Identifying temporal and spatial variations in the binding properties of transition metal-organic ligand complexes in cave waters using organic inclusions in speleothem calcite:

Implications for paleoclimate research

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ROBERT BRODNAX

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1. Abstract

Speleothems such as stalagmites, stalactites and flowstones, contain paleoclimate information encoded in their geochemical properties. One of these properties, transition metal abundance in speleothem calcite, is tightly controlled by complexation reactions in cave water with dissolved organic matter (DOM). Organic compounds are also preserved in speleothem deposits. DOM structure varies from cave system to cave system and over time, but whether these structural variations meaningfully alter the way DOM controls trace metal incorporation into calcite is unclear. This is relevant to the development of speleothem transition metal content as a paleoclimate proxy, particularly over large spatial and temporal scales where DOM structure variations are likely. This study identifies variations in the binding properties of DOM preserved in speleothem calcite over time and space by extracting organic compounds of known age and origin from speleothems collected from New Zealand, Niuean, British, and Australian cave systems. The kinetic properties of DOM complexes from these extracts were measured using small volume diffusive gradients in thin films (SV-DGT) and the variation in the binding strength of complexes with Cu, Co, and Ni was compared across time and space. DOM binding of Cu and Co were found to vary significantly depending on the cave system from which the DOM originated. Whether this variation was linked to climate changes represented in the age of the DOM or to changes in system characteristics related to geographical setting was unclear. Despite this, it is apparent that there are factors related to the origin of DOM in cave systems that influence the kinetic restrictions applied to transition metals in cave water. This variability should be considered when linking trace element contents in cave precipitates to paleoclimate change.

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5. Introduction

5.1 Background

Speleothems (from the Greek *spelaion*, cave, and *thema*, deposit) (Moore 1952) are a type of mineral deposit that occur naturally in cave systems. Speleothems form from meteoric water that enters cave systems through pores and fractures in the rock. As this water travels through the soil above the cave system, it dissolves CO_2 and becomes acidic. It then travels downwards, dissolving carbonate-based minerals in the soil and epikarst (the stone above a cave system) and becomes enriched in basic CO_3^{2-} and HCO_3^- ions. This process is most common in limestone systems (karst systems), in which rocks are largely comprised of $CaCO_3$ minerals such as calcite ($CaCO_3$) and aragonite ($(MgCa)CO_3$) (Fairchild & Baker, 2012). In these systems, the dissolution of $CaCO_3$ in the epikarst widens cracks in the rock, forming chambers, and eventually whole cave systems. The water that continues to flow through these cracks is rich in both Ca^{2+} and CO_3^{2-} ions. As it flows into these caves, newly emerging water degasses CO_2 to readjust to the new atmospheric equilibrium with the cave air, which, in turn forces dissolved Ca^{2+} and CO_3^{2-} to precipitate out of solution, depositing $CaCO_3$ on cave surfaces and walls as speleothems (Qian et al., 2012; White, 2019).

Over the last 50 years, research on speleothems and their formation has revealed them to be invaluable resources for understanding past environments (Fairchild and Baker, 2012; Wong & Breeker, 2015). Speleothem formation is directly related to the hydrological and atmospheric factors that affect the chemistry and flow dynamics of water in cave systems. These factors include regional weather, such as local rainfall and evaporation (Karmann et al., 2007; Li et al., 2005), larger scale climate cycles and oscillations (Huang et al., 2022; Nava-Fernandez et al., 2020), global climate factors such as glacial-interglacial and CO_2 cycles (Lachniet, 2009) and environmental factors such as surface soil conditions, local vegetation cover and base rock composition (Blyth et al., 2013; Fairchild et al., 2000; Heidke et al., 2021). Due to the sheltered conditions within a typical cave system, speleothem formations can remain undisturbed for hundreds of thousands of years, making them an ideal archive of paleoclimate information (McDermont, 2004). Consequently, a large portion of modern speleothem research is focused on

deepening understanding of the relationships between specific compositional traits of a speleothem and the paleoclimate factors that influenced them during formation.

Speleothems can take a range of shapes and sizes, depending on the flow from which they form. Calcite precipitated from flowing water forms in layers over cave surfaces called flowstones. Dripping water leaves behind many fine layers on cave ceilings that eventually form soda straws and stalactites and builds up in layers on cave floor surfaces as stalagmites. Pooling water produces cave coral and other similar branching structures as it either evaporates or gradually degasses CO_2 to adjust to cave atmospheric conditions (Baker et al., 1998).

Local climate factors, such as seasonal rainfall, will affect the flow rate of cave water and impact speleothem growth, crystal structure, and the transport of trace elements and organic compounds across the speleothem surfaces (Borsato et al., 2007; Hartland et al., 2011; Karmann et al., 2007; Wong & Breecker, 2015). Local soil factors, such as surface vegetation or soil type, can sometimes be found represented in the abundances and compositions of the organic compounds and trace elements transported from the soil by water infiltrating into the epikarst (Blyth et al., 2016; Heidke et al., 2021; McDermott, 2004). Global climate variables, such as mean global temperature can occasionally be linked to isotope compositions of speleothem carbonate. The ratio of ^{18}O to ^{16}O in surface water for example, varies over long timescales depending on the relative rates of evaporation and precipitation in the upper latitudes. The heavier isotope of oxygen ^{18}O tends to be preferentially incorporated into ice and snow, so is depleted from global circulation during glacial periods, allowing $\delta^{18}O/^{16}O$ in cave rock to be used as a paleoclimate proxy for glacial extent, as well as global temperature in some specific circumstances (Daëron et al., 2019; Dansgaard, 1964; Lachniet, 2009).

Few relationships between any given speleothem property and a corresponding climate variable are simple. Myriad complicating factors affect most potential paleoclimate proxies, with complex relationships between variables altering the way that speleothem properties respond to a forcing by any given climate factor (Fairchild et al., 2006). Potentially straightforward proxies such as isotope ratios in speleothem calcite are affected not only by the abundance of isotopes in the water source but also by in-cave processes such as evaporation and prior calcite precipitation. These factors can affect cave water differently, depending on the

location of the speleothem in a cave or the paths taken by groundwater in the overlying rock (Daëron et al., 2019, Karmann et al., 2007). Deepening our understanding of the relationships between climate processes and speleothem composition allows us to account for these complicating factors and, as the field has developed, has allowed for an increasingly broad range of speleothem properties to be used as proxies for paleoclimate and paleoenvironmental conditions. Speleothem studies are now a cornerstone of paleoclimate research, and thanks to the increasing precision of U/Th dating (Cheng et al., 2013; Wong & Beeker, 2015), speleothem paleoclimate proxies have greatly expanded our understanding of climate dynamics on inter-annual and millennial timescales. As our understanding of speleothem forming processes expands, researchers continue to link more and more geochemical features of speleothems to paleoclimate factors increasing the flexibility of speleothem research in paleoclimate contexts.

5.2 Aim

This study aims to test the suitability of trace metal abundance in stalagmites as a proxy for paleoclimate rainfall. Previous studies (Hartland & Zitoun, 2018; Linderman et al., 2022) have demonstrated that organic complexation of transition metals limits the availability of free transition metals [Cu'], [Co'] and [Ni'] for incorporation in speleothems. This suggests that the incorporation of metals into speleothem calcite is partially controlled by the gradual, time dependent, release of metals from organic complexes. Hartland and Zitoun (2018) proposed that this time-dependence made residence-time of water droplets on the surface of a speleothem a core control on the number of metal ions that are able to substitute into speleothem calcite. This residence-time is dependent on cave water drip rate. This, by extension, should be related to locally effective rainfall.

This potential relationship was first identified by Hartland & Zitoun (2018) and research attempting to link these variables in modern systems is ongoing (Höpker, 2023). A complicating factor in the development of this proxy is that the binding properties of organic compounds are known to vary based on their structure and composition, which can in turn vary depending on biogeographic factors (Filella, 2008; Blyth et al., 2016). In this way, the source of dissolved organic matter (DOM) in cave water has the potential to affect the complexation and dissociation reactions that control the incorporation of metal ions into speleothem calcite. This source may

vary over time and space, providing additional uncertainty to the use of trace metals in paleoclimate research, or as a proxy in paleoclimate rainfall.

To test the extent of this remaining uncertainty, and to identify potential limitations on new trace metal paleoclimate proxies, this study aims to extract organic matter from speleothems of different ages (from various locations) and to test if the binding properties of these extracts change based on their origin. Samples were obtained from thirteen different caves in Niue, Australia, New Zealand, and the UK, and were used determine experimentally the variation in complexation properties of cave water organic matter-transition metal complexes over time and space. This will help to establish the extent to which this variation affects the application of transition metal abundance in speleothems as a paleoclimate proxy and allow for more accurate estimations of error when linking transition metals in speleothems to paleoclimate factors.

6. Literature Review

6.1 Introduction

Speleothems form when rainwater dissolves limestone in karst landscapes, forming calcium carbonate-rich groundwater that flows down into cave systems and enters as cave drip water through cracks and pores in the rock. Upon entering a cave system, drip water adjusts to lower cave air CO_2 levels, degassing CO_2, and precipitating calcite (Quian et al., 2012). The resulting cave precipitates, (speleothems) contain paleoclimate information encoded in their geochemical properties, as their formation, and thus their composition, is affected by myriad paleoclimate and paleo-environment factors (Fairchild & Baker 2012). Of the properties investigated by paleoclimate scientists as potential archives, transition metal abundance in speleothem calcite has great potential to be a useful proxy for paleoclimate rainfall (Hartland & Zitoun, 2018), but potential variation in binding interactions between transition metal ions and dissolved organic matter in cave water (Hartland et al., 2011; Hartland et al., 2012; Lindeman et al., 2022) make the stability of such a proxy uncertain.

This literature review aims to summarize the research that has been foundational to the development of trace element abundance in speleothems as a paleoclimate proxy, as well as the research by which organic carbon was identified as an important component in aqueous trace element equilibria in cave systems and an important component in speleothems.

6.2 Speleothem Formation

While etymologically the term “speleothem” originally referred to any type of cave deposit, (Moore, 1952) in modern contexts, and for the purposes of this study, “speleothem” has come to refer more specifically to mineral structures, precipitated from aqueous solution by mineral rich groundwater as it adjusts to a cave atmosphere. Most of these speleothem forming minerals are calcium based, the most common being calcite ($CaCO_3$), with aragonite, a polymorph of calcite, and gypsum ($CaSO_4$) speleothems also occurring (Fairchild & Baker, 2012). Calcium carbonate minerals are the most common substrate for speleothem formation due to an unstable equilibrium that exists between solid calcium carbonate and aqueous CO_2 in groundwater. High partial pressures of CO_2 in the soil, due to CO_2 produced

by respiration in decomposing plant matter, supersaturates soil water with CO_2. Aqueous CO_2 acts as an acid, reacting with calcium carbonate in limestone to form CO_3^{2-}, HCO_3^{+}, and H^{+} ions.

$$\mathrm{CaCO_{3\,(s)}} + \mathrm{CO_{2\,(aq)}} + \mathrm{H_2O_{\,(aq)}} \rightarrow \mathrm{Ca^{2+}_{\,(aq)}} + 2HCO_3^{-}{}_{\,(aq)} \qquad \ldots (1)$$

The mineral enriched solution continues to flow through the host rock until it reaches the open air of a cave system. The partial pressure of CO_2 in cave air is typically lower than in the soil above the cave, causing CO_2 to be drawn out of solution into the cave air to reestablish chemical equilibrium. As the [CO_2] in the drip water lowers, the aqueous equilibrium will shift to favour solid $CaCO_3$ over aqueous HCO_3^{-}. This process produces H_2O, gaseous CO_2 and solid $CaCO_3$.

$$\mathrm{Ca^{2+}_{\,(aq)}} + 2HCO_3^{-}{}_{\,(aq)} \rightarrow \mathrm{CaCO_{3\,(s)}} + \mathrm{CO_{2\,(g)}} + \mathrm{H_2O_{\,(l)}} \qquad \ldots (2)$$

In karstic systems (which contain large, permeable limestone aquifers) these processes result in the formation of cave systems and the precipitation of speleothems (Qian et al., 2012). Speleothems thus occur anywhere where karst landscapes form and liquid water is freely available, on every continent, in a wide range of climate zones (Williams, 2009; Fairchild and baker 2012). As a result, speleothem formations can be found almost anywhere on the planet, with ages spanning most of the quaternary period. This wide temporal and special distribution is one the factors that makes speleothems such valuable paleoclimate archives. The fact that their formation is directly related to climate processes allows for changes in speleothem composition to be linked to a wide range of different paleoclimate and environmental changes. Their ubiquity in karst environments anywhere on the planet, allows comparison of speleothems across wide spatial and temporal scales (Fairchild & Baker, 2012; Wong & Breeker 2015).

6.3 Speleothems as Paleoclimate Archives

The potential for speleothems to contain paleoclimate information was recognised as early as 1968 (Hendy & Wilson, 1968; Wong & Breecker, 2015) but this remained largely inaccessible until the late 1980's, when developments in the field of U/Th mass spectrometric techniques (Cheng et al., 2013) allowed for speleothem layers and deposits to be dated at high resolutions. As a result of this innovation, there has been a substantial increase in speleothem research over the course of the

last twenty years (Fairchild et al., 2006; Wong & Breecker, 2015). Contemporary speleothem science uses a range of speleothem properties as proxies for paleoclimate variables. Occasionally these proxies may be used to derive paleoclimate information from a speleothem in any cave system with only minimal adjustments for local factors (Fairchild & Treble, 2009), but often speleothem traits can only be used to derive paleoclimate information under specific conditions (Daëron et al., 2019). Some examples of speleothem properties commonly used paleoclimate proxies include:

- Stable Isotope Ratio (SIR) proxies. Such as $\delta^{18}O$, ^{13}C, and ^{2}H (Mc Dermott 2004).
- Trace Element Proxies, including Mg, Sr, S, Cu, Zn, Co, and Ni (Fairchild & Treble, 2009).
- Organic matter inclusions and biomarkers, including humic compounds, lignin, and long chain alkenes (Blyth et al., 2016).

Trace elements in speleothem calcite, specifically trace transition metals (Hartland & Zitoun 2018, Lindeman et al., 2022) and the closely interconnected property of organic compound inclusions (Lee et al., 2005; Hartland et al., 2011; Lindeman et al., 2022), are of particular interest to this study, due to their potential to provide a direct proxy for paleoclimate rainfall (Hartland & Zitoun, 2018).

6.3.1 Trace element abundance

Trace element analysis is one of the younger techniques used in speleothem science, being relatively unexplored until the late 90's (Gascoyne, 1992). Trace element analyses use the relative abundances of trace elements and their isotopes, particularly trace metals, in speleothem calcite to derive information about the processes that either enriched their abundance in cave water, or the processes that enhanced their incorporation into speleothem calcite. Trace elements are found in low concentrations, typically in the ppm or ppb range, and are largely derived from bedrock and regolith overlying a cave system (Fairchild & Treble, 2009). As water passes through bedrock and topsoil it mobilises trace element ions which are transported into cave systems through groundwater flow, and subsequently incorporated into speleothems alongside the $CaCO_3$ matrix, either via substitution with Ca and CO_3 ions in the crystal lattice, by ternary complexation with organic

matter, or as part of nodules or other particulate inclusions (Lee et al., 2005; Hartland & Zitoun, 2018).

Trace elements in cave drip waters are derived from the soil above the cave system, from natural impurities in either the limestone bedrock from which the cave forms, from rocks overlying the cave system or, in the case of very recent speleothems, from anthropogenic sources such as fertilisers and fossil fuels (Fairchild et al., 2006; Frisia et al., 2005). Additionally, as trace elements are incorporated into speleothems through the medium of cave drip water, their incorporation into speleothems can also be dependent on climatological and hydrological factors such as water temperature, flow rate, and other ionic inclusions (Fairchild & Treble, 2009; Hartland & Zitoun, 2018; Lee et al., 2005). This means that trace element analysis has the potential to provide a diverse range of paleoclimate information. As trace elements are largely derived from local sources however, these types of analysis tend to be more narrowly applicable when compared to $\delta^{18}O$ isotope ratio analysis, which often has strong links to climatological cycles such as quaternary glacial and interglacial cycling (Fairchild et al., 2006).

The most commonly studied trace element proxies in cave systems are trace metals. Amongst these, the most frequently used are the alkaline earth metals, such as Mg, Sr, and Ba. The ratios of these elements in speleothems can vary based on a range of geochemical and paleoclimate processes, and they are incorporated into speleothem minerals by substituting readily with Ca in calcite. For the alkaline earth metals, this process is reasonably consistent between systems, with the partitioning and reactivity of these elements in cave waters being well understood (Fairchild et al., 2000; Fairchild & Treble, 2009). Mg/Ca ratios in speleothems have previously been implemented as a proxy in paleoclimate temperature studies, (Gascoyne, 1992) but this proxy is often limited by other factors impacting Mg/Ca precipitation; in particular, prior calcite precipitation.

More recently, trace alkali-earth metals have been linked to flow conditions that result in enhanced dissolution of dolomite ($CaMg(CO_3)$) in the epikarst (Fairchild et al., 2000) or changes in aridity and seasonal rainfall that result in enhanced precipitation of either dolomite or calcite affecting Mg/Ca, Sr/Ca or Ba/Ca ratios in cave water (Owen et al., 2016; Karmann et al., 2007). Trace metal isotope ratios such as $^{87}Sr/^{86}Sr$ (Li et al., 2005) have also been used alongside Sr/Ca Mg/Ca ratios

and $\delta^{18}O$ isotope proxies (Huang et al., 2022) to link glacial cycles and monsoon conditions in some select cave systems.I In very special cases they have been used to identify transport of trace metal rich sediments in overlying surface systems (Goede et al., 1998). Non-metal trace element proxies, such as trace phosphorus or trace sulphur incorporation into speleothem calcite can also been employed as proxies. Phosphorus in speleothems has been linked to bush fires in Western Australia (McDonough et al., 2022), and Sulphur abundance in speleothems being linked to regional increases in fossil fuel usage since the industrial revolution (Fairchild & Treble, 2009; Frisia et al., 2005; Wynn et al., 2013) as well to increases in atmospheric sulphur abundance caused by volcanic activity (Badertscher et al., 2014), and even to seasonal pH changes in drip waters related to cave ventilation and transport (Wynn et al., 2018; Wynn et al., 2013).

Despite the wide range of applications for trace elements as paleoclimate recorders, these proxies are frequently only narrowly applicable, often varying between cave systems or even within a cave system based on factors independent of the surface climate (Fairchild et al., 2006; Rutlidge et al., 2014). The limitations of proxies such as Mg/Ca ratios in calcite are well known. The amount of Mg or Ca in a speleothem is dependent on factors such as host rock composition and prior calcite precipitation (Fairchild et al., 2000; Fairchild & Treble, 2009; Karmann et al., 2007). The limitations on the relatively untested proxy of transition Metals, Cu, Co, and Ni in speleothem calcite (Hartland & Zitoun, 2018; Lindeman et al., 2022) are less well established, and they are thus increasingly of interest in paleoclimate studies. Currently, the use of these elements as a proxy for paleoclimate rainfall (Hartland & Zitoun, 2018; Lindeman et al., 2022) is being tested.

Just like Mg/Ca and Mg/Sr, the transport of these elements in cave systems has and their incorporation into calcite been linked to changes in cave system flow dynamics (Hartland et al., 2012), unlike other proxies however, the availability of transition metals is strictly controlled by complexation between transition metals and dissolved organic matter ligands (DOM) (Hartland et al., 2011, Hartland & Zitoun 2018; Lindeman et al., 2022). This results in the availability of transition metals in cave waters being controlled primarily by the dissociation kinetics of organic complexes, rather than prior calcite precipitation, or the abundance of these elements in the soil and limestone above a system.

6.3.2 Dissolved organic inclusions.

Like trace metals, dissolved organic matter (DOM) is present in most aquatic systems, and like trace metal ions, DOM from soils can be incorporated into speleothem calcite (Blyth et al., 2016; van Beynen et al., 2001). DOM particles in caves are incorporated into speleothems by adsorbing onto calcite surfaces (Fairchild & Baker, 2012; Lee et al., 2005) or as fluid inclusions (Pearson et al., 2020).

DOM is a broad, operational category, comprised of a diverse range of molecules, in a diverse range of sizes and structures. These molecules exist on a spectrum, ranging in size and shape from large, complex, humic molecules containing many functional groups and interlinked chains and rings, to smaller, simpler molecules consisting of only a few carbon groups and chains (Filella., 2008).

In aqueous systems, DOM plays a key role in facilitating transport of metal ions. Organic molecules generally have multiple negatively charged groups at pH's between 6 and 11, which can bind with cations in drip water. This allows DOM to act as vector for the transport of trace metals, scavenging metal cations from charged sites on soil surfaces, and retaining them in solution as groundwater travels through the vadose zone (Blyth et al., 2016. Borsato et al., 2007; Hartland et al., 2011; 2012). In cave systems, the binding of cations by organic ligands inhibits incorporation of transition metal cations (Cu, Co, and Ni) between aqueous solution and calcite (Hartland & Zitoun, 2018, Lindeman et al., 2022), by limiting the availability of free metal ions in solution.

In addition to impacting the incorporation of trace elements into calcite, DOM in speleothems can be analysed as a paleoclimate proxy in and of itself (Blyth et al., 2013b; Blyth et al., 2016; Heidke et al., 2021). Changes in the DOM in speleothems have been to be linked to factors such as global carbon cycles and surface photosynthesis through changes in DOM $\delta^{13}C$ ratios (Blyth et al., 2013b), and linked to changes in cave and surface ecosystem processes by analysing organic biomarkers present in DOM calcite (Blyth et al., 2014; Blyth et al., 2016; Heidke et al., 2021). Paleoclimate researchers have known about the organic components of speleothems since the 1950's (Gilson and MCarnthey., 1954) but only recently, with developments in analytical techniques, have specific organic structures been characterised in enough detail that the potential for these inclusions to act as proxies

been explored. DOM proxies can provide information about the ecosystems above the cave system, (Blyth et al., 2016; Heidke et.al., 2021) and can also be linked to climate factors affecting the transport of DOM in the vadose (Pearson., 2020).

