Aspects of the Interaction of Trace Metal Ions with Calcium Carbonate

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Abstract

The interaction of high-Mg calcite and aragonite with a number of different trace metals was studied, in particular with Cu$^{2+}$, Ni$^{2+}$ and Co$^{2+}$. Laboratory experiments into the initial sorption of these three metal ions onto the surfaces of artificial aragonite and high-Mg calcite were performed for a pH range of 6.8-8.2, in buffered solutions saturated with respect to calcite. Below a pH of 8.2, substantial dissolution of the unstable polymorph occurred, with little uptake of Co$^{2+}$ or Ni$^{2+}$. At a pH of 8.2, uptake was rapid, reaching a plateau in less than 5 min for all three metals. A higher proportion of the available Cu$^{2+}$ was taken up than of the available Co$^{2+}$ or Ni$^{2+}$.

Two environmental situations were also investigated. Firstly, samples of water, rock and aragonitic speleothems from Bohemia Cave, Mt. Owen, central Nelson, were examined for major and trace element concentrations. Water samples from the area were found to have very low trace metal ion concentrations, and little consistent relationship between the level of interaction with the environment and the trace metal concentration was seen. Concentration factors of trace metals into the speleothems were much larger than concentration factors of minor or major elements. Metal concentrations in the speleothems were lower than in the surrounding rocks, due to the slow deposition rate of the aragonite crystals, and the exclusion of small cations from the lattice of aragonite. Speleothem samples were analysed by electron microscopy, and structures are described.

Secondly, uptake of sub-lethal concentrations of Cu$^{2+}$, Ni$^{2+}$ and Co$^{2+}$ by Corallina officinalis (Corallinales, Rhodophyta) was studied, from a chemically-defined artificial seawater medium. Cu$^{2+}$ was found to be toxic at even low levels of contamination. All three metal ions were taken up by the live seaweed, in proportion to the metal concentration of the medium. Partitioning of the metal ions was examined, between the inorganic phase of high-Mg calcite, deposited in the plant’s cell walls, and the organic material of the plant. Killed seaweed were also “cultured”, and found to contain considerably less metal ion than live seaweed. The presence of an increasing excess of Ni$^{2+}$ had a negative effect on the uptake of Cu$^{2+}$, but no interaction was seen between Co$^{2+}$ and the other metal ions. Concentration factors were greatest for Co$^{2+}$, followed by Cu$^{2+}$ and Ni$^{2+}$. C. officinalis has potential as a bio-monitor of pollution levels in seawater, but its usefulness requires further assessment.
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Part I

Introduction
Chapter 1

Introduction

1.1 Introduction

This thesis describes a study of aspects of the interaction of three transition metal ions, Co$^{2+}$, Ni$^{2+}$ and Cu$^{2+}$, with calcite and aragonite, the two most common polymorphs of calcium carbonate. In this chapter, an introduction is given to calcium carbonate, its polymorphism and its growth. Following this is a discussion of the nature of surfaces in solutions, metal adsorption to oxide surfaces, and the surface of calcium carbonate. The theory discussed here provides a basis for understanding the interactions described in the remainder of the work.

1.2 The Nature of Calcium Carbonate

Calcium carbonate is best known as limestone, or, after metamorphism, as marble. The majority of the world's extensive CaCO$_3$ deposits are biological in origin, making CaCO$_3$ the most common biominal. Many marine, and some freshwater, organisms deposit calcium carbonate as skeleton or shell, including foraminifera, molluscs, corals and some algae. Inorganic deposition is much less substantial, and includes cement between grains of organic CaCO$_3$, diagenetic recrystallization, and secondary deposits in hot springs and caves, such as stalactites.

Calcium carbonate occurs naturally as several different polymorphs (Table 1.1), minerals with the same atomic formula, but different spatial positioning of the ions (in this case Ca$^{2+}$ and CO$_3^{2-}$). The most common polymorph is calcite (Figure 1.1), five different forms of which are known (McCauley and Roy, 1974; Tucker et al., 1990). Calcite is trigonal with R3c symmetry, and can be described as having a distorted NaCl structure, in which each anion site is occupied by a carbon atom surrounded by three oxygen atoms. The oxygen atoms form an equilateral triangle, orientated perpendicular to the body diagonal which has become compressed when the structure was distorted from NaCl (Lippmann (1973); details of the transformation are described in Hyde and Andersson (1989)). This morphological (or cleavage) cell contains four CaCO$_3$ units, but does not completely describe the crystal's symmetry; if the same cell
Chapter 1. Introduction

(a) calcite

(b) aragonite

Figure 1.1: A plan view of the calcite and aragonite structures, showing that the difference between them is the orientation of the calcium atoms to the carbonate group. From Stillwell (1938).

shape is used, a 32-unit cell is needed, which is considered too large to be a convenient description. Acute rhombohedral and hexagonal cells are also possible, and the most commonly used is a hexagonal cell, with six CaCO$_3$ units, which emphasizes the layers of CO$_3$ and Ca alternating along the c-axis. Each Ca$^{2+}$ ion is coordinated by six oxygen atoms, and each oxygen atom coordinates two calcium ions.

Table 1.1: Some data on the polymorphs of calcium carbonate.

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Space Group</th>
<th>Unit cell dimensions (nm)</th>
<th>$G_f^0$ (kJ/mol)$^a$</th>
<th>$-\log^b K_{sp}^b$</th>
<th>Density (g/cm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>calcite</td>
<td>R3c$^a$</td>
<td>0.4995$^a$ 0.49611$^d$ 0.413$^a$</td>
<td>1.7075$^a$</td>
<td>-1128.8</td>
<td>2.71$^c$</td>
</tr>
<tr>
<td>aragonite</td>
<td>Pcmn$^d$</td>
<td>0.79672$^d$ 0.715$^a$</td>
<td>0.57407$^d$</td>
<td>-1127.7</td>
<td>2.93$^c$</td>
</tr>
<tr>
<td>vaterite</td>
<td>Pbnm$^e$</td>
<td>0.848$^a$</td>
<td>-1125.1</td>
<td>7.91</td>
<td>2.65$^e$</td>
</tr>
</tbody>
</table>

$^a$Driessens and Verbeeck (1990a)
$^b$Plummer and Busenberg (1982)
$^c$Lippmann (1973)
$^d$Jarosch and Heger (1986)
$^e$Dickson (1990)

Aragonite (Figure 1.1) is the second most common polymorph. It has an orthorhombic, distorted NiAs structure, with Pcmn symmetry (Jarosch and Heger, 1986) where Ca$^{2+}$ ions are exchanged for arsenic and the carbonate ion for nickel (Lippmann, 1973, pg. 55). This is a more dense structure than that of calcite (Lippmann, 1982), with less distance between atoms. Similarly to calcite, aragonite contains layers of CO$_3$ and Ca groups parallel to the c-axis, but whereas in calcite neighbouring calciums are
1.2. The Nature of Calcium Carbonate

1.2.1. The Nature of Calcium Carbonate

Calcite and aragonite are two members of the AB\textsubscript{2}O\textsubscript{3} group, with B\textsubscript{2}O\textsubscript{3} planar. The calcite structure occurs for compounds with small A ions, such as LiNO\textsubscript{3}, NaN\textsubscript{2}O, and YB\textsubscript{2}O, and the aragonite structure for those with larger A ions, such as KNO\textsubscript{3} and LaB\textsubscript{2}O\textsubscript{3} (Wyckoff, 1964). Seven other naturally-occurring metal carbonates (CdCO\textsubscript{3}, MnCO\textsubscript{3}, FeCO\textsubscript{3}, CoCO\textsubscript{3}, ZnCO\textsubscript{3}, MgCO\textsubscript{3} and NiCO\textsubscript{3}) are known to have the calcite structure, and three naturally-occurring carbonates (SrCO\textsubscript{3}, PbCO\textsubscript{3} and BaCO\textsubscript{3}) and synthetic EuCO\textsubscript{3}, have the aragonite structure (Dickson, 1990).

The work described in this thesis has considered the interaction of transition metal ions in solution with both aragonite and calcite. First, adsorption of Co\textsuperscript{2+}, Ni\textsuperscript{2+} and Cu\textsuperscript{2+} onto both aragonite and high-magnesium calcite powder in a laboratory setting is discussed (Part II). These results are then compared with two environmental situations. The first situation was secondary aragonite formed in a cave in the Mt Owen Marble, Murchison, New Zealand (Part III). Cave and surface water, speleothems and rocks were analysed for major and trace metal concentrations. Speleothem structures were studied using scanning electron microscopy, and the questions of speleothem mineralogy and modes of formation are discussed. The second environmental situation studied was the uptake of Co\textsuperscript{2+}, Ni\textsuperscript{2+} and Cu\textsuperscript{2+} ions by Corallina officinalis grown in a chemically-defined culture medium developed for this purpose (Part IV). C. officinalis is a red
macro-alga, common on rocky shores throughout New Zealand, which deposits calcite within its cell walls. Worldwide, coralline algae are significant contributors to coral reefs, particularly to the high wave energy reefs common in the Pacific Ocean.

The third naturally-occurring polymorph of calcium carbonate, vaterite, is of Pbnm symmetry (Driessens and Verbeeck, 1990a), also with a distorted NiAs structure. It differs from aragonite in that, in vaterite, calcium ions replace nickel and carbonate ions replace arsenic (Lippmann, 1973, pg.68). In this work, vaterite was not considered, as it is not naturally found in the systems of interest.

The -logK_{sp} data presented in (Table 1.1) indicate that calcite is the least soluble form of CaCO_{3}, with a K_{sp} value of 3.8x10^{-9}, and vaterite is the most soluble of the three (K_{sp} = 1.7x10^{-8}). Calcite has the most negative Gibb's free energy of formation of the three polymorphs, making it marginally the most thermodynamically stable form of calcium carbonate. From a calcium bicarbonate solution at ambient temperature and pressure, it would be expected that calcite would preferentially crystallize from solution and that any aragonite or vaterite which formed would be transformed gradually to calcite. However, not only do the other polymorphs sometimes form initially, but transformation of aragonite to calcite is often very slow, giving aragonite significant kinetic stability (metastability). Aragonite is the high temperature-high pressure polymorph of calcium carbonate (Figure 1.3), and dry aragonite is always metastable below approximately 300°C. At standard temperature and pressure it will only convert to calcite by dissolution and recrystallization (Curl, 1962). Even at higher temperatures and pressures, transformation of calcite to aragonite may be extremely slow, depending on the environment and the particular stress placed on the mineral (Snow and Yund, 1987). The apparent stability of aragonite can be sufficiently high that aragonitic shells of Pennsylvanian age (280–320 million years old) are known (Brand, 1989).

Calcite is thermodynamically more stable than aragonite at pressures up to approximately 3 kbar at ambient temperature (Figure 1.3), and the thermodynamic stability of aragonite decreases with increasing temperature (Lippmann, 1982). Therefore greater thermodynamic stability cannot explain preferential deposition of aragonite in solution.

Calcium carbonate can be precipitated from solutions of soluble Ca^{2+} and CO_{3}^{2-} salts, such as CaCl_{2} and Na_{2}CO_{3},

\[
\text{CaCl}_{2} + \text{Na}_{2}\text{CO}_{3} \rightarrow \text{CaCO}_{3(s)} + 2\text{NaCl}
\]  

(1.1)

or from supersaturated Ca(HCO_{3})_{2} solutions, by loss of CO_{2},

\[
2\text{HCO}_{3}^{-} \rightleftharpoons \text{CO}_{2(\text{aq})} + \text{CO}_{3}^{2-} + \text{H}_{2}\text{O}
\]  

(1.2)

and

\[
\text{CO}_{3}^{2-} + \text{Ca}^{2+} \rightleftharpoons \text{CaCO}_{3(s)}
\]  

(1.3)

Whether calcite or aragonite is deposited from either of these systems will depend on other properties of the solutions. In the laboratory, aragonite precipitates from some highly-supersaturated solutions (Sabbides and Koutsoukos, 1993), from some solutions at high temperature, and from solutions containing minor amounts of some metal ions (Section 2.4). Aragonite is deposited in some limestone caves (Murray, 1975), in the
1.2. The Nature of Calcium Carbonate

Figure 1.3: P-T stability diagram of the calcium carbonate minerals, showing the pressure and temperature at which each polymorph is thermodynamically stable. I–V are polymorphs of calcite, with I–III stable at high pressure and low temperature. From Dickson (1990).

shells of many molluscs, in some coral and in some algae (Lowenstam, 1954; Lewis and McConchie, 1994). Much research has focused on the nature of these systems and on the deposition of calcium carbonate within them.

It was observed by Curl (1962) and Lippmann (1982) that for aragonite precipitation to occur the solution must be supersaturated with respect to aragonite (and therefore more highly supersaturated with respect to calcite, as calcite is less soluble). The precipitation of metastable aragonite once high supersaturation levels have been reached has been explained by Nancollas (1983) as a consequence of the Ostwald-Lussac Law of Stages, which states that if supersaturation is high enough for several possible polymorphs to form, the one which is most soluble will crystallize first. This is the opposite conclusion to that obtained intuitively, and is explained by noting the higher hydration energy of the most soluble polymorph. While its lattice energy will be smaller than that of the other polymorph/s, it will form a more hydrated, more stabilized crystal surface, losing less of the hydration energy of the free ion, and therefore will be more energetically favourable. Busenburg and Plummer (1986) concluded that the absence of aragonite in freshwater systems could be explained simply by calcite's substantially faster growth rate. Lippmann (1982) also observed that calcite nucleation and/or growth must be in some way inhibited for a Ca(HCO₃)₂ solution to become supersaturated with respect to aragonite. Factors that significantly affect the polymorphic ratio will be those which significantly affect the amount of calcite crystallizing from solution, either stabilizing or destabilizing calcite relative to aragonite.

It has also been shown that small changes to the experimental conditions of precip-
itation can have a large effect on the polymorphic ratio. For example, Bills (1985) found that after an unstirred solution of calcium bicarbonate had stood for 20 hours a significant amount of aragonite had formed, whereas from a stirred solution, otherwise identical, only calcite formed. Interestingly, Reams (1974), also studying calcium bicarbonate solutions, found that more aragonite was formed from solutions which had been stirred. He proposed that rapid loss of CO₂ increased the level of supersaturation, which promoted aragonite formation. It is necessary for each individual researcher to standardize his or her experimental conditions as far as possible, and there would be merit in an international standardization to facilitate the comparison of results. It seems likely that most of the contradictory reports in the literature are due to variation in experimental systems.

1.3 Calcium Carbonate Growth

To understand the effect of impurity ions on the surface of CaCO₃, or on its polymorphism, it is necessary to consider the effect they have on the nucleation and growth of the crystals. Although a significant amount of work has been done on the crystallization of calcite and aragonite, growth mechanisms are not yet adequately understood. Less work has been done on mechanisms of nucleation, which are more complex, and difficult to examine.

1.3.1 Nucleation

In a pure solution, crystal growth begins with the aggregation of ions into "nuclei", on which the crystal can grow. This process, nucleation, involves first the collision of several hydrated ions, which lose their water molecules of hydration to form a cluster. Clusters can either break apart again or grow, and growing clusters eventually reach a critical size, at which the probability of growth equals the probability of disintegration (Driessens and Verbeeck, 1990c). Once critical nuclei have formed, the process of crystal growth is considered to have begun. The size of the critical nucleus decreases with increasing supersaturation of the solution. Nucleation is faster in more supersaturated solutions, as an increase in solution concentration (of at least one component of the crystal, not necessarily both) moves the system further from equilibrium. Several authors (Simkiss and Wilbur, 1989; Li et al., 1993) state that a certain level of supersaturation must be reached before the nucleation rate becomes high enough for crystals to form, resulting in a "metastable region" of supersaturation. The existence of this metastable region is refuted by Driessens and Verbeeck (1990c), who argue from fundamental nucleation theory that crystals will form from any pure supersaturated solution, given enough time.

Curl (1962) comments on the large number of growth sites in a nucleus, per unit mass, and observes that as they are very disordered they are very susceptible to the presence of impurities. Thus nucleation may readily occur on dust particles, as well as by epitaxy, growth on any suitable particles present. McCauley and Roy (1974) note
1.3. Calcium Carbonate Growth

that epitaxy requires at least two-dimensional structural similarity between an impurity and the crystal precipitating. They consider that in a natural water containing \( \text{Ca}^{2+}, \text{Mg}^{2+} \) and \( \text{CO}_3^{-2} \) such particulate impurities are likely to be available. Isomorphism is not necessary. In \( \text{Mg} \)-containing water, for example, McCauley and Roy propose \( \text{MgCO}_3 \cdot 3\text{H}_2\text{O} \), which occurs readily, as the nucleus for epitaxial precipitation of \( \text{CaCO}_3 \). This is in response to other authors (e.g. Curl, 1962) who have stated that \( \text{Mg}^{2+} \) could not epitaxially promote aragonite, because \( \text{MgCO}_3 \) is not isomorphous with aragonite.

The induction time, the time taken for a critical nucleus to form, is influenced by any factors that alter collision rate and effectiveness, such as temperature, pressure and level of supersaturation. In a solution supersaturated with respect to both aragonite and calcite, it is likely that the form which will dominate is that which nucleates first. Epitaxial nucleation of the less stable polymorph can occur, or nucleation of the more stable polymorph may be prevented. Lippmann (1982) proposes a mechanism for \( \text{CaCO}_3 \) nucleation in which aragonite is stabilized by increasing temperature, as formation of the aragonite precursor has a less negative entropy change. At ambient temperature and pressure, he also observed that spontaneous nucleation is more difficult for aragonite than calcite. As only aragonite grows from aragonite-seeded solutions, even with a small mass of seeds, growth of aragonite must occur readily at supersaturations low enough to prevent rapid calcite nucleation.

Pytkowicz (1964) examined the induction time for \( \text{CaCO}_3 \) nucleation in natural and artificial sea-water and \( \text{Mg} \)-free artificial sea-water. Generally, the nucleation rate increased with increasing added carbonate, but for \( \text{Mg} \)-containing water there was a minimum time before nucleation occurred (approximately 3 hours). Unexpectedly, adding more carbonate than needed to cause minimum nucleation time slowed nucleation. This minimum was missing for \( \text{Mg} \)-free water and nucleation occurred much more quickly. From these results, Berner (1975) assumes that \( \text{Mg}^{2+} \) retards aragonite nucleation, which would contradict the results of McCauley and Roy (1974). However it is worth noting that Pytkowicz does not give the polymorphic ratios formed from his solutions, saying only that “mostly aragonites” were formed. It would be possible that calcite was precipitated rapidly from the \( \text{Mg} \)-free water, as the system was at ambient temperature, and aragonite from the water containing \( \text{Mg}^{2+} \). The results of Reddy and Nancollas (1971) suggest that calcite is expected to precipitate immediately upon solution mixing, as long as supersaturation is high enough. Pytkowicz also notes that in sea-water there is considerably more free \( \text{Mg}^{2+} \) than \( \text{Ca}^{2+} \), and that \( \text{MgCO}_3 \cdot \text{CaCO}_3 \) or \( \text{MgCO}_3 \cdot \text{MgCO}_3 \) pairs are more likely than the \( \text{CaCO}_3 \cdot \text{CaCO}_3 \) pairs needed for non-epitaxial \( \text{CaCO}_3 \) nucleation. Formation of these \( \text{Mg} \)-containing clusters is the mechanism he proposes for \( \text{Mg}^{2+} \) inhibition of \( \text{CaCO}_3 \) precipitation, although he does not discuss the question of whether calcite would epitaxially grow on the \( \text{MgCO}_3 \) clusters. Magnesium is readily incorporated into the calcite structure, and high-Mg calcite, defined as containing more than 4 % \( \text{MgCO}_3 \), is precipitated by many organisms (Lewis and McConchie, 1994).

Other authors have proposed \( \text{CaCO}_3 \)-type precursors for pure calcite or aragonite. Kitano and Hood (1962) suggested that “a gelatinous unstable compound” was initially formed which later tranformed to the observed polymorph, but he did not suggest a formula for this compound. Lippmann (1982)’s temperature stabilization mechanism
involves calcite- or aragonite-like precursors, and Nancollas (1983) states that such precursor particles are homogeneously nucleated and consist of amorphous hydrates. These particles slowly dissolve and reform on crystals. A number of authors have found that vaterite forms initially, but is rapidly converted to calcite (Sabbides and Koutsoukos, 1993). Further evidence of the ease of epitaxial growth is given by Sabbides and Koutsoukos (1993), who seeded artificial seawater with calcite, aragonite or vaterite crystals. In all cases, aragonite was the only phase to grow, and they proposed that the solution composition (in this case, the presence of Mg$^{2+}$) was the deciding factor in polymorph formation, rather than the available surface for growth.

1.3.2 Crystal Growth

Once nucleation has occurred, or a seed crystal has been added to the solution, growth continues smoothly in layers. This is shown by the regular faces and geometric shapes of crystals: random growth would result in a random shape. New layers grow in one of two ways: either each layer is spontaneously nucleated from the bulk solution, which will only occur in highly supersaturated solutions (Nancollas, 1983), or imperfections in the crystal surface act as growth "steps", with new ions adsorbed (see Section 2.1.2) to the surface at these points only (MacInnis and Brantley, 1992; Driessens and Verbeeck, 1990c). Imperfections may be point defects, such as kinks, or line defects, such as a screw dislocation (Figure 1.4). They may be caused by impurity ions or, as a feature of a pure CaCO$_3$ crystal, as a result of a change in experimental conditions. Imperfections offering a higher contact area with the surface will be energetically favourable, so addition at a kink site stabilizes the added growth unit more than addition at a step. Grigor’ev (1965) (in Simkiss and Wilbur (1989)) reports the order of relative energies of different sites on the surface of a cubic mineral, such as halite, as being kinks (0.8738), edges of steps (0.4941), crystal corners (0.2490), steps (0.1806), crystal edges (0.0903) and surface sites (0.0662). Once adsorbed onto the surface, an ion or molecule may migrate towards a more favourable growth site (Gutjahr et al., 1996), and be incorporated at that point into the three-dimensional crystal lattice. Growth therefore involves the two steps: adsorption and incorporation. The rate of calcium carbonate growth is surface-controlled, by one of these processes, or others such as dehydration, rather than controlled by diffusion of ions in the bulk solution (Nancollas and Zawacki, 1984; Gutjahr et al., 1996).

In 1992 Hillner et al. observed calcite growth and dissolution by Atomic Force Microscopy (AFM), in the first real-time study of growth of a mineral (Hillner et al., 1992a,b). They found that calcite growth occurred by monomolecular steps advancing at steady speed across the surface. Step velocities varied with orientation (averaging 0.9 nm/s), which tended to straighten the steps, and the interior of pits grew more quickly than the surrounding flat plateaus, tending to smooth the growing surface. Steps were unable to cross cracks in the surface, a finding which supported the conclusion of Snow and Yund (1987) that calcite and aragonite growth did not cross grain boundaries. At the high pH and low supersaturation of these experiments, Hillner et al. did not see direct evidence for screw dislocations, such as spiral growth (see Figure 1.5). However, they were unable to determine the source of the growth steps, which may have been caused by screw dislocations. Step density was low, which is consistent with
Figure 1.4: An idealised structure of the surface of a growing crystal, showing kinks (a), the edge of a step (b), a corner of the crystal (c), steps (d), an edge of the crystal (e), surface nucleation sites (f), and a screw dislocation (g). Individual cubes show growth units attaching at each different site. Based on Driessens and Verbeeck (1990c) and Simkiss and Wilbur (1989).
a screw dislocation mechanism (Nancollas, 1975). In a later article (Gratz and Hillner, 1993), growth of calcite was observed to be proceeding from a few spiral centres.

In conclusion, the rate of crystal growth is likely to be dependent on the availability and ease of formation of surface flaws and dislocations. Excepting at very high supersaturations, growth will not occur on a smooth surface. In a similar manner, dissolution occurs through loss of material at defects (MacInnis and Brantley, 1992). If an impurity ion could block these defect sites, it could hinder the growth or dissolution of a polymorph significantly. This model is also consistent with the possibility of an impurity ion favouring the growth of a polymorph, by formation of lattice defects, especially in low concentrations.

1.4 The Solid–Solution Interface

The interaction of dissolved metal ions with CaCO₃ is, at least initially, interaction of the ions in solution with the solid surface of the CaCO₃. Most of the investigations into the solid–solution interface, and the interactions of dissolved species with surfaces, have been concerned with various oxide or hydroxide minerals. Many of the models and findings are also applicable to calcium carbonate surfaces, so this section will first summarize oxide and hydroxide minerals, before considering calcite and aragonite.

1.4.1 Oxide Surfaces

The surface of a mineral can be modelled chemically, by consideration of surface groups, and electrically, by consideration of charges at and near the interface. When wet, or in a humid environment, oxide surfaces act as Lewis acids and coordinate water. The water molecules then dissociate, forming surface hydroxyl groups S–OH, where S represents the surface (Figure 1.6). These surface hydroxyl groups are amphoteric, undergoing both acid and base reactions:

\[
S\text{-OH} + H^+ \rightleftharpoons S\text{-OH}_2^+ \quad (1.4)
\]

\[
S\text{-OH} \rightleftharpoons S\text{-O}^- + H^+ \quad (1.5)
\]

It should not be assumed that all surface atoms have single S–OH bonds. There is evidence that other possibilities also exist, including

Using reactions such as Equations 1.4 and 1.5, surface acid dissociation constants can be defined (Schindler and Stumm, 1987):

\[
K_{a1}^s = \frac{[S\text{-OH}][H^+]}{[S\text{-OH}_2^+]} \quad (1.6)
\]
1.4. The Solid-Solution Interface

Figure 1.5: (a) The surface of growing calcite, showing sharp steps, spiralling to form a pyramid (Gratz and Hillner, 1993). Each side of the image is $3 \mu m$ in length. (b) Plan and (c) side views of the expected surface of a crystal which has grown by a spiral mechanism from a single dislocation in the centre of the crystal face (Simkiss and Wilbur, 1989).
Figure 1.6: A pictorial depiction of the formation of surface hydroxyl groups. In (a), the wet surface of an oxide mineral is shown with coordinated water molecules. In (b), dissociative chemisorption has lead to the formation of S–OH groups. Small solid circles represent the cations of the metal oxide, and large open circles oxygen atoms. From Schindler and Stumm (1987).

\[
K_{a2}^s = \frac{\{S-O\}^+[H^+]}{\{S-OH\}}\exp\left(-\frac{zF\Psi_{\text{surface}}}{RT}\right)
\]

where \( \{ \} \) denotes activities of surface species, the subscript \( a \) indicates these constants are acidity constants, and the superscript \( s \) indicates they are surface constants.

The values of the surface acidity constants depend on the surface charge, and decrease as the degree of dissociation increases.

1.4.2 Electrostatic Models

As a charged particle approaches the amphoteric surface, it will interact not only with a single S–OH group, but will be affected by surface charge and other complexed species. The sorption energy can be conceptually separated into an “intrinsic” chemical interaction and an electrostatic term. The intrinsic term is independent of surface charge. The electrostatic term is variable, and dependent on surface potential \( \Psi \) (Dzombak and Morel, 1990). The surface acidity constants can be rewritten as (Drever, 1997a):

\[
K_{a1}^s = \frac{\{S-OH\}^+[H^+]_{\text{bulk}}}{\{S-OH^+\}}\exp\left(-\frac{zF\Psi_{\text{surface}}}{RT}\right)
\]

and

\[
K_{a2}^s = \frac{\{S-O\}^+[H^+]_{\text{bulk}}}{\{S-OH\}}\exp\left(-\frac{zF\Psi_{\text{surface}}}{RT}\right)
\]
1.4. The Solid-Solution Interface

\[ \Omega \equiv \sigma_0 - \sigma_d \]

\[ \psi(x) \]

\[ \psi_0 \]

\[ \psi_d \]

distance, \( x \)

Diffuse-layer model

Figure 1.7: Three different models for the solid-solution interface of an oxide mineral. \( \psi \) is the potential at a given charge \( \sigma \), with the subscript \( 0 \) representing the surface. From Drever (1997a).

where \( \{ \} \) denotes activities of surface species,
the subscript \( a \) indicates these constants are acidity constants,
the superscript \( s \) indicates they are surface constants,
z is the charge on the ion,
\( F \) is Faraday’s constant,
\( \psi_{(surface)} \) is the surface potential,
\( R \) is the ideal gas constant, and
\( T \) is the temperature in Kelvin.

The surface potential (\( \sigma \)) can be described as a function of the surface charge:

\[ \sigma = f(\psi) \tag{1.10} \]

A number of models for this function \( f \) occur, ranging from the mathematically straightforward “constant capacitance” model to the complex “triple layer” model. These two models, plus the commonly-used diffuse double-layer model, are shown diagrammatically in Figure 1.7, and are briefly described below.

The Constant Capacitance Model

This model considers the surface-solution interface as a pair of parallel plates with a constant capacitance between them. One plate consists of the surface, adsorbed ions,
and protonated and deprotonated sites. The other plate is the bulk solution, at a distance \( x \) from the surface, where \( \Psi_{\text{(surface)}} \) is zero.

The capacitance is (Westall, 1987):

\[
C = \frac{\varepsilon \varepsilon_0}{d}
\]

(1.11)

where \( \varepsilon \) is the dielectric constant of water,
\( \varepsilon_0 \) is the permittivity of free space, and
\( d \) is the distance between the two planes.

and

\[
\sigma_{\text{surface}} = C\Psi_{\text{surface}}
\]

(1.12)

This model can be considered physically reasonable when it is describing solutions with a high electrolyte concentration.

The Diffuse Double-Layer Model

In this model, the relationship between surface charge and potential is described in terms of ionic strength \( I \), rather than capacitance. The solid-solution interface is considered as two layers, one the surface layer containing all adsorbed ions, and the other a diffuse layer of countercations (Dzombak and Morel, 1990). The diffuse layer will have a Gouy-Chapman distribution of ions, giving (Morel and Hering, 1993; Goldberg, 1995):

\[
\sigma = (8RT\varepsilon_0 I)^{1/2} sinh\left(\frac{zF\Psi_{\text{surface}}}{RT}\right)
\]

(1.13)

For small potentials at 25°C this relationship reduces to

\[
\sigma = 2.3I^{1/2}\Psi_{\text{surface}}
\]

(1.14)

This model can be considered physically reasonable for solutions of low ionic strength, when the concentration of adsorbing ion is also low.

The Triple-Layer Model

Both the constant-capacitance and the diffuse double-layer models only consider inner-sphere complexes, which are dehydrated and strongly adsorbed to the surface. The triple-layer model adds an additional layer between the surface and the diffuse layer; a layer of hydrated outer-sphere complexes. By altering the parameters of the diffuse layer and/or the layer of outer-sphere complexes, the model can be reduced to a constant-capacitance or diffuse double-layer model.
1.4. The Solid-Solution Interface

These models differ in the number of unknowns which must be calculated, for example, in the number of unknown potentials. Westall (1987) outlines different equations for calculating these unknowns, and emphasizes that care must be taken in interpreting the physical nature of the surface from them. All the models contain empirically-fitted parameters, and can be adjusted to adequately describe the experimental data.

1.4.3 Metal Adsorption onto Surfaces

Metal adsorption onto an oxide or hydroxide surface is generally described as coordination of the metal ions by proton displacement from the surface hydroxyl groups described above. In these reactions the amphoteric surface is acting as a Lewis base, such as (Westall, 1987):

\[
S-OH + M^{2+} \rightleftharpoons S-OM^{+} + H^{+} \tag{1.15}
\]

and

\[
S-OH + M^{2+} \rightleftharpoons S-O\overset{\mu}{-}M + 2H^{+} \tag{1.16}
\]

Anions may be complexed by ligand exchange, with the amphoteric surface acting as a Lewis acid (Schindler and Stumm, 1987; Westall, 1987):

\[
S-OH + L \rightleftharpoons S-L^{+} + OH^{-} \tag{1.17}
\]

or

\[
S-OH + ROH \rightleftharpoons S-OR + H_{2}O \tag{1.18}
\]

A metal complexed by such a surface group may still have properties of a Lewis acid, making coordination of other ligands possible. Examples of this coordination are:

1. Surface proton dissociation (Yasunaga and Ikeda, 1986)

\[
Al-OH + M(H_{2}O)_{n}^{2+} \rightleftharpoons Al-O-M(H_{2}O)_{n-1}^{+} + H_{3}O^{+} \tag{1.19}
\]

Loss of a proton from M restores the surface hydroxyl group:

\[
Al-O-M(H_{2}O)_{n}^{+} + H_{2}O \rightleftharpoons Al-O-M-OH(H_{2}O)_{n-2} + H_{3}O^{+} \tag{1.20}
\]

2. Ligand addition (Schindler and Stumm, 1987)

\[
S-OH + M^{z+} + IL \rightleftharpoons S-OML_{l}^{(z-1)+} + H^{+} \tag{1.21}
\]

Such complexes, with the metal bonded between the ligand and the surface, are referred to as type A ternary surface complexes. Polydentate ligands may also form type B ternary surface complexes, in which the ligand is inserted between the surface and the metal:

\[
S-OH + L + M^{z+} \rightleftharpoons S-LM^{(z+1)+} + OH^{-} \tag{1.22}
\]
Chapter 1. Introduction

Figure 1.8: The surface of a calcite crystal, imaged at atomic level by AFM by Hillner et al. (1992a). The image is 8x7 nm in size.

Examine "adsorption" of potential-determining ions onto various mineral surfaces, Yasunaga and Ikeda (1986) concluded that adsorption onto TiO₂, Fe₂O₃, Fe₃O₄ and α-zirconium phosphate was probably diffusion-controlled. On the other hand, rate constants for adsorption onto γ-zirconium phosphate and silica-alumina were an order of magnitude lower, indicating control by a chemical adsorption step.

As considerable research has been applied to oxide minerals, these models are reasonably well-developed. The next section will discuss the surface of calcium carbonate, making use of similar concepts, and Chapter 2 will examine metal adsorption on calcium carbonate in detail.

1.4.4 The Surface of Calcite and Aragonite

It is generally accepted that surface functional groups will occur on the surface of calcite, but the role that these groups play in structural incorporation of ions at growth sites is not clear (Paquette and Reeder, 1995). Therefore many authors consider it more valid to model ion incorporation as a function primarily of lattice ions on the surface. In this section the surface’s atomic structure will be discussed, as revealed by imaging, followed by consideration of work on possible surface complexes.

As well as observing growth and dissolution of calcite with AFM (Section 1.3.2), Hillner et al. (1992b) imaged the atomic structure of the calcite cleavage plane. A representative image is shown in Figure 1.8, probably of the 1014 surface, with clearly-visible vertical columns spaced 0.39 nm apart, intersecting (at 94±3°) less-obvious horizontal rows spaced 0.43 nm apart.

Paquette and Reeder (1995) also imaged 1014 growth faces of calcite (Figure 1.9(a)), finding they contained large-scale (several hundred micrometers) hillocks. Hillocks were
1.4. The Solid-Solution Interface

Figure 1.9: (a) A DIC (differential interference contrast) micrograph of the 10\(\bar{1}4\) face of a synthetic calcite crystal, showing a single hillock. The hillock summit is indicated by an arrow, and the symmetry of the vicinal faces by the letters a, a', b and b' as for (b). Macrosteps are visible on the top 2 vicinal faces. (b) The geometry of a growth hillock, with the c-axis at approximately 45° toward the top of the diagram. From Paquette and Reeder (1995).

also reported on the 01\(\bar{1}2\) faces of calcite by Paquette and Reeder (1995) and on its 10\(\bar{1}1\) faces by Higuchi et al. (1992). On the 10\(\bar{1}4\) surface, each hillock consisted of 3-4 vicinal faces (which deviated by only approximately 1° from a flat 10\(\bar{1}4\) face) rising in steps to a point a few micrometers above the overall surface. Figure 1.9(b) shows the geometry of one hillock, indicating that sides a and a' (and b and b') grow from parallel steps moving at different speeds, in opposite directions. Sides a and b are symmetrically equivalent, as are sides a' and b'.

The PBC (periodic bond chain) model, which considers the presence of chains of stoichiometrically linked strongest bonds, was used to explain the existence of the vicinal faces on the 10\(\bar{1}4\) faces. The 10\(\bar{1}4\) faces have two non-parallel 4\(\bar{4}1\) PBCs, with different local coordination at kink sites, leading to preferential incorporation of cations of different size at steps moving in different directions. Preferential incorporation of calcium ions explained the more rapid growth of the a and b vicinal faces in Figure 1.9. While they recognized the probable existence of surface complexes, the success of their coordination model at explaining the observed effects lead them to suggest that surface reconstruction at or near kink sites was minimal.

House (1981) studied a range of proposed rate equations for calcite growth and dissolution. For dissolution, he was most convinced by the "mechanistic model" of Plummer et al. (1978), which describes the rate as dependent on pH and stirring rate at low pH, dependent on pH and \(P_{\text{CO}_2}\) at moderate pH, and essentially independent of pH.
or P_{CO_2} at high pH (and low P_{CO_2}). This model describes the surface of the crystal as having reactive sites, and proposes an adjacent adsorption layer, in which ions are relatively immobile. Between this adsorption layer and the bulk of the solution is a hydrodynamic boundary layer, across which ion mobility gradually increases to its bulk value. Most of the rate models developed for calcite growth have assumed some such double layer at the solution-surface interface, similar in nature to the models of oxide surfaces.

A hydrated surface layer several monolayers thick was proposed by Davis et al. (1987) and Fuller and Davis (1987), but the LEED (low-energy electron diffraction) studies of Stipp et al. (1992) indicated that the surface of wet calcite was hydrated to a depth of no more than one monolayer, showing normal calcite lattice structure below this depth. Likewise, Comans and Middelburg (1987) and Zachara et al. (1991) found little evidence of a thick surface layer. As ions are incorporated into the lattice they must dehydrate, and the point or points at which dehydration occurs is unsure.

Sigg (1987) proposed hydrated surface hydroxyl and carboxylic groups as binding sites for metals onto algal surfaces. Possibilities included

\[ S - R - C\overset{\text{O}}{\text{O}} + M^{2+} \rightleftharpoons S - R - C\overset{\text{O}}{\text{O}}M^+ \]  \hspace{1cm} (1.23)

and

\[ S - R - C\overset{\text{O}}{\text{O}}H + M^{2+} \rightleftharpoons S - R - C\overset{\text{O}}{\text{O}} + 2H^+ \]  \hspace{1cm} (1.24)

Intuitively, it would seem reasonable that such groups could occur on CaCO_3 surfaces. Similarly, Stipp and Hochella Jr. (1991) examined calcite surfaces by XPS (X-ray photoelectron spectroscopy) after exposure to water, and found evidence of S-CaOH and S-CaCO_3H surface groups.

Carbonate and calcium ions determine the potential of the surface of calcite (and presumably aragonite), as they are the lattice ions. Zeta potentials are also dependant on pH, but D. W. Fuelstenau and Herrera-Urbina (1992), studying calcite potentials in sodium carbonate solutions, found that the surface remained positively charged at pH as high as 11. The isoelectric point reported for calcite has varied from 8.2 to 11.5, and would appear to depend on individual experimental conditions and mineral samples.

