universität wien

MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

The impact of Cu(II) on DNA-goethite interactions

verfasst von / submitted by

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angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien, 2023 / Vienna 2023

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	UA 066 815
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Masterstudium Erdwissenschaften UG2002
Betreut von / Supervisor:	Univ.-Prof. Dipl.-Geol. Dr. Stephan Krämer

The impact of Cu(II) on DNA-goethite interactions

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Master's Programme, Earth Sciences, 120 ECTS credits

Date: August 24, 2023

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German title: Der Einfluss von Cu(II) auf die Wechselwirkungen zwischen DNA und Goethit

Acknowledgements

I would like to express my sincere gratitude to my advisors, Prof. Dr. Stephan Krämer and Dr. Richard Kimber, for their invaluable guidance and support throughout my master's thesis. Their expertise and encouragement helped me to complete this research and write this thesis. I would also like to thank my colleagues, Florian Exler Bsc and Veer Vikram Msc, for their time and willingness to share their experience. This work would not have been possible without their contribution.

Abstract

Since the technological advance of DNA sequencing, the field of DNA-related studies has boomed. Sequencing ancient DNA (aDNA), which can be hundreds of thousands of years old, is the key to understanding human heritage, helping to identify early population migrations, and ethnicity exchanges. DNA is expected to degrade under environmental conditions and so it is important to understand mechanisms that lead to its long-term preservation. Recent studies have revealed that DNA retrieval might not exclusively be associated with bones, but can also be extracted directly from sediments, in the absence of observable bone material (Slon et al., 2017). Adsorption to different mineral surfaces in these sediments may protect and preserve DNA (Cai et al., 2006a; Freeman et al., 2020). However, simply looking at the binding and preservation of DNA at mineral surfaces is not sufficient, as in nature, these compounds rarely exist in isolation in environmental systems. Metal ions are ubiquitous in nature and they are well-known to interact with mineral surfaces (Brown et al., 1999; Caporale and Violante, 2016; Gier and Johns, 2000). Their impact on DNA adsorption to mineral surfaces has been the focus of several studies (Cai et al., 2006a; Freeman et al., 2020; Sheng et al., 2019a), but key questions regarding the impact of metal ions on DNA preservation and recovery at mineral surfaces have not been answered yet. In this project, the goal was to investigate the impact of Cu(II) on DNA interactions in a DNA-goethite system. The interaction of the DNA with goethite in the presence and absence of Cu(II) was assessed using adsorption studies. The adsorption kinetics for DNA adsorption onto Cu(II)-free goethite are described by fast initial adsorption followed by a slowdown and reaching of equilibrium, which complies with pseudo-second-order kinetics. The adsorption data was described best with the Langmuir model, suggesting a monolayer adsorption mechanism. The efficiency of DNA desorption from the goethite surface using a phosphate buffer and EDTA was investigated. Degradation experiments demonstrated that goethite can protect DNA against enzymatic degradation even after using DNase I concentrations as high as 200 µg/ml. It was hypothesized that Cu-DNA interactions at the gothite surface would enhance DNA protection. In this study, it was shown that Cu(II) on the goethite surfaces lowers DNA adsorption. The desorption results correlated well with the adsorption results, with increasing Cu(II) concentrations leading to greater desorption of DNA from the goethite surface. However, the presence of Cu(II) on the goethite surface did not show any effect on the enzymatic degradation of DNA. Although the adsorption of Cu(II)on the goethite surface hasn´t been shown to improve DNA protection, it has been experimentally proven that Cu(II) does not harm the DNA and can protect DNA against enzymatic degradation while in solution. Overall, this study provides novel insights into the complex interactions between goethite, DNA, and a model metal ion (Cu(II)), with potential implications in anthropology and ecology.

Zusammenfassung

Seit dem technologischen Fortschritt bei der DNA-Sequenzierung ist das Gebiet der DNA-bezogenen Wissenschaftsdisziplinen regelrecht explodiert. Die Sequenzierung alter DNA (aDNA), die mehrere hunderttausend Jahre alt sein kann, ist der Schlüssel zum Verständnis des menschlichen Kulturerbes und hilft dabei, frühe Völkerwanderungen und den Austausch von Ethnien zu identifizieren. Es ist klar ersichtlich, dass sich DNA unter Umwelteinfluss abbaut, weshalb es wichtig ist, die Mechanismen zu verstehen, die zu ihrer langfristigen Erhaltung führen. Jüngste Studien haben gezeigt, dass DNA nicht nur aus Knochen, sondern auch direkt aus Sedimenten extrahiert werden kann, in denen kein Knochenmaterial zu sehen ist (Slon et al., 2017). Die Adsorption an verschiedenen mineralischen Oberflächen in solchen Sedimenten kann die DNA schützen und erhalten (Cai et al., 2006a; Freeman et al., 2020). Die bloße Untersuchung der Bindung und Konservierung von DNA an Mineraloberflächen reicht jedoch nicht aus, da solche Verbindungen in der Natur nur selten isoliert in Umweltsystemen vorkommen. Beispielweise Metallionen sind überall in der Natur vorhanden und es ist bekannt, dass sie mit mineralischen Oberflächen interagieren (Brown et al., 1999; Caporale und Violante, 2016; Gier und Johns, 2000). Ihre Auswirkungen auf die Adsorption von DNA an Mineraloberflächen standen im Mittelpunkt einiger Studien (Sheng et al., 2019), aber die wichtigsten Fragen zu den Auswirkungen von Metallionen auf die Konservierung und Extraktion von DNA an Mineraloberflächen wurden noch nicht beantwortet. Ziel dieses Projekts war es, die Auswirkungen von Cu(II) auf die Bewahrung von DNA in einem DNA-goethit-System zu untersuchen. Die Wechselwirkung der DNA mit Goethit in An bzw. Abwesenheit von Cu(II) wurde anhand von Adsorptionsstudien bewertet. Die Adsorptionskinetik für die DNA-Adsorption an Goethit wird durch eine schnelle anfängliche Adsorption beschrieben, gefolgt von einer Verlangsamung und dem Erreichen des Gleichgewichts, was mit der Kinetik pseudo-zweiter Ordnung übereinstimmt. Die Adsorptionsdaten konnten am besten gemäß dem Langmuir-Modell beschrieben werden, was auf einen Monoschicht-Adsorptionsmechanismus hindeutet. Die DNA-Desorption von der Goethit-Oberfläche wurde mit einem Phosphatpuffer und EDTA untersucht. Degradationsexperimente zeigten, dass Goethit die DNA vor enzymatischem Abbau schützen kann, selbst bei DNase I-Konzentrationen von bis zu 200 µg/ml. Es bestand die Hypothese, dass Cu-DNA-Wechselwirkungen an der Goethit-Oberfläche den DNA-Schutzmechanismus verstärken würden. In dieser Studie wurde gezeigt, dass Cu(II) auf der Goethit-Oberfläche die DNA-Adsorption verringert. Die Ergebnisse zum Desorptionsfähigkeit waren ähnlich, wobei steigende Cu(II)-Konzentrationen zu einer stärkeren Desorption der DNA von der Goethit-Oberfläche führten. Allerdings hat die Anwesenheit von Cu(II) auf der Goethit-Oberfläche keine Auswirkungen auf den enzymatischen Abbau der DNA gezeigt. Obwohl die Adsorption von Cu(II) auf der Goethit-Oberfläche den DNA-Schutz zwar nicht verbessert, wurde experimentell nachgewiesen, dass Cu(II) die DNA zumindest nicht schädigt und sie in Lösung sogar vor enzymatischem Abbau schützen kann. Insgesamt bietet diese Studie wertvolle Einblicke in die komplexen Wechselwirkungen zwischen Goethit, Cu(II) und DNA, und wirft ein neues Licht auf mögliche Anwendungen in Anthropologie und Ökologie.

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Nomenclature

Symbols and Abbreviations

Latin symbols

Symbol	Definition	Unit
A	Absorbance	OD
c	Concentration	ppm
d	Distance	cm
I	Intensity	$W \cdot m^{-2}$
K_L	Langmuir equilibrium constant	
K_f	Freundlich equilibrium constant	
k	Adsorption rate constant	min^{-1}
M	Molarity	mol/l
t	Time	min
Q	Amount of adsorbed DNA (surface normalized)	ng/cm²

Greek symbols

Symbol	Definition	Unit
λ	Wavelength	nm
ε	Molarity extinction Coefficient	$L\ mol^{-1}\ cm^{-1}$

Abbreviations

Abbreviation	Definition
AFM	Atomic Force Microscopy
DNA	Deoxyribonucleic acid
dsDNA	Double-stranded DNA
EDTA	Ethylenediaminetetraacetic acid
HEPES	N-(2-Hydroxyethyl) piperazine-N′-(2-ethanesulfonic acid)
ICP-MS	Inductively Coupled Plasma - Mass Spectrometry
Milli-Q	Water purified using the Millipore Milli-Q lab water system
PZC	Point of Zero Charge
ROS	Reactive Oxygen Species
SSA	Specific Surface Area
UV	Ultraviolet Light
XRD	X-ray diffraction

Chapter 1 Introduction

DNA, short for deoxyribonucleic acid, is the hereditary material in organisms that contains the biological building plan and instructions for multiplying. The chemical structure is the same for every organism, the difference is in the specific arrangement of the base pairs, which are the building blocks of DNA. These differences in the sequence of base pairs, make it possible to identify, populations or individuals (Calladine, 2004). Environmental DNA (eDNA) is plasmid, nuclear or mitochondrial DNA that is released into the environment by organisms. Typical sources can be shed skin, hair, and carcasses (Darling and Mahon, 2011; Takahara et al., 2012; Thomsen et al., 2012). Recently, eDNA has become an important tool in ecology for monitoring species in aquatic environments(Dejean et al., 2012; Ficetola et al., 2008; Jerde et al., 2011). However, despite the importance of eDNA, little is known about mechanisms that result in its preservation and persistence in environmental systems.

DNA interacts with the environment and might take post mortem damage, degrade into short DNA fragments (Pääbo, 1989), mutate (Duncan and Miller, 1980), or mix with other eDNA (DNA contamination)-these reactions and changes make DNA preservation challenging. The most successful way to retrieve ancient DNA from the environment is from bones or teeth. Among these, petrous bone proved to be the best host material for ancient DNA, since its dense structure offers ideal conditions for DNA preservation (Pruvost et al., 2007). However, finding fossils and bones is difficult, since their formation and discovery depend on a variety of ecological and geological events that occur over time. Following death, the majority of primate corpses undergo disassembly and consumption by predators, scavengers, and microorganisms. Alternatively, they undergo a process of chemical decomposition, resulting in the scattering of their molecules throughout the ecosystem. Only when conditions are favorable such as in environments with neutral pH, or an abundance of microorganisms, the organic components within the primate's bones and teeth begin the process of mineralization, which favors DNA protection (Chen and Nedoluzhko, 2023).

The long-term preservation of DNA is of particular interest in evolutionary anthropology. Only recently it became possible, thanks to technical development in DNA sequencing (Haile et al., 2007; Hofreiter et al., 2003), to recover and sequence ancient DNA (aDNA). Ancient DNA typically refers to DNA that is at least a hundred years old. The aDNA research started with the extraction and sequencing of DNA from dried muscle from a 100-year-old museum specimen of the extinct quagga quagga (Higuchi et al., 1984). This set the base for further research like that from Pääbo (Pääbo, 1989) who published the first sequence of ancient Human DNA, a 340 bp long DNA fragment isolated from an Egyptian mummy. He received in 2022 the nobel prize "for his discoveries concerning the genomes of extinct hominins and human evolution". Since then, the record for oldest DNA has constantly been broken, with the oldest ever DNA recovered now being 2 million years old (Kjær et al., 2022). Furthermore, recent advances in DNA extraction and sequencing have enabled the recovery of aDNA directly from sediments in the absence of visible bone material (Slon et al., 2017). This suggests that aDNA preservation may not be limited to bone preservation, and that sediment components (e.g., mineral or organic phases) could also potentially preserve DNA. The findings of ancient DNA from bacteria and plants (Gutaker et al., 2019; Priscu et al., 1999) also suggest that the preservation potential may not be exclusive to bones.

However, the mechanisms and microenvironments that can preserve DNA are not fully understood. Therefore, currently, there are no guiding points in sampling DNA for sequencing

purposes. Thus, sampling is both very inefficient and expensive, with the majority of samples not containing DNA. Recent efforts strive to narrow down sampling to specific minerals or soils where DNA preservation is expected to be enhanced. Hence, studies have been conducted on minerals to find out their influence on DNA preservation. Several studies have shown that DNA can indeed survive in the presence of minerals (Cai et al., 2006a; Freeman et al., 2020; Sheng et al., 2019a). Despite that, the exact mechanism by which the DNA is protected by the minerals is not yet fully understood.

Furthermore, minerals do not exist in isolation in environmental systems. For example, metal ions, often accompany minerals in nature. Their impact on DNA-mineral interactions has been the focus of recent studies (Cai et al., 2006a; Freeman et al., 2020; Sheng et al., 2019a). Metals have been found to damage DNA (Hou et al., 2014a) but also have shown improvement in the adsorption of DNA on mineral surfaces (Freeman et al., 2020; Sheng et al., 2019a). Metals can even change the structure of the DNA (Day et al., 2015). Due to these different effects, currently, there is a lack of scientific knowledge on the exact influence of metals on DNA-mineral interactions. Thus, more research needs to be done to enable more efficient aDNA recovery. Furthermore, studies on DNA-metal-mineral systems could benefit other scientific branches like ecology (Dejean et al., 2012) or medicine (Castro-Smirnov et al., 2020).

1.1 The structure and stability of deoxyribonucleic acid

To include the genetic instructions in every living cell, requires a complex molecule structure. The basis of the DNA molecule consists of three groups: a negatively charged phosphate group, sugar group-which together form a long phosphate sugar chain-, and bases that attach to this chain. These three molecules together form the building block of a DNA nucleotide. The nucleotides, connected by covalent phosphodiester bonds, are arranged into two long strands, the two strands are linked together with a certain directionality, ‘5-prime to 3-prime’, to form a spiral called a helix. The two strands run in opposite directions. The molecule has to build a structure where the soluble sugars and phosphates are placed outside and the hydrophobic bases have to be positioned in the center to avoid water in order to form a stable structure. At the same time, the molecule wants to avoid unnecessary gaps, which occur automatically due to the difference in length between the phosphate-sugar bonds and the thickness of the bases. This helix structure provides a way to separate the bases by 3.3 Å but leaves enough space for the phosphates to be 6 Å apart. The DNA can be further differentiated into three forms if one follows the stacking patterns of the phosphate sugar in 3D. All DNA helices have 10-12 phosphates per helical turn. Where the A form of the DNA has 11 phosphate the B form of the DNA has 10 and the Z form has 12 phosphates (Calladine, 2004). In nature the A and B forms are more abundant, which are the more stable forms and have a right-handed form. The term comes from when the right hand is held out the thumb represents the axis of the double-helix and the fingers curled around it represent the sugar-phosphate backbones. The stability of the double-helix is also dependent on a stable core which is built up from the connections of the different bases. James Watson and Francis Crick presented their double helix model by setting rules for the exact base pairings that were possible. There are four bases in DNA: adenine, thymine, guanine, and cytosine. Cytosine and thymine are both structurally pyrimidines, a hexagon shaped aromatic ring that has two nitrogen atoms built in. The difference is that the nucleobases also have additional

side groups (=O, –CH3, and –NH2) added to the ring. Adenine and guanine have a different structural backbone. Both adenine and guanine are essentially purines where a hexagonal ring and a pentagonal ring with two N atoms in both rings are fused. In the nucleobases, chemical side groups (=O and –NH2) have been added in different combinations. Watson and Crick said that the most stable pairing is A-T and G-C. Where A-T are connected by two H-bonds and G-C are bonded by three H-bonds The base pairings A-T, T-A, G-C, and C-G have the same size and fit precisely inside the double helix. Also the Watson and Crick model with these base-pairings explained the duplication of genes. During cell division, an enzyme splits the DNA into two strands and another enzyme can use these strands as templates for the synthesis of new strands according to the Watson-Crick rules.

1.2 The stability of DNA

1.2.1 UV radiation and DNA

Thanks to its unique structure of a double Helix, DNA is a relatively stable molecule. However, several processes can damage this structure. One source can be UV light. The impact of UVB-radiation from solar light has already been studied on environmental DNA. In a recent investigation (Andruszkiewicz et al., 2017), scientists positioned dialysis bags at distinct depths within a marine environment, resulting in varying degrees of sunlight exposure. The outcome of their study revealed no impact of UVB radiation on the degradation of environmental DNA (eDNA). In a similar endeavor conducted in a non-saline setting, Pilliod et al. (Pilliod et al., 2014) demonstrated that eDNA decay exhibited a swifter rate of DNA degradation when the containers were positioned in sunny locations compared to shaded areas. On the other hand UVA has not exhibited damage to DNA even in the presence of filter feeders (Mächler et al., 2018).

1.2.2 Thermal damage

The copying of genetic information naturally involves some errors. By heating DNA denaturation occurs in which the DNA strands start to open up because the hydrogen bonds between the bases melt. This process is used not only by nature to copy information, but it is an essential element of the PCR process. Heat also seems to have an effect on DNA survival, Kistler et al (Kistler et al., 2017) studied 185 datasets from different locations around the world and compared DNA survival with environmental parameters like temperature and sample age. They found a correlation between increased DNA fragmentation and geographic locations with high humidity and high thermal fluctuation. Other authors have also reported that temperature plays an important role in DNA survival. Smith et al. (Smith et al., 2001) were comparing the thermal age (number of years at constant 10 degrees) of Neanderthal bones from the pleistocene and holocene. They found that the bones that had a thermal age greater than 17000 usually were less likely to contain ancient DNA.

1.2.3 Enzymatic degradation

There is a natural enzyme that cuts DNA into smaller pieces. As such, DNase is part of a group of glycoprotein endonucleases, which are enzymes that catalyze the hydrolysis of DNA backbone phosphodiester connections. These groups of enzymes target the DNA and snip the DNA strand at the phosphodiester bonds creating mono and oligodeoxynucleotides with ‘5-phosphate and 3‘-OH groups (Vanecko and Laskowski, 1961). There are two types of DNases: DNase I and DNase II. The latter works best at acidic pH and is mainly found in the eye lens to clear it from DNA. The more common enzyme for DNA reparations or excess DNA management used throughout in nature is DNase I. This enzyme uses Ca^{2+}, Mg^{2+} and

Mn^{2+} as cofactors; without Ca^{2+} it cannot work. The exact cutting pattern is determined by the available cofactors. Single-strand 'nicks' occur in the presence of Mg^{2+} ions and double-strand breaks occur in the presence of Mn^{2+}. The optimal conditions for enzyme activity are at pH 7.8 at 35-37 °C and in the presence of the mentioned cofactors (Kunitz, 1950). DNase-induced degradation is most likely the biggest threat to DNA in the environment. DNase can be released by microorganisms, therefore locations with less microorganisms or factors that actively lower or inhibit enzyme activity increase DNA survival. At low temperatures enzymes are vulnerable to cold degradation and enzyme activity is greatly reduced (Privalov, 1990). Additionally, there is a restricted number of species that thrive in cold environments, decreasing microbial DNase release in such environments. This suggests a lower possibility of enzymatic DNA degradation in colder environments and thereby probably a greater chance of aDNA survival.