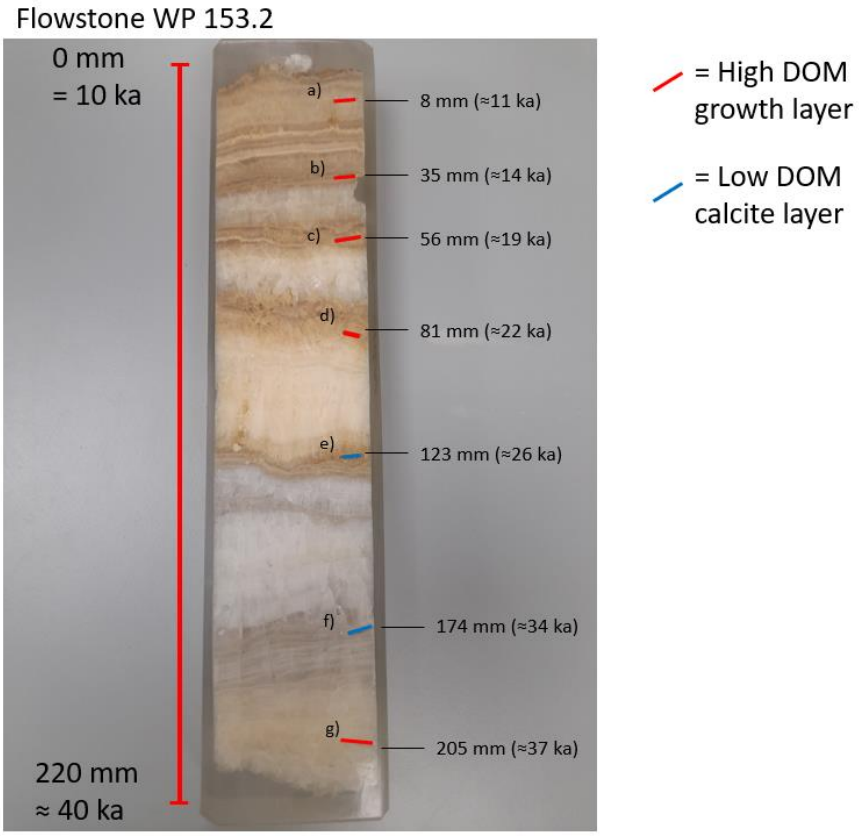

Figure 1: High DOM and low DOM growth layers in a well dated cross section of a flowstone core from Waipuna Cave (Hartland & Zitoun, 2018). Red lines represent high DOM calcite growth layers, and are primarily in brown-dark brown material, blue lines represent low DOM calcite growth, and tend to be comprised of paler calcite.

In cave systems, DOM is thought to fall into one of two main categories, (Blyth et al., 2016; Fiella., 2009; Rutlidge et al., 2014). Allochthonous DOM originates from the ecosystems overlying the cave system, while autochthonous DOM is derived from microbial systems either within the cave itself (autochthonous DOM) or within the vadose zone above (Blyth et al., 2016). The majority of DOM in cave systems is allochthonous, sourced from soil above the cave system (Blyth et al., 2016). This DOM tends to contain a diverse range of sizes and structures, from large, hydrophobic chains and lignin to hydrophilic structures such as organic acids and smaller, simple DOM ligands. Autochthonous DOM, on the other hand is generated by aquatic micro-organisms, either in the cave system itself, or in the vadose zone between the cave system and the soil from the degradation of surface derived DOM (Einsiedl et al., 2007).

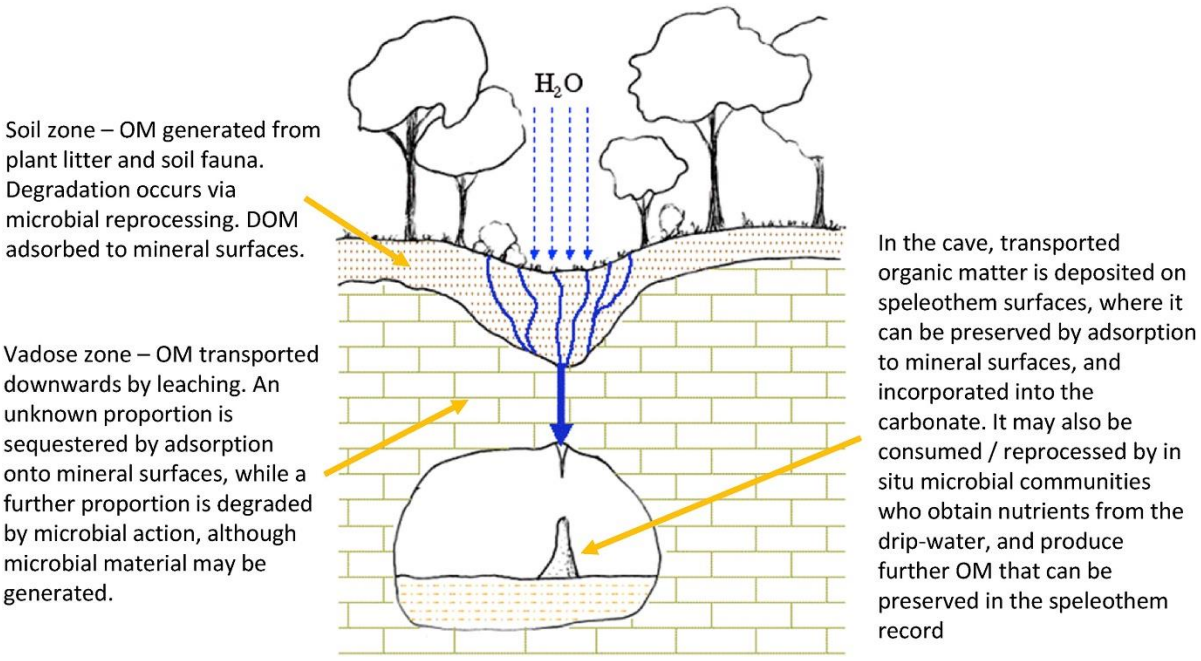

Figure 2: Example pathways of DOM entering a cave system through the vadose zone, influenced both by transport pathways into the system, and by OM in the soil related to soil and environmental conditions above the cave. From Blyth et al., (2016).

The characteristics of DOM in aqueous systems tend to vary depending on the origin of the DOM (Blyth., et al., 2016; Filella., 2008; Filella., 2009). In cave systems, water in cave dripwaters is incorporated into speleothem calcite, with the DOM preserved in speleothems being representative of the types of DOM present in drip water during formation (Heidke et al 2021; Pearson et al., 2020).

In cave systems the majority of DOM is allochthonous, so it follows that surface ecosystem will have a substantial impact on the types and structures of DOM in present in a system. Surface ecosystem isn't the only factor affecting DOM, however. The proportion of allochthonous DOM in cave dripwaters for example, has been demonstrated to vary with depth in some systems, as heavier, more hydrophobic, allochthonous organic matter is consumed by microbial respiration in groundwater during transport, or captured by speleothem calcite, resulting in an increased fraction of smaller, degraded, autochthonous DOM (Einsiedl et al., 2007; Heidke et al., 2021). The conversion of allochthonous DOM by microbes in the vadose zone to autochthonous DOM results in a fraction of more highly degraded, low mass DOM that is increased in abundance in drip waters with long residence times (Blyth et al., 2016, Einsiedl et al., 2007). These residence times can also be affected by climate factors, with flash flows and high infiltration events resulting in a higher abundance of Allochthonous DOM in groundwater (Einsiedl et al., 2007).

The factors that influence the abundance of these two components are complex and how the relative abundance of each of these components contributes to the behaviour of DOM in cave waters is currently unclear (Blyth et al., 2016)

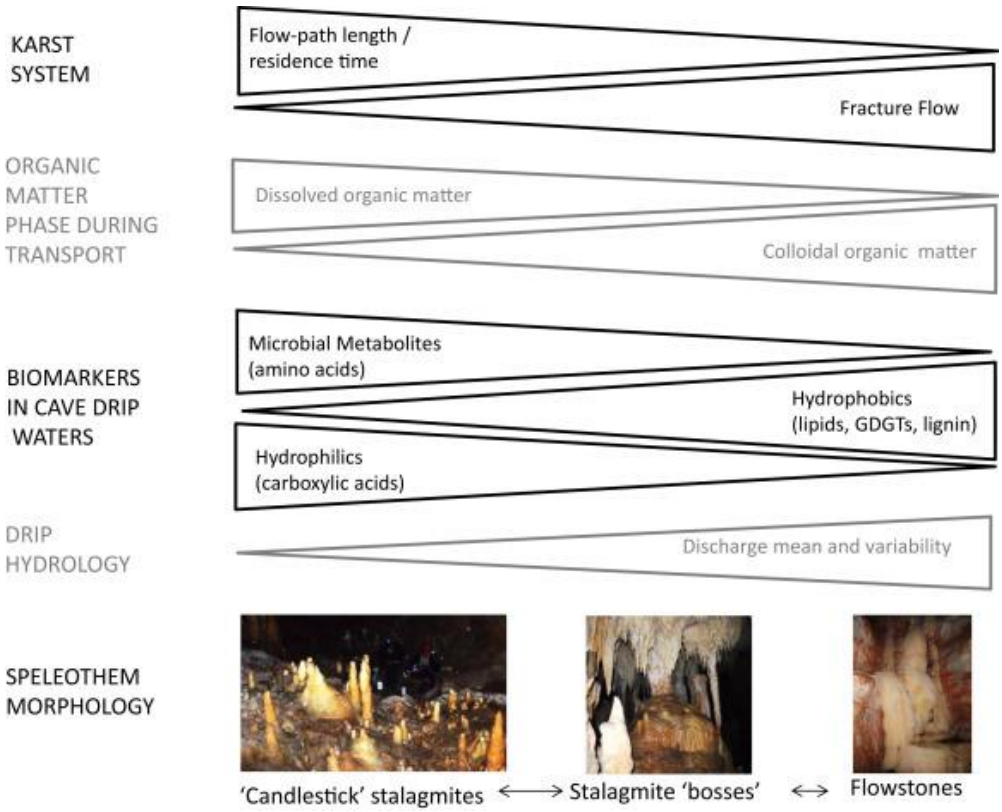

Figure 3: Relationships between water source, residence time, and drip water hydrology and DOM content and morphology in speleothems. The thickness of the bar represents the comparative influence of each factor and the theoretical abundance of each DOM component. Figure from Blyth et al., 2016.

Though there is good evidence that DOM structures change from system to system, and even within the same system, there is limited research on whether changes in DOM structure affect its reactivity in cave waters. Changes in DOM structure don't seem to affect its incorporation into calcite (Pearson, et al., 2020; Hiedke et al., 2021) but it is unclear if changes in DOM structure affect the way it controls the mobility trace elements in solution (Hartland et al., 2011; 2012).

6.4 Organometallic Interactions and Speleothems

The tendency for organic compounds to form complexes with trace element ions (particularly trace metals) is a central control on trace element chemistry in any system in which the two co-exist (Filella, 2008). In soil systems, organic colloids bind trace elements and nutrients to soil particles, as well as facilitate the transfer of aqueous trace elements from soil surfaces into the water column, and the uptake of trace elements by micro-organisms (Boiteau et al., 2018). In cave

systems DOM ligands act as a vector facilitating transport of metal ions from the surface by dissociating them from soil colloids and retaining them in solution as water infiltrates through the vadose zone (Hartland et al., 2012). Once in the cave, DOM goes on to influence trace metal - calcite interactions by inhibiting the exchange of transition metal ions such as Cu^{2+}, Co^{2+}, and Ni^{2+} with Ca^{2+} in calcium carbonate, thus limiting incorporation of these elements into speleothems (Hartland et al., 2014; Hartland and Zitoun, 2018; Lindeman et al., 2022).

6.4.1 Variations in DOM properties

The term dissolved organic matter (DOM) is a procedurally defined term for all organic particles in a solution that remain in dissolved after the filtration of water at 0.45 μm. DOM is comprised of a range of components that are highly diverse in size, structure, and composition, from simple, short chain alkanes to complex carboxylic acids, aromatics, and amines, to complex fragments of lignins, lipids, and other large, complex molecules (Filella; 2009). In this sense, the majority of naturally occurring DOM is heterogeneous, meaning that it is comprised of many, complex, molecular structures containing a variable, difficult to quantify number of binding groups (Filella., 2008). Smaller, organic compounds, for which the binding strengths are known or measurable, will be present to some extent in any system, but the relative abundances of different compounds can be difficult to quantify without extracting each compound from solution individually. The diversity of unique constituents in DOM in any given aqueous system is so great that even though the system technically contains a finite number of components, it is impractical to characterise and quantify all of them (Filella, 2009). This leads to many environmental science studies treating DOM as a conglomerate of “humic compounds” with homogenous properties that are the average of their most commons components. If compounds are analysed, it is typically only select groups of compounds that can be easily identified and extracted from the general solution (Filella, 2008; Filella, 2009).

The way that DOM binds metals is dependent on its physical properties, which can vary depending on its origin. As DOM properties can vary based on factors relating to their transport, time in solution and origin, studies of DOM kinetics often produce results that are only applicable in specific systems, making it difficult to intercompare samples over a wide range of time and space.

Field studies on the binding properties of DOM typically must quantify organic matter concentrations in units of mass per unit volume, as the molar masses of the individual organic components, and thereby their molarities in solution cannot be routinely determined. Such approaches also tend to simplify calculations by assuming all DOM in a system is homogenous, with the same number of binding groups per gram of organic carbon, typically between 3 and 6 mmol g^{-1} (vanLoon and Duffy; 2017; Filella 2008), with a binding strength that is representative of the most common binding groups in solution. The common assumption is that in most DOM, carboxyl (C=O-H groups) will be in the highest abundance, followed by phenols (C_6H_5-O-H), followed by Quinnones, ketones, alcoholic hydroxyl groups as well as nitrogen and sulphur groups. Different functional groups, and configurations of functional groups have different binding strengths and affinities to different ions and mineral surfaces (Filella. 2008, Zhang et al., 2019) depending on the shape, structure, and shielding of adjacent organic groups.

To add additional complexity, the behaviour of these groups can vary differently in response to other solution properties. Organic-mineral binding of different organic functional groups responds to changes in ionic strength and pH of a solution, (Newcomb et.al., 2017), with the degree of variation in binding also change from system to system. This is relevant in cave settings, where pH and ionic strength are high compared to freshwater systems (Fairchild & Baker, 2012), preventing studies on the reactivity of organic complexes in fresh water from being directly applied to cave conditions.

6.4.2 Cave water applications of organometallic kinetics

In cave systems, transition metal ions originate from soil and are transferred into the cave through groundwater by complexation with binding ligands (Hartland et al., 2012). As metals are transported in an aqueous equilibrium with DOM, the concentration of binding groups [L] in a solution will never be exceeded by the total concentration of trace metals in solution [M_T] (Hartland and Zitoun, 2018). These complexes exist in an equilibrium between free, “available” trace metals [M’] and ligand-metal complexes [ML] where:

$$[M_T] = [ML] + [M'] \quad \dots (3)$$

This available fraction, [M'] can be further subdivided into the fraction of ions bound in simple inorganic complexes, [MX_{IN}] and free cations [M^{2+}] where:

$$[M'] = [MX_{IN}] + [M^{2+}] \quad \dots (4)$$

These inorganic complexes tend to be much less stable than their organic counterparts and dissociate readily in the presence of a suitable sink (Shafaei Arvejeh et al., 2013), as such the free metal concentration in a solution [M'] will be the fraction available for substitution from calcite. This means that in cave systems,

$$[L] > [MT] > [ML] >> [M'] \quad \dots (5)$$

with only a small fraction of free metals [M'] in inorganic [MX_{IN}] complexes and free ions [M^{2+}] being available to react (Hartland and Zitoun, 2018).

The ions that make up [M_T] will vary from system to system, dependant on the relative abundances of the ions in the soil and rock through which cave water is sourced, (Borsato et al., 2007) but also depending on the affinities of each of the ions to DOM ligands (Hartland et al., 2012). Different trace element ions have different affinities to different DOM binding sites, due to variations in ionic radius, shielding by valence electrons, and electron affinity. Similarly, different functional groups can have different affinities for different ions due to changes in functional group charge, configuration, and shielding by other organic structures around the binding site (Dudev & Lim, 2014). Different DOM forming processes can produce DOM structures that contain different functional groups in unique configurations. Complex, high molecular weight DOM ligands, for example, have been shown to have higher affinities to ions such as Cu and Ni when compared with low molecular weight counterparts (Milne et al., 2003; Hartland & Zitoun 2018) and have been theorised to preferentially transport these ions in karst systems (Hartland et al., 2012; Blyth et al., 2016; Hartland & Zitoun 2018).

In cave systems, factors like the degree of degradation of DOM, whether the DOM is allochthonous or autochthonous (Blyth et al., 2016), and the dominant vegetation in the ecosystem from which the organic matter was derived (Blyth et al., 2014; Heidke et al., 2021) thus have the potential to determine the properties of DOM in solution by affecting the abundance of binding groups in drip water. This in turn

has the potential to alter the interactions between transition metals and DOM complexes and the incorporation of trace metals into speleothem calcite.

6.5 Implications

Recent research by Hartland & Zitoun (2018) and Lindeman et al., (2022) has identified that due to the vast majority of trace metals in cave systems existing in complexes with organic ligands, the availability of free metal ions [Cu'], [Co'], and [Ni'] is relatively independent of the total concentration of metals in solution [MT]. The majority of transition metals incorporated into speleothems are incorporated as free ions, (Hartland et al., 2018) which, following their incorporation into calcite, must be replenished in solution through the dissociation of DOM-transition metal complexes. This dissociation rection is a somewhat slow, time dependent process which raises the possibility that the residence time of drip water on a speleothem surface directly controls the number of transition metal ions able to exchange with speleothem calcite (Lindeman et al., 2022), in a way that is independent of the total concentration of transition metals in cave water [MT].

The residence time of drip waters on a speleothem surface is directly related to cave water flow (i.e., stalagmite drip rate), and thereby related to, and a potential proxy for, effective rainfall. If this process could be proven to be consistent to hold spatially, this bypasses the main disadvantage of other trace element proxies, which can be influenced by factors that affect the total concentration of trace elements in cave water (Fairchild et al., 2000). This could establish transition metal cation abundance in speleothem calcite as the first direct proxy for paleoclimatic rainfall.

A potential obstacle to the development of this proxy, is the highly variable, near unquantifiable diversity in DOM properties (Filella, 2008; 2009). Studies such as Heidke et al., (2021), Hartland et al., (2012), and (Einsiedl et al., 2007), have demonstrated that DOM structure size and structure in cave water can vary across different systems, as well temporally within individual cave systems (Hartland et al., 2012), but whether these changes impact the applicability of trace metal paleoclimate proxies remains unclear. Hartland and Zitoun (2018) proved that binding of trace Copper was relatively consistent in modern drip waters from temperate New Zealand, but their study did not establish whether these controls were stable over a wider spatial and temporal timescale, or for different elements. If the effect of DOM ligand binding properties on trace metal availability varies

significantly enough across time and space, this could result in inconsistencies in the speed at which transition metals dissociate from complexes on speleothem surfaces, impacting the confidence with which transition metal incorporation in calcite can be correlated with residence time on speleothem surfaces, and therefore rainfall.

For transition metals to be directly linked to paleoclimate change, it's necessary to determine whether the controls on transition metal incorporation into speleothems can be generalised over time and space. As DOM extracted from speleothem calcite is representative of the DOM present in the cave water when the speleothem was forming, (Heidke et al 2021; Pearson et al., 2020) measuring variations in the kinetic properties of DOM-trace element complexes created using DOM extracted from speleothems can provide information on how the kinetics of these complex changes across different systems and on geologic timescales. This thesis aims to establish whether this control on trace metal abundance in speleothem calcite is consistent for use in paleoclimate proxies on a global scale. This will be achieved by extracting DOM from the calcite of a variety of different speleothem growth layers representing a range of geological periods, continents and cave systems. This DOM will be used to create DOM-trace metal complexes, which can be tested to see if DOM binding of trace metals in cave systems has varied over time and space.

6.6 Conclusion

Transition metals in speleothem calcite have great potential as paleoclimate proxies, due to tight controls on its availability for incorporation into speleothem calcite imposed by complexation reactions with cave water DOM. A particularly promising proxy is that of transition metal abundance in speleothem calcite and drip water residence time and paleoclimate rainfall. This proxy functions because complexation of Cu, Co, and Ni with DOM in cave water limits mobility of these ions, slowing their incorporation into speleothem calcite, meaning the abundance of these elements in speleothems is dependent on the residence time of water droplets on speleothem surfaces. The speed at which the dissociation reaction occurs however, will be dependent on binding strength of the DOM-transition metal complexes in cave water. Should the strength of these complexes vary over time and space, this would be an additional factor affecting trace element content of speleothem calcite, impacting the confidence with which transition metals could be

linked to drip water residence times. DOM in speleothem calcite is incorporated into speleothems from cave drip water, and originates from surface soils after traveling through the epikarst. The composition of DOM in cave waters, and thereby in speleothems, varies depending on surface environmental conditions, and depending on the conditions of its transport into cave systems, but whether these variations in structure have a discernible impact on trace element kinetics in cave drip waters remains unclear. As such, it is necessary to test whether changes in DOM-trace element binding kinetics in cave systems change over large spatial and temporal scales. This thesis will aim to test the limitations of transition metals in speleothems as a paleoclimate proxy, by identifying variations in the DOM kinetic processes that have controlled transition metal incorporation into calcite over the quaternary, and across large spatial scales.

7. Variations in speleothem derived organic matter kinetics.

7.1 Introduction

Over the past 20 years, speleothems such as stalagmites, stalactites and flowstones have been explored for their potential to record information about climatic and environmental changes (Blyth et al., 2016; Fairchild & Baker, 2012; Wong & Breecker 2015). Speleothems form slowly, on ten, to hundred-thousand-year timescales, from the precipitation of calcite from $CaCO_3$ saturated groundwater in caves. This calcite is derived from the surrounding rock, dissolved in water flowing from surface rainfall, acidified in soil, and containing trace metals and organic compounds that are continuously being dissolved, precipitated, and altered as cave waters flow down through the epikarst. As a result of this, a wide variety of climate and environmental factors leave their mark on the composition of speleothems (Gascoyne, 1992; Fairchild & Baker 2012), and these formations have the potential to preserve a massive variety of paleoclimate information, encoded in their chemical properties and preserved safely in cave systems (McDermont, 2004). To decode this chemical information into meaningful paleoclimate data, it is the role of speleothem scientists to discover the links between speleothem properties, and the environmental factors that inform them (Fairchild et al., 2006). Any given speleothem property is influenced by many climate and environmental factors, which means that deepening our understanding of the relationships between speleothem compositional traits and their forcing functions is a necessary step in the development of paleoclimate proxies.

Transition metal abundance in speleothem calcite has great potential as a recorder of paleoclimatic change, particularly paleoclimatic rainfall. This is because transition metal incorporation into speleothems is limited by binding with DOM ligands in cave drip water (Hartland et al., 2011; 2014). This kinetic limitation results in a delayed exchange reaction where transition metals take time to be incorporated into speleothem calcite, meaning that the residence time of drip water on speleothem surfaces, and hence drip water flow rate and rainfall, are controls on trace metal ions incorporation into speleothems (Hartland & Zitoun; 2018; Lindeman et al., 2022). One complicating factor and major unknown in the

application of this proxy, and potentially other trace element proxies, is how binding properties of DOM vary between cave systems, and over time. This means that the controls imposed on trace metals in drip waters by DOM, that make trace metals such a promising proxy for past rainfall, also have the potential to make trace metal proxies variable over time and space.

Due to the range of structures and kinetic properties that have been observed in DOM in modern systems (Filella, 2008; 2009), and large changes in surface ecosystems over the course of the last 200,000 years, (Lorrey & Bostock, 2017) it cannot be assumed that DOM structure in drip water systems has remained consistent over the course of the Quaternary. What is unclear is whether DOM structural changes between cave systems and across time have resulted in significant variability in DOM binding properties which hinders their application in paleoclimate studies.