Atomistically, the surface of aragonite cannot be expected to have the same shape as that of calcite, but no images of the aragonite surface have been found in the literature. Surface complexes could be expected to be similar to those of calcite, although Lippmann (1982) suggested that precipitation of aragonite required loss of two waters of hydration from a Ca^{2+}_{(aq)}, and calcite only one, because to bond to aragonite, Ca^{2+} must bond to two O atoms from the same CO_3 group.
Part II

Sorption of Co$^{2+}$, Ni$^{2+}$, and Cu$^{2+}$ onto Calcite and Aragonite
Chapter 2

The Interaction of Transition Metals with Calcite and Aragonite

2.1 Introduction

2.1.1 Background

The interaction of trace metals and calcium carbonate has long been of interest to chemists and geologists. Initially interest arose from discussion in the early 1800's (see Lippmann, 1982) as to whether strontium, dissolved in the solution calcium carbonate precipitates from, would affect the polymorphic ratio of the precipitate, providing a possible explanation for the strangely high quantity of aragonite in marine organisms.

While Rose (1837) concluded strontium was not a major factor in the production of metastable aragonite (but see Section 2.4), research rapidly focused on magnesium. The magnesium ion also offered the advantage of being present at relatively high concentrations in seawater (1270 ppm (Fergusson, 1982)) and so was readily measured and manipulated by early technology.

As the effects of alkaline earth elements were clearly determined, interest spread to other metals and to the mechanism of the observed effects. From the mid-1960's an increasing number of studies were made on transition metals, including the effect of their co-precipitation with calcium carbonate (Tsusue and Holland, 1966), their natural occurrence in carbonate rocks and modern carbonate systems (Harriss, 1965) and their adsorption onto the surface of calcite and aragonite (Kitano et al., 1976).

New techniques for examining surface and near-surface processes and a new appreciation of the complexity of these systems (Angus et al., 1979) have caused a rush of practical and theoretical studies in the 1980's and '90's. A number of models have been proposed and analyses for trace metals have been applied to a wide range of geochemical problems, from the timing of cementation of a carbonate (Nicolaides and Wallace, 1997) to tracing the distribution of metals in different sediment size fractions (Šurija and Branica, 1995). The scope of the topic has also widened. Two recently-studied areas have been metal pollution, which has concerned biologists (e.g. Hanna and Muir,
1990) and chemists (e.g., van der Weijden et al. (1994) studied Cd$^{2+}$ with a view to determining the speciation of anthropogenic cadmium), and materials science, including the work of Walsh and Mann (1995), who grew micro-metre scale hollow porous shells of aragonite from solutions of Ca(HCO$_3$)$_2$ and MgCl$_2$.

Transition metals have partially-filled d-orbitals in at least one of their oxidation states. In their interaction with calcium carbonate, it appears that the oxidation state and the ionic radius are the two most important characteristics of the transition metal, characteristics which are a consequence of the electronic structure, but which are not necessarily restricted to transition metals. The question of which metals to examine in a study is therefore dependent on the aims of the study, with some authors focusing on a single element (Barber et al., 1975), some on the most common elements (Erel and Katz, 1990), some on metals of concern in pollution (Fuller and Davis, 1987) and some on those metals which can readily be detected by a certain technique (Paquette and Reeder, 1995). A number of studies report the detailed composition of a carbonate or carbonate sediment as part of a description of it (Shaw and Bush, 1978).

The work described in this thesis has been primarily concerned with the Groups 9–11 metals cobalt, nickel and copper. However, as results from studies of other elements also need to be considered to gain an understanding of the phenomena involved, this chapter will focus on the interaction of the transition metals and zinc, elements in which electrons in d-orbitals occupy t$_{2g}$ levels, with both calcite and aragonite.

### 2.1.2 Modes of Trace Element Incorporation

Experimental studies generally fall into one of two broad categories: coprecipitation or adsorption. Coprecipitation experiments begin with a solution containing the ions of interest which is supersaturated with respect to calcium carbonate. Calcite or aragonite precipitate, with or without the help of seed crystals. Such methods have been the most common approach for studying growth rates of calcite and factors on which it depends, and for considering which polymorph(s) will be precipitated. Paquette and Reeder (1995) is one of a number of recent studies to use this approach.

The interaction of transition metals with the surface and near-surface regions of calcite and (less-commonly) aragonite has more often been studied by "adsorption" experiments. Trace ions can be incorporated into a growing crystal by surface adsorption (Zachara et al., 1988), by separation into occlusions, or by solid-solution formation (McIntire, 1963; Böttcher and Gehlken, 1997). Precipitates of transition metal carbonate or of other mineral phases can also form on the surface (Pingitore, Jr. et al., 1988; Angus et al., 1979). Adsorption is defined as "the accumulation of matter at the interface between the aqueous solution phase and a solid adsorbent without the development of a three-dimensional molecular arrangement" (Sposito, 1986). Adsorbing ions may be bonded to surface groups, such as those discussed in Section 1.4.4, may exchange with another ion on the surface, or may be attracted by the surface charge, without chemical reaction. Occlusions contain material which was trapped as the crystal grew, and may contain trace elements, a separate mineral phase, or the parent liquid. Solid solution is the formation of a mixed cation phase, most commonly with the trace cation substituting for a host cation at a lattice site. Distortion of the
lattice may or may not occur, depending on the charge/radius difference between the trace and host cations and the percentage substitution. Distinguishing these modes of incorporation experimentally is difficult, and the term "sorption" is frequently used to cover them all.

2.1.3 Partition Coefficients

Sorption has traditionally been described by distribution (or partition) coefficients, a relationship proposed by Berthelot in 1872 and developed mathematically by Nernst in 1891 (McIntire, 1963). Using a system containing calcium carbonate and a single divalent metal cation $M^{2+}$ as an example, for the reaction;

$$M^{2+} + CaCO_3 \rightleftharpoons Ca^{2+} + MCO_3, \quad (2.1)$$

the Berthelot-Nernst equation simply states that the ratio of $M_S$, the concentration of the trace metal in the the host solid, to $M_{aq}$, the concentration of the trace metal in the solution, is a constant:

$$k = \frac{M_S}{M_{aq}} \quad (2.2)$$

The form of the partition coefficient most commonly used now (D) was proposed by Henderson and Kracek in 1927, in which (for the $CaCO_3$ system)

$$D = \frac{(M/Ca)_s}{(M/Ca)_{aq}} \quad (2.3)$$

where $s =$ solid phase,

aq = aqueous phase,

$M$ is the concentration of the metal ion, and

$Ca$ is the concentration of the calcium ion.

This equation is equivalent to

$$D = \frac{M_S/M_{aq}}{Ca_s/Ca_{aq}} \quad (2.4)$$

and is related to $k$ by

$$D = k \frac{g/ml \ of \ Ca_{aq}}{g/ml \ of \ Ca_s} \quad (2.5)$$

As distribution coefficients are developed from equilibrium relationships, the use of concentrations in these ratios is an approximation for activities. Molarity in the solution phase and mole fraction in the solid are reasonable approximations of activities only if the system adequately approximates to the ideal state. In solution the ideal state can either be when all solutes are infinitely dilute (i.e. pure water for the $H_2O-CO_2$ system), which is useful for considering fresh water systems, or when the solute of interest is infinitely dilute in a solvent of given and unchanging ionic strength, which is useful for considering seawater systems (Stumm and Morgan, 1996). In the solid phase the ideal state is normally the pure compound, either $CaCO_3$ or $MCO_3$. A few systems, in which host and trace elements have very similar ionic radii and electronegativities,
and therefore very similar activity coefficients in solution, form ideal solid solutions, and distribution coefficients are independent of concentration. Otherwise, k or D will be dependent on trace element concentration and solution ionic strength, making comparison of constants determined at different concentrations impossible. Dromgoole and Walter (1990) examined three published reports on the partition coefficient of Mn$^{2+}$ in calcite (Mucci, 1988; Lorens, 1981; Pingitore, Jr. et al., 1988) and concluded that $D_{Mn^{2+}}$ was independent of the concentration of Mn$^{2+}$ over the range studied.

If the ratio of the activities of the trace and host ions in solution changes during crystallization, the logarithmic distribution law of Doerner and Hoskins (1925) is a more appropriate description. This law assumes that only the surface layer, rather than the bulk, of the growing crystal is in equilibrium with the solution it grows from (Reddy and Nancollas, 1971). Doerner and Hoskins (McIntire, 1963) showed that at a given stage of crystallization

$$\lambda = \frac{\ln(N_{M^+}/N_{M^+})}{\ln(N_{Ca^2+}/N_{Ca^2+})}$$

(2.6)

where $\lambda$ is the logarithmic partition coefficient, $N_{M^+}$ and $N_{Ca^2+}$ are the initial M and Ca concentrations in the solution and $N_{M^+}$ and $N_{Ca^2+}$ are the M and Ca concentrations in the solution at a given stage of crystallization.

A logarithmic partition coefficient was used by Pingitore, Jr. et al. (1988) to describe Mn$^{2+}$ adsorption onto calcite because they found that Mn$^{2+}$ concentration was depleted more than Ca$^{2+}$ concentration during crystallization. Most other authors have used a homogeneous partition coefficient, and most commonly D. Some experimentally-determined values of D and $\lambda$ are given in Table 2.1.

### Table 2.1: Partition coefficients of various transition metals with calcite.

Note that the range of values presented for Mn$^{2+}$ arises from variation of $D$ or $\lambda$ with precipitation rate (see section 2.2.2)

<table>
<thead>
<tr>
<th>constant used</th>
<th>ion</th>
<th>value of $D$ or $\lambda$</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>Fe$^{2+}$</td>
<td>1.9–3.7</td>
<td>Dromgoole and Walter (1990)</td>
</tr>
<tr>
<td>D</td>
<td>Mn$^{2+}$</td>
<td>3.8–16</td>
<td>Dromgoole and Walter (1990)</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>Mn$^{2+}$</td>
<td>1.33–18.8</td>
<td>Pingitore, Jr. et al. (1988)</td>
</tr>
<tr>
<td>D</td>
<td>Mn$^{2+}$</td>
<td>6–50</td>
<td>Lorens (1981)</td>
</tr>
<tr>
<td>D</td>
<td>Cd$^{2+}$</td>
<td>1240±300</td>
<td>Tesoriero and Pankow (1996)B</td>
</tr>
</tbody>
</table>

### 2.2 Solid Solutions

#### 2.2.1 Introduction

Recent studies have found that when seed crystals are exposed to a solution of metal ions, sorption occurs in two stages (van der Weijden et al., 1994; Fuller and Davis, 1987;
2.2. Solid Solutions

<table>
<thead>
<tr>
<th>Ion</th>
<th>Radius [pm]</th>
<th>CN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe$^{2+}$</td>
<td>61</td>
<td>6</td>
</tr>
<tr>
<td>Co$^{2+}$</td>
<td>65</td>
<td>6</td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>67</td>
<td>6</td>
</tr>
<tr>
<td>Ni$^{2+}$</td>
<td>69</td>
<td>6</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>72</td>
<td>6</td>
</tr>
<tr>
<td>Cr$^{2+}$</td>
<td>73</td>
<td>6</td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>73</td>
<td>6</td>
</tr>
<tr>
<td>Zn$^{2+}$</td>
<td>74</td>
<td>6</td>
</tr>
<tr>
<td>Cd$^{2+}$</td>
<td>95</td>
<td>6</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>Pb$^{2+}$</td>
<td>119</td>
<td>6</td>
</tr>
<tr>
<td>Sr$^{2+}$</td>
<td>126</td>
<td>8</td>
</tr>
<tr>
<td>Ba$^{2+}$</td>
<td>142</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 2.2: Ionic radii of a number of divalent metal ions. CN is the coordination number of the ion, and radius is in picometers. Cr$^{2+}$ and Cu$^{2+}$ structures are distorted. From Lide (1996).

Kornicker et al., 1985). Rapid uptake occurs for the first few hours, followed by slow, steady uptake over a period of weeks or months. It is generally agreed that the first step involves adsorption onto a surface layer, and the second stage involves incorporation of the adsorbed ions into the body of the growing crystal. Further evidence for the latter solid solution formation was found by Qian et al. (1994), who used X-ray standing waves to examine naturally-occurring manganese cations and artificially-sorbed lead cations within a crystal of Iceland spar calcite. All the manganese and two-thirds of the lead was found to be highly ordered within the 1014 planes of the crystal (shown in Figure 1.2), indicating substitution for Ca$^{2+}$. The incorporation of Pb$^{2+}$ ions into lattice positions in the calcite structure was unexpected, even replacing only about 5% of the surface calcium, as Pb$^{2+}$ is substantially larger than Ca$^{2+}$ (Table 2.2), and shows that the calcite lattice can accommodate a substantial amount of localized strain.

2.2.2 Kinetic Effects

Partition coefficients (D) have been shown to be strongly dependent on kinetic factors. Dromgoole and Walter (1990) showed that homogeneous distribution coefficients for Fe$^{2+}$ and Mn$^{2+}$ incorporation into calcite increased as precipitation rate decreased, i.e. more of the trace metal ions were incorporated into the slowly-grown crystals. Pingitore, Jr. et al. (1988) discovered a very similar trend in partition coefficient rate dependence for Mn$^{2+}$ (see Table 2.1). The rate dependence observed is not explained by standard models of solid-solution formation (McIntire, 1963), but electron spin resonance (ESR) found no evidence for occlusions or surface precipitation, indicating instead that Mn$^{2+}$ ions were substituting for Ca$^{2+}$ in trigonal lattice sites, forming a solid solution. In a short article, Pagonis et al. (1997) found evidence of manganese moving "in and out of clusters" during heating of calcite, which suggests
a more complex positioning than an ideal random solid solution. Dependence of partitioning on precipitation rate of the calcite was confirmed by Tesoriero and Pankow (1996) for Sr\(^{2+}\), Ba\(^{2+}\) and Cd\(^{2+}\). In 1994, van der Weijden et al. studied both sorption and desorption of Cd\(^{2+}\) onto calcite, discovering a dependence on time, aqueous free Cd\(^{2+}\) activity, and pH. More Cd\(^{2+}\) was adsorbed at pH 8.3 than at pH 7.9, but irreversibility of sorption after one hour was greater at the lower pH. They considered it unlikely that the irreversibility was explained by an increase in solid solution formation, which would require a greater migration of ions into the crystal while fewer were becoming available on the surface, and instead suggested that at lower pH Ca\(^{2+}\) ions were more competitive so Cd\(^{2+}\) ions only adsorbed onto particularly favourable sites (such as kinks and steps). As time progressed, small calcite crystals dissolved and recrystallized on the surface of larger crystals, burying the Cd\(^{2+}\) ions. Burial both prevented these Cd\(^{2+}\) ions being removed by exchange with Ca\(^{2+}\) and provided a fresh calcium carbonate surface on which more Cd\(^{2+}\) could be sorbed.

The Ca/Cd exchange reaction was found to be ideal, but during the first 48 hours the experimental exchange constant was approximately twice the expected exchange constant \(k_{\text{ex}}\):

\[
k_{\text{ex}} = \frac{K_{\text{SP(calcite)}}}{K_{\text{SP(otavite)}}}
\]

where \(K_{\text{SP(calcite)}}\) is the solubility product of calcite, \(K_{\text{SP(otavite)}}\) is the solubility product of otavite, and otavite is CdCO\(_3\).

This discrepancy indicates either that a solid solution formed but was not in equilibrium with the growth solution, or that another process, possibly proton replacement, was the major process during this early stage of sorption.

Vein calcites (which have grown in secondary fissures in a rock) often exhibit zoning, in which the concentration of trace metals in individual calcite crystals varies along the crystal radius, giving a banded effect. Concentrations can increase or decrease steadily, or can oscillate. Zoning is generally explained by variations in fluid compositions (and the presence of zoning is used to draw conclusions about fluid composition), but Möller et al. (1991) have proposed a changing flow rate as another possibility. Because veins grow in flowing water, material (both trace and major ions) is carried to the crystallization site, making growth rate dependent on flow conditions. Möller et al. (1991) argued mathematically that for elements \((\lambda > 1)\) which became enriched in the solid \((\lambda > 1)\) the M/Ca ratio decreased as the solution flowed further into the vein system at low flow rates and increased at high flow rates, whereas for elements which became depleted in the solid \((\lambda < 1)\) the M/Ca ratio always decreased as the solution flowed further into the vein. By knowing which elements are enriched and which are depleted in calcite (see Table 2.1), and measuring M/Ca ratios for a number of elements, relative flow speed can be deduced. The veins on which Möller et al. (1991) tested their model demonstrated that as much as a threefold variation in M/Ca ratio could be due to changes in flow rate. Caution is obviously needed in assessing the cause of trace element zoning, and in drawing conclusions from it.

Möller et al. (1991) also noted an unexpectedly low Zn\(^{2+}\) concentration in the calcite, which they suggested could be explained by the very high Cl\(^-\) concentration of
5 mol L\(^{-1}\) in this particular hydrothermal fluid. Formation of \(\text{ZnCl}^{(x-2)-}\) complexes in such a fluid would result in a very low free \(\text{Zn}^{2+}\) concentration in solution.

Reddy (1976) observed that ion-pair formation, e.g. of \([\text{CaCO}_3]_{(aq)}\), can reduce the concentrations of free \(\text{CO}_3^{2-}\) and \(\text{HCO}_3^-\) in solution, which will reduce supersaturation levels and the rate of calcite growth.

Rapid adsorption of zinc on calcite was observed by Zachara \textit{et al.} (1988). Surprisingly, they found adsorption to be independent of pH and the concentration of \(\text{CO}_2(g)\), except in so far as both factors influence \(\text{Ca}^{2+}\) activity and \(\text{Zn}^{2+}\) complexation in solution. Adsorption seemed to be a process of exchange of \(\text{Zn}^{2+}\) and \(\text{ZnOH}^+\) with surface \(\text{Ca}^{2+}\), with a lack of surface charge effects suggesting partially-covalent bonding. These results contradict several studies discussed above, where trace metal adsorption was strongly dependent on pH and surface charge. Similarly, Fuller and Davis (1987) found adsorption of \(\text{Cd}^{2+}\) to be dependent on pH in the presence of EDTA, because of changes to the Cd-EDTA speciation.

Data obtained by Kornicker \textit{et al.} (1985) for \(\text{Co}^{2+}\) was consistent with Zachara \textit{et al.}'s concepts of surface exchange, with a strong emphasis on site competition with \(\text{Ca}^{2+}\) in both cases and no indication of formation of a separate surface \(\text{CoCO}_3\) or \(\text{ZnCO}_3\) phase.

### 2.2.3 Models of Solid-Solution Formation

In 1987, Davis \textit{et al.} and Fuller and Davis modelled \((\text{Cd,Ca})\text{CO}_3\) solid solution formation as a three-step process with:

1. \(\text{adsorption of } \text{Cd}^{2+}\),
2. \(\text{diffusion of the adsorbed } \text{Cd}^{2+} \text{ to "additional sorption sites or into surface films of hydrated calcium carbonate"}
3. \(\text{solid solution formation on the recrystallizing calcite surface.}\)

As noted above (van der Weijden \textit{et al.}, 1994), recrystallization of the smallest micro-crystals would aid solid solution formation.

The model of Davis \textit{et al.} (1987) requires the formation of a thick (many monolayers) hydrated surface layer, the existence of which has found little support from other researchers. Stipp \textit{et al.} (1992) proposed a similar model for \((\text{Ca,Cd})\text{CO}_3\), but with only a thin hydrated surface layer. \(\text{CdCO}_3\) (otavite) was grown over the 101 surface of calcite, and the resulting \(\text{CdCO}_3\) layer was found to be hydrated to a depth of no more than one monolayer with \(-\text{OH}\) and \(-\text{CO}_3\text{H}\) species. When the crystal was stored in an ultrahigh vacuum, solid state diffusion of Ca into the Cd layer and Cd into the bulk crystal occurred. Zachara \textit{et al.} (1991) were also unable to confirm the existence of a thick surface layer. They found \(\text{M}^{2+}\) and \(\text{Ca}^{2+}\) exchange on cation-specific surface sites, with Cd and Mn dehydrating soon after adsorption and Zn, Co and Ni forming hydrated surface complexes which persisted until the cations were incorporated into the crystal. A wide range of metals was examined, and the degree of sorption was found to vary in the order: \(\text{Cd} > \text{Zn} \geq \text{Mn} > \text{Co} > \text{Ni} \gg \text{Ba} = \text{Sr}\).
Comans and Middelburg (1987) proposed a surface precipitation mechanism which seems not to require a thick surface layer, occurring as four separate reactions:

1. Adsorption of $M^{2+}$ on the $CaCO_3$ surface, transferring $CaCO_3$ from the monolayer to the solid:

$$CaCO_3_{(monolayer)} + M^{2+}_{(aq)} + HCO_3^-_{(aq)} \rightleftharpoons CaCO_3_{(solid)} + MCO_3_{(monolayer)} + H^+; \quad (2.8)$$

2. Precipitation of $M^{2+}$:

$$MCO_3_{(monolayer)} + M^{2+}_{(aq)} + HCO_3^-_{(aq)} \rightleftharpoons MCO_3_{(solid)} + MCO_3_{(monolayer)} + H^+; \quad (2.9)$$

3. Adsorption of $Ca^{2+}$ on the $MCO_3$ surface:

$$MCO_3_{(monolayer)} + Ca^{2+}_{(aq)} + HCO_3^-_{(aq)} \rightleftharpoons MCO_3_{(solid)} + CaCO_3_{(monolayer)} + H^+; \quad (2.10)$$

4. Precipitation of $Ca^{2+}$:

$$CaCO_3_{(monolayer)} + Ca^{2+}_{(aq)} + HCO_3^-_{(aq)} \rightleftharpoons CaCO_3_{(solid)} + CaCO_3_{(monolayer)} + H^+. \quad (2.11)$$

Each of these reactions involves the exchange of a divalent cation for a proton at the surface, which explains the pH dependence. Rather than considering adsorption and solid-solution formation as two separate phases, this model provides a continuum over time. However, it, and all similar models, requires that adsorption occur directly onto $MCO_3$ or $CaCO_3$ on the surface, rather than adsorption occurring via interaction of dissolved ions with the $-OH$ or $-CO_3H$ groups on the surface.

An alternative system has been proposed by Wang and Merino (1992) to explain oscillatory zoning in calcites. Oscillatory zoning is concentric zoning in which the concentration of trace elements oscillates along the crystal radius rather than gradually changing as the outside edge is approached. As mentioned, it is generally interpreted as being due to frequent sudden changes in solution composition, which is not always likely.

The mechanism proposed by Wang and Merino (1992) involves self-organizational feedback driven by $H^+$ buildup at the crystal surface, in a highly supersaturated solution. The isoelectric point of calcite is about 8.3. If the pH is lower than the isoelectric point at the surface interface, the surface is positively charged, and rejects cations, leading to zoning. As adsorption of certain cations inhibits calcite growth (see Section 2.4.2), calcite will grow more rapidly during this phase. As calcite grows rapidly more $H^+$ ions are produced at the surface by the reaction

$$Ca^{2+} + HCO_3^- \rightleftharpoons CaCO_3 + H^+ \quad (2.12)$$
2.3. Adsorption and Coprecipitation

Surface pH and the trace metal concentration is lowered still further. Eventually growth becomes fast enough that the supply of Ca$^{2+}$ ions is depleted, growth slows, the H$^+$ ions are able to diffuse away and surface pH increases to above the isoelectric point. More trace cations are adsorbed, slowing calcite growth and allowing H$^+$ to accumulate until pH again becomes lower than the isoelectric point and the cycle repeats. This process is shown diagrammatically in Figure 2.1. If the solution is not sufficiently supersaturated, Ca$^{2+}$ desorption occurs as well as Ca$^{2+}$ incorporation (ie. the reverse of the above reaction must be considered) and H$^+$ becomes involved in the rate law directly. Also at high supersaturation the affinity of Ca$^{2+}$ ions for the growing surface overcomes the electrostatic repulsion of the surface’s positive charge. As trace cations have less affinity for the surface than Ca$^{2+}$, trace ions are rejected when Ca$^{2+}$ ions are not.

Wang’s theory was tested by Wogelius et al. (1997) in vein calcite from the Mendip Hills, UK, by examining the oscillations of a number of different trace elements within the vein. Sr was noticeably different to the other trace elements. Pb and Zn co-varied strongly together, and co-varied with Mn and Fe less strongly. Periodicity of Sr was very different to that of the other ions. A consequence of Wang’s model is that all poisoning M$^{2+}$ should behave similarly, and in particular that they should all correlate highly at, or close to, the “starting position” where lag of each is zero. A negative correlation at lag of zero would be impossible, and in this example Sr$^{2+}$ is negatively correlated with the other cations at that point. Isotopic evidence suggested at least three different types of fluid were involved in this vein. Calcite at the vein centre was typical of marine fluids (high Sr and low Pb, Zn, Mn and Fe concentrations). Away from the vein centre, towards the oldest calcite, calcite was typical of meteoric or groundwater, and in nearby veins typical of hydrothermal water which pulsed in through fractures. Trace metals indicated that mixing of these waters was important, and that differences in water composition account for about 95% of the oscillatory zoning in this case. This example indicates the level of detail to which a particular rock can be studied.

Another form of zoning, intrasectoral zoning, was examined by Paquette and Reeder (1995). In intrasectoral zoning different concentrations of trace metals are seen within different regions of a single crystal face. In the case of calcite, differential incorporation of Mn$^{2+}$ and Sr$^{2+}$ occurred on different vicinal faces on the 1014 faces of crystals (Section 1.4.4), which could be explained by differences in coordination of calcium at kink sites on the surface. Sectors of the crystal associated with sides a’ and b’ (Figure 1.9) were enriched in Mn and depleted in Sr$^{2+}$ relative to sides a and b.

2.3 Adsorption and Coprecipitation

A number of authors have found evidence for coprecipitation of either $\text{CaCO}_3$ or another mineral phase on the calcite surface. In a second study on zinc, Zachara et al. (1989) found a precipitate which was probably hydrozincite or its hydrate formed from Zn-containing suspensions of calcite, once the Zn concentration exceeded the solubility of zinc carbonates. Similarly, Papadopulos and Rowell (1988) found that Cd$\text{CO}_3$ precipitated on the surface of calcite at Cd concentrations > 1 $\mu$mol/g. A layer of
CdCO₃ covering the surface prevented the surface CaCO₃ from equilibrating with the solution, as shown by a lowered solution pH. The solution would otherwise be buffered by the CaCO₃(surface)−CO₂−H₂O system.

Barber et al. (1975) precipitated calcium carbonate from a synthetic seawater of NaCl, CaCl₂ and Na₂CO₃, doped with Co²⁺. Depending on the Co²⁺ concentration, initial solution pH varied from 6.3 to 9.3. At all concentrations of Co²⁺ (from 0.0005 to 0.01 mol L⁻¹) an amorphous violet phase formed, probably basic cobaltous carbonate (2CoCO₃ · Co(OH)₂ · H₂O).

Angus et al. (1979) used electron spin resonance (ESR) to probe the environment of a large number of trace metals in calcite. They concluded that:

- Fe²⁺ had little effect on the ESR parameters, probably because it formed a solid solution;
- Ni²⁺ caused increasing lattice distortion and strain with increasing Ni²⁺ concentration. Much of the Ni²⁺ formed a solid solution, but they also found some evidence for nickel carbonate inclusions;
- Cu²⁺ caused notable changes to the ESR parameters even at low concentrations and probably formed a solid solution, distorting the lattice;
- Zn²⁺ caused aragonite to form. The authors suggested Zn could be causing sufficient structural perturbation to destabilize the calcite lattice;
- Cd²⁺ formed complex inclusions, and probably also solid solutions;
- Mn²⁺ (the probing ion) showed an increase in axial distortion of the host site with increasing concentration, and was incorporated into well-defined sites in the calcite lattice.
It can be seen from this list that the behaviour of a trace element in a particular system is very dependent on the nature of the element, in particular upon ion size and the stability of other carbonate phases in the system. Therefore it is necessary to assess each element individually. The application of these results to natural systems is also fraught with problems, because Mn\(^{2+}\) in the synthetic calcite they grew was in more-defined sites than in even very high quality natural samples. The reasons for this were unknown.

It has been suggested that manganese carbonate overgrowths on calcite tests (the shell or supporting skeleton of an organism) may form a major oceanic sink for manganese. To examine this, Mucci (1988) studied manganese overgrowths on calcite seeds in artificial seawater. A "pseudokutnahorite" was deposited, of formula approximating to Ca\(_{1.5}\)Mn\(_{3}\)(CO\(_3\))\(_2\), with between 5 and 10 mole % MgCO\(_3\) incorporated into the mineral, but without the dolomite-like ordering of kutnahorite. It is quite realistic that such compounds could immobilize a large proportion of the trace metals present in seawater. However, immobilization may only be in the short term, as the solubility of these unstable compounds is uncertain. Much less manganese would be expected to be deposited in the final stable sediment, and if this was deposited within the same time scale as the pseudokutnahorite, it would provide a source of manganese to counter the temporary manganese sink.

The effect of oxidation state after incorporation was examined by Komorsky-Lovrić et al. (1997), who precipitated calcite and aragonite from manganese-containing solutions. In aragonite, no Mn–Ca solid solution was observed, but instead MnO\(_2\) (which has Mn in the IV oxidation state) and possibly MnCO\(_3\) formed. Both these compounds also formed in calcite, but a solid solution was the major phase. When the precipi-
tated aragonite was heated in the presence of H₂ a solid solution did form, but when it was heated in the presence of O₂ the main product was the higher oxidation state MnO₂. Measurements were done at 20°C, indicating that the compounds persisted after cooling. This complex variability is shown in Figure 2.2.

Incorporation of trivalent metals into calcite was studied by Kai et al. (1993). Most of the M³⁺ ions examined (Al³⁺, Sc³⁺ and Y³⁺) were incorporated into the calcite lattice with water (because they were hydrated) and OH⁻ (to maintain the charge balance), possibly also forming separate crystals with OH⁻ within the calcite crystals. Trivalent iron, however, was reduced to Fe²⁺, entered into the calcite lattice, and then was gradually reoxidized on exposure to air. Change in oxidation state may form by removal of centres: hole-trapped centres may accept an electron as Fe²⁺ is converted to Fe³⁺, and electron-trapped centres may lose their excess electron to Fe³⁺, converting it to Fe²⁺. Loss of these centres suppresses CO₂⁻ and sulphite radicals in ESR analysis, making the signal due to these radicals unsuitable for carbonate dating.

2.4 Aragonite versus Calcite

2.4.1 Observations and Trends

Alkaline earth metal ions such as Mg²⁺ and up to 2 ppm Sr²⁺ (Kitano, 1962) strongly favour aragonite formation from solution, as does the SO₄²⁻ ion (Kitano, 1975). Larger amounts of Sr²⁺ promote calcite formation, but some disagreement exists as to the required concentration (McCauley and Roy, 1974). The presence of large amounts of Ba²⁺ favours vaterite (McCauley and Roy, 1974) and alkali chlorides enhance calcite (Kitano and Hood, 1962), and inhibit aragonite, growth. Fluoride ions also have this effect (Kitano, 1980). Kitano et al. (1976) also reported that very high or very low concentrations of CO₃⁻ or Ca²⁺ will increase calcite formation, and that HCOS⁻ ions favoured aragonite.

It is interesting to observe as a general trend that if a higher proportion of a bivalent metal ion is incorporated into one polymorph than the other, it tends to promote the formation of the other. An exception to this is Pb²⁺. Aragonite has a higher Pb²⁺ concentration than calcite although Pb²⁺ favours aragonite formation (McCauley and Roy, 1974). Kitano (1980) stated that the presence of bivalent metal ions with an ionic radius less than that of calcium (100 pm) favoured the formation of aragonite, and greater than that of calcium favoured the formation of calcite (see Table 2.2 for ionic radii). This largely agrees with the results of Chen (1977), who found that Mg²⁺, Cu²⁺, Zn²⁺, Ni²⁺, Co²⁺ and Pb²⁺ favoured aragonite formation, and that Mn²⁺, Ca²⁺, Cd²⁺ and Hg²⁺ favoured calcite formation. The ionic radii of the latter are between 80 pm (Mn²⁺) and 110 (Hg²⁺), comparable to that of calcium. However, no mechanism for these results was suggested, and they do not explain, for example, why the presence of small amounts of Sr²⁺ favour aragonite formation, why Pb²⁺ favours aragonite, with an ionic radius of 120 pm, why Ba²⁺ favours vaterite, or why only significantly lower concentrations of transition metal ions than of magnesium ions are required to promote aragonite formation (e.g. Lippmann (1973) showed that a solution
2.4. Aragonite versus Calcite

Mg:Ca ratio of 5.7:1 was required to significantly favour aragonite; for a similar system Barber et al. (1975) found a Co:Ca ratio of 0.11:1 was sufficient.

Chen (1977) examined the effect of adding up to 140 ppm Cu$^{2+}$ to Ca(HCO$_3$)$_2$ solution and found that a peak aragonite concentration of approximately 75% formed at a solution Cu$^{2+}$ concentration of 80 ppm. In equivalent experiments for Zn$^{2+}$, he found a sharp peak of 100% aragonite formation at approximately 45 ppm. As he does not describe the other products of the precipitation at higher concentrations of M$^{2+}$, mixed-metal carbonates, or carbonates of the impurity ion, rather than calcite may have formed. His results for addition of Ni$^{2+}$ are more decisive, showing a local maximum of approximately 25% aragonite formation at approximately 8 ppm Ni$^{2+}$, after which the proportion of aragonite decreased again. It then increased to a high of approximately 45% at 36 ppm. Chen does not consider the mechanism by which a metal ion could have a different effect on the polymorphism at different concentrations. The addition of Sr$^{2+}$, as has already been described, appeared to exhibit this same phenomenon in.

Lippmann (1973) stated that at least 30% of a typical impurity ion is needed to thermodynamically stabilize aragonite with respect to calcite. This makes it unlikely that those impurity ions which promote the formation of aragonite do so by increasing its thermodynamic stability. Instead they appear to operate in one of two ways. Some ions promote epitaxial crystallization of a particular polymorph and others "poison" one polymorph, generally calcite, thereby allowing growth of the other.

2.4.2 Calcite Poisoning

A number of metal ions are known to reduce calcite growth rates, including Mn$^{2+}$ (Wang and Merino, 1992) and Zn$^{2+}$ (Dromgoole and Walter, 1990). Hamdona and Khader (1994) studied the effectiveness of Mg$^{2+}$, Pb$^{2+}$, Zn$^{2+}$ and Cu$^{2+}$ at slowing calcite growth. They found that all four cations slowed calcite growth, with effectiveness of inhibition of precipitation increasing with increasing adsorption affinity, in the order Mg$^{2+}$, Pb$^{2+}$, Zn$^{2+}$ and Cu$^{2+}$. It is interesting to note that they used solutions with M:Ca ratios as high as 0.2, but report no aragonite formation. Their results suggest that poisoning of the growing crystal was simply by adsorption onto active growth sites, as those cations which were most readily adsorbed were the most effective poisons. It is worth noting that ions smaller than or approximately the size of Ca$^{2+}$ are most readily adsorbed, providing a possible explanation for the trends observed by McCauley and Roy (1974); Kitano (1980); Chen (1977) and described above (Section 2.4.1).

Wada et al. (1995) precipitated calcium carbonate in gel, in the presence of Fe$^{2+}$, Mg$^{2+}$, Ni$^{2+}$, Cd$^{2+}$, Co$^{2+}$, Cu$^{2+}$ and Zn$^{2+}$. A source of carbonate ions was allowed to diffuse through the gel in one direction and of Ca$^{2+}$ in the other, so that the two ions met in the gel. Observation of the time and position at which nucleation occurred allowed them to calculate the effectiveness of a given impurity at slowing crystal growth.

At the high M:Ca ratios in solution which they used (3.3–50%), they found:

1. The presence of Cd$^{2+}$ in the solution had no effect upon calcite growth, probably because CdCO$_3$ precipitated instead;
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2. The presence of any of the other six metal ions in solution promoted the growth of aragonite over calcite;

3. At a $M:Ca$ ratio of 3.3%, $Co^{2+}$, $Zn^{2+}$, $Fe^{2+}$ and $Mg^{2+}$ lead to the formation of 23, 42, 44 and 78% vaterite respectively.

Examination of the $Ca^{2+}$ and $CO_3^{2-}$ concentrations at which aragonite precipitated in Wada et al.'s system indicated that the calcite precipitates were originally aragonite, which then converted to a calcite-metal carbonate solid solution. If the calcite-metal carbonate solid solution contained more than a certain percentage metal carbonate (7.2% in the case of $Mg^{2+}$), aragonite was more stable, which prevented the conversion of the original aragonite to calcite. Another effect of the presence of the metal ions was the slowing of aragonite growth, which would maintain the system at a relatively high supersaturation level. A high level of supersaturation then may also promote the formation of aragonite over calcite. Snow and Yund (1987) studied the high-pressure transformation of aragonite to calcite, and suggested that Fe and Mn impurities could destabilize aragonite by increasing its internal energy. As both Fe and Mn are easily accommodated by calcite, they did not expect their presence to affect the growth rate of calcite, which has since been contradicted by Wang and Merino (1992).

Reddy (1976) found that $Mg^{2+}$ inhibited calcite growth at the rather high concentration of $10^{-3}$ molL$^{-1}$. A much lower concentration of $Fe^{2+}$ and $Fe^{3+}$ is effective, as shown by Katz et al. (1993), who studied solutions at a range of pH between 7 and 8. Only $8\times10^{-5}$ molL$^{-1}$ $Fe^{2+}$ and $5\times10^{-6}$ molL$^{-1}$ $Fe^{3+}$ was required to completely block the Ca growth sites. Kink sites were blocked first, as these have the greatest attraction to the cation, having three sites of attachment to the calcite surface. Cations adsorbed on the surface will gradually migrate to these sites. Step sites were next most attractive, and when growth was completely blocked all kink and step sites were poisoned by trace cations. $Fe^{2+}$ will form complexes with the $-CO_3H$ and $-CaOH$ groups on the surface of wet calcite, but $Fe^{3+}$ was probably incorporated as colloidal $Fe(OH)_3$ particles.

An interesting application of calcite poisoning is the controversial magnetic water treatment to reduce scale buildup in pipes by passing the water through a magnetic field first. Sometimes this strange method works, and scientists have been at rather a loss as to how and why. Herzog et al. (1989) suggested that the magnetic field may encourage $Fe^{2+}$ or $Fe^{3+}$ ions to form, which would then poison the calcite scales, allowing aragonite to grow instead. Aragonite forms fine needles which do not build up as scales but are swept out of the system. They did not suggest a method by which the magnetic field could release $Fe^{2+}$ or $Fe^{3+}$ into the water.

However, Ho Lysz et al. (1994) examined the adhesion of $CaCO_3$ particles to the walls of glass capillaries, and found that the greatest adhesion occurred not when the precipitate was only calcite, aragonite or vaterite, but was a mixture of calcite and aragonite. They suggested adhesion to glass was caused by unsaturated forces on the $CaCO_3$ surface, which were higher when the system was in transition from one polymorph to another, when crystal structure was less defined.
2.4. Aragonite versus Calcite

2.4.3 Heterogeneous Nucleation

The initial step in crystal formation is the nucleation of a crystal precursor (see Section 1.3.1). Heterogeneous nucleation refers to nucleation where the precursor is a different mineral to the final form, but normally isomorphous with it. As described in Section 1.2, carbonates of those cations with an ionic radius larger than that of Ca\(^{2+}\) (see Table 2.2), including Pb\(^{2+}\), Ba\(^{2+}\) and Sr\(^{2+}\), tend to take the aragonite structure, in which the cation is coordinated to nine oxygen atoms. Carbonates of those cations with a radius less than Ca\(^{2+}\) take the calcite structure. Many of these small cations are readily adsorbed onto the calcite surface, poisoning the lattice.

