Spring 4-25-2014

Biosorption of Heavy Metals onto the Surface of Bacteriophage T4

Zheng Huan Tan
University of Nebraska-Lincoln, legendarydragoons@hotmail.com

BIOSORPTION OF HEAVY METALS ONTO THE SURFACE OF BACTERIOPHAGE T4

By

Zheng Huan Tan

A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Biological Sciences

Under the Supervision of Professor Karrie A. Weber

Lincoln, Nebraska

May, 2014

Biosorption of heavy metals onto the surface of bacteriophage T4

Zheng Huan Tan, M.S.

University of Nebraska, 2014

Advisor: Karrie A. Weber

Biosorption of heavy metals by bacterial and eukaryotic cell surfaces and the subsequent transport in aqueous environments is well recognized. However, very little is known about the roles viruses play in biosorption. Viruses outnumber prokaryotes and eukaryotes in environmental systems. These organisms represent abundant nanoparticulate organic colloids with reactive surfaces. Here we conducted a series of experiments to assess the biosorption potential of *Escherichia coli* bacteriophage T4. Adsorption of a heavy metal, Zn^{2+}, to the surface of phage T4 was tested in a series of purified phage/metal solutions (0 μM – 1000 μM at 23°C). The Langmuir isotherm reasonably describes the sorption data, with an R-square of 0.8116. The Langmuir constant was determined to be 0.01265 which demonstrates that the adsorption of zinc onto the surface of phage T4 does occur, but not at a rapid rate. Studies have shown that the phage T4 capsid proteins possess negatively charged binding sites, which are the C-terminus for Soc and the N-terminus for Hoc. These two sites were proven to be biologically active and are able to bind certain proteins and antibodies. Thus, it is likely that these sites adsorb cations. Zeta potential analysis demonstrated phage T4 (10^{10} VLPs mL^{-1}) not exposed to zinc at pH 7.0 to be approximately -11.48 ± 1.16 mV. These results demonstrate the surface of phage T4 is naturally electronegative, which supports the capability of the surface of phage T4 to adsorb metal cations. This was subsequently

demonstrated when the zeta potential shifted to -2.96 ± 1.60mV at pH 7.0 and exposure of 10^{10} VLPs mL^{-1} to 150μM Zn^{2+}, which suggests that adsorption of Zn^{2+} ions onto the phage resulted in the neutralization of negative charges on the phage surface. The effects of pH have been determined to have an effect on the adsorption of Zn^{2+} onto the surface of phage T4. Zn^{2+} adsorption is at a minimum when exposed to acidic pH and the amount of Zn^{2+} adsorbed increases with the rise of pH until a pH of 7.5, where precipitation of zinc hydroxide begins to occur and interferes with the adsorption process. Phage decay can alter the available surface area for metal adsorption. Interestingly, the presence of 150 μM Zn^{2+} significantly increased infectivity relative to unamended controls (ANOVA $p<0.05$), which indicates that Zn^{2+} enhances phage T4 infectivity. Together, the results suggest that the sorption of metals to the surface of viruses could not only contribute to nanoparticulate metal transport but also enhance infectivity that contributes to cell lysis in environmental systems.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor, Dr. Karrie Weber for accepting me as her graduate student and allowing me to complete my research in her lab. Her advice and guidance have helped me immensely throughout the three years of working in the lab. I never would have been able to accomplish all these smoothly without her direction and supervision. Besides that, I would also want to extend my gratitude to my committee members, Dr. Hideaki Moriyama and Dr. Leilani Arthurs for the helpful comments and suggestions they have provided for me during the course of my research. I am especially appreciative of the extra time they have to spend in order to evaluate and help improve my writing and research techniques. In addition, thank you to Dr. Moriyama for allowing me to use his centrifuge, his device benefitted me greatly throughout my research.

There are also a several other individuals that I would like to thank for their ongoing support and continuous assistance. Ph.D. student Donald Pan had provided me with the most aid and advice throughout my entire time at the Weberlab and even before I was a graduate student. He helped me solve many problems that occurred from time to time and my research work would have been much more difficult to complete without him. Many thanks to Abigail Heithoff, our lab technician who helped me a great deal when I had difficulties with certain instruments in the lab. Dr. Javier Seravalli, the person in charge of the mass spectrometry facility at the Beadle Center runs the ICP-MS analysis for most of my samples. I would not have obtained all those valuable data without his expertise at running the instrument. A big thank you to our collaborator, Dr. Yusong Li for allowing me to use her zeta potential analyzer and providing help with the modelling

of the adsorption isotherm. Negin Kananizadeh from Dr. Li's lab who modelled the zinc adsorption isotherm for my adsorption data. Also, Jaewoong Lee from Dr. Li's lab who showed and trained me how to use the zeta potential analyzer which allowed me to run my zeta potential analyses. Thank you to the other graduate students in the Weberlab, Olivia Healy and Jason Nolan for their support and suggestions. Jason helped me by training me in using both the ion chromatograph and gas chromatograph instruments. He also aided me a great deal by spending a huge amount of time at helping me in the geochemical modelling of graphs. Those graphs were able to strengthen the data from my experiments.

Last but definitely not least, I would like to express my gratitude to my family and friends, who have supported me all the way until this point of my life. Their encouragement and constant guidance throughout the years have helped me immensely when things go rough.

This research was supported by United States Department of Energy and University of Nebraska-Lincoln's School of Biological Sciences.

TABLE OF CONTENTS

Page

INTRODUCTION

Viruses are the most abundant organisms on Earth, these biological entities are present almost everywhere life is. They are estimated to be 10^{31} viral like particles in the ocean, outnumbering bacteria by at least a 10:1 ratio and they are capable of infecting all types of organisms, from eukaryotes to archaea (Debenedetti & Stanley, 2003; Fuhrman, 1999). Yet, a majority of these viruses are those that infect bacteria, or more appropriately known as bacteriophages (Suttle, 2007; Weinbauer, 2004). These microbial predators are omnipresent, be it inside vast marine environments or even deep down under the ground, they can be found everywhere and with that, there is little doubt that their presence provide a significant impact on biogeochemical cycles throughout all these environments. However, their role in such important aspects of life is overlooked most of the times, in which many questions or issues where viruses come into play are still left unanswered.

Viruses are submicron particles that are made up of single-stranded or double-stranded DNA or RNA covered by a protein capsid, most phages however, contain double-stranded DNA (Calendar, 2006). Enterobacteria phages T4 are one of the most common bacteriophages that infect the bacteria *Escherichia coli*. Phage T4 belong to the order of *Caudovirales* or more commonly known as tailed phages. These type of phages possess a tail structure that are used for attachment onto their bacterial hosts and also the injection of their genetic materials into their hosts (Ackermann, 2003). Although all viruses in the *Caudovirales* order have the tail structure, bacteriophage T4 which belong to the family *Myoviridae* possess a much more complex tail compared to the other viruses in the similar order, in which the tail is comprised of more than 20 proteins that are

present throughout the structure (De Rosier & Klug, 1968). When the virus is infecting the host cell, the baseplate structure of the tail is utilized to attach itself to the surface of the host and this causes the virus to undergo conformational change which enables the injecting of its DNA into the host (Rossmann, Mesyanzhinov, Arisaka, & Leiman, 2004). This baseplate structure consists mainly of fibrous proteins (Kostyuchenko et al., 2003) and the locations and shapes of various baseplate proteins are determined, in which several crystal structures of such proteins having been established in previous researches (Kanamaru et al., 2002; Kostyuchenko et al., 1999).