1.2.4 Reactive oxygen species

Reactive Oxygen Species (ROS) are oxygen molecules containing extra electrons which are unpaired and act as free radicals, thereby being highly reactive. Due to the unfavored state or unpaired electrons, these free radicals usually donate or take away another electron thereby often causing chain reactions in which a radical generates another radical. This process is stopped once two radicals react with each other. The most common radicals are superoxide anion (•O2−) and hydroxyl radical (OH−). They are usually created by ionizing radiation or during the folding of proteins. One mechanism through which free radicals induce DNA damage is through oxidation. This process leads to the formation of DNA lesions called oxidative DNA damage. As oxidation takes place in the bases, the structure alters and oxygen atoms are added onto the bases which cause damage. Out of the four bases, guanine gets oxidized the easiest compared to the other 3 bases, because guanine has a high oxidation potential.(Juan et al., 2021). Free radicals are common in nature. They can be generated by UV or metal ions (Balanikas et al., 2020; Valko et al., 2006) which makes them another primary mechanism of DNA degradation in the environment. Studies by Keyer et al (Keyer and Imlay, 1996) indicate that superoxide may speed up DNA damage by extracting iron from storage proteins. This liberated iron could then attach to the DNA's surface, where it might facilitate the production of DNA oxidants with the help of other electron donors.

1.2.5 Hydrolysis

Hydrolysis is a double decomposition reaction involving water. In organic compounds, this process involves the breaking up of organic molecule and the formation of smaller molecules. Esthers for example produce a carboxylic acid and an alcohol after the hydrolysis reaction (Cleaves, 2011). In the case of DNA, deamination of the bases is one of the main processes (Marrone and Ballantyne, 2010) Deamination can only affect cytosine, adenine, and guanine. During the deamination process, the exocyclic amino group breaks off and results in base transformation There are several ways that hydrolytic reactions occur in the environment. As already mentioned above, the release of DNases in the environment can catalyze the hydrolysis reaction and cause significant damage. Additionally, in highly acidic or basic environments the phosphodiester bonds in DNA can get hydrolyzed, causing strand breaks and DNA fragmentation.

1.3 Minerals and DNA

1.3.1 Clay minerals

There are a variety of clay minerals, but they all share the same key characteristics. Clay minerals are aluminosilicates, i.e., their crystalline structure is composed of silica tetrahedrons and aluminum oxide/hydroxide octahedrons. The stacking pattern of these two groups (silica tetrahedrons and aluminum octahedrons) results in a sheet-like structure with flat surfaces and relatively big interlayer distances. There have been several studies already on clay and DNA interactions, for example Cai et al. (Cai et al., 2006a) found that Clay minerals including Montmorillonite ($(Na, Ca)_{0.3}(Al, Mg)_2Si_4O_{10}(OH)_2 \cdot nH_2O$) and Kaolinite ($Al_2Si_2O_5(OH)_4$) can protect DNA against enzymatic degradation. Greaves et al. (Greaves and Wilson, 1969) studied the effect of pH on the adsorption behavior of DNA to montmorillonite. They found that below a pH of 5 the DNA adsorption was more likely to take place in the interlayer space and the adsorption increased with decreasing pH. Above pH 5 they suggested an adsorption on the clay mineral surface. They also reported that the usage of divalent cations (Ca^{2+},Mg^{2+}) almost doubled the DNA adsorption compared to a clean mineral surface .Hou et al. (Hou et al., 2014) studied the effect of heavy metals (Cd^{2+},Hg^{2+}) on DNA and found out that rectorite was able to reduce the damage done by the heavy metals to DNA adsorbed to the mineral surface compared to DNA in solution. Since clay minerals naturally have flat surfaces, they are optimal for AFM studies. For example, via AFM studies it was shown that the change of DNA conformation on mica in response to cationic content, ionic strength, and increased ionic potential (charge/density) promotes adsorption, which indicates the classical behavior of outer sphere bonding (Zhai et al., 2019).

1.3.2 Calcite

Cave environments have also been a focus of DNA-mineral studies. Speleothems are secondary mineral formations that usually accumulate in caves. It has been shown that they can preserve organic matter (Ramseyer et al., 1997) and DNA (Blyth et al., 2008). Speleothems receive a particular focus because some forms, like stalagmites, experience equilibrium conditions throughout their life span and cave environments provide a semi-closed system. These conditions are especially valuable for base studies. Speleothems are mainly built up from the calcium carbonate mineral calcite ($CaCO_3$). Only few studies have been conducted using calcite as the base mineral for DNA studies. Stahlschmidt et al. (Stahlschmidt et al., 2019) used metagenomic techniques to retrieve DNA from late quaternary stalagmites from the solkota cave in Georgia. They were able to successfully retrieve aDNA from mammals (bear, deer, bats) and plants (hazelnut, chestnut, flax). Notably most of the DNA samples contained the characteristic deamination damage that is typical for ancient DNA. Sand and his team (Freeman et al., 2020) investigated the adsorption of plasmid DNA onto calcite in the presence of (Na^+, Mg^{2+}, Ni^{2+}) and compared the results to the AFM studies done on mica. They found that the main adsorption factor is the surface charge, and the main adsorption area is the step edges of the calcite surface. In contrast to DNA adsorption to mica the ionic potential of the cations adsorbed on the calcite did not seem to influence DNA adsorption.

1.3.3 Hydroxyapatite

The best-recovered DNA samples are commonly from teeth and bone fragments. Hydroxyapatite (HAp) is the main mineral component of bones. Hydroxyapatite ($Ca_5(PO_4)_3OH$) is a calcium phosphate mineral that is scarcely spread in nature. However, its formation can be regulated by DNA in the process of biomineralization (Valle et al., 2014). It has a hexagonal crystal structure build-up from Ca^{2+} and PO_4^{-3} DNA can attach to the Ca^{2+} at the surface. There have already been studies that used synthesized hydroxyapatite as a model mineral for DNA adsorption. For example, Valle et al (Valle et al., 2014) evaluated the capacities of different HAp surfaces to adsorb biomolecules by incubating particles with plasmid DNA. The results showed that all HAp particles adsorbed DNA, but the concentration of adsorbed DNA varied based on the HAp properties, namely, HAp samples with higher zeta potential values adsorbing the most DNA. Computer simulations also showed that the (001) surface of HAp is favorable for binding DNA and maintaining its B-DNA double helix conformation, while the (010; Ca^{2+}–OH^-) surface causes structural deformations in DNA.

1.3.4 Iron oxides

Ferrihydrite and goethite are the most common iron oxide minerals. Ferrihydrite ($Fe_{10}^{3+}O_{14}(OH)_2$) is an amorphous iron hydroxide that is found in various environments from marine environments to space. It forms rapidly from iron-rich aqueous solutions, but due to its instability, it slowly transforms into more stable minerals like hematite or goethite. Goethite is an iron hydroxide (FeOOH) that is very common in soils and sediments. It has an orthorhombic crystal structure, where the iron inhabits the octahedral sites and forms paired chains. These iron ion chains are joined by the hydroxide ions. Ferrihydrite has not been investigated in DNA mineral studies, whereas goethite has already been found to interact with DNA. Cai et al. (Cai et al., 2006a) studied the effect of $MgCl_2$ concentration and pH on DNA adsorption to goethite. They found that the increase in ionic strength and the decrease in pH led to higher DNA adsorption. Schmidt and Martínez et al. (Schmidt and Martínez, 2017) studied the bonding mechanism between DNA and goethite. They reported an inner sphere coordination between the phosphate backbones of the DNA and the goethite surface. These studies, combined with the common occurrence of goethite and other iron oxides in sediments and their use as pigments in cave paintings (MacDonald et al., 2019), suggest that iron oxides have great potential for ancient human DNA preservation.

1.4 Interaction forces between minerals and DNA

1.4.1 Electrostatic interactions

Electrostatic interactions play a significant role in DNA adsorption to minerals. The process is strongly influenced by the diverse surface properties of different minerals. Some minerals exhibit structural charge, which arises primarily from isomorphic substitutions in layer silicates and usually has a negative charge, while others possess variable charge due to pH-dependent ionization of surface functional groups. For instance, clay minerals like kaolinite possess both structural and variable charge but are usually dominated by structural charge, (hence their negative charge even at relatively low pH), while oxides like goethite and hematite have variable charge (Kosmulski 2009, Freeman 2020). The point of zero charge (PZC) is the indicator for the charge of the surface. Going below the PZC results in net positive surface charge, overstepping the PZC by increasing the pH results in a net negative surface charge. DNA can attach to a surface when the mineral surface is positively charged since the sugar-phosphate backbones are negatively charged. These oppositely charged interactions result in electrostatic attractions. Recent studies showed (Freeman et al., 2020) that local mineral surface charge, an effect caused by mineral surface topography differences, also affects DNA adsorption positively. DNA fragments tend to adsorb more strongly to the edges and steps rather than flat terraces surfaces since these structural changes break the surface water layer and DNA can bind directly to the mineral surface.

1.4.2 Influencing factors on DNA adsorption

DNA attaches to the mineral surface via adsorption. Adsorption is a chemical process where ions and molecules (adsorbates) attach to the surface of an adsorbent (for example, minerals). It is a surface-based process that is influenced by many factors including surface area, pH, and ionic strength. Each mineral has different surface properties and, therefore different adsorption capacities. The specific surface area (SSA) of each mineral is a good indicator of surface properties. The adsorption capacity of a mineral is proportional to the specific surface area. The SSA is defined as the total surface area of solid material per unit of mass. Thus, the SSA depends on the size of the particles and the porosity of the sample. Although SSA is a crucial factor in controlling adsorption extent, ultimately it comes down to chemical interactions that control the adsorption process. The most important linear relationship is between surface charge and pH. Saeki et al. (Saeki et al., 2010) showed that DNA adsorption onto andosols decreased by raising the pH from pH 3.0 to pH 9.0. At neutral pH, clays and other silicates tend to have a negative surface whereas oxides, hydroxides, and carbonates have a positive surface charge (Kosmulski, 2009). The way an adsorbate attaches to the mineral surface can be categorized into two larger mechanisms. When the adsorbate attaches directly to the mineral surface it is called an inner sphere complex; when the adsorbate has a water surface interface and attaches through hydrogen bonds to the mineral surface, it is called an outer sphere complex.

1.4.3 Ionic strength

Ionic strength also plays an important role in adsorption processes, as it can affect the stability of the adsorbed species and the interactions between the adsorbent and the adsorbate. Ionic strength is a measure of the concentration of ions in solution, and it is influenced by factors such as the concentration of the electrolyte, the charge of the ions, and

the dielectric constant of the solvent. In general, higher ionic strength can decrease the extent of adsorption, due to the increased repulsion between charged species in solution. However, this effect can be counteracted by the formation of ion pairs or complexes in solution, which can enhance adsorption through electrostatic interactions. In the case of inner sphere complexes, high ionic strength can decrease the stability of the complex and reduce the extent of adsorption. This is because the charged species in the solution can compete with the adsorbate for binding sites on the surface, leading to weaker binding and lower adsorption. (Manning and Goldberg, 1996).In the case of outer sphere complexes, high ionic strength often enhances adsorption. This is because the increased concentration of ions in solution can shield the charges on the surface, reducing the repulsion between the adsorbate and the surface and promoting adsorption. This works only if the ionic strength is not too high, in which case the excess ions can compete with the adsorbate for binding sites, leading to decreased adsorption. Therefore, DNA adsorption is also influenced by ionic strength. Lorenz and Wackernagel (Lorenz and Wackernagel, 1987) showed that an increase in ionic strength leads to DNA retention from the adsorbate (sand filled glass columns in a flowthrough system). Opposing results were achieved by Romanowski et al (Romanowski et al., 1991; Yu et al., 2013), who found that an increase in $MgCl_2$ concentrations led to an increase in DNA adsorption to chemically pure sand in a flowthrough environment. They also suggested that divalent cations are much more effective in enhancing DNA adsorption than monovalent cations, due to the formation of cation bridges.

1.5 DNA-metal-mineral interactions

Metals are widely abundant in soils and often present on mineral surfaces. As already mentioned, metal ions have been reported to influence the ionic strength and thereby the extent of DNA adsorption. Sheng et al (Sheng et al., 2019a) investigated DNA adsorption to montmorillonite saturated with Na^+, Ca^{2+}, or Fe^{3+}. DNA adsorption was found to increase for saturated montmorillonite in the order of $Ca^{2+} < Na^+ < Fe^{3+}$. Their results and modeling work suggest a bridging function of the metals between the oxygen of the montmorillonite and negative moieties of the DNA phosphate group. They also identified that this bridging function depends on valency. Saturation with divalent cations compared to monovalent cations increased the DNA adsorption to the mineral surface. Cai et al. (Peng Cai et al., 2006a) studied the effect of DNA concentrations and the effect of varying pH on the adsorption, on different clay minerals. They found that clays can protect DNA against damage from Hg and Cd metals. These opposing results, (some metals increasing DNA adsorption, some metals damaging DNA) show that each metal has a unique interaction with the different minerals and each metal reacts differently with DNA. In this work, to better understand, DNA-metal-mineral interactions, Cu(II)-s effects on DNA preservation were studied. Cu(II) is one of the first metals that were mined and used by ancient human settlements. Thereby has enormous relevance for sites where ancient DNA may be expected to be present. Additionally, Cu(II) is a divalent cation which shows potential for DNA bridging, a mechanism which was reported by Sheng et al. Cu(II) is also well known for its antibacterial effects and could potential inhibit enzymatic degradation of DNA and help preserve aDNA. In contrast, Cu(II) nanoparticles have already been reported to induce reactive oxygen species and thereby damage DNA (Angelé-Martínez et al., 2017). These properties of Cu(II) suggest that Cu(II) could potentially damage DNA-as has been observed for Pb or Cd, - or enhance DNA preservation through enhanced adsorption facilitated by cation bridging and potential protection of DNA from enzymatic degradation, - as was observed for Mg^{2+} or Ca^{2+}.

1.6 Desorption

Although there are several DNA adsorption studies in the literature (Cai et al., 2006b; Freeman et al., 2020; Sheng et al., 2019a; Sodnikar et al., 2021) the desorption process is rarely discussed in detail or even mentioned at all. To quantify the damage caused during degradation experiments, it is necessary to be able to retrieve DNA from the mineral surface in a quantitative manner. Multiple chemical desorption methods can be used for DNA desorption studies.

1.6.1 Solvent extraction

Here, the goal is to dissolve the adsorbate and separate it from the adsorbent. This method, however, has several drawbacks for mineral DNA-related studies, as it only works for minerals like hydroxyapatite or calcite which dissolve easily. To dissolve silicates or iron oxides, concentrated acids are necessary which are harmful to the DNA (Direito et al., 2012)

1.6.2 Chelation

Chelation is a type of chemical bonding involving metal atoms and multidentate ligands. During chelation, the ligand forms two or more coordinative bonds with the metal atom in the center. Chelating agents are often used for metal remediation purposes (Gulcin and Alwasel, 2022). This method has the potential for DNA desorption when metal cations are preabsorbed to the mineral surface. The principle behind the method is that the DNA is bound to the metal cation, such that, when the metal cation is desorbed via chelation, the DNA is desorbed with it. However, this method does not consider competitive adsorption, it is possible that during chelation, the DNA re-adsorbs to the mineral surface. There is the possibility of ligand adsorption which would reduce the effectiveness of the ligand in chelating and desorbing the metal. EDTA has already been applied for DNA extraction during DNA metal-mineral studies by Yang and Gao et al. (Yang and Gao, 2019) They reported successful desorption with no harm to DNA. Their results showed that DNA strands form layers which are disintegrated by the removal of the metal ions, thereby helping desorption. An increase in desorption time and EDTA concentration resulted in a higher desorption yield.

1.6.3 Acid/Base treatment

This method is especially effective when electrostatic forces cause adsorption. When applying acid/base treatment the pH change will affect the surface charge and enable repulsion (Saeki et al., 2010). When using this method for mineral DNA studies, it is important to consider each mineral's surface charge.

1.6.4 Competitive adsorption

Competitive adsorption occurs when several compounds compete for the same free sites on adsorption surfaces. Two key concepts are

- Affinity: Describes the attraction towards the site of the surface. High affinity means a higher likelihood of adsorption. Affinity can be influenced by the polarity of the surface.
- Displacement: Displacement occurs once a compound with higher affinity replaces the adsorbate.

This method is frequently used in DNA studies, especially with phosphate buffer (Direito et al., 2012; Guerra et al., 2020; Tanaka et al., 2009). Phosphate is neutral towards DNA and since DNA also attaches to surfaces via the phosphate sugars, it is effectively replacing DNA on the surface. Moreover, phosphate buffer can be prepared at different pH levels so even the desorption effects of base treatment can be used as well.

1.7 Significance

Currently, the protective effects of minerals on DNA preservation are not well understood. Although there are several studies investigating DNA interactions with clay minerals (Cai et al., 2006a; Hou et al., 2014b; Scappini et al., 2004; Yu et al., 2013) and iron oxides (Freeman et al., 2020; Schmidt and Martínez, 2017; Sodnikar et al., 2021), the particular mechanisms which control DNA preservation at these surfaces are not known. There are only a few studies on clay minerals but no studies with iron oxides on DNA degradation with DNase I. A systematic approach is needed to elucidate the protective capability of different mineral phases to better understand DNA degradation in nature. Secondly, most adsorption studies do not report desorption data, and a lot of DNA mineral interactions are studied under AFM which do not require desorption. Therefore, it is crucial to develop desorption methods that can retrieve a significant amount of DNA from the mineral surface without inducing damage. Improving desorption methodology helps to better quantify the loss of DNA amount during degradation experiments thereby limiting errors. Method development in DNA desorption from minerals may also lead to more efficient recovery methods for aDNA extraction from sediments. Thirdly, there are many contrasting results regarding the impact of metals on DNA-mineral systems. With current knowledge it cannot be stated if metals further improve or reduce DNA protection. The answers can only be achieved when using a model mineral system and testing it with specific metals. This way the differences in protective effects can be better quantified, which also helps to amass data on DNA-metal-mineral interactions which currently is not available.

1.8 Objectives

The goal of this work is to better understand DNA-metal-mineral interactions. Several open questions remain unanswered after studying the relevant literature. The most important ones that build the objectives of this work are summarized in the following research questions:

1. Is goethite able to protect DNA from enzymatic degradation?
2. What are effective methods for DNA desorption?
3. What is the effect of Cu(II) on DNA adsorption and desorption?
4. Does Cu(II) damage the DNA or enhance protection against enzymatic degradation?

To answer these questions, a model mineral system with goethite was developed. With this model system, DNA adsorption, adsorption kinetics, desorption, and degradation of DNA at the goethite surface have been studied. Furthermore, a nondestructive desorption protocol based on competitive adsorption with phosphate was developed for enhanced DNA desorption yields from goethite. Finally, to better understand the effect of Cu(II) on DNA preservation; adsorption, desorption, and degradation at the goethite surface, studies were also performed in the presence of goethite with a varying mass of Cu(II) pre-adsorbed to the surface. Results were analyzed and compared between Cu(II)-exposed and Cu-free goethite.

Chapter 2 Methods

Table 1.8 List of Materials

Name	Formula	Company
Calcium carbonate	$CaCO_3$	Millipore
Calcium nitrate tetrahydrate	$Ca(NO_3)_2 * 4(H_2O)$	Merck
Copper(II) chloride dihydrate	$CuCl_2*2(H_2O)$	Millipore
Di-Ammonium hydrogen phosphate	$(NH_4)_2HPO_4$	Millipore
EDTA	$C_{10}H_{14}N_2Na_2O_8\ 2(H_2O)$	Sigma aldrich
HEPES	$C_8H_{18}N_2O_4S$	Roth
Iron(III) chloride hexahydrate	$Fe^{III}Cl_3*6\ (H_2O)$	Sigma aldrich
Iron(III) nitrate nonahydrate	$Fe(NO_3)_3* 9\ (H_2O)$	Millipore
Potassium hydroxide	KOH	Millipore
Sodium chloride	NaCl	Millipore
Sodium dihydrogen phosphate	$Na_2H_2PO_4^-$	Honeywell
Sodium hydroxide	NaOH	Roth
Sodium monohydrogen phosphate	$Na_2HPO_4^{2-}$	Millipore

2.1 Mineral synthesis

The minerals used were either synthesized in our lab or bought from suppliers. Synthesized or purchased minerals were homogenized into single batches of each mineral, for use in each experiment in this study.