Kornicker et al. (1985) performed experiments on the adsorption of Co\(^{2+}\) on calcite and aragonite and found that its affinity for calcite was about an order of magnitude higher than for aragonite.

Large laboratory-grown crystals of aragonite were found by McCauley and Roy (1974) to contain heterogeneous regions in their centres, evidence that the phase initially forming was not pure aragonite. Sr-induced aragonite crystals contained SrCO\(_3\) centres, and Mg- and Ni-induced aragonite also contained central regions rich in Mg or Ni.

It seems likely that the poisoning of calcite crystals allows sufficient time and energetically favourable conditions for aragonite to nucleate, either homo- or heterogeneously.

Another model is the metal-chelate concept of Mirsal and Zankl (1985), an attempt to unite the biological and inorganic precipitation approaches. It has been shown that many biomineralizing organisms exert a large amount of control over their mineralization (see Section 7.2), but the mechanisms seem to involve an organic molecular framework and have little to do with trace ions in the water. Mirsal proposed that a citrate ligand, for example, could form a chelate with a metal ion. The chelate would then be attacked electrophilically by hydrated Ca\(^{2+}\) or Mg\(^{2+}\), forming a hydrogen-bonded complex, which would contain a carbonate group bonded to one metal ion and two CO\(_3\)(Mg or Ca)OH\(_2\) groups. Each oxygen of the carbonate group would be coordinated to two of the three groups. Dehydration would result in the removal of the OH\(_2\) groups, leaving the CO\(_3\) bonded to one metal and two Ca or Mg carbonates, giving M-O-CO\(_2\) bonds. Weakening of these bonds would eventually result in the complex breaking up into Ca- or Mg-carbonate and a M\(^{2+}\) chelate. The nature of the original chelate would control the geometry of the resulting calcium or magnesium carbonate phase, giving a mechanism for either aragonite or calcite formation with different organic chelates. While this is an interesting model, it does not seem to have become popular, and no more recent references to it were found.

Much less work has been done on the adsorption of metals onto aragonite than calcite, partly because the ESR signal of aragonite cannot be resolved enough to give useful information on bonding environments. The tendency for trace quantities of calcite to form and trace ions to be preferentially partitioned into them is also a problem (Brečecić et al., 1996).
2.5 Summary

Transition metals ions are incorporated into growing calcite crystals by initial rapid adsorption onto a disordered surface layer which has not yet been described with certainty. They then diffuse, possibly to stable surface kinks and steps, in some cases dehydrating. Finally ions are slowly incorporated into the crystal lattice, onto specific lattice sites (in the case of Mn and Pb, certain sites on the 10\text{I}4 faces). In some circumstances, H\textsuperscript{+} production at the surface may control trace metal partitioning, as seen by a dependence on pH and precipitation rate. Adsorption of transition metal ions into kink and step sites prevents Ca\textsuperscript{2+} being adsorbed there and poisons the growing crystal. Heterogeneous nucleation of a phase isomorphous with aragonite may then encourage aragonite to form instead of calcite.

At high enough supersaturations, coprecipitates of M\textsubscript{CO\textsubscript{3}} or hydrated mineral phases will form on the surface, and cations with more than one stable oxidation state may be oxidised or reduced by electron-poor or -rich sites on the growing surface.

Dependence of partition coefficients on kinetic effects rather than equilibria limits their applicability to natural systems. Laboratory-determined coefficients may not reflect the complexity of a geochemical sample at all, and predicting partition coefficients accurately has so far proved almost impossible even in the laboratory where most variables are known and controlled. Determination of a natural partition coefficient, where the only variables known are the Ca\textsuperscript{2+} and M\textsuperscript{2+} mole fractions in the rock, may be little better than guessing (Morse and Bender, 1990).
Chapter 3

The Adsorption of Co(II), Ni(II) and Cu(II) onto High-Mg Calcite and Aragonite Powder

3.1 Introduction

Very few studies have been reported on the adsorption of metal ions onto either aragonite or high-Mg calcite. This chapter describes the methods and materials used to examine short-term (initial) sorption of $\text{Co}^{2+}$, $\text{Cu}^{2+}$ and $\text{Ni}^{2+}$ onto both these minerals. Sorption was performed at varying pH, buffered with $\text{HCO}_3^-$ or with HEPES/KOH (HEPES = N-[2-Hydroxyethyl]piperazine-N’-[2-ethane-sulfonic acid]), a biological buffer known not to complex either $\text{Ca}^{2+}$ or the metal ions of interest.

The majority of experiments into sorption of a metal ion onto a surface are performed for a period of some hours or days, at varying metal ion concentrations. Results are then mathematically modelled using an adsorption isotherm, frequently a simple $K_d$ (see Section 2.1.3), or the more complex Langmuir adsorption coefficient ($K_{\text{Lang}}$), which is calculated from plotting $1/m_i(\text{ads})$ versus $1/m_i(\text{soln})$, where $m_i$ refers to the molarity of metal ion i, the subscript “ads” to the concentration of surface sites on which the metal ion is sorbed, and the subscript “soln” to the solution concentration. This graph gives a straight line, with slope $1/K_{\text{Lang}}m_i(\text{ads,max})$, where “ads,max” indicates the maximum possible concentration adsorbed by the substrate.

This approach was not taken in this study. The problems of dissolution of unstable polymorphs made long-term adsorption experiments problematic, as was the addition of very high concentrations of acidic metal ion solutions, which would lower the pH and promote solid dissolution. The technique chosen for solution analysis, flame atomic absorption spectroscopy, made the examination of very low metal ion concentrations unfeasible. The two questions of interest in this study were the short-term (less than 5 min) uptake of metal ions onto these minerals, and the effect of pH on the adsorption. Results cannot be directly compared with long-term rate measurements, because it has generally been found that uptake of any metal ion onto calcium carbonate during the first hours of adsorption is considerably greater than the long-term exchange.
3.2 Materials and Methods

3.2.1 Preparation of Aragonite

A number of authors have reported simple syntheses of aragonite (Ho Lysz et al., 1994; Wray and Daniels, 1957; Tomoyama and Kitano, 1984), involving precipitation from either CaCl₂ or Ca(NO₃)₂ and (generally) Na₂CO₃. To force the formation of aragonite rather than calcite, one of these methods has been employed:

- the level of supersaturation has been made very high, and either
- the temperature is at least 70°C, or
- Mg²⁺ is added to the solutions.

A number of these methods were tested, for reliability and exclusiveness of aragonite production.

Ho Lysz et al. (1994) found aragonite was formed almost exclusively at temperatures higher than 65–75°C, when precipitated from CaCl₂ and Na₂CO₃ solutions of varying Ca : CO₃ ratios. Similarly Wray and Daniels (1957) combined 1 mol L⁻¹ Ca(NO₃)₂ or CaCl₂ with 0.1 mol L⁻¹ Na₂CO₃, and obtained increasing aragonite proportion with increasing solution temperature. A large excess of Ca salt over Na₂CO₃ seemed to more effectively promote aragonite formation. It has been suggested that the presence of Na⁺ promotes calcite formation (Kitano and Hood, 1962), although not as strongly as Mg²⁺ favours aragonite.

Tests were performed in 28 mL glass vials, with 10 mL of each solution heated to the desired temperature and combined rapidly. Different initial reactant concentrations and different temperatures were assessed. Precipitates were vacuum-filtered immediately and initially dried by sucking air through them, then in an oven at 80°C. The mineral form was identified by Fourier-Transform Infrared Spectroscopy (FTIRS). A small amount of each sample was combined with dried KBr and pressed into a pallet for analysis. No attempt was made to quantify the polymorphic ratio, so the ratios given in Table 3.1 are only approximate. Some samples were analysed before drying to check conversion of aragonite to calcite was not occurring, and some were examined under a microscope to confirm the presence or absence of calcite rhombs and acicular aragonite.

More aragonite was found with increasing temperature, and more with (NH₄)₂CO₃ as the source of CO₃²⁻ than with Na₂CO₃, but results were sporadic, and in no case was 100% aragonite formed. The simple presence of Mg²⁺ was not able to cause exclusive formation of aragonite. Particularly high supersaturation levels resulted in a higher proportion of calcite, rather than aragonite; however all the experiments were highly supersaturated.

A number of authors have made aragonite at, or close to, room temperature, by adding Mg²⁺ ions to the solution, generally as MgCl₂. For example, Tomoyama and Kitano (1984) combined solutions containing 1 or 0.5 g L⁻¹ Ca²⁺, 2 g L⁻¹ Mg²⁺, 7.26 or 6.37 g L⁻¹ Cl⁻ and 0.61 g L⁻¹ HCO₃⁻, and produced 100% aragonite with loss of CO₂.
### Table 3.1: Assessing the effectiveness of different reported methods of synthesizing aragonite

<table>
<thead>
<tr>
<th>Source of Ca$^{2+}$</th>
<th>Conc. of Ca$^{2+}$ (mol L$^{-1}$)</th>
<th>Source of CO$_3^{2-}$</th>
<th>Conc. of CO$_3^{2-}$ (mol L$^{-1}$)</th>
<th>Temp. ($^\circ$C)</th>
<th>% calcite</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaCl$_2$</td>
<td>0.1</td>
<td>Na$_2$CO$_3$</td>
<td>0.1</td>
<td>95</td>
<td>90+</td>
</tr>
<tr>
<td></td>
<td>0.025</td>
<td></td>
<td>0.025</td>
<td></td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td></td>
<td>0.01</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>CaCl$_2$</td>
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<td>Na$_2$CO$_3$</td>
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<td>60</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>70</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>80</td>
<td>90+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>90</td>
<td>90+</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>1</td>
<td>Na$_2$CO$_3$</td>
<td>0.1</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td>CaCl$_2$ plus</td>
<td>0.1</td>
<td>Na$_2$CO$_3$</td>
<td>0.1</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>MgCl$_2$</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CaCl$_2$ plus</td>
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<td>Na$_2$CO$_3$</td>
<td>0.1</td>
<td>95</td>
<td>40</td>
</tr>
<tr>
<td>2 mol L$^{-1}$ MgCl$_2$</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CaCl$_2$</td>
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<td>(NH$_4$)$_2$CO$_3$</td>
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<td>60</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td></td>
<td>0.2</td>
<td>70</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>80</td>
<td>90+</td>
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<td></td>
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<td>90</td>
<td>90+</td>
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<td></td>
<td></td>
<td>variable from 0–90+</td>
</tr>
<tr>
<td>Ca(NO$_3$)$_2$</td>
<td>0.2</td>
<td>NaCO$_3$</td>
<td>0.067</td>
<td>95</td>
<td>70</td>
</tr>
</tbody>
</table>
A modification of this method was found to reliably produce aragonite. \( \text{Ca(HCO}_3\text{)}_2 \) solution was made by bubbling \( \text{CO}_2 \) (from sublimation of dry ice) through triply-distilled water plus BDH AnalAr-grade \( \text{CaCO}_3 \) (calcite), at 5°C for 8–10 hours. Solutions were filtered through a porosity 4 glass frit and stored under a \( \text{CO}_2 \) environment at 3°C until use. The \( \text{Ca}^{2+} \) concentration was determined by titration with ethylenediaminetetraacetic acid (EDTA), using eriochrome black-T as an indicator, and solutions were diluted to 0.4 gL\(^{-1}\) \( \text{Ca}^{2+} \).

This diluted \( \text{Ca(HCO}_3\text{)}_2 \) solution was filtered through a 0.025 \( \mu \text{m} \) filter onto the appropriate masses of \( \text{MgCl}_2 \) and \( \text{CaCl}_2 \) to give a \( \text{Ca}:\text{Mg} \) ratio of 1:4, and the following concentrations:

- \( 0.4 \text{ gL}^{-1} \text{ Ca}^{2+} \) from \( \text{Ca(HCO}_3\text{)}_2 \),
- \( 1.2 \text{ gL}^{-1} \text{ HCO}_3^- \) from \( \text{Ca(HCO}_3\text{)}_2 \),
- \( 4 \text{ gL}^{-1} \text{ Mg}^{2+} \) from \( \text{MgCl}_2 \),
- \( 11.6 \text{ gL}^{-1} \text{ Cl}^- \) from \( \text{MgCl}_2 \),
- \( 0.6 \text{ gL}^{-1} \text{ Ca}^{2+} \) from \( \text{CaCl}_2 \), and
- \( 1.0 \text{ gL}^{-1} \text{ Cl}^- \) from \( \text{CaCl}_2 \).

The solution was filtered onto the salts because, with exposure to vacuum for filtering, loss of \( \text{CO}_2 \) caused almost immediate formation of \( \text{CaCO}_3 \) crystallites. In the absence of \( \text{Mg}^{2+} \), these crystallites were calcite.

The solution was left for 2 weeks in an open container, covered with tissue paper to reduce dust levels on the surface, before collection of aragonite. Many studies (Angus et al., 1979) have found that laboratory-produced aragonite frequently contains trace calcite contamination, at levels too low to be detected by X-ray diffraction spectroscopy. Microscopic and FTIRS examination confirmed that the precipitate was completely aragonite. The precipitate consisted of clusters of very fine acicular needles, and is shown in Photo 1.

### 3.2.2 Preparation of High-Magnesium Calcite

The experiments into production of aragonite described in the previous section showed that a substantial amount of \( \text{Mg}^{2+} \) could be added to a \( \text{CaCl}_2 + \text{Na}_2\text{CO}_3 \) solution, without causing the formation of aragonite. 25 g of high-Mg calcite were made in one batch by combining 5 L of 0.1 mol L\(^{-1}\) \( \text{Na}_2\text{CO}_3 \) solution with 5 L of 0.1 mol L\(^{-1}\) \( \text{CaCl}_2 \) plus 0.05 mol L\(^{-1}\) \( \text{MgCl}_2 \) solution. The mixed solution was stirred occasionally for 1 hr, then filtered. Some of the filtrate was added to the precipitate, and it was heated in an oven for 3 hrs at 80°C, to age the precipitate, and convert any unstable hydrated \( \text{CaCO}_3 \) present to high-Mg calcite. After filtering again, the precipitate was dried to constant weight at 80°C. Mineralogy was determined by FTIRS and X-ray diffraction to be calcite. The magnesium content of three random 0.006 g samples was measured by flame atomic adsorption spectroscopy, and the \( \text{MgCO}_3 \) concentration of the calcite was found to be 11.22±0.14% by mass.

One parameter which can be expected to be significant in surface reactions, including metal ion sorption by a mineral, is the number of surface flaws (Katz et al., 1993). To allow comparison of different experiments, only one substantial batch was made of each
3.2. Materials and Methods

Photo 1: The aragonite manufactured for use in this study.

Photo 2: The high-Mg calcite manufactured for use in this study.
of the high-Mg calcite and the aragonite used for these experiments. The assumption is that the flaws in a single batch of material will be evenly distributed throughout the sample. Morse (1986) indicated that it was important to prevent particles being under 0.1 μm, as below this size the surface free energy increases sufficiently to substantially increase solubility. Electron microscopy of the particles produced indicated all were above this size (Photo 2).

3.2.3 Preparation of Buffers

Experiments were initially unbuffered, but the lowering of pH on addition of the acidic transition metal chloride solutions lead to partial dissolution of aragonite. An attempt was made to avoid this pH change by adding the salts as solids, as a mixture of acidic CuSO₄ and basic Cu(NH₃)₄SO₄, but this procedure was unsuccessful. Not only did the pH change on addition of CuCl₂, but infrared spectra of the solid material after addition indicated the formation of a sulphate compound. A KH₂PO₄/Na₂HPO₄ buffer was tested, and it was found that 0.1 mol L⁻¹ was sufficiently concentrated to prevent pH change, but caused the formation of a phosphate solid phase. Likewise, an imidazole/HCl buffer produced surface precipitates at too low a concentration for adequate buffering. A HEPES/KOH buffer was both successful in buffering the addition of the required amounts of added metal ion, and did not produce any additional solid phase.

3.2.4 The Problem of Dissolution of Unstable Polymorphs

A difficulty which is encountered in all experimental work involving calcium carbonate in solution is the increase in solubility of calcium carbonate with increasing partial pressure of carbon dioxide. This difficulty is particularly apparent in work such as that described in this chapter, which involves adsorption onto the surface of the less stable forms of calcium carbonate. It is not possible to produce a solution containing aragonite powder and supersaturated with respect to aragonite, without promoting the conversion of aragonite to calcite, or the nucleation of calcite in the solution. Likewise, magnesium-containing calcite will at least partially convert to low-magnesium calcite by a process involving surface dissolution and recrystallization on the calcite grains. In this work, all solutions were saturated with magnesium-free calcite, and a supply of dry air blown over the top of reaction containers to standardize the atmosphere in which reactions occurred. As the duration of all experiments was short, and equilibration of solutions with atmospheric carbon dioxide is a slow process, significant change to the amount of carbon dioxide dissolved in the solution was not expected to occur.

Solutions saturated with low-Mg CaCO₃ were produced as follows. Triply-distilled water was brought to equilibration with BDH AnalaR-grade CaCO₃, simply by stirring the two together for a period of two weeks at 25°C. This solution was stored in an open container at 25°C, and filtered through a 0.025 μm filter before use. It had a final pH of 8.3. HEPES/KOH buffer solutions of the desired pH were made from this solution, and the results of adsorption onto aragonite and high-Mg calcite in buffers of varying pH is given in Section 3.3.1.
Speciation calculations were performed to determine the initial state of the solutions, and the complexes involved.

### 3.2.5 Generalised Adsorption Procedure

A generalized procedure was followed for all adsorption experiments. Experiments were performed in a vial or beaker of minimal volume, so that a large air space did not occur in the container above the solution. The appropriate mass of solid was weighed accurately into the container, and 10 mL of buffer was added with a pipette. The required amount of $M^{2+}$ was added as 25–50 μL of 3000–15000 ppm metal chloride solution. Precise concentrations of the metal chloride solutions were measured by atomic adsorption spectroscopy, because the salts were hygroscopic and could not be weighed accurately. The ionic strength of the buffer was 0.1 M. The solution was stirred under the air flow described above for 30 sec–5 min with a magnetic flea, filtered through a 0.45 μm filter, and quickly rinsed with two 1 mL portions of triply-distilled water. Rinsing removed the remaining $M^{2+}$ solution from around the CaCO$_3$ surface, so that this $M^{2+}$ was not counted as adsorbed, and was done rapidly, in 10–15 seconds, to avoid further adsorption. Even if the surface had reached saturation with $M^{2+}$, further $M^{2+}$ could be taken up to replace surface $M^{2+}$ as (if) migration of ions into the solid occurs (Stipp et al., 1992). The retenate was transferred to a collecting vial, and acidified by addition of 200 μm of concentrated HNO$_3$ per 10 mL. Ten mL of 10% HNO$_3$ was pipetted into the top of the filter vessel, and swirled for 30 sec to dissolve the CaCO$_3$ powder. This solution was then sucked through the filter, and collected. The filter and unit were rinsed in two portions of triply-distilled water before the next sample.

Both the concentration of $M^{2+}$ which remained unadsorbed and the concentration of adsorbed $M^{2+}$ were measured by flame atomic adsorption spectroscopy, except in the case of Co$^{2+}$ adsorption onto aragonite. For this case, adsorption was low enough that the subtle changes in the concentration of the initial solution could not be detected, and only the concentration of Co$^{2+}$ in the solid was measured. The Ca$^{2+}$ concentration of the solutions after adsorption was also measured, primarily to assess the effect of the experiment on the solubility of the CaCO$_3$. Metal ion standards were prepared from BDH commercial standard solutions, and were acified to the same level as the samples to be analysed. For analysis of dissolved CaCO$_3$, an appropriate mass of BDH AnalaR-grade CaCO$_3$ was also dissolved in the standard solution. Calcium standards were prepared by dissolution of BDH Analar-grade CaCO$_3$.

### 3.2.6 The Effect of Solution pH

Solution pH is a major factor in ion adsorption, with adsorption onto γ-Al$_2$O$_3$ varying from zero to almost 100% as the pH increases over a range of approximately two units (Yasunaga and Ikeda, 1986). The pH at which adsorption becomes significant is dependant on the ion, decreasing in the order: Co$^{2+}$ (pH 6.8), Zn$^{2+}$ (pH 6.2), Pb$^{2+}$, Cu$^{2+}$ (pH 5), the order of the ions' hydrolysis constants.
The effect of pH on adsorption onto both aragonite and high-magnesium calcite was studied, within the working pH range of the HEPES/KOH buffer. For each of Co\(^{2+}\), Ni\(^{2+}\) and Cu\(^{2+}\), eight different pHs were tested: 6.8, 7.0, 7.2, 7.4, 7.6, 7.8, 8.0 and 8.2. For the Cu\(^{2+}\) experiments 0.0100 ± 0.0002 g of the appropriate polymorph was weighed into a 14 mL vial; for Ni\(^{2+}\) and Co\(^{2+}\) 0.1 g of the polymorph was used. 10 mL of HEPES/KOH buffer saturated with low-magnesium calcite was pipetted into each container, and 25 \(\mu\)L of 3000 ppm CuCl\(_2\) solution, or 50 \(\mu\)L of 15000 ppm NiCl\(_2\) or CoCl\(_2\) was added. The solution was treated as described above (in Section 3.2.5), with a stirring time of 5 min.

### 3.2.7 Short-term Sorption

The adsorption of Cu\(^{2+}\), Co\(^{2+}\) and Ni\(^{2+}\) ions onto both the unstable polymorphs was assessed over a time period of 30 sec to 5 min. Solutions were made as described in Section 3.2.4, buffered with both HEPES/KOH and HCO\(_3^-\). The pH of the experiments was 8.2, and the generalized adsorption protocol was followed.

Adsorption of Cu\(^{2+}\) onto aragonite over a period of several hours was also assessed, at pH 8.2, in HCO\(_3^-\) buffer.

### 3.3 Results and Discussion

#### 3.3.1 Adsorption at Varying pH

Adsorption of Cu\(^{2+}\), Ni\(^{2+}\) and Co\(^{2+}\) onto both high-Mg calcite and aragonite with varying pH is given in Tables 3.3, 3.4 and 3.5, and is graphed in Figures 3.1, 3.2 and 3.3. Solutions were buffered with 0.1 mol L\(^{-1}\) HEPES/KOH, saturated with CaCO\(_3\). Initial Cu\(^{2+}\) concentration was 7.5 ppm, and initial Co\(^{2+}\) and Ni\(^{2+}\) concentrations were 75 ppm, to give a metal ion:solid ratio of 7.5 mg/g for each of the three metal ions. The results presented are the means of three repetitions, and error bars give the range of actual values found. The Ca\(^{2+}\) concentration of the solution from which adsorption had occurred is also given, as a percentage of the initial Ca\(^{2+}\) concentration, to show the increases in Ca\(^{2+}\) concentration which occurred.

The speciation of the metal ion solution was determined for Cu\(^{2+}\) (for which appropriate carbonate complexation data was available), using the data given in Table 3.2. The subprogram “Species” within the program “Soleq” (Pettit and Powell, 1999) was used to calculate distribution curves, and equilibrium constants were taken from “SC-database” (Pettit et al., 1999). Total carbonate, and calcium, concentrations were 0.45 mM, Cu\(^{2+}\) concentration was 0.126 mM, Cl\(^-\) concentration was 0.25 mM, temperature was 25°C and ionic strength was 0.1 mol L\(^{-1}\). The distribution curves, shown in Figure 3.5, indicate that over the pH of interest (7.6–8.2) the Cu\(^{2+}\) in solution changes from predominantly free Cu\(^{2+}\) (73.2% at pH 7.6) to a mixture of free Cu\(^{2+}\) and CuCO\(_3^0\) (59.2% at pH 8.2).
3.3. Results and Discussion

Table 3.2: The complexes and log$\beta$ or log*$\beta$ values used for calculation of speciation of Cu$^{2+}$ in a 0.1 mol L$^{-1}$ solution of HEPES/KOH.

<table>
<thead>
<tr>
<th>complex</th>
<th>log$\beta$</th>
</tr>
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<tbody>
<tr>
<td>H$\text{CO}_3$^-</td>
<td>10.3</td>
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<tr>
<td>H$_2$CO$_3$</td>
<td>16.8</td>
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<tr>
<td>CuCO$_3$</td>
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<td>CaCO$_3$</td>
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<tr>
<td>CaHCO$_3^+$</td>
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<tr>
<td>CuCl$^+$</td>
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<tr>
<td>CuOH$^+$</td>
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<td>Cu$_2$(OH)$_2^{2+}$</td>
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<tr>
<td>Cu(OH)$_2$(s)</td>
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</table>

Table 3.3: The uptake of Cu$^{2+}$ onto high-Mg calcite and aragonite at varying pH; and the Ca$^{2+}$ concentration of the solutions, as a percentage of the starting Ca$^{2+}$.

<table>
<thead>
<tr>
<th>pH</th>
<th>aragonite</th>
<th>calcite</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Cu$^{2+}$ adsorbed</td>
<td>Ca$^{2+}$ %</td>
</tr>
<tr>
<td>6.8</td>
<td>42.0</td>
<td>332.7</td>
</tr>
<tr>
<td>7.0</td>
<td>48.7</td>
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<td>71.6</td>
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<td>75.8</td>
<td>102.9</td>
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Table 3.4: The uptake of Ni$^{2+}$ onto high-Mg calcite and aragonite at varying pH; and the Ca$^{2+}$ concentration of the solutions, as a percentage of the starting Ca$^{2+}$.

<table>
<thead>
<tr>
<th>pH</th>
<th>aragonite</th>
<th>calcite</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Ni$^{2+}$ adsorbed</td>
<td>Ca$^{2+}$ %</td>
</tr>
<tr>
<td>6.8</td>
<td>9.23</td>
<td>313.5</td>
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<td>7.0</td>
<td>9.65</td>
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<td>11.8</td>
<td>102.9</td>
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Table 3.5: The uptake of $\text{Co}^{2+}$ onto high-Mg calcite and aragonite at varying pH; and the $\text{Ca}^{2+}$ concentration of the solutions, as a percentage of the starting $\text{Ca}^{2+}$.

<table>
<thead>
<tr>
<th>pH</th>
<th>% $\text{Co}^{2+}$ adsorbed</th>
<th>$\text{Ca}^{2+}$ %</th>
<th>% $\text{Co}^{2+}$ adsorbed</th>
<th>$\text{Ca}^{2+}$ %</th>
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<tr>
<td>6.8</td>
<td>2.78</td>
<td>321.3</td>
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<td>348.6</td>
</tr>
<tr>
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<td>7.2</td>
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<td>243.9</td>
</tr>
<tr>
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<td>235.6</td>
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<td>2.91</td>
<td>164.5</td>
<td>3.72</td>
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</tr>
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<td>2.67</td>
<td>137.6</td>
<td>3.64</td>
<td>172.4</td>
</tr>
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</table>

Figure 3.1: The % of available $\text{Cu}^{2+}$ adsorbed onto aragonite and high-Mg calcite at varying pH.
3.3. Results and Discussion

**Figure 3.2:** The % of available Ni$^{2+}$ adsorbed onto aragonite and high-Mg calcite at varying pH.

**Figure 3.3:** The % of available Co$^{2+}$ adsorbed onto aragonite and high-Mg calcite at varying pH.
Chapter 3. Adsorption onto CaCO₃ Powder

**Figure 3.4:** The calcium concentration (as a percentage of the initial calcium concentration of the solution) of the solutions after adsorption of Cu²⁺ onto aragonite and high-Mg calcite at varying pH.

**Figure 3.5:** The speciation of 0.126 mM Copper(II) in the solution from which the adsorption experiments were performed.
3.3. Results and Discussion

Photo 3: The aragonite manufactured for use in this study, in greater detail.

The initial solution from which the buffers were made was in equilibrium with CaCO$_3$ at a pH of 8.4 and an ionic strength of close to zero, and had an initial Ca$^{2+}$ concentration of 17.9±0.3 ppm. Making the buffer solutions decreased the pH and increased the ionic strength of the CaCO$_3$-“saturated” solution, both of which altered the calcite solubility (Section 4.4). Below a pH of 8.0 for Cu$^{2+}$, the amount of metal ion adsorbed increased steadily, and the Ca$^{2+}$ concentration in the solution also increased steadily. At a higher pH, initial metal ion adsorption levelled out. Up to a pH of approximately 8.0, the initial adsorption of both Ni$^{2+}$ and Co$^{2+}$ was low and steady, and increased at higher pH. A much greater proportion of the available Cu$^{2+}$ was adsorbed than of the available Ni$^{2+}$ or Co$^{2+}$, perhaps because Cu$^{2+}$ is a harder acid than the other two (Bersuker, 1996), making it more attracted to oxygen-containing ligands. Another possibility is that Cu$^{2+}$ was more able to distort to fit specific active sites, due to the Jahn-Teller effect.

The high-Mg calcite manufactured for this project contains 11.22 mass % MgCO$_3$. This is equivalent to a mole % of 13.4% Mg. According to Lippmann (1973), high-Mg calcite with Mg mole % of 10% has a solubility approximately the same as aragonite in pure water. A slightly higher increase in the Ca$^{2+}$ concentration of the solutions containing calcite over those containing aragonite was seen, indicating the solubility of high-Mg calcite was higher than that of aragonite.

Solubility is also dependent on surface characteristics of the crystals. The crystals were examined by electron microscopy; overviews are shown in Photos 1 and 2, and more detailed views in Photos 3 and 4. The form of the two polymorphs is very different, with the aragonite being the clusters of acicular needles typical of the mineral. The calcite, on the other hand, formed twinned “doughnuts”, instead of the rhombohedrons of pure calcite generally seen. Visual examination of the electron micrographs of the two
different minerals suggests that the surface area of the aragonite is considerably larger than that of the calcite. However, surface area is a less significant factor in adsorption than the number of unstable active sites. The aragonite crystals were grown over a period of two weeks, so may have fewer surface flaws, and therefore fewer active sites per square metre than the calcite crystals, which were only aged in solution for three hours. The similarity of the degree of initial sorption of \( \text{Co}^{2+} \), \( \text{Ni}^{2+} \) and \( \text{Cu}^{2+} \) onto aragonite and high-Mg calcite with varying pH may indicate that the two minerals’ solubilities were similar, in spite of their different morphologies.

### 3.3.2 The Variation of Sorption with Time

Adsorptions of \( \text{Cu}^{2+} \), \( \text{Co}^{2+} \) and \( \text{Ni}^{2+} \) onto both high-Mg calcite and aragonite for 30 sec to 5 min are given in Tables 3.6, 3.7 and 3.8, and are graphed in Figures 3.6, 3.7 and 3.8. Experiments were performed at pH 8.2, as the experiments on the influence of pH showed that by this pH mineral dissolution was not occurring.

The linear region of uptake of each metal was fitted to a line of best fit equation. Equations were

- For \( \text{Cu}^{2+} \) adsorption onto aragonite:
  \[ \% \text{ Cu}^{2+} \text{ adsorbed} = 0.20 \times \text{time} + 22.6. \]

  For \( \text{Cu}^{2+} \) adsorption onto high-Mg calcite:
  \[ \% \text{ Cu}^{2+} \text{ adsorbed} = 0.16 \times \text{time} + 40.4. \]
### Table 3.6: The uptake of Cu\(^{2+}\) onto high-Mg calcite and aragonite after varying exposure times at pH 8.2.

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<th>time (seconds)</th>
<th>aragonite</th>
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<tr>
<td></td>
<td>% Cu(^{2+}) adsorbed</td>
<td>% Cu(^{2+}) adsorbed</td>
</tr>
<tr>
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<tr>
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### Table 3.7: The uptake of Ni\(^{2+}\) onto high-Mg calcite and aragonite after varying exposure times at pH 8.2.

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</thead>
<tbody>
<tr>
<td></td>
<td>% Ni(^{2+}) adsorbed</td>
<td>% Ca(^{2+})</td>
</tr>
<tr>
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Table 3.8: The uptake of Co$^{2+}$ onto high-Mg calcite and aragonite after varying exposure times at pH 8.2.

<table>
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<tr>
<td>300</td>
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</table>

Figure 3.6: The uptake of Cu$^{2+}$ onto high-Mg calcite and aragonite after varying exposure times at pH 8.2.
3.3. Results and Discussion

Figure 3.7: The uptake of Ni$^{2+}$ onto high-Mg calcite and aragonite after varying exposure times at pH 8.2.

Figure 3.8: The uptake of Co$^{2+}$ onto high-Mg calcite and aragonite after varying exposure times at pH 8.2.
Chapter 3. Adsorption onto CaCO₃ Powder

Figure 3.9: The calcium concentration (given as a percentage of the initial calcium concentration of the solution) of the solutions after adsorption of Ni²⁺ onto aragonite and high-Mg calcite for varying lengths of time at pH 8.2.

- For Ni²⁺ adsorption onto aragonite:
  \[ \% \text{Ni}^{2+} \text{ adsorbed} = 0.024 \times \text{time} + 4.78. \]

- For Ni²⁺ adsorption onto high-Mg calcite:
  \[ \% \text{Ni}^{2+} \text{ adsorbed} = 0.028 \times \text{time} + 6.80. \]

- For Co²⁺ adsorption onto aragonite:
  \[ \% \text{Co}^{2+} \text{ adsorbed} = 0.009 \times \text{time} + 0.92. \]

- For Co²⁺ adsorption onto high-Mg calcite:
  \[ \% \text{Co}^{2+} \text{ adsorbed} = 0.021 \times \text{time} + 0.80. \]

Calcium(II) concentrations of each solution after adsorption were also measured, and were found to be slightly higher than before adsorption. In the case of Ni²⁺, for which the greatest mass of M²⁺ was taken up, a definite increase in Ca²⁺ concentration with increasing uptake occurred. This increase was examined by EDTA titration of the Ca²⁺ concentration of the solutions, because sufficient accuracy could not be achieved with flame atomic absorption spectroscopy. Results are incorporated into Table 3.7, and plotted in Figure 3.9. The increase in number of atoms of calcium in the solution was only 11% of the decrease in number of atoms of nickel. Exchange of Ni²⁺ with surface Ca²⁺ ions could only explain part of the initial metal ion uptake.

A much higher proportion of the available Cu²⁺ was taken up than of the available Ni²⁺ or Co²⁺, as for the experiments with adsorption at changing pH. This order of preference was also seen for the Corallina officinalis algae investigated in this work (Section 9.3.3).
3.3. Results and Discussion

As summarized in Section 1.4.4, Stipp and Hochella Jr. (1991) found evidence of the existence of S-CaOH and S-CaCO₃H groups on the surface of calcite. Consideration of the mechanism for formation of surface hydroxyl groups (Figure 1.6) leads to the observation that, while oxide surfaces contain Lewis acid sites and so attract the O atoms of water molecules, the surface of CaCO₃, with the isoelectric point occurring at a pH of at least 8.2, is more likely to exhibit Lewis basicity and coordinate to water through the H atoms. Figure 3.10 gives a possible depiction of the formation of surface hydroxyl groups for a calcium carbonate surface. While adsorption onto such complexes would not explain the loss of Ca²⁺ from the mineral during adsorption, it is plausible that more than one mechanism of sorption was occurring simultaneously, with exchange of Ca²⁺ from particularly unstable sites. Both polymorphs showed similar trends of sorption, suggesting that uptake was dependent on characteristics which were common to both, such as surface hydroxy or carboxy groups. However, as both minerals had similar solubilities, their surface concentrations of unstable sites may have also been similar.

### 3.3.3 Longer-term Cu²⁺ Adsorption onto Aragonite

The adsorption of Cu²⁺ onto aragonite was measured over several hours at pH 8.3, buffered with 0.1 M ammonium bicarbonate solution. Ammonium bicarbonate solution was chosen to reduce the dissolution of CaCO₃, which dissolves by reaction with aqueous CO₂ to give Ca(HCO₃)₂. The presence of excess HCO₃⁻ at the buffered pH drives the equilibrium of the dissolution reaction to the left, reducing surface exchange of CaCO₃.
Chapter 3. Adsorption onto CaCO₃ Powder

Figure 3.11: Percentage of available Cu(II) adsorbed onto aragonite powder versus time of adsorption, in minutes.

The percentage of available Cu²⁺ is given in Figure 3.11, and shows an interesting maximum after 30 minutes. It is possible that copper ions are initially adsorbed onto unstable sites on the aragonite surface and are removed from some of these sites with time. The large spread of values after an adsorption time of 30 minutes may also suggest this. Percentage adsorption is approximately half that of HEPES/KOH buffered solutions after 5 min. This may indicate that reduction of Ca²⁺ exchange between the surface and the solution reduced the adsorption, or that the effect may be due to competition for adsorption sites between the metal ions and the ammonium ion.

3.4 Summary

Measurements of the effect of pH on initial adsorption of Cu²⁺, Ni²⁺ and Co²⁺ onto the unstable CaCO₃ minerals, aragonite and high-Mg calcite, suggested that dissolution of the mineral may have had a major effect on initial metal ion uptake. Under natural HCO₃⁻-rich fresh water systems, such as cave waters, it is possible that the chemistry of the metal interaction with unstable polymorphs is dominated by dissolution of the mineral, rather than by sorption onto the surface of the polymorph.

The very short-term adsorption of these three transition metal ions was studied at pH 8.2, 25°C and atmospheric CO₂ partial pressure. While actual solution metal ion concentrations varied between the three metals, the metal ion:solid ratio was the same for all three. Rates of uptake were similar on both polymorphs, and increased in the order of Co²⁺ < Ni²⁺ ≪ Cu²⁺. The Ca²⁺ concentrations of the solutions also increased, indicating adsorption may have been due in part to exchange with surface Ca²⁺ ions.
Part III

Studies of Aspects of the Chemistry of Bohemia Cave, Murchison
Chapter 4

Calcium Carbonate Cave Systems, and Bohemia Cave, Mt Owen

4.1 Dissolution of Calcium Carbonate

Limestone and marble caves form on land when CaCO₃ is dissolved by CO₂ in meteoric water. Meteoric water is water which originated as rainfall over land, and can exist as rain, surface or sub-surface stream water, or ground water. The process of cave formation (speleogenesis) requires both that the water must be undersaturated with respect to CaCO₃ and that the water must be flowing, to remove dissolved material (Lohmann, 1988).

