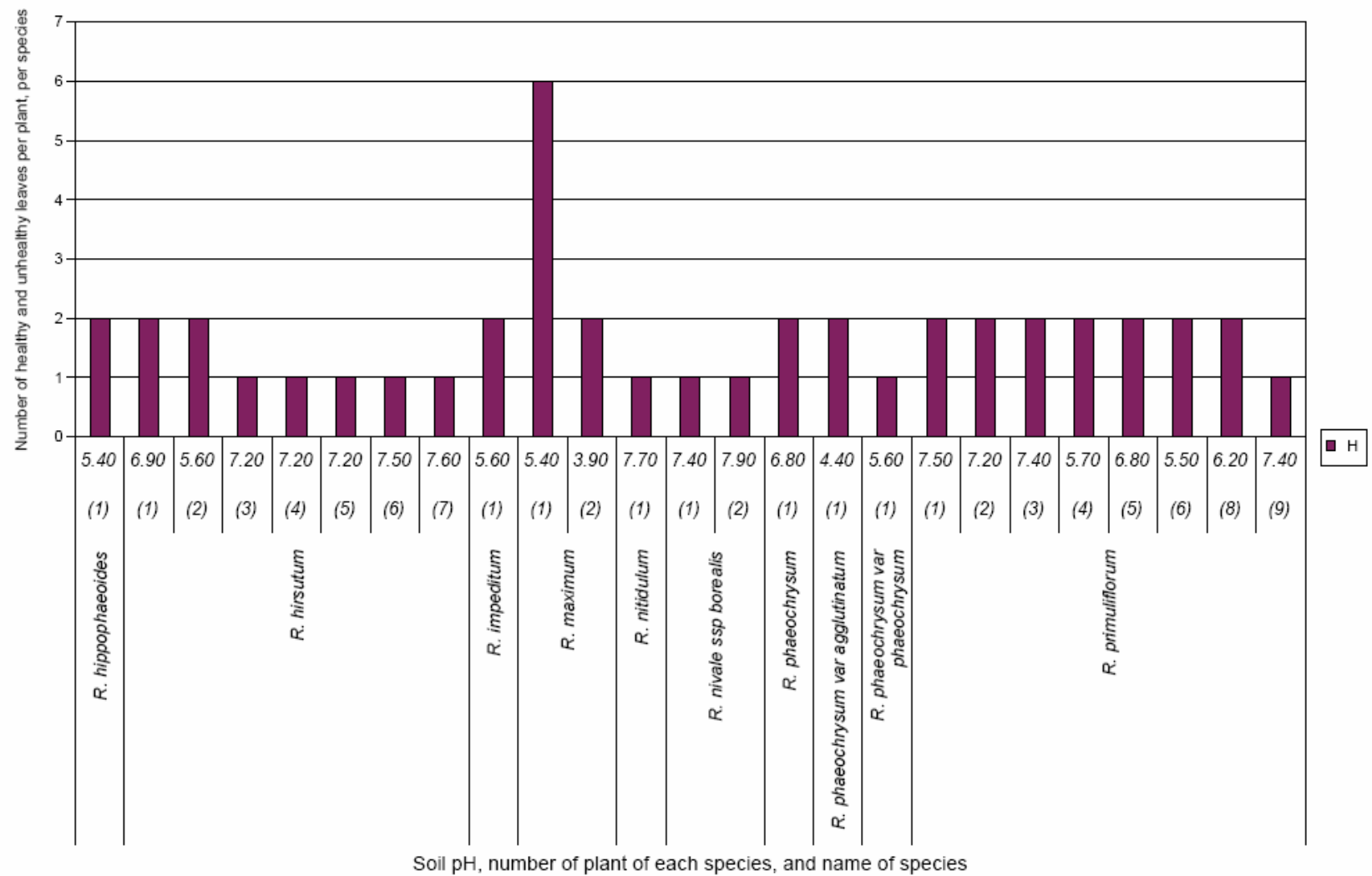
CP 4b Ratio of [Mn] (L) / [Mn] (S) for current-year leaves (0) for each *Rhododendron* plant with variation in leaf size.



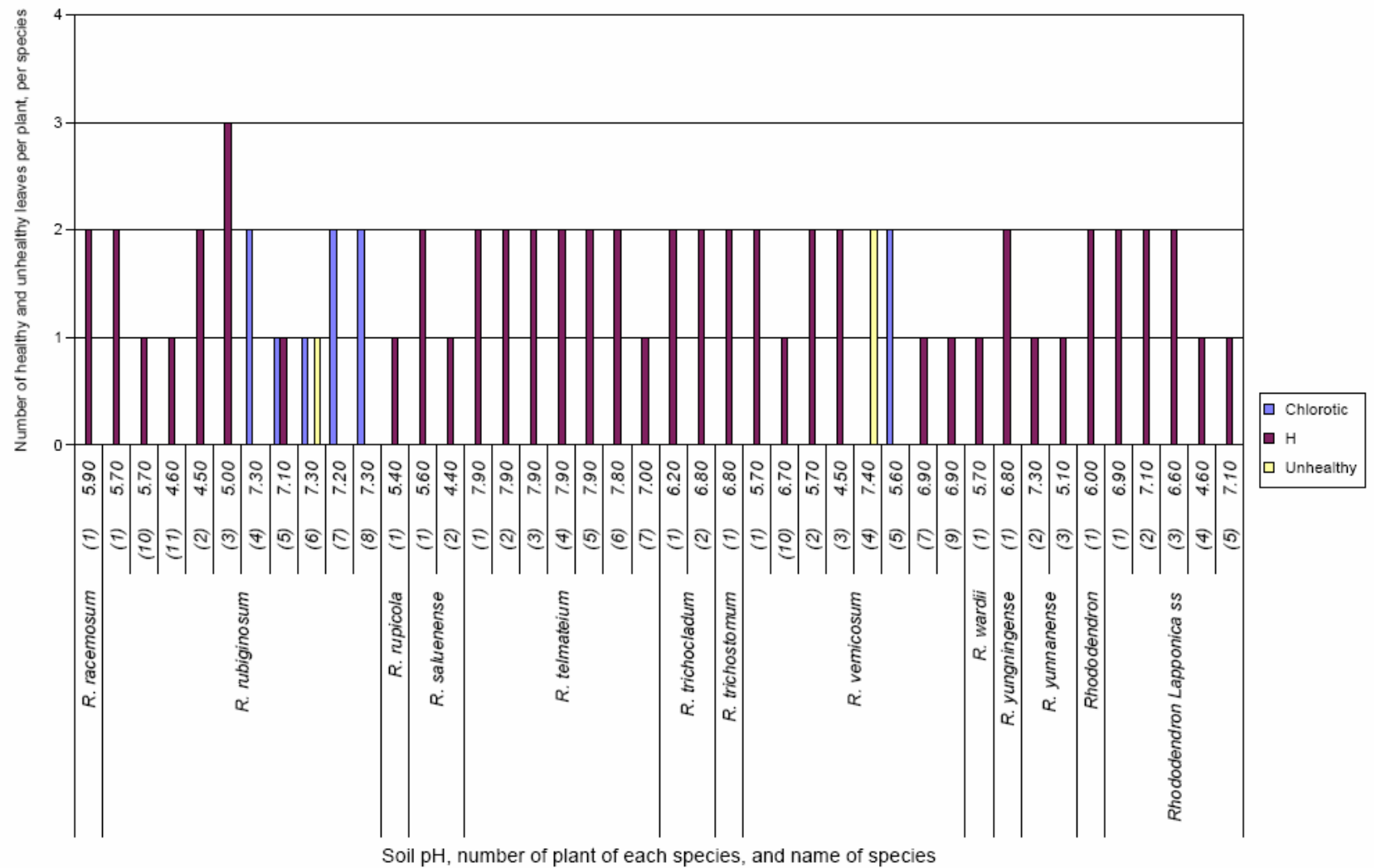
CP 4c Ratio of [Mn] (L) / [Mn] (S) for one (1), two (2), three (3), four (4) and five-year-old (5) leaves for each *Rhododendron* plant with variation in leaf size.