A mature virus is made up of more than 40 polypeptides which not only include the baseplate and tail, but other structural components as well, such as the head or capsid, the neck and a several other parts (Karam & Drake, 1994). The double-stranded DNA of the bacteriophage is encapsulated within the capsid, which is essentially a protein shell that consists of a major capsid protein, the pentameric vertex protein, a connector protein and two accessory proteins (Karam & Drake, 1994). These protein coated surfaces of viruses are reactive surfaces that are able to adsorb heavy metals/radionuclides (HMR) present in the environment, in which certain amino acid groups and carboxyl groups on the surfaces or cell walls of bacteria have already been proven to adsorb metals (Beveridge & Murray, 1980; Borrok, Fein, & Kulpa, 2004; Fein, Daughney, Yee, & Davis, 1997; Yee & Fein, 2001). Two major proteins, the Hoc and Soc capsid proteins for bacteriophage T4 possess negatively charged binding sites, which are the C-terminus for the Soc protein and the N-terminus for the Hoc protein. These two sites have been proven to be biologically active in studies and that they are able to bind certain proteins and antibodies (Ren & Black, 1998). Thus, it is possible for positively charged metal

cations to adsorb onto these negatively charged sites. Numerous studies have been conducted in the past on adsorption of metals onto bacterial surfaces, both gram positives and gram negatives (Beveridge & Koval, 1981; Beveridge & Murray, 1976), thus proving the possibility of metal adsorption onto viruses in a similar environment. Besides that, metal-ion protein interactions have been demonstrated to occur on the nucleocapsid of retroviruses, in which there is a high binding affinity between the proteins on the capsid with divalent metal cations, such as Co^{2+} and Zn^{2+} (Green & Berg, 1990).

Many other bacteriophages that are similar to the T4, such as the T2 and T6 or now just categorized as the "T-even" bacteriophages can be found in many different habitats from all over the globe, in which they possess similar gene sequences of essential genes, regulatory mechanisms and a few other structural aspects (Karam & Drake, 1994). Their presences are so ubiquitous that they infect various types of bacteria, ranging from not only those in the gut of mammalian species, to bacteria in aquatic environments and even those that can be found in our food (Calendar, 2006; Hagens & Loessner, 2007). This shows that HMR adsorption can occur in almost every possible environment as long as both HMRs and these viruses are present. In actual fact, conventional transmission electron microscopy techniques have relied on using heavy metals and uranium, which is a radionuclide for viral stains (Arnott & Smith, 1968).This further confirms the possibility of HMRs adsorbing onto viruses. HMRs that adsorb onto the surface of these viruses in can be mobilized in the forms of nanoparticulate colloids, travelling to various locations. These HMRs can then be transported not only to ground water from soils and sediments, but to and from any aqueous environments when adsorped onto viral surfaces (Drewry & Eliassen, 1968; Novikov et al., 2006). On the other hand, there were studies

done on iron precipitation onto marine viruses in the sea which suggests that adsorption of metals onto viruses in marine environments are possible as well (Daughney et al., 2004).

The mechanism and occurrences of HMR adsorption onto viruses is still poorly understood but the effects can no doubt bring upon significant impacts to several aspects in the environment. The first and foremost would be the effects that HMR adsorption have on the viruses' rate of infectivity on their bacterial hosts, mainly by affecting the attachment phase during the infection process. Protein interactions that occur during the attachment causes conformational changes in the baseplate and sheath structures (Kellenberger, 1980; Leiman, Kanamaru, Mesyanzhinov, Arisaka, & Rossmann, 2003), tail fibers that are stored in the whiskers are also ejected for irreversible attachment onto the bacterial surfaces, by recognizing sites on the lipopolysaccharide layers (Goldberg, Grinius and Letellier 1994) or certain outer membrane proteins depending on the type of viruses involved (Henning and Hosseini 1994). If HMRs were to adsorb onto the surface proteins on the tails or baseplates structures of the viruses, they might interfere with the recognition mechanisms and subsequently, hinder the attachment phase and vice-versa, in which certain HMRs might actually impede attachment of the viruses onto the bacteria. On the other hand, Mn^{2+}, Co^{2+}, Zn^{2+} and several other divalent metal cations are believed to be able to stabilize the structures of bacterial viruses, such as phage T5 by forming a complex between the phage structure and the metal ion, in which the rate of phage inactivation was seen to decrease when the viruses were incubated with the metal cations (Adams, 1949b). Besides that, divalent cations such as Ca^{2+} have been demonstrated to possess protective effects on bacteriophages, such as phage T4, T5, Xp12 and perhaps

several other viruses (Adams, 1949a; Chow, Lin, & Kuo, 1971). In both cases, HMR adsorption will ultimately affect the rate of infection of the viruses on their bacterial hosts.

The variation in rates of viral infection on microbes will bring about significant impacts in the environment and especially the biogeochemical cycles that are already in place that keep microbial life around us going. The availability of not just carbon but many other nutrients are extremely dependent upon microbial mortality, in which the latter can be highly influenced by viral induced lysis (Weitz & Wilhelm, 2012). Lysis of microbes in the ecosystem will result in the release of organic carbon and other nutrients back into the biogeochemical cycles that are present in the environment. This can both further drive or impede these cycles depending upon the amount or types of nutrients available. On the other hand, viral induced lysis may also increase the rate of horizontal gene transfer among microbial communities in the environment (Anderson, Brazelton, & Baross, 2013). Transformation can occur when free floating genetic materials released during microbial lysis is taken up by other microbes within the vicinity. Viruses can also aid in the occurrences of transduction by transferring genetic materials between the microbial communities present in a given environment or ecosystem.

Therefore, a model for HMR adsorption onto viruses will better allow the quantification of HMR adsorption and also a more in depth understanding of its effects on the rate of infections on their bacterial hosts. So, I propose to study the adsorption of a non-redox sensitive divalent metal cation, zinc and and other potential HMRs, such as lead and uranium, onto the surface of *Escherichia coli* bacteriophage T4 in order to elucidate a model for HMR adsorption onto viruses. Not only will we be able to get a better grasp about the true nature of virus-metal ion interaction, it will also be possible to

further determine the implications of these interactions on the fate of biogeochemical cycles and other microbially mediated processes in the vast world that we live in.