Fresh rainwater is in equilibrium with atmospheric CO₂ (P_{CO₂} = 10^{-3.5}) in the air, and has a pH of approximately 5.7. This equilibrium is represented by the solution of CO₂ in rainwater (Lohmann, 1988):

\[ \text{CO}_2(\text{g}) \rightleftharpoons \text{CO}_2(\text{aq}) \quad K = 10^{-6.4} \quad (4.1) \]

and the equilibrium of the dissolved CO₂ with H₂CO₃ (Fergusson, 1982; Snoeyink and Jenkins, 1980; Butler, 1982):

\[ \text{CO}_2(\text{aq}) + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \quad K \approx 10^{-3} \quad (4.2) \]

It can be seen from the equilibrium constants of these two reactions that almost all CO₂ in rainwater is present as CO₂(aq). Total CO₂ concentration (represented by H₂CO₃⁺) is commonly given as the sum of the H₂CO₃ and the dissolved CO₂. For a given water, H₂CO₃⁺ can be obtained from P_{CO₂} by Henry’s Law:

\[ [\text{H}_2\text{CO}_3^+] = K_H P_{\text{CO}_2} \quad K_H = 10^{-1.5} \quad (4.3) \]

The dissolved CO₂ reacts further to give bicarbonate ions (Stumm and Morgan, 1996):

\[ \text{H}_2\text{CO}_3^+ \rightleftharpoons \text{HCO}_3^- + \text{H}^+ \quad \text{pK}_1 = 6.35 \quad (4.4) \]
This reaction can also be written as:

\[ \text{CO}_2^{(aq)} + 2\text{H}_2\text{O} \rightleftharpoons \text{H}_3\text{O}^+ + \text{HCO}_3^- \]  (4.5)

The resulting \(\text{HCO}_3^-\) also undergoes reaction (Stumm and Morgan, 1996):

\[ \text{HCO}_3^- \rightleftharpoons \text{H}^+ + \text{CO}_3^{2-} \quad \text{pK}_2 = 10.33 \]  (4.6)

The fraction of each species present at a given pH can be determined from these equilibria, using the concentration condition, that

\[ C_T = [\text{H}_2\text{CO}_3^+] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}] \]  (4.7)

The fraction of a component \(X\) (\(\alpha_X\)) is

\[ \alpha_X = \frac{[X]}{[C]_T} \]  (4.8)

\([\text{H}_2\text{CO}_3^+], [\text{HCO}_3^-]\) and \([\text{CO}_3^{2-}]\) can be rewritten in terms of \(\text{H}^+, \text{K}_1\) and \(\text{K}_2\). The resulting equations are:

\[ \alpha_{\text{H}_2\text{CO}_3^+} = \frac{[\text{H}^+]^2}{[\text{H}^+]^2 + \text{K}_1[\text{H}^+] + \text{K}_1\text{K}_2} \]  (4.9)

\[ \alpha_{\text{HCO}_3^-} = \frac{\text{K}_1[\text{H}^+]}{[\text{H}^+]^2 + \text{K}_1[\text{H}^+] + \text{K}_1\text{K}_2} \]  (4.10)

and

\[ \alpha_{\text{CO}_3^{2-}} = \frac{\text{K}_1\text{K}_2}{[\text{H}^+]^2 + \text{K}_1[\text{H}^+] + \text{K}_1\text{K}_2} \]  (4.11)

A distribution diagram such as Figure 4.1 can be constructed, giving the relative amount of each species at each pH.

In soil, oxidation of organic material and plant respiration (e.g. of soil algae) increases the \(\text{P}_{\text{CO}_2}\) of the air considerably, to values of approximately \(10^{-1.5}\) atm. The total \(\text{CO}_2\) concentration becomes

\[ [\text{H}_2\text{CO}_3^+] = \text{K}_\text{P}_{\text{CO}_2} = 10^{-1.5} \times 10^{-1.5} = 10^{-3}\ \text{molL}^{-1} \]  (4.12)

As \(10^{-3}\ \text{molL}^{-1}\) is a relatively high concentration, soil water has a relatively high capacity to dissolve \(\text{CaCO}_3\). Dissolution occurs by

\[ \text{H}_2\text{CO}_3^+ + \text{CaCO}_3 \rightleftharpoons \text{Ca}^{2+} + 2\text{HCO}_3^- \]  (4.13)

The formation of \(\text{HCO}_3^-\) in this dissolution reaction increases the pH of the solution, by production of \(\text{OH}^-\):

\[ \text{HCO}_3^- + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 + \text{OH}^- \]  (4.14)

\(\text{K}_\text{b}\) for this reaction is \(2.4\times10^{-7}\), which is considerably higher than \(\text{K}_\text{a}\) for the competing reaction,

\[ \text{HCO}_3^- + \text{H}_2\text{O} \rightleftharpoons \text{H}_3\text{O}^+ + \text{CO}_3^{2-} \quad \text{K}_\text{a} = 4.8\times10^{-11} \]  (4.15)

Therefore waters high in dissolved \(\text{CaCO}_3\) are slightly alkaline.
4.2 Karst Landscapes

With most rock types, features of the landscape reflect processes of weathering and mechanical abrasion. In more soluble rocks, such as CaCO$_3$, dolomite (CaMg(CO$_3$)$_2$) or the evaporites (such as gypsum (CaSO$_4$·2H$_2$O) and halite (NaCl)), solution features dominate the terrain. Large-scale features include caves, dolines (large closed depressions), and towers and canyons, made by very sharp downcutting of surface water through the soluble rock. Generally only very pure limestones (90% or more calcite) are cave-forming. Soil is often thin and sometimes absent, with bedrock exposed on the surface. Collapse of the sides of depressions, or of near-surface cavern roofs, or deeper roofs which are later exposed by uplift, can fill depressions and hollows with breccia. On a smaller scale, surfaces of rocks are often complexly scalloped (or fluted), and dissolved CaCO$_3$ may be reprecipitated as travertine on the surface (generally in hot springs), as cement between grains and particles of rock, and as speleothems inside caves. Most of the names applied to formations inside caves, such as “stalactite” or “flowstone”, are morphological names, primarily indicating shape. The terms “speleothems” and “cave formations” are generalized names, referring to any secondary mineral deposits within a cave. Landscapes formed in soluble rock, exhibiting features such as these, are termed “karst”, which has been defined as (Esteban and Klappa, 1983)

a diagenetic facies, an overprint in subaerially exposed carbonate bodies, produced and controlled by dissolution and migration of calcium carbonate in meteoric waters, occurring in a wide variety of climatic and tectonic settings, and generating a recognizable landscape.

Photo 5 shows typical large-scale alpine karst, with steep slopes, thin or absent soil, and many deep cracks, which have formed along joints perpendicular to the moderately-dipping surface. Photo 6 focuses more closely on the karst field itself. Some of the
cracks here are partly filled with soil, providing a sheltered environment for plants. Cracks in this karst field vary from approximately one to five metres deep, and drain surface water towards the underlying Bulmer Cave. Note also that a number of the depressions within the cracks are approximately right-angled, indicating solution along intersecting joints. The solution nature of the depressions is shown particularly clearly in the detail of Photo 6.

A single surface of the karst field is shown in Photo 7, with surface fluting, and a narrow developing crack. The flutes are on a centimeter scale, and almost knife-sharp, with the steep face of each scallop pointing in the direction of water flow. Fluting occurs on the surface of impermeable rocks, such as this marble, because water flows off the sloping surface instead of pooling and soaking into it (James and Choquette, 1988), and may be a useful paleoflow indicator inside an abandoned cave.

4.3 Cave Genesis

4.3.1 Cave Aquifers

Water entering an aquifer can come from two significantly different sources: either from localized point sources or diffuse percolation sources. In a non-limestone aquifer diffuse sources are most common, with water percolating through soil and then underlying rock pores, and being stored between grains of sediment or within connected pores in a permeable rock. Massive limestones and marbles are generally well-cemented, resulting in very low porosity (a measure of the void space between crystals or grains in the rock) and intergranular permeability (a measure of pore interconnection and therefore of the ability of a fluid to flow through the rock). Secondary permeability through fissures
4.3. Cave Genesis

Photo 6: Karst field. Mt Owen, central Nelson, New Zealand. The small photograph is a detail of the larger, and shows a single solution depression approximately 5 m wide.

Photo 7: A single rock of the karst field, showing surface fluting. Mt Owen.
is normally far more significant, and the volume of these can be huge, with large cave river systems the extreme example. Every cave system includes an unknown number of passages which are too small to enter, and in a highly fractured limestone it is possible for no one fracture to develop to a greater size than any other fracture, leading to a highly permeable network of tiny fissures. For a carbonate rock groundwater system, point sources are very common, particularly dolines (or sinkholes) which provide rapid access of surface water to the groundwater system. Water from a point source is less likely to be in equilibrium with the soil, a fact which is particularly significant when considering dissolved CO$_2$ concentrations. The high flow rate of groundwater along fissures reduces residence time within the aquifer, making such water more susceptible to retaining some forms of short-term pollution, because slow chemical or biological processes lack the time to neutralize pollutants. Water from a point source such as a doline or streamsink, is also unlikely to have passed through a thick soil filter before entering the aquifer, although some streams are sourced at the soil–limestone interface rather than at the surface. Soil in a limestone terrain is frequently thin or absent, especially within dolines. Water within a carbonate groundwater system cannot be assumed to behave in the same way as water in a non-calcareous aquifer.

### 4.3.2 Open and Closed Systems

When groundwater reacts with CaCO$_3$, two possible situations occur:

1. An open-CO$_2$ system, in which the water maintains contact with soil CO$_2$ while dissolving CaCO$_3$, and
2. A closed-CO$_2$ system, in which water is isolated from the soil CO$_2$.

In a closed system, as CaCO$_3$ is dissolved, CO$_2$ is consumed and the H$_2$CO$_3$ concentration decreases. In an open system, the reacting CO$_2$ is constantly replaced from the soil air, and H$_2$CO$_3$ concentration is maintained. In Figure 4.3 the plots of $[\text{HCO}_3^-]$ versus pH show that
4.4 The Effect of Other Dissolved Ions in the Solution

There are three significant ways in which the presence of additional dissolved ionic substances can affect CaCO$_3$ solubility in a HCO$_3^-$ solution.
Chapter 4. Calcium Carbonate Cave Systems

1. The Ionic Strength Effect:
   The presence of any foreign electrolyte raises the ionic strength of the solution, which lowers the activity coefficients of all ions present. The result is lower activities and increased CaCO₃ solubility. In seawater, the ionic strength effect almost doubles CaCO₃ solubility, from what it would be on the basis of a carbonate medium alone.

2. The Common Ion Effect:
   Any other Ca²⁺ or CO₃⁻-containing salt dissolved in the solution will lower CaCO₃ solubility. This effect occurs because CaCO₃ solubility is the product of the Ca²⁺ and CO₃⁻-activities, independent of the source of the ion. In seawater, or if a cave water contains a high MgCO₃ concentration from passing through dolomitic layers, CaCO₃ solubility will be reduced.

3. Ion Pair Formation:
   If MgCO₃ is present, not only is the common ion effect noticeable, but Mg²⁺ also forms ion pairs with HCO₃⁻ (MgHCO₃⁻) and CO₃⁻ (MgCO₃). Formation of ion pairs promotes CaCO₃ dissolution, by Le Chatelier's effect, reducing the activity of the ion-paired anion. Because of this ion pair formation, Mg²⁺ in solution reduces the common ion effect to a small extent. The presence of MgCl₂, as opposed to MgCO₃, will increase CaCO₃ solubility by ion pair formation and the ionic strength effect.

The overall interaction of these effects can be complex. Picknett et al. (1976) reported that 0–10% Mg²⁺ reduces Ca²⁺ solubility, and >10% increases it.

4.5 Speleothems

4.5.1 Speleothem Formation

Speleothems are secondary minerals formed within caves. CaCO₃ speleothems are deposited when water enters a cavity containing air with a lower P CO₂ than the water, and loses CO₂ to the cave atmosphere.

\[
2\text{HCO}_3^- \rightleftharpoons \text{CO}_2(\text{aq}) + \text{CO}_3^{2-} + \text{H}_2\text{O} \quad (4.16)
\]
\[
\text{CO}_3^{2-} + \text{Ca}^{2+} \rightleftharpoons \text{CaCO}_3(\text{s}) \quad (4.17)
\]

Deposition by evaporation plays at most a minor role in speleothem formation. Most caves are highly humid, reducing evaporation, and the large difference between cave atmospheric CO₂ concentration (\(P_{\text{CO}_2} \approx 10^{-2.5} \text{ atm}\)) and dissolved CO₂ in cave waters (\(P_{\text{CO}_2} \approx 10^{-1.5} \text{ atm}\)) results in substantial loss of CO₂ when the water comes into contact with the air of the cave. Canning (1988) modelled straw growth from two Australian caves, and found that growth rates from de-gassing were 100–1000 times greater than rates from evaporation.
4.5. Speleothems

Figure 4.4: A cross-section of a typical stalactite, showing the orientation of calcite crystals within the original straw, and on the outside of the stalactite. From Onac (1997).

Most cave formation types were named at a time when little was known of their mineralogy or modes of formation. For this reason, and because of the difficulty of non-destructive field identification of stalactite mineralogy, the different categories of speleothem are named by their appearance and growth position. Stalactites, in particular, can be formed from many different minerals, including silicates.

The three most common forms of speleothem are stalactites and stalagmites (both dripstone) and flowstone. All three forms occur only under relatively high rates of water flow.

Stalactites, which are suspended from the ceiling, begin by forming “straws”, hollow tubes with the diameter of a drop of water (2–9 mm). The drop of water at the tip of the straw loses CO₂ by degassing to the air until it becomes supersaturated with respect to calcite, when a layer of calcite forms on the bottom of the drop. As the drop grows it oscillates, and surface tension causes the layer of calcite to move up and attach to the wall. After some time, it is likely that the straw’s internal conduit will become blocked, causing water to seep through the porous tube to flow over the outside, and thicken it to a typical carrot shape instead. During this phase of growth, the c-axes of the calcite crystals most commonly grow radially outward, perpendicular to the original straw (Figure 4.4). Stalactites generally increase in length no more than a few millimetres a year.

Stalagmites are formed on the floor of the cave from water which has dripped from the ceiling. The force of the impact causes CO₂ degassing, and also spreads the drop outwards. Stalagmites are broader than stalactites, and form cylindrical onion-skin-like layers. Vertical growth rates are less than 1 mm per year.

The third common formation type is flowstone, which forms from a thin layer of water flowing over a surface, generally the wall of the cave. Speleothem “waterfalls” can be formed, many metres high. Water which flows through a long narrow crack, or off a ledge on a wall, forms “draperies”, thin transparent sheets suspended from the shelf or ceiling, intermediate in nature between flowstone and stalactites.
4.5.2 Aragonitic Speleothems

Aragonite is the third most common mineral in cave formations world-wide, after calcite and gypsum (Fullerton, 1983), but very little has been found in New Zealand. Recently-discovered caves in Northwest Nelson have been an exception to this. Small quantities of aragonite speleothems have been reported in three caves in the Mt. Arthur Marble: Summit Cave on Takaka Hill (Cody, 1979), and Windrift (Worthy, 1987) and Nettlebed Caves (Cody, 1980) nearby. More significantly, Bulmer (Patterson, 1988) and Bohemia (Tásler, 1991) Caves in the Mt. Owen region contain a number of pure aragonite speleothems, some large and spectacular. The only other area in New Zealand where aragonite speleothems have been reported is in Lime Cave, a small cave in Cenozoic limestones in the Waitomo area (Worthy, 1984).

The most common crystal form of speleothem aragonite is acicular (needle-shaped). The needles of aragonite crystals can grow to be oriented at about 60° to the longitudinal axis of the growing stalactite, forming “spathites”, thin cones of aragonite joined together in an undulating tube. The formation of a cone from a water drop is shown in Figure 4.5.

Helictites, one of the most common speleothems in Bohemia Cave, occur where the water flow rate is too slow to form a drop. In this situation water flow is not gravity-induced, but instead the tips of the helictite are fed by seepage under pressure through a central capillary. Thickening occurs from a water film on the outside, and from secondary capillaries which branch off from the central canal. As capillary flow is not controlled by gravity it can be in any direction, and a wide variety of erratic speleothem forms have been described. Most helictites are calcite, but those in Bohemia Cave are aragonitic.

Anthodites (from the Greek word for “flower”) sometimes have a similar appearance to helictites. They branch outward from stalactites, and are mostly aragonitic. As with helictites, they form when water supply is so slow a drop doesn’t form. Humidity must be high enough to maintain a water film, from which mineral growth occurs.

Loose masses of hydrated magnesium carbonates can be deposited on the tips of aragonite speleothems, suggesting aragonite was formed because of high Mg concentrations (Hill and Forti, 1997), which increased as the aragonite deposited (Mg is not taken up by the aragonite structure).
4.6 Bohemia Cave

4.6.1 Location

Bohemia Cave has formed in the Owen Massif, Murchison, New Zealand, at an altitude of 1250 m. It is one of a number of caves in the area, with many smaller caves in its immediate vicinity. Nearby is Bulmer, the second-deepest cave in the Southern hemisphere. The Mt. Owen karst field is New Zealand’s only example of alpine karst, and both karst and vegetation are well-preserved. The area is part of a State Forest Park, and is under the jurisdiction of the Department of Conservation.

The base for exploration of the karst area of interest is Lake Bulmer, a shallow eutrophic lake located at the bottom of a deep basin, at 1400 m. A eutrophic lake is fed only by rainfall, and has no streams draining it. Access to the lake is from Mr Ken Farrell’s farm, on the Owen River, and involves a 5–6 hr walk. The route begins by following the Owen River, then turns up Bulmer Creek, and follows it to the bottom of a 200 m bluff. It then climbs (sometimes very steeply) up the bluff, onto a forested slope, which leads, mostly climbing an old stream bed, to Lake Bulmer. The route to Bohemia Cave turns off this track near the lake, and takes approximately 45 min.

4.6.2 Geological Setting

The Mt. Owen region was mined for gold and silver during the 1880’s, and its geology was studied then by Park and Hector (as summarized in Newman (1979)). Substantial mapping was carried out by H. E. Fyfe in the 1920’s, and published in Fyfe (1968). Only one substantial study of the geology of the Mt. Owen area has been performed since that time, a Masters of Science thesis in geology by Newman (1979). He focussed primarily on the mineralization of the area, particularly of the rocks underlying the marble, where some gold and silver had been found and mined. At about the same time, the New Zealand Geological Survey updated the geological maps of the area (Coleman, 1981), and as part of that work, some of the rocks were dated more accurately (Simes, 1980). Since then, a summary of the geology has appeared in the New Zealand Speleological Bulletin (Wopereis, 1988). In 1990, Bohemia Cave was discovered by a expedition of cavers from the Czech Speleological Society (Tásler, 1991), who also made some geological observations in the area of the cave, and produced an outline geological map, which is reproduced in Figure 4.6. The description below is summarized from these sources.

Caves of the Owen Massif have developed in the Arthur Marble, of Late Middle Ordovician age. The Ordovician period spanned 500–435 million years before present (BP). At Mt. Owen this marble is overlain, by the Wangapeka Formation, and overlays the Owen Formation. The lower 100 m of the Wangapeka Formation consist of thin grey sandstone and quartzite beds, interbedded with finely-laminated, dark, commonly carbonaceous siltstone. Erosion has removed almost all of the originally thick Wangapeka Formation on the Mt. Owen massif, and it now occurs only very thinly, if at all, in the area of the caves. A stratigraphic column is shown in Figure 4.7, and the stratigraphic terminology formalized by Coleman (1981) is used in the following description.
1 Periodical streams 7 Loamy-stony and stony slope debris
2 Vertical rock steps 8 Thick accumulation of loams
3 Quartzites 9 Bedding and dip
4 Schists 10 Faults
5 Marbles 11 Underground cave system
6 Fine-grained sandstones

Figure 4.6: An outline geological map of the area surrounding Bohemia cave, which can be seen in black towards the top of the diagram. From Tásler (1991).
4.6. Bohemia Cave

**WANGAPEKA FORMATION**
(Upper Ordovician)
600m +

- Pyritic sandstone,
- Quartzite,
- Carbonaceous mudstone

**ARThUR MARBLE FORMATION**
(?Upper Ordovician to Lower Ordovician)
250-500m

- Phosphatised top, Conodont fauna
- Flint-banded marble
- Poor conodont fauna
- Massive blue-grey marble
- Impure fissile marble

**OWEN FORMATION**
(Lower Ordovician to ?Cambrian)
750-1200 +

- Cherty quartzite
- (Beilby Quartzite)
- Dolomitic mudstone
- Black, pyrrhotitic, siliceous dolomite
- Cleaved dolomitic sandstones and mudstones, with sparse carbonate lenses.

---

**Figure 4.7:** A stratigraphic column, showing the rocks of the Mt. Owen region. Modified from Newman (1979).
Chapter 4. Calcium Carbonate Cave Systems

The Owen Formation consists of up to 1200 m thickness of quartzitic sandstone, and sericitic mudstone, with dolomitic horizons. The unit immediately underlying the Arthur Marble is the Beilby Quartzite (Newman, 1979), a complex mix of chert, coarse sandstone, and a chert granule sandstone. Thin shale and dolomitic marble laminates occur within it. The Owen Formation was originally referred to as the Flora Formation, until mapping of the Mt. Arthur region revealed that, at the type locality of this formation, it was a facies of the Wangapeka Formation. Confusion had arisen because at Mt. Arthur two limestones occur, separated by the Wangapeka Formation, whereas at Mt. Owen, only one thick marble is present. The term “Owen Formation”, for the rocks known as the Flora Formation at Mt. Owen, was proposed by Newman (1979), and was formally adopted by Coleman (1981). Newman felt that the Flora Formation at Mt. Owen may possibly be equivalent to the Pikikiruna Schist Formation, which underlies the Arthur Marble at Takaka Hill, 65 km north, but the Pikikiruna Schist is so much more altered than the formation at Mt. Owen that he considered using the same name to be unjustified. Tásler (1991) referred to the underlying rocks by this term, combined with the obsolete classification, as

... phyllites belonging to the Pikikiruna Schist Formation and graptolitic schists to phyllites of the Flora Formation ...

The Arthur Marble at Mt. Owen is 250–500 m thick, and is mainly massive. The marble is generally blue-grey to black in colour, with no macro- and few microfossils, and is a crystallized calcitic marble. Tásler (1991) found that the CaCO$_3$ content (of an unstated number of samples) varied from 85–98%, and the MgCO$_3$ content from 1.8–4%. Its origin is likely to have been biomicritic, as indicated by the lack of stratigraphic features. Unlike the bulk of the unit, the basal approximately 60 m contains finely-laminated silicious shale and dolomite, in increasing proportions as the base is reached, appearing to conformably (or mainly conformably) grade into the Beilby Quartzite.

A low level of metamorphism in all units, but particularly in the Owen Formation, has been caused by the Separation Point Granite, which is in close proximity to the Mt. Arthur Complex. Extensive mineralization and metamorphism has not occurred in the Wangapeka Formation or the Mt. Arthur Marble at this location. Newman attributed this to the Owen Formation being closer to the intrusion, and also to the “capping” effect of the overlying, more impermeable marble.

4.6.3 Development of Bohemia Cave

Bohemia Cave is large and complex, with at least three different levels of passages, and many intersections. A simplified map, showing the main passages and the sampling sites for this project is in Figure 4.8. Passages in the top levels have generally formed along bedding joints, except in the Emmenthaler area (the most structurally complex of the cave), where they follow minor faults. Below the first shaft, labelled P7 on the map, is a fault trace, visible from a 30–50 cm crushed zone and slickensides. Below this, the majority of the passages follow joints, as can be seen by the sharp angles of
Figure 4.8: A simplified map of parts of Bohemia cave. Modified from Tasler (1991).
passage intersections. The major passages at the top of the cave are phreatic, and in the middle are phreatic-vadose, with minor rifting. Ceiling collapse occurs at some passage intersections, but is not common. At the lowest levels of the cave are a series of huge domes, named the Dream of Alberice Cavers (DAC), which are vadose features developed along the steeply-dipping contact between the Arthur Marble and the Beilby Quartzite. The absence of carbonate rocks below this level means the cave does not extend lower than this contact. The domes exhibit extensive roof collapse, being filled with debris ranging from 10 cm³ to more than 500 m³ in size.

Tásler (1991) propose three phases of cave development. First, the area of the Emmenthaler was developed below the water table. Then a local lowering of the erosional base caused a substantial stream to flow through the cave, forming the Old Passage, and flowing through the (now gravelled-filled) passage. This passage has recently been partially dug out, to provide an easier access route to the large domes. This phase would have lasted for some time with little tectonic activity. Finally, a period of rapid uplift caused the paleostream to cut down to the base of the Mt. Arthur Marble, forming the huge domes. The development of the long meander passage and the Highway Tunnel it runs into was from separate vadose streams. The Old Passage is proposed to be older than Pliocene (at least 5 million years old), by comparison with a passage formed under similar conditions at a similar altitude in Bulmer Cave, from which gravel samples have been dated. The lower levels of Bohemia Cave are proposed to be of Pleistocene age (2–5 million years).

4.6.4 Speleothems of Bohemia Cave

Above the Old Passage, only a small number of dead stalactites exist. In the Old Passage, decoration becomes more extensive, with stalactites up to 70 cm long, columnar stalagmites up to 1.5 m tall, and eccentric aragonite formations (Photo 8). Some of the formations can perhaps be described as anthodites, consisting of fine radiating acicular aragonite. The other unusual speleothems which occur at this level are three calcite "trees" about 20 cm high (Photo 9). These are among the few calcite formations in the cave.

The next area with substantial speleothem decoration is the Black and White Minstrels chamber, towards the bottom of the cave. This area contains stalactites, stalagmites and columns (in which a stalactite and stalagmite have grown together), with a chaotic tangle of twisting erratic forms growing off them. The chamber is even more impressive because some sides of the formations are coloured black and some white. Photos 10, 11 and 12 show some of the formations.

Further aragonitic decoration within the DAC domes, and the tunnel leading up to them, includes clusters of straws up to 1 m in length, large stalactites and stalagmites, and eccentric bushes up to 1 m in diameter. Individual aragonite fibres in these bushes are up to 10 mm in length.

Features similar to some of these speleothems are found in the other caves in the Mt. Arthur Marble. Otherwise, they seem to be unique in New Zealand, and are of international significance, both for scientific and aesthetic reasons.
4.6. Bohemia Cave

Photo 8: Stalactites and eccentric formations, Old Passage, Bohemia Cave.

Photo 9: A calcite “tree”, Old Passage, Bohemia Cave.
Chapter 4. Calcium Carbonate Cave Systems

**Photo 10:** Stalactites, stalagmites and columns. Black and White Minstrels, Bohemia Cave.

**Photo 11:** A particularly erratic speleothem. The horizontal section of the formation is about 20 cm long, and the photo is the correct way up. Black and White Minstrels, Bohemia Cave.
Photo 12: Straw stalactites with helictites. Black and White Minstrels, Bohemia Cave.
Chapter 5

Trace Elements and Calcium Carbonate in Bohemia Cave: Experimental

5.1 Introduction

In this section of the project, speleothem samples, rocks and water were collected from Bohemia Cavern, Murchison, New Zealand. Samples were analysed for major and trace element concentrations, to describe element partitioning. Crystal morphology of speleothem samples was examined by electron microscopy, and mineralogy determined by X-ray diffraction.

5.2 Water Samples

The procedure used for collecting water samples was a modification of a protocol developed at the University of Otago for sampling of water from unpolluted rivers (Ahlers et al., 1990). Great care must be taken in the analysis of natural waters for trace metal analysis, to avoid contamination, particularly in the collection of samples, when it is easy to also sample dirt, hair, sweat, metal from collecting equipment, and particularly airborne dust particles. The collection protocol of Ahlers et al. is designed to avoid these problems. Unpolluted natural waters were found by the Otago University group to pose similar contamination problems to remote seawater, requiring a level of care in collection which is seldom found. The cave environment is particularly difficult to sample, with small air volumes, restricted air movement and often mud around the edges of pools and streams.

Cleaning of equipment, and all processing of samples for trace analysis was performed in a positive-pressure cleanroom, to avoid contamination. The cleanroom consists of an anteroom, without its own filtered air supply, in which workers put on lab coats, caps and overshoes. The anteroom leads into a Class-10000 cleanroom, designed for
Chapter 5. Trace Elements and Calcium Carbonate in Bohemia Cave: Experimental

![Photo 13: The ETAAS, showing the protective garb worn by workers in the cleanroom.](image)

**Table 5.1:** Specifications of Class-10000 and Class-100 cleanrooms

<table>
<thead>
<tr>
<th>Class</th>
<th>Maximum particle number (particles/ft³)</th>
<th>Particle size</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>100</td>
<td>0.5 μm and larger</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>5 μm and larger</td>
</tr>
<tr>
<td>10000</td>
<td>10000</td>
<td>0.5 μm and larger</td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>5 μm and larger</td>
</tr>
</tbody>
</table>

instrumental work, containing a GBC 908 AA electrothermal atomic adsorption spectrometer, connected to a GBC PAL 3000 autosampler (Photo 13), an EG & G Princeton Applied Research Model 384B Polarographic Analyzer, connected to a Model 303 SMDE hanging mercury drop electrode, and a milli-Q water system. From the Class-10000 cleanroom, a Class-100 cleanroom can be reached, which is used for sample preparation and storage of samples and cleaned equipment. The only pieces of metal equipment in use in the Class-100 cleanroom are a microwave and a hotplate, for sample digestion. The layout of the complex is shown in Figure 5.1. All people entering the room first remove their shoes, and any clothing or jewellery which is particularly likely to contaminate the room (such as metal watches or fluffy jerseys). They don lint-free lab coats, caps and overshoes, and, once inside the room, disposable non-powdered plastic gloves. The rooms are cleaned weekly, to avoid build-up of dust, and regularly tested to check conformity with industry standards of cleanliness, which are given in Table 5.1.
Figure 5.1: The cleanroom complex in the Chemistry Department, University of Canterbury.
5.2.1 Preparation of Equipment

Water samples were collected in 1 L or 250 mL high-density polyethylene bottles. Bottles were initially washed by soaking for two weeks in 50% AnalaR HNO₃ (diluted with triply-distilled water). They were then rinsed in triply-distilled water, transferred to the cleanroom, and rinsed four times in milli-Q water. Plastic bags and a sheet of polythene for collecting rainwater (if possible) were also acid-washed, and thoroughly rinsed. Each bottle was filled with milli-Q water and spiked to 1% Aristar HNO₃, to provide a further cleaning stage by leaching the interior. Bottles were then wrapped in two acid-washed thick plastic bags, and three thin ones, and stored for two weeks. Before the cave was visited, ten bottles were unwrapped (in the cleanroom), rinsed well, and filled with milli-Q water, to be available as sample blanks. Two of these bottles were left in the cleanroom, one of which was spiked with 10 ppb Zn²⁺ and Fe³⁺, and 20 ppb Al³⁺, to assess whether loss of elements to the bottle walls or leaching from the bottle walls occurred. The bottles were packed into a light-coloured plastic tub (light-coloured to reflect sunlight, minimizing temperature changes) lined with black polythene (black to reduce algal growth by providing no sunlight), and the lid taped on. The tub was transported by car to the Owen Valley, at the start of the track to the cave area, and by helicopter to the campsite about 45 min from the cave entrance. Five layers of plastic bag were used to protect each bottle, because they were to be transported to and from the cave in dirty caving packs, and because a substantial storage time at the campsite was anticipated before collection by helicopter.

5.2.2 Sampling Sites

As far as possible, the intention was to collect water from near speleothems inside the cave, surface water which was a possible source of cave water, and rainwater. Two samples were collected from shallow pools within the cave, fed by small waterfalls from chamber roofs, one in the upper and one in the lower levels of the cave. Two samples of surface water were also collected: one a snow plug on the limestone plateau above the cave, and the other the shallow eutrophic lake just above the level of the cave. There is no flowing surface water in the vicinity of the cave, none could be collected. The rainwater was collected during heavy rain in an area of sparse bush just below the bush line. This position was still above the cave level, and the cave water would be sourced from both rainfall and high snow plugs in the limestone platform above the cavern. Rainwater provided the cave with diffuse recharge, whereas snow plugs fill dolines and are localized point sources. The rainwater would be modified more than the snow before entry to the cave, by contact with soil.

The upper of the sampled cave streams originates from two springs in the high northernmost part of the cave. Its flow rate was not measured in this study, but was reported by Tásler (1991) to vary from 0.2-0.5 Ls⁻¹, with the rate dependent on surface rainfall. The largest stream in the cave, with a flow rate up to 6 Ls⁻¹, runs through the section named the Highway down through the Dream of Alberice Cavers domes, and was beyond the author’s skill as a caver to reach. The lower of the sampled streams joins the main stream in the third dome, and was more major than the upper stream.
Tásler (1991) visited the cave in autumn, when source water from snow plugs would be minimal.

### 5.2.3 Sampling Protocol

#### Cave Water

The sampling procedure was practiced beforehand at the easily-accessable Profanity Cave, Inangahua Junction. Photo 14 shows the two workers, with person 2 lifting a bottle to drain it. A calcite stalactite was also collected from this cave, and used for comparison purposes with the aragonitic stalactites from Bohemia Cave.

On arrival at each sampling spot, a sheet of polythene was laid by the pool. Both samplers put on hats, booties, masks, and plastic ponchos. Ponchos were worn rather than the disposable paper suits of the practice session, because the cave environment was cold and wet, with water dripping from the roof. The sampling procedure was sufficiently time-consuming that care had to be taken to avoid hypothermia.

The layout of the lower collecting site is shown in Figure 5.2, and Photo 15 is the upper site. In the 2-person protocol, person 1 only handled the plastic bags, and stood downstream of person 2, who only handled the bottles. A 1 L and a 250 mL sample were collected at each site. The procedure was:

1. Person 1 opened the outside two layers of plastic bags, while not wearing gloves.

2. Person 1 put on disposable plastic gloves and opened the three inner layers, replacing gloves at each layer.
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Figure 5.2: The lower of the two collecting sites within Bohemia Cavern. Note that person 1 stood downstream of person 2, who stood downstream of the water source.

Photo 15: The upper of the two collecting sites within Bohemia Cavern.
3. When person 1 had nearly finished, person 2 put on shoulder-length gloves. She waited until then to minimize contact time of gloves with the atmosphere.

4. Person 2 took the bottle out of the bag, touching the inside of the bag as little as possible.

5. Person 1 held the bags shut, to minimize contact with the environment.

6. Person 1 emptied the bottle downstream, and rinsed it by filling it upstream, capping it, holding it above her head to drain (so that water did not run off her gloves onto the bottle), and emptying it downstream. The pools were too shallow to submerge the bottle, so it was tilted so that water gently ran in.

7. The bottle was rinsed again, then filled in the same way and capped securely.

8. Person 1 replaced the bottle in the innermost plastic bag.

9. Person 2 reshut the plastic bags, replacing gloves before handling each of the inner three layers, and knotting the outer layers shut.

A 1L blank was exposed in the same way and for the same length of time as the samples.

Rainwater

Rainwater was collected by taping a large sheet of acid-washed plastic in a funnel shape between two branches, and placing a rinsed empty collecting bottle at the funnel's end. A blank was formed by opening one of the bottles of milli-Q water and placing it under the shelter of the piece of plastic, so that rain could not fall into it. The water was collected overnight, an approximately 12 hour time period, during which it rained steadily. The two-person protocol described above was used to open and re-close both the sample and blank bottles.

Snow Plug

The alpine limestone plateaus have a number of permanent snowplugs, which have formed inside cracks and dolines, and melt only partially in summer. One of the largest (and therefore most accessible safely) of these was sampled. A clean area was chosen, with no visible footprints or debris. A sheet of polythene was laid on the snow, on which both samplers stood, and the two-person protocol of the cave sampling regime was used to unwrap each bottle. Wearing clean gloves, person 2 scraped the top surface of the snow away and rejected it. She then rinsed the collecting bottle in milli-Q water twice, changing gloves each time, and, wearing clean gloves, scraped snow into the bottle. While the lower levels of snow were icy, the top layers had softened and were easy to collect. A second clean area was selected for the 250 mL bottle, in case the first had been contaminated by the collection.
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Lake Water

The expedition campsite was at one end of the eutrophic lake, so water was collected from the far end (Photo 16). Sampler 1 stood on the shore, downwind from person 2, who stood on a rock just offshore. The lake was approximately 50 cm deep, which made it possible for the sampler to submerge the bottles fully. The two-person protocol of the cave sampling regime was used to unwrap each bottle, which was emptied onto the shore. The bottle was then recapped, held under water, uncapped, filled, and recapped under water. Two rinses were emptied onto shore before the sample was collected.

5.2.4 Chemical Measurements on the Water Samples

Measurement of pH in near-neutral natural water of low ionic strength poses problems, primarily with the liquid junction of the reference electrode (Davison, 1990). Many of these problems can be overcome by use of a flow-cell in which the glass electrode is held in a column, and the water sample steadily flows past it. A plastic flow-cell was made for this work (Figure 5.3). The water to be measured was steadily poured into the large compartment and flowed past the probe at a reasonably constant rate. Several minutes were required for the pH reading to stabilize.

Low ionic strength buffers were used to calibrate the pH meter before each sampling session. A pH 4.008 at 25°C buffer was prepared from 10.1254 g L\(^{-1}\) dried potassium hydrogen phthalate (COOH\(_{6}\)H\(_{4}\)COOH). A pH 7.413 at 25°C phosphate buffer was prepared from 1.179 g L\(^{-1}\) KH\(_{2}\)PO\(_{4}\) and 4.302 g L\(^{-1}\) Na\(_{2}\)HPO\(_{4}\) (Bates, 1964). Both buffers were placed in the water for long enough to cool to ambient temperature, and their recorded pH was adjusted for the temperature of the water sampled.
Temperature of the cave water was measured using the temperature probe of the pH meter. Humidity was determined with using a wet bulb–dry bulb hygrometer, which was whirled vigorously until readings were stable.

5.2.5 Filtration

All water samples, plus the remaining unopened blank, were re-packed carefully into the plastic tub, well-wrapped in the black polythene. The lid was firmly taped on, and the tub stored under a tarpaulin for several weeks, until collected by helicopter. The tub was transported to Christchurch, and the samples were stored still wrapped in a laboratory until analysis.

At that stage the outside two layers of plastic bag were removed, and the bottles were then unwrapped in the cleanroom, using clean gloves for each layer. The outside of each bottle was washed with milli-Q water. Filters were soaked overnight in 5% Aristar HNO₃, and for six hours in milli-Q water. Each sample was filtered through an acid-washed 0.025 µm millipore filter. Filters were prepared by soaking overnight in 5% Aristar HNO₃, and for six hours in milli-Q water. The bottle was rinsed twice in milli-Q water and the sample poured back into the bottle and acidified to 0.2% with Aristar HNO₃. The samples were then left for 48 hours before analysis, to allow leaching of metal from the container walls. Filtration on site had been considered to contain too high a risk of contamination, and therefore acidification of samples immediately after collection had been impossible. When a long storage time is unavoidable, as in this case, acidification immediately after collection is likely to result in excessive leaching of metal ions from the sample bottle walls (K. Hunter, pers. comm.).

5.2.6 Electrothermal Atomic Absorption Spectroscopy

All the water samples were analysed for iron, copper and aluminium by graphite furnace atomic absorption spectroscopy. Copper standards of 0.05–0.20 ppb were prepared from the nitrate salt, and were acidified to 1% with Aristar HNO₃, as were the samples for copper analysis. Iron standards of 5.0–25.0 ppb were made from ferrous ammonium sulphate, and both standards and samples were acidified to 0.5% with HNO₃. Aluminium
standards of 5.4–13.5 ppb were from potassium aluminium sulphate (KAl(SO₄)₂), and were acidified to 1%.

5.2.7 Anodic Stripping Voltammetry

Zinc, cadmium and lead were analysed by Square-wave Anodic Stripping Voltammetry. 10 mL samples were buffered to pH 5 with an ammonium acetate buffer, made from 0.5 mL of 1 M Aristar ammonium acetate and 5 mL of isopiestically-distilled acetic acid. Samples were deposited onto the mercury drop for 10–20 min. The method of standard additions was used to determine concentrations.