This study aims to test the consistency of binding properties of cave DOM trapped in speleothems from a number of different climates over the course of the last 180 Ka, a period that spans the Holocene, the last ice age, the Eemian (the last interglacial), and the penultimate glacial period. To test this variability, we will compare the variability in the binding of cave DOM-metal complexes across large temporal and spatial scales to variability over shorter timescales that are localised within a single growth layer. This study will also aim to identify trends and changes in DOM binding affinity for trace metals between cave systems and over geological periods.

Small volume diffusive gradient thin films (SV-DGT's) were used to measure the relative binding affinities of speleothem DOM extracts for Cu, Co, and Ni (Welikala et al., 2018). These transition metals were selected for analysis based on previous research by Hartland et al., (2011; 2014), Hartland & Zitoun (2018), and Lindeman et al., (2022) which indicated that these elements tend to bind more strongly to DOM in karst settings, making any potential variations in the binding of these elements easier to detect.

The variations in binding observed across time and space were then compared to the variation observed in both a lab calcite blank and the binding observed in a single, homogenised speleothem layer from Hodge Creek cave, Mt Arthur, New Zealand (Pearson et al., 2020). The lab calcite blank contained no DOC,

representing the mobility of metals in the absence of organic complexes, while the variation in the Hodge Creek standard was used to test the error of the method, and to act as a against which binding from other sites could be compared.

7.1.1 Principles of SV-DGT

Small volume diffusive gradient thin film (SV-DGT) is a technique used for quantifying the lability of ions when bound in solution by complexing ligands (Welikala et al., 2018). In this technique, a solution containing ions and complexing ligands is exposed to a pair of permeable layers of known thickness. The first contains no binding compounds, and is known as the *diffusive layer*, the second contains a binding compound with a high affinity for the target ions, known as the *binding layer*. Upon exposure to the diffusive layer, the ions in the solution begin to diffuse toward the binding layer through the diffusive layer. A concentration gradient then becomes established between the solution and the binding layer forming a *diffusive boundary layer* in the solution, perpendicular to the concentration gradient across which the solution is steadily depleted of ions (Welikala et al., 2018). The concentration of ions remaining in the solution (C_t) in an SV-DGT after time (t) will depend on the initial concentration of the ion (C_o), the surface area of the diffusive layer (A), the diffusion coefficient of the ion through the boundary layer (D^{DL}), the volume of the solution (V_{sol}), and the thicknesses of each of the boundary layer, diffusive boundary layer, and binding layer, collectively referred to as the apparent diffusive boundary layer, with an apparent path length through the layer of (δ_A). Where:

$$C_t = C_o \times e^{\left(-\frac{D^{DL} \times A}{\delta_A \times V_{sol}} \times t\right)} \quad \dots (6)$$

Quantifying the concentrations of metals at the beginning and end of SV-DGT experiments (C_o and C_t), allows for the estimation of δ_A by solving equation 4. If the trace metals are present in complexes with binding ligands, the availability of trace metal to be transferred to the diffusion layer is limited by the rate at which it's complexed species can dissociate, and thereby provides a measure of the complexes' binding strength. In an SV-DGT, all conditions (D^{DL}), (V_{sol}), (A), and (t) are controlled for, so the presence of binding ligands will affect only the apparent path length (δ_A), with complexes that exhibit higher binding strength having a larger (δ_A). This allows for semi-quantitative estimates to be made of the

relative kinetic properties of binding ligands in solutions. If (δ_A) can be estimated in a control solution containing no ligands, where most metals exist as free ions (δ_{AC}), then compared to (δ_A) in a test solution containing ions in kinetically restrictive complexes (δ_{AT}), the ratio (δ_{AT}/δ_{AC}) can serve as a comparative measurement of ligand binding strength. Conversely, solutions where all metals exist as free ions this ratio should approach unity ($\delta_{AT}/\delta_{AC} = 1$). To find this value, the kinetic equation for the test DGT:

$$C_{tT} = C_{oT} \times e^{\left(-\frac{D^{DL} \times A}{\delta_{AT} \times V_{sol}} \times t\right)} \quad \ldots (7)$$

Can be solved for δ_{AT}, where:

$$\delta_{AT} = \frac{-D^{DL} \times A \times {V_{sol}}^{-1} \times t}{\mathrm{Ln}\left(\frac{C_{tT}}{C_{oT}}\right)} \quad \ldots (8)$$

The same logic can be used to solve for apparent path length (δ_{AC}) in a control DGT. In a well-constructed DGT trial, parameters, (D^{DL}), (V_{sol}), (A), and (t) will be the same for both the control and the trial DGT. Under these assumptions, solving for the path length ratio (δ_{AT}/δ_{AC}) results in the following expression:

$$\delta_{AT}/\delta_{AC} = \frac{\mathrm{Ln}(C_{tC}/C_{oC})}{\mathrm{Ln}(C_{tT}/C_{oT})} \quad \ldots (9)$$

Where the relative path length of a solution containing a complex can be expressed in terms of the natural log of the ratio of metal remaining in solution after applying the DGT for any given time (t).

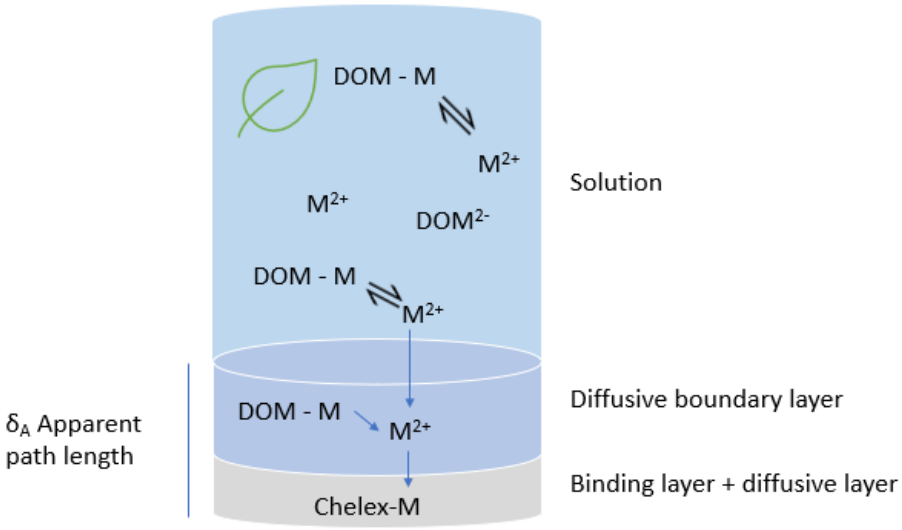

Figure 4: Simple model of an SV-DGT. Width of diffusive boundary layer (dark blue) and thereby apparent path length δ_A will depend on the strength of DOM-M complexes. Adapted from Welikala et al., (2018)

Testing a range of metals together will allow for the identification of competitive inhibition, where one or more metals are restricted preferentially in a DGT over others. This is possible due to the fact that different metals have different affinities with different ligands (Boiteau et al., 2018; Dudev & Lim, 2014; Filella., 2008; Zhang et al., 2019), caused by conformational differences and variations in the stability of ligand binding sites. When testing multiple metals at once, it is important to note that δ_{AT}/δ_{AC} is a measurement of the relative inhibitive effect a ligand amendment has on the mobility of the trace metal component. It is not a measurement of the overall lability of a trace metal in solution. A high ratio of δ_{AT}/δ_{AC} thus indicates that an amendment in the test solution binds the metal tightly, it does not indicate whether that trace metal is bound more tightly than other metals in solution. To compare the overall lability of a component, it is better to instead use C_{tT}/C_{oT}.

Herein, SV-DGT is used to compare the binding properties of DOM extracted from a range of New Zealand, Australian, British and Niuean Speleothems of different ages. The relative strength of these binding properties is then used to identify differences in the binding properties between cave systems and climatological zones, thereby determining if DOM metal binding properties vary with parameters such as cave location, overlying vegetation, speleothem age, and DOM composition.

7.1.2 Adapting DGT's for use on speleothem extracts

Small volume diffusive gradient thin films (SV-DGT's) not yet been applied to quantify the relative labilities of aqueous ions and binding ligand complexes in cave systems, but rather have been on the binding of DOM complexes in conditions analogous to surface waters and soils (Welikala et al., 2018). These environments differ from cave systems in that they are typically lower pH, and have much lower total ionic strengths, with fewer dissolved ions, while cave waters are almost always saturated with $CaCO_3$, and typically have basic pH's and higher ionic strengths. The techniques outlined in Welikala et al., (2018) thus have to be adjusted so that they are able to measure binding in higher pH, higher ionic strength conditions to be applicable in cave water environments.

Making these adaptations presents a few challenges. In order to create subsamples from a stalagmite growth layer that represent narrow age ranges, speleothem powders often need to have low masses, between 10 and 50 mg. Speleothem-derived DOM is also typically found in much lower abundances in speleothem calcite than in soil (Blyth et.al., 2016; Pearson et al., 2020), with this abundance varying depending on factors such as surface soil conditions, rainfall, the geological setting of a cave and other geomorphological or climatic factors affecting the cave drip waters during speleothem formation. To complicate things further, in order to ensure that the DGT's are measuring the binding strength of DOM as it would be in cave water, both the ionic strength and pH of the DGT solution need to be as high as possible.

Finally, cave dripwaters typically contain DOM ligands ([L]) in much higher abundance than trace metals, with upwards of 99% of all metals [M^{2+}] bound in complexes with organic matter (**Equation 5.**) (Hartland & Zitoun, 2018). The low concentrations of DOM in cave calcite means that creating a DGT solution where [M^{2+}] << [L] is difficult without creating a solution where [M] after application of a DGT is too low for quantification using ICP-MS. To provide an accurate estimate of the binding strength of the ligands in solution, [L]:[M^{2+}] needs to be kept relatively constant across all extracts. Keeping this ratio relatively constant by keeping [M^{2+}] << [L], as in Welikala et al., 2018, isn't possible in a speleothem extract, as DOC yields are so low. As a result, [L] needs to be estimated for each

extract before DGT is applied, so [M^{2+}] can be added to each extract in a concentration that ensures [M^{2+}]: [L] is consistent between samples.

7.1.3 Adapting extraction techniques for application of DGT

In an ideal SV-DGT, the ratio of [L] to [M^{2+}] will be as consistent as possible between ligand amendments, and the pH will be as consistent as possible with the environment the trials are supposed to be representing. Maintaining a constant ratio of [L] to [M^{2+}] minimises potential effects of ligand concentration on metal mobility. Maintaining a constant pH minimises the effects of pH on ligand function (Milne et al., 2003; Welikala et al., 2021). Thus, for the purposes of this study, the speleothem DOM extracts for SV-DGT must have a quantifiable number of DOM ligand binding groups, and a consistent, high pH, as close as possible to cave water.

Pearson et al., (2020) achieved quantitative estimates of the concentration of DOM in speleothem calcite by dissolving 5 mg speleothem powder in 0.025 M HCl then removing excess powder using a syringe filter. This produces an unsaturated solution of $CaCO_3$ and DOM suitable for DOM quantification using excitation-emission matrix (EEM) fluorescence analysis. By using an approximation for the number of ligand binding sites [L] in 1 mg of DOM [L] = 2-6 mol mg^{-1} DOM (VanLoon & Duffy, 2017; Zhang et al., 2019), it is possible to turn this DOM concentration into an estimate of ligand functional group concentration [L]. Pearson et al., (2020)'s method does not produce a saturated solution of $CaCO_3$, or a solution with high enough pH to avoid pH effects on the function of the organic ligands in the speleothem DOM (Milne et al., 2003; Welikala et al., 2021). To adjust for this, keep a consistent extraction pH and to ensure the SV-DGT had conditions as close as possible to cave water, it was necessary to extract DOM form an excess speleothem powder.

7.2 Methods

7.2.1 Extracting DOM from speleothem calcite.

A collection of thirty-two cave precipitates, including twenty sectioned stalagmites and seven sectioned flowstone cores from Australia, Aotearoa-New Zealand, and Niue, as well as a collection of nine drip water precipitate powders collected from Pooles Cavern in the United Kingdom were sourced from the University of Waikato and University of Auckland geological archives **(Table 1)**. Samples with precise

U-Th dates and organic stained, yellow-brown (Van Beynen et al., 2001) growth layers were prioritised. Speleothem powders were collected from each sample by drilling trenches 1-2 cm long and 3 mm deep along growth layers of known age in each cross section using a Sherline 5401 micro-mill for small, narrow speleothems and cores, or a Dremel 110 electric stylus for cross sections and cores too large to fit on a micro mill stage. A total of 120 powders were produced, representing just under 200 ka of speleothem growth across 17 cave systems (Pearson et al., 2020).

Enough speleothem extract was used to produce a saturated solution of $CaCO_3$, with a Ph of near 7. This value was calculated in PHREEQC 3.0, and the necessary mass of speleothem ($CaCO_3$) to produce a saturated $CaCO_3$ solution with pH 6.81, in 5 mL of 0.025 M HCl (Pearson et al., 2020) was determined to be 8.23 mg. This simulated value was tested practically by dissolving a series of 8-mg to 20-mg aliquots of speleothem powder from Hodge Creek cave in 5 mL of 0.025 M HCl. It was determined that using an excess of 20 mg of $CaCO_3$ was the best way to ensure that extractions consistently produced saturated solutions, which tended to have pH's in the range of 6.98 ± 0.2.

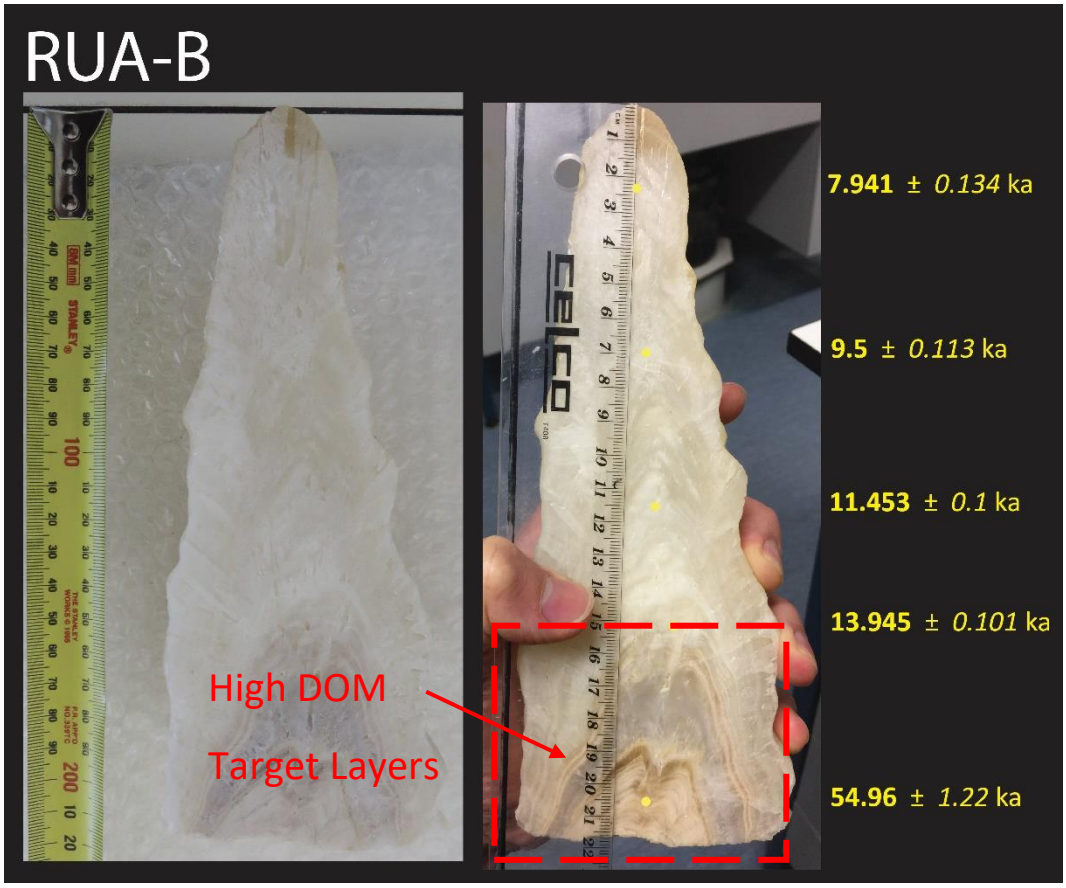

Figure 5: Example stalagmite cross section "Ruakuri – A" from Ruakuri Cave in Waitomo. High DOM target layers highlighted in red. On loan from the University of Aukland. Photo taken By Jeffery Lang.

The speleothem powders were then dissolved in 5 mL of 0.025 M HCl, (Pearson et al., 2020). For each speleothem extract, 20 mg weights of each of these powders were weighed into a 20 mL polypropylene vial and digested in 5 mL 0.025 M HCl to produce saturated solutions of $CaCO_3$ with pH 6.98 ± 0.2. This process was repeated for 15 aliquots of high DOM speleothem powder milled from a single growth layer Hodge Creek (Pearson et al., 2021), which were used to test method reproducibility, and for 15 aliquots of lab grade $CaCO_3$ containing no DOM, which served as a method blank and control. These extracts were shaken for 30 seconds then left for 16 hours to allow the $CaCO_3$ to fully dissolve. The extracts were then centrifuged for 3 minutes at 4000 rpm to separate out any remaining $CaCO_3$ powder, and 4 mL of the supernatant for each extract was transferred to another 20 mL polypropylene vial for DOM quantification.

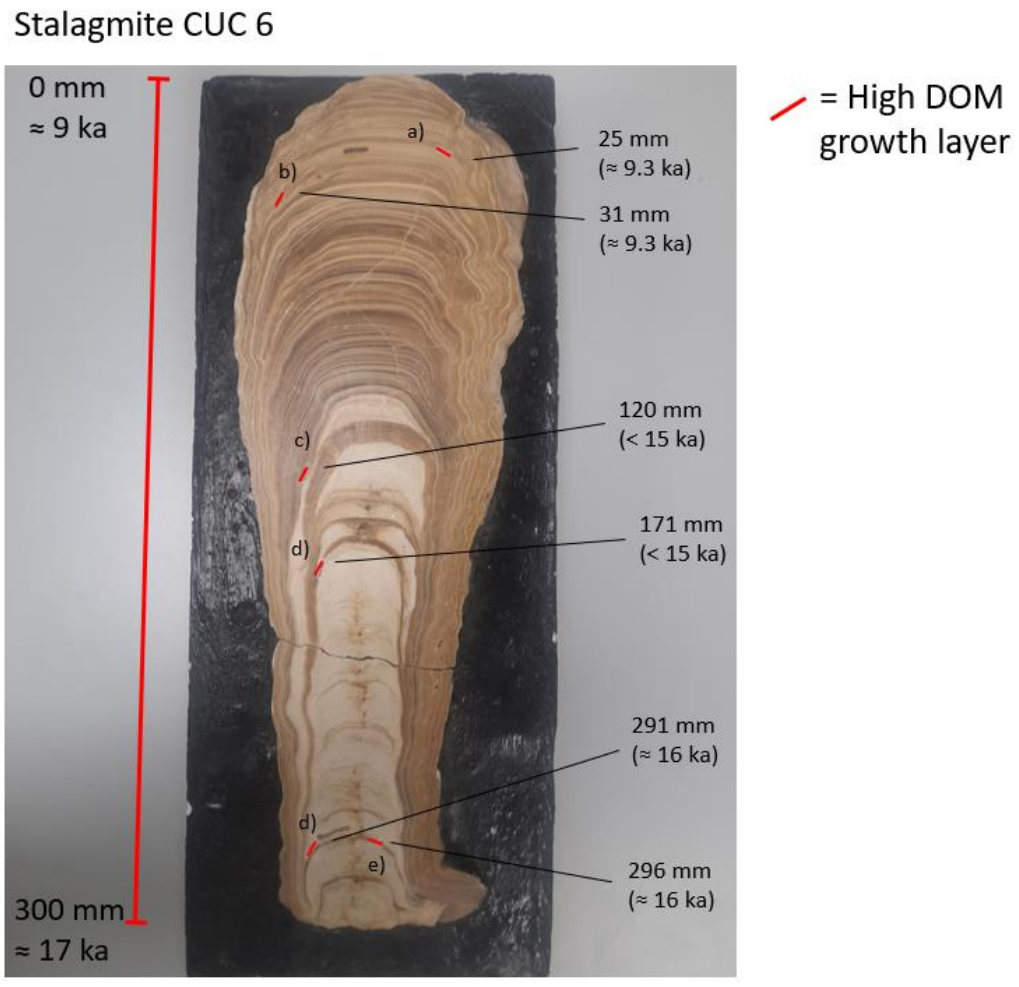

Figure 6: Targeted sampling of high DOM growth layers in a stalagmite from Cutta Cutta cave, Cutta Cutta caves park, Northern Territory, Australia. This speleothem has low accuracy on its U-Th dates, due to high local U and Th abundances. The lower, lighter calcite represents growth from during the last glacial, while the more recent, darker brown calcite represents growth during the early Holocene.