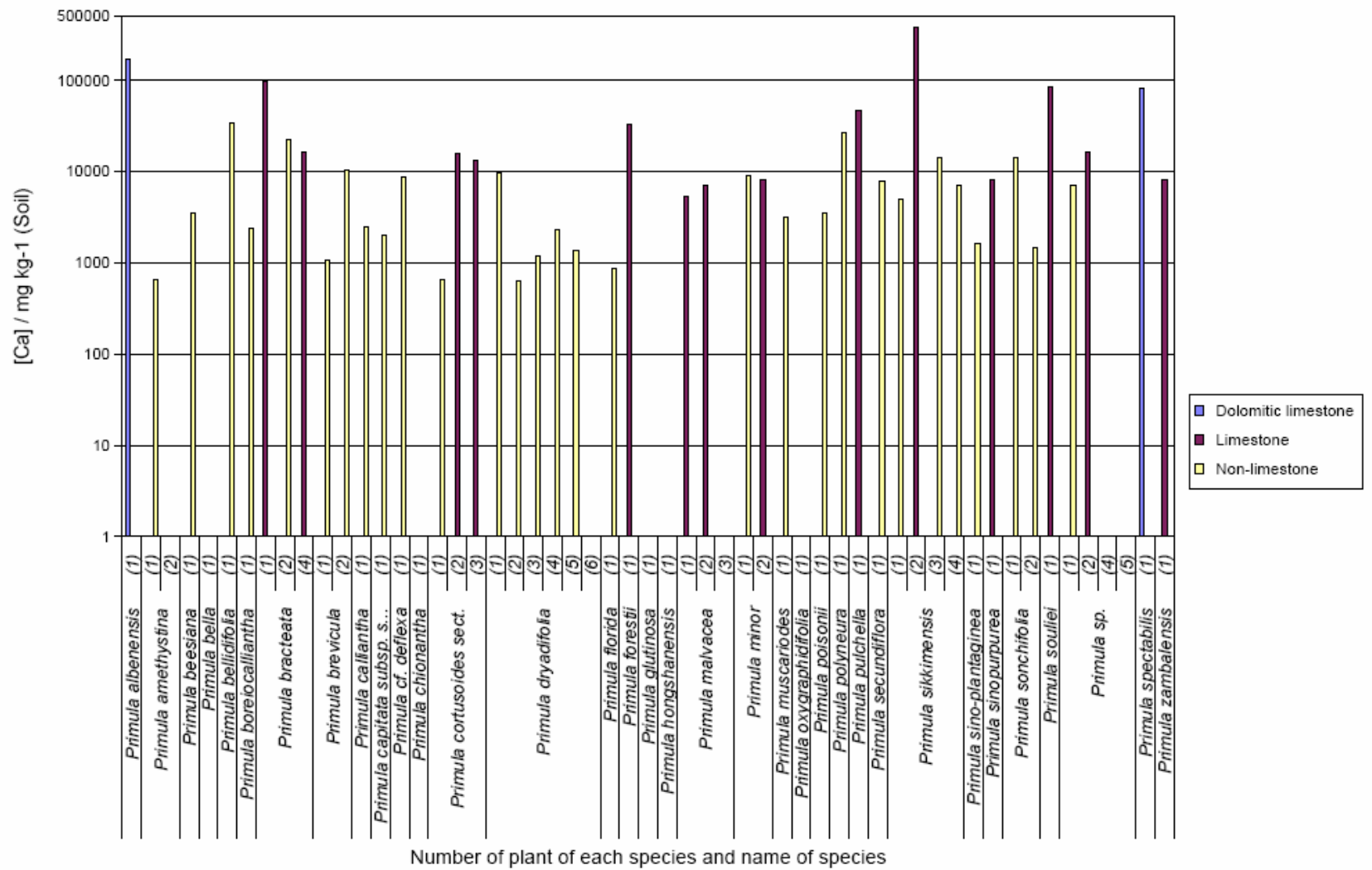
CP 5a Number of healthy and unhealthy leaves, as a sum of all leaves, of all ages, collected for each *Rhododendron* plant of species with names from A-F, and soil pH.



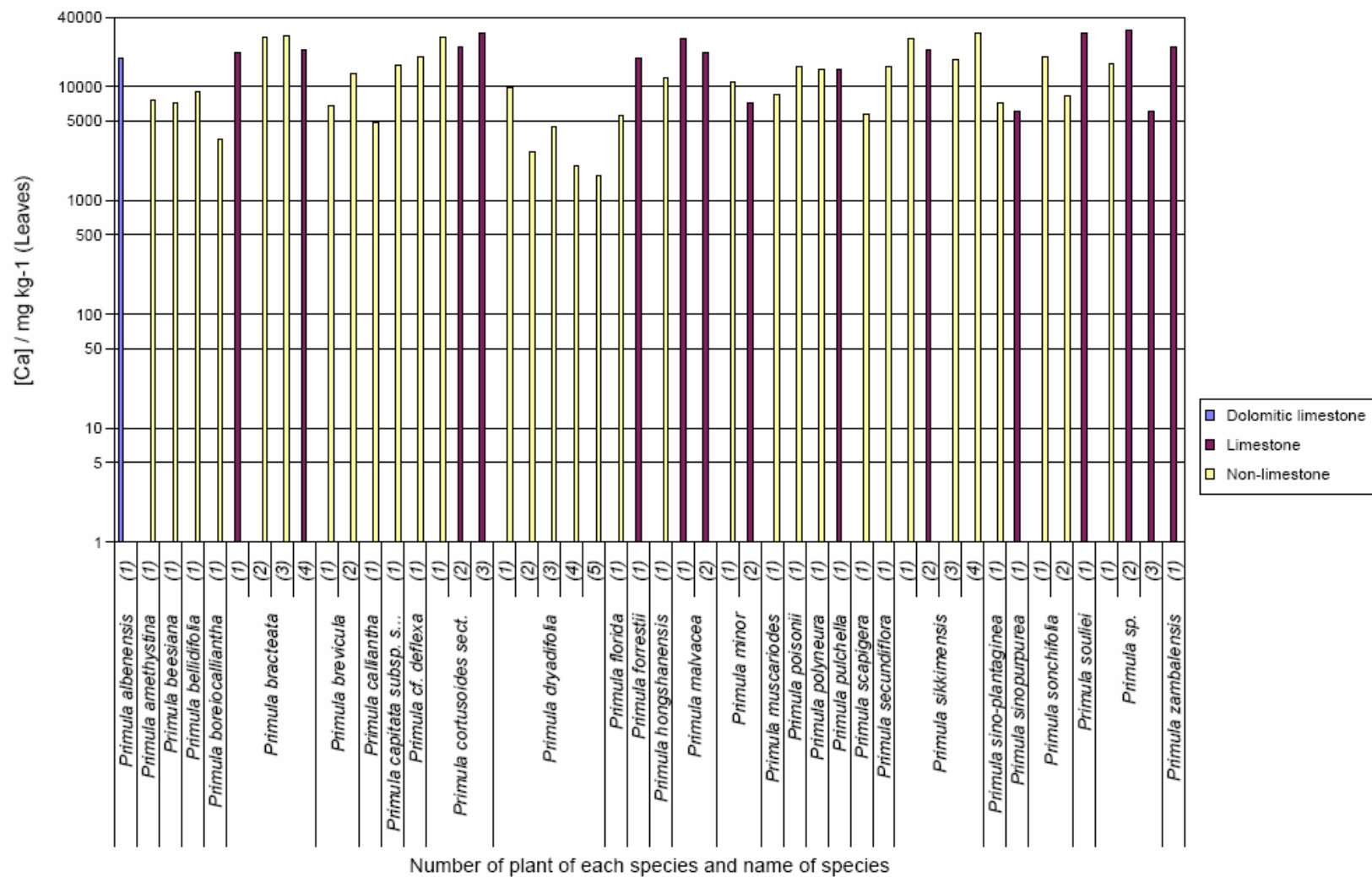
CP 5b Number of healthy and unhealthy leaves, as a sum of all leaves, of all ages, collected for each *Rhododendron* plant of species with names from G-P, and soil pH.



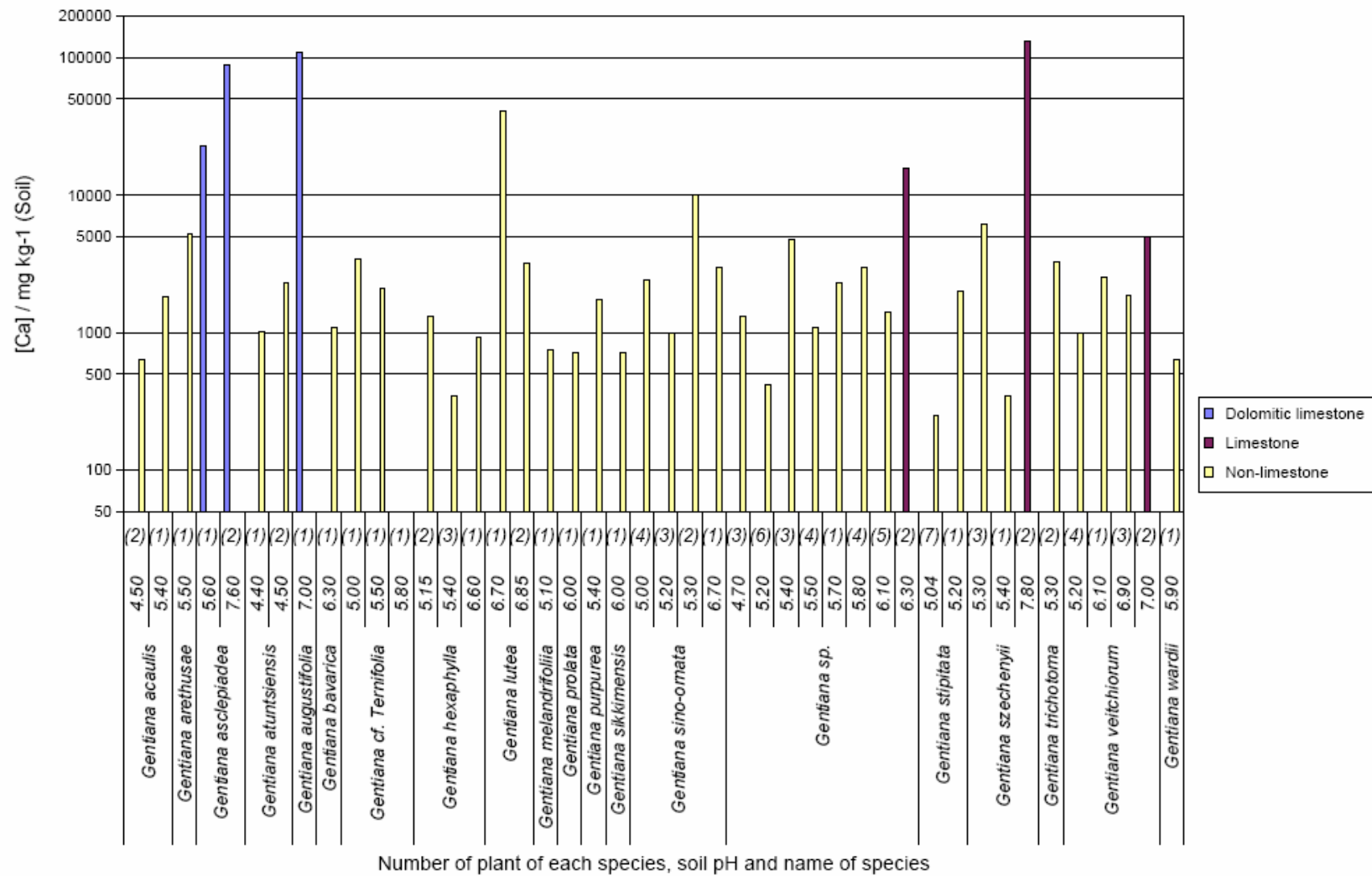
CP 5c Number of healthy and unhealthy leaves, as a sum of all leaves, of all ages, collected for each *Rhododendron* plant of species with names from Q-Z, and soil pH.