5.2.8 Flame Atomic Absorption Spectroscopy

Magnesium and calcium were analysed by Flame Atomic Absorption Spectroscopy, and sodium by Flame Atomic Emission Spectroscopy. Standards were made from commercially-available BDH solutions, and were acidified to 0.2% with nitric acid, to match the sample acidification.

5.3 Rock and Speleothem Samples

5.3.1 Collection Sites

Permission to collect speleothem and rock samples from within the cave was granted by the Department of Conservation. Two rock samples were collected from the passage leading down to the upper water sampling site, from the rock layer which formed the roof the water trickled down from (samples 2a and 2b). One sample of rock which had fallen from the roof of the lower sampling pool was collected (sample 1), and two samples from a passage at the level of the roof (samples 1a and 1b). One of these rock samples came from the roof of the passage beside a small, dead stalactite which was collected. This level of the cave no longer had effectively forming speleothems, and those which remained were covered in a layer of clay material. A live stalactite and a small helictite were collected from the level of the roof of the lower water sampling site, in a passage nearby. Also, a broken fragment of stalactite was collected from the Black and White Minstrels chamber. Speleothems were collected in such a way as to avoid damage to surrounding decoration, or to the overall appearance of the area. Disposable gloves were worn, to avoid dirtying the samples, and they were packed into acid-washed polyethylene vials for transport. Because flowing stream water generally is sufficiently acidic to dissolve CaCO₃, and flowing too rapidly to deposit it as speleothems instead, speleothems and flowing water are seldom found in the same cave passage. The areas of Bohemia cave which I sampled contain few stalagmites, and water is generally not visible dripping off speleothems on the roof.
5.3.2 X-ray Diffraction

Samples of rocks and speleothems were analysed by X-ray diffraction, to determine their mineralogy. All rock samples were dusty, and were scrubbed well with distilled water. One of the two rocks from the passage at the lower level was covered in an overgrowth of soft material, and was first soaked in HCl to remove this layer. None of the rock samples were totally homogeneous, and the following samples were analysed:

- rust-brown vein material from sample 1,
- the matrix of sample 1,
- acid-resistant material from sample 1a (light-coloured),
- non-resistant from sample 1a (darker-coloured),
- the light-coloured band from sample 1b,
- the darker-coloured band from sample 1b,
- the matrix material of sample 2a,
- the matrix material of sample 2b, and
- the pink-coloured material from sample 2b,

All speleothem samples were analysed, including the black coating on the outside layer of the sample from the Black and White Minstrels chamber. Samples were ground to a fine powder before analysis in a tungsten carbide mill.

5.3.3 X-ray Fluorescence Spectroscopy of Rock Samples

10 g of each sample was powdered with a tungsten carbide ring mill. Five samples were present in sufficient quantities for XRF analysis:

- the matrix of sample 1,
- acid-resistant material from sample 1a,
- non-resistant from sample 1a,
- the matrix material of sample 2b, and
- the dark-coloured band from sample 1b,

Both the acid-resistant and less-resistant samples from rock 1a contained brown veins. XRF is a multi-element technique and Si, Ti, Al, Fe(III), Mn, Mg, Ca, Na, K, P and S concentrations were determined.
5.3.4 Electron Microscopy of Speleothems

Eight speleothem samples were examined with scanning X-ray microscopy. For the two growing samples from the lower section of the cave, a horizontal cross-section and the outside of the growing tip were examined. For the dead stalactite from the upper section of the cave, the inside of the outer clay layer, and a section of the core were chosen. A horizontal and a vertical cross-section of the broken sample from the Black and White Minstrel chamber were also examined. Samples were coated in carbon before microscopy.

5.3.5 Chemical analysis for Major and Trace Elements

Dissolution of Samples

Randomly-chosen samples of both rocks and speleothems, for major ion analysis, were ground in the tungsten carbide ring mill to a fine powder. They were then heated gently in minimal volumes of a 7:3 mixture of concentrated HNO₃ and concentrated HClO₄ until all carbonate material had dissolved. For Ca²⁺ measurement, 0.01 g was dissolved, for Mg, Na and Al 0.1 g was required, and for Fe 1.0 g was extracted. For Cu²⁺ analysis, 0.1 g samples were treated similarly, in Aristar acids, in the cleanroom. Powdering these samples cleanly was not possible, so they were reduced to small fragments with an agate mortar and pestle. After dissolution, all samples were diluted, and centrifuged to remove undissolved material, from which the metal ions had been extracted.

Calcium, Mg, Na, Al and Fewere determined by flame absorption (emission for Na and Fe) spectroscopy, after dilution if necessary.

Copper was determined by graphite furnace atomic absorption spectroscopy, after complexation with ammonium pyrrolidine dithiocarbamate (APDC) at pH 5.0 and extraction into 4-methyl-2-pentanone (MIBK), to remove the interfering Ca matrix (Mathieson, 1995).
Chapter 6

Trace Elements and Calcium Carbonate in Bohemia Cave: Results and Discussion

6.1 Measurements on the Water

The temperature and pH of the cave water samples, and the humidity of the chambers they were collected from are given in Table 6.2. Table 6.1 names the different sites samples were collected from, and lists the samples from each site. Figure 4.8 contains an outline map of the cave, showing the sites. Water temperature was very low, and will be equivalent to the annual surface mean temperature, if the water has had sufficient contact with the rock to be in thermal equilibrium with it. Cave air temperatures were approximately the same as the water temperature.

As the pH of pure water saturated with calcite at atmospheric pressure and 25°C is 8.26, the pH of these waters is rather low. Lower values indicate that with the lower temperatures, and higher \( \text{CO}_2(\text{atm}) \) of the cave, more \( \text{CO}_2 \) is dissolved in the water.

6.2 Elemental Concentrations of the Water Samples

A selection of major, minor and trace cations were determined for the water samples collected. Trace element concentrations are given in Tables 6.3 and 6.4, and major and minor elements in Table 6.5.

Lake Bulmer contains approximately 25 ppb Fe, assumed to be \( \text{Fe}^{3+} \) because all the waters measured were adequately oxygenated. This is the highest concentration of all the samples, as expected, because the lake pH was low enough to prevent widespread formation of insoluble iron hydroxides. The moderately high concentration in the snow plug also demonstrates this speciation effect. Rainwater has little \( \text{Fe}^{3+} \), and
### Table 6.1: The different collecting sites, and the samples collected from them.

<table>
<thead>
<tr>
<th>Site</th>
<th>Speleothem</th>
<th>Rock</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bohemia Cave</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black and White Minstrels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Boh2 stalactite</td>
<td>2a and 2b</td>
<td>upper samples a and b</td>
</tr>
<tr>
<td></td>
<td>Boh1 stalactite</td>
<td>1, 1a and 1b</td>
<td>lower samples a and b</td>
</tr>
<tr>
<td></td>
<td>Boh1 helictite</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B+W speleothem</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lake Bulmer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rainwater</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snow plug</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Profanity Cave</td>
<td>calcite stal</td>
<td></td>
<td>lake samples a and b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rainwater</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>snow samples a and b</td>
</tr>
</tbody>
</table>

### Table 6.2: Temperature, pH and humidity data for the two cave sampling sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Temperature</th>
<th>pH</th>
<th>Humidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>upper</td>
<td>3.6°C</td>
<td>7.87</td>
<td>93%</td>
</tr>
<tr>
<td>lower</td>
<td>3.9°C</td>
<td>7.84</td>
<td>96%</td>
</tr>
</tbody>
</table>

### Table 6.3: Iron(III), aluminium and copper concentrations of the water samples, determined by Electrothermal Atomic Absorption Spectroscopy.

<table>
<thead>
<tr>
<th>Site</th>
<th>Fe(III) (ppb)</th>
<th>Al(III) (ppb)</th>
<th>Cu(II) (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bohemia lower site, sample a</td>
<td>5.3</td>
<td>9.7</td>
<td>nd</td>
</tr>
<tr>
<td>Bohemia lower site, sample b</td>
<td>26.1</td>
<td>23.7</td>
<td>0.17</td>
</tr>
<tr>
<td>Bohemia lower site, blank</td>
<td>0.4</td>
<td>2.2</td>
<td>0.026</td>
</tr>
<tr>
<td>Bohemia upper site, sample a</td>
<td>2.4</td>
<td>7.6</td>
<td>nd</td>
</tr>
<tr>
<td>Bohemia upper site, sample b</td>
<td>6.1</td>
<td>9.4</td>
<td>nd</td>
</tr>
<tr>
<td>Bohemia upper site, blank</td>
<td>0.1</td>
<td>0.81</td>
<td>nd</td>
</tr>
<tr>
<td>snow plug, sample a</td>
<td>10.1</td>
<td>7.3</td>
<td>nd</td>
</tr>
<tr>
<td>snow plug, sample b</td>
<td>14.6</td>
<td>15.6</td>
<td>2.3</td>
</tr>
<tr>
<td>snow plug, blank</td>
<td>0.4</td>
<td>1.3</td>
<td>nd</td>
</tr>
<tr>
<td>rainwater</td>
<td>6.6</td>
<td>7.0</td>
<td>0.034</td>
</tr>
<tr>
<td>rainwater blank</td>
<td>0.4</td>
<td>0.81</td>
<td>nd</td>
</tr>
<tr>
<td>Lake Bulmer, sample a</td>
<td>24.7</td>
<td>2.7</td>
<td>0.0095</td>
</tr>
<tr>
<td>Lake Bulmer, sample b</td>
<td>26.4</td>
<td>4.0</td>
<td>nd</td>
</tr>
<tr>
<td>Lake Bulmer, blank</td>
<td>0.4</td>
<td>0.54</td>
<td>nd</td>
</tr>
<tr>
<td>opened blank</td>
<td>2.4</td>
<td>1.1</td>
<td>nd</td>
</tr>
<tr>
<td>spiked blank</td>
<td>8.9</td>
<td>18.7</td>
<td>nd</td>
</tr>
<tr>
<td>average stream</td>
<td>40</td>
<td>50</td>
<td>7</td>
</tr>
</tbody>
</table>
6.2. Elemental Concentrations of the Water Samples

Table 6.4: Zinc, cadmium and lead concentrations of the water samples, determined by Anodic Stripping Voltammetry.

<table>
<thead>
<tr>
<th>Site</th>
<th>[Zn] (ppb)</th>
<th>[Cd] (ppb)</th>
<th>[Pb] (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bohemia site 1, sample a</td>
<td>5.2</td>
<td>1.4</td>
<td>nd</td>
</tr>
<tr>
<td>Bohemia site 1, sample b</td>
<td>22</td>
<td>5.8</td>
<td>0.37</td>
</tr>
<tr>
<td>Bohemia site 1, blank</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Bohemia site 2, sample a</td>
<td>1.4</td>
<td>0.34</td>
<td>0.49</td>
</tr>
<tr>
<td>Bohemia site 2, sample b</td>
<td>3.9</td>
<td>1.9</td>
<td>nd</td>
</tr>
<tr>
<td>Bohemia site 2, blank</td>
<td>3.5</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>snow plug, sample a</td>
<td>7.3</td>
<td>nd</td>
<td>0.058</td>
</tr>
<tr>
<td>snow plug, sample b</td>
<td>25</td>
<td>nd</td>
<td>0.41</td>
</tr>
<tr>
<td>snow plug, blank</td>
<td>3.3</td>
<td>nd</td>
<td>0.23</td>
</tr>
<tr>
<td>rainwater</td>
<td>21</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>rainwater blank</td>
<td>5.7</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Lake Bulmer, sample a</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Lake Bulmer, sample b</td>
<td>6.6</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Lake Bulmer, blank</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>unopened blank</td>
<td>1.4</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>spiked blank</td>
<td>10.6</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>average stream</td>
<td>30</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

the more alkaline cave waters also contain little dissolved Fe\(^{3+}\). The relatively high concentrations of Fe\(^{3+}\), Al\(^{3+}\) and Cu\(^{2+}\) in sample b of the lower site suggest that that sample was contaminated.

The lowest aluminium concentration was found in Lake Bulmer. This result was surprising, as the lake contained a base layer of sediment, which was expected to be relatively high in aluminium. However, the lake was sampled two days after 36 hours of heavy rainfall, in an area with a relatively rocky bottom, probably reducing the water's contact with soil or clay. The lower of the two values (2.7 ppb) may not be significantly above the procedure's detection limit (blank values varied from 0.54 to 2.2 ppb, although the highest blank was from the lower cave site, the site with the greatest potential for contamination). The rainwater and snow plug, the cavewater sources, both contain approximately 7 ppb Al\(^{3+}\). This is a similar value to the second (higher) site in the cave, but may be significantly lower than that for the first site, suggesting that water had flowed through relatively aluminium-rich rock, such as limestone with clay inclusions.

Copper concentrations in almost all samples and blanks were very low. With multiple injections, the detection limit of the method, expressed as \(\bar{x} \pm 3\sigma\) of four measurements on the blank, was found to be 0.0095 ppb. Too few of the samples contained measurable copper for any conclusions to be drawn about its distribution throughout the system.

Zinc is environmentally ubiquitous, and poses major contamination problems in analysis. The inconsistency of the measured Zn\(^{2+}\) concentrations, and the high values for
several of the blanks, suggest contamination occurred in these cases. If the lowest values are taken as most accurate, the lowest concentrations are in the cave water, and the higher in the surface waters, suggesting that contact with the external atmosphere is the main source of zinc in the samples. The high concentrations in both the rainwater and the rainwater blank may be due to the prolonged collecting time of those samples.

As with the zinc results, the concentrations of lead are highly inconsistent, and were not considered to be significant. The inconsistency is probably a result of low concentrations, close to the detection limit of the method, with occasional contamination. The only samples with measurable quantities of cadmium were in the cave water. The lower site contained significantly more than the upper site, but both were low.

Overall, the water near and in Bohemia Cave was unpolluted, with very low concentrations of all the trace metal ions determined. Drever (1997b) reports average surface stream concentrations, which are tabulated in each of the tables, for comparison with the Bohemia samples.

Adsorption of metals onto surfaces is highly pH-dependent, varying from almost zero to almost 100% as pH increases over a range of 1–2 pH units (see Section 3.2.6). A result of this phenomenon is that metal concentration in a natural water will vary with pH (Salomons and Förstner, 1984) which, in the case of a carbonate-rich cave water, will vary with dissolved CO$_2$ concentration. If the water has not had time to come to equilibrium with the CaCO$_3$ it is flowing through, for example in conditions of high flow rate during or immediately after high rainfall, less of the dissolved metal present may be adsorbed, and free metal ion concentration may be higher. The research described in Section 3 also indicates that it may be possible that adsorption onto suspended CaCO$_3$ solids in non-equilibrium situations is primarily influenced by dissolution of the solid, rather than by adsorption onto the available surfaces. Adsorption of dissolved metal is also dependent on solid concentration, with the adsorption edge occurring at a lower pH for higher solid concentrations (Yasunaga and Ikeda, 1986). As flood waters carry a higher solid load than normal water flows, the effect of solid concentration will be counter to the effect of lower dissolved CO$_2$ concentration, and the dependence of adsorption on flow rate may be complex. Water samples from the upper site of the cave contained slightly lower trace metal ion concentrations than those from the lower site. This difference could be due to the water flowing through more pure limestone, to it being sourced from an area with thinner soil, or to the samples having been collected within 48 hrs of heavy rainfall.

Analysis of the blank which had been left in the clean room indicated that slight further leaching of trace metals off the walls occurred. Recoveries from the milli-Q water sample which was spiked and left in the clean room, then filtered, acidified and analysed with the other samples, were good. For Fe$^{3+}$, 8.9 of 10 ppb was recovered, for Al$^{3+}$ 18.7 of 20 ppb and for Zn$^{2+}$ 10.6 of 10 ppb.

Statistical analysis indicated no correlation between the concentrations of the different trace metals. However, the concentrations of Fe$^{3+}$, Al$^{3+}$ and Zn$^{2+}$ show an interesting trend. These three metal ions were present at high enough concentrations to be measured with reasonable accuracy, but low enough levels for contamination during collection, processing and/or analysis to possibly be significant. For all three metals, concentrations in sample “b” are higher than in sample “a”. Samples labelled “a” were
Table 6.5: Calcium, magnesium and sodium concentrations of the water samples, determined by Flame Atomic Absorption.

<table>
<thead>
<tr>
<th>Site</th>
<th>[Ca] (ppm)</th>
<th>[Mg] (ppm)</th>
<th>[Na] (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bohemia lower site, sample a</td>
<td>23.3</td>
<td>4.1</td>
<td>0.75</td>
</tr>
<tr>
<td>Bohemia lower site, sample b</td>
<td>19.6</td>
<td>4.1</td>
<td>0.90</td>
</tr>
<tr>
<td>Average</td>
<td>21.5</td>
<td>4.1</td>
<td>0.83</td>
</tr>
<tr>
<td>Bohemia upper site, sample a</td>
<td>31.1</td>
<td>0.55</td>
<td>0.84</td>
</tr>
<tr>
<td>Bohemia upper site, sample b</td>
<td>28.4</td>
<td>0.55</td>
<td>0.88</td>
</tr>
<tr>
<td>Average</td>
<td>29.8</td>
<td>0.55</td>
<td>0.86</td>
</tr>
<tr>
<td>Lake, sample a</td>
<td>18.9</td>
<td>1.05</td>
<td>0.23</td>
</tr>
<tr>
<td>Lake, sample b</td>
<td>18.8</td>
<td>1.08</td>
<td>0.20</td>
</tr>
<tr>
<td>Average</td>
<td>18.9</td>
<td>1.07</td>
<td>0.22</td>
</tr>
<tr>
<td>Snow, sample a</td>
<td>0.32</td>
<td>nd</td>
<td>0.55</td>
</tr>
<tr>
<td>Snow, sample b</td>
<td>0.30</td>
<td>nd</td>
<td>0.42</td>
</tr>
<tr>
<td>Average</td>
<td>0.31</td>
<td>nd</td>
<td>0.49</td>
</tr>
<tr>
<td>Rainwater</td>
<td>0.13</td>
<td>0.12</td>
<td>0.76</td>
</tr>
</tbody>
</table>

collected first, in new (although thoroughly cleaned) 1 L bottles. Samples labelled “b” were collected second, in either old or new 250 mL bottles. Blanks were exposed last, and were old 1 L bottles.

There are several possible causes of the difference in results. One possibility is that more metal ions were absorbed by the walls of the new bottles that samples “a” were collected in. The spiked blank left in the clean room indicated slight absorption for Fe^{3+} and Al^{3+}, but this was in an old bottle, so the result cannot be directly compared. Another possibility is that the higher surface:volume ratio for samples “b” resulted in greater leaching from the walls of the 250 mL bottles. Unfortunately, no spiked 250 mL sample was examined, so this possibility cannot be assessed. A third possibility is that environmental levels of contaminant metals were greater for samples “b” than for “a”. It is worth noting that the greatest difference between the two samples occurs in the lower cave site, the dirtiest and most confined of the four sites, and the least difference between the two lake samples, the most open of the sites. Preparing the collecting equipment, exposing, rinsing and filling a sample bottle and rewrapping that bottle took approximately 45 minutes, so by the time sample “b” was collected the cave air had been exposed to the collectors for a significant length of time. Ahlers et al. (1990) found it necessary to stand downwind of the collecting site to avoid contamination, which was not possible in a cave.

The Ca^{2+}, Mg^{2+} and Na^{+} concentrations of the source waters are all substantially lower than concentrations in the cave waters, with the exception of the Na^{+} concentration of the rainwater. The cave site was located approximately 50 km from the ocean, and isolated by a mountain range, making sea spray an unlikely source of the high Na^{+} concentration. Measurement of one rainfall event is insufficient to allow conclusions to be drawn about the Na^{+} concentration.

The Ca^{2+} concentration of the upper site (even though it was collected soon after
Chapter 6. Trace Elements and Calcium Carbonate in Bohemia Cave: Results and Discussion

Figure 6.1: The concentration of the major cations in the sampled water, plotted against increasing exposure to the environment.

heavy rainfall) is higher than that of the lower site, which is unlikely to be saturated with Ca$^{2+}$ at the temperature of 4°C. The change in major ion concentrations with increasing exposure to the environment is plotted in Figure 6.1, and the change in trace metal ion concentration in Figure 6.2. An increase in Mg$^{2+}$ and Ca$^{2+}$ concentrations with increasing exposure is seen, but the situation is more complex for Na$^+$. The lowest Na$^+$ concentration is seen in the lake water, with concentrations in the rain being almost as high as concentrations in the cave waters. The marble contained very little Na$^+$ (Table 6.7), resulting in little increase in concentration after water had flowed through it. As the lake had a high population of aquatic plants, and Na$^+$ is an essential element, it may have been removed from the lake water by the organisms within it. Little correlation between exposure to the environment can be seen for the trace metal ion concentrations.

The Ca$^{2+}$ to Mg$^{2+}$ ratio of the water a stalactite grows from has been widely found to be the most common cause of aragonite formation, instead of the stable calite polymorph. However, Hill and Forti (1997) indicates that a Mg/Ca ratio of 0.4 (at pH 7.0 and 10°C) is required for aragonite formation to occur. Much laboratory work has been done on this topic, and the large body of literature which exists is in reasonable agreement. The Mg/Ca ratios of the two cave waters sampled here are 0.018 and 0.19, for the upper and lower sites respectively. The CaCO$_3$ towards the base of the Mt. Arthur Marble is much less pure than that towards the top of the formation, although dolomitization does not occur above the Owen Formation, and water flow in conduits through these less pure marbles may have resulted in the higher Mg$^{2+}$ concentration of water from the lower site. Neither of these streams sampled are water from which speleothems were growing. Minor and trace metals may be dissolved less in the streams, because of their higher flow...
Figure 6.2: The concentration of the trace cations in the sampled water, plotted against increasing exposure to the environment. Error bars are given where the average of two analyses is plotted, and span the range of the two measurements.

rates, or trace metal constituents may be higher in the streams because of the greater dissolution effectiveness of turbulent flow. Water concentrations of Mg$^{2+}$ may have been higher in the past, when the Mt. Arthur Marble was more substantially overlaid by the Whangapeka Formation, so the now-dead aragonite stalactites on the upper level may have been deposited from water with a much higher Mg$^{2+}$ concentration. While no speleothem formation is now occurring at that level, the mid–upper regions of the cave are not now depositing substantial calcite, with almost all speleothem deposition being aragonitic. In a limestone as pure as the Mt. Arthur Marble, it would seem to be premature to conclude that Mg$^{2+}$ is the cause of the aragonitic speleothem deposition, without undertaking measurements of the water speleothems are actually depositing from. The very low (or non-existent) rate of water drop formation on these speleothems makes sampling water off them difficult.

Another proposed cause of aragonite formation in caves is the rate of deposition of the mineral (Hill and Forti, 1997). Very fast deposition is thought to result in vaterite, which rapidly transforms to the less unstable aragonite. In a cave, low humidity and high wind levels could cause rapid de-gassing of the solution and therefore rapid deposition. High humidity, low temperature and still, CO$_2$-rich air would result in slow de-gassing, and under conditions of very slow deposition the probability is higher for formation of an aragonite nucleus than a calcite one (Lippmann, 1982). Humidity is high and the temperature low in Bohemia Cave (Table 6.2), and deposition from capillary seepage slow, so this mechanism, in combination with the presence of Mg$^{2+}$ and other metal ion impurities, may provide the mechanism for aragonite formation. The speleothems examined consisted of large pure crystals, indicative of slow growth under
stable conditions (see Section 6.3.3).

### 6.3 Analysis of the Rock and Speleothem Samples

#### 6.3.1 Mineralogy of the Rocks and Speleothems

The following rock samples were analysed by X-ray diffraction and Fourier transform infrared spectroscopy, to determine their mineralogy.

- The matrix of rock number 1, a slab of fallen ceiling from the chamber the lower cave water sample was collected in. By both XRD and FTIRS, no minerals were detected other than calcite. Aragonite would have been observable in the FTIRS at approximately 1% by mass.

- Rust-brown vein material from rock number 1, which also occurred in all the other rocks. No mineral other than calcite was detected in either the XRD or FTIRS spectra of this sample. The veins were probably iron-rich calcite which had deposited after the rock became fractured during uplift.

- A sample of the acid-resistant layers from rock 1a. This band was pale cream-grey in colour, and contained quartz and calcite. Banding in the two rocks 1a and 1b occurred on a centimeter scale, and was sometimes discontinuous over cross-cutting veins in the rock. It was probably due to original bedding, rather than to metamorphic changes.

- A sample of the non-acid-resistant layers from rock 1a. This band was darker-coloured, and contained a much higher level of calcite than quartz.

- A sample of the light-coloured bands from rock 1b, which was high in quartz, as was the equivalent band in rock 1a. No minerals other than quartz and calcite were detected.

- A sample of the darker-coloured bands from rock 1b, which was primarily calcite, with some quartz. A region of transparent crystals several millimeters in size was also analysed by FTIRS, and found to be calcite.

- The matrix material of rock 2a, which was from the upper site in the cave, and therefore in a more pure region of the Mt. Arthur marble. This sample was homogenenous in colour, lacking the dark and light layering of rocks from the lower regions, but had been highly fractured, with substantial rust-brown veining. Its mineralogy was calcite, with no detectable quartz. A white vein running through the rock was also analysed by FTIRS, and found to be calcite.

- The matrix material of rock 2b, which was removed from the edge of the wall on which the upper collected stalactite had grown. This rock was much less fractured, and contained both calcite and quartz.
6.3. Analysis of the Rock and Speleothem Samples

Photo 17: The stalactite collected from the lower sampling site of Bohemia Cave.

- A centimeter-scale region with pink discolouration from rock 2b, which was almost pure quartz.

In terms of mineralogy, the five rocks analysed were very pure. They contained no detectable minerals but calcite and quartz, even in post-depositional features, such as veins and larger crystals.

The collected speleothems are shown in Photos 17–21, at approximately actual size. The tips of the two samples from the lower site were covered in a white powdery deposit (Section 6.3.3), and were otherwise lustrous in appearance and cream in colour. Some of the thin branches of the helictite showed the radiating petal-like structure of spathites (Figure 4.5). The dead stalactite from the upper site was brown-yellow, with the infilled tube of its original straw forming a preserved solid core, and the outer layers of material severely eroded. The sample from the Black and White Minstrels chamber had a thin black-brown layer on one side of its exterior surface, and was cream on the other side and white in cross-section. An infilled remnant straw could clearly be seen in a horizontal cross-section, but the proturbances on the speleothem at right angles to this original straw suggest that later capillary growth has also occurred. The Black and White Minstrels chambers contained straw stalactites, large columns, and helictites, with the helictites frequently growing off the surface of the larger speleothems.

All the speleothem samples were analysed by both XRD and FTIRS. The core of the stalactite collected from the upper region, the stalactite from the lower region, and the helictite from the lower region were all completely aragonite. A powder rich in the brown-black colouration of the Black and White Minstrels sample was examined separately from the powdered stalactite interior. Both were found to contain primarily aragonite, with some calcite. No other mineral was detected in the black-brown layer, but isolating this very thin layer was difficult, and the sample may have been too diluted.
Photo 18: The helictite collected from the lower sampling site of Bohemia Cave.

Photo 19: The stalactite collected from the upper sampling site of Bohemia Cave. Both the intact remnant straw and the broken and partially dissolved external layers are shown.
6.3. Analysis of the Rock and Speleothem Samples

Photo 20: The brown-black-coloured side of the speleothem from the Black and White Minstrels chamber.

Photo 21: The cream-coloured side of the speleothem from the Black and White Minstrels chamber.
6.3.2 Elemental Concentrations of the Rocks and Speleothems

Five rock samples were analysed by X-ray Fluorescence Spectroscopy: the matrix of rock 1, the acid-resistant band of rock 1a, the less acid-resistant band of rock 1a, the darker band of rock 1b, and the matrix of rock 2b. Rocks numbered 1 came from the lower sampling site; the rock numbered 2 came from the upper site. For the carbonate minerals (containing the ions Ca\(^{2+}\), Mg\(^{2+}\), Ti\(^{2+}\) and Mn\(^{2+}\)) concentrations of the oxide were converted to the carbonate, using the formula (for CaCO\(_3\)):

\[
\text{CO}_2 \text{ from CaCO}_3 = \frac{M_r(CO_2)}{M_r(CaO)} \times \% \text{ CaO}
\]

and

\[
\% \text{ CaCO}_3 = \% \text{ CaO} + \% \text{ CO}_2.
\]

where \(M_r(x)\) is the relative molar mass of compound \(x\). The concentrations obtained are tabulated in Table 6.6.

These rocks, and the speleothems, were also analysed by flame atomic adsorption or emission spectroscopy, for Ca\(^{2+}\), Mg\(^{2+}\), Na\(^+\), Al\(^{3+}\), Fe\(^{3+}\) and Cu\(^{2+}\) (Tables 6.7 and 6.9).

Magnesium carbonate: calcium carbonate (R %) ratios were calculated for the rocks and stalactites as

\[
R \% = \frac{\% \text{ MgCO}_3}{\% \text{ CaCO}_3} \times 100.
\]

Ratios are given in Table 6.8, from both the X-ray fluorescence and the flame atomic adsorption spectroscopy analyses. Magnesium carbonate percentages in the rocks are highly consistent, indicating little change between the two levels of the cave. The increased silica content of the lower rock samples has not influenced their magnesium concentration significantly. The calcite stalactite from Profanity Cave has a much higher Mg\(^{2+}\) concentration than the aragonitic speleothems from Bohemia Cave, which is due to the higher capacity of the calcite lattice to incorporate Mg\(^{2+}\). The dead corroded stalactite from the upper site had the highest Mg\(^{2+}\) concentration, and the sample from the Black and White Minstrels the lowest. The powder on the two samples from the lower sampling site had a high Mg\(^{2+}\) concentration (see Section 6.3.3).

With the exception of Na\(^+\), all minor and trace elements were present in the rocks at much greater concentration than in the speleothems.

Concentration factors for partitioning of the metal ions between the stalactites and the cave water were calculated, for Fe\(^{3+}\), Al\(^{3+}\), Na\(^+\) and Mg\(^{2+}\), and are tabulated in Table 6.10. The formula used was

\[
\text{CF} = \frac{[M] \text{ in the water}}{[M] \text{ in the rock}}
\]

where the units of concentration were parts per million for both the water and the speleothem samples. Concentration factors are presented to 3 or 4 significant figures, and the trace element concentrations of water sample b from the lower site were rejected, due to probable contamination.
Table 6.6: Percentages of each mineral in the rock samples analysed by XRF.

<table>
<thead>
<tr>
<th>Sample</th>
<th>SiO₂</th>
<th>CaCO₃</th>
<th>MgCO₃</th>
<th>Fe₂O₃</th>
<th>TiCO₃</th>
<th>MnCO₃</th>
<th>Al₂O₃</th>
<th>Na₂O</th>
<th>K₂O</th>
<th>P₂O₅</th>
<th>SO₃</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrix 1</td>
<td>1.19</td>
<td>95.18</td>
<td>0.88</td>
<td>0.14</td>
<td>0.04</td>
<td>0.03</td>
<td>0.22</td>
<td>&lt;0.1</td>
<td>0.06</td>
<td>0.02</td>
<td>&lt;0.01</td>
<td>97.80</td>
</tr>
<tr>
<td>Acid-res. 1a</td>
<td>45.81</td>
<td>51.63</td>
<td>0.54</td>
<td>0.36</td>
<td>0.09</td>
<td>0.02</td>
<td>0.96</td>
<td>&lt;0.1</td>
<td>0.32</td>
<td>0.05</td>
<td>&lt;0.01</td>
<td>99.88</td>
</tr>
<tr>
<td>Less-res. 1a</td>
<td>9.61</td>
<td>87.81</td>
<td>0.82</td>
<td>0.48</td>
<td>0.09</td>
<td>0.03</td>
<td>0.75</td>
<td>&lt;0.1</td>
<td>0.25</td>
<td>0.05</td>
<td>&lt;0.01</td>
<td>100.02</td>
</tr>
<tr>
<td>Darker 1b</td>
<td>28.91</td>
<td>69.54</td>
<td>0.52</td>
<td>0.13</td>
<td>&lt;0.02</td>
<td>0.34</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>0.11</td>
<td>0.09</td>
<td>&lt;0.01</td>
<td>99.74</td>
</tr>
<tr>
<td>Matrix 2b</td>
<td>6.80</td>
<td>91.20</td>
<td>0.96</td>
<td>0.27</td>
<td>0.06</td>
<td>&lt;0.01</td>
<td>0.42</td>
<td>&lt;0.1</td>
<td>0.15</td>
<td>0.03</td>
<td>&lt;0.01</td>
<td>99.98</td>
</tr>
</tbody>
</table>

Table 6.7: Percentages of Ca²⁺, Mg²⁺ and Na⁺ in the rock and speleothem samples, as measured by flame atomic adsorption spectroscopy, with comparisons with concentrations measured by XRF.

<table>
<thead>
<tr>
<th>Sample</th>
<th>[Ca(II)] % by flame AAS</th>
<th>% CaCO₃ by flame AAS</th>
<th>% CaCO₃ by XRF</th>
<th>[Mg(II)] % by flame AAS</th>
<th>MgCO₃ % by flame AAS</th>
<th>MgCO₃ % by XRF</th>
<th>[Na(I)] ppm by flame AAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>rock 1</td>
<td>38.5</td>
<td>96.3</td>
<td>95.18</td>
<td>0.24</td>
<td>0.83</td>
<td>0.88</td>
<td>226.6</td>
</tr>
<tr>
<td>1a acid-res</td>
<td>21.9</td>
<td>54.9</td>
<td>51.63</td>
<td>0.14</td>
<td>0.49</td>
<td>0.54</td>
<td>274.5</td>
</tr>
<tr>
<td>1a less-res</td>
<td>33.5</td>
<td>83.8</td>
<td>87.81</td>
<td>0.22</td>
<td>0.75</td>
<td>0.82</td>
<td>298.4</td>
</tr>
<tr>
<td>2a</td>
<td>37.5</td>
<td>93.7</td>
<td>93.4</td>
<td>0.25</td>
<td>0.85</td>
<td>0.96</td>
<td>296.2</td>
</tr>
<tr>
<td>2b</td>
<td>37.3</td>
<td>93.4</td>
<td>91.20</td>
<td>0.26</td>
<td>0.90</td>
<td>0.96</td>
<td>260.4</td>
</tr>
<tr>
<td>cal straw</td>
<td>38.8</td>
<td>97.0</td>
<td>0.040</td>
<td>0.137</td>
<td>0.057</td>
<td>300.5</td>
<td></td>
</tr>
<tr>
<td>B+W</td>
<td>39.5</td>
<td>98.7</td>
<td>0.017</td>
<td>0.057</td>
<td>338.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boh1 stal</td>
<td>39.8</td>
<td>99.5</td>
<td>0.021</td>
<td>0.073</td>
<td>311.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boh1 hel</td>
<td>39.3</td>
<td>98.3</td>
<td>0.022</td>
<td>0.076</td>
<td>309.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boh2 stal</td>
<td>38.5</td>
<td>96.3</td>
<td>0.011</td>
<td>0.098</td>
<td>287.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 6.8: R% or MgCO₃:CaCO₃ ratios of the rocks and speleothems.

<table>
<thead>
<tr>
<th>Sample</th>
<th>XRF %MgCO₃</th>
<th>AAS %MgCO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrix 1</td>
<td>0.92</td>
<td>0.86</td>
</tr>
<tr>
<td>Acid-res. 1a</td>
<td>1.05</td>
<td>0.89</td>
</tr>
<tr>
<td>Less-res. 1a</td>
<td>0.93</td>
<td>0.89</td>
</tr>
<tr>
<td>Darker 1b</td>
<td>0.75</td>
<td>0.91</td>
</tr>
<tr>
<td>Matrix 2a</td>
<td>1.05</td>
<td>0.96</td>
</tr>
<tr>
<td>calcite stal</td>
<td></td>
<td>0.14</td>
</tr>
<tr>
<td>B+W</td>
<td></td>
<td>0.058</td>
</tr>
<tr>
<td>Boh1 stal</td>
<td></td>
<td>0.073</td>
</tr>
<tr>
<td>Boh1 hel</td>
<td></td>
<td>0.077</td>
</tr>
<tr>
<td>Boh2 stal</td>
<td></td>
<td>0.102</td>
</tr>
</tbody>
</table>

### Table 6.9: Concentrations of trace elements in the rock and speleothem samples from Bohemia Cave, plus a calcite stalactite from Profanity Cave.

<table>
<thead>
<tr>
<th>Sample</th>
<th>[Fe(III)] ppm, by flame AAS</th>
<th>[Fe(III)] ppm, by XRF</th>
<th>[Al(III)] ppm, by flame AAS</th>
<th>[Al(III)] ppm, by XRF</th>
<th>[Cu(II)] ppm, by furnace AAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>rock 1</td>
<td>301.8</td>
<td>200.2</td>
<td>400.9</td>
<td>415.7</td>
<td>10.1</td>
</tr>
<tr>
<td>1a light</td>
<td>596.5</td>
<td>514.7</td>
<td>1955.2</td>
<td>1813.9</td>
<td>36.8</td>
</tr>
<tr>
<td>1a dark</td>
<td>537.3</td>
<td>686.3</td>
<td>1447.2</td>
<td>1417.1</td>
<td>37.0</td>
</tr>
<tr>
<td>2a</td>
<td>488.1</td>
<td>1031.2</td>
<td>5.6</td>
<td></td>
<td>39.4</td>
</tr>
<tr>
<td>2b</td>
<td>545.4</td>
<td>386.0</td>
<td>705.1</td>
<td>793.6</td>
<td>34.4</td>
</tr>
<tr>
<td>calcite stal</td>
<td>42.7</td>
<td>93.2</td>
<td></td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>B+W</td>
<td>54.3</td>
<td>136.7</td>
<td></td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>Boh1 stal</td>
<td>65.6</td>
<td>81.5</td>
<td></td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>Boh1 hel</td>
<td>63.9</td>
<td>98.3</td>
<td></td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>Boh2 stal</td>
<td>55.6</td>
<td>194.5</td>
<td></td>
<td>5.0</td>
<td></td>
</tr>
</tbody>
</table>

### Table 6.10: Concentration factors for partitioning of metal ions between the cave waters and the speleothem samples.

<table>
<thead>
<tr>
<th></th>
<th>Fe³⁺</th>
<th>Al³⁺</th>
<th>Na⁺</th>
<th>Mg²⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>lower site</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>helictite</td>
<td>12060</td>
<td>8402</td>
<td>373</td>
<td>53.7</td>
</tr>
<tr>
<td>stalactite</td>
<td>12380</td>
<td>10130</td>
<td>375</td>
<td>51.2</td>
</tr>
<tr>
<td>upper site</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>stalactite</td>
<td>13080</td>
<td>22880</td>
<td>335</td>
<td>200</td>
</tr>
</tbody>
</table>
The two trace elements Fe$^{3+}$ and Al$^{3+}$ were concentrated into the speleothems much more than were the minor elements. Concentration factors were highly consistent between the two samples from the lower sampling site, but more aluminium and magnesium was concentrated into the sample from the upper site. Increased aluminium and magnesium uptake may be due to the decay of the speleothem since its growth stopped, especially if it has been subjected to flooding in the past.