Table 1: Aotearoa-New Zealand Speleothems sampled for DOM by region and cave

Location	Cave	Speleothem	Description
Waikato	Ann's Cave	ANN1	Large, brown-dark brown stalagmite with many, clearly visible growth layers, highly fragmented, with heavy crystallisation at the base. **(2) (79-81 Ka)**
	Gardener's Gut	GG1	Medium stalagmite, with several distinct, brown growth layers. Moderate crystallisation in white calcite. (Low DOM)
		GG2	Very tall, narrow, white stalagmite, few, distinct brown to grey growth layers in between moderately crystalised white calcite. (Low DOM)
		GG5	Medium stalagmite, predominantly white with significant secondary crystallisation, except in a single dense brown growth layer. (Low DOM)
	Majumba	MAJ1	Elongated stalagmite, many cream, translucent growth layers. (Low DOM)
	Ruakuri	RUA-A	Medium cream stalagmite, heavy crystallisation around growth axis, dark brown layers near the base (Low DOM)
		RUA-B	Medium cream stalagmite, moderate crystallisation around growth axis, distinct brown layers at the base. **(2) (55 Ka)**
		RUA-E	Broad white stalagmite, heavy crystallisation with a distinct red-brown growth layers near the base. (Low DOM)
	Twin Forks	C172	Short, wide, white-cream speleothem, few, thick distinct growth layers with significant crystallisation. (Low DOM)
	Simms Cave	C174	Short, narrow, white-cream speleothem. (Low DOM)
	Waipuna	WP152	Thin flowstone with very many, weakly defined pale brown growth layers and intermediate crystallisation. **(1) (0.7 Ka)**
		WP153	Flowstone, many well defined growth layers fading red brown to yellow. Heavy Secondary crystallisation. **(4) (14-22 Ka)**
Eastland	Te-Reinga	TR2	Broad, yellow-brown stalagmite, moderate secondary crystallisation, and few distinct growth layers. (Low DOM)
		TRA	Medium white stalagmite, many cream-black growth layers, no secondary crystallisation. (Low DOM)
		TRC	Tall, elongated stalagmite. Many, tight, translucent-white, growth layers, indistinct. (Low DOM)
Nelson	Hodge Creek	HC15.3	Deep flowstone core, many growth layers with significant crystallisation, increasing down the core. **(5) (85-87 Ka)**

		HC1.1	Thick flowstone, many distinct, white to yellow growth layers darkening to few, crystalised yellow to cream growth layers towards the base. **(3) (10-14 Ka)**
	Wairau Valley	CH180	Very short stalagmite stub with few indistinct-yellow-white growth layers. No crystals. **(2) (0.7-1.7 Ka)**
	Nettlebed	NB1.4	Short flowstone core with few distinct white-cream growth layers. High crystallisation throughout. **(1) (126 Ka)**
		NB3.2	Moderately thick flowstone core, many distinct yellow-brown growth layers. Moderate crystallisation. (Low DOM)
Fiordland	Aurora Cave	AA3	Medium stalagmite, heavy crystallisation at the base with many fine white growth layers. **(2) (79-81 Ka)**

Table 2:Other, international speleothems, sampled for DOM by Country/Region of Origin

Location	Cave	Speleothem	Description
Niue	Anapala	ANA191	Squat yellow brown stalagmite, many, distinct, yellow – brown growth layers. **(2) (1.0-3.0 Ka)**
		ANA199	Small, yellow-brown stalagmite with numerous, distinct, brown-dark brown growth layers. **(4) (4.0 – 9.5 Ka)**
	Anatoloa	C135	Medium stalagmite, darkening towards the tip, many, fine growth layers, minimal secondary crystallisation. **(2) (120-150 Ka)**
	Mataga	C132	Tall, dark red-brown stalagmite, many distinct yellow brown growth layers with moderate crystallisation. **(3) (5.5-70 Ka)**
		C134	Narrow cream-brown stalagmite, with very many distinct white-brown growth layers. No crystalistation. **(2) (60-70 Ka)**
	Ulupaka	ULU19	Tall, narrow, dark brown stalagmite with few visible growth layers, heavy crystallisation. **(4) (n.d.)**
Australia (Northern	Cutta Cutta	CUC3	Wide, stocky stalagmite with many, clearly defined growth layers. Weak crystallisation. **(4) (0.1-2.0 Ka)**
Territory)		CUC6	Thick, wide, brown-black speleothem with few, well defined growth layers, overlying much older, narrower, squarish white speleothem. **(3) (10-16 Ka)**
UK (East Midlands)	Pooles Cavern	Pcpe	Orange, hyperalkaline precipitate harvested from drip loggers, poached egg chamber, Poole's Cavern UK. **(4) (2011, CE)**
		PCrc	Pale yellow, hyperalkaline precipitate harvested from drip loggers, roman chamber, Poole's cavern UK. **(5) (2011, CE)**

7.2.2 Quantification of speleothem DOM

In order to control for the abundance of DOM in each extract when measuring the DOM kinetic properties, the concentration of DOM in each speleothem powder extract needed to be measured. These measurements were made using a linear function of 3D-EEM fluorescence factors vs TOC, calibrated using a peat water sample from the Kopuatai peat dome on the North Island of New Zealand. Water from the peat dome was filtered three times using Whatman grade 6 filter paper, and its total organic carbon content (TOC) was determined to be 26.65 ppm (mg C L^{-1}), using an O.I. Analytical, Aurora 1030 TOC analyser. A series of eight calibration standards with TOC concentrations ranging 0.266 ppm (1% peat water) to 13.325 ppm (50% peat water) were created from the peat water and high purity (> 18 mΩ) deionised (DI) water.

To quantify the DOM in the speleothem extracts, for each speleothem extract, method blank, peat water standard, and internal standard, organic fluorescence was measured using a Horiba Aqualog spectrophotometer. The fluorescence spectra for each of the eight calibration standards were taken, then the fluorescence of the speleothem DOM extracts was analysed in three separate runs, each containing forty speleothem powders, alongside three to nine lab carbonate controls and five internal standards of Hodge Creek Flowstone, HC-15-3 (Pearson; 2020), a high DOC flowstone formed during the last interglacial (≈ 87 ka). Three-dimensional fluorescence excitation-emission matrices (3D-EEM) were created for each extract, as well as for the peat water calibration standard, by measuring fluorescence of each solution within a range of excitation wavelengths between 240 and 800 nm at 3 nm increments, with a 0.5 s integration time and an emission measurement range of 245-825 nm. Each matrix was corrected for inner filter effects (IFE), Rayleigh scatter lines were masked, and then spectra were normalised using the Raman spectrum of DI water.

The resulting 3D EEM for each of the samples, controls, standards, and the calibration standards were analysed using PARAFAC analysis in MATLAB (Stedmon & Bro, 2008), employing drEEM N-way toolboxes (Murphy et al., 2013). To account for potential contamination between runs, four component PARAFAC analysis was used to separate out the humic fluorescence factors between 300 and 400 nm, and the phenol-protein fluorescence factor between 450 and 500 nm, (Lee

et al., 2021). Each of these components was regressed against the concentration of TOC in the peat water standard, producing a series of linear graphs of the relationship between the PARAFAC component scores and the TOC concentration in the peat water standards. For each run of the experiment, the fluorescence factor with the lowest sum of squared residuals amongst the HC-15-2, Hodge Creek internal standard across all three runs, and the lowest baseline fluorescence in the pure calcite method blanks, was selected to be used as an analogue for TOC content in the remaining speleothem extracts.

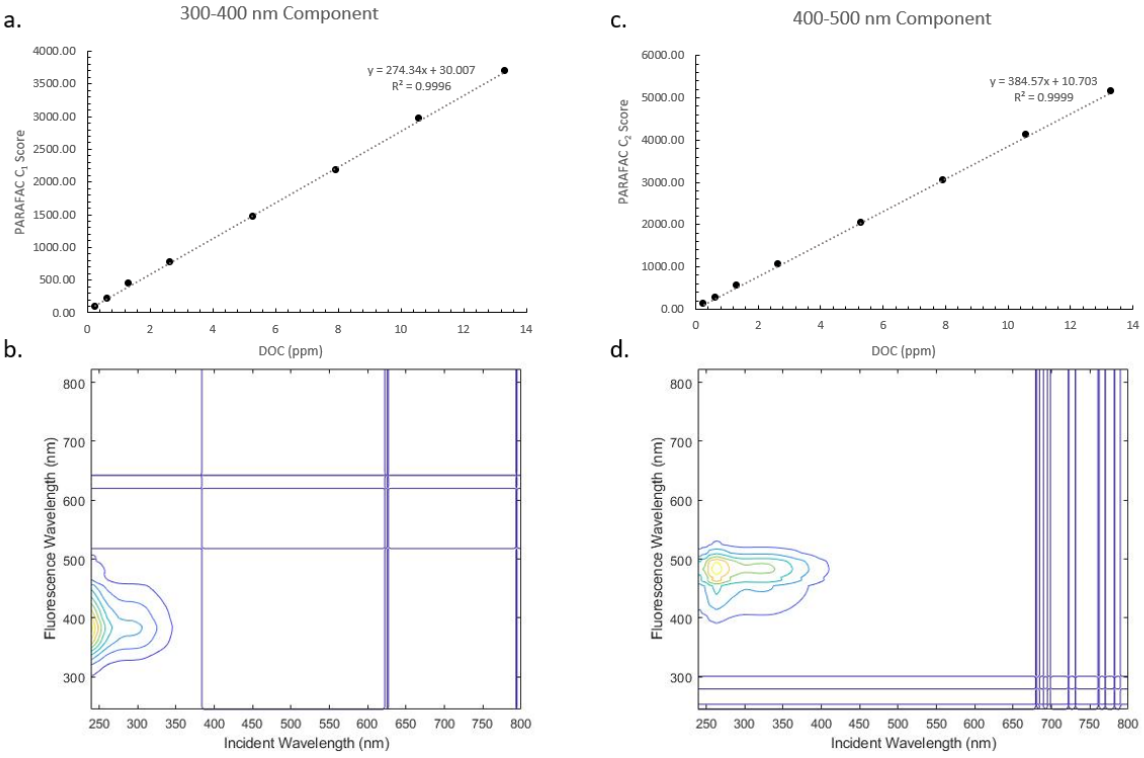

Figure 7. (a, c) Example calibration curves of PARAFAC responses vs TOC of peat water standards for each of the factors displayed in the corresponding 3D Excitation-emission matrices (EEM) displayed below (b, d). Equations like those in examples (a) and (c) were used to calculate DOM concentrations in the speleothem extracts across the three runs.

For each factor in each run, a linear regression of PARAFAC component score vs TOC in each of the peat water standards was created in Microsoft Excel. The linear function that provided the most consistent estimation of DOM content in the HC-15-2 Hodge Creek internal standard using a least squares method, and provided the lowest baseline estimate of the DOM content of the lab carbonate method blanks, was used to estimate the concentration of DOM in each speleothem extract.

7.2.3 Determination of DOM binding potential

After the DOM in each speleothem extract had been quantified, speleothem extracts that contained a total of < 0.2 pm DOM were discarded, as spiking these samples with trace metals would produce solutions containing trace metals too near to the limit of detection of the Aligent 8900 ICPMS used in this study for reliable quantification.

Speleothem extracts containing > 0.2 ppm DOM were set aside and refrigerated, then spiked with Co^{2+}, Cu^{2+} and Ni^{2+}. To ensure a consistent ratio of DOM to $[M]^{2+}$ in each extract and internal standard, 3 mL of each extract was transferred to a clean plastic vial and spiked with 10 μL of 0.1 mmol L^{-1} [Co, Cu, Ni]NO_3 for each μg of DOM, then the solutions were made up to 10 mL with DI water. Assuming that there are 2-6 mM of functional groups in ever gram of DOM in the extracts (VanLoon & Duffy, 2017), this method resulted in solutions containing 1 M of each trace metal for each 2-6 M (VanLoon & Duffy, 2017) of binding sites in each extract, with a ratio of $[M^{2+}]$:[L] somewhere between 3:2 and 1:2. This process was then repeated for each of the Hodge Creek internal standards. For each of the lab grade $CaCO_3$ method blanks, 3 mL of extract was transferred to a clean vial then spiked with with 20 uL [Co, Cu, Ni] NO_3 then made up to 10 mL using DI water. These 10 mL spiked solutions containing the speleothem extracts, blanks, and internal standards were left to equilibrate for a period of at least 48 hours before being transferred to SV-DGT's to quantify the mobility of the spike in each solution.

Small volume diffusive gradient thin films (SV-DGT's) were used to determine the lability of trace Co^{2+}, Cu^{2+} and Ni^{2+} ions when complexed with the speleothem DOM extracts, using a variation on the method described by Welikala et al., 2018. SV-DGT's were prepared using 400 mg Bio-Rad 200-400 mesh Chelex 100 resin in Na^{+} form, previously hydrated for 1 hr in 0.2 mL of DI water. The resin was weighed into the bottom of a clean 26mm diameter 30ml polypropylene vial. Into each vial 1 mL of liquid 1.5% agarose was pipetted to form a 2 mm thick layer (henceforth referred to as the binding layer) containing the Chelex-100 and the agar. Vials were shaken to incorporate the Chelex and establish a cast, then placed in a drying oven for 45 minutes at 45^{o}C until set. A second agarose layer (the diffusive layer) was then cast by pipetting 1.5 mL of 1.5% agarose gel over the top of the

binding layer, the DGT was shaken, and the cast set following the same procedure as before (Amery et.al., 2010.)

Once the SV-DGT's were prepared, 5 mL of each of the 10 mL spiked and diluted extracts were pipetted into each of the SV-DGTs. For each extract, the remaining 5 mL was transferred to a falcon tube and acidified to pH 1 with conc. HNO_3. The DGTs were then left for 16 hours at room temperature, after which, the resulting solution was acidified to pH 1 with conc. HNO_3. Both the DGT samples and the original extracts were sent to be analysed by triple quadrupole (QQQ) inductively coupled plasma mass spectrometry (ICP-MS) using an Aligent 8900 ICP-MS with an SPS 4 autosampler.

ICP-MS was used to quantify both the initial concentration of trace metals in each of the 10 mL spiked solutions, as well as the concentration of Co^{2+}, Cu^{2+} and Ni^{2+} remaining in each extract after the SV-DGT experiment. The concentration of the initial metal spike remaining in the extract test solution before and after applying DGT for 16 hours (C_{oT} and C_{16T}) and the mean concentrations in the method blanks before and after DGT for 16 hours (C_{oC} and C_{16C}) were then used to calculate the relative inhibitive strength (δ_{AT}/δ_{AC}) of each DOM fraction extracted from speleothems, including the Hodge Creek internal standard, using ***equation 6***. To provide an indicator of the spread expected in the inorganic binding of trace metals by lab carbonate in the control, the relative inhibitive strength for each lab carbonate control extract ($\delta_{AC}/\delta_{A\bar{C}}$) was calculated against the average DOM binding across all controls ($\delta_{A\bar{C}}$ = 1). The spread of these values was used to provide an indication of when a metal was not bound by cave DOM (i.e., binding in a solution containing DOM could not be distinguished from that in lab calcite)

7.2.4 Statistical analysis

The variation in binding strength for each metal in the speleothem extracts (δ_{AT}/δ_{AC}) was compared to the variation observed in the (temporally and spatially stable) Hodge Creek internal standard, as well as to the variation in binding observed in the pure calcite control solution ($\delta_{AT}/\delta_{AC} = 1 \pm 0.15$). Spatial and temporal differences in the binding strength of the speleothem DOM extracts were then identified by comparing the extracts based on their cave of origin, the environment above their cave of origin, and the glacial period during which they were formed (Holocene/Aranui, last glacial/Otira, Emian interglacial/Kaihinu, etc).

The variation of DOM binding strength with the age of the DOM extract (ka), the concentration of DOM in the extract (ppm) and the wavelength of the highest intensity fluorescence peak observed under EEM were also evaluated. Where binding varied significantly between extracts and the internal standard multivariate corrections were applied for, age, glacial period of origin, and cave of origin.

Statistical analysis was carried out both in R and in Microsoft excel. Excel was used for basic data processing, such as outlier checking and for imaging the spread of the data. The significance in the differences in DOM binding between cave systems, ecosystems and across geological periods, as well as the significance of linear relationships between speleothem and DOM properties were determined in R. Tests conducted in R included simple linear regressions and T-tests to detect differences in binding across systems, multiple linear regression models and mixed effects analysis using lme4 (Bates et al., 2015) and analysis of variance (ANOVA) conducted using both lme4 and CRAN (Fox & Weisberg, 2023).

7.3 Results

7.3.1 Dissolved organic carbon extracts.

In total, 120 speleothem powders were sampled over the course of this study, representing 120 growth layers from 39 different speleothems and 12 different

systems. Of these samples, a subset of 53 had high enough DOM to be usable in the SV-DGT experiments.

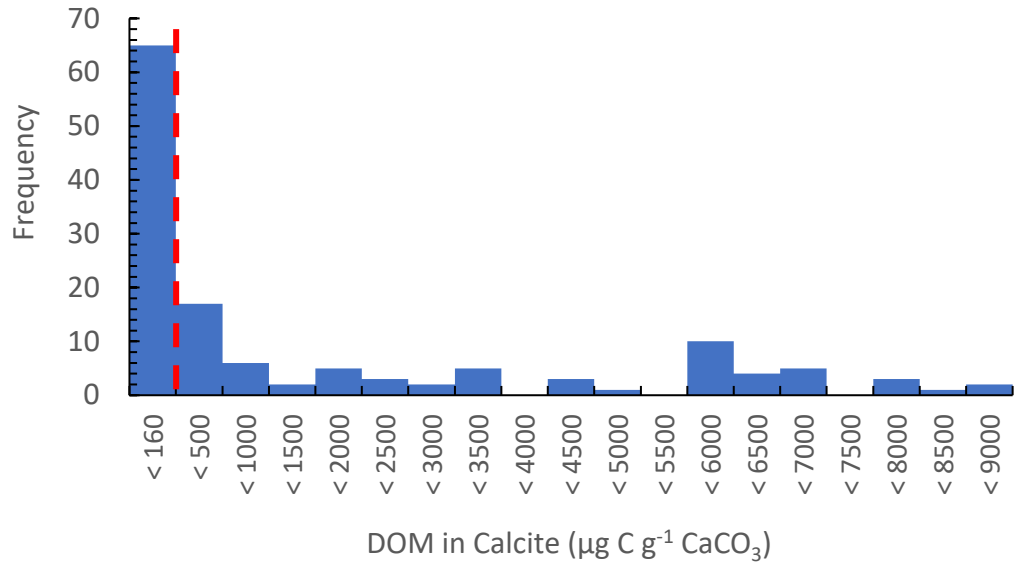

Figure 8: Histogram of the frequency of DOM contents across all speleothem DOM extracts. DOM contents lower than 160 ppm were unusable in DGT, as they contained insufficient DOM for the trace metal spike to be added in concentrations detectable using ICPMS.

The abundance of DOM extracted from speleothem calcite varied widely across time and space. There was a significant positive skew to the data, with the majority of speleothems containing very low concentrations of DOM. Amongst the speleothems sampled in this study, the highest DOM extracts were obtained from the tropical speleothems. The highest DOC extracts originated from Anapala, in Niue with an abundance of 8793 µg DOM per g of carbonate (0.8% OM), while the lowest concentrations of DOM were found in bright white Waipuna and Nettlebed flowstones with DOM concentrations too low to be used in DGT trials (DOM < 160 ppm). The average DOM content of all the speleothems sampled in this study was 1173 ppm (µg C g $CaCO_3^{-1}$), while the median DOM was much lower at 133 ppm, indicating a positive skew to the data. Overall variance was very high, there was wide range of DOM abundances across different systems, with a standard deviation across all subsamples of 2099 ppm.

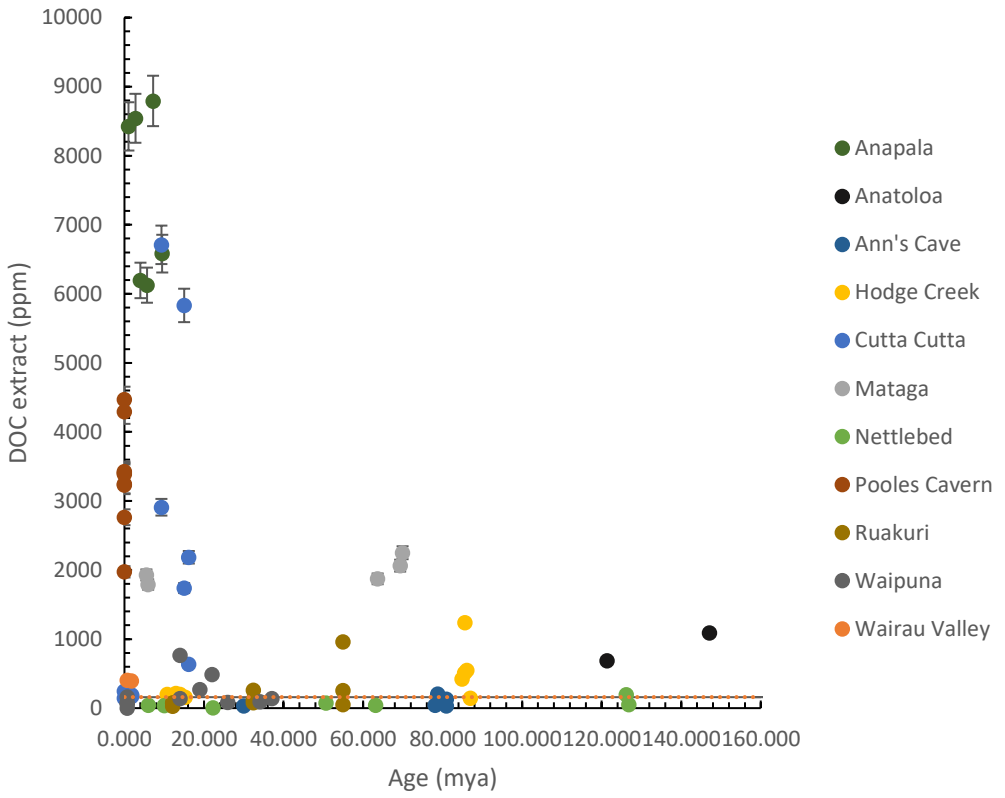

Figure 9 Scatter plot of total DOM content of speleothem powders extracted in this study by cave of origin, in units of μg C per gram of speleothem powder ($CaCO_3$). The dotted yellow line is the DOM abundance of 160 ppm. Extracts containing less DOM than this could not be analysed using DGT without trace metal spikes being too low concentration to detect using ICP-MS.

Amongst the speleothems sampled in this study, there were no significant temporal changes observable in speleothem DOM content across the last two glacial cycles. Speleothem DOM content varied most significantly with site, with several key systems consistently producing higher DOM extracts compared to others. Tropical and Savanah environments, and sites nearer to the equator, more commonly produced high DOM extracts. Anapala, Mataga and Ulupaka Caves from Niue, Cutta Cutta Cave in the Northern Territory, Australia, and Pooles Cavern in the UK had significantly higher DOM contents than were typical amongst New Zealand Speleothems, ($p < 0.05$).

7.3.2 Variation of DGT results

In an SV-DGT, the binding of ions with ligands in a test solution decreases their mobility, thereby reducing the rate at which ions diffuse into the binding layer, becoming immobilised. The presence of binding ligands in a test solution therefore increases the immobile fraction of ions that remain in solution after a SV-DGT experiment. These differences in mobility are observable as the variation in apparent path length δ_A in each experiment. By comparing the apparent path length (δ_{AT}) in a test solution containing ions bound in complexes with organic ligands, to the apparent path length (δ_{AC}) in a control solution containing the ion of interest in only inorganic complexes, SV-DGT studies can provide a semi-quantitative estimate of the inhibitive effects of binding with a particular compound on ion mobility.

Table 3: Measures of central tendency for the labilities of each of the three trace metal amendments (Co, Ni, Cu) in the Hodge Creek internal standard, lab carbonate controls and the general speleothem population expressed as a percentage of the trace metals remaining in the DGT after a given reaction time (t). A higher % remaining corresponds to a lower lability. DGT run 3 excluded from this dataset due to higher baseline binding for Co, as a result of an experimental inconsistency.

Co Lability (% bound after time, *t*)	**Hodge Creek Growth Layer**	**Emsure Lab Grade $CaCO_3$**	**Speleothem Population**
Mean	14.4	12.2	20.6
Median	14.2	11.9	17.7
Variance	2.62	0.549	62.4
SD	1.62	0.741	7.90
Ni Lability (% bound after time, *t*)	**Hodge Creek Growth Layer**	**Emsure Lab Grade $CaCO_3$**	**Speleothem Population**
Mean	42.2	36.6	39.9
Median	44.0	37.3	42.2
Variance	69.9	17.4	92.6
SD	8.36	4.17	9.62
Cu Lability (% bound after time, *t*)	**Hodge Creek Growth Layer**	**Emsure Lab Grade $CaCO_3$**	**Speleothem Population**
Mean	31.3	30.2	28.7
Median	31.2	29.9	26.9
Variance	11.2	27.1	121
SD	3.35	5.21	11.0

The lability of each trace metal amendment in the extracts can be approximated using the percentage of the trace metal spike remaining in solution following the aplication SV-DGT. A lower % retention for any given metal in the DGT after time

(t) equates to a lower inhibition of that metal in solution, and a higher lability, as that metal was more free to be depleted from complexes. Cobalt was the most labile metal in the majority of extracts. In lab cabonate, only 12% ± 0.7% of cobalt remained in solution, this increased to an average of 14% ± 2.6 of cobalt remaining in solution in the presence of Hodge Creek standard DOM, and further increased to higher than 20% remaining in the general speleothem population. Due to a high variance in their mobility, mean % binding of copper and nickel were statistically similar when compared using a simple student T test comparing the populations, though mean Ni binding appeared higher overall. In a lab calcite solution containing no organic ligands, as much as 30% ± 5.4% of Cu and 37% ± 4.4% of Co was retained after DGT were applied. In the presence of Hodge Creek standard DOM, this fraction remaining in solution was very similar for Cu at 31% ± 3.4%, and increased slightly, but not significantly, for Ni to 42% ± 8.3%. The immobile fraction for each of these extracts varied widely in presence of speleothem DOM from other sites. For Cu the immobile fraction ranged as low as 8% to as high as 50%, and for Ni binding ranged from 20% to 60%. The average bindings for these elements across all speleothems were similar to those in Hodge Creek ($p >> 0.05$), but the range of bindings was far greater.