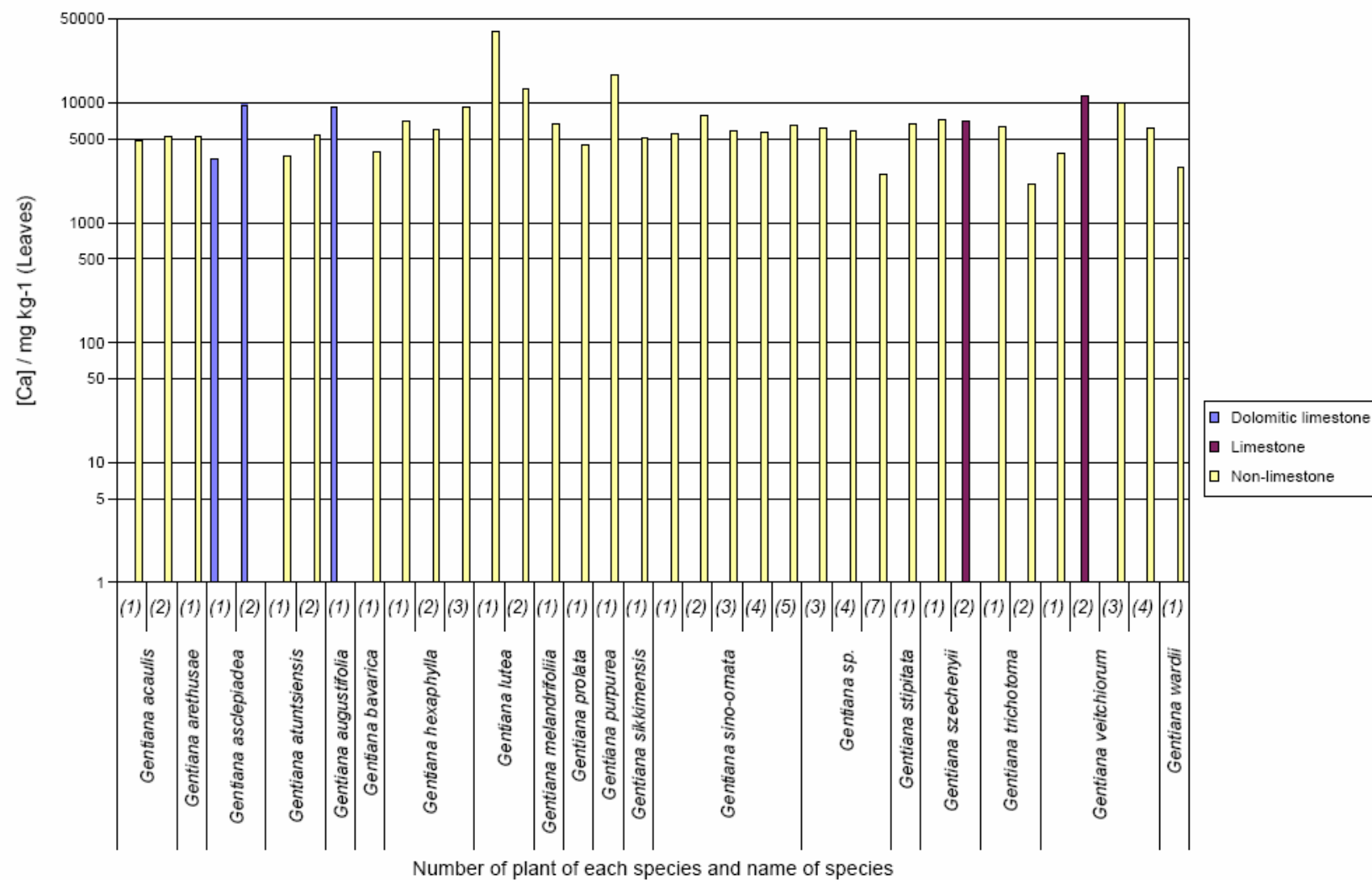
CP 6a [Ca] in soils for *Primula* plants.



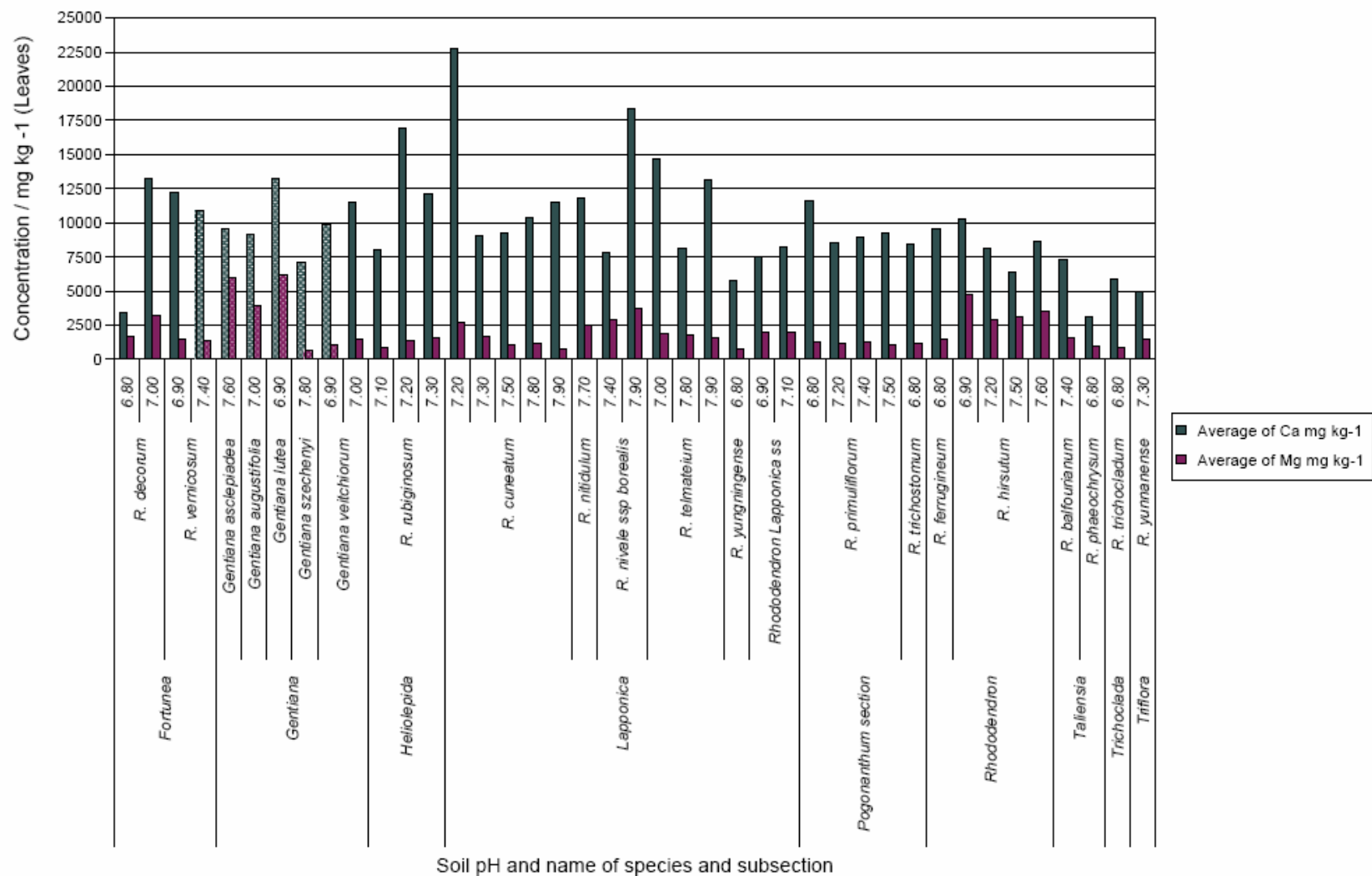
CP 6b [Ca] in the leaves of *Primula* plants.