2.1.1 Calcite synthesis

The Kitano method for calcite synthesis was followed (Kitano, 1962). 8g of powdered $CaCO_3$ were suspended in 5 L of Mili-Q water. To the suspension, CO_2 gas was added for 40h while continuously stirring on a magnetic stirring plate. After the CO_2 bubbling, the solution was supersaturated with $Ca(HCO_3)_2$. The solution was then filtered with a 40 μm Wattman filter to separate it from the remaining $CaCO_3$ crystals, which could not dissolve. After filtering the solution was stored in the fridge to avoid the formation of aragonite and vaterite which tend to form at higher temperatures. The calcite crystallization process was arduously slow: after leaving the solution in the fridge for months, only 2 mg of calcite had formed.

2.1.2 Goethite synthesis

Goethite was prepared from a 2-line ferrihydrite system (Schwertmann and Cornell, 1991) from two solutions:
A) 1 M $Fe(NO_3)_3$solution B) 5 M KOH solution.
To begin 100 mL of solution A was transferred into a 2 L polyethylene flask, and 180 mL of solution B was added rapidly while stirring. During the reaction red-brown 2-line ferrihydrite precipitates. The suspension was diluted to 2 L with Mili-Q water and held in a closed polyethylene flask at 70 °C for 60 h. During the 2.5-day heating process, the deep red-brown suspension of ferrihydrite is converted to a yellow-brown precipitate of goethite. After that, the goethite was removed from the oven, centrifugated, washed, and dried.

2.1.3 Ferrihydrite synthesis

Two-line ferrihydrite was synthesized by titrating a 104 mM aqueous solution of ferric chloride hexahydrate ($Fe^{III}Cl_36(H_2O)$) to a pH of 7–7.5 with 1 M sodium hydroxide (NaOH) (Schwertmann and Cornell, 1991). After that, the precipitates were centrifuged and the ferrihydrite particles were washed (5–7 times) with Milli-Q water and then freeze-dried.

2.1.4 Hydroxyapatite synthesis

A 0,2 M solution of $Ca(NO_3)_2$ and 0,2M solution of $(NH_4)_2HPO_4$ were prepared then filtered (0,45 μm Wattman filter) and adjusted to pH 11.0 with ammonia. $(NH_4)_2HPO_4$ solution was quickly added to $Ca(NO_3)_2$ solution while stirring. The mixture was left under continuous homogenization on a stirring plate for 24 h. After 24 h the precipitate was washed with Mili-Q water 3x times and ultrasonicated for 10 min. (Li et al., 2020)

2.1.5 Clay minerals

Montmorillonite and kaolinite were not synthesized due to the difficulty and the time span of the synthesis process. Store-bought, already available, minerals from Sigma-Aldrich were

used for the experiments. However, to create a homogeneous surface, the surface was saturated with sodium. For that, the minerals were suspended in 1 M NaCl, and the NaCl solution was exchanged 3x times. Finally, the suspension was filled in dialysis tubes and was set up for dialysis. During dialysis, the conductivity was measured, and the Milli-Q water frequently changed. Once the conductivity value stopped sinking, samples were freeze-dried.

2.2 Characterization

2.2.1 XRD

X-ray diffraction method with a RIGAKU mini flex benchtop X-ray diffractometer (University of Vienna, Austria) was used to determine the exact mineralogical composition of the synthesized minerals.

The principle of XRD is based on the scattering of X-rays from the crystal atoms in a material, resulting in a characteristic diffraction pattern. The scattering of X-rays from crystal atoms is primarily due to the interaction between X-rays and the electrons of the atoms, known as elastic scattering. This type of scattering from a regular array results in a regular array of spherical waves, which are superimposed on each other, resulting in constructive interference in certain directions. This interference is governed by Bragg's law (eq. (1)), which is described by the spacing between diffracting planes d, the incident angle θ, the beam wavelength λ, and the integer n

(1) $$2d \sin\theta = n\lambda$$

The directions of constructive interference correspond to distinct spots on the diffraction pattern, which are called reflections. Thus, X-ray diffraction patterns are created from the interaction of electromagnetic waves with a regular array of scatterers in a material. The wavelength of X-rays is of the same order of magnitude as the spacing between crystal planes, ranging from 1-100 angstroms, making X-rays an ideal tool for probing the crystal structure of materials. (Bunaciu et al., 2015)

This instrumental analysis helped to confirm the purity of the synthesized minerals. All samples were measured on glass holders, where the sample was weighed and then pressed uniformly, resulting in an even distribution. The 2 θ angles were set between 5° and 80° and the scan speed was set to 0.7 deg/min in continuous scan mode. The measurement was set for a long scan lasting for 6 hours. The software, smart-lab Studio II, which also controlled the measurement process, was used to interpret the XRD patterns. The software can perform phase identification by running an algorithm that checks the peaks of the sample and compares it with multiple databases such as ICDD, PDF-2, PDF-4+, COD.

2.2.2 ICP-MS

Inductively Coupled Plasma Mass Spectrometry (7000D Quadrupole MS/MS-EI-Paket, Agilent, University of Vienna) was used to determine the concentration of the three $CuCl_2$ stock solutions (1 ppm, 25 ppm, and 50 ppm) and determine the amount of copper left in the solution after adsorption to the goethite surface. The samples were diluted 1: 1000 with 2x boiled HNO_3 the matrix liquid that the ICP uses. The Cu-goethite suspension samples were centrifuged at 20000 rpm, and the supernatant was diluted with the 2x boiled HNO_3. Internal standards were used for calibration with concentrations of 1, 10, 100 ppb.

2.3 DNA Quantification

2.3.1 DNA adsorption

A goethite stock solution (4 mg/ml) was prepared at pH 7.5 and allowed to hydrate for at least 24 hours. DNA stocks were prepared by dissolving double-stranded DNA from salmon sperm (lyophilized powder, ≤5% protein, Sigma-Aldrich) in a background solution of 3 mM Hepes and 30 mM NaCl. This was left for 3.5 h of equilibration on 30 rpm on an overhead shaker. From this highly concentrated (>300 ng/µl) DNA stock, dilutions were prepared with DNA concentrations of 40, 80, 100, 120, 140, 200 and, 300 ng/µl. From the goethite stock solution 500 µl aliquot was pipetted to 2ml Eppendorf tube. 500 µl of DNA stock was added to give a final volume of 1 ml containing 2mg/ml goethite and DNA concentrations of 20, 40, 50, 60, 70, 100 and, 150 ng/µl. After adding the DNA to the Mineral, the mixture was left to equilibrate on an overhead shaker for 6 h at 30 rpm. During the shaking, the tubes were covered with aluminum foil to prevent interaction with sunlight. After 6 h, the samples were centrifuged at 20000 rpm and the supernatant was measured with Nanoquant plate reader and Qubit. Nanoquant Infinite M200 Pro plate reader measures concentrations through absorbance. It has wavelength settings between 260 nm (5 nm bandwidth), 280 nm (5 nm bandwidth), and 310 nm (5 nm bandwidth) for reference. After the measurements of DNA with the NanoQuant plate, the software automatically calculates in excel the DNA concentrations according to the Lambert-Beer law(eq. (2)).

(2) $$A = \varepsilon * \mathrm{dc}$$

Where A= Absorbance, ε=Molarity Extinction Coefficient (L mol^{-1} cm^{-1}), d=Distance (path length in cm), c=Concentration (mol L^{-1})

DNA purity is monitored in the background in the following way:

(3) $$A\lambda = \log 10\left(\frac{I_0}{I}\right)\ [OD]$$

where "I" denotes the light intensity at a particular wavelength that traversed through the specimen, while "I_0" represents the intensity of the incident light prior to its interaction with the sample. The absorbance of the sample is directly related to both the sample's thickness and the concentration of the absorbing entities present within it. The concept of absorbance is logarithmic in nature, with its unit expressed as OD (Optical Density).

The UV spectroscopy method measures the maximal absorbance of nucleic acids; so it does not distinguish between dsDNA, single-stranded DNA (ssDNA), and RNA. In contrast, fluorescence spectroscopy quantifies the quantity of undamaged dsDNA, and the resulting measurement output diminishes in correspondence with the degree of DNA fragmentation and denaturation. (Georgiou and Papapostolou, 2006) Qubit 4 from Invitrogen is a fluorometer. It uses dyes that fluoresce upon interactions with the DNA. The fluorometer picks up this fluorescence signal, which is directly related to the concentration of DNA in solution.

The amount of DNA adsorbed on the goethite surface was calculated from the measured results in the following way(eq. (4)):

(4) $$Q = \left\{\frac{cDNA\ initial - cDNA\ final}{cGoethite * SSA\ Goethite}\right\} * 10^2$$

where Q [ng/cm²] is the DNA adsorbed on the goethite surface, cDNA initial [ng/µl] is the concentration of DNA before adding it to the goethite suspension, cDNA final [ng/µl] is the concentration of DNA after 6 h of adsorption, cGoethite [mg/ml] is the amount of goethite suspension used for the reaction, and SSA Goethite [m²/g] is the specific surface area of goethite.

2.3.2 Adsorption kinetics

To better understand the rate of the DNA adsorption to the goethite system, kinetics experiments were performed. The setup was as described in section 2.3.1. Here 100 ng/µl DNA was added to 4 mg/ml of goethite, the reaction volume was modified to 5 ml instead of the 1ml. During the experiment, 300 µl aliquots were taken from the sample at the time points 1 min, 3 min, 10 min, 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, and 8 h. The experiment was carried out in a windowless room and samples were covered with aluminum foil. The timepoint samples were centrifuged at 20000 rpm and measured on Qubit and plate reader. Based on these data, all future adsorption experiments were run for 6h time.

2.4 Cu(II) Adsorption

The concentrations used in the paper from Peacock (Peacock and Sherman, 2004) 1 ppm, 25 ppm, and 50 ppm of $CuCl_2$ were in this work also implemented. Cu(II) adsorption was performed by mixing 6 ml of $CuCl_2$ in concentrations of 1 ppm, 25 ppm and 50 ppm with 6 ml of 8 mg/ml goethite in 25 ml low-bind Eppendorfer tubes, and the pH adjusted to 6.5. Following that, the suspension was left in a box for 24 h on an overhead shaker for shaking at 30 rpm. After 24 h the pH was checked and readjusted to 6.5 if necessary. DNA concentrations (40, 80, 120, 140, 200, 300 ng/µl) were added as in the previous adsorption experiments with Cu-free goethite. The samples were left for 6 h on the shaker at 30 rpm, covered with aluminum foil and then the supernatant was measured by Qubit and Tapestation. The use of Plate reader was neglected since Cu influences the measurement.

2.5 Desorption experiments

After the successful adsorption experiments, a DNA desorption method needed to be developed. This was mainly necessary for the upcoming degradation experiments where it was important to be able to identify the damage that was done by the enzyme. Unfortunately, there is no uniform desorption protocol that is widely used for desorption purposes. The papers that raised the main questions this work wanted to focus on did not provide a desorption yield or method (Hou et al., 2014) especially regarding the damage/protection as described in metals-focused papers (Sheng et al., 2019a; Wu et al., 2014). There were two possible options to go with:

i) take a commercial extraction kit where little information is available on its production methods and the chemical composition of the extraction kit and
ii) self-develop a desorption method that produces the best yield for the goethite system.

Option two (ii) was chosen and two methods were tested:
A) Multiwash method: Here a 250 mM phosphate buffer was prepared containing 0.0786 mol of sodium dihydrogen phosphate ($Na_2H_2PO_4^-$) and 0.1713 mol of sodium monohydrogen phosphate ($Na_2HPO_4^{2-}$) at pH 7, pH 8 and pH 9. This was added to the mineral pellet and was ultra-sonicated for 1 min and placed up for shaking at 60 rpm for 15 min. After that the samples were centrifuged and the supernatant was decanted into a separate Eppendorfer tube and the DNA concentration was measured on Qubit and Plate reader. This procedure was repeated 4x times and then a final wash was performed using 250 mM EDTA and shaken for 12 h while samples were covered with aluminum foil.

B) Single wash method: A 20 mM phosphate buffer and 0.1 mM EDTA mixture at pH 9 was added to the samples and set up for shaking for 24 h at 30 rpm, while samples were covered with aluminum foil. After that, the DNA concentration in the supernatant was measured with Qubit and Tapestation.
The desorption percentage was calculated with the following formula (eq. (5)) where DNA adsorbed [µg] is the known amount of DNA on the goethite surface and DNA desorbed [µg] is the amount that could be retrieved with the desorption method.

(5) $$Des\ \% = \frac{DNA\ adsorbed}{DNA\ desorbed} * 100$$

2.5.1 Desorption experiments with pre-adsorbed Cu(II)

For the desorption experiments with Cu(II), only the single wash method was used since it had not show DNA degradation during the desorption process (see Appendix 6.9; Figure 6.9.1). After adsorbing 50 ng/µl DNA for 6 h; the same desorbing solution (as used for the Cu free goethite) of 20 mM phosphate buffer and 0.1 mM EDTA mixture was used and shaking time was set up for 24 h. An additional time point after 12 h was added, where the DNA concentration of the supernatant was measured.

2.6 Degradation experiments

Experiments with the enzyme DNase I were performed to test the protective capabilities of goethite with DNA adsorbed onto its surface. The main measurements were done on an Agilent Tapestation 4150 automated gel electrophoresis instrument. This instrument was used to determine the length of DNA and was used to monitor degradation processes. Most of the experiments were conducted with the genomic screen tape where the assay can measure DNA between 200 and 60000 base pairs. For some experiments, the d1000 screen tape was used, where the assay can measure between 35 bp and 1000 bp. Sigma Aldrich Deoxyribonuclease I from bovine pancreas (lyophilized powder, Protein ≥85 %, ≥400 Kunitz units/mg protein) was used. Dnase I (2 mg) was weighed into a 2ml Eppendorf tube. To that, 1ml of 6x concentrated 0.5 mM $CaCl_2$, 2.5 mM $MgCl_2$, 10 mM Tris solution was added. The tubes and reagents were always on ice during the preparation process to prevent early activation of the enzyme. The suspension was set up for shaking for 10 min at 30 rpm to equilibrate. From this, a serial dilution to DNase concentrations of 1200, 600, 60, 6 µg/ml was made. From the DNase dilutions 200 µl was added to 1ml of goethite sample that had been preequilibrated with 100 ng/µl DNA to allow DNA to absorb to the mineral prior to addition of the enzyme. The tubes were set up for shaking at 30 rpm for 1 h at 37 °C in a temperature-controlled table shaker. The table shaker was set to 0 rpm only the heating was configured, and an overhead shaker was put inside it. After 1 h hour of shaking, immediately 1,25 ml 0.5 M EDTA was added, and the samples were quickly transported into a 75 °C-preheated water

bath to quench the reaction. The samples were centrifuged for 10 min at 20000 rpm. Then DNA concentration of the supernatant was measured with Qubit and discarded. Before the desorption process, an additional step of washing with 3mM Hepes Buffer with 30mM NaCl was performed. The final step was the addition of 1ml of the desorbing solution, a 20 mM Phosphate, and 0.1 mM EDTA mixture, and set up for shaking at 30 rpm for 24 h. After 24 h the samples were centrifuged at 20000 rpm and the DNA concentration was measured with Qubit and Tapestation. The amount of DNA lost was calculated from the Qubit data. The calculations were made with the following formula (eq. (6)):

(6) $$Loss\ \% = \frac{DNA\ adsorbed - DNA\ desorbed}{DNA\ adsorbed} * 100$$

2.6.1 Degradation experiments with preadsorbed Cu(II) on goethite

The workflow was very similar to that used for the experiments with Cu-free goethite. Cu(II) adsorption followed was done as described in section 2.4. Then 500 µl of 100 ng/µl DNA was added to 500 µl of the Cu(II)exposed goethite suspension. This followed the degradation step where 200 µl of 60 µg/ml DNase I (resulting in a final concentration of 10 µg/ml DNase I) was added to the DNA-Cu(II)-goethite suspension and shaken for 1h at 30 rpm at 37 °C. After 1h hour of shaking, immediately 1.25 ml 0.5 M EDTA was added, and the samples quickly transported into a 75 °C-preheated water bath to quench the reaction. The samples were centrifuged for 10 min at 20000 rpm. Then the supernatant was measured with Qubit and discarded. Before the desorption process, an additional step of washing with 3 mM Hepes Buffer with 30 mM NaCl was performed. The final step was the addition of 1 ml of the desorbing solution, a 20 mM Phosphate, and 0.1 mM EDTA mixture, and setting up for shaking at 30 rpm for 24 h. After 24 h the samples were centrifuged at 20000 rpm and the DNA concentration was analyzed with Qubit and Tapestation.

Chapter 3 Results

3.1 Kinetics

The reaction kinetics of DNA adsorption to mineral surfaces are not fully understood. Here, a batch experiment was performed to provide initial data on DNA adsorption kinetics to goethite. The kinetic curve of this experiment is depicted in Figure 3.3.1 A. Initially, a fast adsorption process was observed up until 1 h after which it slows down towards reaching equilibrium after 3 h. One possible mechanism for DNA adsorption to goethite is through electrostatic interactions. The experiment was performed at pH 7.5 where the goethite surface is expected to have a net positive charge (goethite PZC = 7.85) so the negatively charged phosphate sugar bones can electrostatically attach to the mineral surface. As DNA was still measured in solution, following the establishment of equilibrium conditions, this suggests that equilibrium did not result from depletion of DNA in solution but rather due to saturation of all available goethite surface sites. The best kinetic rate order was determined to be pseudo-second-order kinetics (see Appendix 6.2; Figure 6.2.1). The first 1 h has a kinetic rate with a k value of 6.51 x 10^{-2}(µg mg^{-1} min^{-1}). After 1 h the rate slows down to 7.70 x 10^{-3} (µg mg^{-1} min^{-1}). The goodness of fit (R^2) has a value of 0.995 for the pseudo-second-order kinetics.
Mathematically the pseudo-second-order kinetics model is defined in Table 3.1.1 accordingly:

Table 3.1.1 Kinetic Model

Kinetic Model	Formula	Constants	Goodness of Fit
Pseudo-second-order	$qt = \frac{K_2 q_e^2 t}{1 + K_2 q_e t}$	K_2= 7.70 x 10^{-3} (µg mg^{-1} min^{-1}) q_e= 20.064 µg/mg	R^2 = 0.995

Where qt and qe (µg/mg) are the adsorptive removal capacity at equilibrium and variable time(t). and K_2 is the respective rate constant.