For this study, the binding strength of the speleothem derived organic carbon within each extract was tested for each of the Cu, Ni and Co amendments, and is expressed as the difference in apparent diffusive path length (δ_{AT}) for each element. The (δ_{AT}) values from SV-DGT experiments containing both the binding organic ligands and trace metal spikes are then compared to the apparent diffusive path length δ_{AC} in a control DGT containing only saturated calcium carbonate and trace metal spikes where trace metals are expected to behave as free metal ions.

Table 4 Measures of central tendency of DOM binding of each of the trace metal amendments (Co, Ni, Cu) in the Hodge Creek internal standard, lab carbonate controls and the general speleothem population. Expressed using the relative inhibitive effect of each solution on ion mobility δ_{AT}/δ_{AC}.

δ_{AT}/δ_{AC} (Co)	Hodge Creek Growth Layer	Eamsure Lab Grade $CaCO_3$	Speleothem Population
Mean	1.15	1.01	1.32
Median	1.11	1.04	1.22
Variance	0.0159	0.0484	0.102
SD	0.126	0.220	0.320
δ_{AT}/δ_{AC} (Ni)	Hodge Creek Growth Layer	Eamsure Lab Grade $CaCO_3$	Speleothem Population
Mean	1.12	1.03	1.13
Median	1.02	1.12	1.14
Variance	0.0477	0.0730	0.0700
SD	0.218	0.270	0.265
δ_{AT}/δ_{AC} (Cu)	Hodge Creek Growth Layer	Eamsure Lab Grade $CaCO_3$	Speleothem Population
Mean	1.00	1.01	1.03
Median	0.988	1.01	0.968
Variance	0.0102	0.0336	0.113
SD	0.101	0.182	0.336

Of the three ion complexes observed in this study, only Co was generally more tightly bound across all speleothem extract solutions containing DOM when compared to the $CaCO_3$ method blank. In the Hodge Creek internal standard, cobalt was also the only trace metal that was bound more strongly by speleothem DOM than it was by inorganic complexes in the lab grade $CaCO_3$ control solutions ($\delta_{AT}/\delta_{AC} > 1$). Using a student T-test to compare the datasets, binding of Co by speleothem DOM was significantly higher ($p = 0.0442$) amongst the single growth layer Hodge Creek internal standards than it was in pure $CaCO_3$ ($1.15 \pm 0.09 > 1.01 \pm 0.15$). Amongst speleothem DOM extracts from all cave systems, the average inhibitive effect exerted by DOM ligands on Co was also significantly higher than in the lab carbonate controls ($1.32 \pm 0.09 >> 1.01 \pm 0.15$), ($p = 0.001$). The mean binding effect on Co across all DOM extracts was also higher than mean binding effect observed in the Hodge Creek internal standard but was not significantly higher. In contrast, the mean inhibitive effect of speleothem DOM on the mobility of Ni (δ_{AT}/δ_{AC} (Ni)) and Cu (δ_{AT}/δ_{AC} (Cu)) was not significantly greater than the inhibition observed pure $CaCO_3$ controls. In the case of Ni this seems the result of inconsistent binding of Ni across all extracts, with high variance and wide standard deviation exhibited in the binding of Ni in the standards and lab calcite method

blanks **(Table 3)**. For binding of Cu this seems to be the result of a low average affinity for Cu in speleothem DOM, (δ_{AT}/δ_{AC} (Cu) = 1.02 ±0.09 ≈ 1). This low, average binding is a result of Speleothem DOM increasing the mobility of Cu compared to lab calcite in some circumstances, with δ_{AT}/δ_{AC} (Cu) < 1.

The range of inhibitive effects observed amongst speleothem extracts in this study was wide, particularly for Co and Cu. Co inhibition by DOM ranged between 0.8 and 2.2 times that of lab calcite, and the inhibitive effect of DOM on Cu ranged from 0.4 and 2.2 times that in pure calcite. The range of bindings for Ni was between 0.4 and 1.6 times than the average binding in pure calcite.

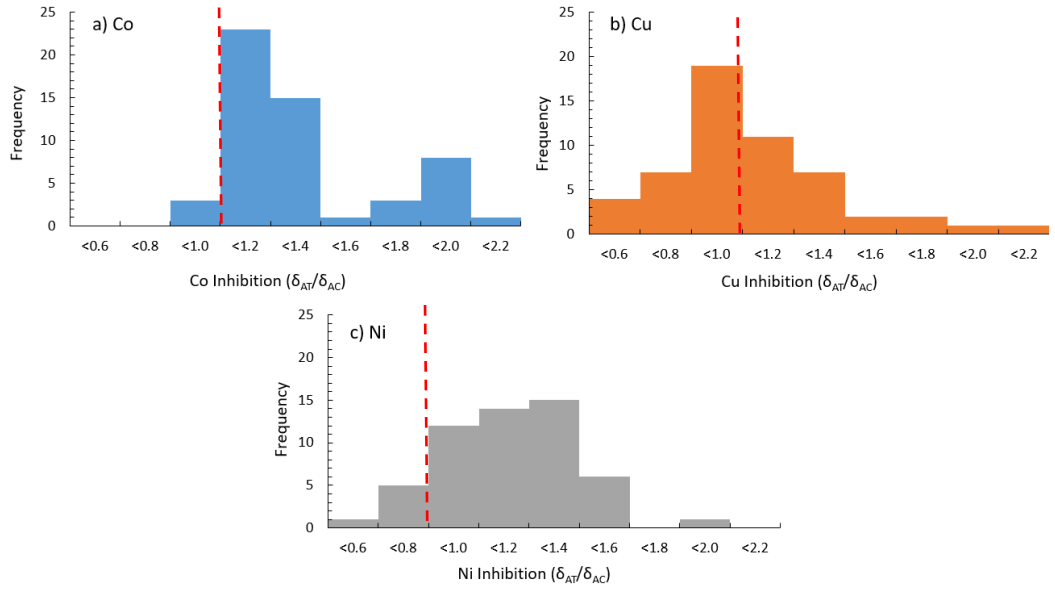

Figure 10: Histograms describing the spread of binding strengths for a) Co, b) Cu and c) Ni, observed in DOM extracted from speleothem calcite. Dashed red line demarcates the equivalent inhibition as the $CaCO_3$ blank.

The shape of the spread in DOM inhibition across all speleothem extracts was compared to the spread of DOM inhibition in the temporally stable Hodge Creek internal standard using the Jarque Bera test for normality in Excel. The variation in binding for DOM with Ni appeared normally distributed, ($p = 0.946 > 0.05$) while the spread of binding for both Co ($p = 0.0050$) and Cu ($p = 0.00059$) binding by speleothem DOM exhibited non-normal distributions. Specifically, both binding of Co and Ni had strong a positive skew, with Co binding showing potential bimodality, with inhibitive affects between 1.8 times and 2.0 times that of lab carbonate being comparatively common compared to the median binding of 1.2. The distribution of DOM binding strengths exhibited by the temporally stable Hodge Creek extract, as well as for the lab calcite internal standard were

comparatively normal across all three transition metals trailed in this study, with p values for the Jarque Bera test > 0.05.

Comparing the variance of the samples to the variance of the standards, using Levene's test for the equality of two variances in CRAN, the variance in the binding strength of Cu-DOM ligand complexes extracted from all speleothems (Var.S = 0.113) was significantly higher than the variance observed in the temporally and spatially stable Hodge Creek internal standard (Var.HC = 0.010 << 0.113; p = 0.0360). This indicates that the variation in Cu binding strengths is significantly wider across larger spatial and temporal scales. The range of binding strengths observed for Co-DOM ligand complexes in DOM extracts observed across different systems and speleothems (Var.S = 0.102) was also wider than the variance observed within the Hodge Creek internal standard (Var.HC = 0.016 < 0.102; p = 0.102) but these variances did not pass Levene's test for unequal variation (p < 0.05). It cannot be stated with confidence that there was a wider variation in Co binding across all speleothems than within a single growth layer. Binding of Ni showed slightly higher variation across all speleothem extracts (Var.S = 0.700) when compared to the spatially and temporally consistent Hodge Creek internal standard (Var.HC = 0.477), but the difference between the variance in the population and the standards was insignificant (p = 0.472) using the Levene test for equal variances.

In combination, these tests indicate that DOM binding of Cu and Co is unstable over large spatial and temporal scales, as the distributions of binding affinities for these elements vary in shape and size over time and space. These differences take the form of a significantly wider variance in the binding of Cu (p < 0.05) when considering the wider range of samples compared to the standards, and a significant skew in the binding of Cu and Co, with speleothem DOM often exhibiting strong binding of Co or Cu under certain circumstances that do not occur within the single growth layer standard. Speleothem DOM-Ni binding had similar variance to the lab carbonate control and the Hodge Creek extract internal standard method blanks (p >> 0.05), with it being difficult to distinguish between the binding of Ni in the presence of speleothem extracts and the binding of Ni in pure lab carbonate.

7.3.3 Regional and temporal differences in DOM binding properties

To provide insight into the nature of the variation observed in DOM ion binding properties, DOM binding of Co, Cu and Ni can be compared across different cave

systems and geological periods. The inhibitive effect of DOM on the three trace metals in this study can also be correlated with the DOM composition, as well as the composition of the speleothems from which the complexes originate. Of the sites sampled in this study, six sites provided five or more high DOM samples from one or more speleothems, this allowed the typical binding affinities of DOM from these sites to be compared statistically, thereby allowing for the identification of significant variations in binding over time and space. These sites were Hodge Creek Cave (Hartland and Zitoun, 2018; Pearson et al., 2020), a deep, subalpine cave under beach forest in Kahurangi National Park, near Nelson, in the South Island, New Zealand; Waipuna Cave (Hartland & White, 2019; Nava-Fernandez et al., 2020; Pearson et al., 2020), another deep cave system under thick, organic soils near Waitomo in the Waikato region of New Zealand's North island; Anapala and Mataga Caves (Höpker, 2023) situated in eastern region of Polynesia in the Pacific ocean in Niue, both under tropical rainforest, with Anapala cave being shallower than the New Zealand examples; Cutta Cutta cave under scrub/savannah, in the Cutta Cutta caves park in the Northern Territory of Australia; and Pooles Cavern (Hartland et al., 2011; 2014), a shallow, cave with hyperalkaline dripwaters near Derbyshire in the West Midlands in the United Kingdom, beneath deciduous woodland scattered with the remains of lime kilns, which alter the groundwater in this system and cause pH values in cave drip waters to be exceptionally high (pH 11+) (Hartland et al., 2010). Differences between these cave systems and other systems that produced fewer than five high DOM aliquots were also investigated, but due to smaller sample sizes, it is harder to interpret these differences with confidence.

To determine whether a speleothem extract exhibited a meaningful binding response, T-tests were first used to compare the binding of DOM in each speleothem extract to the range of bindings observed in by inorganic complexes in the lab carbonate controls (Shafaei Arvejeh et al., 2013). Amongst the speleothems sampled from each of these systems, speleothems from Anapala Cave, Pooles Cavern, Waipuna Cave and Mataga Cave contained DOM that had an inhibitive effect on the mobility of Cu and Co that could easily be distinguished from that of pure calcite. The inhibition effect of DOM-Ni complexes was effectively indistinguishable from the binding of Ni in pure $CaCO_3$ solutions. Specifically, speleothem-derived DOM from Anapala, (δ_{AT}/δ_{AC} Co = 1.529), Pooles Cavern

(1.369), Mataga (1.285), and Waipuna (1.637) exhibited significantly stronger binding of Co than was observed in the absence of DOM ($p < 0.05$). DOM from Anapala and Pooles cavern also demonstrated significantly weaker binding of Cu (δ_{AT}/δ_{AC} Cu = 0.6666 and 0.8742) than was observed in inorganic complexes in lab calcite ($p = 0.00135$ and 0.0259), with Cu being more mobile in DGT's containing DOM from these cave sites than it would be expected to be in DGT's containing no DOM.

Comparing the binding of DOM across extracts from different locations provided an indication of the types of circumstances in which DOM binding of transition metals changed between cave systems. DOM from Anapala cave in Niue and Waipuna cave in Waitomo had a significant inhibitive effect on the mobility of Co in SV-DGT's, with mean Co binding in Anapala of 1.53 ± 0.32, and a mean Co binding in Waipuna of 1.65 ± 0.62. While these caves both produced DOM with a strong inhibitive effect on Co, they had very different inhibitive effects on Cu. DOM from Anapala cave was found to bind Cu significantly less tightly than was standard (δ_{AT}/δ_{AC} Cu = 0.67 < 1.01, $p = 0.0118$). In contrast, DOM from Waipuna Cave tended to bind Cu more strongly than in Hodge Creek (1.31 > 1.01, $p = 0.0405$).

Of the six systems with high enough DOM to produce 5 or more extracts, not one produced an extract that had a significant effect on the mobility Ni. DOM binding of trace metals was also found to vary significantly between a few of the less heavily sampled systems. DOM binding of Ni from the four stalagmite powders from Ulupaka cave in Niue was found to be significantly lower than the rest of the systems, and binding of Cu with DOM from two stalagmite powders from Ann's cave in Waitomo was also significantly higher than in Hodge Creek.

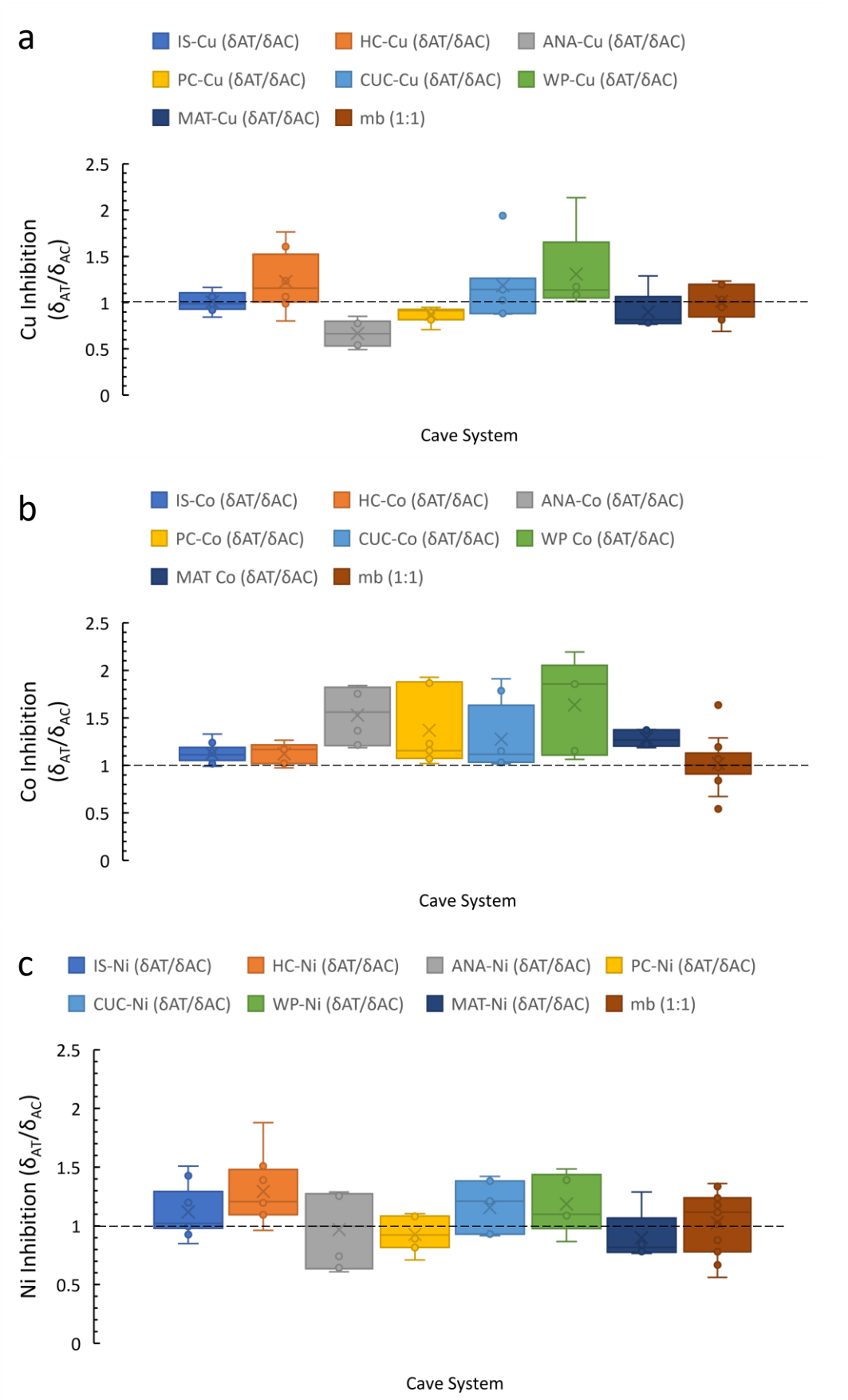

Figure 11: DOM inhibition of Cu (a), Co (b), and Ni (c) by speleothem derived DOM across cave systems (Hodge Creek, HC; Anapala, ANA; Pooles Cavern, PC; Cutta Cutta, CUC; Waipuna, WP; and Mataga, MAT) as well as the Hodge Creek internal standard (IS) representing the intrinsic variability (error) of the method, and lab calcite (mb) where $\delta_{AT}/\delta_{AC} = 1$ is representing no DOM effect on metal ion mobility.

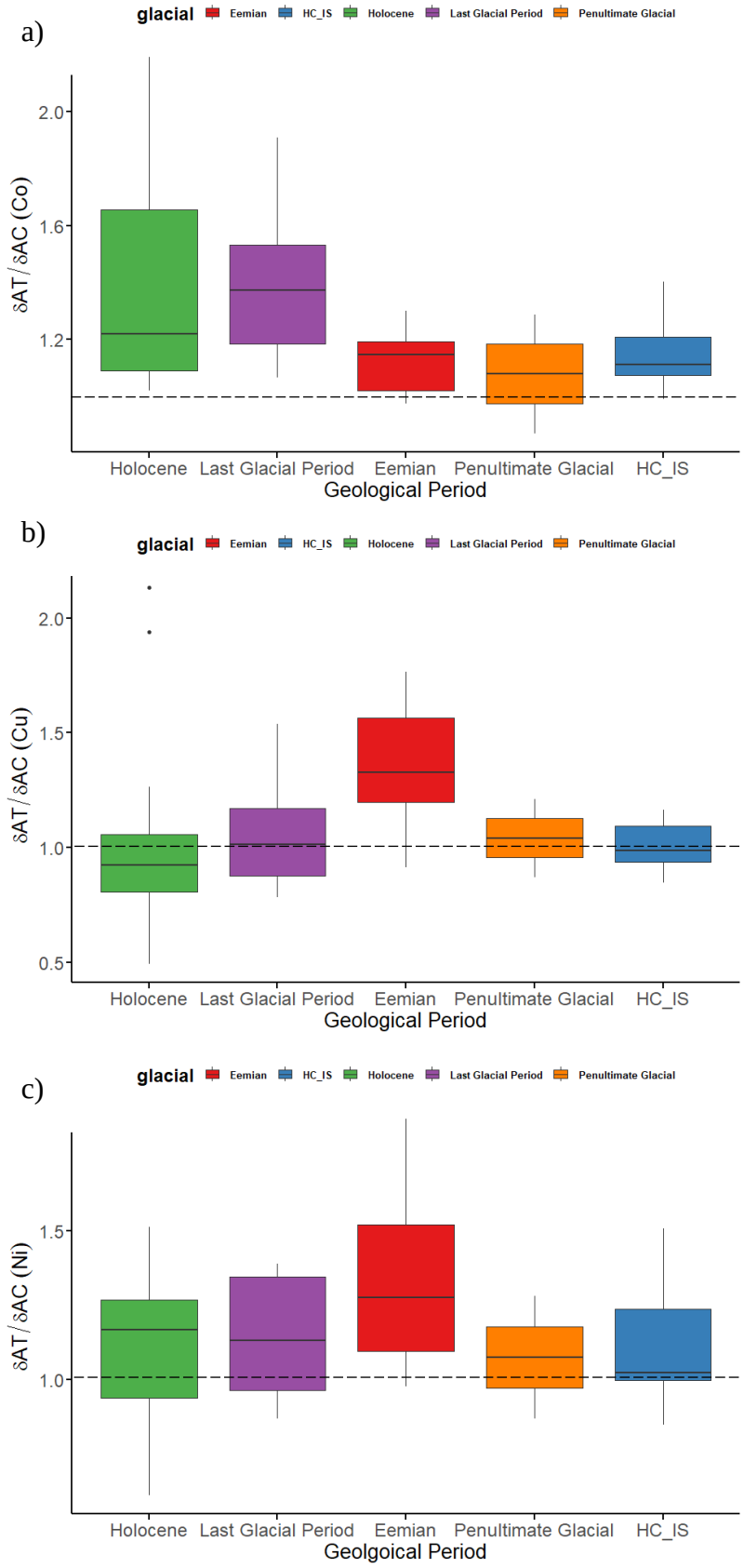

Figure 12: Variation in relative kinetic inhibition for a) Co, b) Cu, c) Ni, with geological period over the last 160 Ka, Holocene (0-15 ka), Last Glacial Period (15-75 ka), Eemian (75-125 ka) and Penultimate Glacial Period (125-180 ka), each compared to the Hodge Creek internal standard (≈13 Ka) representing the intrinsic variability of the method. All periods up until the Waimea Glacial represented by at least 6 different extracts, with only two DOM extracts collected older than 120 Ka (Waimea Glacial).

The ligand binding properties of DOM extracts were also observed to vary with the geological period from which the DOM in the DGT trial originated. Copper in particular, was significantly more tightly bound in DOM originating from the glacial period before the last ice age (Eemian/Kaihinu interglacial) when compared to both the DOM originating from more recent, Holocene (Aranui) speleothem extracts ($p < 0.05$), and the DOM in the Hodge Creek internal standard ($p = 0.0255$). The mean δ_{AT}/δ_{AC} (Cu) for DOM originating in the Eemian (Kaihinu) being 1.391, compared to δ_{AT}/δ_{AC} (Cu) of 0.965 for Holocene (Aranui) DOM. In the case of Co, the mean Co affinity of DOM ligands appears higher amongst samples extracted from calcite originating in the Holocene (Aranui) (δ_{AT}/δ_{AC} (Co) = 1.346) interglacial and last glacial period (Otira) (δ_{AT}/δ_{AC} (Co) = 1.389) when compared to the earlier Eemian (Kahinu) interglacial and the penultimate glacial maximum (Waimea). This difference is not statistically significant ($p > 0.05$). Binding of Ni varied in a similar manner to Cu, but the high variance of Ni binding amongst the organic extracts in this study meant that there were no statistically significant changes identified.