MATERIALS AND METHODS

Zinc Adsorption Experiment

A frozen stock of host bacteria (*Esherichia coli* (Migula) Castellani and Chalmers ATCC® 11303™) were inoculated in a flask containing Luria broth media and incubated in a shaking incubator at 37°C to mid log phase. After that, a frozen stock of E.coli phage T4 (T4 Bacteriophage ATCC® 11303-B4™) was added to the host bacteria and incubated once more for virus production. The optical density (OD) of both host bacteria and virus production was obtained by using a spectrophotometer and thus, determining the growth phase of the host bacteria and also confirmation of virus production (indicated by the decrease of OD). In order to separate the viruses from cells and cell debris, they were subjected to centrifugation at 10,000x g for 20 minutes. The supernatant was then filtered through a 0.2µm PVDF filter to remove remaining cells and cell debris from the viruses (<0.2 µm) and concentrated using a 100kDa molecular weight cutoff filter (Amicon® Ultra-15 Centrifugal Filters). The concentrated viral fraction (<0.2 µm to 100 kDa) was added to PIPES buffer, pH 7.0 that contained the Zn^{2+} (final concentrations ranging from 0 µM – 1000 µM) to a final abundance of (ca.10^7 VLPs/mL). The experiment was conducted in duplicate and the phage/metal solutions were incubated on an orbital shaker at 100 rpm for 18 hours at 23°C. Two types of samples were collected for each experimental tube, a filtered one and an unfiltered one. The filtered sample was prepared by filtering 0.5 mL of sample through a 0.025µm mixed cellulose ester filter membrane into 0.5 mL of nitric acid (Omni*Trace* Ultra™ 67-70% Nitric acid). We chose to use these types of filter membranes because of the negligible amount of zinc that adsorbed

onto the filter membranes (Figure A-4). The unfiltered sample was obtained by pipetting 0.5 mL of a similar sample into 0.5 mL of nitric acid.

After obtaining the data, we calculated the amount of zinc for the filtered and unfiltered samples. The amount for the filtered samples will always be lower than that of the unfiltered samples and were calculated in terms of μmoles. In order to quantify the amount of zinc adsorbed onto the viruses, we would just deduct the value of the filtered samples from the unfiltered samples. For all zinc adsorption experiments, there was always an increasing trend in the amount of zinc adsorbed, where the higher amount of zinc is provided, the more zinc is adsorbed onto the surface of viruses. These values are calculated and recorded on a Microsoft Excel worksheet.

We would then proceed to normalize the amount of zinc adsorbed onto the viruses to 10^{10} viral like particles per ml (VLPs/mL). In order to do that, the enumeration of viruses was done via flow cytometry, where the samples within the range of 200-1000 events per second are recorded and saved. The amount of viral like particles for every sample were then obtained by calculating the total number of gated events, while taking into account the dilution factor and flow rate for each respective sample and background noise generated by a negative control.

Sorption isotherm models were applied to fit the sorption of zinc onto the viral surfaces. Both the Freundlich and Langmuir isotherm were able to fit the sorption data. MATLAB 2013b Curve Fitting Toolbox 3.4 (The MathWorks, Inc) was used to conduct the curve fitting. Trust Region algorithm for nonlinear least squares optimization was

applied to minimize the least absolute residuals. This step was performed by our collaborator, Dr. Yusong Li and her doctoral student, Negin Kananizadeh.

There are a few assumptions that were taken into consideration during the modelling of the Langmuir isotherm. Firstly, all of the adsorption sites are assumed to be equivalent and each of these sites can only accommodate one molecule. Second, the surface where adsorption occurs is energetically homogeneous and the adsorbed molecules do not interact with each other. Third, there are occurrences of phase transitions. And the last one, adsorption only occurs on localized sites on the surface, not with other adsorbates (Czepirski, Balys, & Komorowska-Czepirska, 2000).

pH Zinc Adosorption Experiment

The experiment was conducted in almost exactly the same way as the regular zinc adsorption experiment described above. The only difference was that these sets of experiments were conducted in a series of different pH instead of a varying range of Zn^{2+} concentrations. The concentrated viral fraction was added to tubes that have varying buffers ranging from pH 4- pH 9, where pH 4 & 5 (20 mM PIPPS), pH 6, 7 & 7.5 (20 mM PIPES) and lastly pH 8 & 9 (20 mM HEPES). Each experimental tube was amended with 150 μM zinc as we have determined that amount of provided zinc to produce the least variability from the results of previous zinc adsorption experiments. The rest of the experiment and data collection procedure were carried out just as described above.

Epifluorescence Microscopy

Right after the viruses were concentrated with the 100 kDa molecular weight cutoff filter, they were prepared for enumeration via epifluorescence microscopy (Hobbie, Daley, & Jasper, 1977; Noble & Fuhrman, 1998). Approximately 5 µL of the concentrated viruses were extracted and serially diluted to 10^{-4} dilution with TE buffer (10 mM Tris, 1mM EDTA, pH 8.0). The diluted samples were then filtered onto a 0.02 µm Anodisc filter using a filter tower apparatus (Figure 1). Once the viruses were collected on the filter, the filter was stained with SYBR Green I dye (1:40 dilution) for 12 minutes. Excess dye is washed off the filter with deionized water. An antifade solution (1µL 10% phenylenediamine solution + 99µL of glycerol-phosphate buffer saline mixture) was applied onto an empty microscope slide before placing the stained filter atop of it, in order to prevent the permanent loss of fluorescence due to prolonged exposure to high intensity light sources. The filter was then covered with a cover glass after another 20µL of antifade solution was applied on top of the filter. The filter was observed under the 100x objective lens, where the viral-like particles (fluorescent particles under the microscope) were counted in 10 random fields (20-80 fluorescent particles) per field on the filter (with a Zeiss Axioskop 40 upright microscope that is equipped with an epifluorescent light source and phase contrast ring). The total number of fluorescent particles was back calculated in order to estimate the number of viruses concentrated in the molecular weight cutoff filter (Equation 1).

$$\frac{Number\ of\ VLPs}{Number\ of\ fields\ counted} x \frac{Number\ of\ fields\ counted}{Area\ of\ each\ field} x \frac{Area\ of\ filter\ with\ viruses}{Volume\ of\ viruses} \quad (Equation\ 1)$$

Flow Cytometry

Samples from the zinc adsorption experiment were removed preserved between 2-3 hours after the viruses were added to the zinc/metal solutions for the viruses to homogenize within the solution and immediately fixed with glutaraldehyde (0.5% final concentration). This was done where 200 μL of each sample was pipetted into separate 1.5 mL centrifuge tubes and treated with glutaraldehyde (0.5% final concentration) for 15-25 minutes at 4°C before being flash frozen with liquid nitrogen and finally, stored at -80°C. The samples were enumerated within the week of sample collection.

Immediately prior to flow cytometry analysis, frozen samples were thawed and diluted in TE buffer(10 mM Tris, 1mM EDTA, pH 8.0) and sstained with SYBR Green I dye (1:2000 dilution). Stained samples were heated (70-80°C for 10 minutes) and cooled for 5 minutes at room temperature (23°C). The samples were then enumerated with a BD FACSCantoII flow cytometer (488 nm, 20-mW solid state laser; Franklin Lakes, NJ, USA). The trigger was set to green fluorescence with a one minute acquisition time at a rate of 200-1000 events per second (Brussaard, 2009), with a detection limit between 10^3-10^4 viruses per mL. Fluorescence from a negative control in the form of SYBR Green stained TE buffer (without a virus addition) was subtracted from the sample counts in order to remove enumerated background events. This technique had been demonstrated to provide viral counts that are consistent with plaque assays or phage titers in other studies (Gates et al., 2009).