3.2 Adsorption

The most standard way to visualize data from adsorption-related experiments is through adsorption isotherms. For the adsorption isotherm, the temperature, mineral mass, and pH are fixed so the only variable left is the concentration of the adsorbate under study. To reduce the complexity, and the time span of this work and to focus more on the desorption and degradation experiments, a model mineral was chosen. Goethite had the highest adsorption capacity (weight normalized) of the six minerals that were tested, and it showed the least pH drift during the buffer-pH test (see Appendix 6.3; Appendix 6.4). Considering these results goethite became the obvious choice as a model mineral. By using increasing adsorbate concentrations, the aim was to determine the maximum DNA adsorption capacity of goethite. This point is reached when, regardless of increasing the concentrations of the added DNA, the isotherm reaches a plateau. This plateau represents the point where the DNA adsorbed to the goethite surface is in equilibrium with the DNA in solution, and hence, the surface is saturated. Based on the previous kinetic data, 6 h was chosen as the reaction time to ensure equilibrium would be reached. As Figure 3.3.1 B shows, the adsorption equilibrium is reached at around 70 ng/cm². Small solution concentrations up until 30 ng/µl were

completely adsorbed, and there is no DNA measured in the supernatant after the 6 h equilibration time. Continued adsorption was seen at the goethite surface for DNA solution concentrations between 10 and 52 ng/µl but after this, equilibrium is soon reached between DNA at the surface and DNA in solution. Previous experiments (see Appendix 6.3) revealed that buffer concentration can influence the extent of adsorption. As such a low buffer concentration 3 mM, was chosen to limit the effect of the buffer on the adsorption data. This concentration was low enough to limit the influence of Buffer on DNA adsorption but high enough to inhibit pH shifts (see Appendix 6.4). Several papers have studied the photochemical effects of goethite (Jung et al., 2021; Rodríguez et al., 2011). These papers show that at low pH, solar radiation can induce the formation of Hydroxyl radicals which degrade organics. To prevent the production of ROS or DNA contact with UV that could induce potential damage to the DNA directly (Wang, 2022), the samples were always covered in aluminum foil or put in a card box to protect them from light. To understand more about the adsorption behavior of DNA, Freundlich and Langmuir isotherm models were fit. The two models were fit based on the mathematical linearization of Langmuir (Eq. (7)), (see Appendix 6.5; Figure 6.5.1) and Freundlich (Eq. (8)), (see Appendix 6.6; Figure 6.6.1) referring to the isotherm equations:

(7) $$N_{ads} = N_m * \left(\frac{K_L * C_e}{1 + K_L * C_e}\right)$$

(8) $$N_{ads} = K_f \ * \ {C_e}^{1/n}$$

The terms KL, Kf, Nm (maximum adsorption capacity), and n, are constants at fixed temperature. The term Ce is the equilibrium concentration of DNA in solution [µg/ml]. The best fit after linearization resulted in the Langmuir model having an R^2 value of 0.997. Since the R^2 values of the two linearized models cannot be compared directly, a nonlinear fit was also applied which gave the best fit for the Langmuir model (see Appendix 6.7; Figure 6.7.1).

Table 3.2.1 Adsorption models

Adsorption model	**Fitted adsorption parameters**	**Correlation Coefficient**
Langmuir	N_m = 26.860 µg/mg K_L= 0.757	Linear: R^2 = 0.997 Nonlinear: R^2 = 0.778
Freundlich	K_f =30.056 n=4.153	Linear: R^2 = 0.592 Nonlinear: R^2 = 0.517

3.3 Desorption

To quantify the extent of damage to DNA adsorbed to minerals, it was necessary to develop an efficient DNA extraction protocol. Few methods have focused on DNA desorption from minerals described in the literature (Direito et al., 2012; Yang and Gao, 2019). One method is to use a chelating agent (Yang and Gao, 2019). A chelating agent is referred to as an organic or inorganic compound that has two or more free electron pairs and can form a coordinative binding with a central (metal)-ion. This way a chelator is capable of binding and fixing divalent or multivalent cations in stable ring-shaped complexes. This method is particularly

effective in breaking cation bridges that bind DNA to the mineral surface, and was considered here since future experiments are planned with Cu(II). Another desorption mechanism can be achieved by modifying the surface charge. Since the DNA is probably bound to the mineral surface by electrostatic attraction, changing the surface charge to the opposite (more negative surface) can lead to repulsion and desorption. A change in the surface charge can for example be achieved by changing the pH. In the case of goethite this would mean increasing the pH above the PZC (pH 7.85). Another potential desorption method is competitive adsorption. This method works by applying an adsorbate that is more reactive at the same pH conditions and replacing the adsorbed DNA. Competitive adsorption with phosphate buffer was already used by Direito et al. (Direito et al., 2012). Since they have done desorption experiments with different minerals, and achieved more than 50% desorption with goethite, this paper was a good start for developing a desorption protocol. The first experiments used several washing steps. Each step consisted of adding 250 mM phosphate buffer and letting it equilibrate under continuous stirring for 15 minutes. This was repeated four times. For a final washing step, 250 mM EDTA, a chelating agent, was used for 12h. The solutions after each washing step were analyzed by Qubit to measure the concentration of DNA. The DNA concentrations from each washing step were combined to determine the total extent of DNA desorbed during the entire procedure. The solutions were also analyzed with the Tapestation to monitor any potential degradation of the DNA that may have occurred either during the initial adsorption process or the subsequent desorption.

As Figure 3.3.1 C shows, the multi-wash method was performed at two different pHs, pH 6.2 and pH 8.2. As a starting point, pH 6.2 was chosen, based on future adsorption at pH 6.5 for Cu experiments and because the phosphate buffer had a pka of 7.2 meaning the two pH values were exactly 1 pH unit away from the pka of phosphate. At pH 8.2, there was a 7-10 % increase in DNA desorption compared to the results at pH 6.2. The results also show that the first 2 washing steps mobilize most of the DNA and that the remaining wash steps only contribute to a few more percent of DNA that can be retrieved from the surface. The last washing step with the EDTA which had more time to react with the surface, could not desorb any more DNA from the surface. However, since the total amount of DNA that could be desorbed was only 70 %, it suggested that there should be still DNA left on the goethite surface. After analyzing the samples of the different washing steps at the Tapestation, the results showed degradation (see Appendix 6.8; Figure 6.8.1). The Tapestaion makes detecting degradation easy by comparing the results to the DNA that was measured before the experiment, and a DNA control. In normal cases, double-stranded DNA is stable for 24h at room temperature (see Appendix 6.11; Figure 6.11.1). Both wash 1 and wash 2 samples show much lower basepair sizes between 100 - 3000 bp and lower intensity compared to the 100 ng/µl DNA stock where the average bp length is between 4000-48500 bp (see Appendix 6.8-6.9). This was not the only issue with this multi-wash method. By applying such high concentrations of the phosphate buffer the results from the Qbit became influenced so a correction was applied to the calculations. (see Appendix 6.12) This was not ideal, but it was not the reason why the Tapestation showed degradation. After some tests and literature research (Yang and Gao, 2019), it became obvious that instead of the concentration of the buffer, reaction time is the most important factor. Additionally, some small tests have shown that using the highest rpm settings to homogenize the suspension after adding the desorption agent potentially contributed to the degradation of DNA.

To try and limit the extent of DNA degradation, a new approach based on a single-wash method was used. Here, the first test determined the pH and concentration to use for the

experiment. After adsorbing the DNA to goethite, the supernatant was removed and washed once with Hepes buffer. Then 1 ml of a) 1 mM phosphate and 0.1 mM EDTA mixture or b) 10 mM phosphate and 0.1 mM EDTA mixture, each at pH 7, 8, 9 was added and left on the shaker for 24 h. Finally, the DNA concentration in the supernatant was analyzed. The results in Figure 3.3.1.D show that the pH effect in the single-wash method was similar to that was observed with the multi-wash method. Increasing the pH again resulted in a higher % of DNA desorption. Likewise, the results also show that by increasing the concentration of phosphate, the removal of DNA from the surface becomes more efficient. An increased concentration leads to more competition for the surface sites; this was the primary idea in the first place for using such high phosphate concentrations in the multi-wash method. However, here (single wash method) the highest phosphate concentration was 20 mM since this was also the threshold limit that did not interfere with Tapestation analysis. In this method (single wash method), there was no purification with spin columns necessary before doing measurements. This not only saved time but was also more cost-effective, 20 mM phosphate did not affect the Qubit either so no more corrections in the calculations were necessary. The results also show that at pH 9 the 1 mM phosphate washed samples also increased in efficiency but still the desorption yield was too poor to in order to be able to use such low buffer concentrations effectively. Based on these data, a desorption buffer of 20 mM phosphate and 0.1 mM EDTA at pH 9 was used for 24 hours for all future desorption purposes.

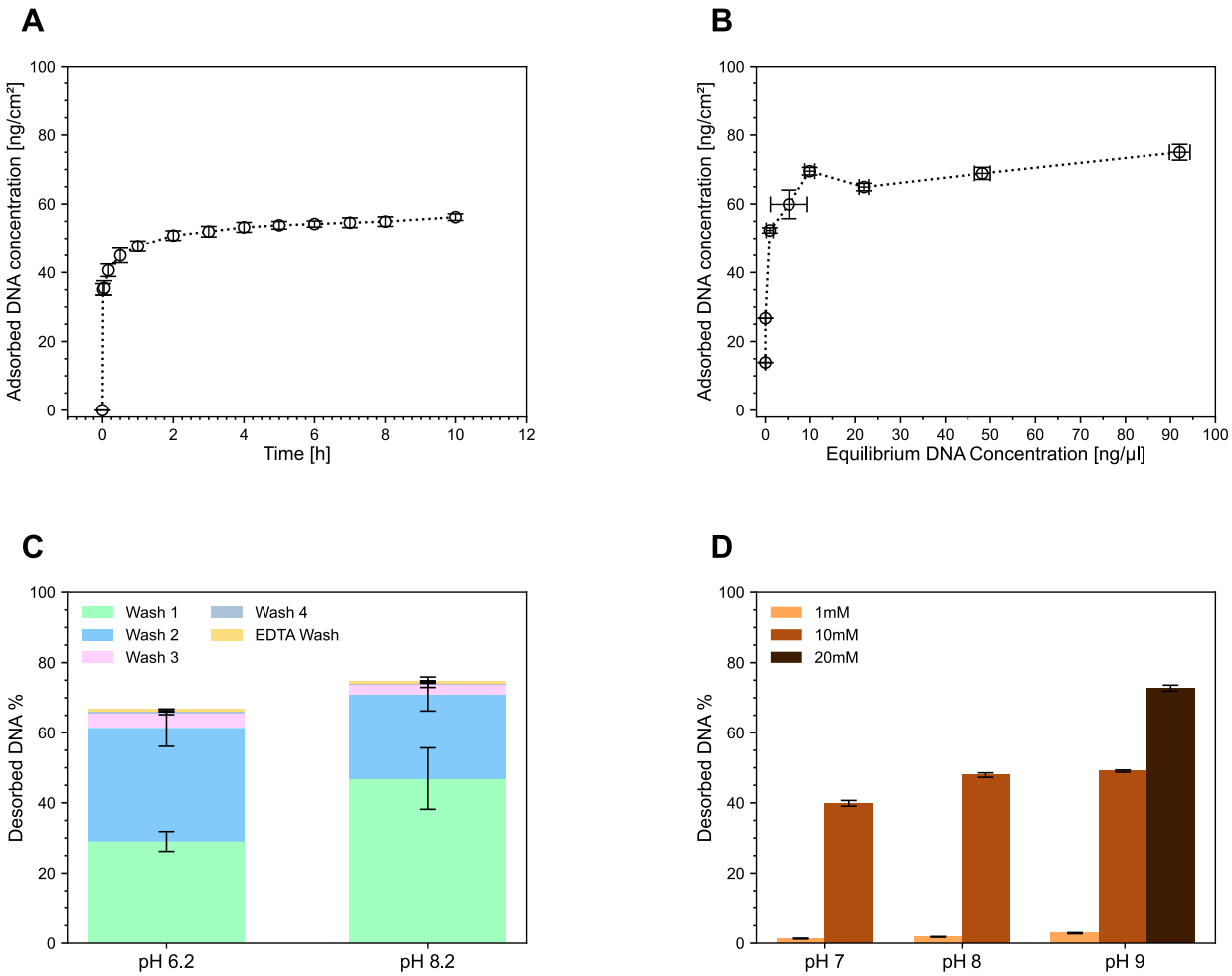

Figure 3.3.1 ***A)*** *Kinetics of DNA adsorption onto goethite* ***B)*** *DNA adsorption isotherm onto goethite* ***C)*** *DNA desorption from goethite with a multi-wash method:* ***D)*** *DNA desorption from goethite with a single wash method*

3.4 Degradation

To assess the stability of DNA on the goethite surface, a method was needed to destroy the DNA. Although UV (Scappini et al., 2004) and X-rays (Ciaravella et al., 2004) naturally damage the DNA, however, exposure to sunlight is not always the biggest threat to DNA preservation. In cave sediments or soils DNase activity is one of the more likely ways of degradation to which the DNA would be exposed. After deciding on using DNase I as a degradation agent, the first experiments were performed to find an optimal concentration. This meant a sufficient DNase concentration where degradation can be observed, but not so high that everything is degraded too rapidly that the relative degradation cannot be monitored in each system. An experimental trial was initiated with DNA at a concentration of 100 ng/µl. The DNA was allowed to pre-equilibrate with goethite initially present at a concentration of 4 mg/ml. Following this, 200 µl of DNase I stock was introduced to the mixture, resulting in DNase I concentrations of 1, 10, 100, or 200 µg/ml. After DNase addition, the solutions were shaken at a constant temperature (37°C) at 20 rpm for 1 hour. After 1 h, the reaction was quenched via addition of EDTA to chelate the Mg^{2+} and Ca^{2+} required by the DNase, thus inhibiting further enzymatic activity. The DNA concentration of the samples were then measured on Qubit and Tapestations. Figure 3.4.1-Figure 3.4.2 shows these results. When interpreting results from the Tapestation it is important to know that the machine always generates a reference peak for the genomic tape at 100 bp and for the D1000 tape at 25 bp. These peaks are not from the samples. In Figure 3.4.1, one can see that the 1 µg/ml DNase shows no effect on DNA in the presence of goethite. At 10 µg/ml of DNase, there is a drastic shift in base pair lengths instead of the high peak around 30000 bp most DNA is in sizes between 100-1500 bp. When going to higher DNase concentrations of 100-200 µg/ml the genomic tape shows complete degradation of the DNA. Or at least it cannot differentiate between the samples that contain goethite-DNA-Dnase and the controls that had no but only the different DNase concentrations and 50 ng/µl DNA in solution.

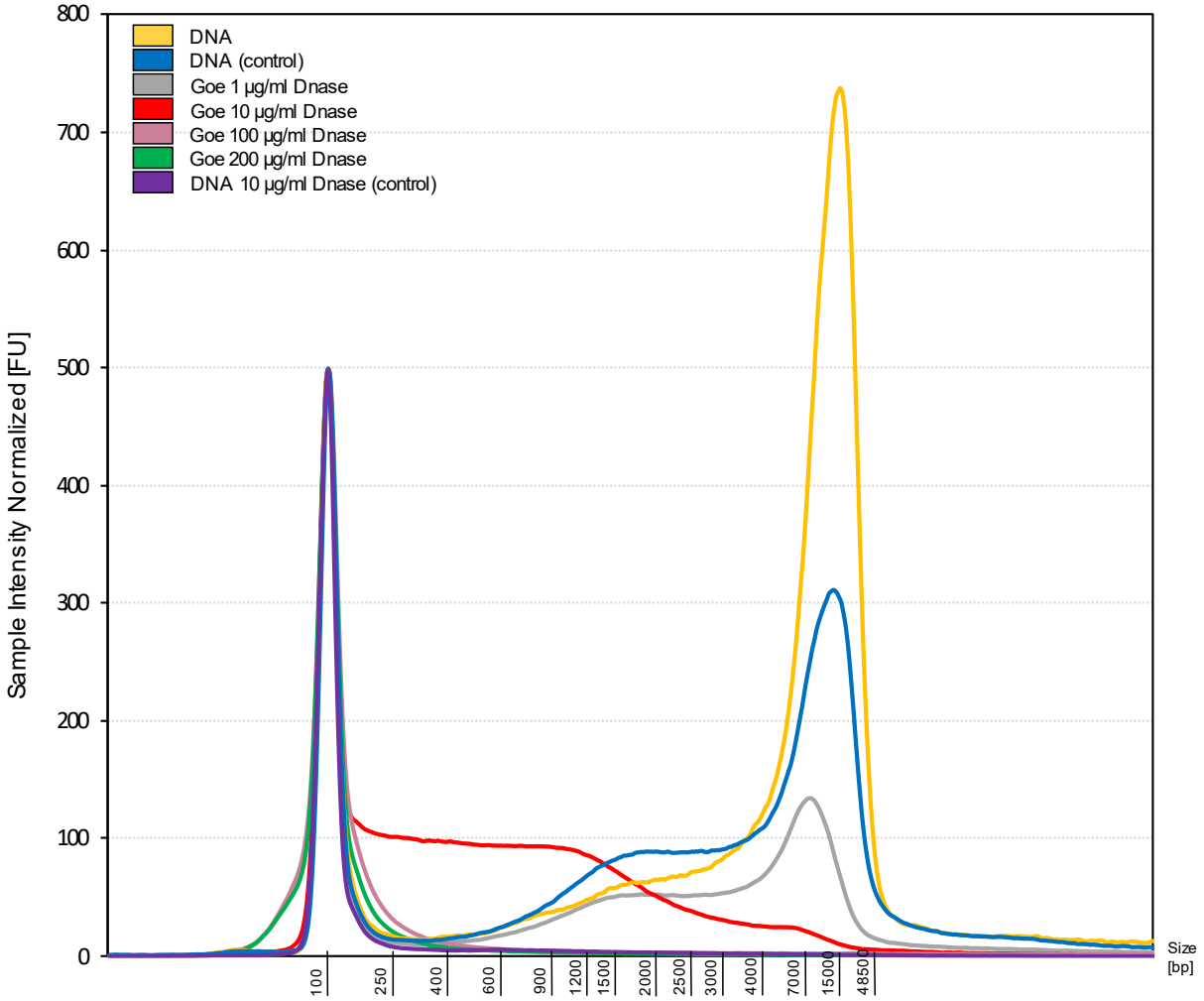

Figure 3.4.1 Degradation after using 1-200 µg/ml DNase I. DNA control (blue) is the goethite and DNase free DNA after 24 h. The other control (purple) contains no goethite.

Since there was a possibility to measure even smaller base pair sizes, the same samples were measured with a d1000 tape, which can measure DNA concentrations as low as 0.1 ng/µl. To do an extra measurement with the smaller bp tape was indicated by the Qubit results which showed that there was still around 15 % of the DNA left on the goethite surface even after using 200 µg/ml of DNase. Figure 3.4.2 shows these Qubit results of the comparison between the extent of DNA degradation (% DNA lost) by DNase when DNA was in solution or adsorbed to goethite. The figure shows that although there is an elevation in degradation of DNA as the DNase concentration is increased (1 µg/ml-200 mg/ml DNase), there is no complete degradation for the samples containing

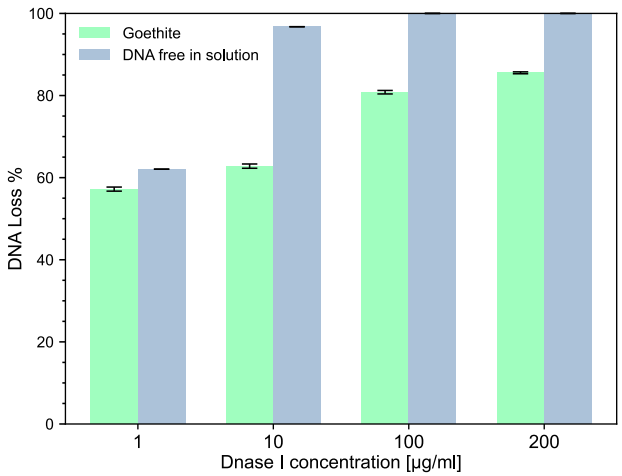

Figure 3.4.2 Comparison of DNA on the goethite surface and free in solution after DNase I treatment. Grey bars = DNA free in solution after DNase I treatments with different concentrations. Green bars = DNA desorbed from goethite after DNase I treatments with different concentrations

goethite, rather, there is still a significant amount of DNA that could be desorbed from the goethite.
Similarly, or even exceeding the first degradation results Figure 3.4.3 shows the Tapestation results with the d1000 tape. On the d1000 bp tape the 2 flat lines of the controls C1 and C2 show that there is no detectable DNA, whereas all the samples that had DNA adsorbed to goethite treated either with 100 or with 200 µg/ml DNase, showed base pair sizes between 25 bp and 200 bp. The results confirm the data from the Qubit (Figure 3.4.2) measurements in the presence of goethite, DNA is protected from enzymatic degradation. Without goethite, 95 % of the DNA is exterminated when using 10 µg/ml DNase, and at a higher concentration, there is no more detectable DNA concentration. This not only shows that indeed DNase is effectively degrading DNA in solution but also that goethite can successfully prevent the complete degradation of DNA if the DNA is adsorbed onto the mineral surface. The results also highlight the importance of looking at data from different perspectives. Although Qubit is accurate in measuring DNA concentrations it only shows the overall concentration on the sample and gives no information on the base pair size of the DNA strand. Here lies the importance of the Tapestation because now the results can be interpreted in more detail.