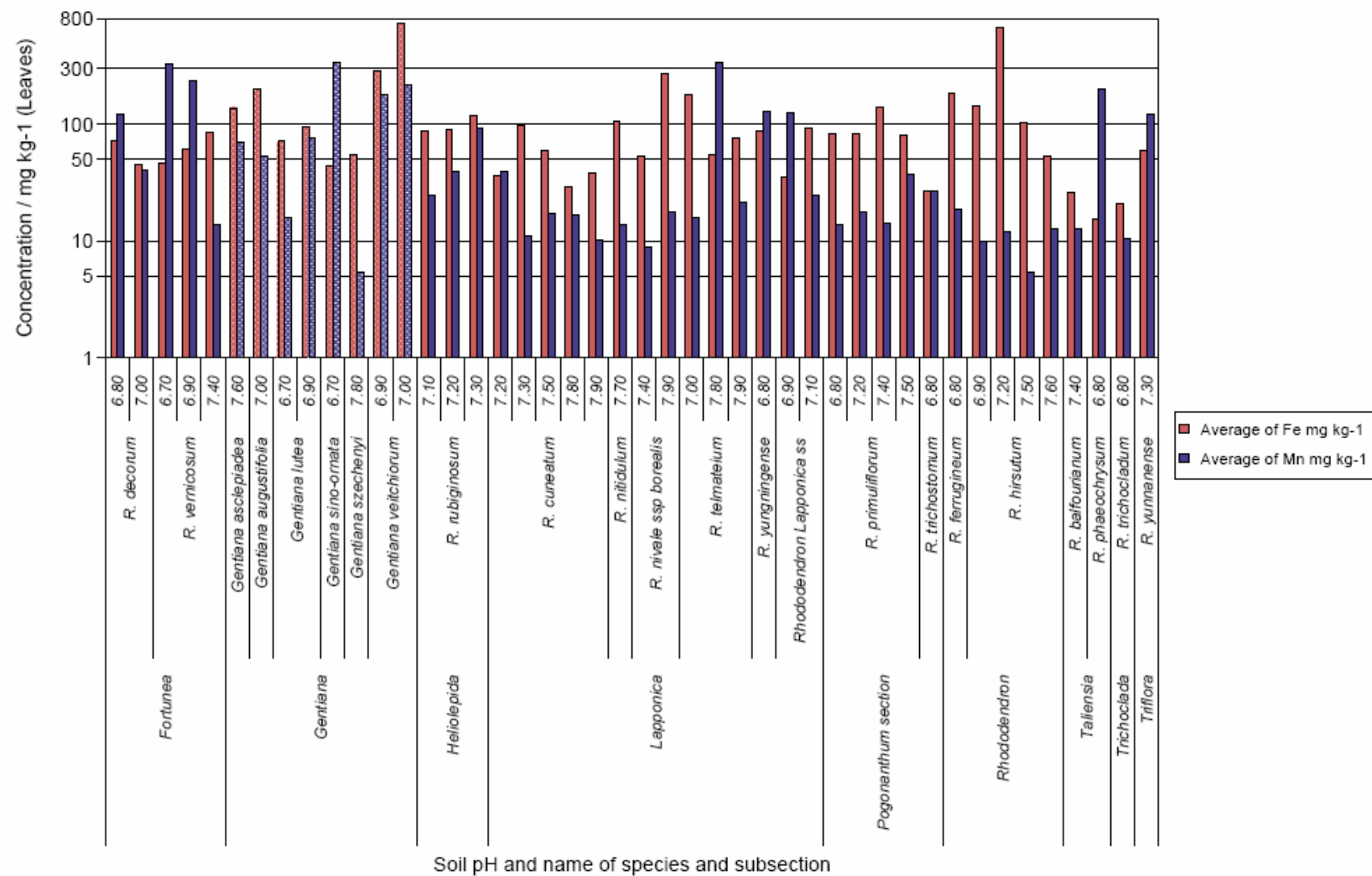
CP 7a [Ca] in soils for *Gentiana* plants.



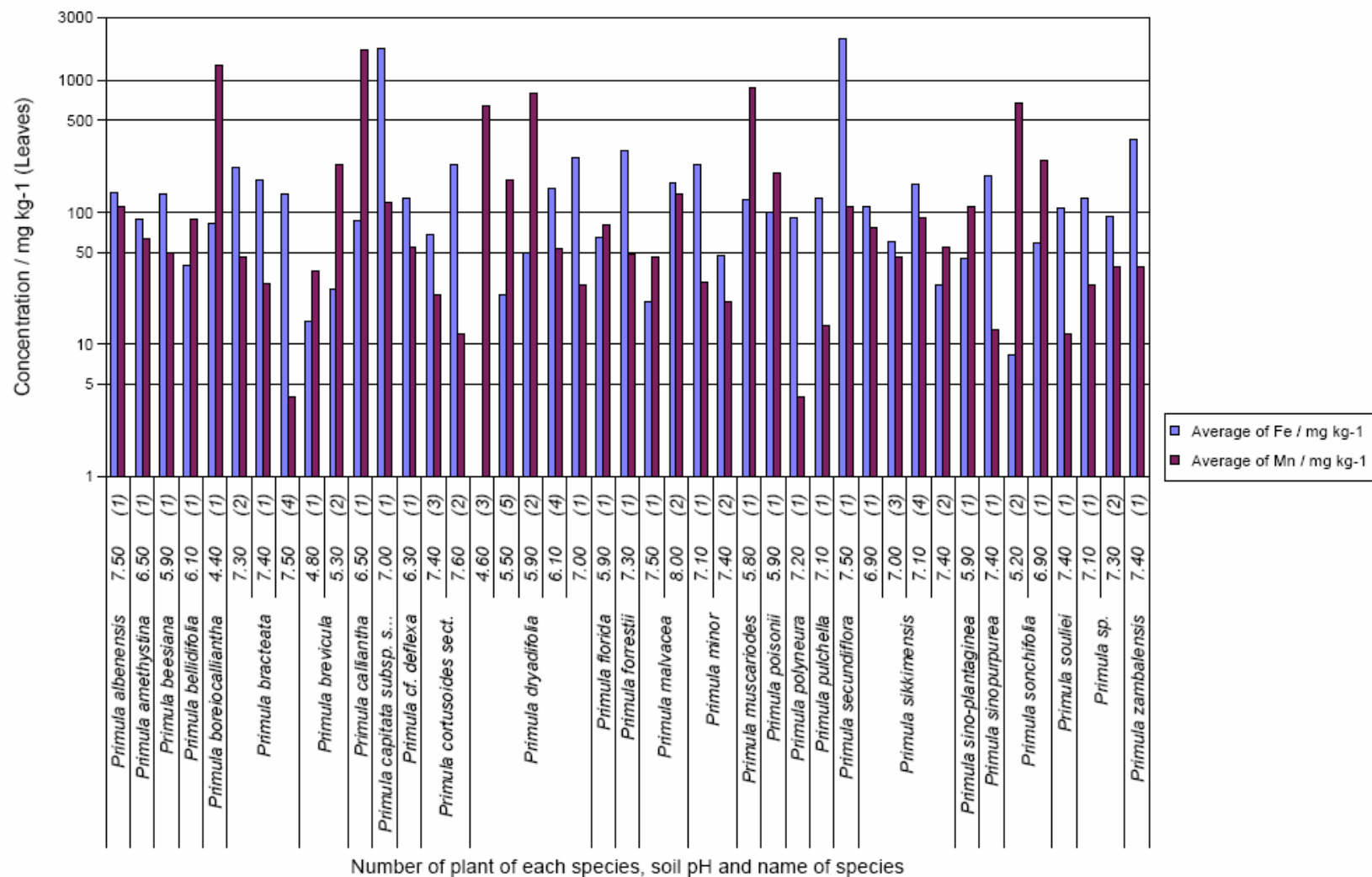
CP 7b [Ca] in the leaves of *Gentiana* plants.



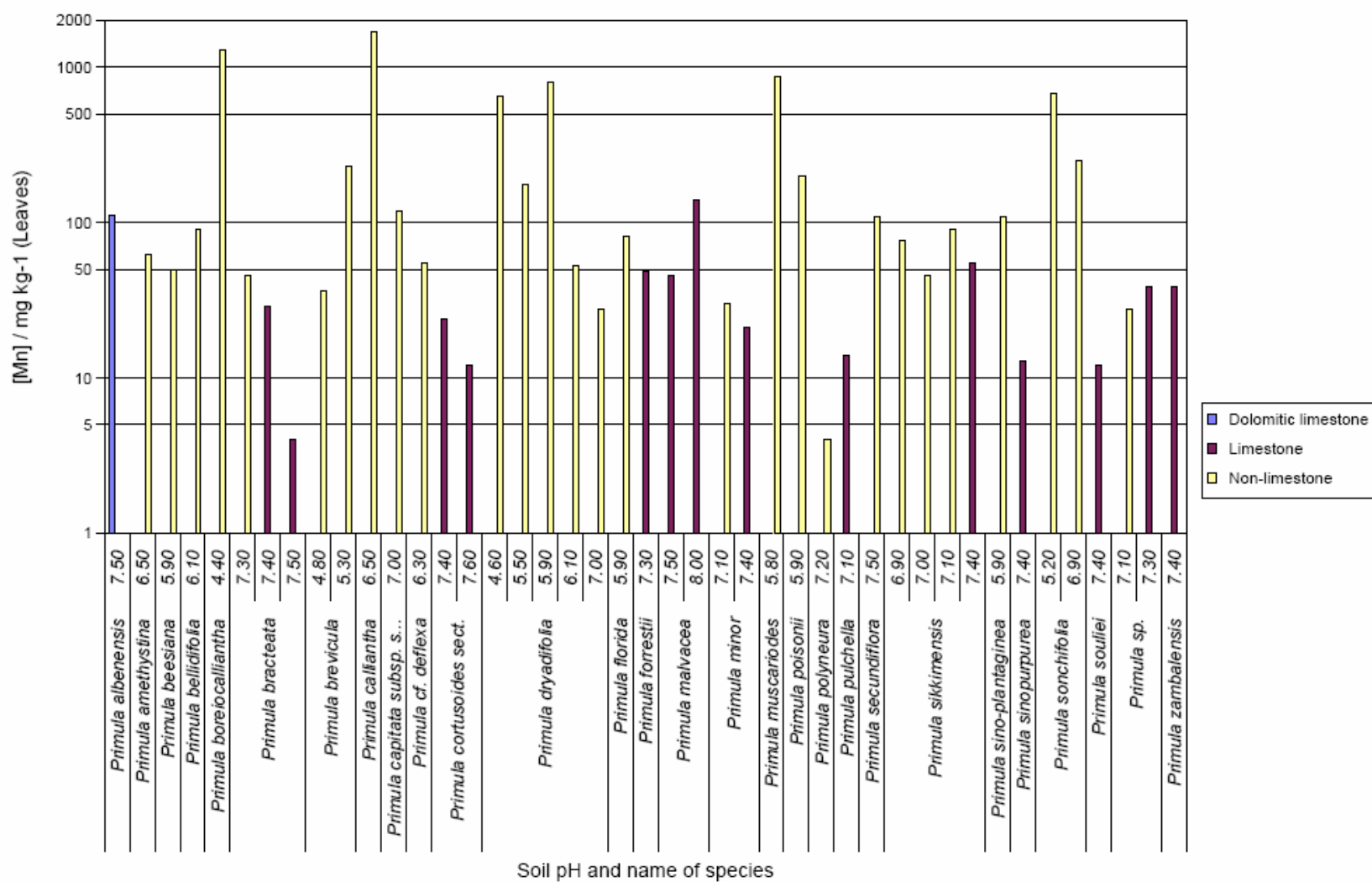
CP 8a [Ca] and [Mg] in the leaves of *Gentiana* and *Rhododendron* plants growing in alkaline soils at pH > 6.8.