Bacteriophage Titer

Bacteriophage titers were performed to assess the infectivity of viruses following metal exposure. The viruses were cultivated as described above (Zinc Adsorption Experiment), purified, and added to experimental tubes that consisted of four different treatments, negative control (20 mM PIPES buffer at pH 7), 150µM Zn, 150µM Zn + 300µM EDTA and 150µM NaCl. Virus-metal solutions were incubated at room temperature (23°C) for 18 hours, the viruses were extracted and serially diluted.

Bacteriophage titers were performed using the double agar overlay method on the serial dilutions with *Escherichia coli* as the host bacteria, initially grown to mid-log phase (Dulbecco & Vogt, 1953; Kropinski, Mazzocco, Waddell, Lingohr, & Johnson, 2009). Both the host bacteria and viruses at the desired dilution were added to a tube of soft overlay agar (agar final concentration 0.4-0.6%). The contents of the tube were then poured onto a hard underlay agar (agar final concentration 1.5%) and allowed to cool for 5-10 minutes at room temperature (23°C). The procedure was repeated for all samples and replicates that were to be studied before the plates are placed in a 37°C incubator for 2 hours.

After incubation, the number of clearings or plaque forming units (PFUs) was counted and and converted to PFUs/mL as noted in Equation 2.

$$\frac{PFUs}{mL} = \frac{PFUs\ x\ Dilution\ factor}{Volume\ of\ viruses\ added} \quad \text{(Equation 2)}$$

The results were then compared using a 2-way ANOVA analysis in order to demonstrate the effect of each treatments on the infectivity of the viruses.

The virus-metal solution was incubated on an orbital shaker for 18 hours to ensure that all the adsorptive sites on the viruses were fully saturated. However, phage decay could generate artifactually low titers (Gersberg, Lyon, Brenner, & Elkins, 1987). (Gersberg et al., 1987)In order to assess the significance of phage decay under the experimental conditions noted above I conducted a decay experiment over a 48 hour time period. The experiment was conducted in duplicate where the double agar overlay method described above was performed just for untreated viruses. Samples were collected and bacteriophage were enumerated at 6 time points over a 48 hour period, 0, 4, 8, 19, 24 and 48 hours .

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

The samples extracted from the zinc adsorption experiment mentioned above were then subjected to analysis via ICP-MS, in order to quantify the amount of zinc or other metals that have adsorbed onto the viruses. The samples are diluted 20 fold with a prepared dilution solution (52.6 ppb Ga in 0.1% nitric acid) by Dr. Seravalli from the Redox Biology Center in the Beadle center, who subsequently performs the analysis of our samples. The instrument used for this purpose was the Agilent Technologies, Inc. ICP-MS Model 7500 series, with a lower detection limit for zinc analyses to average around 0.22 μM.

Zeta Potential Analysis

Samples for the zeta potential analysis were prepared in triplicate. Each experimental tube that contain 150 μM of Zn^{2+} was amended with 10^{10} viruses/mL and vortexed in order homogenize the solutions. Approximately 3 mLs of sample were measured and poured into a cuvette, then ran using a NanoBrook ZetaPlus zeta potential analyzer from Brookhaven Instruments. The instrument is capable of determining zeta potentials for any given sample via phase analysis light scattering. The data from the zeta potential analysis is obtained from the computer connected to the instrument and recorded. The recorded results were analayzed and the average values of zeta potential for the samples were calculated.

RESULTS

Zinc Adsorption Experiment

The surface of bacteriophage T4 will adsorb Zn^{2+}. The amount of Zn^{2+} adsorption to the T4 surface increased with the addition of higher concentrations of Zn^{2+} (Figure 2). Maximum adsorption was observed with the addition of 619.57 μM of Zn^{2+} resulting in the adsorption of 7.5 μmoles/10^{10} VLPs. This data suggests that the negatively charged available sites are filled with cations. Experimentally the addition of 1274.56 μM of a ZnCl solution suggested significantly higher adsorption. But, precipitation of zinc hydroxide from the excess zinc in the solution would possibly lead to artifactully high adsorption data (Albrecht, 2011). There is indeed variability among the data throughout the various adsorption points of varying concentrations. This may be due to an artifact caused by some amount of zinc adsorbed onto the filters and at higher concentrations, there may be a higher amount of zinc adsorbed to the filters.

Both a Langmuir and a Freundlich isotherm was modelled for the biosorption of zinc onto the surface of bacteriophage T4 (Figure 3). The data point for the addition of 1274.56 μM of ZnCl was not included from the modelling of the adsorption isotherms. It was determined through nonlinear regression analysis that that data point was a statistical outlier that possesses a huge error bar. With the removal of that data point, the Langmuir was determined to be a better fit for the adsorption isotherm with an R^2 of 0.8116 when compared to the Freundlich which only possesses an R^2 of 0.7608. The Langmuir isotherm has a constant of 0.01265 where it portrays a slow adsorption rate of Zn^{2+} onto the surface of phage T4 until the point where it plateaus at 8.693 μmoles/10^{10} VLPs. This was determined to be the maximum adsorption point where all the adsorption sites on the

viral surface have been filled with Zn^{2+}. The four assumptions of the Langmuir isotherm may or may not entirely apply with the adsorption onto the viral surface of phage T4 since biological entities rarely have perfect surfaces, which is a part of nature.

pH Zinc Adsorption Experiment

The pH of the aqueous solution was determined to have an effect on the adsorption of Zn^{2+} to the surface of bacteriophage T4. The adsorption of Zn^{2+} was evaluated from pH 4-7.5. Adsorption of Zn^{2+} to the surface of bacteriophage T4 increased with an increase in pH (Figure 4). The lowest amount of zinc adsorbed was observed at pH 4, with an average of 2.4 μmoles/10^{10} VLPs adsorbed onto the viruses. On the other hand, the highest amount of zinc adsorption was observed at pH 7.5 which was an average of 6.3 μmoles/10^{10} VLPs adsorbed. Above a pH of 7.5 significant precipitation, presumably zinc hydroxide, resulted in the form of aqueous Zn^{2+} (Figure A-2).. The Bjerrum plot of the aqueous medium (20 mM PIPPS buffer, 20 mM PIPES buffer and water) modeled a decrease in Zn^{2+} at a pH above 7.5. This decrease in Zn^{2+} is concurrent with the precipitation of zinc hydroxide (Figure A-3).

$$Zn^{2+} + 2H_2O \rightarrow Zn(OH)_2 + 2H^+ \ (Equation\ 3)$$

This is consistent with visual observations of precipitates in the tube as well as the experimental data (Figure A-2). Thus, due to the precipitation of zinc hydroxide, we were unable to accurately measure the adsorption of Zn^{2+} to the surface of bacteriophage T4 at pH values exceeding 7.5. This demonstrates that the excess protons in the acidic

solutions are competing with the Zn^{2+} for the negatively charged sites on the surface of the viruses and thus, the decrease in the amount of zinc adsorbed with the decrease in pH.