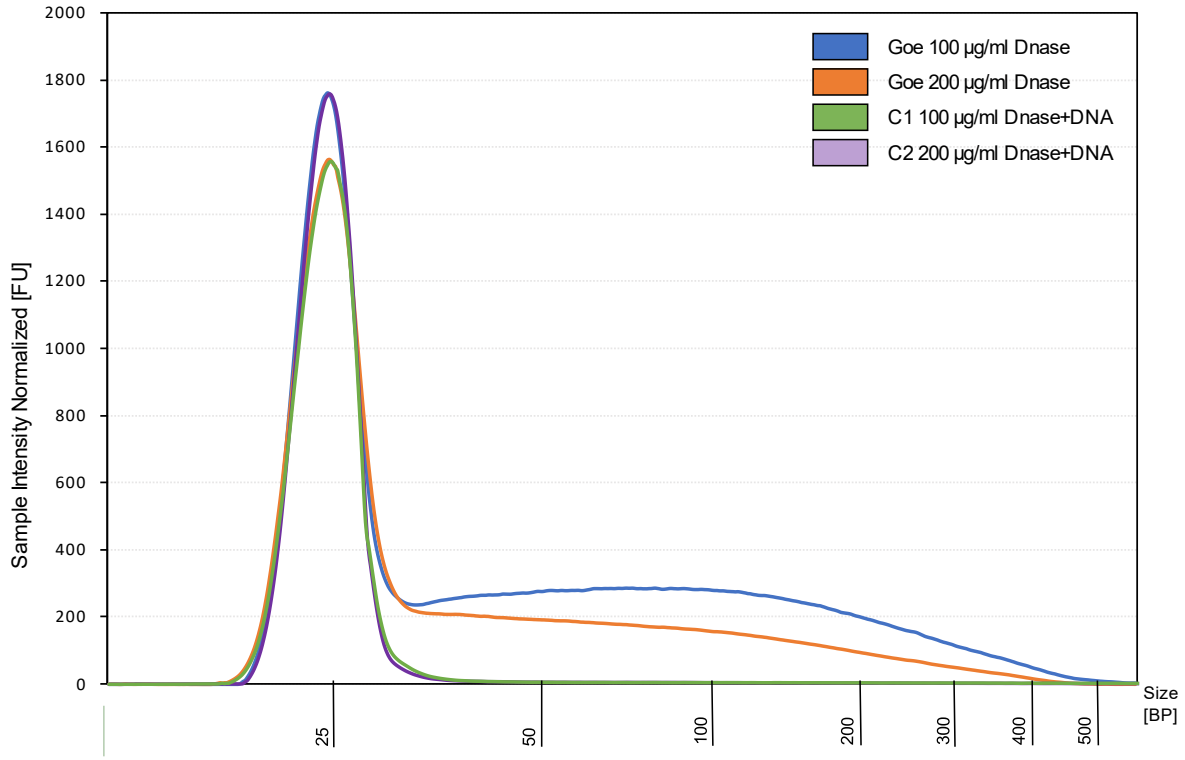

Figure 3.4.3 DNA after 1–200 µg/ml DNase I treatment on the 1000bp tape. Samples are the same 100 µg/ml and 200µg/ml DNase I treated samples as in Figure 3.4.1

3.5 Effect of Cu(II) on DNA Adsorption

The Cu experiments were based on the same adsorption protocol that was developed for the Goethite DNA experiments. The only issue was that the literature research (Peacock and Sherman, 2004) predicted the formation of Cu(II) hydroxides at pH 7 so the pH was set for all copper-related experiments to pH 6.5. Before starting the adsorption experiments with DNA, the formation of Cu(II) hydroxides(see Appendix 6.13.1) and the extent of Cu(II) adsorption to goethite was determined by Phreeqc Modelling (see Appendix 6.13.2) and ICP-MS measurements. ICP-MS measurements confirmed (see Appendix 6.10) for the 1 ppm

solution concentrations a complete adsorption of Cu(II). For the 25ppm and 50ppm Cu(II) the ICP results indicate that 94% of the initial Cu(II) that existed in solution has now been found to be adsorbed onto the mineral surface. The goal was to investigate how increasing copper concentration affect the DNA-goethite interactions and the degradation of DNA. Therefore, 3 different copper concentrations were used 1 ppm, 25 ppm, and 50 ppm. These allowed to investigate three different initial solution concentrations of Cu(II) without the risk of Cu hydroxide formation at the surface(Peacock and Sherman, 2004). After the Cu was adsorbed to the mineral surface, different DNA concentrations were added (40, 80, 120, 140, 200, 300 ng/µl) and left for shaking for 6 h. The results are shown in Figure 3.5.1 A. For comparative measurements, the data for goethite and DNA (no Cu) at pH 7.5 was added to the graph. The highest DNA adsorption is to the Cu-free goethite at pH 6.5 showing increased adsorption compared to the pH 7.5 data. At pH 6.5 adsorption equilibrium is reached around 80 ng/cm^2 that's a 10 % increase compared to the results obtained at pH 7.5. There is a clear trend that can be observed with increasing Cu(II) adsorbed to the goethite surface, there is a decrease in the adsorption of DNA. The samples initially supplied with 1 ppm Cu and 25 ppm Cu have very similar patterns both reaching equilibrium (plateau) at 70 ng/cm^2.

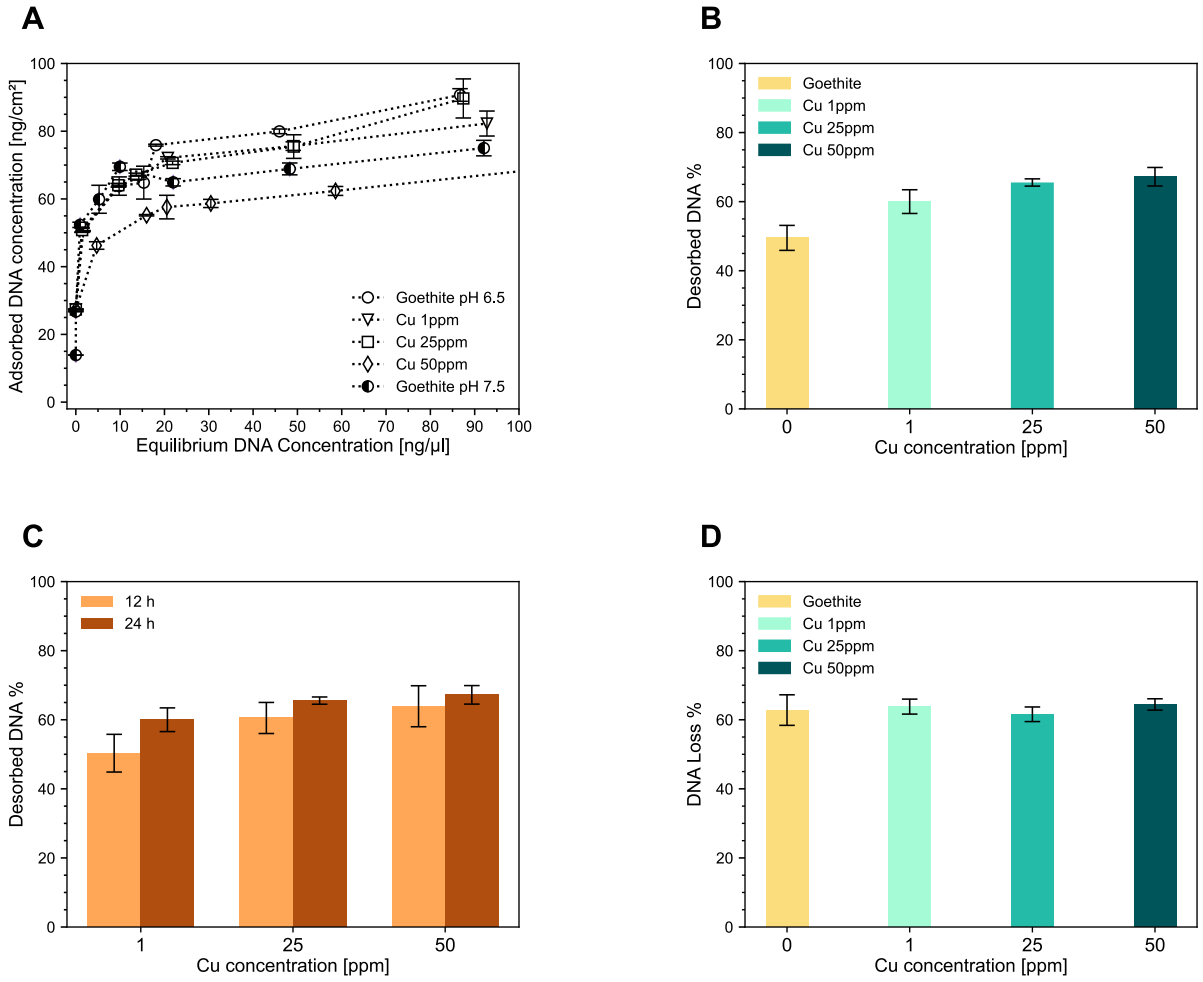

Figure 3.5.1 ***A)*** *Adsorption of DNA onto goethite and goethite exposed with Cu(II).* ***B)*** *DNA Desorption comparison between Cu(II) exposed goethite and Cu(II) free goethite.* ***C)*** *DNA Desorption comparison between Cu(II) exposed goethite samples after 12h and 24 of reaction time. The same sample conditions as in Figure B.* ***D)*** *Comparison of DNA loss due to enzymatic degradation between Cu(II) exposed goethite and Cu(II) free goethite.*

These results are still close to the plateau of the Cu free goethite at 80 ng/cm^2, however, for the samples with initial solution concentration of 50 ppm Cu(II) there is a significant drop in adsorption of DNA. The equilibrium state is reached at 55 ng/cm^2 for the 50 ppm Cu(II) exposed samples, which is a 25 % decrease in adsorption compared to the samples where there was no Cu adsorbed to the goethite.

3.6 The effect of Cu(II) on DNA desorption

Before doing the degradation experiments the influence of Cu(II) on DNA desorption needed to be determined to identify any potential bias in the desorption efficiency as a function of Cu(II) at the surface. This was necessary information to avoid false interpretations of the degradation experiments. The desorption experiments were carried out the same way as with the Cu-free system using the single wash method. First, 50 ng/µl DNA was adsorbed for 6 h on the Cu(II) exposed goethite after taking off the supernatant it was washed for 24 h with a 20 mM phosphate buffer and 0.1 mM EDTA mixture. Then the DNA concentration of the samples were analyzed with Qubit. At this point, UV/VIS spectrometry measurements with the plate reader were not possible anymore since Cu had such a huge distortionary effect on the results. Figure 3.5.1 B and C shows the results of the desorption experiments with Cu(II) exposed goethite.

As the Figure 3.5.1 C shows there was an extra time point at 12 h which was added, this was for the reason to see how much difference it makes to leave the desorbing agent longer to react. The results show it is true that the reaction time is important and contributes to higher desorption efficiency. This effect gets smaller with increasing Cu concentration. For example, at the highest Cu concentration, the 12 h longer reaction time only contributes two 2%-3% increase in desorption which statistically is not a significant difference. In Figure 3.5.1 B the desorption efficiency was compared after 24 h of desorption with the single wash method. The desorption rate is increasing from 60 % at Cu 1 ppm, 65.54 % at Cu 25 ppm to 67.21 % at Cu 50 ppm. The experimental results indicated a notable increase in the desorption rate in the presence of Cu(II). This supports the hypothesis that in the presence of Cu on the goethite surface the DNA can´t attach that strongly to the mineral surface as without Cu. In the case of competitive adsorption, the DNA gives up its place on the goethite surface much easier to phosphate compared to when the goethite surface has no Cu(II).

3.7 The effect of Cu(II) on DNA degradation

With already seeing the different effects that the presence of Cu(II) causes in the adsorption and desorption mechanisms, there were high expectations of also seeing effects in the degradation experiments. The hypothesis was that the presence of Cu on the goethite surface leads to less adsorption and easier desorption which means that the DNA is bound less strongly to the mineral surface. This suggests that the DNA retrieved from the Cu-exposed goethite should show more degradation than the Cu -free goethite. The Degradation experiment was based on the same protocol as for the Cu -free goethite. After the Cu was adsorbed to the mineral surface, 100 ng/µl DNA was added and left for shaking for 6 h. This followed the degradation step, after 6 h 10 µg/ml DNase was added to the suspension and left on the shaker for 1 h at 37 C°. Following, immediately 1.25µl 0.5 M EDTA was added, and the samples were put for 15 min in a 75 °C Water bath to quench the enzyme. This followed the desorption step with the single wash method and the measurement of the DNA concentrations of the samples with Qubit and Tapestation. Figure 3.7.1 shows the Tapestation results of the DNase -treated DNA desorbed from Cu(II) exposed and Cu -free goethite.

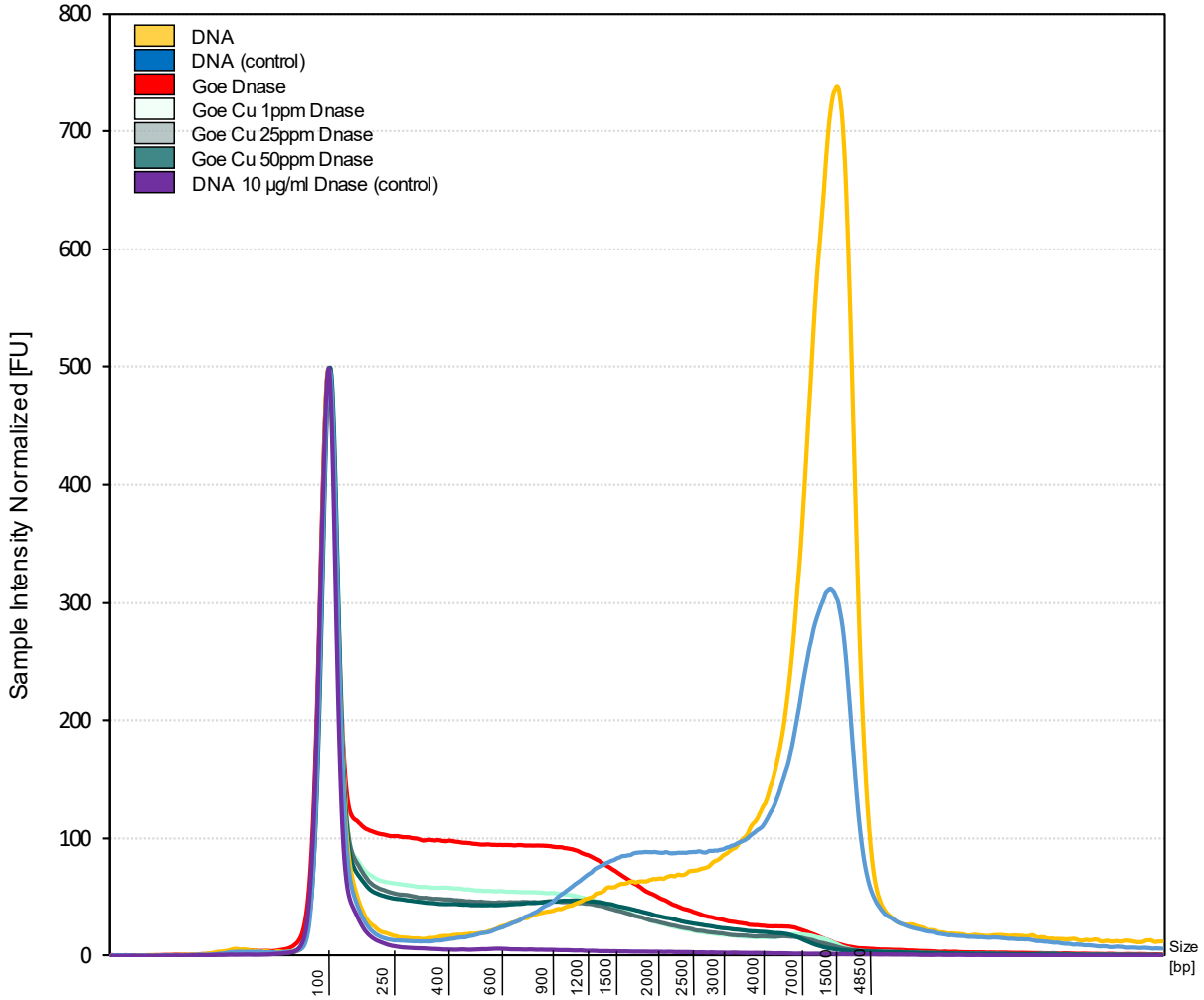

Figure 3.7.1 Comparison of DNA Degradation between Cu(II) exposed goethite and Cu(II) free goethite. DNA control (Blue) is the goethite and DNase free DNA after 24 h

As the results show (Figure 3.7.1), in the presence of 10 µg/ml DNase, DNA degradation was observed in all cases, with only very limited degradation in the DNase free control. The majority of the degraded DNA is between 100–2000 bp. There are also some bigger DNA pieces between 15000–2000 bp but the intensity is getting less and less with larger bp sizes. However, despite effecting extent of DNA adsorption and desorption presence of Cu does not appear to effect the degradation of DNA at the surface. Qubit data Figure 3.5.1.D shows no difference between the goethite with Cu and without Cu. One more observation must be noted, the DNA control (blue line Figure 3.7.1) after 24 h showed some degradation. This was unexpected since there were previous test results from where it was known that DNA does not degrade on its own in 24 h (see Appendix 6.11). The only difference from previous experiments was the 75 °C water bath. This suggests that a small amount of DNA is partially degraded due to the heat treatment.

3.8 The effect of Cu(II) on DNA without the presence of mineral

Before Cu(II) adsorption on goethite, the question occurred what effect Cu(II) can have in solution on the degradation experiment. From the ICP data (see Appendix 6.10; Table 6.10.1), it was obvious that there should be little to no Cu(II) in the solution rather everything on the goethite surface. Another experiment was carried out where no goethite was involved just the Cu(II) and DNA in a Hepes buffer. This experiment had two purposes: a) to reconfirm

that Cu(II) is not damaging DNA on its own and b) to see what happens if we added the DNase. The first question was reconfirmed. The second experiment was more interesting since it was known from previous experiments that DNA gets completely degraded in free solution when 10 µg/ml DNase is added. However, in the case when Cu(II) was in solution with DNA, degradation was inhibited Figure 3.8.1 shows the results of this experiment. Here we can see that the protective effect of Cu(II) is only working with increasing Cu(II) concentrations. At 1 ppm Cu(II) solution concentration still all the DNA is degraded. However, at 25 ppm Cu(II) solution concentration the bp length almost hasn´t changed still the majority of DNA is between 12000–48500 bp but the relatively flat intensity lines indicate that degradation is happening. At 50 ppm Cu(II) solution concentration most of the DNA has a smaller base pair length than the samples which contained 25 ppm Cu. These results indicate that Cu can protect DNA in solution however it cannot inhibit complete degradation.