Speleothem DOM with similar binding properties often occurred at similar latitudes, in similar regions and environments. The latitude in which the cave was based will have had a significant effect on the type of vegetation common above the cave system during the last 100,000 years of speleothem growth. Most regions have been dominated by only one or two vegetation types over the course of the Holocene: with only one region, the Nelson area in the North of the South Island, prominently featuring two different ecosystems overlying cave-forming rocks, alpine scrub, above Nettlebed cave, and beech forest above Hodge Creek cave.

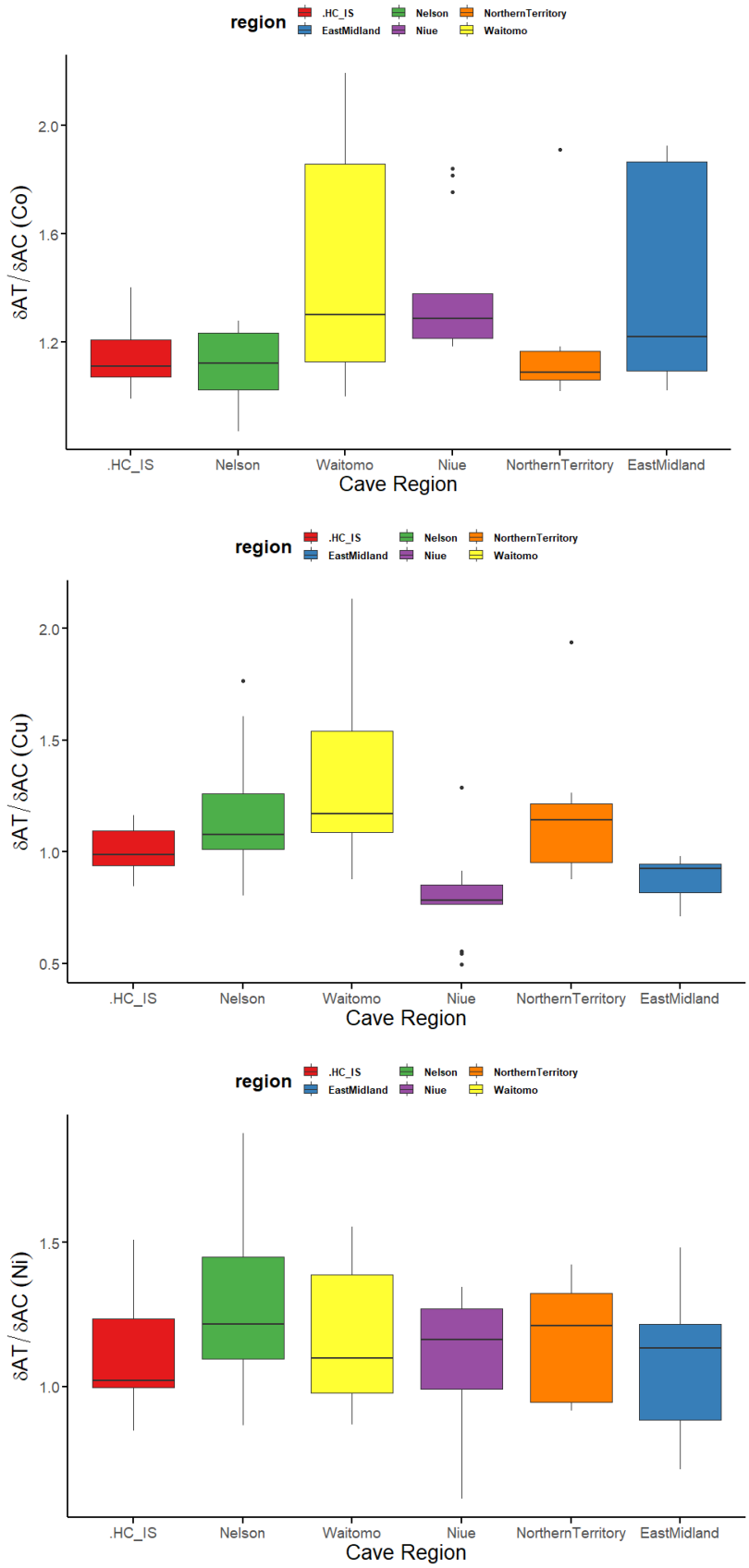

Figure 13: Distribution of DOM inhibition for a) Co^{2+}, b) Cu^{2+}, c) Ni^{2+}, by origin region, Nelson, in the North of the South Island, New Zealand. Waitomo, in the Central North Island New Zealand, Niue, in Central Polynesia, and The East Midlands, in the UK Also displayed is the Hodge Creek internal standard (HC_IS) from Hodge Creek Cave in Nelson.

Binding of Co was significantly higher amongst samples from the Waitomo Region of New Zealand's North Island, when compared to the internal standard, ($p = 0.0194$) or to samples from South Island of New Zealand. Similarly, binding of Cu was significantly elevated amongst Waitomo samples compared to the Hodge Creek internal standard ($p = 0.0101$) and was significantly lower amongst Niuean speleothems ($p = 0.0417$). It is important to note that these regional values are likely heavily influenced by the unique binding traits of the most heavily sampled systems from those regions. Sites like Anapala and Waipuna produced DOM with distinct qualities and DOM extracts from these sites were also most numerous from their respective regions. Likewise, the only Northern Territory site was Cutta Cutta cave, and the only cave sampled from the UK was Pooles Cavern.

7.3.4 Multivariate Corrections of Significance

Though many of the above differences in binding of DOM across cave systems, geological period, and surface environment are statistically significant, it is impossible to interpret potential causal relationships between variables without correcting for biases in sampling, and confounding variables.

Multivariate corrections (Bates et al., 2015) can be applied to a dataset to account for biases in sampling. They function by identifying and accounting for secondary correlations between numerical and categorical variables. Applying multivariate corrections is particularly important in datasets like this one, where cave systems were sampled by convenience, not selected systematically. For example, in this study, many systems experienced the majority of their high DOM speleothem growth over specific time periods, and some time periods are represented by only one or two systems. The samples representing the last/Eemian interglacial were primarily from Hodge Creek cave, making it difficult to determine whether the significant differences detected in the properties of DOM from this period are the result of factors related to their age, or of factors related to geographic origin.

Applying multivariate corrections to correct for potential sampling biases substantially lowers the significance of all the correlations identified, with no significant differences ($p > 0.05$) remaining following corrections. This suggests that while there are significant differences in DOM function depending on its origin, it is impossible to determine what specific forcing factors could be causing these differences without more data.

7.3.5 Trends and relationships in DOM binding properties

Categorical comparisons between different speleothem groups revealed that unique speleothem forming circumstances can produce discrete binding conditions. Identifying linear trends in DOM binding properties was more difficult, and trends tended to be weak, if they occurred at all. There were no clear relationships between the binding characteristics of a DOM extract and its age, or between the binding of an extract and its fluorescence characteristics, with R^2 values for these relationships being less than 0.05 across the board. The most prominent linear relationships observable within the data were between the observed binding of certain elements and the abundance and fluorescence of DOM in the source mineral, as well between different affinities.

There was a moderately strong, positive, linear relationship between the inhibitive effects of speleothem DOM on Cu and Ni ($R^2 = 0.412$). Extracts with a high affinity for Cu tending to also have a high affinity for Ni, and vice versa.

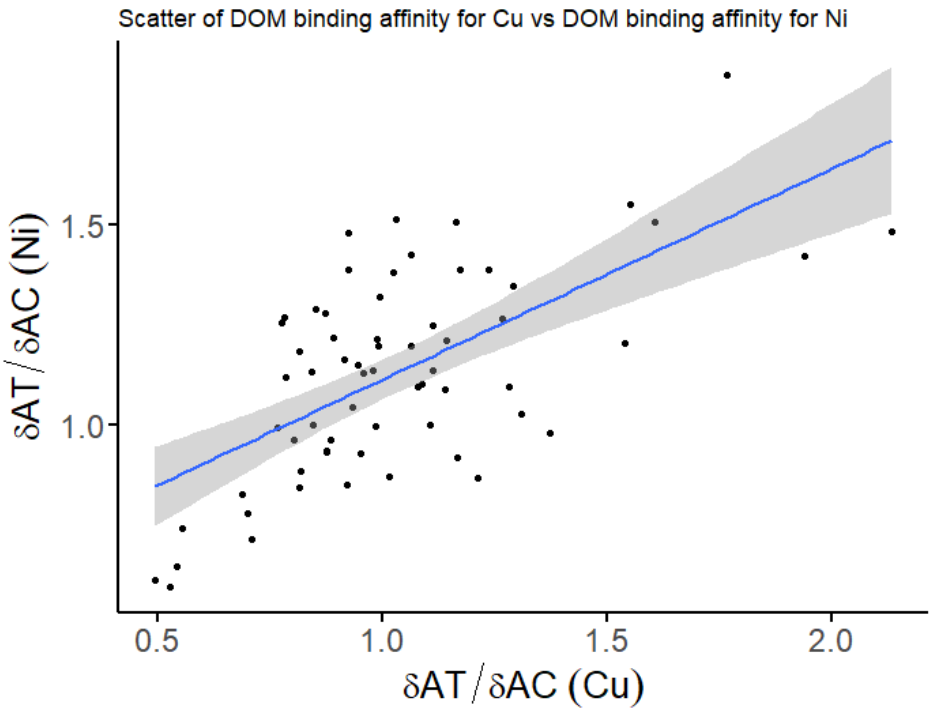

Figure 14: Linear regression between the inhibitive effect of DOM on the mobility of Ni Vs the inhibitive effect of DOM on the mobility of Cu in DGT extracts containing speleothem DOM. The confidence interval on the linear gradients ($p < 0.05$) represented by grey shading.

In comparison, the tendency of DOM to bind Co was not strongly interrelated with binding of the other two metals. Speleothem DOM seems to bind Co in a manner that is independent of its binding of the other elements. With there being no clear linear relationship between DOM binding of Co and Cu ($R^2 = 0.0026$)

and only very weak, positive linear correlation between DOM binding of Co and Ni (R^2 = 0.091).

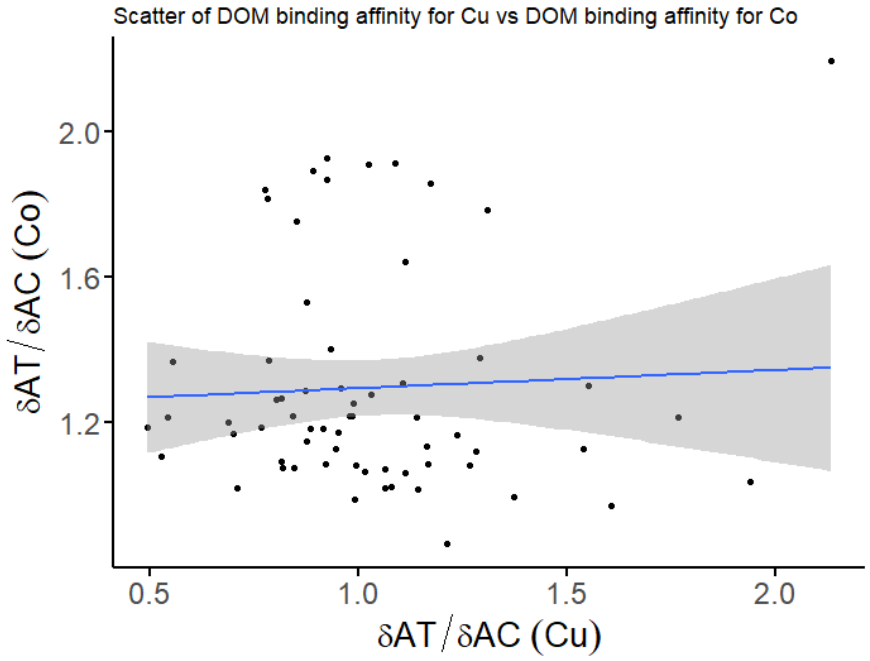

Figure 15: Linear regression between the inhibitive effect of DOM on the mobility of Co Vs the inhibitive effect of DOM on the mobility of Cu in DGT extracts containing speleothem DOM. The confidence interval on the linear gradients ($p < 0.05$) represented by grey shading.

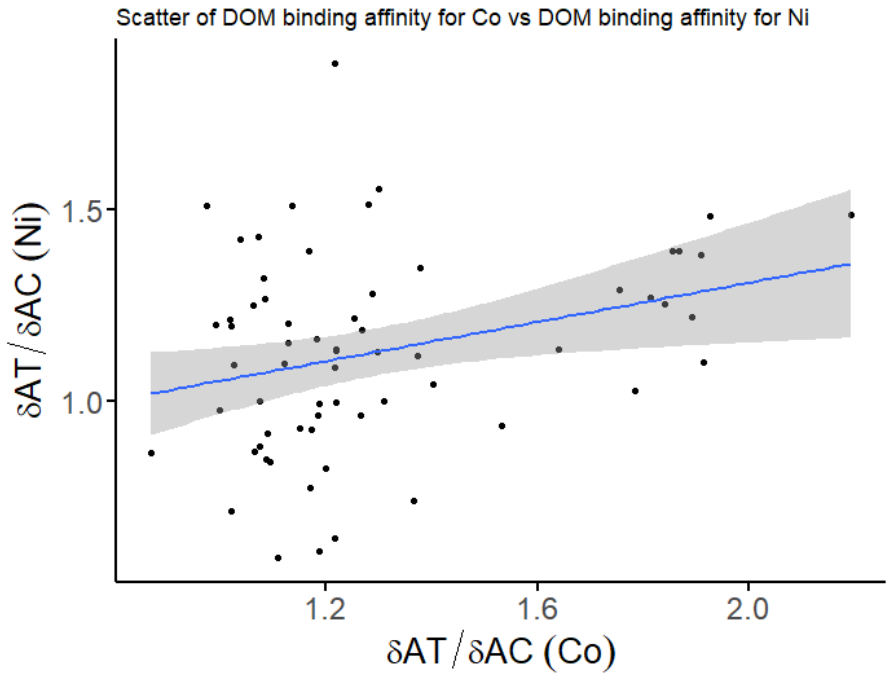

Figure 16 Linear regression between the inhibitive effect of DOM on the mobility of Ni Vs the inhibitive effect of DOM on the mobility of Co in DGT extracts containing speleothem DOM. The confidence interval on the linear gradients ($p < 0.05$) represented by grey shading.

There were also occasionally strong correlations between the amount of DOM in a speleothem extract and the kinetic properties of that DOM. With DOM from certain high DOM speleothems tending to have higher relative affinity for certain elements. As the extracts were spiked with trace metal amendments in a way that maintained a consistent ratio of DOM to trace metal ions, barring major systemic errors in the methodology, these relationships represent a link between the conditions that produce high DOM speleothems and the binding properties of the DOM incorporated in these high DOM conditions.

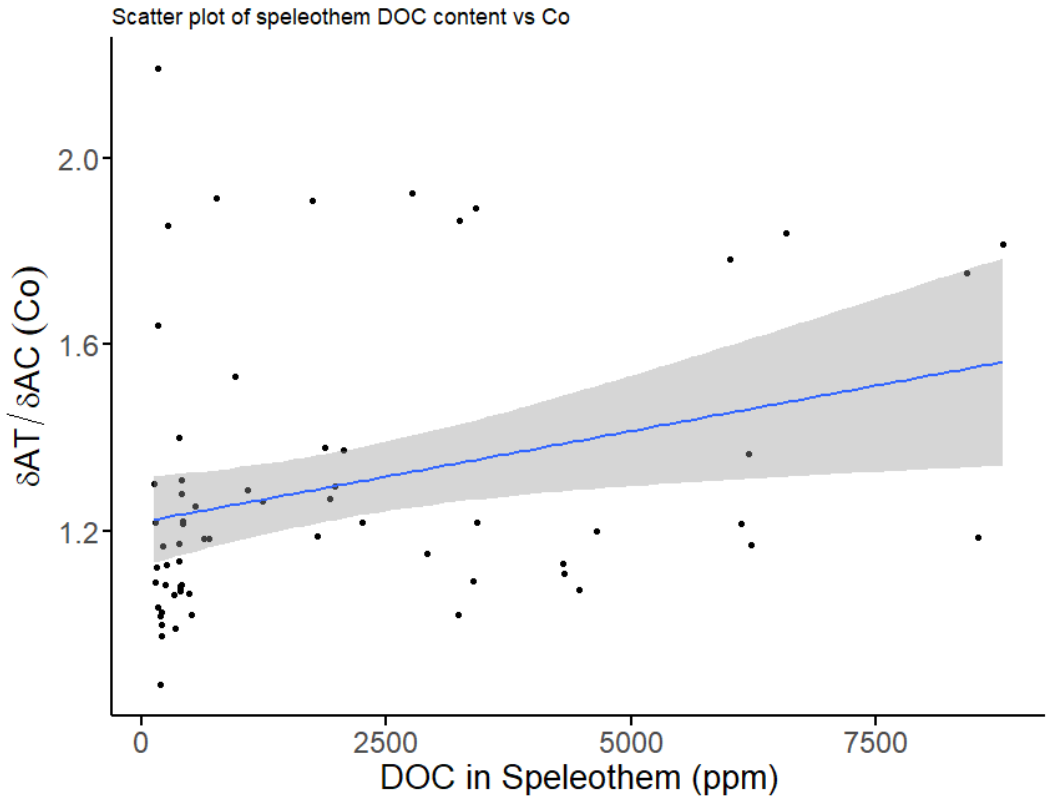

Figure 17: Linear regression between DOM content in speleothem calcite (ppm OC) and apparent path length for the diffusion of Co^{2+} in DGTs containing DOM extracts. The confidence interval on the linear gradients ($p < 0.05$) represented by grey shading.

Kinetic inhibition of Co had a statistically weak (R^2 = 0.065), shallow to very shallow positive relationship with DOM content in the source speleothem with a linear equation of $\delta_{AT}/\delta_{AC}\,(Co) = 0.00003\ \mathrm{DOM} + 1.25$.

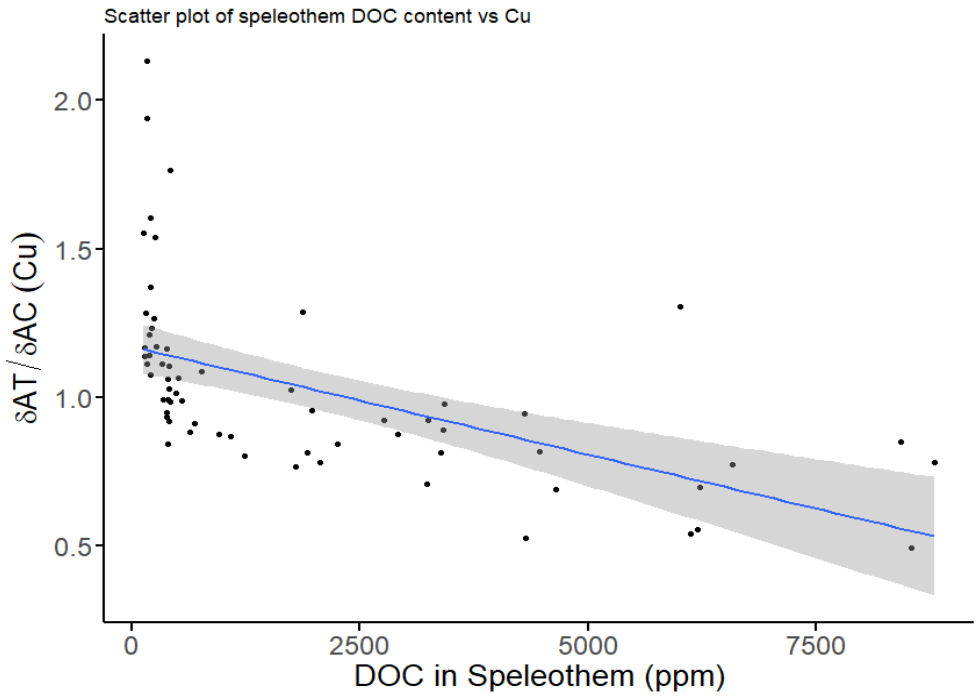

Figure 18: Linear regression between DOM content in speleothem calcite (ppm OC) and apparent path length for the diffusion of Cu in DGTs containing DOM extracts. The minimum and maximul linear gradients for this data ($p < 0.05$) represented by grey shading.

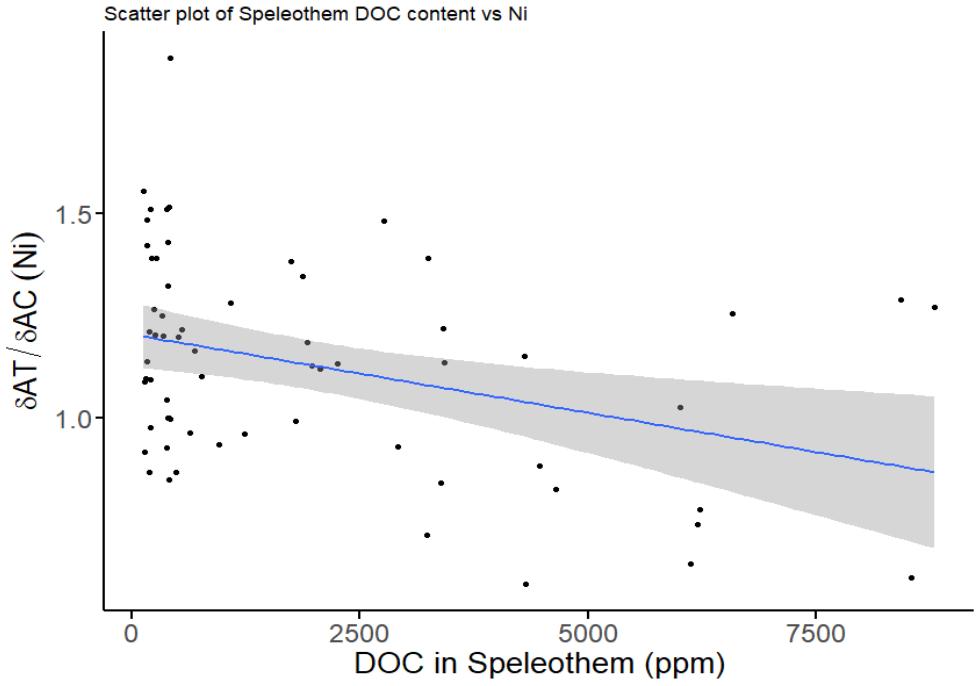

Figure 19: Linear regression between DOM content in speleothem calcite (ppm OC) and apparent path length for the diffusion of Ni in DGTs containing DOM extracts. The minimum and maximul linear gradients for this data ($p < 0.05$) represented by grey shading.