Bacteriophage Titers

Bacteriophage titer results from our phage decay experiment indicate that phage decay wasn't a significant issue within a 24 hour time period for our adsorption experiments. We counted the number of PFUs (plaque forming units that can be observed visually on the plates) from the titer plates, then converted the amount to PFUs/mL (unit for infective viruses per mL) and plotted a graph in order to visualize the data (Figure A-1). At t=0, there was only approximately 10^7 PFUs/mL, which was much lower compared to the other time points. We allowed the viruses to homogenize within the solution before performing another titer at t=4 and discovered the number of PFUs/mL to be much more consistent after that, which was around 10^8 PFUs/mL. The PFUs/mL at t=4, 8, 18 and 20 were not significantly different and were determined to be approximately 10^8 PFUs/mL. Thus, we were able to dispel our earlier concerns about any data inaccuracies that may have been caused by phage decay. A significant drop in the number of PFUs/mL at t=48 suggests that significant phage decay only occurs at least after 24 hours of incubation.

The adsorption of Zn^{2+} onto the surface of bacteriophage T4 increases its infectivity on bacterial host, *E.coli*. The number of PFUs counted from the plates in the triplicate experiment at both time points, t=4 and t=20 showed that at both time points, the counts for the 150 μM Zn yielded at least 2 times more counts compared to the other treatments (Figure 5). The number of PFUs for the -5 dilution plates was then converted

to PFUs/mL (Figure 6). The bar graph shows that at both time points, viruses that were treated with only 150µM Zn had much higher PFUs/mL than all the other treatments. The results from the plaque assays have indicated that the adsorption of zinc onto the surface of phage T4 enhances the infectivity of the virus on its bacterial host. Bacteriophage T4 when exposed to 150µM Zn has a significantly higher infectivity when compared to the other treatments, which are the negative control, EDTA and 150µM NaCl. The results show that when exposed to only zinc, the bacteriophage titer yielded around 10^8 PFUs/mL, whereas all the other treatments only yielded around 10^7 PFUs/mL. Based on a 2-way ANOVA anaylsis of the results, the difference of a single magnitude between the two treatments has demonstrated the effect of zinc to be significant on the infectivity of phage T4. The EDTA treatment was added in order to chelate the zinc away from the surface of the viruses and to show that zinc was indeed the one that's increasing the infectivity, which was successfully shown in the results. The NaCl treatment was included in order to demonstrate that the high salt content in the Luria broth growth media used to cultivate both the hosts and viruses was not the cause of increase in phage T4 infectivity, where a higher salt content did not result in a significant enhancement of phage infection.

Zeta Potential Analysis

Zeta potential analysis revealed the surface of bacteriophage T4 to be negatively charged. The analysis from the results demonstrated the zeta potential of phage T4 (10^{10} VLPs mL^{-1}) not exposed to zinc at pH 7.0 to be approximately -11.48 ± 1.16 mV. These

results demonstrate the surface of phage T4 is naturally electronegative, which supports the capability of the surface of *E. coli* bacteriophage T4 to adsorb positively charged ions or cations. This was subsequently demonstrated when the zeta potential shifted to -2.96 ± 1.60 mV at pH 7.0 and exposure of 10^{10} VLPs mL^{-1} to 150µM Zn^{2+}, which suggests that adsorption of Zn^{2+} ions onto the phage resulted in the neutralization of negative charges on the phage surface.

DISCUSSION

The results from the zinc adsorption experiments are able to show that there are indeed adsorption sites on the surface of bacteriophage T4. These sites are found to be negatively charged through the zeta potential analysis and thus, demonstrating the capabilities of these viruses at adsorbing positively charged metal cations, such as zinc for our experiment. It is highly possible that other heavy metals, such as copper, lead and various other metals that form cations in aqueous environments are able to adsorb onto the surface of viruses similar to the surface of bacteria, which have been extensively studied on. The Fruendlich isotherm that was modeled based on our zinc adsorption data is also strongly supported by the identification of two different proteins, Hoc and Soc, on the outer surface of the capsid. The Hoc and Soc capsid proteins of phage T4 possess negatively charged binding sites, which are the C-terminus for the Soc protein and the N-terminus for the Hoc protein(Ren & Black, 1998).(Ren & Black, 1998) (Ren & Black, 1998)These two sites have been proven to be biologically active in studies and that they are able to bind certain proteins and antibodies (Ren & Black, 1998). Thus, it is likely that these negatively charged binding sites adsorb positively charged metal cations. And if this were to be true, there will be quite a number of significant impacts on the environment and ecosystem that house the microbial life which consists of not only just the bacteria and viruses.

First and foremost, the adsorption of heavy metals onto the surface of viruses will play a major role in contaminant mobility in subsurface environments. With the fact that the amount of viruses outnumbers the amount of bacteria by a 10:1 ratio, it is highly evident that viruses will impact the environment more significantly than bacteria

(Fuhrman, 1999). It is known that biogeochemical cycles highly influence the presence of heavy metals and other chemicals in the soil and the microbes that drive these cycles contribute greatly to the mobility of these metals and chemicals in the subsurface environment (Hesterberg, 1998). Studies have also shown that microbes can be responsible for both the mobilization and immobilization of heavy metals in the subsurface, which influences the storage and release of these metals in the environment that these microbes are present in (Boult, Hand, & Vaughan, 2006). This demonstrates the potential of viruses which are much more abundant than bacteria, to be a potential player in contaminant mobility in subsurface environments.

The heavy metals in soils and subsurface environments may not just be present due to natural causes or biogeochemical cycles, as there are also instances where soils are contaminated by accidental spills or improper disposals by certain individuals. Bioaccumulation of these heavy metals and also biosorption by microbial biomass have been determined to influence the mobility of such contaminants present in the soils (Ahmad, Hayat, & Pichtel, 2005). Viruses that are ubiquitous in these environments and possess reactive surfaces would promote adsorption of heavy metals onto their surfaces and subsequently, have a major effect on the mobilization of these metals. Biosorption of heavy metals and radionuclides have long been associated with microbially mediated bioremediation techniques (McHale & McHale, 1994). Further enhancements to any of these techniques could be discovered with the added knowledge of viruses as a prospective tool that can be utilized in any biosorption strategies of these contaminants in the environment.

The biosorption of heavy metals onto the viruses affect their infectivity in different ways. Our results from the phage titer experiments demonstrate that the adsorption of zinc onto the viruses increases the infectivity of its bacterial host significantly. This agrees with the findings of most studies out there which have proven that most divalent metal cations are able to help increase or enhance the infectivity of bacteriophages (Adams, 1949b). When the infectivity of viruses increases, this means that there will be a rise in the occurrences of bacterial lysis in the ecosystem, which could bring about the release of organic carbon and nutrients into the environment. This will strongly impact the biogeochemical cycles that consist of not just prokaryotic organisms, but eukaryotic ones as well. There are various ecological effects that could stem from viral activities in numerous ecosystems. The role of viruses in biogeochemical cycles that are present in marine systems have been studied and discussed for a long period of time, but the actual impact that they bring about have not been quantified on a global scale(Fuhrman, 1999). Their roles include not only the regeneration, release and storage of organic carbon and other nutrients that drive the biogeochemical cycles that are already in place since the beginning of time, it is evident that viruses are no doubt major players in all of these environments (Weitz & Wilhelm, 2012).