6.3.3 Electron Microscopy of Stalactite Samples

In the calcite stalactite sample, collected from Profanity Cave, electron microscopy indicated that a number of minerals were present as solid inclusions. While aluminium-rich clays, quartz, pyrite, and zircon were found in the inclusions, random EDAX sampling of calcite crystals showed no measurable trace metal concentration in the calcite crystals which made up the bulk of the stalactite. In contrast to this, no inclusions were observed in the Bohemia stalactites. Trace metal levels of randomly-selected aragonite crystals were also below the detection limit of the EDAX technique, indicating that metal concentrations were less than approximately 1%. Total trace metal ion concentrations of the calcite stalactite were approximately the same as for the aragonite speleothems. However, because some of the trace metals in the calcite sample were present in separate solid inclusions, this may indicate that fewer trace metal ions were incorporated in the calcite lattice than in the aragonite lattice. Without knowledge of the concentrations of metal ions available at each site, the flow rates involved, or the actual metal ion concentration in the calcite crystals, this possibility cannot be confirmed.

Each of the four speleothems from Bohemia Cave was examined by electron microscopy, in horizontal and vertical cross-section. External surfaces were also studied, particularly the tips of the samples, where active crystal growth was occurring. A number of interesting features were observed, and are discussed for each speleothem.

Speleothems from the Lower Site

In both horizontal and vertical cross-section, the stalactite from the lower collecting site was seen to consist of large featureless crystals, hundreds of microns in length (Photo 22). Cleavages were sharp, as can be seen in the top right-hand corner of Photo 23. The aragonite crystals consisted of long, thin flattened needles (10–30 μm thick), and end-on views are shown in Photos 24 and 25. These two micrographs are views of the tip of the stalactite, showing the interlocked pattern the crystals have grown in. A crystal with a wedge-shaped tip is clearly visible in the bottom right-hand corner of Photo 25. As well as the aragonite crystals, Photo 26 focuses on one of a number of flat layered crystals growing on the outside of the stalactite tip, perpendicular to the aragonite needles. This crystal was found to contain Mg and Si, and may be a layered calcium magnesium silicate or carbonate. Because these elements are not taken up by the aragonite lattice, they can become concentrated in the water from which the speleothem is growing, eventually forming a separate phase on its surface. The presence of sufficient Mg$^{2+}$ to do so in these circumstances is evidence in favour of the solution containing sufficient Mg$^{2+}$ to influence the mineralogy of the speleothems.
Photo 22: A vertical cross-section of the stalactite sample collected from the lower site in Bohemia Cave.

The tip of a branch of the helictite showed a similar pattern of interlocking wedges (Photos 27, 28 and 29), but without the randomness of orientation of the tip of the stalactite. The layered Mg-containing surface material also occurred in this sample, with a typical crystal in the top left-hand corner of Photo 28. Crystals in the helictite were smaller than in the stalactite, but were still hundreds of microns long. Photo 30 portrays a broken-off horizontal cross-section, showing needles cleaved on the inside of the speleothem, and flaring out to terminate on the surface of the speleothem. A magnified view is given in Photo 31, showing unbroken crystals terminating in wedges. The lustrous appearance of aragonitic speleothems is a consequence of these flared crystals, resulting because some of the length of the crystals can be seen. In the centre of this sample, a region approximately 100 μm in diameter was observed which was partially filled with fibrous crystals (Photo 32), and was most likely to be a remnant capillary.

The Stalactite from the Upper Site

The remnant infilled straw core of this sample consisted of ordered crystals with few surface features, and sharp jagged cleavage at right angles to the length of the straw (Photos 33 and 34). Towards the outside of the core, corrosion has occurred, and crystals are jagged and partially dissolved (the left of Photo 33). The outer layers of the stalactite are severely corroded (Photos 35 and 36), with dissolution occurring from the edges of the crystal plates.

The Black and White Minstrels

A cleavage surface of a horizontal cross-section of the Black and White Minstrel speleothem is shown in Photo 37. As with the other speleothems collected, this Black and
6.3. Analysis of the Rock and Speleothem Samples

Photo 23: A single surface of the vertical cross-section of the stalactite sample collected from the lower site in Bohemia Cave.

Photo 24: The tip of the stalactite sample from the lower site of the cave.
Photo 25: A close-up of the centre of the tip of the stalactite from the lower sampling site.

Photo 26: Layered platy crystals on the surface of the tip of the stalactite from the lower sampling site.
6.3. Analysis of the Rock and Speleothem Samples

Photo 27: The tip of a branch of the helictite sample collected from the lower sampling site. Note the flattened "s"-shape formed by some of the crystals, which extends from the centre of the sample towards the right.

Photo 28: A closer view of the tip of the helictite branch. The wedge-shaped ends of the aragonite crystals can be seen.
Photo 29: Another view of the tip of the helictite, showing the crystals interweaving.

Photo 30: The edge of the helictite collected from the lower sampling site.
6.3. Analysis of the Rock and Speleothem Samples

Photo 31: A close-up of the edge of the helictite, showing both cleaved and unbroken aragonite crystals.

Photo 32: A remnant capillary tube in the helictite, filled with fine needles.
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Photo 33: The edge of a horizontal cross-section of the stalactite collected from the upper sampling site.

Photo 34: A single surface of a crystal from the stalactite collected from the upper sampling site.
6.3. Analysis of the Rock and Speleothem Samples

Photo 35: The inside surface of the corroded outer material of the stalactite collected from the upper sampling site.

Photo 36: A close-up of the crystals of the corroded outer material from the stalactites.
White Minstrel sample consisted of long flattened aragonitic needles, but its crystal size was considerably less (Photo 38), and the speleothem was not so homogeneous in internal structure. Photo 39 is a fan-shaped cleavage feature, consisting of very thin needles (Photo 40).

Black layering on speleothems has most commonly been explained in the literature as due to Mn, often birnessite Na$_4$Mn$_{14}$O$_{27}$·9H$_2$O). The rock samples from Bohemia Cave contain minor manganese, and manganese nodules can be seen in Bulmer Cave nearby. Therefore the outer layer of the Black and White Minstrels sample was examined for the presence of manganese, with the electron microscope's EDAX facility, which results in a map of the position of any metal ions present, on the surface or within a depth of a few microns, at at least 1%. No signal was observed for manganese; however, manganese oxides are highly discolouring, so minor surface contamination with less than 1% manganese could be sufficient to produce this strong discolouration. The non-uniform distribution of the colouring is difficult to explain if it is a manganese deposit.

Photos 41 and 42 focus on the edge of a horizontal cross-section of the specimen, showing that the surface consists of both long flattened aragonite needles (above the scale bar), and a fine-grained plate-like material. This second material is best seen in the top left-hand corner of Photo 42, and contained up to 5 atomic% silicon, 2% aluminium and 1% magnesium. Such a mineral could be a clay, but it is also difficult to explain its preferential deposition on one side of most of the observed samples. Often, the colouring was observed to be on the top of speleothems (see Photo 10), and could be the result of an air-borne contaminant such as ash, a fungus or another carbonaceous deposit (Hill, 1982).
6.3. Analysis of the Rock and Speleothem Samples

Photo 38: A detail from a vertical cross-section of the Black and White Minstrel speleothem.

Photo 39: A fan-shaped feature in the Black and White Minstrels speleothem.
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**Photo 40:** A close-up of the crystals of the fan-shaped feature in the Black and White Minstrels speleothem.

**Photo 41:** A view of the edge and outside surface of the Black and White Minstrels speleothem.
6.4 Summary

The Bohemia Cave system waters sampled, and the surrounding water, were unpolluted, being lower than an average stream in all trace elements. Likewise, the rock and speleothem samples contained only low concentrations of trace elements, and were mineralogically simple. The rocks consisted of calcite and a variable amount of quartz, and all but one of the stalactite samples was pure aragonite. Magnesium concentrations of the sampled rocks and water were too low to decisively conclude that the cause of aragonite formation was the presence of magnesium, and it is proposed that slow deposition rates may also be a factor in the deposition of aragonite in this cave system.

Examination of the speleothem samples under an electron microscope indicated that they were formed of long flattened needles of aragonite, which radiated outwards to terminate on the edges of the speleothem. The thin brown-black coating on the Black and White Minstrel sample cannot be explained by the presence of a manganese mineral, and may instead be due to a carbonaceous deposit.
Part IV

The Interaction of $\text{Co}^{2+}$, $\text{Ni}^{2+}$, and $\text{Cu}^{2+}$ with \textit{Corallina officinalis}
Chapter 7

Biomineralization and Coralline Algae

7.1 Corallina officinalis Linnaeus

7.1.1 Description and Ultrastructure

Corallina officinalis Linnaeus is a coralline alga, a member of the division Rhodophyta (the red algae), belonging to the class Rhodophyceae, of the subclass Florideophycidae, of the order Corallinales\(^1\). The most obvious distinguishing feature of the coralline algae is their biomineralization, with (mainly) high-Mg calcite deposited within their cell walls (Kerkar, 1994). While many other marine algae also deposit CaCO\(_3\), few do so to the extent of the coralline algae, and aragonite is the most common biomineral in marine algae, rather than calcite. Coralline algae are frequently categorized as either crustose or erect, and erect forms as either articulated or non-articulated. C. officinalis is an articulated erect species, which attaches to a solid substrate by a calcareous holdfast. Adult plants (Figure 7.1) are small (generally 1–6 cm long, although up to 12 cm in tide pools), branched and dull purple-red. Articulation is provided by the existence of two different types of cells: tiers of calcified cells 60–90 \(\mu\)m long the intergenicular segments, and uncalcified genicular cells. Branching occurs at the genicular cells, which form after every 14–18 tiers of calcified cells, and grow to 250 \(\mu\)m long.

The ultrastructure of the whole plant of C. officinalis was examined by Borowitzka and Vesk (1978) and Cabioch and Giraud (1986), and of the epithallial (outermost) cells by Pueschel et al. (1996). Borowitzka and Vesk distinguished between three different kinds of cells: epidermal cells (equivalent to the epithallial cells of Pueschel et al., on the surface of the thallus; the internal thallus cells, which form the intergenicular segments, and the genicular cells. The term “thallus” is used instead of terms like “stem”, to describe plant material which is not differentiated into leaves and stems. Scanning electron micrographs of the plants collected for this study are shown in Photos 43 (a

\(^{1}\)An older classification scheme, still frequently used, has the coralline algae as a family (Corallinaceae) within the order Cryptonemiales
Figure 7.1: A sketch of *Corallina officinalis*, showing details of a single branch. From Adams (1994). The single branch shown in the detail is 0.5–1 cm in length.

The epidermal cells have thick cell walls which sometimes form ingrowths into the cells. Borowitzka and Vesk proposed that the cell wall ingrowths promote solute transport, by increasing the surface area of the wall in contact with the cell contents. The walls are calcified, except for the section which is in contact with the surface of the thallus. The surface layer of cells is periodically replaced, with the pit connection between the forming cell and the old cell being blocked off by cell wall, and the dead cell eventually sloughing off. Pueschel *et al.* (1996) found that, while several layers of epithallial cells could exist at once, only one layer had a functioning pit connection between its cells and the next-most inner layer. The epidermis was effectively one cell thick. Loss of the outer layer of cells could be a mechanism to reduce build-up of epiphytes on the plant, as any organisms growing on its surface would be lost with the shed cells.

The internal thallus contains cortical cells and medullary cells. Medullary cells form an inner core connecting the genicula. They contain starch grains, and have few chloroplasts or other organelles, and tend to be dead in the oldest parts of the plant. Inner cortical cells are similar, though with more organelles. As cells become closer to the surface, the amount of starch they store reduces, and the number of chloroplasts increases. The presence of thick calcified walls acts to reduce the level of light reaching internal cells, making them more suitable for energy storage than production. Calcium carbonate crystals in the outside of the cell walls (closest to the cell interior) are rods approximately 350 nm long, oriented with their c-axes perpendicular to the cell surface. Further inside the cell wall, the crystals are larger, and there is an inner layer of wall which is not calcified.

The flexibility provided by the thick-walled, uncalcified genicular cells protects the
7.1. *Corallina officinalis* Linnaeus

**Photo 43:** A vertical cross-section of *C. officinalis*.

**Photo 44:** A section showing the cell wall of *C. officinalis*. 
Chapter 7. Biomineralization and Coralline Algae

7.1.2 Habitat

Coralline algae, including *C. officinalis*, often dominate the subtidal and lower intertidal region on rocky shores. *C. officinalis* and other species can form "meadows" on the sides of rock pools (Photo 45). They are tolerant of a wide range of light conditions, although some of this tolerance is by formation of sun and shade morphotypes (Häder *et al.*, 1997), and they grow best under lower light conditions. Plants are long-lived and slow-growing, with *C. officinalis* found to have a mean growth of almost 3 mm after six weeks in culture (Colthart and Johansen, 1973). *C. officinalis* are ubiquitous in the temperate zone, growing in water temperatures up to >20°C. They are not tolerant of desiccation, with only those species which can trap sufficient moisture surviving out of water. Substantial water movement seems to be a requirement, with plants growing well in areas where wave action is pronounced.

7.2 Biomineralization

Biomineralization may favour an organism in a number of ways. A hard skeleton, either internal or external, provides strength and protection. For the coralline algae, in which up to 85% of the plant’s dry mass is CaCO₃, the strength provided may allow the plants to thrive in areas with high wave action. A high mass of undigestible CaCO₃ also gives the algae a low calorific value, which make them less palatable to grazing animals. Meyer and Paul (1995) studied Neomeris annulata, a green alga...
which deposits aragonite, and also produces a brominated sesquiterpene, which fish find unpalatable. The study concluded that the combination of the two defences was effective at reducing grazing of the alga. Deposition of Ca and Mg in the cell wall also provides a method of controlling intracellular concentrations of these elements, and may function as a detoxification technique.

The coralline algae have a major effect in terms of world-wide production of CaCO₃. According to Adey (1998), 50–80% of the carbonate mass of a “coral” reef consists of gaps filled with carbonate sand. Particularly for deeper reefs, this carbonate sand is dominated by fragments of Halimeda species (a green alga), and coralline algae. Crustose coralline algae play a very significant role within coral reefs in cementing carbonate debris, without which erosion rates would be greatly increased.

Medakovic et al. (1995) examined the composition of the biominerals of nine species of coralline algae from the Adriatic Sea, with X-ray diffraction, and found that magnesium-containing calcite was the dominant phase in almost all of the species. The percentage of Mg was dependent primarily on water temperature, and varied from 16–20% in their study area. In two samples, aragonite was dominant, and one sample contained 5% dolomite. For most species, and especially Pseudolithophyllum expansum, the mineral deposited varied, with different results from different sites. C. officinalis was found to contain at least 99% high-Mg calcite. Findings of Kerkar (1994) were similar, with the coralline algae examined depositing almost totally high-Mg calcite. Interestingly, he also found minor amounts of quartz in one coralline species, and in other (non-coralline) calcifying algae, found halite, illite, and feldspar. He attributed these minerals to contamination of the plant by detritus or epiphytic organisms.

Biomineralization is an interesting combination of the chemical principles of nucleation and crystal growth, with biological mediation by such mechanisms as ion pumps and organic matrices. A good summary of both the chemical and biological principles is given in Simkiss (1986). Mann and his colleagues (Mann et al., 1989; Walsh and Mann, 1995) are one of a number of groups which have focussed on the practical applications of biominerals, using the same principles to produce matrix-mediated aragonite precipitates of controlled shape.

The mechanisms of biomineralization vary with the organism, and have been conveniently divided into two broad groups (Simkiss, 1986). The first category has been termed “biologically-induced”, implying a high level of control by the depositing organism, and the second “organic matrix-mediated”, in which the shape and polymorphism of the mineral are affected (to a greater or lesser degree) by the organic material which forms the framework of the area of deposition. The distinction between the two categories is extremely blurred. An algal example of biologically-induced biomineralization is formation of the plates which make up the skeletons of coccolithophorids, which are formed in the golgi apparatus of the cell, and transported in vesicles to their final positions. Deposition of high-Mg calcite in coralline algae is an example of matrix-mediated deposition.

Crystals in the cell walls of coralline algae are in close association with the organic material making up the cell wall. Cabioch and Giraud (1986) describes the crystals in the youngest, outermost parts of the plants as needles parallel to the polysaccharide fibrils of the cell walls, with plates rather than needles forming towards the base of
the outermost cells. These crystals are oriented tangentially to the cell. Further into the thallus, secondary crystals also form between polysaccharide fibrils, and are radial rather than tangential. As the centre of the plant is approached, calcification increases, reflected by an increase in the radial crystals, with the tangential crystals still produced. The walls of mature cells are made up of these two parts, a thin "primary" layer, with tangential polysaccharide fibrils, and a "secondary" part, with radial polysaccharides. The orientation of the high-Mg crystals reflects the orientation of the organic matrix.

Algal biomineralization is also strongly dependent on photosynthesis (Mueller and Haywick, 1995), with calcification rates in the light being 3–4 times those in the dark (Johansen, 1981). Also, a direct relationship was found between the amount of $^{14}$C taken up by photosynthesis and the amount incorporated into calcite (with a ratio of 1.03). Four models have been proposed to explain this photosynthetic uptake effect.

1. The simplest, and oldest, model suggests deposition of CaCO$_3$ is a consequence of the utilization of CO$_2$ from seawater around the alga, during photosynthesis. For the reaction:

$$Ca^{2+} + 2HCO_3^- \rightleftharpoons CaCO_3(s) + H_2O + CO_2$$

(7.1)

removal of CO$_2$ pushes the equilibrium of the reaction to the right, promoting CaCO$_3$ formation.

There are two major objections to this theory. Firstly, it predicts that all photosynthesizing algae and water plants should calcify, because they would all produce this local reduction in CO$_2$ concentration by the plants. Secondly, it does not allow for plant mediation of mineral shape, morphology or polymorphism.

2. A second theory is the "electrophoretic proton uptake model", similar to the above theory, but used to explain calcification in Chara corallina. Chara corallina is a giant-celled alga, which is very convenient for the study of calcification, because its cells consist of non-calcified acidic bands alternating with calcified alkaline bands. In the electrophoretic model, both HCO$_3^-$ and Ca$^{2+}$ diffuse towards the calcifying area from the surrounding water. HCO$_3^-$ then reacts to give CO$_3^{2-}$ and H$^+$, a process which is driven by cellular transport of H$^+$, which is taken up by HCO$_3^-$ in the acid band to form CO$_2$ for photosynthesis. The removal of H$^+$ on the surface of the alkaline band results in sufficient CO$_3^{2-}$ to cause local supersaturation with CaCO$_3$, which precipitates. This model is shown on the left-hand side of Figure 7.2.

A problem with the model is that it does not allow for transport of any ions but H$^+$ through the cell. When McConnaughey (1995) added $^{14}$C-labelled HCO$_3^-$ to only the acid bands of C. corallina, he found that $^{14}$C was incorporated into the deposited calcite in the alkaline bands. The only way this would have been possible is if cellular transport of C occurred. He estimated that at least 74% of the carbon for calcification came from the acid bands.

3. A third theory was proposed by Digby (1977), and involves control of cellular pH by reduction of H$^+$. The first step is the photosynthetic splitting of water to give molecular oxygen, protons and electrons:

$$H_2O \rightarrow \frac{1}{2}O_2 + 2H^+ + 2e^-.$$  

(7.2)
7.2. Biomineralization

Electrophoretic H\(^+\) Uptake

\[
\begin{align*}
\text{HCO}_3^- & \rightarrow \text{CO}_2^- + \text{H}^+ \\
\text{Ca}^{2+} + \text{CO}_3^- & \rightarrow \text{CaCO}_3
\end{align*}
\]

ATP powered Ca\(^{2+}/2\text{H}^+\) Exchange (CORAL)

\[
\begin{align*}
\text{Ca}^{2+} + \text{CO}_2 + \text{H}_2\text{O} & \rightarrow \text{CaCO}_3 + 2\text{H}^+ \\
2\text{HCO}_3^- + 2\text{H}^+ & \rightarrow 2\text{CO}_2 + 2\text{H}_2\text{O}
\end{align*}
\]

\[
\begin{align*}
\text{HCO}_3^- + \text{H}^+ & \rightarrow \text{CO}_2 + \text{H}_2\text{O}
\end{align*}
\]

**Figure 7.2:** A comparison of the Electrophoretic H\(^+\) Uptake and the CORAL models for the interaction of biomineralization and photosynthesis in *Chara corallina*. Modified from McConnaughey (1995).

The \(\text{O}_2\) diffuses out of the cell, and the electrons are transported through the cell by metabolic action. They are recovered from the reducing pool, and reacted with the \(\text{H}^+\), producing hydrogen radicals:

\[
\text{H}^+ + \text{e}^- \rightarrow \text{H}^- \quad (7.3)
\]

Digby proposed that this reaction involves oxidases, and produces excess \(\text{OH}^-\), which reacts with \(\text{HCO}_3^-\) to produce \(\text{CO}_3^{2-}\).

\[
\text{OH}^- + \text{HCO}_3^- \rightarrow \text{CO}_3^{2-} + \text{H}_2\text{O} \quad (7.4)
\]

Other ions also react with the electrons, including \(\text{HCO}_3^-\):

\[
2\text{HCO}_3^- + 2\text{e}^- \rightarrow 2\text{H}^- + 2\text{CO}_3^{2-} \quad (7.5)
\]

The \(\text{CO}_3^{2-}\) diffuses out of the cell, where it partly dissociates, to re-form \(\text{HCO}_3^-\), and raise the pH:

\[
2\text{CO}_3^{2-} + \text{H}_2\text{O} \Leftrightarrow 2\text{HCO}_3^- + 2\text{OH}^- \quad (7.6)
\]

The raising of the pH creates local supersaturation with \(\text{CaCO}_3\) in the solution in the cell walls, and calcium carbonate is deposited by

\[
\text{Ca}^{2+} + \text{HCO}_3^- + \text{OH}^- \Leftrightarrow \text{CaCO}_3 + \text{H}_2\text{O} \quad (7.7)
\]

or by

\[
2\text{Ca}^{2+} + 2\text{CO}_3^{2-} \Leftrightarrow 2\text{CaCO}_3 \quad (7.8)
\]
This model adequately explains both the evidence for cellular transport of ions, and the organism's control over the nature of the crystals formed.

4. A fourth model was developed by McConnaughey, and is called the CORAL (CO₂ Rate Limited) hypothesis. It was initially used to study partitioning of isotopes in corals, and was adapted to Chara corallina in McConnaughey (1995). The model involves ATP-driven exchange of Ca²⁺ and H⁺ at the alkaline sites of calcification, instead of simple diffusion. Two H⁺ exchange for one Ca²⁺, and are transported to the acid band, where they react with 2HCO₃⁻ to give CO₂. One of these CO₂ molecules is used for photosynthesis, the other moves to the alkaline band, and is incorporated in the deposited CaCO₃. The observed 1:1 ratio between photosynthesis and calcification is thus explained. The CORAL model is shown on the right-hand side of Figure 7.2.

All of these models include an explanation of how supersaturation with respect to CaCO₃ can be reached in the solution from which the crystal is actually growing, one of the vital chemical requirements for deposition to occur. Cabioch and Giraud (1986) observed that coralline algae in culture may be less calcified than those which grow in the ocean. It has been suggested that the lower level of Ca²⁺ in many culture media is responsible for this effect. For example, Hepperle and Krienitz (1997) found that the zoospores of Phacotus lenticularis (Phacotaceae, Chlorophyta), a lake-dwelling unicellular alga, required that the medium in which they were formed be supersaturated with respect to CaCO₃, for calcification to occur. The level of calcification was dependent on the degree of supersaturation.

There is evidence that the concentration of HCO₃⁻ and CO₃²⁻ may be the limiting factor for calcification in seawater, rather than the Ca²⁺ concentration, or the total dissolved CO₂ (Gao et al., 1993). An increase in the CO₂ concentration of an enhanced seawater medium lowered both pH and calcification rate for the alga Corallina pilulifera. Elevation of the dissolved CO₂ concentration in seawater at constant pH caused an increase in deposition of CaCO₃.

### 7.3 Metal Cation Uptake by Algal Cells

Copper is an essential element for marine algae, including the Rhodophyta (Kain and Norton, 1990), as a component of such enzymes as amine oxidase and cytochrome c oxidase (Gledhill et al., 1997). Cobalt is also required by many red seaweeds, in the form of vitamin B₁₂, and increases the speed of ion uptake in the light (Kain and Norton, 1990). In land plants, excess cobalt inhibits growth (Liu et al., 1998). A requirement for nickel has not been demonstrated.

Copper is highly toxic in excess, but what concentration is "in excess" is not clear for macroalgae. The optimal concentration of free Cu²⁺ for phytoplankton has been found to be 10⁻¹³–10⁻¹¹ mol L⁻¹, but macroalgae may be more tolerant. A free Cu²⁺ concentration higher than 1 nM is likely to be detrimental to the health of algae. Toxicity of complexed Cu²⁺ is dependent on the nature of the complex, with labile inorganic
7.3. Metal Cation Uptake by Algal Cells

![Diagram of metal cation uptake by algal cells]

Figure 7.3: The interaction between free and complexed Cu$^{2+}$ in seawater, with Cu$^{2+}$ taken up by an algal cell. Cu$\text{L}_I$ is Cu$^{2+}$ complexed to a carrier complex, and Cu$\text{L}_I$ to an internal ligand. From Gledhill et al. (1997).

Three possible mechanisms for trace metal uptake into aquatic plant cells have been proposed (Morrison, 1989).

- Some metal species may directly diffuse through the cell membrane. This route is probably only important for small lipid-soluble species, such as the highly toxic organo-mercury species.

- Cu$^{2+}$, and other essential metal ions, may be taken up actively into the cell, through complexation by "transfer" ligands across the cell membrane (see Figure 7.3). Once inside the cell, the cell concentration of Cu$^{2+}$, which is much higher than the concentration in seawater, is maintained by complexation of Cu$^{2+}$ with internal binding agents (Williams, 1981). The precise nature of either the transport or internal ligands has not been determined. Morrison (1989) describes lasolocid A, a chain molecule which has both lipophobic and lipophilic conformations, as a possible example.

- An active pathway for major nutrients exists, involving transport by ATPase through an ion channel. This pathway may also transport trace nutrients (Morrison, 1989).

Algae may also release compounds such as polyphenols into the seawater which bind Cu$^{2+}$ and similar metals, reducing their toxicity.

Starý and Kratzer (1984) studied dried cells of Scenedesmus obliquus, a chlorophyte microalga, using radio-labelling to examine uptake of metal cations. They concluded...
that cell walls behave like weakly acidic cation exchangers. In natural waters they found the cell walls would be mainly coordinated with Ca\(^{2+}\) and Mg\(^{2+}\), and that trace elements, such as transition metal ions, would be accumulated by other ligands than those responsible for uptake of the alkali and alkaline earth cations. In acidic media, metal ions would be replaced by H\(^+\). Release of these H\(^+\) into solution as the pH increases both explains the buffering capacity of a microalgal-containing solution, and illustrates the potential complexity of a chemical system containing them. Sigg (1987) notes that Cu\(^{2+}\) will preferentially bind to amino acid groups, followed by carboxylic or hydroxo groups. While zinc, silver, magnesium and cadmium showed mainly electrostatic effects in exchange with calcium on the surface of Vaucheria (Heterokontophyta, Xanthophyceae), copper and lead had a more covalent style of bonding.

No studies have been found on the uptake of metal ions into the biominerals of coralline algae. McConnaughey (1995) reported that Mn\(^{2+}\) and Sr\(^{2+}\) were deposited along with Ca\(^{2+}\) when the culture medium contained Mn\(^{2+}\) and Sr\(^{2+}\). In a study of rhodophytes at Antikyra Gulf (Greece), Malea et al. (1994) found Corallina elongata concentrated Cu\(^{2+}\) by 100-2100 times, and another coralline alga, Jania rubens, concentrated it 200-17700 times. These concentration factors were higher than for non-calcifying rhodophyte species. Total concentrations were measured, rather than concentrations of either the organic material or the biomineral. The coralline algae (and the species of Liagora, an aragonite-depositing alga) also had higher Ca\(^{2+}\) and Mg\(^{2+}\) concentrations, presumably due to their fixation as biominerals, and lower Fe, Na\(^+\) and K\(^+\) concentrations than the other species studied.

Another localised study of seawater and algal metal concentrations (Muse et al., 1999) found that the green algae Ulva lactuca and Enteromorpha prolifera showed a positive correlation between plant and seawater concentration for zinc, copper, chromium, and lead. The rhodophyte Porphyra columbina showed a positive correlation for only copper and zinc, with no detectable uptake of lead. These results indicate that, while marine algae may be useful biomonitors of metal ion pollution, each individual species needs to be assessed, probably at each individual site. Similarly, Leal et al. (1997) assessed the usefulness of two Enteromorpha and two Porphyra species for biomonitoring. They found that variability of metal concentrations within a particular sampling site was low, and that variability between different sites was high. Concentrations of metals in the seawater and the seaweed both showed seasonal variation (except for copper in the seaweed), but the trends were different. A reverse relationship existed for cadmium, and no apparent relationship for copper, mercury or lead. However, when variation between each of the locations was considered, the mean concentration of metal in the seaweed (averaged for the whole 8 months of the study) correlated well with seawater concentrations. It has been suggested (Muse et al., 1999; Gledhill et al., 1997) that some of the differences between seawater and seaweed metal concentrations may simply be due to the speciation of metal in seawater. Clearer results may be yielded by studies which compare algal concentrations with the bioavailable (free and labile) metal in the water, rather than with total metal concentrations.
Chapter 8

Culturing *Corallina officinalis* in a Defined Medium Containing Co(II), Ni(II) or Cu(II): Experimental Methods

8.1 Introduction

In this project, *Corallina officinalis* were grown in a chemically-defined artificial seawater medium polluted with either Cu$^{2+}$, Co$^{2+}$ or Ni$^{2+}$ ions, in order to assess the level of cation uptake. Uptake by growing plants was compared with uptake by killed plants, to consider the effect of cellular processes on cation concentration within the cell and cell walls. Uptake from a medium also containing the level of calcium found in seawater was compared with uptake from a solution containing only half as much calcium, to consider the effect of calcium carbonate saturation level.

8.2 Seaweed Collection

8.2.1 Kaikoura

Initially, algae for this project were collected from the Banks Peninsula area, east of Christchurch. These plants were very small, even from unpolluted beaches, and contained many epiphytic plants and animals. Therefore, algae for later stages of the project, including the final growth runs performed, were collected from First Bay on the Kaikoura peninsula, 180 km north of Christchurch.

The Kaikoura peninsula shoreline consists of wide shelving reefs (Photo 46), with large rockpools developed in the cracks and the ends of channels in the reefs. *Corallina officinalis* are very abundant and well formed, with large healthy plants relatively free of epiphytes (Photo 47). The Kaikoura peninsula is a rural area, focusing on tourism,
Chapter 8. Culturing Corallina officinalis: Experimental

Photo 46: One of the many cracks in the Kaikoura shore, showing rock pool formation.

and with very little industrial activity, is unpolluted. The bays are small, and rapidly open to the Pacific Ocean, with the Kaikoura Canyon flowing into the deep Hikarangi Trough just offshore. No major rivers flow into the ocean in this area, making natural input of metal ions low.

8.2.2 Concentrations of Trace Metals in Kaikoura Seawater

Samples of seawater were collected from one of the rocky pools containing Corallina officinalis, and analysed by hanging mercury drop stripping voltammetry, to determine the initial trace metal concentration of the water the algae had been growing in.

Clean procedures were adhered to during collection. Acid-washed high density polyethylene bottles were filled with milli-Q water and wrapped in several layers of plastic bag in the cleanroom. Beside the pool, two people put on shoulder length plastic gloves, one to unwrap each bottle, the other to rinse and fill it. The bottles were re-wrapped in the plastic bags.

Each sample was filtered through a 0.45 μm filter and acidified with Aristar HNO₃ to 0.2%. 10 mL samples were digested under a high intensity UV lamp for 30 minutes, to break down organic metal complexes. The concentration of Pb(II) and Cu(II) were determined on undiluted samples by anodic stripping voltammetry at pH 5. Samples were purged with oxygen-free N₂ for 10 min to remove oxygen, and were deposited for 10–15 min onto the mercury drop electrode. Samples were then complexed with dimethylglyoxime and buffered to pH 9 with isopiestically-distilled NH₃/NH₄Cl buffer. Co(II) and Ni(II) were adsorbed onto the mercury drop electrode as the dimethylglyoxime complex (prepared from doubly-sublimed dimethylglyoxime), and reduced by a cathodic scan (method of Gilbert et al. (1988)). The method of standard additions was used to determine metal ion concentrations.
8.3 The Growth Medium

Determination of the concentration of Pb(II) in the seawater gave an indication of heavy metal contamination levels. Initial concentrations of Cu(II), Co(II) and Ni(II) in the synthetic medium used in this study were made the same as the M(II) concentrations in the seawater, so that the effect of an increasing level of pollution could be assessed.

8.3 The Growth Medium

The artificial growth medium was designed to be similar to seawater, particularly in major and trace element concentrations. It was based upon two published media, Aquil (Morel et al., 1979) and MCM (Woelkerling et al., 1983). MCM was specifically designed and tested for the growth of coralline algae, and recommended a low concentration of $\text{B}^{3+}$, $\text{Mg}^{2+}$ and $\text{Ca}^{2+}$, and a reduced concentration of $\text{Na}^{+}$. As biomineralization is dependent on the saturation state of the seawater, the medium for this work was assessed in both Woelkerling et al.'s recommended concentration of 5mM $\text{Ca}^{2+}$, as well as the standard 10.3mM $\text{Ca}^{2+}$ concentration of seawater. Br$^-$ and F$^-$ were found to be unnecessary for the growth of coralline algae, and silicate was not included in the medium, to discourage diatom growth. MCM also contained high concentrations of trace metal ions, which were undesirable for this project.

Aquil was specifically developed for work in trace metal analysis, for use with micro-algal suspensions. In general, it bore more resemblance to seawater than MCM, particularly in concentrations of trace metal ions. It also recognized the necessity of cleanliness, particularly the need to remove contaminant heavy metals such as Pb and Cd from the medium, to avoid poisoning the algae. Trace metal ions in Aquil were complexed with EDTA, to simplify speciation and in particular to avoid precipitation of iron hydroxides. As trace metals complexed with EDTA have reduced bioavailability,
this approach was considered unsuitable for this project. Speciation of the developed medium was calculated, and is reported in Section 9.2.

Table 8.1 gives a comparison of the initial concentrations of the ions in MCM, Aquil and the medium developed for this project, with total concentration of each ion in seawater. Most seawater concentrations were obtained from Stumm and Morgan (1996).

8.3.1 Initial Concentrations

Concentrations of \( Na^+ \), \( SO_4^{2-} \) and \( Cl^- \) in the medium were calculated as follows:

- The concentrations of \( SO_4^{2-} \) from \( CuSO_4 \) and \( ZnSO_4 \) were summed and subtracted from the total required \( SO_4^{2-} \). The remaining \( SO_4^{2-} \) was provided as \( Na_2SO_4 \). In practice, concentrations of \( CuSO_4 \) and \( ZnSO_4 \) were so small they were insignificant.

- Similarly, the concentrations of \( Na^+ \) from \( Na_2SO_4 \), \( NaH_2PO_4 \cdot H_2O \), \( NaN_3 \) and \( Na_2EDTA \) were summed. The remaining \( Na^+ \) was provided as \( NaCl \).

- \( Cl^- \) concentration was calculated by summing chloride concentrations from all the \( Cl^- \)-containing salts. Chloride concentration does not seem to be critical for algal health, so variation from other artificial media was not considered important. Concentrations of chloride provided by the micronutrients were also insignificant.

8.3.2 Making the Medium

The masses of chemicals used in the medium are given in Table 8.2, as are the concentration factors involved. The process is given diagrammatically in Figure 8.3.2. The medium was mixed up in 8 L batches, because this volume could be conveniently handled in an acid-cleaned 10 L polyethylene bottle. All stock solutions were made up in milli-Q water, in the clean room, to avoid anion contamination.

1. The appropriate masses of dry \( KCl \), \( NaCl \) and \( Na_2SO_4 \) were dissolved in minimal water in a 2 L volumetric flask. 358 mL of \( MgCl_2 \) stock, 4.5 mL of \( H_3BO_3 \) stock, and one unit of \( Ca(HCO_3)_2 \) stock were added, and the solution volume was made up to 2.25 L. The solution was then passed through a Chelex-100 column (see Section 8.3.4) to remove heavy metals. 2 L of the clean solution was measured into an acid-washed 10 L polyethylene bottle. Excess medium was made in case of loss due to spillage, for cleaning of the column, and for speciation experiments.