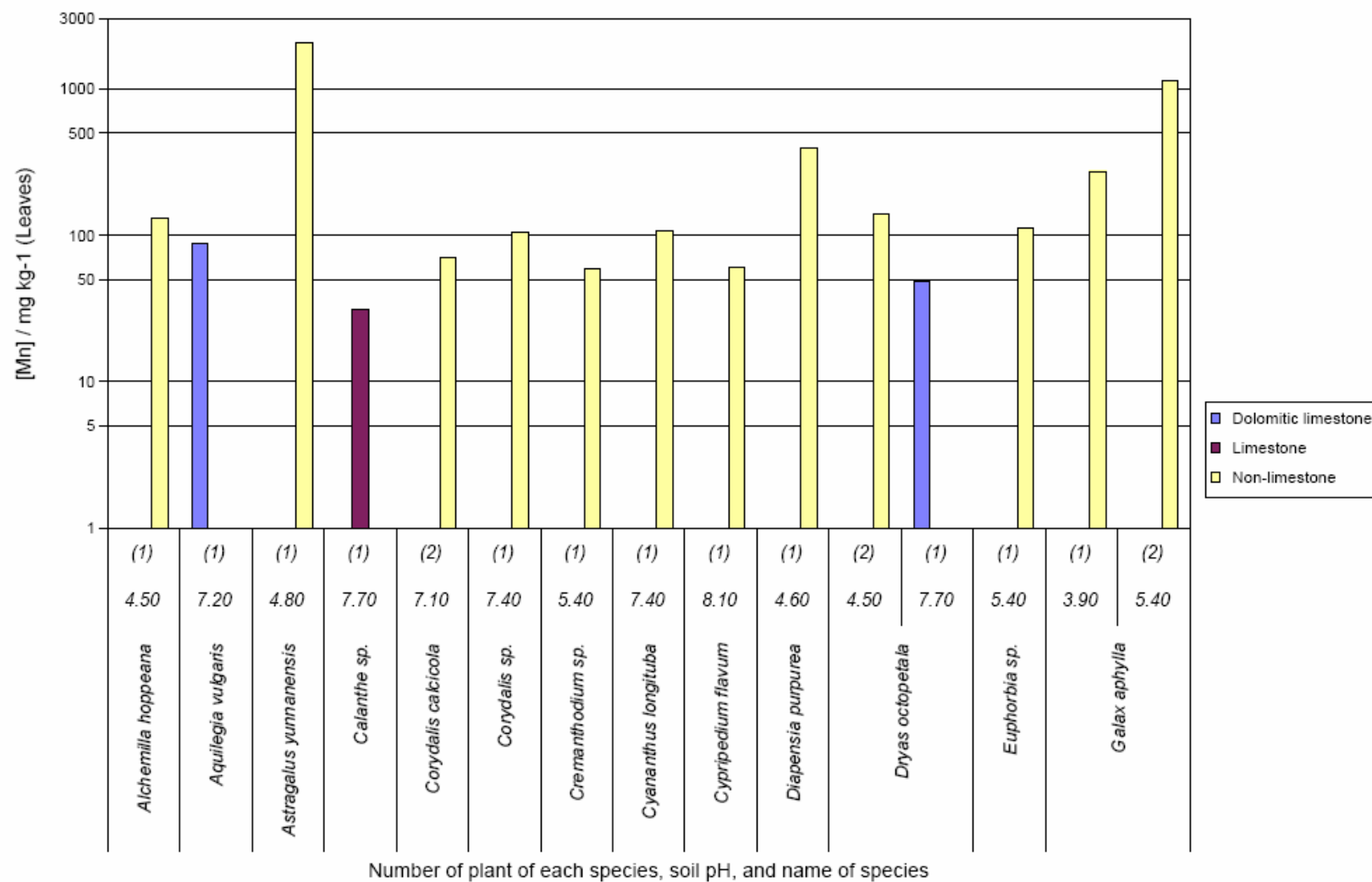
CP 8b [Fe] and [Mn] in the leaves of *Gentiana* and *Rhododendron* plants growing in alkaline soils at pH > 6.8.



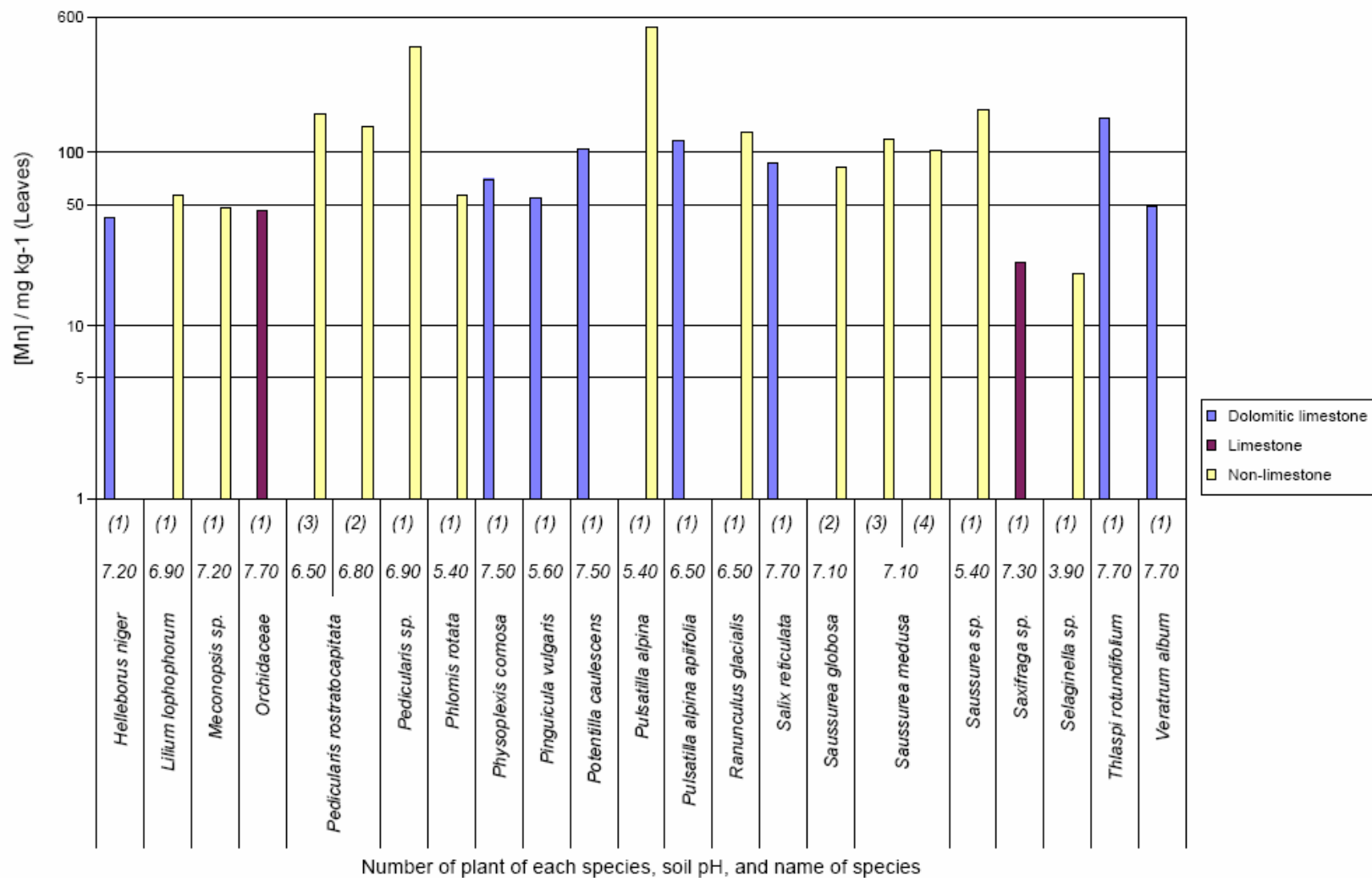
CP 9 Variation of [Fe] and [Mn] in the leaves of *Primula* plants growing on all types of soil.