The abundance of microbial communities may also be affected viral lysis when a species of microbes are targeted by bacteriophages in the environment (Thingstad & Lignell, 1997). So with knowing the fact that the adsorption of heavy metals onto the surface of viruses will enhance infection of their bacterial hosts, this will significantly impact an environmental system, especially where viral lysis is a predominant cause of bacterial mortality (Wilhelm, Brigden, & Suttle, 2002; Winget & Wommack,

2009).(Thingstad & Lignell, 1997) It is also known that marine planktons are dependent upon the bioavailability of iron that can be increased significantly with the viral activity in these systems, such as viral release of iron and the cycling of iron that are mediated by viruses (Poorvin, Rinta-Kanto, Hutchins, & Wilhelm, 2004).

On top of that, the presence of viruses is usually tied with the concept of horizontal gene flow in the ecosystem. Viruses are able to facilitate the transfer of genetic materials between the microbial communities present in all systems through direct transformation, transduction or even pathways that have yet to be discovered (Syvanen, 1994). The rise in infection rates due to the adsorption of heavy metals onto the surface of these viruses may very well increase the probability of horizontal gene flow occurring within these microbial communities. Various studies have shown the presence of alien genes within the genome of microbes that may have only been acquired from bacteriophages through horizontal gene transfer, one example would be a significant deviation found in the genome of E.coli from 15% of this organism's general pattern of codon usage (Medigue, Rouxel, Vigier, Henaut, & Danchin, 1991). This shows that the enhanced infectivity of viruses through the adsorption of heavy metals will cause their presence to be a major part in horizontal gene transfer between microbial communities in almost every single ecosystem present on Earth.

My thesis presents the initial findings that address the issue of biosorption of heavy metals onto the surface of viruses, using zinc and bacteriophage T4 as a model. We've been able to determine that adsorption of heavy metals onto the surface of virsues does indeed occur. Hopefully from there onwards, our findings will be able to help expand research throughout this field, where the adsorption of other metals and

radionuclides can be assessed in order to determine any significant impacts it will have in the environment.

BIBLIOGRAPHY

Ackermann, H. W. (2003). Bacteriophage observations and evolution. *Res Microbiol, 154*(4), 245-251. doi: 10.1016/S0923-2508(03)00067-6

Adams, M. H. (1949a). The calcium requirement of coliphage T5. *J Immunol, 62*(4), 505-516.

Adams, M. H. (1949b). The stability of bacterial viruses in solutions of salts. *J Gen Physiol, 32*(5), 579-594.

Ahmad, I., Hayat, S., & Pichtel, J. (2005). *Heavy metal contamination of soil : problems and remedies*. Enfield, NH: Science Publishers.

Albrecht, T. W. J. A.-M., Jonas; Fornasiero, Daniel (2011). Effect of pH, concentration and temperature on copper and zinc hydroxide formation/precipitation in solution *In: Chemeca 2011: Engineering a Better World: Sydney Hilton Hotel, NSW, Australia, 18-21 September 2011. Barton, A.C.T.: Engineers Australia, 2011: [2100]-[2110]*.

Anderson, R. E., Brazelton, W. J., & Baross, J. A. (2013). The Deep Viriosphere: Assessing the Viral Impact on Microbial Community Dynamics in the Deep Subsurface. *Carbon in Earth, 75*, 649-675. doi: DOI 10.2138/rmg.2013.75.20

Arnott, H. J., & Smith, K. M. (1968). Electron microscopic observations on the apparent replication in vivo of a plant virus. *Virology, 34*(1), 25-35.

Beveridge, T. J., & Koval, S. F. (1981). Binding of metals to cell envelopes of Escherichia coli K-12. *Appl Environ Microbiol, 42*(2), 325-335.

Beveridge, T. J., & Murray, R. G. (1976). Uptake and retention of metals by cell walls of Bacillus subtilis. *J Bacteriol, 127*(3), 1502-1518.

Beveridge, T. J., & Murray, R. G. (1980). Sites of metal deposition in the cell wall of Bacillus subtilis. *J Bacteriol, 141*(2), 876-887.

Borrok, D. M., Fein, J. B., & Kulpa, C. F., Jr. (2004). Cd and proton adsorption onto bacterial consortia grown from industrial wastes and contaminated geologic settings. *Environ Sci Technol, 38*(21), 5656-5664.

Boult, S., Hand, V. L., & Vaughan, D. J. (2006). Microbial controls on metal mobility under the low nutrient fluxes found throughout the subsurface. *Sci Total Environ, 372*(1), 299-305. doi: 10.1016/j.scitotenv.2006.08.043

Brussaard, C. P. (2009). Enumeration of bacteriophages using flow cytometry. *Methods Mol Biol, 501*, 97-111. doi: 10.1007/978-1-60327-164-6_11

Calendar, R. (2006). *The bacteriophages* (2nd ed.). Oxford ; New York: Oxford University Press.

Chow, T. Y., Lin, Y. T., & Kuo, T. T. (1971). Stability of Phage Xp12. *Botanical Bulletin of Academia Sinica, 12*(2), 57-&.

Czepirski, L., Balys, M. R., & Komorowska-Czepirska, E. (2000). Some generalization of Langmuir adsorption isotherm. *Internet Journal of Chemistry, 3*(14), U3-+.

Daughney, C. J., Châtellier, X., Chan, A., Kenward, P., Fortin, D., Suttle, C. A., & Fowle, D. A. (2004). Adsorption and precipitation of iron from seawater on a marine bacteriophage (PWH3A-P1). *Marine Chemistry, 91*(1–4), 101-115. doi: http://dx.doi.org/10.1016/j.marchem.2004.06.003

De Rosier, D. J., & Klug, A. (1968). Reconstruction of three dimensional structures from electron micrographs. *Nature, 217*(5124), 130-134.

Debenedetti, P. G., & Stanley, H. E. (2003). Supercooled and glassy water. *Physics Today, 56*(6), 40-46. doi: Doi 10.1063/1.1595053

Drewry, W. A., & Eliassen, R. (1968). Virus movement in groundwater. *J Water Pollut Control Fed*, Suppl:257-271.

Dulbecco, R., & Vogt, M. (1953). Some Problems of Animal Virology as Studied by the Plaque Technique. *Cold Spring Harbor Symposia on Quantitative Biology, 18*, 273-279.

Fein, J. B., Daughney, C. J., Yee, N., & Davis, T. A. (1997). A chemical equilibrium model for metal adsorption onto bacterial surfaces. *Geochimica Et Cosmochimica Acta, 61*(16), 3319-3328. doi: Doi 10.1016/S0016-7037(97)00166-X

Fuhrman, J. A. (1999). Marine viruses and their biogeochemical and ecological effects. *Nature, 399*(6736), 541-548. doi: 10.1038/21119

Gates, I. V., Zhang, Y., Shambaugh, C., Bauman, M. A., Tan, C., & Bodmer, J. L. (2009). Quantitative measurement of varicella-zoster virus infection by semiautomated flow cytometry. *Appl Environ Microbiol, 75*(7), 2027-2036. doi: 10.1128/AEM.02006-08

Gersberg, R. M., Lyon, S. R., Brenner, R., & Elkins, B. V. (1987). Fate of viruses in artificial wetlands. *Appl Environ Microbiol, 53*(4), 731-736.

Green, L. M., & Berg, J. M. (1990). Retroviral nucleocapsid protein-metal ion interactions: folding and sequence variants. *Proc Natl Acad Sci U S A, 87*(16), 6403-6407.