In contrast, kinetic inhibition of Ni and Cu had similarly shallow, but more statistically significant, negative relationships with DOM abundance in the source mineral. With $\delta_{AT}/\delta_{AC}\,(Cu) = -0.00008\,\mathrm{DOM} + 1.19$ (R^2 0.334) and $\delta_{AT}/\delta_{AC}\,(Ni) = -0.00004\,\mathrm{DOM} + 1.23$ (R^2 0.16). While R^2 values are low, the

minimum and maximum potential gradients for these trends ($p < 0.05$) are all somewhat negative, indicating the potential for a negative correlation between the amount of DOM in calcite and its kinetic properties.

The existence of these trends could imply one of two things, either that the conditions that produce high DOM speleothem calcite also have a marked effect on the inhibitive properties of that DOM, or that there is a flaw in the assumption that the number of available of DOM binding groups in an extract scale linearly with DOM concentration.

7.4 Discussion

There is a small but growing body of studies testing variations in DOM binding of trace metals in cave systems. Hartland et al., (2011) was the first study to characterise the relationship between DOM complexes and transition metals in cave water, proving that the availability Cu, Ni, Co, and V were all to some extent controlled by the dissociation kinetics of DOM-transition element complexes in Pooles Cavern, UK. Hartland et al., (2012) detected spatial variability in the DOM facilitated transport of Co, Cu, Ni and Zn when comparing cave drip waters in Pooles Cavern, UK to other sites in Italy and Spain. This variability was attributed to the unique hyperalkaline conditions in Pooles Cavern, rather than to changes in DOM properties between systems. Hartland and Zitoun, (2018) tested variations in DOM binding of Cu in drip waters from four New Zealand Cave systems; Waipuna Cave in Waitomo, and Hodge Creek and Nettlebed near Mt Arthur, and the Luxmore caves In Fjordland. This study found differences in DOM ligand behaviour at Nettlebed, Waipuna, and Hodge Creek DOM under specific circumstances, but its focus was restricted to Cu only, and only on modern drip waters.

There is a distinct lack of information on how these DOM binding properties vary on wider scales, across multiple continents, or have varied across time. By directly measuring ligand binding strength of DOM complexes preserved in calcite from a wide range of different cave systems, this study aimed to record the variability in DOM binding characteristics in cave systems across time and space. This variation was observed as changes in the size and shape of the distribution of typical DOM bindings across systems and across time, as well as significant variations in binding properties between different systems and different geological periods.

To observe changes in the spread of DOM bindings, measures of the shape, skew and spread of the distributions of DOM inhibitive effects in speleothem derived DOM were compared across a range of speleothems (the sample population) as well as to the shape and spread of the distribution of DOM binding strengths on smaller, more strictly defined temporal and spatial scales (i.e., within a single growth layer of a single speleothem).

The binding strengths observed in the internal standard, collected from a single growth layer of a single speleothem from Hodge Creek, represented the range of bindings that could be expected from Homogenous DOM of a single origin. The bindings observed in the internal standard DOM extracts were normally distributed for each of the amendments (Co, Cu, and Ni), and were found within a reasonably narrow range, suggesting that the variation from within the hodge creek standard was the result of random error. In contrast, the distribution of DOM binding of Cu and Co across all speleothems was not normally distributed ($p < 0.05$), with both Co and Cu binding exhibiting a significant positive skew, as well as a wider variance. In particular, the range of DOM binding properties for Cu were significantly wider across all speleothems than in the internal standard.

This significantly wider spread in binding indicates that binding properties of Cu-DOM complexes are highly variable across large temporal and spatial scales. Differences in the variability of DOM binding over time and space, observable as changes in the size and shape of the distribution of Co and Cu binding, indicate the presence of forcing factors affecting the binding of DOM in cave systems (Fairchild et al., 2006). These forcing functions could be related to any number of climate, environmental or geological differences between the conditions under which the extracts were formed.

Another piece of evidence suggesting that the binding of DOM ligand – trace metal complexes change over time and space is that DOM binding of Cu and Co was significantly different in different certain cave systems. There were several systems in which speleothem DOM binding of Co, Cu or Ni varied significantly. Specific cave systems like Anapala and Waipuna consistently produced DOM with either significantly higher or lower than average binding affinity for either Co or Cu, and binding of Cu was significantly higher amongst samples harvested from the last interglacial.

It is important to note that these differences were not significantly different following multivariate corrections vs age and origin. This indicates that speleothem age and system are interrelated to some extent, and effects related to the system from which speleothem DOM originates can't be fully discriminated from potential temporal effects of changing ecosystem and climate over the course of the quaternary. Nevertheless, the existence of statistically significant variations in DOM binding of Cu and Co across time and space implies that DOM inhibition of these elements is temporally and spatially unstable. This has implications for the application of these two elements in particular in paleoclimate studies.

7.4.1 Interrogating the Spread of DOM binding.

Changes and instabilities in the spread of a speleothem compositional trait over time and space can be indicative of forcing by changes in climate, environment, or geological factors (Fairchild et al., 2006). This forcing was most easily observed in the binding affinities for Cu, which varied most widely over time and space, followed by Co, then Ni.

Cu

Amongst the DOM complexes and transition metals investigated in this study, DOM binding of Cu was the least stable over time and space. The average binding of Cu (δ_{AT}/δ_{AC} (Cu)) observed amongst speleothem extracts in this study was ≈ 1.00, functionally the same as in pure calcite, but the spread of bindings was comparatively wide, with three different locations in this study exhibiting distinctly high or low Cu binding properties, and binding of Cu amongst DOM from extracts captured during last interglacial (Eemian/Kaihinu) being higher on average than amongst DOM captured during the current interglacial (Holocene). Binding of Cu was normally distributed in the internal standard, but had positive skew when considering all speleothem extracts, with many DOM extracts strongly binding Cu in solution. Variation in Cu binding affinity based on DOM fraction size and DOM origin was previously detected in caves by Hartland et al., (2011) and Hartland & Zitoun (2018).

Co

In contrast, Co binding also had a non-normal distribution over large timescales, and a normal distribution within the Hodge Creek internal standard, but the spread of the binding strengths for Co was slightly tighter than that of Cu. The inhibitive effect of DOM complexation on the mobility of Co was stronger and more stable than the effect of DOM on Cu or Ni, and in most cases, the presence of speleothem DOM in a solution caused a significant decrease in the mobility of Co was when compared to lab carbonate (δ_{AT}/δ_{AC} (Co) = 1.32 > 1.00). This tighter spread meant that variation in the DOM binding of Co wasn't significantly wider across all extracts than in the Hodge Creek internal standard. Other studies on Co binding by DOM in cave systems (Hartland et al., 2011) have noted that Co binding tightly to DOM in cave water is not a typical behaviour for the element, which tends to be only weakly inhibited by ligands in surface systems. Co was still the most mobile fraction in most solutions in this study, but the degree to which it was inhibited by DOM was unexpected.

Ni

This study found no significant difference between the way that Ni was bound in complexes with DOM (δ_{AT}/δ_{AC} (Ni)) when compared to inorganic complexes with pure lab calcite (δ_{AT}/δ_{AC} (Ni) = $1.13 \pm 0.15 \approx 1.0 \pm 0.2$). Unlike Co and Cu, the distribution of Ni binding had no easy to detect skew, with the distribution's shape being roughly normal regardless of the range of sample used ($p > 0.05$), with both control and test distributions having a similar variance. This means that it is difficult to confirm whether the binding of Ni being observed in the speleothem extracts is by organic complexes, rather than inorganic complexes with CO_3^{2+} in dissolve $CaCO_3$. DOM binding of Ni has been previously observed in cave derived DOM from Pooles Cavern, one of the caves investigated in this study (Hartland et al., 2011; 2014) and in lab settings (Lindeman et al., 2022). In light of this, it is possible that this study's lack of confidence in the observations of Ni binding is a result of insensitivity in the method. Given the positive correlation between DOM facilitated binding Cu and Ni binding amongst DOM extracts, it is also possible that DOM binding of Ni was occurring in the DGT's but was unobservable due to the high margin

of error in Ni binding in lab carbonate controls meant to represent binding of Ni in the absence of DOM (δ_{AT}/δ_{AC} (Co) = 1.0 ± 0.2).

Comparing the DOM binding affinity for the three trace metals trailed in this study, Co was generally the least strongly bound, with the lowest immobile fractions (10%-20% bound) in both test solutions containing organic complexes and in the lab calcite controls. Cu was the next most mobile with an average immobile fraction of approximately 30% across both standards and blanks. Ni was the least mobile element in all SV-DGT's, with 40-50% of Nickel ions in the SV-DGT's remaining in solution following application of a charge gradient across all samples and controls.

Amongst the speleothem DOM extracts in this study, DOM ligands, on average, had a stronger inhibitive effect on the mobility of Co than on Cu and Ni. DOM binding of Cu varied significantly depending on the age of the extract the cave system from which the DOM originates, and binding of Ni by DOM was difficult to distinguish from inorganic binding in lab carbonate controls. There was no strict hierarchy observable in the binding affinities of the DOM extracts for each the three metals investigated in this study. Though the inhibition of Co by DOM was stronger on average than for Cu or Ni, some DOM extracts in this study bound very weakly to Cu, with some DOM extracts even appearing to increase the mobility of Cu compared to lab calcite (δ_{AT}/δ_{AC} (Cu) < 1) while other extracts exhibited similar binding for both metals **(Figure 11)**. The low (δ_{AT}/δ_{AC} (Cu) < 1) binding affinities for Cu that occurred in caves like Anapala and Pooles Cavern could either be the result of competitive exchange at DOM binding sites favouring Co and other ions (Boiteau et al., 2018; Dudev & Lim, 2014) over Cu, or be indicative of DOM facilitating the transport of Cu through the DGT by strongly binding with the metal and diffusing through to the binding layer as organic complexes (Hartland and Zitoun., 2018).

If the observed behaviour is a result of competitive exchange, then this behaviour is atypical of DOM in surface systems, which tends to bind Cu more strongly than Co (Milne et al., 2003), but is consistent with the higher binding affinity of Co for DOM reported in Poole's Cavern dripwaters by Hartland et al., (2011; 2014). If this behaviour is the result of DOM facilitating transport of Cu, the fact that Cu mobility is increased (δ_{AT}/δ_{AC} (Cu) < 1) in some systems compared to others is also

interesting, as it indicates a variation in kinetic controls on Cu mobility between cave drip waters.

Cave derived DOM exhibiting a stronger inhibitive effect on Co, compared to Cu and Ni is not unique to the extracts observed in this study, but is different behaviour to what would be expected in surface systems. Modelling by Hartland et al., (2011) on the transition metal binding tendencies of DOM extracts from Pooles Cavern using WHAM and visual MINTEQ software tended to underestimate binding of Co. Both WHAM and V-MINTEQ models predicted Co to be the least inhibited transition metal in drip waters, and more mobile than Cu or Ni. The models in these trials were calibrated using experimental data from surface systems, such as Warnken et al., (2007) and Milne et al., (2003). In such surface waters, Co tends to be more mobile than Cu or Ni, with Cu and Ni heavily restricted by DOM complexes. Even in groundwater systems, Hartland et al., (2012) found that DOM bound with and transported Cu through limestone epikarst preferentially over Co and Ni, resulting in a larger comparative abundance of Cu over Co in cave drip water than in surface soil solution. According to the Irving Williams series (Mellor & Maley, 1948), in the absence of other effects, Cu tends to bind more tightly to organic functional groups, followed, by Ni, then Co. In cave systems like Poole's Cavern, however, it seems that this paradigm can occasionally be reversed, with DOM placing stronger restrictions on Co compared to other transition metals.

The tendency for DOM to bind transition elements differently is most likely the result of conformational changes in the DOM structures typical in certain cave systems and conditions (Smith et al., 2002; Dudev & Lim, 2014). Hartland et al., (2011; 2012) suggested that high strong inhibition of mobility for Co by DOM in Pooles Cavern could be the result of hyperalkaline conditions in the cave's waters (pH = 11) affecting the conformation of DOM functional groups, resulting in tighter binding of Co. The SV-DGT's employed in this experiment, however, provide evidence that contradicts this. The trials had internal pH's between 6.8 and 7.1, well within the range within which the models employed by Hartland et al, would be expected to apply (Milne et al., 2003). Despite this, DOM from Pooles Cavern in this study still had a slightly stronger inhibitive effect on the mobility of Co compared to Cu. In addition, Anapala Cave, the site in this study which produced the DOM with the strongest affinity for Co and the weakest affinity for Cu, tends to have drip water lower pH values, typical of other karst settings between 7.9 -8.5

(Höpker, 2023). Given that the two sites that produced DOM with a stronger binding for Co than for Cu had such different pH's, and that DOM from these two systems exhibited this tendency even in controlled DGT conditions, it seems likely that variations in DOM binding between these systems are not related to the ion or pH balance in cave drip water, (Hartland et al., 2012) but are rather the result of factors that are directly related to changes in DOM structure changing how DOM binding sites conform with Co. The presence and abundance of organic structures such as benzene rings and long alkene chains can affect the shape, electronegativity, and charge transfer properties of binding site groups on DOM (Dudev & Lim, 2014), altering their affinity for ions with specific ionisation energies and atomic radiuses. For example, Vitamin B_{12} preferentially binds Co over Cu in natural waters (Zhang et al., 2019) because of the structure of its functional groups.

The potential for seasonal and spatial variation in DOM size and structure to control DOM binding of specific ions has been identified before by Hartland et al., (2011) and Hartland & Zitoun (2018), but due to their tighter focus on fewer systems and on drip water DOM specifically, these studies were unable to determine whether these variations were significant over large spatial scales.

7.4.2 Potential spatial origins for DOM binding variation

The potential causes for the significant spatial and temporal differences in DOM binding strength observed in this study fall into two groups. Spatial differences in DOM binding based on regional climatic, environmental, and geological differences between cave systems, and differences resulting from soil and ecosystem change driven by climate processes over the course of the late quaternary. Given that the effects of cave system location and of speleothem age on the binding of the DOM extracts could not be distinguished for these samples following multivariate corrections, both temporally driven climate and ecosystem changes and spatial differences in geological setting and surface climate could be related to the significant regional variations detected in DOM function between extracts from Hodge Creek Cave, Waipuna Cave, Anapala Cave, and Pooles Cavern. Analysing the unique conditions in these systems during the periods in which they underwent the majority of their speleothem growth could give insight into some potential causes for variations in the binding strength of DOM in these systems, and potential help guide future study.

Hodge Creek

Hodge Creek Cave is a high-altitude cave system in the South Island of New Zealand (Pearson, 2020). The cave lies at an altitude of 940 m above sea level (a.s.l.) and at a latitude of 41^{o} South. Hodge creek experiences a cool maritime climate and typically receives 2000 – 2500 mm of annual rainfall with a mean annual temperature of 10^{o}C. The site has been covered in silver beech and fern forest for the majority of the Holocene (Lorrey and Bostock, 2017) and during this period it experienced warmer temperatures and received lower rainfalls than it does today. There is no uniform soil layer overlying the cave, but rather deep crevasses and grykes filled with heavily reduced gley soils overlain by thick leaf litter (Pearson, 2020). The samples from Hodge Creek analysed in this study were all flowstone cores, sampled by Pearson et al., (2020), and were formed over the last 100 ka, either during the early to mid-Holocene, or during the interglacial before (80+ ka). The DOM contents of flowstones from Hodge Creek are well recorded, (Pearson et al., 2020) and as such, Hodge Creek Cave is the cave from which the speleothem DOM internal standard for this study was derived. In this study, DOM from Hodge Creek speleothem extracts exhibited intermediate to low bindings of both cobalt and copper compared to other NZ sites. Which was in line with Hartland & Zitoun (2018), which found that the typically binding affinity of DOM for Cu in Hodge Creek drip waters was slightly lower than average binding in Waipuna and Luxmore drip waters, while being significantly lower than typical Cu binding in Nettlebed. Hartland and Zitoun (2018) did not record Co or Ni binding in this system, which in this study was similar across all Hodge Creek DOM regardless of age, including the internal standard. Binding of Cu in Hodge Creek cave was slightly more variable across all speleothems in this system, which varied widely in age, when compared to the temporally stable internal standard. This indicates some sort of temporal variation in DOM binding was present in this system. We know from Pearson (2020)'s research that humic DOM inputs incorporated in Hodge Creek calcite varied over the Holocene. These changes in humic input may also be linked to structural changes in humic compounds being incorporated into speleothem calcite, but without detailed characterisation of the nature of the organic structures in this system the nature of this link is tenuous.

Waipuna Cave

Waipuna Cave is situated in the Waitomo region of the North Island, New Zealand. It has been used as a comparison site to Hodge Creek cave by both Hartland & Zitoun (2018) and by Pearson (2020). The cave lies at 38^{o} south, and sits 80 m a.s.l. (Hartland & White, 2019; Pearson 2020). It experiences a strongly seasonal climate, affected by the El Niño-Southern oscillation, with a mean annual rainfall of 1,500 mm a year, and a mean annual temperature of 13^{o}C. The site above the cave is densely forested with temperate podocarp forest, and soils overlying this system are thick (> 1 m), well drained, and volcanic, being rich in allophane, a clay mineral that is known to increase the organic carbon storage capacity in soils (Sparks, 2003). Samples from Waipuna in this study were also flowstones, formed from the end of the last ice age and all through the Holocene (0.7 – 20 ka). DOM contents in Waipuna speleothems tended to be lower than in Hodge Creek in this study, and in Pearson., (2020). In this study, Waipuna DOM was found to bind more strongly to both Cu and Co than Hodge Creek DOM, a feature that had previously been recorded to a lesser extent in drip water studies DOM by Hartland and Zitoun, (2018). Hartland and Zitoun (2018) also found that Waipuna DOM tended to bind Cu in complexes with smaller, more degraded hydrophilic DOM as opposed to in complexes with larger humic compounds.

Anapala Cave

Anapala cave is a shallow cave that sits just above sea level under tropical rainforest in South-West Niue (S. Höpker, 2023). The Cave is at an altitude of ≈ 50 m (a.s.l.), and at a latitude of 19^{o} South. It experiences tropical climates, with mean annual rainfalls of 2000 mm a year and an average annual temperature of 26^{o}C. The overlying soil is thin and organic, and the overlying bedrock is less than 10 m thick at its thinnest point, and around 10 m thick above the chamber from which the samples in this study were taken. Dripwaters in this cave are reasonably alkaline, with pH's between 7.9 and 8.5. The site is relatively unstudied, with research and mapping currently ongoing. The speleothems in this study from Anapala were stalagmites and were sampled from the lower chamber. These samples were formed since the start of the Holocene (10 – 1 Kya). As of this study, speleothems from Anapala were found to contain DOM extracts that bound strongly to Co, but seemed to

actively increase the mobility of Cu where they were present. Preferential binding of speleothem DOM with Co over Cu has been observed in cave systems before, specifically Hartland et al., (2011) observed this tendency in drip waters in Pooles Cavern, a temperate, Hyperalkaline cave in Buxton, in the UK.

Poole's Cavern

Poole's cavern is a shallow cave under temperate forest in Buxton, in the East Midlands region of the UK. It sits at a latitude of 53^{o} North, and at an altitude of 330 m (a.s.l.). The climate in and around buxton is cool and temperate, with a mean annual temperature of 9^{o}C and a mean annual rainfall of 1300 mm. This rainfall is partially seasonal, with particularly heavy rainfall falling during late autumn and early winter. The overlying soil is thin (30-60 cm) brown and organic rich, with a thick litter layer. The forest overlying the cave is dotted with historic lime kilns, and the hillside accordingly contains deposits of "lime ashes". These deposits cause dripwaters in the underlying cavern to be hyperalkaline in many places, particularly near the entrance where overburden is thin (Hartland; 2011).

Samples for this study came from two chambers within the system, the shallow Roman chamber, near the entrance, with a 10-20 m overburden, and Poached Egg chamber, farther from the entrance where overburden is larger (up to 50 m). Dripwater in Poached Egg cavern is entirely hyperalkaline (pH 11.6-12.1) due to lime contamination, while in Roman cavern dripwater pH ranges from 7.7-8.4 but can be as high as 12 in places where hyperalkaline contamination seeps through from the surface. All samples from Pooles Cavern were modern hyperalkaline precipitates, formed over the course of the last 20 years.

Pooles Cavern is notable for the purposes of this study as it is the site at which a large portion of existing research on DOM trace metal binding has been conducted (Hartland et al., 2011; 2012; 2014). This site itself is notable due to the similarity between the binding characteristics observed here to those in Anapala, Niue. DOM from Both of these systems exhibits the tendency to preferentially bind Co over Cu, a tendency that was observed by Hartland et al., (2011). In this study, the inhibitive effect of Pooles Cavern DOM on the mobility of Co was widely distributed, ranging between 1.2 and 2 times higher

than lab calcite. In contrast, the movement of Cu was not inhibited by Pooles Cavern DOM whatsoever, with DOM affinity for the metal being between 0.8 and 0.9 times that of lab calcite, (δ_{AT}/δ_{AC} (Cu) < 1).

From the information available at the time of this study, it is not possible to link the differences in DOM binding behaviour observed between these cave systems to any specific system trait. There are, however, some commonalities between systems with similar binding tendencies that have been linked to changes in binding potential in the past, which may be indicative of potential avenues for future study on DOM binding properties in cave systems.

The two most notable systems in this study were Waipuna cave and Anapala Cave, with DOM from both systems exhibiting significantly different binding characteristics of Cu and Co when compared to the lab calcite blanks. Waipuna cave DOM tended to strongly bind both Cu and Co, while Anapala cave DOM inhibited the movement of Co while increasing the mobility of Cu. DOM from Pooles Cavern in the UK (Hartland et al., 2011; Hartland et al., 2014) often has binding properties similar to those of DOM from Anapala, with a stronger than average tendency to bind Co and a lower-than-expected affinity for Cu.

Identifying an exact cause for these binding affinities in Pooles Cavern and Anapala isn't possible within the scope of this study, but there are some similarities between the samples taken from the sites that could suggest potential avenues for further investigation. Extracts from both caves were sourced from comparatively shallow locations within the system, and both sets of extracts were from drip water precipitates such as stalagmites, as opposed to flowstone cores.

Both the depth of a system and differences in the processes involved in the formation of drip water precipitates, as opposed to flowstones, have been linked to variation in the molecular weight and structure of DOM in cave systems (Blyth et al, 2016). In particular, the molecular weight of the DOM in cave drip water is heavily affected by its residence time in the epikarst (Blyth 2016, Einsiedl et al., 2007). Both Hartland & Zitoun, (2018) and Hartland et.al., (2011) have identified a correlation in the binding affinity for Cu and the size and molecular weight of DOM molecules in dripwater, but the exact nature of this relationship is unclear. Hartland et al., (2011; 2012) detected seasonal variations in DOM structures in Pooles Cavern that were linked to seasonal rainfall, with heavy rain in Autumn and

faster groundwater flows linked to an increased abundance of larger, higher weight organic material. This more massive DOM tended to have a stronger than average affinity for Cu than the lower molecular weight fractions. In contrast, Hartland & Zitoun, (2018), found that filtering dripwaters from Waipuna and removing large, colloidal material increased the binding affinity (dissociation constant, *k*) that that DOM had for Cu.

The similarities in DOM binding of Cu between Pooles Cavern and Anapala speleothems could potentially be related to factors influenced by DOM transport in the epikarst, and by extension the depth of the sample formation.