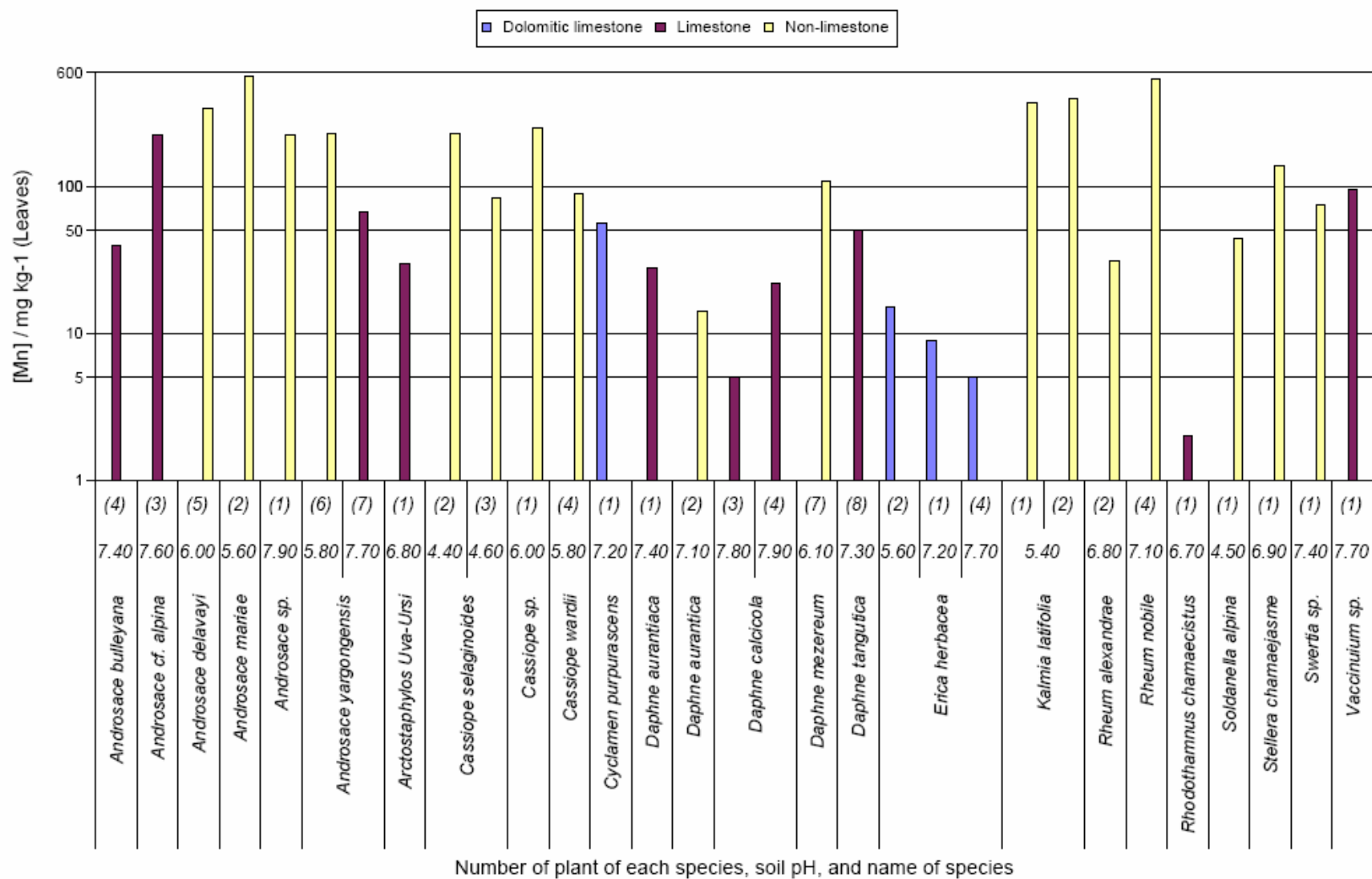
CP 10 [Mn] in the leaves of *Primula* plants growing on all types of soil.



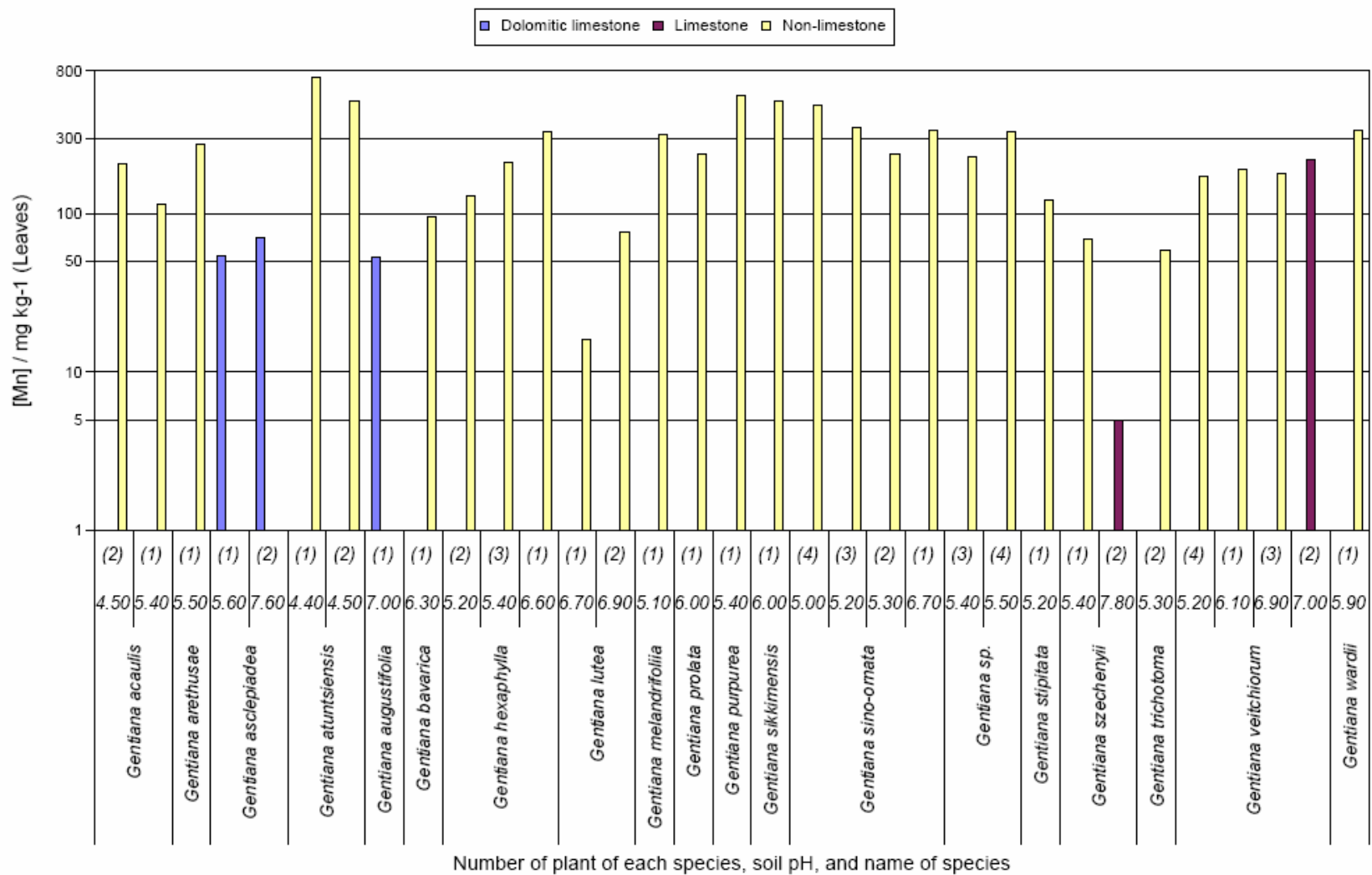
CP 11 [Mn] in the leaves of alpine plants having only one representative leaf sample, with names from A-G.