Hagens, S., & Loessner, M. J. (2007). Application of bacteriophages for detection and control of foodborne pathogens. *Appl Microbiol Biotechnol, 76*(3), 513-519. doi: 10.1007/s00253-007-1031-8

Hesterberg, D. (1998). Biogeochemical cycles and processes leading to changes in mobility of chemicals in soils. *Agriculture Ecosystems & Environment, 67*(2-3), 121-133. doi: Doi 10.1016/S0167-8809(97)00110-2

Hobbie, J. E., Daley, R. J., & Jasper, S. (1977). Use of nuclepore filters for counting bacteria by fluorescence microscopy. *Appl Environ Microbiol, 33*(5), 1225-1228.

Kanamaru, S., Leiman, P. G., Kostyuchenko, V. A., Chipman, P. R., Mesyanzhinov, V. V., Arisaka, F., & Rossmann, M. G. (2002). Structure of the cell-puncturing device of bacteriophage T4. *Nature, 415*(6871), 553-557. doi: 10.1038/415553a

Karam, J. D., & Drake, J. W. (1994). *Molecular biology of bacteriophage T4*. Washington, DC: American Society for Microbiology.

Kellenberger, E. (1980). Control Mechanisms in the Morphogeneses of Bacteriophage Heads. *Biosystems, 12*(3-4), 201-223. doi: Doi 10.1016/0303-2647(80)90018-0

Kostyuchenko, V. A., Leiman, P. G., Chipman, P. R., Kanamaru, S., van Raaij, M. J., Arisaka, F., . . . Rossmann, M. G. (2003). Three-dimensional structure of bacteriophage T4 baseplate. *Nat Struct Biol, 10*(9), 688-693. doi: 10.1038/nsb970

Kostyuchenko, V. A., Navruzbekov, G. A., Kurochkina, L. P., Strelkov, S. V., Mesyanzhinov, V. V., & Rossmann, M. G. (1999). The structure of bacteriophage T4 gene product 9: the trigger for tail contraction. *Structure, 7*(10), 1213-1222.

Kropinski, A. M., Mazzocco, A., Waddell, T. E., Lingohr, E., & Johnson, R. P. (2009). Enumeration of bacteriophages by double agar overlay plaque assay. *Methods Mol Biol, 501*, 69-76. doi: 10.1007/978-1-60327-164-6_7

Leiman, P. G., Kanamaru, S., Mesyanzhinov, V. V., Arisaka, F., & Rossmann, M. G. (2003). Structure and morphogenesis of bacteriophage T4. *Cell Mol Life Sci, 60*(11), 2356-2370. doi: 10.1007/s00018-003-3072-1

McHale, A. P., & McHale, S. (1994). Microbial biosorption of metals: potential in the treatment of metal pollution. *Biotechnol Adv, 12*(4), 647-652.

Medigue, C., Rouxel, T., Vigier, P., Henaut, A., & Danchin, A. (1991). Evidence for horizontal gene transfer in Escherichia coli speciation. *J Mol Biol, 222*(4), 851-856.

Noble, R. T., & Fuhrman, J. A. (1998). Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. *Aquatic Microbial Ecology, 14*(2), 113-118. doi: Doi 10.3354/Ame014113

Novikov, A. P., Kalmykov, S. N., Utsunomiya, S., Ewing, R. C., Horreard, F., Merkulov, A., . . . Myasoedov, B. F. (2006). Colloid transport of plutonium in the far-field of the Mayak Production Association, Russia. *Science, 314*(5799), 638-641. doi: 10.1126/science.1131307

Poorvin, L., Rinta-Kanto, J. M., Hutchins, D. A., & Wilhelm, S. W. (2004). Viral release of iron and its bioavailability to marine plankton. *Limnology and Oceanography, 49*(5), 1734-1741.

Ren, Z., & Black, L. W. (1998). Phage T4 SOC and HOC display of biologically active, full-length proteins on the viral capsid. *Gene, 215*(2), 439-444.

Rossmann, M. G., Mesyanzhinov, V. V., Arisaka, F., & Leiman, P. G. (2004). The bacteriophage T4 DNA injection machine. *Curr Opin Struct Biol, 14*(2), 171-180. doi: 10.1016/j.sbi.2004.02.001

Suttle, C. A. (2007). Marine viruses--major players in the global ecosystem. *Nat Rev Microbiol, 5*(10), 801-812. doi: 10.1038/nrmicro1750

Syvanen, M. (1994). Horizontal gene transfer: evidence and possible consequences. *Annu Rev Genet, 28*, 237-261. doi: 10.1146/annurev.ge.28.120194.001321

Thingstad, T. F., & Lignell, R. (1997). Theoretical models for the control of bacterial growth rate, abundance, diversity and carbon demand. *Aquatic Microbial Ecology, 13*(1), 19-27. doi: Doi 10.3354/Ame013019

Weinbauer, M. G. (2004). Ecology of prokaryotic viruses. *FEMS Microbiol Rev, 28*(2), 127-181. doi: 10.1016/j.femsre.2003.08.001

Weitz, J. S., & Wilhelm, S. W. (2012). Ocean viruses and their effects on microbial communities and biogeochemical cycles. *F1000 Biol Rep, 4*, 17. doi: 10.3410/B4-17

Wilhelm, S. W., Brigden, S. M., & Suttle, C. A. (2002). A dilution technique for the direct measurement of viral production: a comparison in stratified and tidally mixed coastal waters. *Microb Ecol, 43*(1), 168-173. doi: 10.1007/s00248-001-1021-9

Winget, D. M., & Wommack, K. E. (2009). Diel and daily fluctuations in virioplankton production in coastal ecosystems. *Environ Microbiol, 11*(11), 2904-2914. doi: 10.1111/j.1462-2920.2009.02038.x

Yee, N., & Fein, J. (2001). Cd adsorption onto bacterial surfaces: A universal adsorption edge? *Geochimica Et Cosmochimica Acta, 65*(13), 2037-2042. doi: Doi 10.1016/S0016-7037(01)00587-7

Figure 1. Filter tower apparatus set up to filter viruses onto a 0.02 µm Anodisc filter in order to be stained for epifluorescent microscopy. The filter tower consists of alow extractable filter holder, borosilicate glass with a 15mL graduated funnel, anodized aluminum clamp, and a number 5 silicone stopper. It also includes a coarse porosity 40-60um fritted glass support base. VWR Item number: D109495.


Fisher Scient
VORTEX MIXER
SPEED
AUTO
OFF
ON

Figure 2. Zinc adsorption graph generated with the results from the ICP-MS analysis of filtered and unfiltered samples. The concentrations of zinc were evaluated ranging from 0 μM – 600 μM and a single point at 1300 μM. The data reveals that when more zinc is present in the solution, more zinc is able to adsorb onto the surface of the viruses until all the adsorption sites on the viral surfaces are saturated.