2. It was desirable to add all the \( HCO_3^- \) as \( Ca(HCO_3)_2 \), to maintain the Ca speciation such that calcium carbonate would not precipitate in the solution. Sufficient \( Ca(HCO_3)_2 \) for 2.25 L of stock solution was made by dissolving 2.1438 g of \( CaCO_3 \) (in approximately 100 mL of milli-Q. water) in the stoichiometric quantity of \( HCl \).
Table 8.1: A comparison of initial concentrations of ions and vitamins in the artificial media Aquil and MCM with seawater and the medium developed for this work. Note that these concentrations make no allowance for speciation and do not indicate free ion concentrations.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Aquil$^a$</th>
<th>MCM$^b$</th>
<th>Open seawater$^c$</th>
<th>Mine</th>
<th>Based on</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCO$_3^-$</td>
<td>2.38 mM</td>
<td>2.5 mM</td>
<td>2.38 mM</td>
<td>2.38 mM</td>
<td>Aquil</td>
</tr>
<tr>
<td>Br$^-$</td>
<td>0.84 mM</td>
<td>—</td>
<td>0.00673 g kg$^{-1}$</td>
<td>—</td>
<td>MCM</td>
</tr>
<tr>
<td>Bo$_3^{3+}$</td>
<td>0.485 mM</td>
<td>1 µM</td>
<td>B: 0.0045</td>
<td>1 µM$^d$</td>
<td>MCM</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>10.5 mM</td>
<td>5 mM</td>
<td>10.2 mM</td>
<td>545 mM</td>
<td>—</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>559 mM</td>
<td>505 mM</td>
<td>545 mM</td>
<td>545 mM</td>
<td>—</td>
</tr>
<tr>
<td>Co$^{2+}$</td>
<td>2.5 nM</td>
<td>200 nM</td>
<td>0.004–0.05 nM</td>
<td>0.4 nM</td>
<td>Kaikoura seawater</td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>0.997 nM</td>
<td>300 nM</td>
<td>0.4–1.4 nM</td>
<td>14 nM</td>
<td>Kaikoura seawater</td>
</tr>
<tr>
<td>EDTA$^{4-}$</td>
<td>5 µM</td>
<td>6.6 µM</td>
<td>—</td>
<td>1.2 nM</td>
<td>—</td>
</tr>
<tr>
<td>F$^-$</td>
<td>71.4 µM</td>
<td>—</td>
<td>0.0013 g kg$^{-1}$</td>
<td>—</td>
<td>MCM</td>
</tr>
<tr>
<td>Fe$^{3+}$</td>
<td>0.451 µM</td>
<td>1 µM</td>
<td>0.05–0.5 nM</td>
<td>1 nM</td>
<td>coastal seawater</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>54.6 mM</td>
<td>40 mM</td>
<td>53.2 mM</td>
<td>40 mM$^d$</td>
<td>MCM coastal seawater</td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>23 nM</td>
<td>1000 nM</td>
<td>0.08–5 nM</td>
<td>55 nM</td>
<td>—</td>
</tr>
<tr>
<td>Mo$^{2+}$</td>
<td>1.5 nM</td>
<td>1000 nM</td>
<td>107 nM</td>
<td>4.7 nM</td>
<td>—</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>100 µM</td>
<td>1000 µM</td>
<td>0.5–45 µM</td>
<td>100 µM</td>
<td>aquil</td>
</tr>
<tr>
<td>Ni$^{2+}$</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>80 nM</td>
<td>aquil</td>
</tr>
<tr>
<td>PO$_4^{3-}$</td>
<td>10 µM</td>
<td>30 µM</td>
<td>0.03–3.5 µM</td>
<td>10 µM$^d$</td>
<td>aquil</td>
</tr>
<tr>
<td>K$^+$</td>
<td>10.3 mM</td>
<td>10 µM</td>
<td>10.2 mM</td>
<td>10 µM$^d$</td>
<td>MCM</td>
</tr>
<tr>
<td>SiO$_4^{2-}$</td>
<td>12.5 µM</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>MCM</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>480 mM</td>
<td>457 mM</td>
<td>468 mM</td>
<td>457 mM</td>
<td>MCM</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>28.8 mM</td>
<td>1 mM</td>
<td>28.2 mM</td>
<td>10 mM</td>
<td>—</td>
</tr>
<tr>
<td>Zn$^{2+}$</td>
<td>4 nM</td>
<td>1000 nM</td>
<td>0.06–0.24 nM</td>
<td>2.6 nM</td>
<td>coastal seawater</td>
</tr>
<tr>
<td>Vitamins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cyano-</td>
<td>0.46 nM</td>
<td>0.74 nM</td>
<td>0–0.0036 nM$^b$</td>
<td>0.46 nM</td>
<td>aquil</td>
</tr>
<tr>
<td>cobalamin</td>
<td>2.05 nM</td>
<td>4 nM</td>
<td>—</td>
<td>2.05 nM</td>
<td>aquil</td>
</tr>
<tr>
<td>biotin</td>
<td>0.30 µM</td>
<td>1.48 µM</td>
<td>0–0.000059 µM$^b$</td>
<td>0.3 µM</td>
<td>aquil</td>
</tr>
</tbody>
</table>

$^a$Morel et al. (1979)
$^b$Woelkerling et al. (1983)
$^c$Stumm and Morgan (1996)
$^d$These concentrations were recommended by Woelkerling et al. (1983) as particularly effective for the growth of coralline algae.
$^e$See text
Figure 8.1: The procedure for making the medium. See text for details. This diagram is based on a concept in Morel et al. (1979).
8.3. The Growth Medium

3. Stock solutions of the macronutrients, NaH$_2$PO$_4$·H$_2$O and NaNO$_3$, were batch-treated with Chelex-100 resin to remove heavy metals. The NaH$_2$PO$_4$·H$_2$O solution was stored in an acid-washed glass flask, and the NaNO$_3$ solution in a polyethylene Nalgene bottle, in the clean room. To make 8 L of the final medium, 8 mL of each of the two cleaned stock solutions was added to the major salt solution of 1, which was then diluted to 7.7 L in a cleaned polyethylene bottle.

4. The appropriate quantity of calcium stock was added to the diluted major salt and macronutrient solution. As all carbonate was present as bicarbonate, the pH was low enough that calcium carbonate precipitation did not occur.

5. Five separate micronutrient stock solutions were made.

(a) To enable the concentrations of Co$^{2+}$, Ni$^{2+}$ and Cu$^{2+}$ to be varied, a separate stock solution of each of these was prepared, at 100 ppm Ni$^{2+}$, 10 ppm Cu$^{2+}$ and 1 ppm Co$^{2+}$.

(b) An Fe-EDTA stock solution was prepared by combining the desired concentration of FeCl$_3$ with 1.2 times the stoichiometric equivalent of EDTA at a pH of 8.0. To remove any colloidal iron hydroxide which had formed, the solution was filtered through a 0.025 μm filter.

(c) The remaining three transition metals (Mo$^{2+}$, Mn$^{2+}$ and Zn$^{2+}$) were combined into one stock solution.

The Fe(III)-EDTA and the Mo$^{2+}$, Mn$^{2+}$ and Zn$^{2+}$ stocks were both prepared at 1,000,000 x the required concentration, and then diluted to 1000 x the required concentration. To make 8 L of the final medium, 8 mL of each of the trace element solutions was added to the major salts and the macronutrients in the polyethylene bottle.

Additional volumes of Co$^{2+}$, Ni$^{2+}$ or Cu$^{2+}$ stock solutions were added to the medium once the growth containers had been filled.

6. A biotin stock solution was made by dissolving 0.002 g of biotin into 10 mL of milli-Q water at pH 5. 1.2 mL of this biotin stock, 880 μL of 0.25 gL$^{-1}$ cyanocobalamin stock and 0.04 g of thiamine HCl were diluted to 100 mL, to make a mixed vitamin stock containing 2.2x10$^{-3}$ gL$^{-1}$ B$_12$, 2x10$^3$ gL$^{-1}$ biotin and 0.4 gL$^{-1}$ thiamine HCl. 2 mL of mixed stock were dispensed into disposable brown glass vials, and stored frozen. To make 8 L of the final medium, one vial was thawed, rinsed on the outside in milli-Q water, and broken into the polyethylene bottle.

7. The pH of the final medium was carefully adjusted with dilute NaOH to 8.3 (at the ambient temperature of the cleanroom, approximately 20°C). The solution was agitated during addition to avoid the pH rising above 8.8, at which point calcium carbonate would precipitate.

8. 1.6 L of the medium were dispensed into each of the incubation jars, and spiked with Co$^{2+}$, Ni$^{2+}$ and Co$^{2+}$ stock as required.
Table 8.2: A recipe for the medium developed for this work, giving mass of each compound in each 1 L stock solution (where stock solutions were used), the concentration factor of each stock solution, the volume of each stock that was used to make up 8 L of the medium, and the resulting concentration in the medium. Note that a number of the micronutrient salts are actually dissolved into one mixed stock solution, and that a number of the major salts are dissolved directly into the medium.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mass (g)</th>
<th>Conc. factor</th>
<th>Mass (g) used for each 2.25 L stock</th>
<th>Conc. in medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major salts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl&lt;sup&gt;a&lt;/sup&gt;</td>
<td>229.755</td>
<td>0.4368 M</td>
<td>0.4368 M</td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>6.7095</td>
<td>10 mM</td>
<td>10 mM</td>
<td></td>
</tr>
<tr>
<td>Na&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>12.7836</td>
<td>10 mM</td>
<td>10 mM</td>
<td></td>
</tr>
<tr>
<td>CaCO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>2.1438</td>
<td>2.38 mM</td>
<td>2.38 mM</td>
<td></td>
</tr>
<tr>
<td>MgCl&lt;sub&gt;2&lt;/sub&gt;·6H&lt;sub&gt;2&lt;/sub&gt;O&lt;sup&gt;b&lt;/sup&gt;</td>
<td>358</td>
<td>40 mM</td>
<td>40 mM</td>
<td></td>
</tr>
<tr>
<td>H&lt;sub&gt;3&lt;/sub&gt;BO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.1237</td>
<td>1 μM</td>
<td>1 μM</td>
<td></td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;P&lt;sub&gt;4&lt;/sub&gt;O&lt;sub&gt;7&lt;/sub&gt;·H&lt;sub&gt;2&lt;/sub&gt;O&lt;sup&gt;b&lt;/sup&gt;</td>
<td>98</td>
<td>7.92 mM</td>
<td>7.92 mM</td>
<td></td>
</tr>
<tr>
<td>Macronutrients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaH&lt;sub&gt;2&lt;/sub&gt;P&lt;sub&gt;4&lt;/sub&gt;O&lt;sub&gt;7&lt;/sub&gt;·H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>1.5298</td>
<td>1000</td>
<td>10 μM</td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>5.2598</td>
<td>1000</td>
<td>90 μM</td>
<td></td>
</tr>
<tr>
<td>NaN&lt;sub&gt;3&lt;/sub&gt;</td>
<td>8.4994</td>
<td>1000</td>
<td>100 μM</td>
<td></td>
</tr>
<tr>
<td>Micronutrients&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(NH&lt;sub&gt;4&lt;/sub&gt;)&lt;sub&gt;6&lt;/sub&gt;Mo&lt;sub&gt;7&lt;/sub&gt;O&lt;sub&gt;24&lt;/sub&gt;·4H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>0.5553</td>
<td>1000000</td>
<td>0.008</td>
<td>0.429 nM</td>
</tr>
<tr>
<td>MnCl&lt;sub&gt;2&lt;/sub&gt;·4H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>1.2893</td>
<td>1000000</td>
<td>0.008</td>
<td>5 nM</td>
</tr>
<tr>
<td>ZnSO&lt;sub&gt;4&lt;/sub&gt;·7H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>0.0942</td>
<td>1000000</td>
<td>0.008</td>
<td>2.6 nM</td>
</tr>
<tr>
<td>FeCl&lt;sub&gt;3&lt;/sub&gt;·6H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>0.0360</td>
<td>1000000</td>
<td>0.008</td>
<td>1 nM</td>
</tr>
<tr>
<td>Na&lt;sub&gt;2&lt;/sub&gt;EDTA</td>
<td>0.0401</td>
<td>1000000</td>
<td>0.008</td>
<td>1.2 nM</td>
</tr>
<tr>
<td>Vitamins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cyanocobalamin</td>
<td>2</td>
<td>0.55 μgL&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>0.55 μgL&lt;sup&gt;-1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>biotin</td>
<td>2</td>
<td>0.5 μgL&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>0.5 μgL&lt;sup&gt;-1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>thiamine HCl</td>
<td>2</td>
<td>0.1 mgL&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>0.1 mgL&lt;sup&gt;-1&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Some NaCl was added to the NaH<sub>2</sub>P<sub>4</sub>O<sub>7</sub>·H<sub>2</sub>O stock to equalize its Na concentration with that of the NaN<sub>3</sub> stock.

<sup>b</sup>A solution of precisely-known concentration was calculated by the method of McLachlan (1973) (see section 8.3.2)

<sup>c</sup>1 mL of each trace metal stock solution, and of the Fe<sup>3+</sup>/EDTA solution, is first diluted into a combined trace metal solution and then 8 mL of this is diluted again to make 8 L of the final medium. See text for details.
8.3. The Growth Medium

Table 8.3: The manufacturers' maximum allowable concentrations (as percentages) of $\text{PO}_4^{3-}$, $\text{NO}_3^-$, $\text{SO}_4^{2-}$ in the major salts used in the medium, and the percentage this maximum is of the desired anion in the medium. It can be seen that contamination with phosphate from NaCl is of particular concern.

<table>
<thead>
<tr>
<th>compound</th>
<th>% $\text{PO}_4^{3-}$ in salt</th>
<th>% of $\text{PO}_4^{3-}$ in medium</th>
<th>% N in medium</th>
<th>% $\text{SO}_4^{2-}$ in medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl</td>
<td>0.0005</td>
<td>0.35</td>
<td>0.001</td>
<td>0.38</td>
</tr>
<tr>
<td>CaCl$_2$·6H$_2$O</td>
<td>&lt;0.0005</td>
<td>&lt;0.42</td>
<td>0.001</td>
<td>0.46</td>
</tr>
<tr>
<td>Na$_2$SO$_4$</td>
<td>0.002</td>
<td>2.70</td>
<td>0.0005</td>
<td>0.37</td>
</tr>
<tr>
<td>MgCl$_2$·H$_2$O</td>
<td>0.0005</td>
<td>5.57</td>
<td>0.0002</td>
<td>1.21</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.0005</td>
<td>12.05</td>
<td>0.0005</td>
<td>6.54</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td>21.09</td>
<td>8.96</td>
<td></td>
</tr>
</tbody>
</table>

Determination of the Concentration of Hygroscopic Salts

The hygroscopic nature of MgCl$_2$ and CaCl$_2$ salts makes it impossible to accurately weigh them. Instead, accurately-known MgCl$_2$ and CaCl$_2$ stocks were prepared by measuring chloride concentration by the Mohr titration, according to the method of McLachlan (1973). AgNO$_3$ was standardised against KCl, with 5% K$_2$Cr$_2$O$_7$ solution as an indicator. The MgCl$_2$ and CaCl$_2$ stocks were titrated against the standardised AgNO$_3$ solution.

8.3.3 Determination of the Phosphate Concentration of the Major Salts

A significant contamination problem would occur if concentrations of the macronutrients $\text{PO}_4^{3-}$, $\text{NO}_3^-$, $\text{SO}_4^{2-}$ and $\text{SiO}_4^{2-}$ were as high as the manufacturers' maximum allowable limits in the major salts, particularly in NaCl, the most concentrated salt in the medium (Table 8.3.3). Total phosphate from Table 8.3.3 is 21% of the desirable concentration in the medium. While the Chelex-100 resin has a particular affinity for heavy metal cations in the presence of other cations, no resin exists with a particular affinity for anions in the presence of a very high chloride concentration. A 4 kg jar of AnalaR-grade NaCl was obtained, and the level of potential contamination was assessed by measurement of phosphate concentration, as potentially the most serious contaminant, and also the most readily measured.

The “molybdenum-blue” colourimetric method was used, in which a phosphomolybdate complex is reduced with hydrazine to give a blue solution. This method is reliable for the determination of very low concentrations of phosphate, and is not affected by a high NaCl concentration or high ionic strength. An acidic solution of Na$_2$MoO$_4$·2H$_2$O plus hydrazine sulphate was added to the solution to be analysed, and heated. Absorption was measured at 830 m$\mu$. Phosphate standards ranging from 0.1 to 2 ppm were prepared from KH$_2$PO$_4$ in milli-Q water, and treated identically to the samples.
8.3.4 Removal of Undesirable Heavy Metals from the Solution

Commercially-available salts are also potentially a major source of heavy metal ion contamination. A number of heavy metals, such as Cd(II) and Pb(II), are toxic to algae in very low concentrations. Also, in this project, the presence of excess Co^{2+}, Cu^{2+} or Ni^{2+} from the salts used to produce the medium was undesirable.

To avoid these problems, the major salt solution was passed through a column of Chelex-100 cation-exchange resin, and each of the micronutrient stock solutions was batch-treated with Chelex-100. Chelex-100 ion exchange resin is a cellulose-type resin, with bonded aminodiacetate groups. It has a particular affinity for heavy metals over Na\(^+\) or H\(^+\) ions, and is frequently used to quantitatively remove such cations from solution as a preconcentration step.

The resin was equilibrated to the pH of the solution to be treated in it, by titration with 1 M Aristar NaOH or HCl. Flow rate through the column was set to 250 mL per hour. The collecting vessel was covered in gladwrap to avoid concentration of solution by evaporation. NaH_2PO_4.H_2O and NaNO_3 solutions were treated with the same batch of resin, so the ionic strength of the NaH_2PO_4.H_2O solution was adjusted with NaCl to equal that of the NaNO_3 solution.

Concentrations of Pb(II), Cu(II), Co(II) and Ni(II) in the major salt solution were assessed before and after treatment, using the same methodology as for seawater (Section 8.2.2).

8.4 Handling and Cleaning of Algae and Equipment

8.4.1 Equipment

All equipment was soaked for two weeks in 30% HNO_3 in an open laboratory. After four rinses in triply-distilled water, equipment was transferred to the cleanroom, rinsed in milli-Q water, and soaked for one week in 0.1% Aristar HNO_3. It was then rinsed four times in milli-Q water, and soaked for one week in milli-Q water, before being rinsed a final time and air dried. Equipment which was not being used immediately was stored in plastic bags in the cleanroom.

8.4.2 Collection of Algae

Algae were collected on the outgoing tide, 1 to 2 hours before low tide. Disposable plastic gloves were worn during collection and handling. Plants were placed in an acid-washed plastic container lined with a cleanroom wipe, and were covered in a damp cleanroom wipe. To reduce the amount of dust contacting the seaweed, the container was loosely covered with a sheet of plastic. To ensure adequate continued oxygen supply, the seaweed were not transported in water, but only damp. Seawater for later addition was also collected. While it used to be popular to cool algal material
on transport, it has been found that chilling increases shock (Wendy Nelson, pers. comm.), so plants were transported at air temperature.

Upon return to Christchurch, the plastic container of algae was filled with seawater, and an aquarium bubbler used to bubble washed air through it. Plants were maintained in these conditions overnight, and processed for culture the next day.

8.4.3 Cleaning of Algae

Algae were cleaned by gentle rubbing with gloved hands, inside a tub of seawater. Loose epiphytic algae and juvenile animals were removed by hand, and plants which were free from firmly-attached epiphytes and animal by-products such as slime were chosen. Plants were rinsed well in flowing milli-Q water and placed inside the growth containers. This treatment was sufficient to obtain a uni-macroalgal culture.

Sterilization of plants to obtain an axenic culture was considered, but abandoned due to three reasons. Firstly, the difficulty of achieving sterility without killing the plants, and of maintaining sterility in culture, was considered prohibitive. Secondly, the intention of this project was to imitate the natural environment as far as possible, and it was considered that the effect of metal ions on the seaweed may be influenced by the presence or absence of surface bacteria. Thirdly, the sterilization techniques in general use involve steps which cause surface damage (for example, ultrasonification or soaking in iodine solution (McCracken, 1989)) which is likely to affect uptake of metal ions from solution, because metal ion uptake is a surface reaction. A second concern about surface-damaging sterilization techniques was the probable negative effect of wounding on the plants. The cleaning procedure above, combined with careful handling during collection, did not result in plant mortality, but the much rougher handling required to sterilize the surface of plants has generally caused considerable damage to them. It was considered that plants with damaged surfaces would be likely to respond to external stresses such as pollution differently to healthy plants in the natural environment. For example, Fester et al. (1994) studied the response of Acetabularia acetabulum, a giant unicellular alga, to routine laboratory pressure-wounding, and concluded that such wounds substantially prolonged the alga's lifecycle.

8.5 Growth Experiments

8.5.1 Growth Containers

Seaweed were grown inside two litre glass jars (Photo 48), inside a lit and heated polystyrene-walled box. The incubation system is diagrammatically shown in Figure 8.5.1.

Air was supplied at 9.5 L min⁻¹ by a Precision SR-9500 aquarium pump, and was initially passed through a series of gas washbottles, containing first 1% Aristar nitric acid, then 1% Aristar sodium hydroxide, then milli-Q water. As well as cleaning the
Figure 8.2: The incubation system.
8.5. Growth Experiments

Photo 48: One of the two litre containers in which algae were cultured.

air, this system had the advantage of water-saturating it, which reduced problems of evaporation. From the washing system, the air tube (acid-washed 4 mm clear PVC tubing) passed into the polystyrene box through a hole in the front of the box, and was connected to a gas manifold made from 10 plastic aquarium valves (Aquarium Accessories) joined together. Clear PVC tubing ran from each valve to the top of a growth container, and was connected to a glass bubbler made from a coiled glass tube. Air was allowed to escape from the containers through an inverted U-tube, which did not allow the entry of dust or dirt. Contamination of the system was further reduced by the presence of a sheet of acid-washed plastic under the lid of each container. The lids, which were glass, were closed with stainless steel catches.

The light source consisted of two Thorn 18W/D62 daylight fluorescent tubes, mounted on the underside of the wooden lid of the polystyrene-walled box. The incubation system was kept in a cold room at 3-4°C, and heating, thermostated to 15±1°C, was provided by a copper element and a computer fan, mounted between the growth tubes on the lid. A 14:10 day:night cycle was provided by connecting the fluorescent tubes to a timer switch.

Summer temperatures of Kaikoura coastal seawater is approximately 14°C. Colthart and Johansen (1973) assessed the growth of Corallina officinalis at different temperatures, finding that while the alga grew naturally in water warmer than 20°C, C. officinalis collected from cool water would only grow in cool water. Maximum growth occurred between 12-18°C. Therefore, 15°C was chosen as the temperature for this work.

8.5.2 The Growth Experiments

- First, Corallina officinalis was grown for 5 days in solutions containing 5 mM Ca²⁺ polluted with either Co²⁺, Ni²⁺ or Cu²⁺. Concentrations chosen were 1, 3, 6, and 9x the concentration of each metal in the Kaikoura seawater from which the seaweed were collected.

The intention was to assess uptake of sublethal concentrations of metal ions. Literature data on lethal concentrations for algae are sparse (see Section 7.3),
but generally indicated that at >9x the concentrations of the Kaikoura seawater adverse health effects became likely (Xue and Sigg, 1990; Gledhill et al., 1997).

- Second, these experiments were repeated using killed seaweed. Seaweed were first blotted dry with a cleanroom wipe, then immersed in liquid nitrogen to kill them rapidly with minimal tissue damage.

- Third, both sets of experiments were repeated with a \( \text{Ca}^{2+} \) concentration of 10.3 mM.

### 8.5.3 Extraction Protocol

After culturing, the unopened vessels were taken to the clean room, where the jars were opened. Samples of the unpolluted and most polluted media for analysis were removed by siphoning through an acid-washed PVC tube. Then most of the seaweed were removed, leaving the amount required for determination of photosynthesis (see below).

With the exception of the drying and centrifuging steps, and acid digestion of the organic material, all seaweed processing was performed in the cleanroom.

The seaweed was rinsed in a small amount of milli-Q water and blotted dry on a cleanroom wipe. The samples were transferred to acid-washed glass vials, and dried under high vacuum overnight or until constant weight was achieved. Plants were ground in an agate mortar and pestle, and samples of 0.05 g were weighed accurately into 50 mL centrifuge tubes.

2 mL of 0.2 mol L\(^{-1}\) isopiestically-distilled acetic acid were added, and the centrifuge tubes gently shaken until effervescence had completely stopped. The tubes were capped with parafilm and centrifuged for 20 min at 5000 rpm, and the acid collected with a pipette. The pellet of remaining plant material was rinsed in 2 mL of milli-Q water, and centrifuged again. The rinse water was combined with the collected acetic acid extract.

1.4 mL of concentrated Aristar H\(\text{NO}_3\), 0.6 mL of concentrated Aristar H\(\text{ClO}_4\) and 1 mL of milli-Q water were added to the pellet in the centrifuge tube. The tubes were capped with a micro-refluxer and digested in a water bath at 65°C for 3 hours. This digestion was sufficient to fully dissolve the remaining organic material.

One of the aims of the project was to provide as simple an analytical process as possible, but it was found to be impossible to directly analyse the algal extracts because of interferences. Therefore, both the acetic acid and the \( \text{HNO}_3 \) extracts were treated by solvent exchange before analysis by atomic absorption. The acid extracts were complexed with 5 mL of saturated 8-hydroxyquinoline (oxine) solution in 25 mL of milli-Q water, and shaken with 2 mL of xylene at pH 5 to extract the transition metal ions. The \( \text{Ca}^{2+} \) concentration of the inorganic phase of the plant was determined by treating a separate weighed sample with acetic acid, centrifuging the insoluble residue, and drying it to constant weight.
8.5.4 Quality Control

Quality control for the extraction procedure was performed in two ways. First, the completeness of metal extraction by rapid dissolution in 2 mL of 0.2M acetic acid was tested. A number of samples were extracted for 1, 4 or 12 hours in 2 or 3 mL of the acid, and analysed as above. Second, the selectiveness of the split extraction was tested. A standard material for this purpose was not available. Therefore a composite sample was made from a sample of a Porphyra sp. (a red seaweed), which was collected from Kaikoura, combined with a calcite stalactite which had already been analysed for Cu$^{2+}$ concentration. Each part of the composite sample was analysed separately by the protocol above, then they were combined thoroughly and analysed together.

8.5.5 Algal Health

To investigate the possibility of the added metal ions poisoning the algae, and because biomineralization is a by-product of photosynthesis, net photosynthesis was measured before and after culturing.

Approximately 0.2 g (dry weight) of live seaweed was transferred to a well-washed 500 mL clear glass bottle. For measurements before culturing, the initial oxygen concentration of 0.45 µm-filtered seawater, at 15°C, was reduced by bubbling with oxygen-free N$_2$, and measured with an oxygen meter. The glass bottle was filled with a siphon, topped with parafilm, and capped firmly. No oxygen bubbles were trapped in the bottle. For measurements after culturing, the culture were returned to the cold room after removal of plants for analysis. The air supply to the vessels was replaced by oxygen free N$_2$ to reduce the initial oxygen concentration below saturation, oxygen concentration measured, and bottles filled as before.

Bottles were cultured at 15°C in the culturing apparatus for three to four hours. The medium was removed from the bottle by siphon, and the oxygen concentration measured. Net photosynthesis was calculated using (Thomas, 1988):

$$\text{net photosynthesis} = \frac{375.9(\text{final } O_2 - \text{initial } O_2)}{\text{time} \times \text{P.Q.}} \times \frac{1}{\text{volume}} \times \text{mass}$$

(8.1)

where final and initial O$_2$ concentration is measured in ppm, P.Q. is the photosynthetic quotient, and is taken to be 1.2, time is measured in hours, volume is measured in mLs, and mass is measured in grams.
Chapter 9

Culturing *Corallina officinalis* in a Defined Medium Containing Co(II), Ni(II) or Cu(II): Results and Discussion

9.1 Metal Concentrations in the Kaikoura Seawater

The average concentrations of Pb$^{2+}$, Cu$^{2+}$, Ni$^{2+}$ and Co$^{2+}$ determined from the two samples of rock pool water collected were 0.72±0.18 ppb, 0.90±0.29 ppb, 4.6±1.4 ppb and 0.023±0.006 ppb, respectively. Results were determined as the mean of two experiments for each of the two samples, and are ± the standard deviation. Standard deviations are high, particularly for Eu$^{2+}$, which was close to the working limit of the method, and the results should be taken to give only the order of magnitude of the concentration of each metal ion. The blank sample collected at the same time contained no measurable amount of any of the four metals.

Using a molarity scale, the concentration of Pb$^{2+}$ was 3.5 nM, of Cu$^{2+}$ 14 nM, of Ni$^{2+}$ 78 nM, and of Co$^{2+}$ 0.39 nM. Stumm and Morgan (1996) reports coastal concentrations for cobalt of 0.25–1.5 nM and for copper of 3.7–260 nM, commenting that extremely high levels of copper indicate pollution. These figures indicate that the Kaikoura seawater collected from this rock pool, while not pristine, was unpolluted.

9.2 Speciation of Transition Metals in the Medium

Speciation of the transition metals in the artificial medium is important for two reasons. Firstly, the bioavailability of each metal is dependant not on its total concentration, but on the concentration of free metal ion and highly labile complexes. Secondly, the speciation of Fe(III) is particularly significant, because of the stability of iron hydroxide complexes and hydrous ferric oxide, Fe(OH)$_3$·nH$_2$O, at the pH of seawater. If colloidal
Fe(OH)$_3$·nH$_2$O forms in the artificial medium, it will provide a surface for adsorption of trace elements, removing them from solution.

As the initial concentrations of all elements in the medium were known, the speciation of any element could be calculated. The sub-program "Species" within the program "Soleq" (Pettit and Powell, 1999) was used to calculate distribution curves, and most equilibrium constants were taken from "SC-database" (Pettit et al., 1999). Where possible, constants were chosen that had been determined at approximately the ionic strength of seawater, or were adjusted to the ionic strength of the artificial medium (0.63). If the appropriate enthalpy data was available, constants were adjusted to 15°C.

The constants used for calculating trace metal speciation are given in Table 9.1. The computer program used has a limit of 28 constants, so those most likely to affect EDTA and Fe(III) speciation were chosen. Although Cl$^-$ is present in large excess, it complexes Fe$^{3+}$, Cu$^{2+}$, Ni$^{2+}$ and Co$^{2+}$ so little that it was not necessary to include chloride species in the calculations. Fe(III) was supplied to the medium as the EDTA complex, to avoid supplying precipitated Fe(OH)$_3$. This solution to the problem of iron hydrolysis was suggested by Morel et al. (1979), who had a sufficiently high concentration of EDTA in their medium to complex most of the metal ions present. However, EDTA complexation may reduce the bioavailability of the metals, so for this project the EDTA concentration was made much less than the total metal concentration, so that most of the metal would not be complexed by EDTA. While the EDTA was initially provided as Fe(EDTA)$^-$, the speciation calculations show that it was completely complexed by Ni$^{2+}$, which was present in large excess over Fe$^{3+}$. The Fe$^{3+}$ was hydrolysed to Fe(OH)$_3^-$ and Fe(OH)$_4^2$-. The formation of Fe(OH)$_3(s)$, while possible, was considered to be unlikely.

The only constants in Table 9.1 for which small changes in the log$\beta$ values have a significant effect on the speciation are the iron hydrolysis constants, and the solubility product for hydrous ferric hydroxide (Fe(OH)$_3(s)$). Hydrolysis constants for the first three hydrolysis products of Fe(III), and the associated $\Delta H$ values, at the ionic strength of seawater, were taken from Byrne et al. (1999). They were adjusted for temperature using the van't Hoff relationship:

$$\ln\left(\frac{\beta_n(T_2)}{\beta_n(T_1)}\right) = \frac{\Delta H_n}{R} \left(\frac{1}{T_1} - \frac{1}{T_2}\right)$$  \hspace{1cm} (9.1)

where $\beta_n(T_1)$ is the cumulative complexation constant for the reaction under consideration at absolute temperature $T_1$, $\Delta H_n$ is the enthalpy of the reaction, and $R$ is the gas constant.

Constants from Byrne et al. (1999), and the adjusted constants are given in Table 9.2. log $\beta$ for the fourth hydrolysis reaction was estimated from Baes, Jr. and Mesmer (1976).

The remaining key constant for the speciation of iron is the solubility constant for hydrous ferric oxide. Estimates of the $pK_{sp}$ for this meta-stable, at least partially amorphous, precipitate vary from 37 to 39, and depend on the speed of deposition.
Table 9.1: Complexes and \( \log \beta \) or \( \log^* \beta \) values used for calculation of speciation of trace metals and EDTA in the artificial medium.

<table>
<thead>
<tr>
<th>Complex</th>
<th>( \log \beta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeOH(^{2+})</td>
<td>-3.01(^a)</td>
</tr>
<tr>
<td>Fe(OH)(_2^+)</td>
<td>-7.1(^a)</td>
</tr>
<tr>
<td>Fe(OH)(_3^0)</td>
<td>-14.0(^a)</td>
</tr>
<tr>
<td>Fe(OH)(_4^-)</td>
<td>-22.1(^a)</td>
</tr>
<tr>
<td>FeEDTA(^-)</td>
<td>25.0</td>
</tr>
<tr>
<td>FeHEDTA</td>
<td>26</td>
</tr>
<tr>
<td>Fe(OH)EDTA(^3-)</td>
<td>20</td>
</tr>
<tr>
<td>HEDTA(^3-)</td>
<td>9.2</td>
</tr>
<tr>
<td>H(_2)EDTA(^2-)</td>
<td>15.6</td>
</tr>
<tr>
<td>H(_3)EDTA(^-)</td>
<td>18</td>
</tr>
<tr>
<td>CuEDTA(^2-)</td>
<td>17.3</td>
</tr>
<tr>
<td>CuHEDTA(^-)</td>
<td>20.1</td>
</tr>
<tr>
<td>Cu(OH)EDTA</td>
<td>6.8</td>
</tr>
<tr>
<td>CuOH</td>
<td>-6.8(^a)</td>
</tr>
<tr>
<td>Cu(_2)(OH)(_2)</td>
<td>-11.5(^a)</td>
</tr>
<tr>
<td>CaEDTA</td>
<td>10</td>
</tr>
<tr>
<td>CaHEDTA</td>
<td>13.5</td>
</tr>
<tr>
<td>ZnEDTA</td>
<td>15.7</td>
</tr>
<tr>
<td>ZnHEDTA</td>
<td>18.2</td>
</tr>
<tr>
<td>NiEDTA</td>
<td>18.5</td>
</tr>
<tr>
<td>NiHEDTA</td>
<td>21.3</td>
</tr>
<tr>
<td>CoEDTA</td>
<td>16.3</td>
</tr>
<tr>
<td>CoHEDTA</td>
<td>19.4</td>
</tr>
<tr>
<td>HCO(_3^-)</td>
<td>9.62</td>
</tr>
<tr>
<td>H(_2)CO(_3)</td>
<td>15.51</td>
</tr>
<tr>
<td>Fe(OH)CO(_3)</td>
<td>-3.8</td>
</tr>
<tr>
<td>HSO(_4^-)</td>
<td>1.23</td>
</tr>
<tr>
<td>FeSO(_4^+)</td>
<td>2.2</td>
</tr>
<tr>
<td>Fe(OH)(_3)(s)</td>
<td>4.8</td>
</tr>
</tbody>
</table>

\(^a\)\( \log^* \beta \) value

Table 9.2: \( \log^* \beta \) and enthalpy constants used for Fe(III). From Byrne et al. (1999).

<table>
<thead>
<tr>
<th>complex</th>
<th>( \log \beta ) at T(^\circ)C</th>
<th>( \Delta H_n ) (kJmol(^{-1}))</th>
<th>( \log \beta ) at 15(^\circ)C</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeOH(^{2+})</td>
<td>-2.74 at 25(^\circ)C</td>
<td>42.7</td>
<td>-3.01</td>
</tr>
<tr>
<td>Fe(OH)(_2^+)</td>
<td>-6.7 at 25(^\circ)C</td>
<td>70.7</td>
<td>-7.1</td>
</tr>
<tr>
<td>Fe(OH)(_3^0)</td>
<td>-13.7 at 20(^\circ)C</td>
<td>100</td>
<td>-14.0</td>
</tr>
</tbody>
</table>
and the age of the precipitate. Langmuir (1997) gave an initial pK_{sp} of about 37, increasing with age and the consequent partial recrystallization. Byrne and Luo (1999) found a log^*K_{sp} of 4.28 for their fresh precipitates, which, with a pK_{w} of 14.28, would give a pK_{sp} of 38.6. The reported dissolved Fe(III) concentration in seawater varies between 10^{-6.9} and 10^{-8.75} mol L^{-1} (Langmuir, 1997), making it reasonable to conclude that the 10^{-10} mol L^{-1} Fe^{3+} present in the medium of this study would all be in solution. Therefore, for speciation of this medium, which did not involve the possibility of slow aging of a precipitate, and which had a very low initial Fe^{3+} concentration, a log^*K_{so} of at least 4.28 was assumed. Figure 9.1 shows the distribution curves for 10^{-9} mol L^{-1} Fe(III) assuming log^*K_{so} = (a) 4.3 and (b) 4.7, and Figure 9.2 the shows the equivalent curves assuming log^*K_{so} = (a) 4.8 and (b) 6.0. While the curves do not show Fe(OH)_{3-n}H_{2}O , it is present for the pH region where the distribution curve for Fe(OH)_3 is a flat line. Note that if log^*K_{so} \geq 4.7, no solid Fe(OH)_3.7H_2O forms at pH 8.2. These curves are calculated for an Fe^{3+} concentration 10 times the concentration in the artificial seawater because 10^{-9} mol L^{-1} was the lowest concentration that could be inputted into the program. Therefore, the results are highly conservative for the artificial seawater under consideration.

For the other metals in the medium, speciation at pH 8.2 was calculated to be as follows: Co(II) and Zn(II) were 100% free M^{2+}; and Ni(II) was 98% free Ni^{2+} and 2% Ni(EDTA)^{2-}. All of these species are bioavailable, excepting perhaps Ni(EDTA)^{2-}.

Speciation of Cu(II) was calculated separately from the calculation given in Table 9.1, to allow inclusion of carbonate species. This procedure is possible because the total copper concentration is very much less than the ligand concentration. Constants used for the carbonate complexes were logK_1=5.73 and log\beta_2=9.3, and the results are shown in Figure 9.3. Cu(II) was determined to be 53% CuCO_3, 29% CuOH^+, 17% Cu(CO_3)^{2-} and 1% free Cu^{2+}.

Byrne et al. (1988) considered the speciation of a number of metal ions in seawater, using available equilibria and enthalpy data, and examining the effect of pH (8.2 and 7.6) and temperature (25°C and 5°C). The solution chemistry of Cu^{2+} was found to be dominated by complexation with carbonate, and varied only moderately with both pH and temperature (see Table 9.3). Co^{2+} and Ni^{2+} were both weakly complexed in seawater, with a minor increase in percentage of free metal with decreasing pH and/or temperature. Mn^{2+} was also found to be only weakly complexed, and its speciation was almost totally independent of pH and temperature.

These results show noticably less complexation of Ni^{2+} and Co^{2+} with CO_3^{2-} than those of Turner et al. (1981). Turner et al. calculated that approximately 50% of the Ni^{2+} and Co^{2+} was complexed as MCO_3, at 25°C in seawater.

While these published speciation results for seawater are similar to the calculated speciation of metals in the chemically-defined artificial medium, Gledhill et al. (1997)

\[\text{Fe(OH)}_{3(s)} + 3H^+ \rightleftharpoons \text{Fe}^{3+} + 3H_2O \quad (9.2)\]

\[\text{pK}_w \text{ was calculated for 15°C and an ionic strength of 0.63 by the method of Harned and Owen (1958). The equations and constants used are given in Appendix B}\]
9.2. Speciation of Transition Metals in the Medium

Figure 9.1: Distribution diagrams of soluble species for $10^{-9}$ mol L$^{-1}$ Fe(III) at different values of log$^*$K$_{so}$.

(a) log$^*$K$_{so} = 4.3$

(b) log$^*$K$_{so} = 4.7$
Figure 9.2: Distribution diagrams of soluble species for $10^{-9}$ mol L$^{-1}$ Fe(III) at different values of $\log^+ K_{s0}$. 
points out that these speciation models do not allow for metal complexation by dissolved organic material. The actual concentration of free Cu$^{2+}$ has been found to be $10^{-13.4} - 10^{-12.4}$ in seawater, because more than 99% of the copper present is complexed by organic ligands. In contrast to this, free trace metal concentrations calculated for the artificial medium were high. These calculations indicated that no Fe(OH)$_3\cdot n$H$_2$O would form for the added metal ions to be adsorbed onto, and that all (or almost all) of the trace metal ions in solution were in a biologically available form. Therefore the uptake of trace metal ions by C. officinalis presented below can be related directed to the added metal ion concentrations.

9.3 Transition Metal Ion Uptake by C. officinalis

9.3.1 Initial Investigations into the Uptake of Cobalt by C. officinalis

To determine whether bio-concentration of cobalt occurred in measurable quantities, a sample of C. officinalis, which had been soaked overnight in seawater doped with 1000 ppm Co$^{2+}$, was examined under a scanning electron microscope, by EDAX. The plant was rinsed in triply-distilled water and frozen in liquid nitrogen before mounting. Table 9.4 shows which elements were present, and, for the thallus cross-section, the position of these within the plant. In the 2nd and 4th columns of the table, the percentages are determined from signal peak area, and exclude carbon, hydrogen and oxygen which do not produce a signal under electron microscopy. Electron micrographs of these two views are given in Photos 49 and 50.