7.4.3 Temporal origins for DOM binding variation

Unlike spatial variations in DOM binding, which have been previously documented in cave drip waters, there is substantially less information on how the binding properties of DOM in cave systems vary with time. As the extent of certain ecosystems has changed significantly over the last two interglacials, large scale temporal differences between speleothem DOM binding have the potential be linked to changes in the climate and soil conditions in the ecosystem above a system (Lorrey & Bostock, 2017; Pearson et al., 2020). While local climate is also linked to the location of any given cave system, any differences in DOM binding strongly related to location also have the potential to be related to differences in the geological setting of a cave system. Geological factors such as with cave depth, overburden, and flow paths of groundwater and drip-flow aren't directly linked to climate and are amongst the most common factors that prevent the comparison of paleoclimate proxies between systems.

There was only a single significant temporal variation in DOM binding detected in this study. Speleothem derived DOM from the Eemian/Kaihinu interglacial (the last interglacial) produced a stronger than average inhibitive effect on the mobility of Cu. The DOM that produced this effect originated from mostly from deeper, New Zealand caves, being extracted from Hodge Creek cave flowstones in Mt Arthur, Ann's cave stalagmites in Waitomo, and a single subsample from Anatoloa Cave, in Niue, which behaved as an outlier, with a low binding of Cu similar to those observed in Anapala. Conditions in New Zealand during the last interglacial were likely much colder and wetter than they are today (Lorrey & Bostock 2017). In the South Island, forests tended to contain less beech and more scrub dominant heading

into the last ice age. In contrast, North Island forests seem to have been more beech dominated, with southern beech pollen found in sediment records as far North as Auckland. These climate and environmental changes may be related to changes in speleothem DOM incorporation and transport towards the late Eemian. Pearson et al., (2020), linked changes in speleothem DOM abundance in calcite to changes in soil moisture conditions during the Holocene. Hence, changes in DOM incorporation processes have the potential to be linked to changes in DOM kinetic properties **(Figure 18),** but the process by which these may be linked is unclear. An equally likely cause for the variation in **Figure 18** may be a flaw in the assumption that the binding sites available in DOM scale linearly with DOM concentration, with higher concentrations of DOM resulting in changes in the availability of binding sites.

7.4.4 Limitations

This study aimed to identify the existence of variations in the binding properties of DOM across either time or space, and in that it has been successful. Significant variations were detected in the spread of binding and in the typical DOM binding effects between cave sites and across time. This has implications for the use of trace, transition metals in large scale paleoclimate studies over different systems and long timescales, as trace metal incorporation into cave minerals has the potential to be affected differently by changes DOM sources between cave systems. We have also attempted to draw links between these changes and some potential root causes for DOM binding variation but are limited in our ability to draw direct conclusions about the nature of these DOM changes due to the wide initial scope of our study.

Sampling Limitations

This study was designed with the primary aim of establishing the existence of variations in the trace metal binding properties in speleothem DOM over time and space. Identifying potential causes and trends in this variation was a secondary objective, and as such, sampling was conducted in a way that ensured that the widest possible range of systems was sampled, while ensuring that DOM content was as high as possible, so the effects of DOM binding were quantifiable using DGT and ICPMS. So that as many extracts as possible could be analysed, the speleothems used in this study were sampled based on convenience. Only speleothems and powders from pre-existing archives were sampled, with systems that were known

to produce very high DOM calcite such as Pooles Cavern (Hartland et al., 2011), Waipuna Cave (Hartland & Zitoun; 2018), and Hodge Creek (Pearson et al., 2020), sampled more rigorously than systems where speleothem derived DOM was expected to be less common, such as Nettlebed. Speleothems with known chronologies were sampled preferentially over undated archives, and even within speleothems, growth layers with dark brown colouration, indicating organic content, were sampled preferentially over paler growth layers (van Beynen et al., 2001) to ensure as much DOM analyte as possible was extracted. Even with this targeted sampling strategy, more than half of all extracts analysed in this study did not contain enough fluorescent DOM for trace metal spikes to be within LOD of ICPMS and were subsequently discarded. As a result, the speleothems in this study can be expected to contain significantly more DOM than is typical in speleothem calcite.

Another result of the sampling method is the introduction of systemic biases. No significant link between a binding property and a speleothem property detected in this study remained significant after multivariate corrections, indicating bias in the distribution of samples in this study. Some caves only produced high DOM calcite within a specific range of ages, or in a specific type of formation, and some geological periods sampled in this study are represented by only a few samples from specific caves or regions. As a result, links between cave system traits and DOM behaviour cannot be drawn with confidence, only speculated at. Research on DOM behaviour in modern drip water seems to indicate both seasonal climate variation (Hartland et al., 2012) and differences in a cave's geological setting (Hartland et al., 2012; Hartland & Zitoun., 2018; Einsiedl et al., 2007) can be corelated to differences in DOM structure and behaviour, while studies on variation in DOM in calcite have correlated changes in overall speleothem DOM content with large scale temporal climate oscillations above Hodge Creek cave, NZ (Pearson, A., 2020). Climate processes and the environmental factors that inform ecosystem formation, DOM transport and deposition are also heavily interlinked (Hartland et al., 2012; Lorrey & Bostock 2017; Pearson, 2020). As such, it is the most likely case that both spatial and temporal climate driven changes in speleothem DOM properties work in concert to affect the DOM formation and transport in ways that are system specific, making it impossible to fully decouple temporal and spatial influences on DOM properties.

Method Limitations

Beyond sampling limitations, this study was also limited by compromises that had to be made when adapting SV-DGT for application on DOM extracts. In cave waters, trace metal ions will always be present in concentrations lower than the concentration of ligand binding sites, (Hartland & Zitoun., 2018). In this study, solutions were spiked with metal ions based on the concentration of DOM in g C L^{-1}, so that there was 1 mmol of each trace metal amendment in the extract for each gram of carbon. This means, assuming typical DOM contains 2-6 mmol of function groups per gram of DOM (VanLoon & Duffy, 2017), trace elements could theoretically outnumber functional groups in the DGT by ratios as great as 3:2. The pH's of the SV-DGT's in this study were also not directly comparable to those in cave water, with pH of 6.98 ± 0.2. This pH is considerably more acidic than those typical in the cave systems from which the DOM originates (pH > 7.5). These factors together mean that conditions in the DGT's were not directly analogous to those in cave water.

If the Co, Cu and Ni ions in the DGT's outnumber the binding sites in solution, this could lead to competitive behaviour between the ions in the extracts (Boiteau et al., 2018; Dudev & Lim, 2014) where one of the three types of ions is prevented from forming complexes by the other two preferentially bonding to ligand binding groups. This competitive inhibition would not be expected to occur in cave waters. In the SV-DGT's employed by this study, DOM ligand binding of Ni in extracts containing speleothem DOM could not be differentiated from binding of Ni with inorganic complexes in pure calcite controls. This was unexpected, and makes it impossible to ascertain whether the high, stable retention of Ni in DGT's was a result of DOM facilitated binding or not. It may be the case that the majority of binding of Ni observed in the SV-DGT's was inorganic, due to competitive exchange favouring binding of Co and Cu in DGT's.

If the pH in the extract solution is too dissimilar from that in cave water, pH effects on DOM binding (Milne et al., 2003; Welikala et al., 2021) may make the binding potentials observed here unrepresentative of the binding potentials of DOM in natural cave waters. These effects were minimised in this study by ensuring the pH in extract solutions was as high as possible, which appears to have been effective. The relative binding of Cu and Co observed in Pooles Cavern, Waipuna and Hodge

Creek extracts in this study were comparable to binding behaviour observed in drip water from those caves by Hartland et al., (2011) and Hartland & Zitoun (2018).

This study is also limited by the high variance in trace metal inhibition in the lab carbonate controls. There was a high margin of error on the values for $\mathrm{Ln}(C_{tC}/C_{oC})$, and as a result δ_{AT}/δ_{AC} for some samples it was difficult to distinguish binding from the baseline (δ_{AT}/δ_{AC} = 1.0). This seems to be a result of inconsistency in the SV-DGT method adapted from Welikala et al., (2018). The original method was applied to solutions containing organic amendments derived from soil and organic waste, which typically contained functional groups in abundances of 5-60 mmol in 5 mL of solution. The concentrations of organic ligands obtained from speleothem calcite in this study were much lower than this. Assuming 2-6 mmol of ligand functional groups for each g of organic carbon in the DGTs (VanLoon & Duffy, 2007), each DGT contained 10-30 μmol of ligands, a concentration 1000 times lower than in SV-DGT's deployed by Welikala et al., (2018). It's possible that insensitivity in the method is a result of DGT being unsuitable for quantitative analysis on solutions this dilute.

7.5 Conclusion

In cave waters, the movement of transition metals such as cobalt, copper, and nickel is controlled by binding with aqueous organic compounds derived from surface ecosystems. These trace metals can enter speleothem calcite from drip water by substituting for calcium in the calcium carbonate crystal lattice. Their abundance in calcite has potential for use as a proxy for paleoclimate rainfall, but the degree to which binding of trace metals by DOM varies between cave systems and over time is difficult to quantify. This complicates the application of trace elements in calcite as a paleoclimate proxy, as it adds an additional element to the relationship between the incorporation of transition metals into calcite, and cave water flow factors linked to rainfall. It also hinders the comparison of trace metal incorporation into speleothems across different cave systems and over time. By extracting preserved DOM from speleothem calcite of a range of different ages and testing its tendency to bind Co, Cu, and Ni, it was found that the tendency for DOM to inhibit the mobility of Co and Cu varied significantly between cave sites and over time. Binding of Cu by speleothem derived DOM exhibited the most significant variation, with a significantly broader range of bindings displayed over all

speleothem extracts. Co was more stable, with DOM inhibition of cobalt only varying significantly in a few key systems. Binding of Ni in this study was consistently strong, and did not vary significantly over time and space, but it was hard to determine if this binding was all DOM controlled, due to high baseline variance of inorganic Ni binding in lab carbonate controls, meaning this stability may not hold in cave systems where binding of Ni is DOM dominated. As for Co and Cu, it is difficult to link the variations in binding behaviour of DOM for these elements to either climate changes between systems and over time, or to changes inherent to the cave system itself. It is the most likely case that a range of factors, including soil, geological and climate factors have an effect on DOM structure in cave systems and thereby impact binding of Cu and Co in cave water. Potential variations in these factors should be accounted for when comparing transition metal abundance in speleothem calcite between systems, and variations in DOM binding strength over time should be accounted for in when using confidence to link transition metal abundance in calcite to paleoclimate factors.

8. Implications

8.1 Introduction

In a cave system, the composition of speleothems such as stalagmites, stalactites and flowstones depend on climate and environmental factors that influence the composition of groundwater entering the cave system, the flow dynamics of water over speleothem surfaces, and the atmospheric conditions in the cave during speleothem formation (Fairchild & Baker; 2017). The transition of certain trace elements in water from solution into speleothem calcite, for example, is controlled by the presence of surface-derived dissolved organic matter (DOM) ligands (Hartland et al., 2011; Hartland & Zitoun 2018; Lindeman et al., 2022), that form complexes with metal ions in solution.

As a result of the tight controls on transition metal availability by DOM, the availability of free transition metals in calcite is relatively independent of their total concentration in solution and is instead dependent on the dissociation kinetics of DOM-metal complexes (Hartland & Zitoun 2018). To substitute with calcite in the calcite matrix, free metal ions must first dissociate from complexes with DOM. This is a time dependent process, which means that how many trace element ions dissociate from complexes with DOM to be incorporated into cave minerals varies depending on how long the complexes remain in equilibrium with the calcite surface, which is a function of drip water residence time on speleothem surfaces, and thereby a function of drip water flow rate (Hartland & Zitoun, 2018; Lindeman et al., 2022). This link between drip water flow in cave systems and the incorporation of trace metals into speleothem calcite raises the potential for changes trace metal abundance in speleothems to be linked to rainfall driven changes in drip water dynamics, thus acting as a proxy for rainfall.

This proxy is especially promising, as the link between variations in trace metal inclusion into calcite and cave drip water dynamics has the potential to be dependent only on the residence time of DOM on speleothem surfaces, allowing for comparison across multiple different cave systems, and for the creation of high-resolution rainfall records across the duration of the Quaternary (Hartland & Zitoun 2018).

DOM ligand kinetics in drip water are not the only control on the availability of transition metals in drip waters. The rate at which ligands release transition metals, and thereby the number of transition metals available in solution after any given residence time is dependent on the kinetic properties of the DOM-transition metal complexes in cave waters. There is also potential for variations in the metal binding strength of these complexes to cause variability in the availability of trace metals across cave systems. Some DOM complexes bind more tightly than others to certain metals, reducing their incorporation into calcite, or vice versa.

This study identified significant variations in the relative binding strengths of DOM-metal complexes between cave systems in Niue and New Zealand, as well as significant broadening in the variation of these properties over large spatial and temporal scales. It also identified specific circumstances under which DOM binding properties varied significantly between cave systems, with certain systems, speleothems and periods producing DOM with significantly different binding affinities for specific metals when compared to each other. Whether these differences are the result of age differences between the speleothems at these sites or differences in geological setting affecting DOM properties was unclear. These variations suggests that trace element incorporation into speleothem calcite, while still heavily related to cave water flow dynamics and to local rainfall variations, could also be dependent on factors affecting the structure and the binding tendencies of the DOM ligands controlling trace element mobility, leading to variability in the proxy.

8.2 Discussion

This study tests the limitations on transition metal-based paleoclimate proxies by establishing the existence of variations in the controls on their incorporation into speleothem calcite, specifically, by identifying variations in the binding affinities of DOM for the trace metals Cu and Co. This was achieved by measuring how strongly the mobility of these ions is inhibited in the presence of DOM of different origins compared to in a control solution that does not contain DOM (Welikala et al., 2018). This difference was expressed as the relative path length (δ_{AT}/δ_{AC}) for diffusion of ions in a solution where they are in habited by DOM δ_{AT} compared to a solution where they were not inhibited δ_{AC}. This value was recorded in this study as a measure of the inhibitive effect of DOM on trace metal movement in a

controlled setting and can be considered roughly analogues to trace metal availability in cave waters containing the DOM in each of the extracts. The variable δ_{AT}/δ_{AC} is correlated with the dissociation constant k of DOM-trace metal complexes (Welikala et al., 2018) and as such δ_{AT}/δ_{AC} can be used as a measurement of the effect that DOM complexation would on the mobility of trace elements in cave waters.

The effects of the DOM extracted from speleothems on the mobility of Cu, Co, and Ni were tested in this study, but only DOM effects on the inhibition of Cu and Co were significant enough to be distinguished from binding by inorganic complexes in the controls. Inhibition of Ni by speleothem DOM could not be distinguished from the binding of Ni by inorganic complexes in $CaCO_3$, due to a high variance of Ni binding in the controls. Given that the binding of trace Ni has been reported in cave systems before this (Hartland et al., 2011; Hartland et al., 2014; Lindeman et al., 2022) the tendency for DOM binding of Ni in this study to be similar to the binding of Ni in inorganic complexes in pure $CaCO_3$ (Shafaei Arvejeh et al., 2013) is highly unexpected. This could be the result of competitive binding by DOM favouring the other trace metals more reliably (Dudev & Lim, 2014), leading to Ni being bound in similar ways regardless of the presence of DOM. In a cave system, DOM ligand concentration will always exceed the total concentration of trace metals (Hartland & Zitoun, 2018) so competitive exchange of trace metals at binding sites is not likely to be a factor affecting the inhibition of Ni. This makes it difficult to draw conclusions about the stability of DOM-Ni complexes in drip waters from the δ_{AT}/δ_{AC} values in this study, as there is a strong possibility that DGT's are not measuring the strength of the DOM-Ni complexes which control Ni availability in cave water. The fact that binding of Ni by DOM was related to the binding kinetics of DOM ligands in solutions to a limited extent, in that it varied linearly with DOM binding of Cu, suggests that DOM may have affected the binding of Ni in the SV-DGT's under some circumstances, but further study will be necessary to identify the extent of this relationship.

Binding of Co and Cu in the presence of DOM complexes was distinguishable from the inorganic binding in the controls, and this binding changed significantly over time and space. There was a significantly higher variation in DOM binding of Cu across broad time periods than was detected in a single growth layer, and in significantly different binding effects on Co and Cu between sites under sub-alpine

beach forest in Hodge Creek, New Zealand, (Pearson et al., 2020) shallow tropical caves in Niue (Höpker., 2023), and deep sites under podocarp forest with thick organic soils in Waitomo (Hartland & White, 2019; Nava-Fernandez et al., 2020). These differences suggest that temporal and spatial variation in the origin of DOM in cave systems can have an effect on the kinetic properties of corresponding DOM-metal complexes.

The fact that no relationship between DOM properties and climate variables remained significant following multivariate corrections makes it impossible to draw direct correlations between variations in DOM binding properties and variables related to that DOM's formation. Despite this, previous research on variations in DOM driven transport and DOM properties (Hartland et al., 2011; Hartland et al., 2012; Hartland & Zitoun 2018) allows us to speculate on factors that have been related to DOM binding in the past that could help guide future study.

One potential cause for variations is variation in DOM molecular weight, corresponding to differences in the degree of DOM degradation and transport in the epikarst (Blyth et al., 2016; Einsiedl et al., 2007). Research by Hartland & Zitoun et al (2018), found evidence suggesting that binding of Cu could vary between cave systems in Aotearoa, New Zealand, as well as with DOM particle size. Binding of Cu by DOM in cave waters at cave sites in Mt Arthur National Park and around Waitomo varied slightly from region to region, and in Waipuna cave, DOM binding varied with the size fraction of DOM in cave water. DOM molecular weight (roughly corresponding to hydrodynamic radius) has been shown to vary seasonally in some cave systems (Hartland et al., 2011; 2012), and can vary based on the origin of the DOM and its transport in the epikarst (Blyth et al., 2016; Einsiedl et al., 2007).

Due to the wide range and low resolution of ages recorded in this study, any possible seasonal, or even decadal variations in trace metal binding (Hartland et al., 2012), would appear as increased variance in δ_{AT}/δ_{AC} in the sample population when compared to the homogenised, Hodge Creek internal standard. This variation was observed for DOM binding of Cu and to some extent for Co.

On the other hand, variations in DOM binding linked to changes in regional changes in climate, environment or geological setting, or variations in DOM binding linked to glacial-interglacial driven vegetation change would be represented in significant

differences in DOM binding between systems, which were present for Co and Cu, but not Ni. It is important to note that all these variables are interlinked to some extent. Climate change and surface vegetation changes over the last interglacial above cave systems are interlinked (Lorrey & Bostock, 2017) as are DOM transport and surface climate (Hartland et al., 2012; Pearson, 2020) and DOM structure and surface ecosystem (Heidke et al 2021). Given this, it makes sense that it is difficult to discriminate between temporal, spatial and random variation in DOM ligand binding after applying statistical corrections.

Regardless of the cause, the fact that δ_{AT}/δ_{AC} varies with DOM origin in any capacity means, that in the case of Cu and Co, the origin of the DOM in cave waters has the potential to affect trace metal incorporation into speleothem calcite in addition to the effect drip water flow rate (Hartland & Zitoun 2018). This complicates the use of Cu and Co in calcite as a paleoclimate proxy for rainfall. Future studies on trace metal contents in speleothems may now also need to consider the potential for temporal or spatial variations in trace metal binding to affect trace metal abundances in calcite. Given this, the fact that differences in DOM binding were only significant between a few different systems and on some specific timescales is encouraging, and future paleoclimate reconstructions may be able to account for these variations by applying margins of error around estimations of the free metal availability in in cave water solutions [M'] or the drip water residence times required to produce a certain transition metal abundance [t] in speleothem calcite (Hartland & Zitoun, 2018; Lindeman et al., 2022).

8.3 Next Steps

The presence of significant variation in the binding properties of DOM trace metal complexes in cave drip waters has major implications for the application of trace metals as paleoclimate proxies. The potential for change in DOM effects on trace element mobility over time and space presents an additional limitation when linking the trace metal contents of cave minerals to weather or climate conditions. Further research on the exact mechanisms causing changes in binding function in cave water DOM could be a way to circumvent these limitations, so they can be accounted for when comparing systems over large spatial scales or drawing conclusions about climate change over time.

This study was unable to link changes in speleothem DOM to either temporal or spatial variations in DOM properties, but rather detected variations that could potentially be attributed to either changes in the age or spatial origin of the extracts. A more focused study, with sampling stratified to specifically target variations in DOM properties across time *or* space, paired alongside qualitative analysis of DOM structure using spectrographic techniques (Blyth et al., 2016; van Beynen et al., 2001) or some form of analysis of DOM mass and hydrodynamic radius (Hartland et al., 2012; Hartland & Zitoun 2018; Zheng & Price, 2012) could identify if changes in DOM binding tendency could be attributed to the presence of colloids of specific sizes, or the presence of specific organic structures. A paired site study focusing on DOM from Hodge Creek extracts, Waipuna and Ann's cave extracts, and Niuean speleothem extracts from Anapala and Mataga caves would be able to identify whether spatial variations in DOM binding between these cave systems exist across all timescales, while a stratified study targeting speleothems from the late Eemian and Early Holocene at Hodge Creek and Waipuna caves could potentially identify if variations in DOM binding over time exist independent of cave system. Establishing the significance of either of these variations individually could give insights into the kinds of circumstances where it is appropriate to compare trace metals in calcite, allowing studies to bypass the increased margins of error on flow rate estimations arising from accounting for potential variations in of DOM binding.

The inability of this study to determine whether binding of Ni varies spatially and temporally reveals another meaningful gap in the methodology. Cu, Co, and Ni were tested following research by Hartland et al., (2011) which found that these transition metals were among the most consistently complexed by DOM in Pooles Cavern, UK, and research by Lindeman et al., (2022), which proved that DOM strongly controlled the incorporation of these elements into calcite in a laboratory setting. Other transition metals in speleothems, such as V (Hartland et al., 2011) are also controlled by complexation with DOM in cave water and as such may also be linked to paleoclimate rainfall (Hartland & Zitoun 2018). These metals were not investigated in this study, and their mobility may be affected differently by changes in DOM over time and space. Expanding the study by focusing on a larger array of trace metals could potentially identify elements for which the binding properties of DOM ligands in cave water do not vary significantly. Including Ni in such a study

could remove the uncertainty surrounding the variation of DOM binding of Ni observed amongst the extracts in this study. In order to test for this, higher ratios of [L]: [M'] would need to be used to account for potential competitive exchange at binding sites, potentially by using higher masses of speleothem powder without adjusting the final solution concentration, or potentially by only using one metal amendment at a time in each extract.

8.4 Conclusion

This study established the existence of temporal and spatial variance in the binding strength of DOM-transition metal complexes with Co and Cu in cave waters across time and space, by extracting DOM compounds from speleothem calcite and testing their binding strength using SV-DGT. These variations in DOM properties have the potential to corresponds with changes of the availability of metals for incorporation into speleothem calcite over time and space, impacting the application of proxies that rely on the consistency of this availability. The fact that significant differences in DOM binding of Co and Cu only occurred between three key sites however, with many other sites being largely comparable, suggests that with sufficient care these variations can be accounted for in reconstructions. The fact that significant differences in binding were localised also raises the potential for future studies to identify the potential climate, environmental or hydrological conditions responsible for the shifts in DOM binding, allowing for judicious application of transition metal proxies to avoid confounding factors altogether.

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