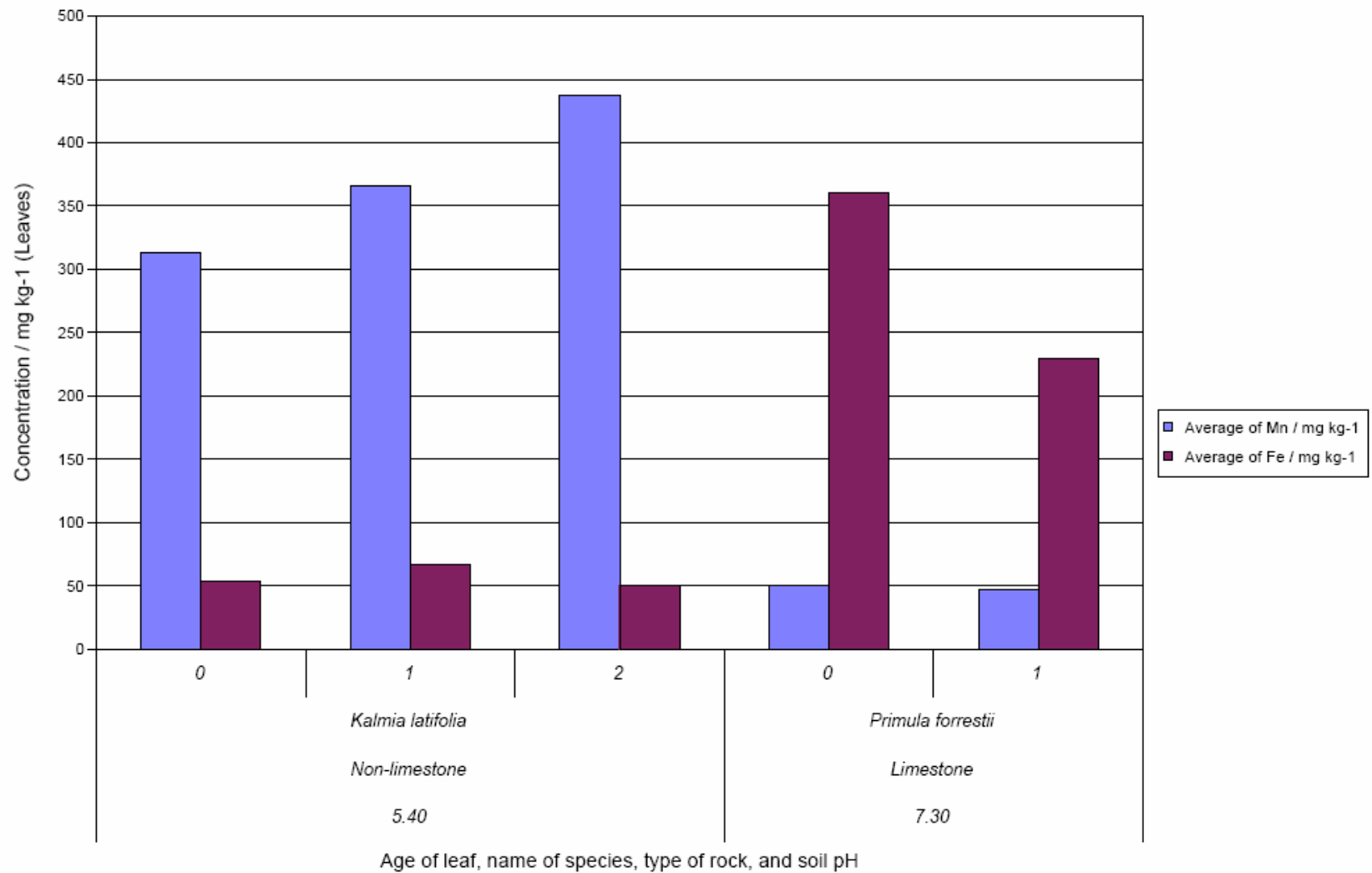
CP 12 [Mn] in the leaves of alpine plants having only one representative leaf sample, with names from H-V.



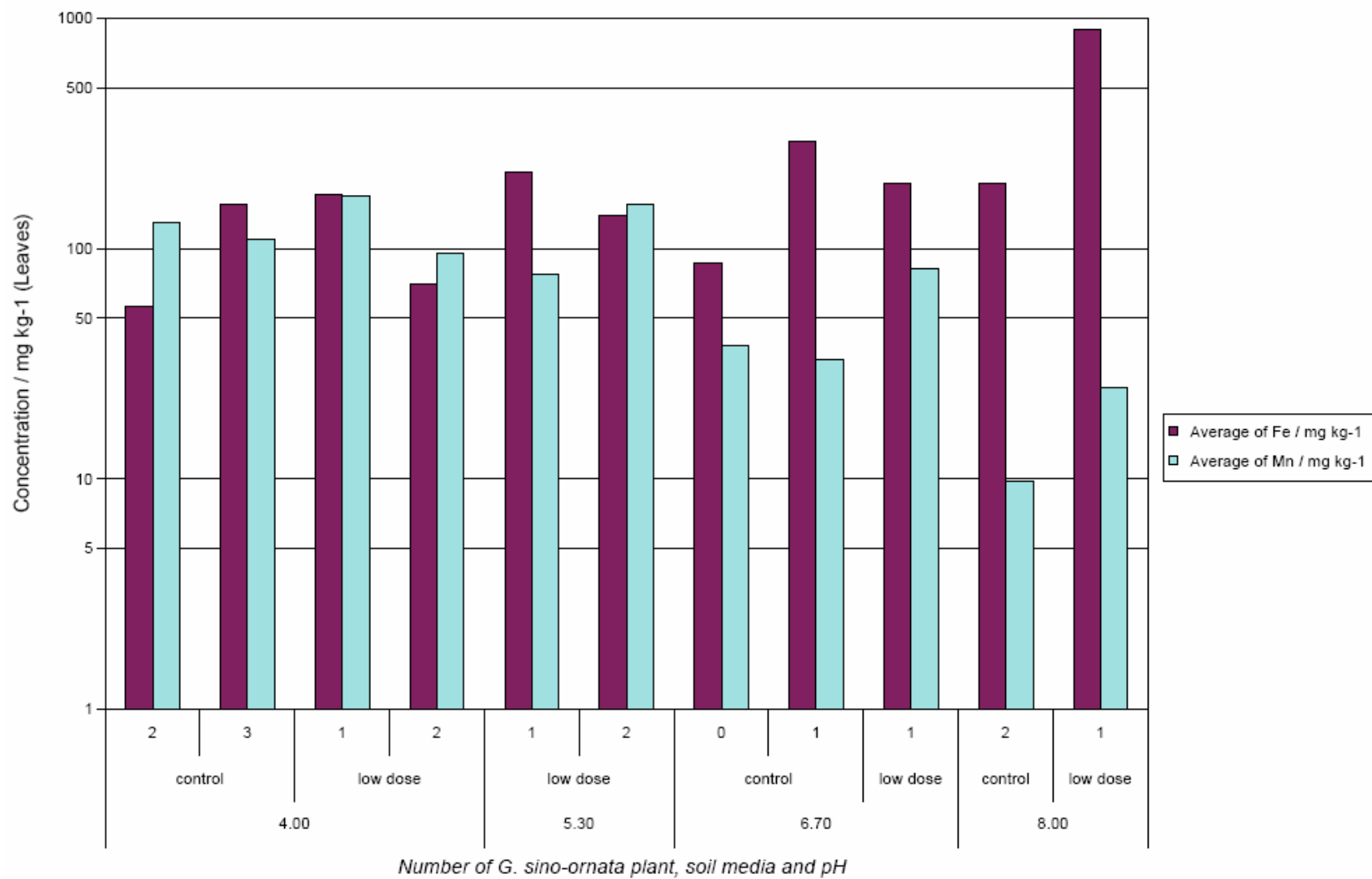
CP 13 [Mn] in the leaves of *Androsace*, *Daphne*, *Ericaceae*, *Rheum* and some *Gentianaceae* plants.



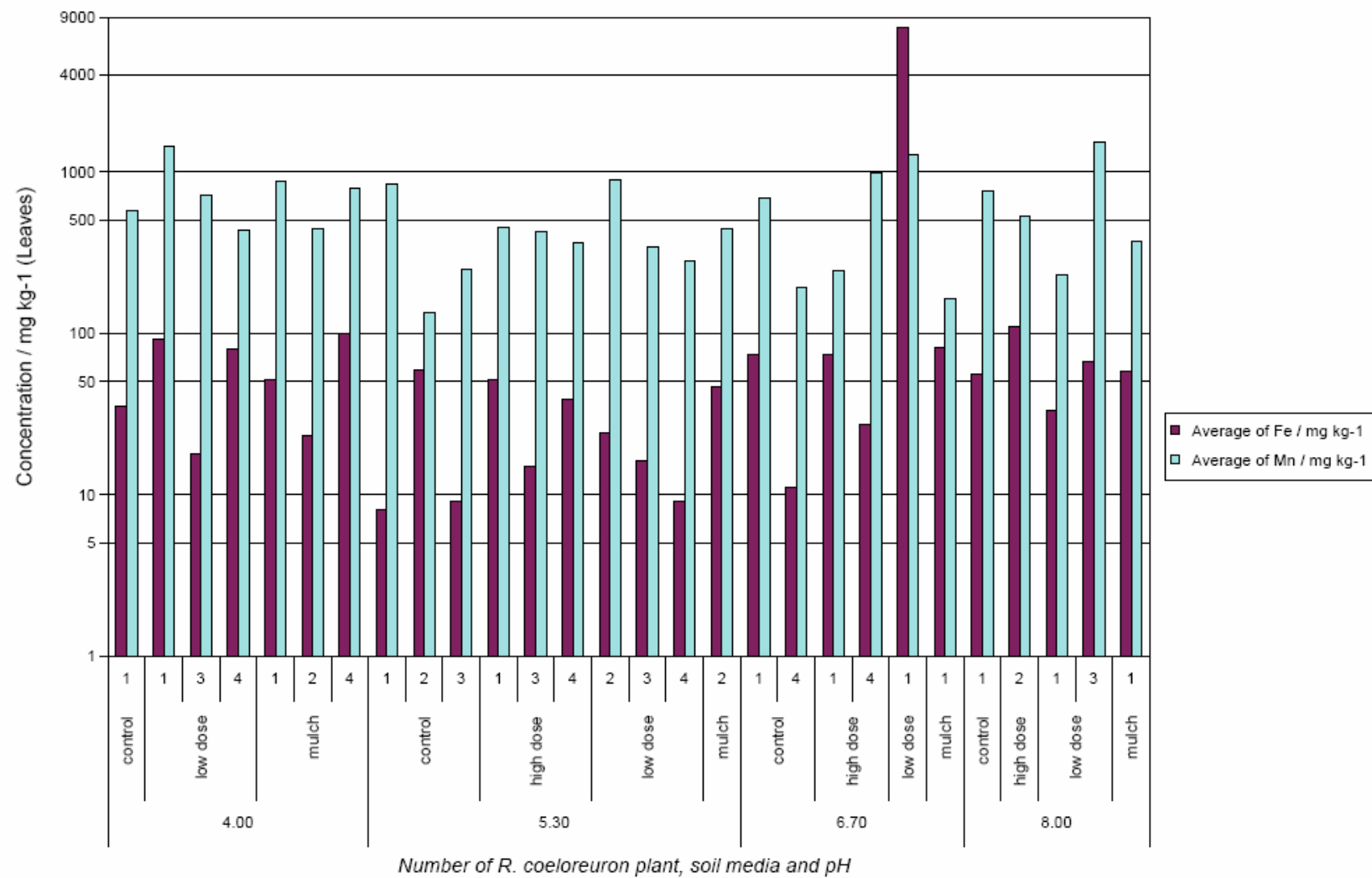
CP 14 [Mn] in the leaves of *Gentiana* plants in all types of soil.