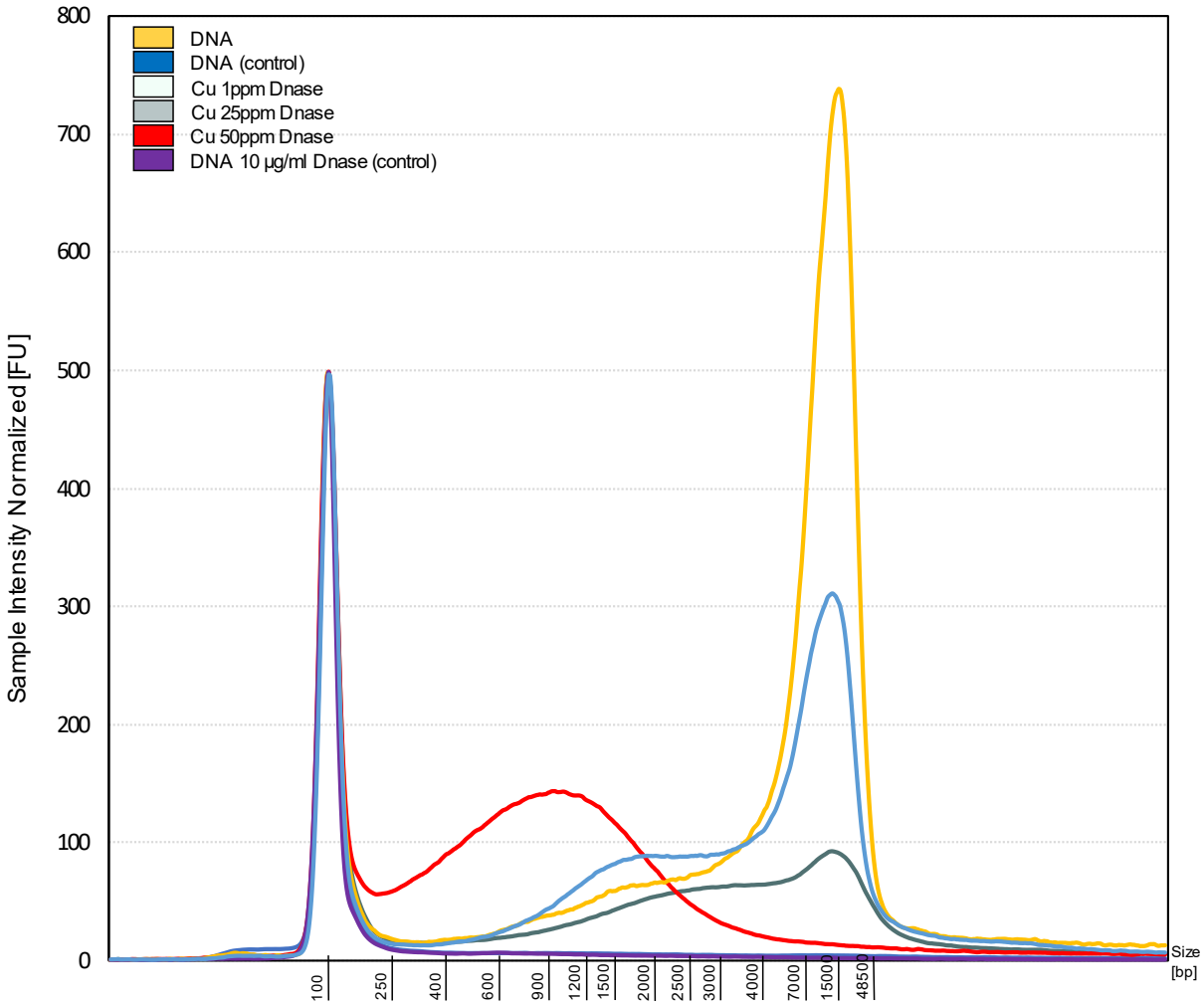

Figure 3.8.1 DNA free in solution with Cu(II) after 10µg/ml DNase treatment. DNA control (blue) is the goethite and DNase free DNA after 24 h

Chapter 4 Discussion

4.1 Goethite and DNA

4.1.1 Adsorption and Desorption of DNA at the goethite surface

Previous works with DNA goethite-related experiments overlapped with the experiments in this study. The kinetic experiment results gave a rapid adsorption mechanism that correlates well with Sodnikars results (Sodnikar et al., 2021). There is a high confidence in the assigned kinetic rate of pseudo-second-order which produced a high R^2 value of 0.995. However, in this study, the kinetics were not the main objective. The primary goal of the kinetic experiments was to identify the time at which adsorption equilibrium was reached.

The adsorption results are also relatively similar to Sodnikar et al results (Sodnikar et al., 2021). The two isotherm models that were fit Langmuir (Langmuir, 1918, 1916) and Freundlich (Freundlich, 1907), are not the only possible options. There are also a variety of other models, such as the linear model, the Sips model (Sips, 1948), the Temkin model (Temkin, 1940), and the Brunauer, Emmett, and Teller (BET) model (Brunauer et al., 1938). Furthermore, the adsorption models can also be categorized by the shapes of the isotherms (S-, L-, H-, and C-shaped) (Limousin et al., 2007). However, this becomes less important since both Langmuir and Freundlich are L-shaped. The Langmuir and Freundlich models were chosen since they are frequently used in adsorption studies and are easy to linearize. Also, these two models show a good comparison between monolayer (Langmuir) and multi-layer (Freundlich) adsorption. The Langmuir model showed a good fit after the linearization and showed the best fit in the non-linear approach. The Freundlich model showed a low R^2 value for the linearization but since the units of the y-axis were different for the two models the R^2 values cannot be compared directly. Thus, a non-Linear fit was also performed. This nonlinear fit gave a better fit for the Langmuir model with an R^2 of 0.778 compared to the Freundlich model with an R^2 of 0.517. These results suggest that the DNA is forming a monolayer on the goethite surface. This is explained by the mechanism of how the DNA attaches to the goethite surface. The goethite surface is positively charged so the negatively charged, phosphate sugar bones of the DNA electrostatically attach to the mineral surface. Once the surface is saturated with DNA, the negative phosphate sugar bones of the adsorbed DNA make further DNA adsorption unfavorable.

The impact of pH on adsorption and desorption was clearly shown in this work. There was an increase in adsorption once the pH was lowered from pH 7.5 to pH 6.5 (Figure 3.5.1 A). Similarly in the case of desorption, since there was no difference in the used phosphate buffer concentrations in the multiwash method but there was a significant increase in adsorption at pH 8.2 compared to pH 6.2 (Figure 3.3.1 C), which shows well how much effect pH has on surface chemistry. By applying a higher pH, the surface of the goethite (PZC=7.85) becomes more negative. Since DNA is attached to the mineral surface through the negative phosphate moieties, a negative surface charge leads to repulsion and forces the DNA off the surface. The phosphate buffer itself is working well as a desorbing agent because it competes with the DNA for the same surface sites and since the phosphate molecules from the phosphate buffer HPO_4^{-2} and PO_4^{-3} are negatively charged. However, in

contrast to the DNA, the phosphate ions compete effectively across a range of pH values(Green, 1933), for the surface binding sites.

In this work, it has also been shown that there is a positive correlation between desorption agent concentration and desorption efficiency. When the concentration of phosphate buffer is increased (Figure 3.3.1 D), more phosphate ions are available in the solution to compete with the phosphate groups on the DNA, for binding sites on the goethite surface. This leads to higher removal of the DNA from the surface and more effective desorption. In the multiwash method, EDTA was also used as a desorbing agent (Figure 3.3.1 C). However, the ineffectiveness of EDTA during the multiwash method (Figure 3.3.1 C) suggests that competitive desorption with prior phosphate washes is more important than breaking cation bridges.

4.1.2 Protection of DNA at the goethite surface

One of the most interesting results from this work is the fact that even after using 100 µg/ml of DNase, the DNA is degraded to 25–300 bp and after doubling the amount of DNase, it shows almost no difference (Figure 3.4.2; Figure 3.4.3). This suggests that after the DNA is degraded to a smaller base pair size, it becomes more capable and durable in withstanding the DNase enzyme. However, this effect only applies if the DNA is adsorbed to a surface. Nevertheless, these results are in good agreement with the ancient human DNA found at Archeological sites. This ancient DNA is also found most of the time in bp lengths below 400 bp (Heintzman et al., 2014; Pääbo, 1989; Warinner et al., 2017). This suggests that goethite - containing soils could be a good host material for finding ancient DNA and environmental DNA. Furthermore, the results highlight the importance of the size dependence of DNA in the ability to survive in the environment. In future experiments, a bigger focus needs to be placed on experiments where DNA with smaller bp lengths is compared to the experiments in this study. If the smaller bp sizes show an even lower or no degradation at all in the presence of the enzyme, it would confirm this hypothesis of size-dependent survival of DNA.

Another observation needs to be mentioned. During the degradation experiments, a small degradation could be observed in the DNA control (Figure 3.7.1). To this control no DNase was added, but the DNA still showed degradation after 24 h. Since DNA is stable at room temperature for 24 h (see Appendix 6.11; Figure 6.11.1) this meant that a different process than enzymatic degradation was causing damage. After rerunning some of the DNase free controls (also some containing goethite) it turned out that the 75 °C water bath is probably causing heat degradation. This suggests that although goethite can effectively reduce enzymatic degradation, it is not able to prevent heat degradation. However, the small extent of the heat degradation does not affect the interpretation of the results.

4.2 Cu(II) on the goethite surface influencing adsorption and desorption

The DNA adsorption and desorption results when Cu was present on the goethite surface were quite unexpected. The decrease in DNA adsorption and possible increase in DNA desorption efficiency due to the influence of Cu(II) at the mineral surface has not been reported before. Previous studies (Cai et al., 2006a; Freeman et al., 2020; Sheng et al., 2019b) observed increased DNA adsorption resulting from cation-saturated surfaces. In those studies, the metal cations act as bridges, neutralizing the charge of the phosphate sugar on the DNA and reducing the tension between the two DNA strands. The first interpretation was that since there was a minimal pH shift of -+0.25 pH, this contributed to the

lower DNA adsorption amounts. However, when comparing to the results obtained at pH 7.5, it became clear that the difference between pH 7.5 and pH 6.5 adsorption is smaller than between Cu-free goethite and 50 ppm exposed goethite (Figure 3.5.1 A). Therefore, the sole contribution of the changes by shifting pH was ruled out. There are two other possible explanations: DNA is forced to form outer sphere complexes (Boukemara et al., 2016; McBride et al., 1998) instead of inner sphere complexes (Bochathay et al., 1997). By limiting the available surface sites, the DNA may adsorb only at sites which Cu(II) does not occupy and thus, adopt a different surface complex to that in the absence of surface-adsorbed Cu. The second possible explanation is that the presence of Cu(II) forces the DNA to change its structure thereby limiting the possibility to attach to the mineral surface. This fact is supported by later experiments where the presence of Cu(II) in the solution (without the presence of goethite) can reduce the enzymatic degradation of DNA. A study at the University of East Anglia conducted experiments that support the fact that Cu salts indeed change the structure of DNA (Day et al., 2015). However, additional work is required to further test these hypotheses. Further studies focusing on Cu-related dsDNA structural change and synchrotron studies for DNA adsorption and desorption from goethite are required to better understand these mineral-metal-DNA interactions. For example synchrotron (EXAFS) data could help better identify the nature of DNA complexation at goethite surface in presence or absence of Cu(II).

4.3 Cu(II) Influence on DNA Degradation

In the adsorption studies, the presence of Cu(II) resulted in a decrease in DNA adsorption and an increase in desorption efficiency at the goethite surface. However, the presence of Cu(II) doesn´t appear to influence the extent of DNA degradation at the mineral surface as there was no difference observed between DNA degradation at the goethite surface in the presence or absence of Cu. At the experiment where DNA was in the solution with Cu(II) Figure 3.8.1 (no goethite was involved) and DNase I was added, Cu could protect the DNA from complete degradation on its own. Surprisingly at the highest Cu(II) solution concentration (50 ppm), the average base pair length was smaller than that of the sample with 25 ppm Cu. These results raised a couple of questions: Why is Cu able to protect DNA in solution but has no positive influence when it is on the goethite surface? Why is it that in the presence of 25ppm Cu(II) in solution we see enhanced DNA protection, but this protection declines with further increasing Cu(II) concentration of 50ppm? Both can be answered with the same approach. First of all, the reason why Cu can protect DNA in solution is mainly because Cu(II) can potentially exchange with other metal cations present in the co-factor inside the DNase enzyme. DNase requires Ca^{2+} and Mn^{2+} or Mg^{2+} as a cofactor, so when Cu(II) is added to the solution, it can occupy the place of the Cofactor and thereby inhibit the activity of the enzyme. The other mechanism that could influence DNA degradation the ability of Cu(II) to change the DNA´s structure and thereby possibly limit the enzyme's ability to interact with the DNA. Day et al (Day et al., 2015) showed that Cu(II) can change the I-motif DNA structure to a Hairpin form. Furthermore, LaMarr et al. (LaMarr et al., 1997) were studying the effect of DNA supercoiling on radical-induced DNA strand breaks. In their studies, they investigated five different oxidizing agents and used both positively supercoiled pUc19 plasmid DNA and negatively supercoiled DNA. They produced radicals with Cu(II)/ H_2O_2 and found out that strand breaks in the positively supercoiled substrate occurred at lower Cu concentrations than in negatively supercoiled DNA. Additionally, the damage response of the negatively supercoiled substrate was absent in the positively supercoiled

DNA. These studies illustrate how DNA reacts differently with Cu(II) depending on its structure; although the above cited authors worked with different DNA structures than the double-stranded DNA used in this work. Nevertheless, when Cu(II) can change different secondary structured DNA, this suggests it can have a similar effect on double-stranded DNA. The potential exchange of Cu(II) with the Cofactors of the DNase enzyme should be the driving factor in DNA protection in solution compared to the ability of Cu(II) to change the DNA´s structure. Since it would be expected that Cu(II) should already have caused structural changes on the DNA at a low Cu concentration. However, at 1ppm Cu(II) concentration there is no evidence of a protective mechanism since everything is degraded (Fig.3.8.1). As already mentioned, at 50 ppm Cu(II) there is more degradation observed than at 25 ppm. This suggests that above certain Cu concentrations there are competing effects that can induce damage to the DNA e.g. Cu(II) inducing ROS (Angelé-Martínez et al., 2017).

Although Cu(II) appears to impact DNA degradation in solution, its influence diminishes once it becomes adsorbed to the goethite surface. When Cu(II) is on the mineral surface, replacing the cofactors is not possible anymore, so the weakening of the enzyme is likely not a factor in DNA degradation on the mineral surface. Also the ability of Cu(II) to change the DNA structure seems to have no effect on enzymatic degradation once the Cu(II) is adsorbed to the mineral surface.

Chapter 5 Conclusions

In this thesis, goethite´s ability to protect DNA against enzymatic degradation was experimentally measured. Kinetic and adsorption studies showed successful adsorption of DNA to the goethite surface. Furthermore, during this work, a desorption method was developed for the goethite DNA system which was also applicable to experiments involving metals. By applying a 20 mM phosphate and 0.1 mM EDTA mixture as a desorbing agent, it was shown that the phosphate successfully competes for the surface sites against DNA. Raising the pH and the phosphate concentration increased desorption efficiency. These increases were likely due to the repulsion caused by high pH induced surface charge changes and increased competition due to higher concentrated phosphate. Although the desorption percentage never reached 100 % this work acts as a solid basis for further improvements in DNA-goethite desorption-related studies. Goethite's ability to protect DNA against enzymatic degradation was experimentally demonstrated. When using 10 µg/ml DNase the DNA samples containing goethite were only partially degraded to less than 400 bp whereas the DNA samples without goethite were completely degraded. It could also be shown that by increasing the DNase concentration to 200 µg/ml the DNA on the goethite surface was still able to survive, albeit in small amounts as low base pair fragments. These results correlate well with the ancient DNA fragment lengths (~30 bp) that can be found at archeological sites. The findings indicate that soils containing goethite, are potential DNA traps for environmental DNA. This knowledge can be useful in aDNA fields such as paleoecology and paleomicrobiology and suggests that goethite-containing soils should be considered as sampling sites for Anthropologists. This work is the first to report Cu(II) influence on DNA-mineral interactions. It was shown that Cu(II) can reduce the extent of DNA adsorption to the goethite surface potentially by forcing the DNA to form outer sphere complexes instead of inner sphere complexes. Also, it was found that higher Cu(II) concentrations on the goethite surface increased DNA desorption efficiency. This correlates well with the adsorption results since a weaker surface attachment would be expected to lead to easier desorption. This study is also the first to address Cu(II) influence on DNA degradation on the goethite surface. It was found that Cu(II) on the goethite surface had no significant effect on DNA protection against enzymatic degradation. However, it was proven that in solution Cu(II) can protect DNA from enzymatic degradation. In solution, Cu(II) is probably occupying the enzyme's cofactor places and inhibits the enzyme functionality, also Cu(II) could change the dsDNA structure thereby limiting the enzyme's attachment. These findings show that metals indeed influence the DNA-goethite system and further studies should be conducted towards finding a positive influence on DNA protection induced by metals.