It can be seen from these results that considerable concentration of metals occurs within C. officinalis. The difference in silicon concentration between the thallus and tips of
Chapter 9. Culturing Corallina officinalis: Results

Photo 49: Electron micrograph of a cross-section of the thallus of a sample of *C. officinalis*.

Photo 50: Electron micrograph of the outside of a tip of a sample of *C. officinalis*. 
9.3. Transition Metal Ion Uptake by C. officinalis

Table 9.3: Speciation of a number of metal ions in seawater, as calculated by Byrne et al. (1988). Cu\(^{2+}\), Mn\(^{2+}\), Co\(^{2+}\) and Ni\(^{2+}\) were also partially present as complexes of Cl\(^-\) and SO\(_4\)\(^{2-}\), with percentages almost unchanged by changes in pH or temperature.

<table>
<thead>
<tr>
<th>Cation</th>
<th>pH</th>
<th>T (°C)</th>
<th>log(M(_T)/[M])</th>
<th>% free metal</th>
<th>% M(CO(_3))(_n)</th>
<th>% M(OH)(_n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(^{2+})</td>
<td>8.2</td>
<td>25</td>
<td>1.32</td>
<td>5</td>
<td>85</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.92</td>
<td>12</td>
<td>80</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.6</td>
<td>25</td>
<td>0.82</td>
<td>15</td>
<td>74</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.50</td>
<td>32</td>
<td>55</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Mn(^{2+})</td>
<td>8.2</td>
<td>25</td>
<td>0.15</td>
<td>72</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.13</td>
<td>74</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.6</td>
<td>25</td>
<td>0.14</td>
<td>73</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.13</td>
<td>75</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Co(^{2+})</td>
<td>8.2</td>
<td>25</td>
<td>0.18</td>
<td>65</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.13</td>
<td>74</td>
<td>6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.6</td>
<td>25</td>
<td>0.13</td>
<td>73</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.11</td>
<td>77</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Ni(^{2+})</td>
<td>8.2</td>
<td>25</td>
<td>0.28</td>
<td>53</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.17</td>
<td>68</td>
<td>16</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.6</td>
<td>25</td>
<td>0.16</td>
<td>70</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.11</td>
<td>77</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

the Corallina species is particularly noteworthy. A cross-section of the tip, although not clear, suggested the silicon may be concentrated on the outside of the tip, with calcium found more on the inside, and may be a consequence of the greater calcification of internal cells. Potassium, chlorine and iron, which are involved in cellular processes, were concentrated on the inside of the sample. At this cobalt concentration of the seawater, considerable uptake of the cobalt occurred, but the metal ion was evenly dispersed across the sample, both internally and externally.

9.3.2 Quality Control of Transition Metal Determination

Before culturing, the initial Cu\(^{2+}\), Ni\(^{2+}\) and Co\(^{2+}\) concentrations of freshly collected and dried C. officinalis were measured for each step of the extraction. Three separate digestions were performed, and three repetitions of each digest were measured automatically by the graphite furnace atomic adsorption spectroscopy program. Results are presented in Table 9.5, as “uncultured alga”, with the errors spanning the range of values measured. It can be seen that concentrations of the transition metal ions are low in the inorganic phase (the acetic acid extract), but are approximately proportional to the concentrations found in the Kaikoura seawater they were collected from. Metal concentrations are significantly higher in the organic material phase (the concentrated HNO\(_3\)/HClO\(_4\) extract, referred to in this chapter as the HNO\(_3\) extract for brevity), and are also associated with the seawater concentration. Concentration factors (CF)
Table 9.4: Element positions and percentages in a cross-section of the thallus, and the tip, of *Corallina officinalis*, determined by scanning electron microscopy. The views are shown in Photos 49 and 50.

<table>
<thead>
<tr>
<th>element</th>
<th>thallus cross-section</th>
<th>tip exterior</th>
</tr>
</thead>
<tbody>
<tr>
<td>calcium</td>
<td>83.7 % on inside of thallus</td>
<td>36.0 %</td>
</tr>
<tr>
<td>silicon</td>
<td>31.1 % on outside of thallus</td>
<td>31.1 %</td>
</tr>
<tr>
<td>potassium</td>
<td>2.72 % on inside of thallus</td>
<td>11.6 %</td>
</tr>
<tr>
<td>aluminium</td>
<td>8.97 % on outside of thallus</td>
<td>7.66 %</td>
</tr>
<tr>
<td>chlorine</td>
<td>2.26 % on inside of thallus</td>
<td>3.03 %</td>
</tr>
<tr>
<td>sulphur</td>
<td>3.47 % evenly dispersed</td>
<td>3.47 %</td>
</tr>
<tr>
<td>iron</td>
<td>0.93 % on inside of thallus</td>
<td>4.94 %</td>
</tr>
<tr>
<td>cobalt</td>
<td>1.48 % evenly dispersed</td>
<td>2.21 %</td>
</tr>
</tbody>
</table>

were calculated as (Malea *et al.*, 1994):

\[
\text{CF} = \frac{\mu g^{-1} \text{dry weight in plant}}{\mu g^{-1} \text{in medium}}
\]  

(9.3)

Into the inorganic phase, concentration factors were 1280 for Cu$^{2+}$, 310 for Ni$^{2+}$, and 6090 for Co$^{2+}$. In the organic phase metals are concentrated by 11010 for Cu$^{2+}$, 4520 for Ni$^{2+}$, and 14780 for Co$^{2+}$. The proportion of metal ion extracted by acetic acid to metal ion extracted by concentrated acid is 0.12 for Cu$^{2+}$, 0.067 for Ni$^{2+}$, and 0.41 for Co$^{2+}$. The observed relationship between metal ion concentrations and seawater concentrations in these samples indicates that *Corallina officinalis* has the potential to be a bio-indicator of heavy metal concentration in coastal seawater, at least in this area, and at the levels observed. Further investigations, including seasonal monitoring and comparisons with other sites, would be needed to determine the universality of this potential application.

Selectivity and completeness of the extraction procedure was shown to be good, both from the measurements of Cu$^{2+}$ in the *Porphyra*-stalactite sample, and from the tests of the effect of adding more acetic acid and/or a longer acetic acid digestion time. As the pH of the acetic acid would remain between approximately 2 and 5, it was not expected that organic plant material would be substantially extracted by it. For the mixed *Porphyra*-stalactite extraction, a recovery of 105.8% was achieved from the inorganic phase, and of 97.0% from the organic phase. Both these results were identical to those determined separately, within experimental error, but may also indicate that a small amount of loosely-bound metal ion within the cell was extracted by the acetic acid. An increase in the amount of acetic acid, or in the extraction time, did not lead to an increase in concentration of any of the three metals in the inorganic extract. The technique of dissolving the calcium carbonate phase of a rock, sediment or organism in dilute or weak acid has been widely used (Esslemont, 1994; Martin *et al.*, 1987). Acetic acid was used in this study because of its higher pH and buffering action.

For the majority of extractions, two separate samples were digested, with three measurements on each digest. The average percentage variation between the two digests
Table 9.5: Cu$^{2+}$, Co$^{2+}$ and Ni$^{2+}$ concentrations of material used for quality control, in parts per million.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>uncultured alga</td>
<td>1.15±0.07</td>
<td>9.91±0.6</td>
<td>1.38±0.2</td>
<td>20.5±0.8</td>
<td>0.14±0.03</td>
<td>0.34±0.06</td>
</tr>
<tr>
<td>2mL 1hr</td>
<td>1.15</td>
<td>9.84</td>
<td>1.42</td>
<td>20.8</td>
<td>0.13</td>
<td>0.35</td>
</tr>
<tr>
<td>3mL 1hr</td>
<td>1.22</td>
<td>9.35</td>
<td>1.50</td>
<td>20.2</td>
<td>0.15</td>
<td>0.32</td>
</tr>
<tr>
<td>2mL 4hr</td>
<td>1.07</td>
<td>10.6</td>
<td>1.45</td>
<td>20.7</td>
<td>0.17</td>
<td>0.38</td>
</tr>
<tr>
<td>3mL 4hr</td>
<td>1.15</td>
<td>9.94</td>
<td>1.38</td>
<td>21.0</td>
<td>0.13</td>
<td>0.37</td>
</tr>
<tr>
<td>2mL 12hr</td>
<td>1.18</td>
<td>10.0</td>
<td>1.41</td>
<td>20.5</td>
<td>0.12</td>
<td>0.36</td>
</tr>
<tr>
<td>3mL 12hr</td>
<td>1.13</td>
<td>10.2</td>
<td>1.44</td>
<td>20.2</td>
<td>0.16</td>
<td>0.32</td>
</tr>
<tr>
<td>Porphyra</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcite stal.</td>
<td>5.2±0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td>5.5±0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 9.6: Average percentage variation in the values determined for each metal.

<table>
<thead>
<tr>
<th>Metal</th>
<th>HAc extraction</th>
<th>HNO₃ extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Nickel</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Cobalt</td>
<td>24</td>
<td>30</td>
</tr>
</tbody>
</table>

is given in Table 9.6, and is particularly high for both extractions of Co²⁺, which had a low concentration in each extract. In the graphs presented in the following sections, error bars give the range of concentrations found, rather than a mean plus standard deviation.

The high-Mg calcite proportion of the collected *C. officinalis* was determined by weighing five samples before and after dissolution. It varied substantially, from 79.9–89.4%, with a mean of 85.1%. Variation was probably due to the particular plant parts included, with more calcification at the base in the older parts of the plants, and less at the branch tips. The Mg²⁺ concentration of the calcite was determined from three extractions to be 13.4±0.5%.

9.3.3 Metal Ion Uptake of the Cultured Algae

The accumulation of Cu²⁺, Ni²⁺ and Co²⁺ after five days of culturing in artificial seawater are presented in Tables 9.7–9.9, and are plotted in Figures 9.4–9.9. Seawater metal ion concentrations are presented as a 3, 6 or 9-fold increase over the concentration of the seawater which the plants were collected from. Transition metal ion concentrations are presented as a ratio of [M²⁺]:mass of CaCO₃ for the acetic acid extracts. This procedure is necessary because of variations in the CaCO₃ percentage of the samples. The organic portion of the plant was assumed to be that which was not soluble in acetic acid, and the metal concentrations for the HNO₃ extractions are presented relative to this mass. This procedure is effective for the live plants, but not for the killed plants, which lost (by visual estimation) half their mass over the culturing period. Metal concentrations in the organic material of the killed plants cannot be directly compared with the concentrations in the organic material of the live plants.

Copper

The concentration of Cu²⁺ increased with increasing artificial seawater Cu²⁺ concentration, in both the acetic-acid-extractable and the concentrated-acid-extractable fractions of the live alga. The increase was greater in the organic phase, almost doubling as the seawater Cu²⁺ concentration was increased 9-fold, and showing the high affinity that Cu²⁺ has for organic material.

An increasing concentration of Co²⁺ in the medium had little effect on Cu²⁺ accumulation by the alga, but a definite drop in Cu²⁺ concentration was seen with increasing
### Table 9.7: Copper concentrations of material from *C. officinalis* after culturing in artificial seawater, in parts per million.

<table>
<thead>
<tr>
<th>Sample</th>
<th>10.3 mol L(^{-1}) Ca(^{2+})</th>
<th>5.0 mol L(^{-1}) Ca(^{2+})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HAc ext.</td>
<td>HNO(_3) ext.</td>
</tr>
<tr>
<td>Live plants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1x</td>
<td>1.27</td>
<td>11.4</td>
</tr>
<tr>
<td>3xCu</td>
<td>1.38</td>
<td>15.3</td>
</tr>
<tr>
<td>6xCu</td>
<td>1.65</td>
<td>23.9</td>
</tr>
<tr>
<td>9xCu</td>
<td>1.83</td>
<td>29.4</td>
</tr>
<tr>
<td>3xNi</td>
<td>1.15</td>
<td>10.7</td>
</tr>
<tr>
<td>6xNi</td>
<td>0.96</td>
<td>9.34</td>
</tr>
<tr>
<td>9xNi</td>
<td>0.72</td>
<td>7.66</td>
</tr>
<tr>
<td>3xCo</td>
<td>1.18</td>
<td>10.9</td>
</tr>
<tr>
<td>6xCo</td>
<td>1.15</td>
<td>11.6</td>
</tr>
<tr>
<td>9xCo</td>
<td>1.13</td>
<td>11.5</td>
</tr>
<tr>
<td>Killed plants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1x</td>
<td>0.33</td>
<td>7.43</td>
</tr>
<tr>
<td>3xCu</td>
<td>0.45</td>
<td>9.28</td>
</tr>
<tr>
<td>6xCu</td>
<td>0.54</td>
<td>10.9</td>
</tr>
<tr>
<td>9xCu</td>
<td>0.68</td>
<td>14.9</td>
</tr>
<tr>
<td>3xNi</td>
<td>0.25</td>
<td>6.19</td>
</tr>
<tr>
<td>6xNi</td>
<td>0.23</td>
<td>5.63</td>
</tr>
<tr>
<td>9xNi</td>
<td>0.20</td>
<td>4.70</td>
</tr>
<tr>
<td>3xCo</td>
<td>0.33</td>
<td>7.35</td>
</tr>
<tr>
<td>6xCo</td>
<td>0.34</td>
<td>6.98</td>
</tr>
<tr>
<td>9xCo</td>
<td>0.34</td>
<td>7.29</td>
</tr>
</tbody>
</table>
Chapter 9. Culturing Corallina officinalis: Results

Figure 9.4: The concentrations of Cu$^{2+}$ (in $\mu$gg$^{-1}$ dry weight) in the inorganic part of the seaweed, extracted by acetic acid.

Figure 9.5: The concentrations of Cu$^{2+}$ (in $\mu$gg$^{-1}$ dry weight) in the organic part of the seaweed, extracted by HNO$_3$. 
9.3. Transition Metal Ion Uptake by *C. officinalis*

Table 9.8: Nickel concentrations of material from *C. officinalis* after culturing in artificial seawater, in parts per million.

<table>
<thead>
<tr>
<th>Sample</th>
<th>10.3 mol L(^{-1}) Ca(^{2+}) HAc ext.</th>
<th>HAc ext.</th>
<th>5.0 mol L(^{-1}) Ca(^{2+}) HNO(_{3}) ext.</th>
<th>HNO(_{3}) ext.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live plants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1x</td>
<td>1.45</td>
<td>20.4</td>
<td>1.36</td>
<td>18.4</td>
</tr>
<tr>
<td>3xNi</td>
<td>1.52</td>
<td>25.6</td>
<td>1.40</td>
<td>22.7</td>
</tr>
<tr>
<td>6xNi</td>
<td>1.55</td>
<td>31.0</td>
<td>1.48</td>
<td>27.5</td>
</tr>
<tr>
<td>9xNi</td>
<td>1.72</td>
<td>34.7</td>
<td>1.59</td>
<td>33.6</td>
</tr>
<tr>
<td>3xCu</td>
<td>1.63</td>
<td>22.6</td>
<td>1.37</td>
<td>19.9</td>
</tr>
<tr>
<td>6xCu</td>
<td>1.56</td>
<td>20.0</td>
<td>1.44</td>
<td>17.4</td>
</tr>
<tr>
<td>9xCu</td>
<td>1.42</td>
<td>21.3</td>
<td>1.27</td>
<td>16.9</td>
</tr>
<tr>
<td>3xCo</td>
<td>1.53</td>
<td>17.9</td>
<td>1.30</td>
<td>18.3</td>
</tr>
<tr>
<td>6xCo</td>
<td>1.66</td>
<td>22.6</td>
<td>1.41</td>
<td>20.6</td>
</tr>
<tr>
<td>9xCo</td>
<td>1.47</td>
<td>21.1</td>
<td>1.46</td>
<td>19.5</td>
</tr>
<tr>
<td>Killed plants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1x</td>
<td>0.49</td>
<td>6.04</td>
<td>0.51</td>
<td>5.55</td>
</tr>
<tr>
<td>3xNi</td>
<td>0.57</td>
<td>10.2</td>
<td>0.54</td>
<td>9.07</td>
</tr>
<tr>
<td>6xNi</td>
<td>0.66</td>
<td>18.3</td>
<td>0.67</td>
<td>19.2</td>
</tr>
<tr>
<td>9xNi</td>
<td>0.78</td>
<td>26.6</td>
<td>0.76</td>
<td>26.0</td>
</tr>
<tr>
<td>3xCu</td>
<td>0.52</td>
<td>6.22</td>
<td>0.46</td>
<td>6.08</td>
</tr>
<tr>
<td>6xCu</td>
<td>0.56</td>
<td>5.95</td>
<td>0.49</td>
<td>5.77</td>
</tr>
<tr>
<td>9xCu</td>
<td>0.48</td>
<td>6.00</td>
<td>0.47</td>
<td>6.18</td>
</tr>
<tr>
<td>3xCo</td>
<td>0.51</td>
<td>5.82</td>
<td>0.44</td>
<td>5.94</td>
</tr>
<tr>
<td>6xCo</td>
<td>0.45</td>
<td>6.07</td>
<td>0.47</td>
<td>5.93</td>
</tr>
<tr>
<td>9xCo</td>
<td>0.54</td>
<td>6.10</td>
<td>0.45</td>
<td>6.14</td>
</tr>
</tbody>
</table>

Table 9.9: Cobalt concentrations of material from *C. officinalis* after culturing in artificial seawater, in parts per million.

<table>
<thead>
<tr>
<th>Sample</th>
<th>10.3 mol L(^{-1}) Ca(^{2+}) HAc ext.</th>
<th>HAc ext.</th>
<th>5.0 mol L(^{-1}) Ca(^{2+}) HNO(_{3}) ext.</th>
<th>HNO(_{3}) ext.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live plants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1x</td>
<td>0.18</td>
<td>0.42</td>
<td>0.18</td>
<td>0.37</td>
</tr>
<tr>
<td>3xCu</td>
<td>0.17</td>
<td>0.40</td>
<td>0.19</td>
<td>0.35</td>
</tr>
<tr>
<td>6xCu</td>
<td>0.21</td>
<td>0.51</td>
<td>0.23</td>
<td>0.49</td>
</tr>
<tr>
<td>9xCo</td>
<td>0.29</td>
<td>0.63</td>
<td>0.30</td>
<td>0.66</td>
</tr>
<tr>
<td>Killed plants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1x</td>
<td>0.10</td>
<td>0.33</td>
<td>0.09</td>
<td>0.31</td>
</tr>
<tr>
<td>3xCu</td>
<td>0.13</td>
<td>0.37</td>
<td>0.12</td>
<td>0.36</td>
</tr>
<tr>
<td>6xCu</td>
<td>0.17</td>
<td>0.44</td>
<td>0.16</td>
<td>0.46</td>
</tr>
<tr>
<td>9xCo</td>
<td>0.20</td>
<td>0.49</td>
<td>0.22</td>
<td>0.52</td>
</tr>
</tbody>
</table>
Chapter 9. Culturing Corallina officinalis: Results

Figure 9.6: The concentrations of Ni$^{2+}$ (in $\mu$gg$^{-1}$ dry weight) in the inorganic part of the seaweed, extracted by acetic acid.

Figure 9.7: The concentrations of Ni$^{2+}$ (in $\mu$gg$^{-1}$ dry weight) in the organic part of the seaweed, extracted by HNO$_3$. 
9.3. Transition Metal Ion Uptake by C. officinalis

Figure 9.8: The concentrations of Co^{2+} (in μg g^{-1} dry weight) in the inorganic part of the seaweed, extracted by acetic acid.

Figure 9.9: The concentrations of Co^{2+} (in μg g^{-1} dry weight) in the organic part of the seaweed, extracted by HNO_{3}.
Ni\(^{2+}\) concentration. It is possible that this drop in Cu\(^{2+}\) concentration is due to competition between the two metal ions for transfer ligands, especially as Ni\(^{2+}\) was present in large excess over Cu\(^{2+}\).

Copper concentrations of both phases were lower for those seaweed which were killed before "culturing". Levels of Cu\(^{2+}\) were approximately 1/3 of the live concentrations in both the inorganic and, allowing for loss of organic material with rotting, the organic material. The concentrations were lower than the initial concentrations of the seaweed, meaning that metal ions must have leached out of the dead plant cells into the surrounding medium. The Ca\(^{2+}\):Cu\(^{2+}\) ratio has been reduced for the calcite phase, which may be a consequence of the fact that intracellular Ca\(^{2+}\) concentrations in live plants are much lower than the surrounding water concentrations, whereas the cell concentration of transition metals is very much higher than that of the surrounding water. If the cell mechanisms for transporting metal ions against a potential gradient are partially broken down, the aqueous system would restore equilibrium by reducing cellular transition metal concentrations. Intact dead cells would be expected to have almost the same metal concentrations as live cells, as up to 90% of the absorption of the metal ion is facilitated by transfer ligands, into the cell, and occurs through cell membranes within the first hour of exposure, whether the plant is alive or not (Sigg, 1987). The remaining 10% of uptake is due to ligand exchange processes within the cell, and occurs more slowly. A decrease in metal ion concentration to approximately 30–50% of the initial concentration occurred in the killed plants for all three of the metals, indicating that the process causing it was independent of the metal ion. The rapid expansion due to killing by immersion in liquid nitrogen could be expected to cause substantial cell damage, and cells which are killed by slow drying may be more intact.

Nickel

A slight increase in Ni\(^{2+}\) concentration in both the calcite and the organic phase was seen with increasing Ni\(^{2+}\) concentration in the medium. Although the Ni\(^{2+}\) concentration of the medium was much higher than the Cu\(^{2+}\) or Co\(^{2+}\) concentrations, proportional uptake of Ni\(^{2+}\) was less. Nickel is not known to be an essential element for marine algae, and therefore may have fewer avenues for entry into the cell than the essential elements.

Increases in the concentration of the other two transition metals had no effect on Ni\(^{2+}\) accumulation. The live algae cultured in artificial seawater containing 5.0 nM Ca\(^{2+}\) have incorporated less Ni\(^{2+}\) than the plants cultured with 10.3 nM Ca\(^{2+}\). Woelkerling et al. (1983) found that a lower Ca\(^{2+}\) concentration resulted in healthier coralline algae in culture. If the plants were healthier, they may have accumulated less of the toxic Ni\(^{2+}\), although no significant increase in photosynthesis was seen at the lower Ca\(^{2+}\) concentration (see Section 9.4). This slight decrease in uptake of Ni\(^{2+}\) was the only observed variation with the varying Ca\(^{2+}\) concentration, indicating that the Ca\(^{2+}\) concentration of the growth medium is not a critical factor in metal incorporation into C. officinalis.
Table 9.10: Net photosynthesis rates of the cultured algae.

<table>
<thead>
<tr>
<th>Medium conc.</th>
<th>10.3 mol L(^{-1}) Ca(^{2+})</th>
<th>5.0 mol L(^{-1}) Ca(^{2+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x</td>
<td>0.37</td>
<td>0.34</td>
</tr>
<tr>
<td>3xCu</td>
<td>0.36</td>
<td>0.22</td>
</tr>
<tr>
<td>6xCu</td>
<td>0.30</td>
<td>0.20</td>
</tr>
<tr>
<td>9xCu</td>
<td>0.19</td>
<td>0.17</td>
</tr>
<tr>
<td>3xNi</td>
<td>0.37</td>
<td>0.32</td>
</tr>
<tr>
<td>6xNi</td>
<td>0.38</td>
<td>0.29</td>
</tr>
<tr>
<td>9xNi</td>
<td>0.37</td>
<td>0.34</td>
</tr>
<tr>
<td>3xCo</td>
<td>0.38</td>
<td>0.30</td>
</tr>
<tr>
<td>6xCo</td>
<td>0.37</td>
<td>0.31</td>
</tr>
<tr>
<td>9xCo</td>
<td>0.37</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Cobalt

There was a similar and significant increase in uptake into the Ca\(_2\)CO\(_3\) of the plants with increasing concentration of Co\(^{2+}\) in the artificial medium, for both killed and live algae, once the cobalt concentration in the medium had more than tripled. No significant changes in cobalt concentrations were seen in the plants cultured in seawater with elevated Cu\(^{2+}\) or Ni\(^{2+}\) levels, with concentrations varying between 0.13 and 0.18 ppm for the acetic acid extracts, and between 1.26 and 1.40 for the nitric acid extracts. A higher proportion of the Co\(^{2+}\) taken up was found to be in the calcite phase, possibly because the cobalt concentrations were sufficiently low to allow deposition of relatively more of the metal with the calcite. This deposition may be a detoxification technique for the alga.

9.4 Photosynthesis of the Cultured Plants

Net photosynthesis of each set of plants was measured before and after culturing, primarily to indicate the health status of the algae. Photosynthesis rates for the algae cultured in 5.0 mM Ca\(^{2+}\) were slightly lower than those cultured in 10.3 mM Ca\(^{2+}\) (Table 9.10, and Figures 9.10 and 9.11), although the variation lies within the experimental uncertainty of the measurements. The pre-culturing photosynthesis rates were 0.30 and 0.36 mg C g\(^{-1}\) dry weight h\(^{-1}\) respectively, and were unchanged after both the Co\(^{2+}\) and the Ni\(^{2+}\) culturing experiments. Unlike Co\(^{2+}\) and Ni\(^{2+}\), Cu\(^{2+}\) showed substantial toxicity, halving photosynthesis at a 9-fold increase in metal ion concentration in the medium.

For the uptake experiments, plants were cultured under two fluorescent tubes. Prior measurements found that photosynthesis rates under one tube were approximately half the rates under two tubes, indicating that photosynthetic saturation levels were not reached.
Figure 9.10: The rate of photosynthesis of *C. officinalis* cultured in artificial seawater containing 5.0 mM Ca\(^{2+}\), *versus* the metal ion concentration of the growth medium, expressed as a 3, 6 or 9-fold increase over the concentration in Kaikoura seawater.

Figure 9.11: The rate of photosynthesis of *C. officinalis* cultured in artificial seawater containing 10.3 mM Ca\(^{2+}\), *versus* the metal ion concentration of the growth medium, expressed as a 3, 6 or 9-fold increase over the concentration in Kaikoura seawater.
9.5 Summary

*C. officinalis* were cultured in a chemically-defined artificial seawater medium, containing either 5.0 or 10.3 mM Ca\(^{2+}\), and spiked with sub-lethal amounts of Cu\(^{2+}\), Ni\(^{2+}\) or Co\(^{2+}\). The bioactivity of Cu\(^{2+}\) was found to be substantially higher than that of Ni\(^{2+}\) and Co\(^{2+}\), with Cu\(^{2+}\) being toxic at the concentrations supplied. As with laboratory adsorption experiments (Chapter 3), more Cu\(^{2+}\) was taken up by the calcium carbonate fraction than Ni\(^{2+}\) or Co\(^{2+}\) was. All three metals were concentrated in both the organic and the inorganic parts of the plants, with uptake increasing as the metal ion concentration increased. Killed plants, which were “cultured” in the same way as the live plants, showed a substantial decrease in metal ion concentrations, particularly in the inorganic fraction. This phenomenon may be due to cell rupture during killing. The seaweed *C. officinalis* shows potential as an indicator of heavy metal concentrations in seawater. Because trends were similar in the organic and inorganic fractions of live plants, it would be unnecessary to separate the fractions for routine analysis, reducing the expense and time of the procedure. However, further tests would be required to assess the practicability of this application. In particular, desorption of metal ions if the seawater metal concentration was reduced, and any seasonal variation in metal uptake, would have to be determined.
Chapter 9. Culturing Corallina officinalis: Results
Appendix A

Initial Experimentation on the Polymorphism of Calcium Carbonate

A.1 Introduction

Initial research for this thesis involved studying the growth of calcium carbonate crystals to attempt to determine which of Cu$^{2+}$, Co$^{2+}$, Ni$^{2+}$ and Zn$^{2+}$ ions promoted aragonite formation, the required concentrations to achieve this effect, and why. This research involved growing crystals in the laboratory with different amounts of metal ion dissolved in the liquid the crystals grew from.

A.2 Materials and Methods

A calcium bicarbonate solution was made by bubbling CO$_2$ gas (from a dry ice source) through a stirred suspension of CaCO$_3$ (AnalaR grade) in water. This was allowed to react for 6-10 hours until the resulting solution had a Ca$^{2+}$ concentration between 380 and 420 ppm. The solution was then filtered through a porosity 4 glass frit and stored under a CO$_2$ atmosphere. Both triply distilled and diluted tap water were used in this solution. Tap water contains a significant quantity of sodium, and the effect of this was investigated. Crystals of CaCO$_3$ were grown by placing 150mL aliquots of the Ca(HCO$_3$)$_2$ solution in conical flasks in waterbaths, generally at 25°C. The aliquots were doped with CoCl$_2$·6H$_2$O, ZnCl$_2$, CuCl$_2$·2H$_2$O, or NiCl$_2$·6H$_2$O solution to give a variety of concentrations of M$^{2+}$ within the solution. Samples were left, open to the atmosphere, to grow for seven days and then were collected, washed with triply distilled water and dried in air. When dry, the crystals were analysed by infrared spectroscopy for calcite/aragonite ratio, and weighed samples were dissolved in dilute HCl and analysed by atomic absorption spectroscopy to determine M$^{2+}$ concentration. In all cases, samples grown in Ca(HCO$_3$)$_2$ solution which was not doped with a metal ion were pure calcite, both the solutions made from triply distilled and tap water. When
Appendix A. Initial Experimentation on the Polymorphism of Calcium Carbonate

solutions were doped with the four metal ions it was expected that with increasing $M^{2+}$ concentration, an increasing amount of aragonite would form. While this did occur in some cases, the situation was seldom this simple. In general, the percentage of $M^{2+}$ in the crystals increased steadily with increased $M^{2+}$ concentration in the initial solution, as expected.

Some general trends were observed, and are reported below, but the polymorphic ratio of the precipitates was inconsistent. From 6–10 repetitions of most experiments, 3–7 significantly different results were obtained. Attempts to standardize the atmosphere under which the crystals grew in a glovebox failed to increase consistency. It was noted that the appearance of the precipitates on the side of the growth containers varied, an effect which thorough prior acid-washing of flasks did not remove. As nucleation onto the sides of the flasks was probably varying, attempts were made to cover the surface with gladwrap, or to coat it with silicone oil. This approach was also unsuccessful, and the research was abandoned.

The system from which the crystals grew could be possibly be sufficiently standardized by growth in a CO$_2$-controlled, temperature-controlled, dust-free environment, which was unavailable, as metal concentrations were too high for a clean room to be used, and by use of new flasks for each experiment. Most successful recent work in the literature was based upon growth in sealed containers or from seeded solutions, and such techniques as the counting of radioactive $M^{2+}$ isotopes.

A.3 General Trends Observed

A.3.1 Nickel

Solutions of Ca(HCO$_3$)$_2$ made from diluted tap water

Solutions with a Ni$^{2+}$ concentration of less than 2 ppm consistently formed calcite, and with a concentration of 4–12 ppm mostly formed aragonite. These aragonite crystals contained only 0.1% nickel, indicating that very little of the metal ion needed to be adsorbed or incorporated into the crystal to have a major effect on the polymorphism. Different sets of solutions with a Ni$^{2+}$ concentration between 14 and 20 ppm showed a large variation in polymorphic ratio. This may be because of the presence of Na$^+$ in the tap water, as Na$^+$ would be expected to enhance calcite formation (Kitano and Hood, 1962), and may counter the effect of Ni$^{2+}$. Crystals grown from solutions of Ca(HCO$_3$)$_2$ made from triply distilled water with a concentration of 14–20 ppm Ni$^{2+}$ and 8 ppm Na$^+$ also varied in polymorphic ratio. This does not explain why reasonably consistent results were obtained from solutions containing 4–12 ppm Ni$^{2+}$ and as much Na$^+$. A peak in aragonite percentage occurred at approximately 20 ppm Ni$^{2+}$, after which an increasing amount of calcite appeared again. This was unexpected, but has been reported before by Chen (1977). Solutions with a Ni$^{2+}$ concentration of greater than 200 ppm frequently formed NiCO$_3$ instead of CaCO$_3$. At this concentration the Ca$^{2+}$:Ni$^{2+}$ ratio was only 2:1, and the solution must have achieved supersaturation with respect to Ni$^{2+}$ before becoming supersaturated with respect to Ca$^{2+}$.
A.3. General Trends Observed

Solutions of Ca(HCO₃)₂ made from triply-distilled water

Solutions with a Ni²⁺ concentration of less than 2 ppm formed calcite, and 4–20 ppm mostly formed aragonite. For 12 ppm and less, this was identical to the situation with tap water. The variation in polymorphic ratio shown by crystals containing sodium ions did not occur as much in those grown from Ca(HCO₃)₂ made from triply distilled water, but results were still inconsistent. As with crystals grown from Ca(HCO₃)₂ made from tap water, a peak in aragonite percentage occurred at very approximately 20 ppm Ni²⁺, after which an increasing amount of calcite appeared again. By this high a Ni²⁺ concentration, the Ni²⁺:Na⁺ ratio may be high enough for the effect of Ni²⁺ to sometimes overwhelm the proposed effect of Na⁺, and both systems behaved similarly above this concentration of Ni⁺.

A.3.2 Copper

Solutions with a Cu²⁺ concentration of less than 12 ppm mostly formed calcite, and greater than 12 ppm mostly formed aragonite. This is six times as great a concentration as required for formation of aragonite to sometimes appear in the presence of Ni²⁺. Copper results were also less variable from set to set at any particular concentration than Ni²⁺ results, and did not show a peak in the aragonite/calcite ratio, as samples containing Ni²⁺ did. In this way, the behaviour of Cu²⁺ differed significantly from that of Ni²⁺. Solutions with a Cu²⁺ concentration between 300 and 500 ppm formed CuCO₃, and with a concentration greater than 600 ppm, an unknown product, possibly a copper hydroxy compound. The infrared spectrum of this compound showed a hydroxy peak, but no carbonate peak. The percentage of copper in these crystals was also significantly higher than the percentage of nickel in crystals doped with nickel. Therefore, if Ni²⁺ and Cu²⁺ affected the growing calcium carbonate surface in exactly the same way, CaCO₃ grown from solution doped with Cu²⁺ would be expected to take up a higher proportion of the metal, and form aragonite at a lower solution concentration than CaCO₃ grown from a solution doped with Ni²⁺. Instead, a higher solution concentration of Cu²⁺ was required.

A.3.3 Zinc

Solutions of Ca(HCO₃)₂ made from diluted tap water

The percentage of Zn²⁺ in the CaCO₃ crystals increased linearly in the range 0–50 ppm. Most of the samples analysed in this range were predominantly calcite, with the exception of 30 ppm Zn²⁺, which varied considerably, and was up to approximately 90% aragonite. This sample still fitted on the linear plot of Zn²⁺ percentage. Six different sets of crystals were grown from solution containing 30 ppm Zn²⁺, and three of these contained at least 90% aragonite. Aragonite percentage then seemed to decrease until about 60 ppm Zn²⁺, after which it sometimes increased until the appearance of ZnCO₃ at a solution concentration of approximately 200 ppm Zn²⁺.
Appendix A. Initial Experimentation on the Polymorphism of Calcium Carbonate

Solutions of Ca(HCO$_3$)$_2$ made from triply-distilled water

Fewer repetitions of these experiments were attempted. Of the two samples grown from solution containing 30 ppm Zn$^{2+}$, one was almost pure calcite and the other almost pure aragonite. Apart from this result, aragonite did not start to form until a solution concentration greater than 100 ppm Zn$^{2+}$ had been reached. Zinc carbonate also formed in solutions containing at least 200 ppm Zn$^{2+}$ and was the only product by a concentration of 600 ppm.

A.3.4 Cobalt

Solutions of Ca(HCO$_3$)$_2$ made from diluted tap water

Solutions with a Co$^{2+}$ concentration of less than 2 ppm formed calcite, but in the range of solution concentration of 2–3 ppm Co$^{2+}$, a peak of 100% aragonite occurred several times. Totally inconsistent results were obtained for concentrations of 4–10 ppm Co$^{2+}$, possibly also due to the presence of Na$^+$ in the tap water. Percentages of Co$^{2+}$ in the crystals were very similar to percentages of Zn$^{2+}$ or Cu$^{2+}$ in crystals obtained from solution doped with Zn$^{2+}$ or Cu$^{2+}$. All three were considerably higher than percentages of Ni$^{2+}$ in crystals doped with Ni$^{2+}$. Solutions with a Co$^{2+}$ concentration of greater than 14 ppm reasonably consistently formed aragonite, with an unknown CoCO$_3$-like compound starting to form when the solution contained about 80 ppm Co$^{2+}$. This was the exclusive product once the solution concentration reached 600 ppm Co$^{2+}$. While the percentage of Co$^{2+}$ in the crystals formed a curve, increasing with increasing Co$^{2+}$ concentration in the original solution, the rate of increase was less at higher initial Co$^{2+}$ concentrations. This may be because of the formation of the CoCO$_3$-like compound at these concentrations, causing Co$^{2+}$ percentage to level off at a maximum value.

Solutions of Ca(HCO$_3$)$_2$ made from triply-distilled water

Solutions with a Co$^{2+}$ concentration of less than 4 ppm formed calcite, and greater than 4 ppm a varying proportion of aragonite. No peak of 100% aragonite at low concentrations was seen. As with crystals grown in Ca(HCO$_3$)$_2$ made from diluted tap water, the CoCO$_3$-like unknown appeared at a solution concentration of approximately 80 ppm. It appears that for Co$^{2+}$, as with all the other metals, the presence of Na$^+$ serves to complicate the polymorphism rather than to simply reduce the effect of the bivalent metal ion.

A.4 Summary

Ni$^{2+}$ and Co$^{2+}$ had the greatest effect on the polymorphic ratio of CaCO$_3$, frequently producing aragonite at low concentrations. Zinc(II) had the least effect, with most samples being mostly calcite. The effect of tap water, or of doping solutions with Na$^+$, was variable.
Appendix B

Determination of $K_w$

Harned and Owen (1958) describe the following empirical equation for calculating $K_w$ in a given medium, at a given temperature and ionic strength.

\[ K_w = [H^+] [OH^-] \left( \frac{\gamma_{H^+} \gamma_{OH^-}}{\{H_2O\}} \right) \]  
\[ \text{(B.1)} \]

where

\[ \log \left( \frac{\gamma_{H^+} \gamma_{OH^-}}{\{H_2O\}} \right) = \frac{-2S A^{0.5}}{1 + A T^{0.5}} + B t + C t^{0.5} \]

The parameters $B$ and $C$ are linearly dependent on temperature, and

\[ S = \frac{1.184 \times 10^6}{(D T)^{1.5}} \]

where $D$ is the dielectric constant and $T$ the temperature in °K.

\[ A = \frac{a^0 x 50.30}{(D T)^{0.5}} \]

\[ B = b^0 + b_1 t \]

and

\[ C = c^0 + c_1 t \]

where $t$ is the temperature in °C.
For NaCl solutions, such as the artificial seawater medium of this study, the following parameters are valid at 15°C:

\[(DT) = 2.3696 \times 10^4\]

\[a^0 = 3.6, \quad b^0 = 0.198 \quad \text{and} \quad c^0 = -0.0085\]

\[b_1 = 2.00 \times 10^{-4} \quad \text{and} \quad c_1 = -2 \times 10^{-4}\]

\[t = 15\]

This calculation gave a \(pK_w\) of 14.286, at an ionic strength of 0.63 and a temperature of 15°C.
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