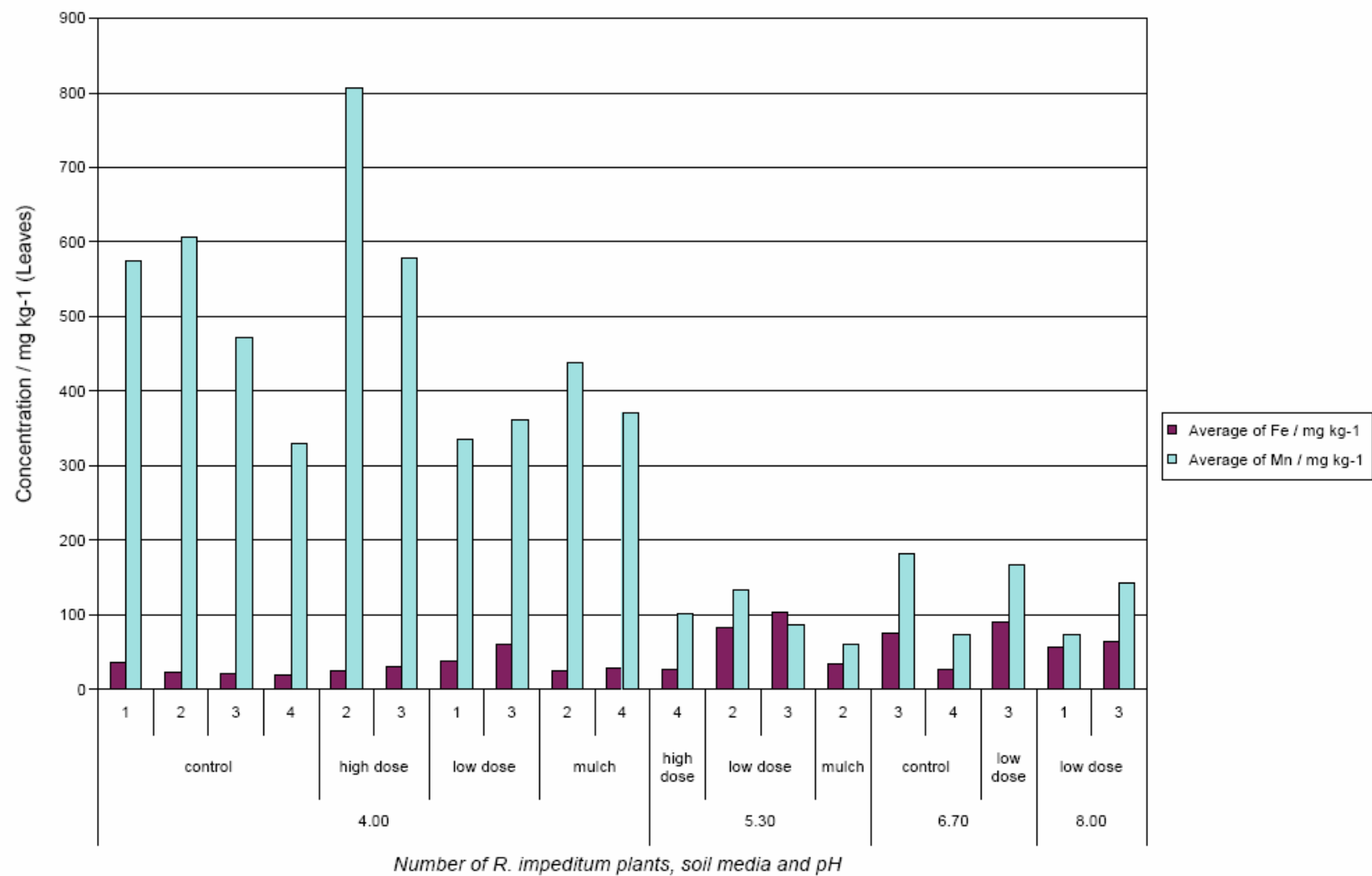
CP 15 Variation of [Fe] and [Mn] in the leaves of a *Kalmia latifolia* and a *Primula forrestii* plant with the age of leaf.



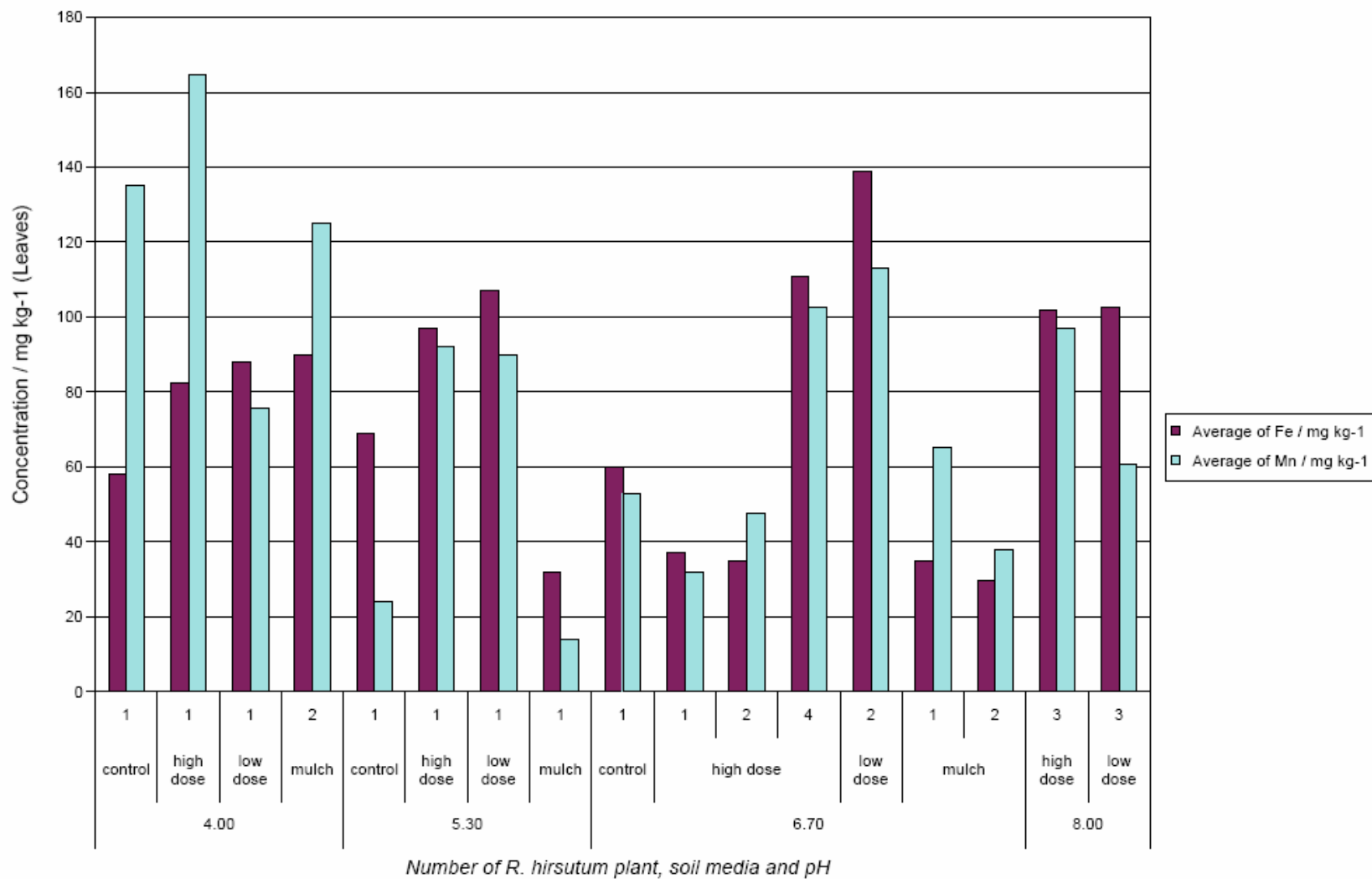
CP 16 Variation of [Fe] and [Mn] in the leaves of *G. sino-ornata* with soil media and pH.



CP 17 Variation of [Fe] and [Mn] in the leaves of *R. coeleureuron* with soil media and pH.



CP 18 Variation of [Fe] and [Mn] in the leaves of *R. impeditum* with soil media and pH.



CP 19 Variation of [Fe] and [Mn] in the leaves of *R. hirsutum* with soil media and pH.

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