Chapter 6 Appendix

6.1 XRD patterns

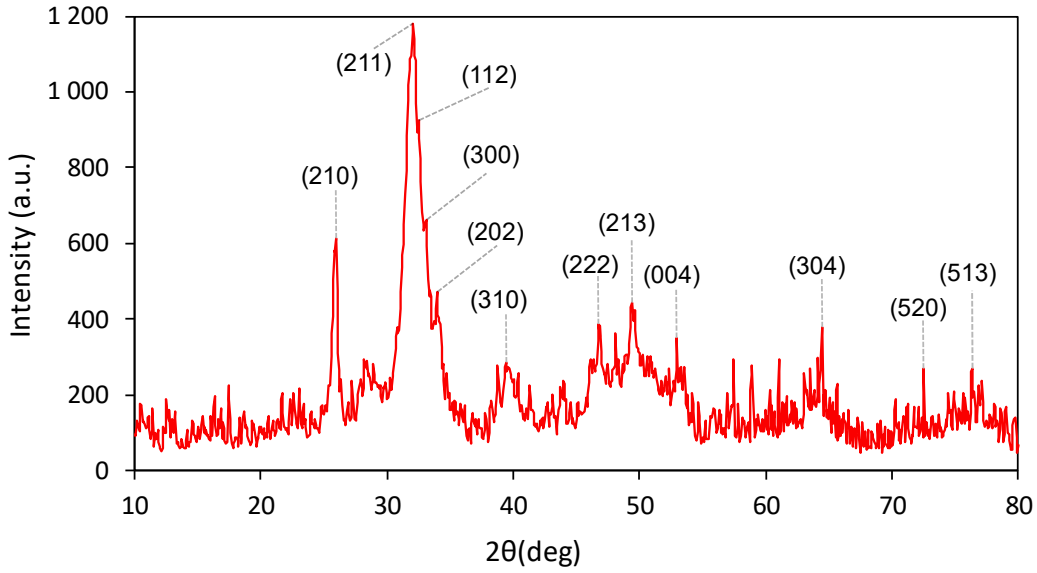

Figure 6.1.1 XRD results of synthesized hydroxyapatite

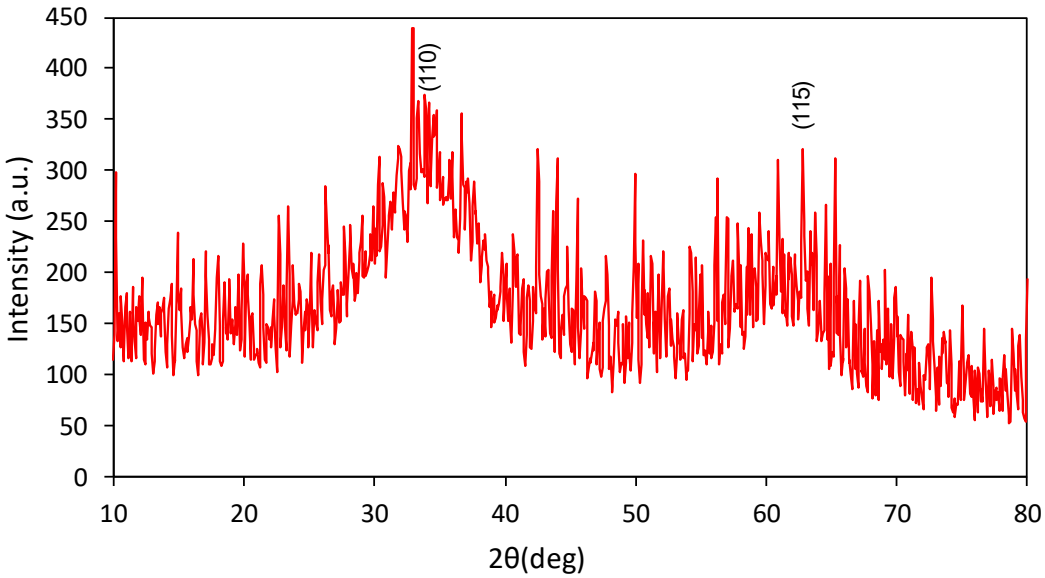

Figure 6.1.2 XRD results of synthesized two-line ferrihydrite

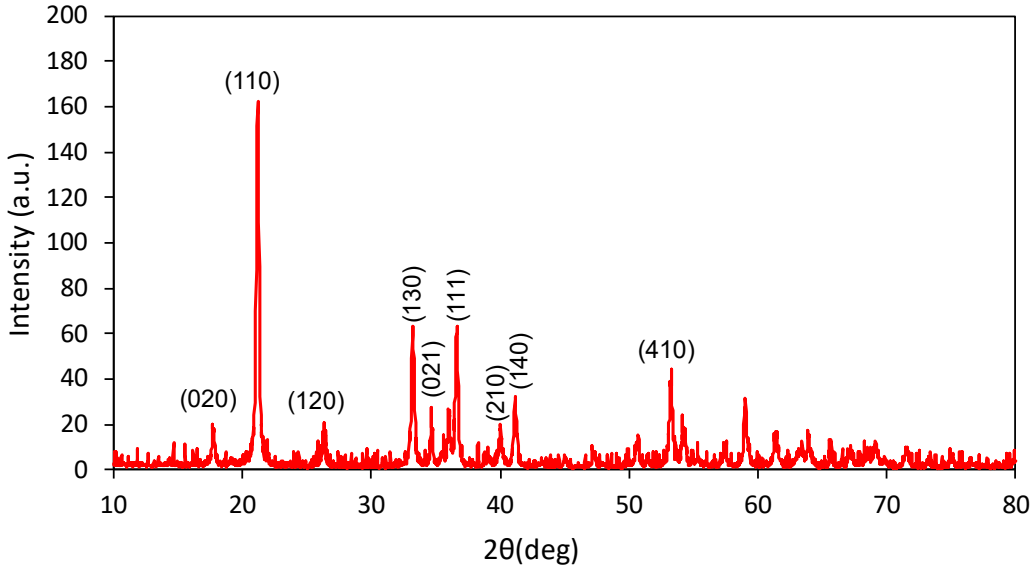

Figure 6.1.3 XRD results of synthesized goethite

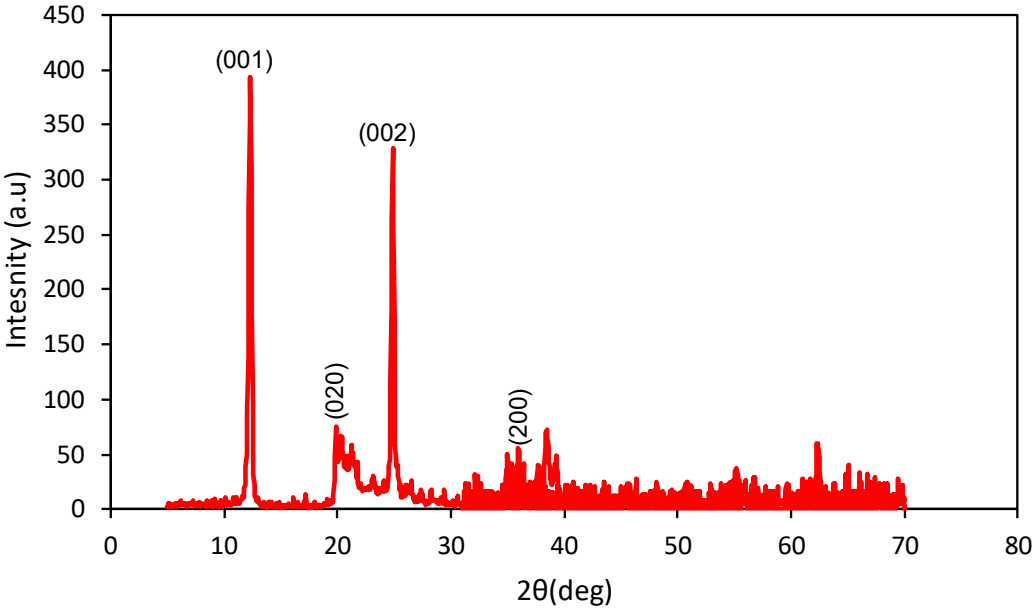

Figure 6.1.4 Na-kaolinite XRD pattern

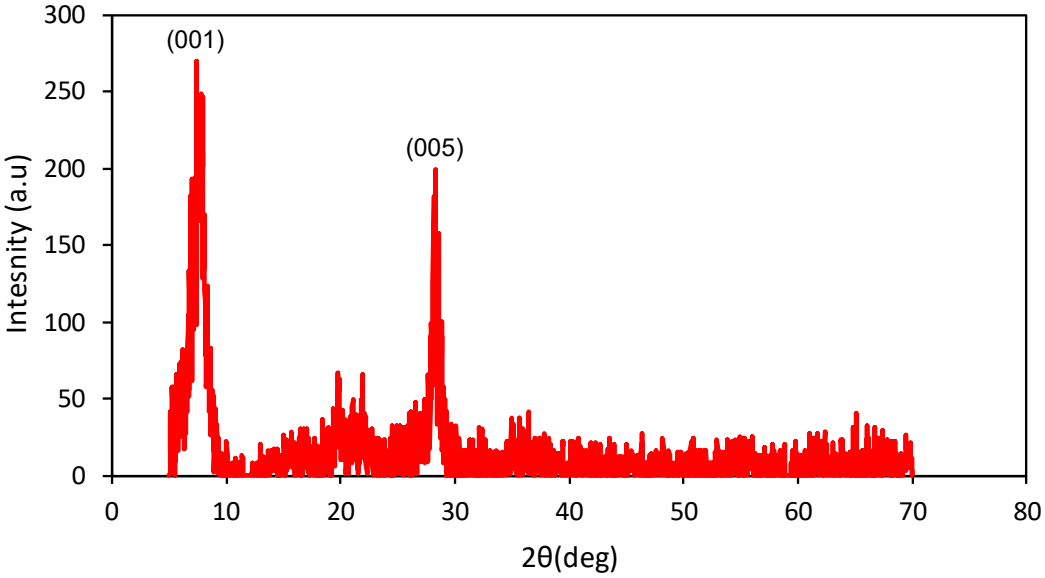

Figure 6.1.5 Na-montmorillonite XRD pattern

6.2 Pseudo-second-order kinetics

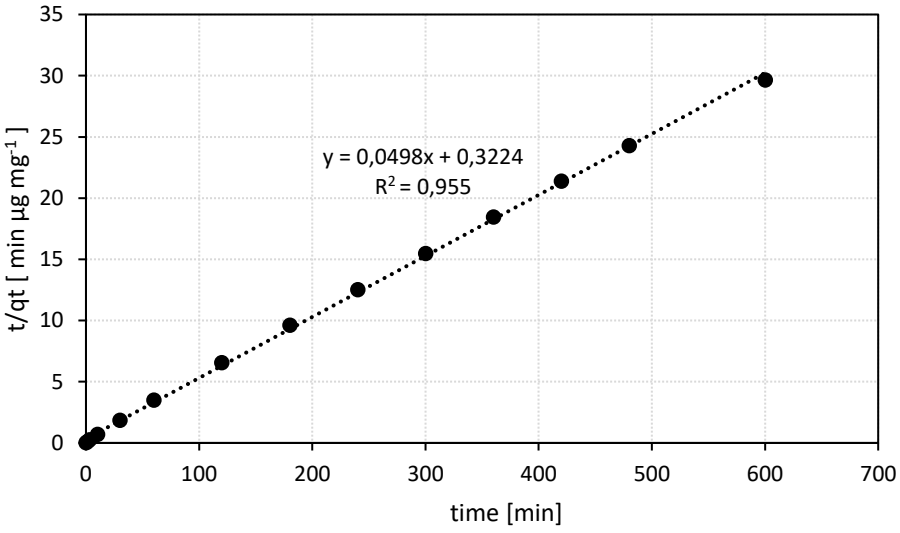

Figure 6.2.1 Linearization of the pseudo-second-order kinetics

6.3 DNA sorption after 24 h on different minerals with different buffer concentrations

This small experiment was carried out to assess the adsorption characteristics of 6 minerals and to see which effects on adsorption the buffer concentration and ionic strength have. The highest adsorption was measured for goethite while Hydroxyapatite also showed high adsorption. Clays showed significantly lower DNA adsorption values. Ionic strength influenced the DNA adsorption in all experiments, the highest DNA adsorption was achieved

when NaCl was in the buffer. Similarly, a higher buffer concentration yielded more adsorption to the mineral surface.

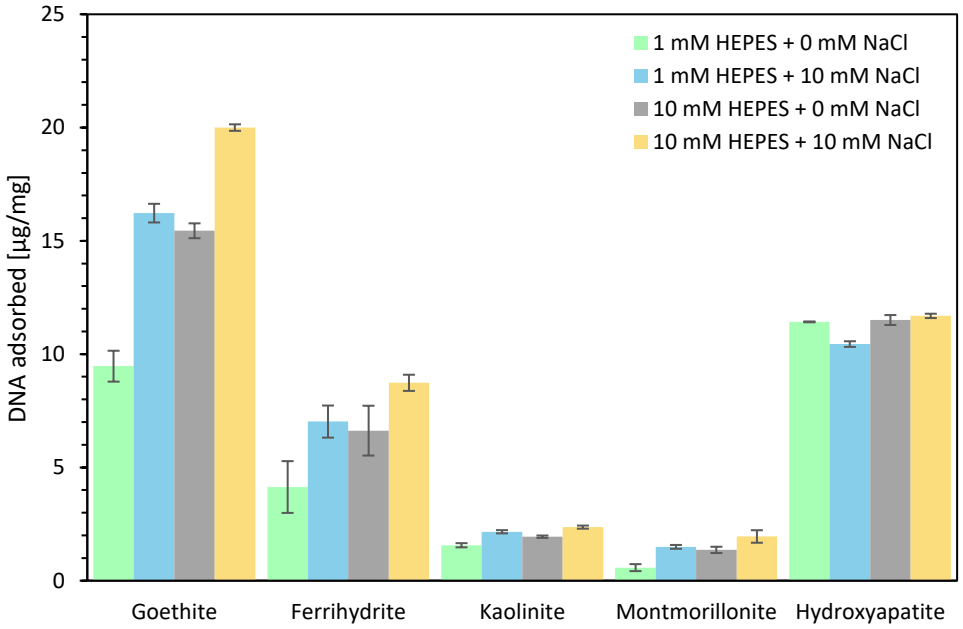

Figure 6.3.1 Buffer, Ionic Strength Influence test on DNA adsorption. Weight normalized

6.4 pH change with different buffer concentrations

During the buffer, ionic strength experiment (Appendix 6.3) the pH was monitored as well. Since the pH influences adsorption, the buffer needed to be able to reduce big pH shifts. The results show that pH shifts were mainly mineral dependent under the same conditions(Figure 6.4.1;Distance of the column heads from the redline shows extent of pH shift). Goethite was relatively stable when using 1 mM Hepes whereas the clays and hydroxyapatite showed pH shifts in several pH units. Results also show that using higher buffer concentrations (10 mM) lowers these pH shifts in all mineral suspensions.

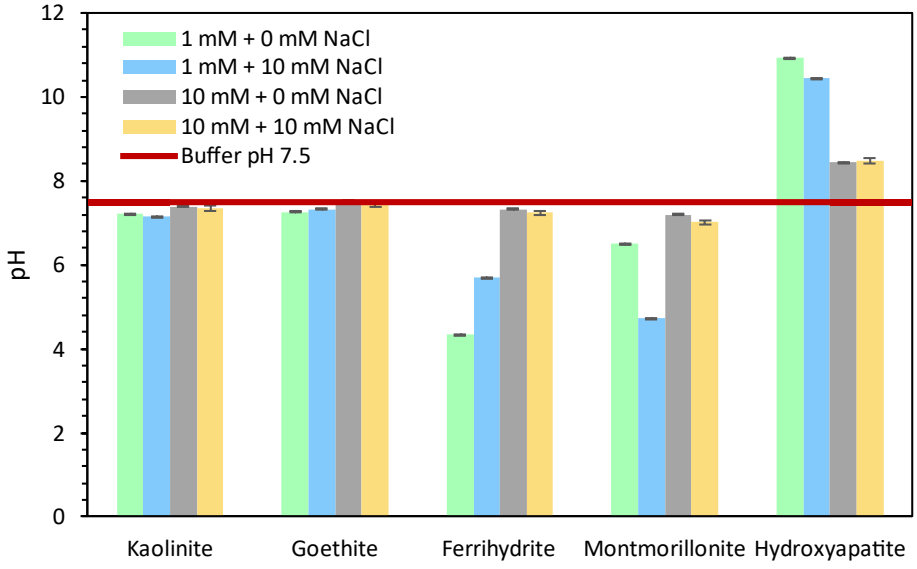

Figure 6.4.1 pH shifts caused by mineral Hydration in different concentrations of Hepes buffer

6.5 Linearized Langmuir fit

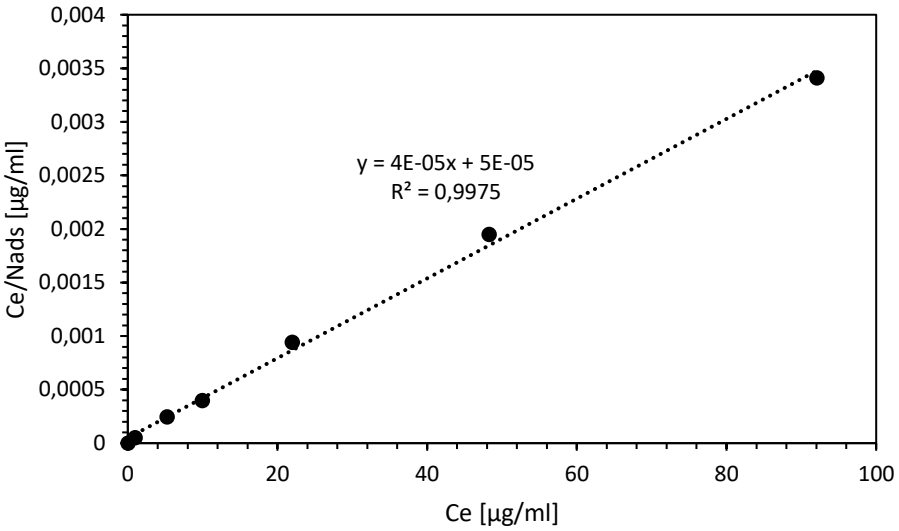

Figure 6.5.1 Linearization of the Langmuir model

6.6 Linearized Freundlich fit

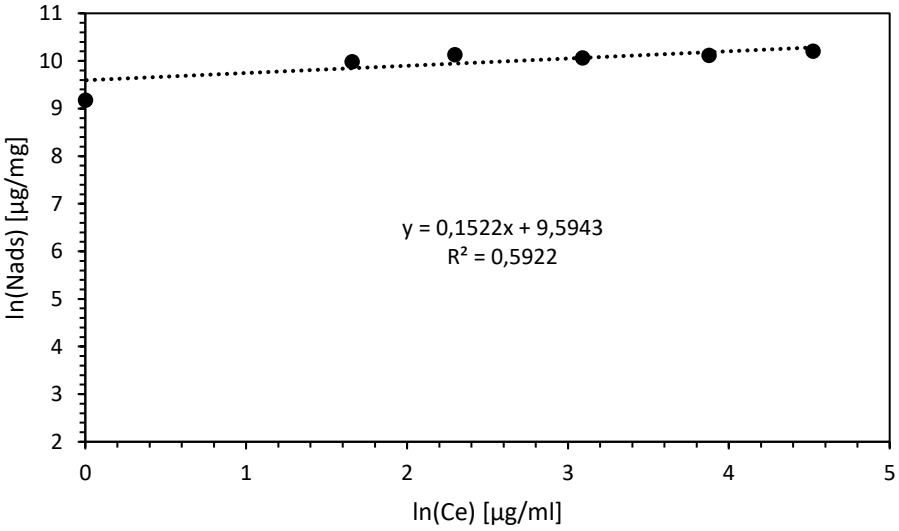

Figure 6.6.1 Linearization of the Freundlich model

6.7 Nonlinear adsorption model fit

Since the linear plots of the Freundlich and Langmuir could not be compared directly a nonlinear fit was also used. Here, Microsoft excels data solver function was applied. The data solver was given a close estimate value of the constants Nm and K_L and for the Freundlich model the constants n and Kf. These constants were then modified by the solver until the best fit was achieved.

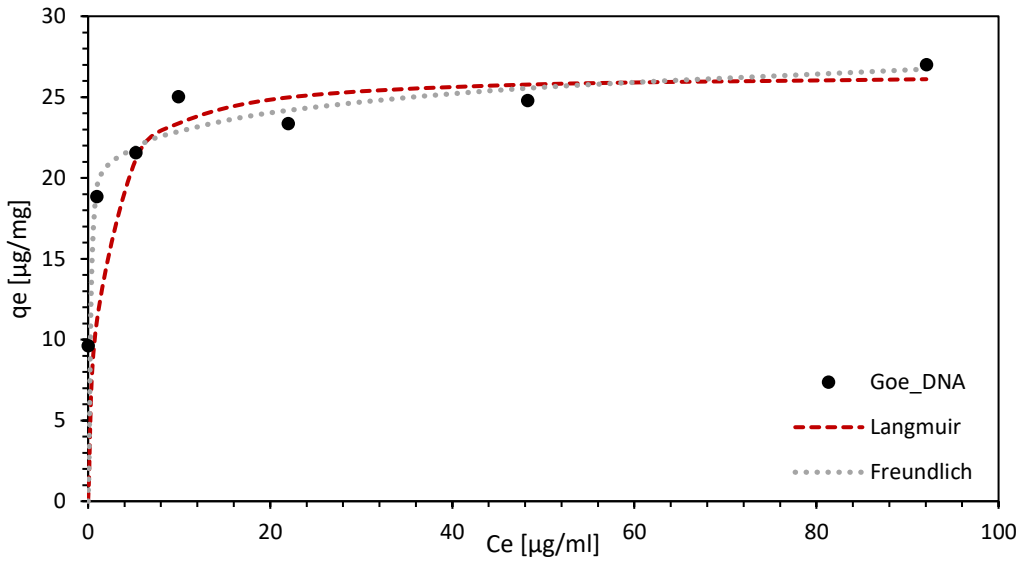

Figure 6.7.1 Non-linear adsorption model fitting to experimental data

6.8 Desorption multiwash method

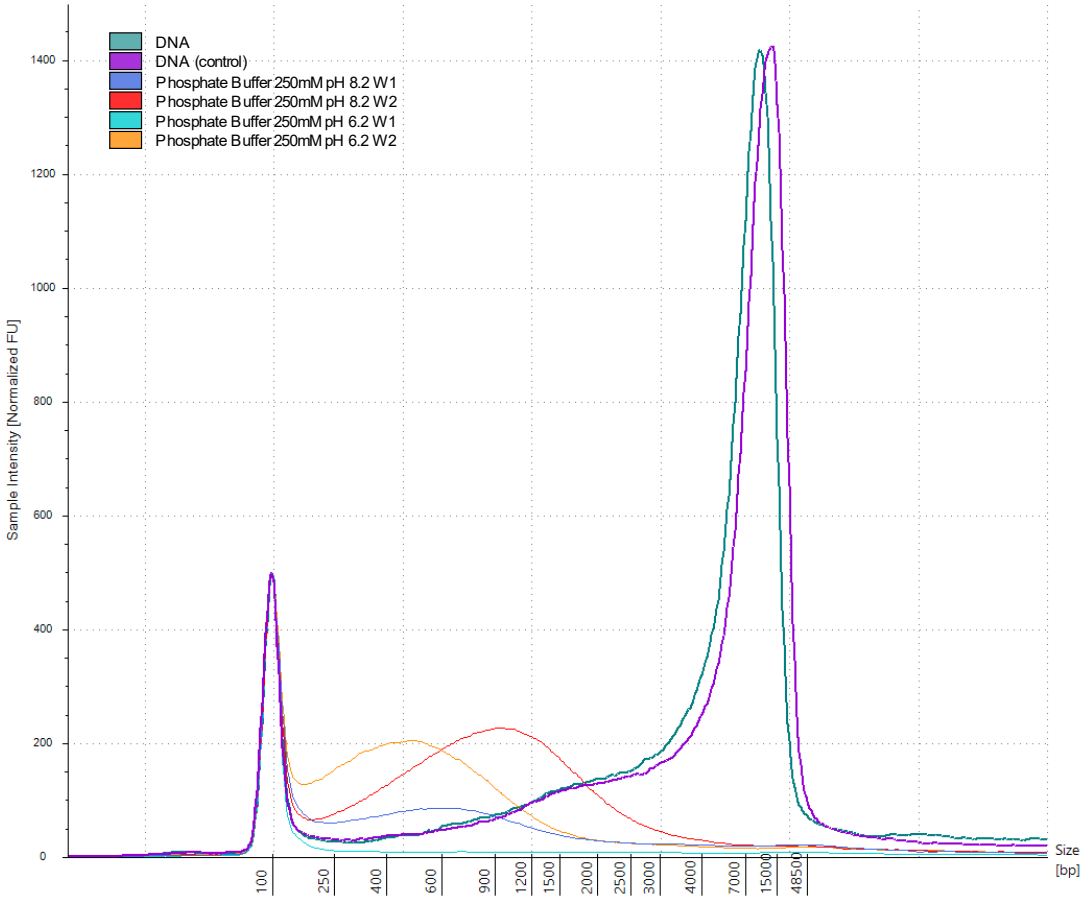

Figure 6.8.1 Multiwash method Tapestation results. DNA control is the goethite free DNA after 24 h

6.9 Desorption singlewash method

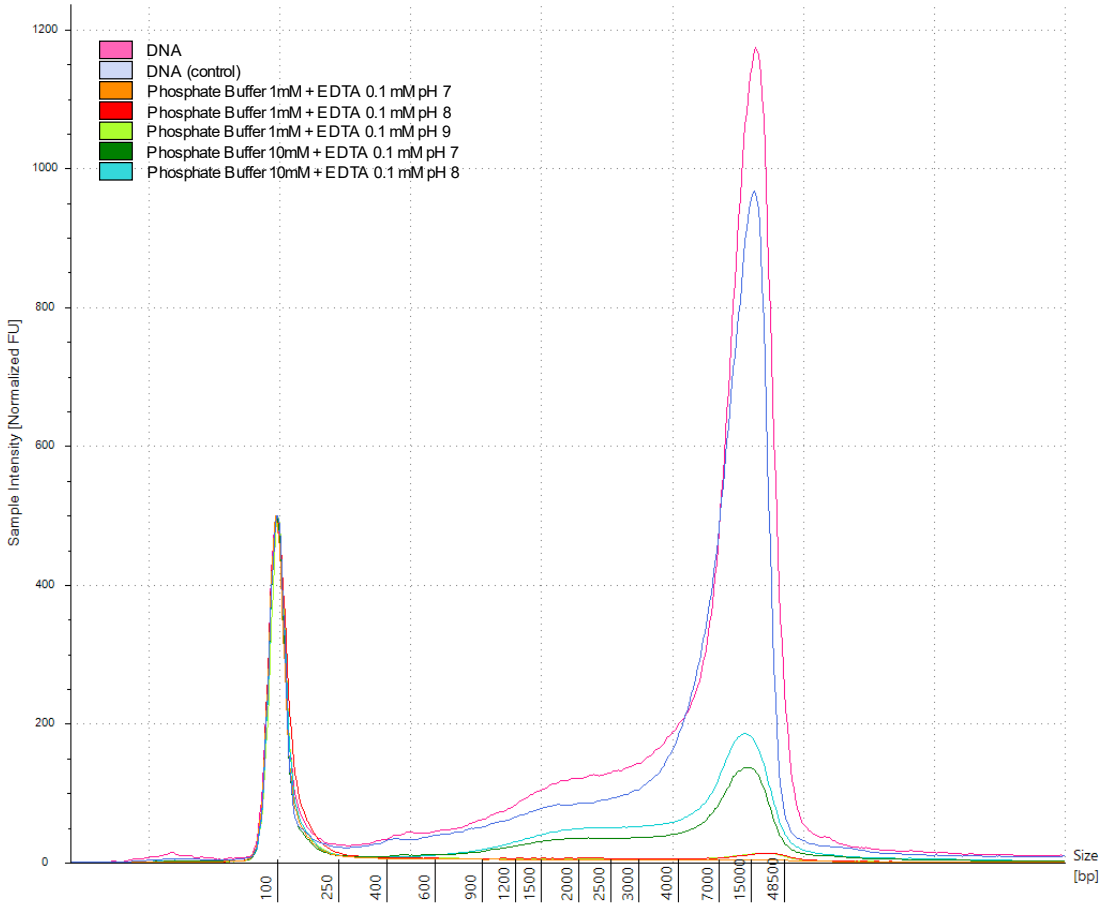

Figure 6.9.1 Singlewash method Tapestation results. DNA control is the goethite free DNA after 24 h

6.10 ICP-MS measurements

Table 6.10.1 ICP-MS data

Sample Name	Initial Cu(II) [ppm]	Final Cu(II) [ppm]	STD
Goe no Cu	0.538	0.599	0.058
Goe 8mg/ml 1ppm Cu	0.895	0.581	0.055
Goe 8mg/ml 25ppm Cu	27.389	1.756	0.207
Goe8mg/ml 50ppm Cu	53.941	2.977	1.280

6.11 DNA stability after 24h

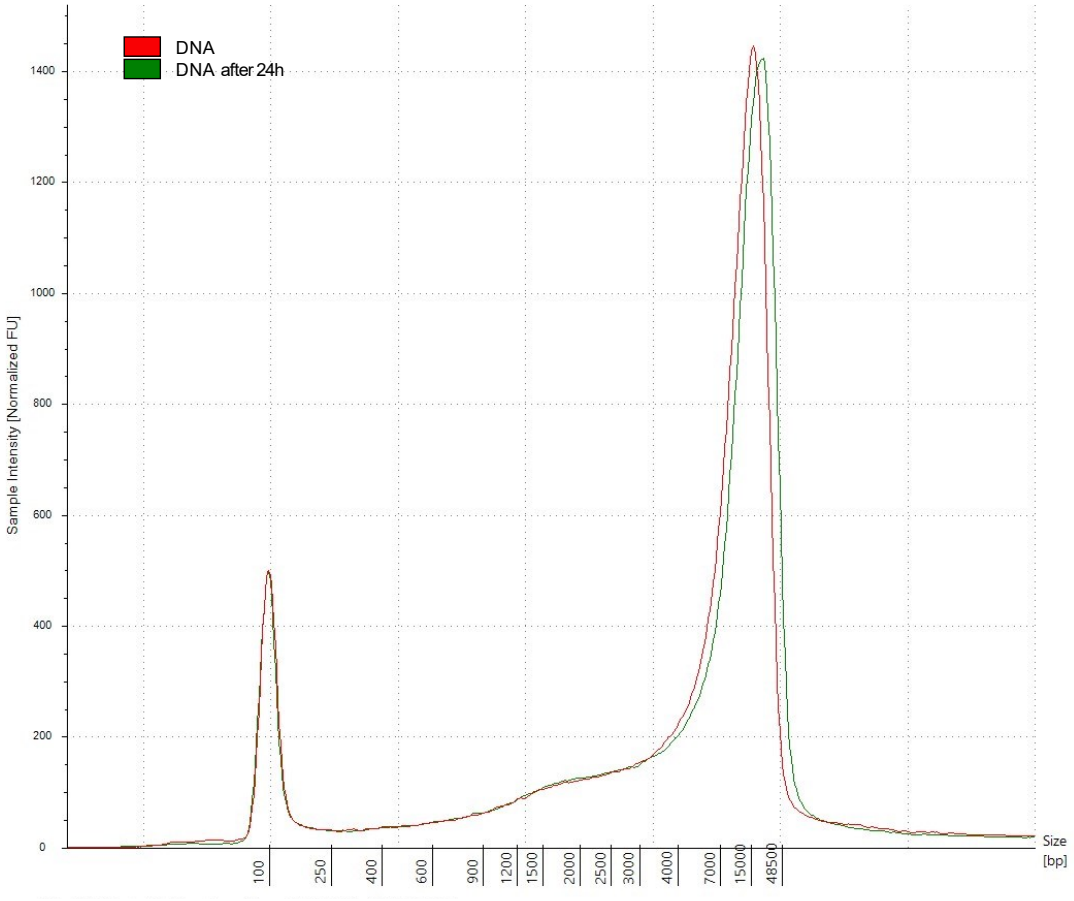

Figure 6.11.1 DNA stability after 24h

6.12 Qubit calibration phosphate

Table 6.12.1 Qubit calibration 0.25 M phosphate

DNA expected [ng/µL]	**DNA in 3mM HEPES [ng/µL]**	**DNA in 0.25 M Phosphate [ng/µL]**
0,5	0.44	0.224
1	0.871	0.625
10	9	6.38
20	17.7	12.6
30	26	18.7
40	34.8	25.8
50	42.6	31.1

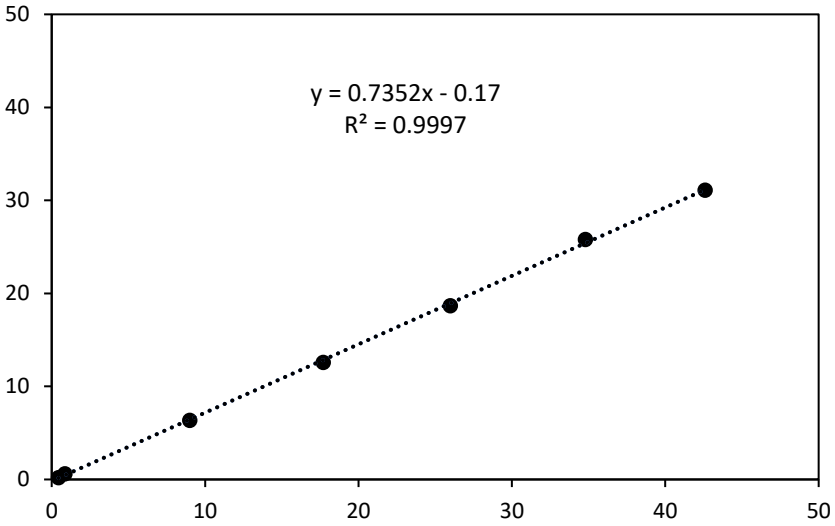

Figure 6.12.1 250mM phosphate pH 8.2 Qubit calibration curve

6.13 Phreeqc modelling

6.13.1 Cu precipitation modelling

Before doing any experiments with Cu(II), the concentrations for the experiments needed to be decided on. The Cu(II) values from the paper by Peackock et al. (Peacock and Sherman, 2004) were based on simulations and tests. Nevertheless, simulations in the software Phreeqc were performed to predict $Cu(OH)_2$ formation. $Cu(OH)_2$ formation would have influenced the adsorption experiments. Results show (Table 6.13.1) no $Cu(OH)_2$ formation for the used Cu(II) concentrations. Although atacamite formation was simulated by Phreeqc, the formation kinetics is far too slow for the experiment timespan. Cu(II)Hydroxide formation Phreeqc simulation

Table 6.13.1 Cu(II)hydroxide formation Phreeqc simulation

Cu Concentration [ppm]	Phase	SI	Log IAP	Log K (298 K, 1 atm)
1	Atacamite $Cu_2(OH)_3Cl$	-5.79	1.60	7.39
	$Cu(OH)_2$	-3.83	4.84	8.67
25	Atacamite $Cu_2(OH)_3Cl$	1.16	8.55	7.39
	$Cu(OH)_2$	-0.35	8.32	8.67
50	Atacamite $Cu_2(OH)_3Cl$	2.90	10.29	7.39
	$Cu(OH)_2$	0.52	9.19	8.67

6.13.2 Cu(II) adsorption onto goethite

Cu adsorption modeling was performed with Phreeqc based on the General Two Layer Surface Complexation model (GTLM) developed by Dzombak and Morrel (Dzombak and Morel, 1991). The application for goethite was developed by MacArthur and Dzombak(Mathur and Dzombak, 2006). The surface complexation constants and the GTLM model used in this work are based on that paper. The model simulates between pH 5- pH 8.5 the adsorption of 1 ppm, 25 ppm, and 50 ppm Cu(II) on 4 mg/ml goethite.

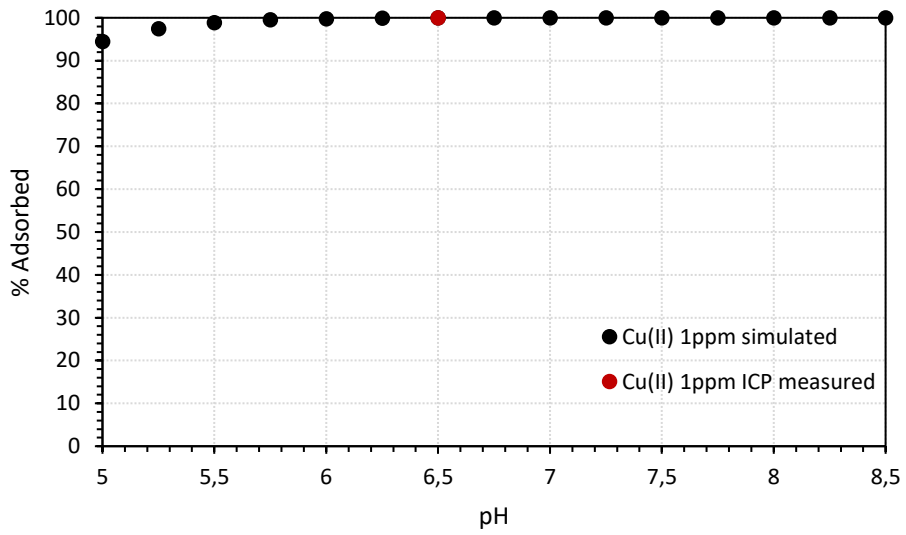

Figure 6.13.1 1 ppm Cu(II) adsorption on goethite phreeqc model

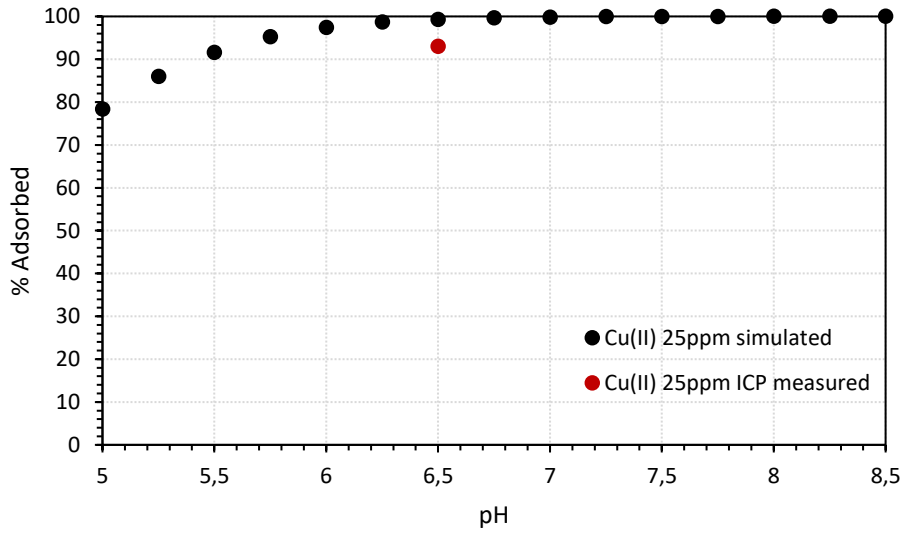

Figure 6.13.2 25ppm Cu(II) adsorption on goethite phreeqc model

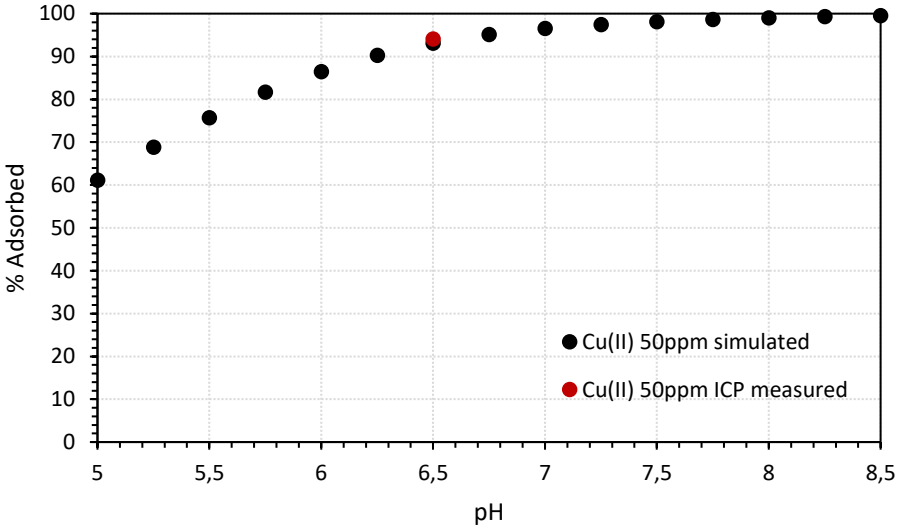

Figure 6.13.3 50ppm Cu(II) adsorption on goethite phreeqc model

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