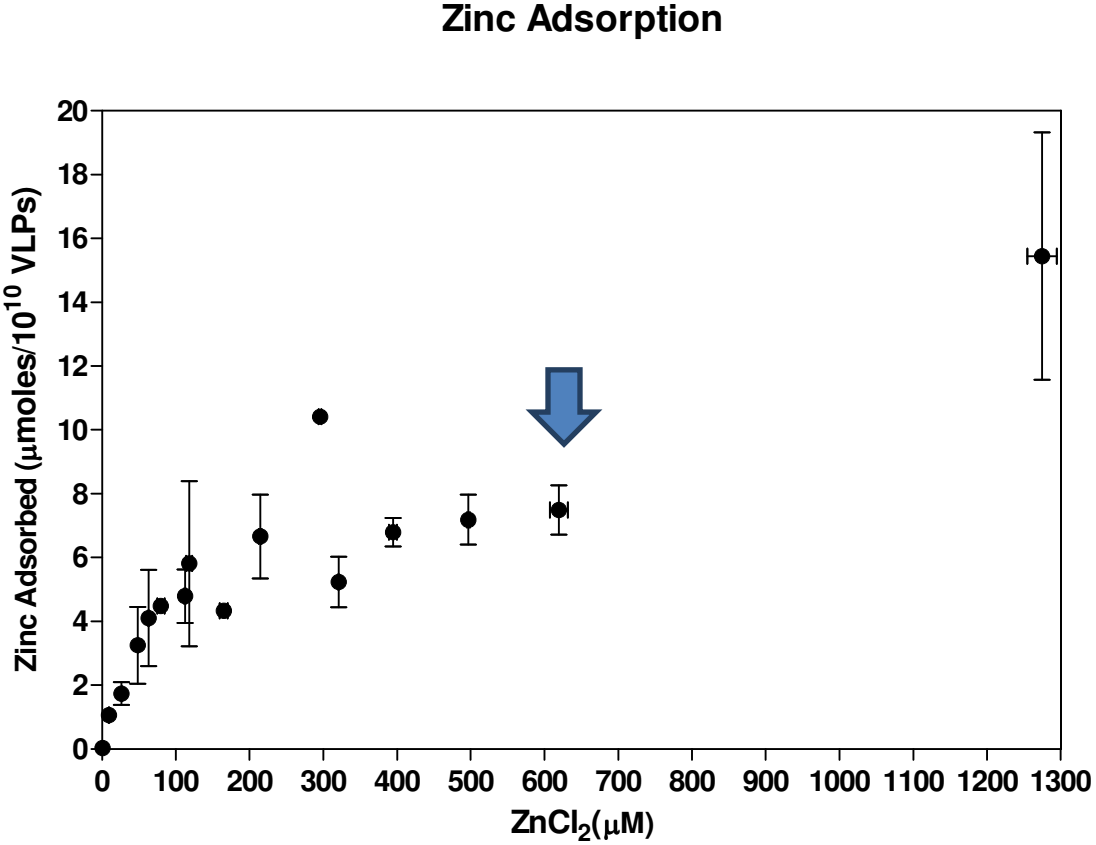

Zinc Adsorption
Zinc Adsorbed (µmoles/10^10 VLPs)
0
2
4
6
8
10
12
14
16
18
20
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
ZnCl2(µM)

Figure 3. Langmuir and Freundlich isotherm generated for the adsorption of zinc onto the surface of bacteriophage T4. The red curve represents the Freundlich isotherm and the green curve represents the Langmuir isotherm. The adsorption isotherms were based on all the data points up to the blue arrow indicated on Figure 2. The data point at 1274.56 μM of ZnCl was determined to be a statistical outlier through nonlinear regression analysis and was thus, excluded from the model (Graph was modeled by our collaborator, Dr. Yusong Li and her doctoral student, Negin Kananizadeh)

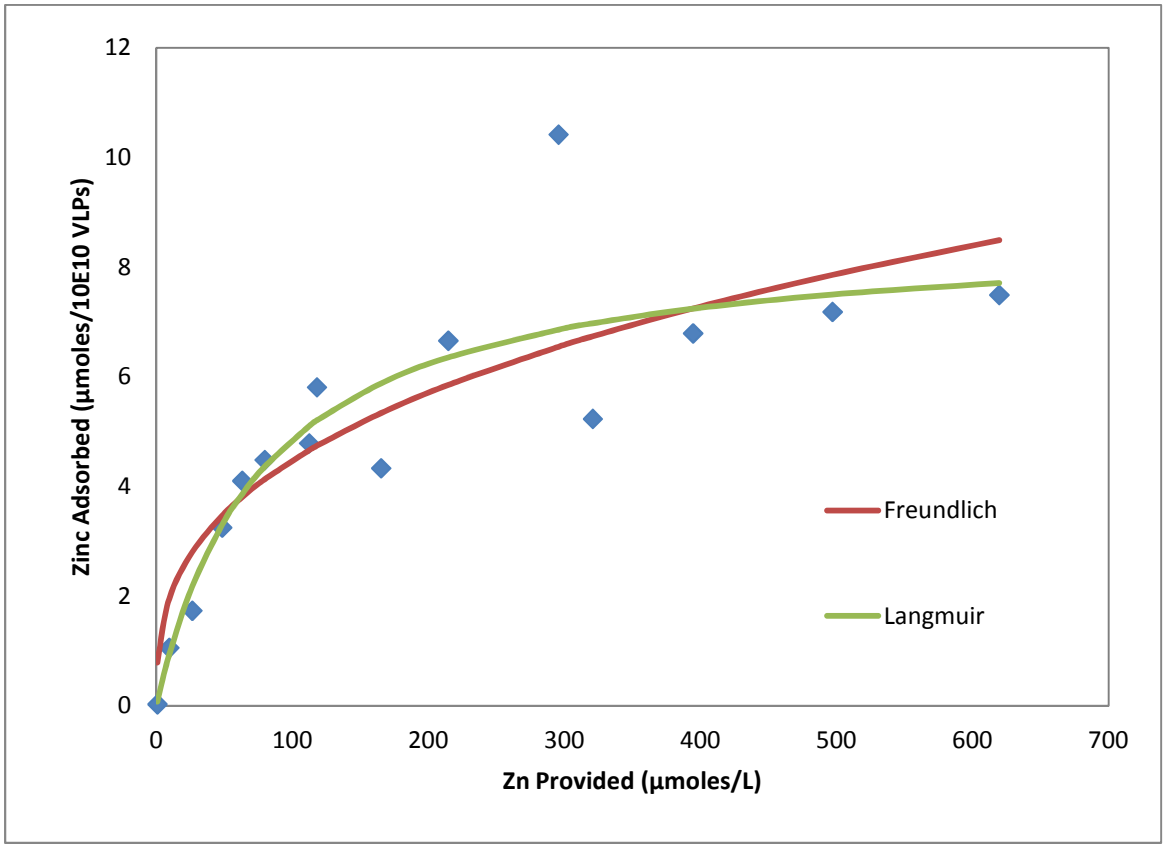

12
10
8
6
4
2
0
Zinc Adsorbed (µmoles/10E10 VLPs)
0
100
200
300
400
500
600
700
Zn Provided (µmoles/L)
Freundlich
Langmuir

Figure 4. Graph depicting the adsorption of zinc onto the surface of bacteriophage T4 at pH4-7.5. Adsorption was conducted in 20 mM PIPPS buffer (pH 4 & 5) and 20 mM PIPES buffer (pH 6, 7 & 7.5). The results show that the adsorption of zinc onto the surface of phage T4 increases with pH and that there is minimal amount of zinc adsorbed at acidic pH.

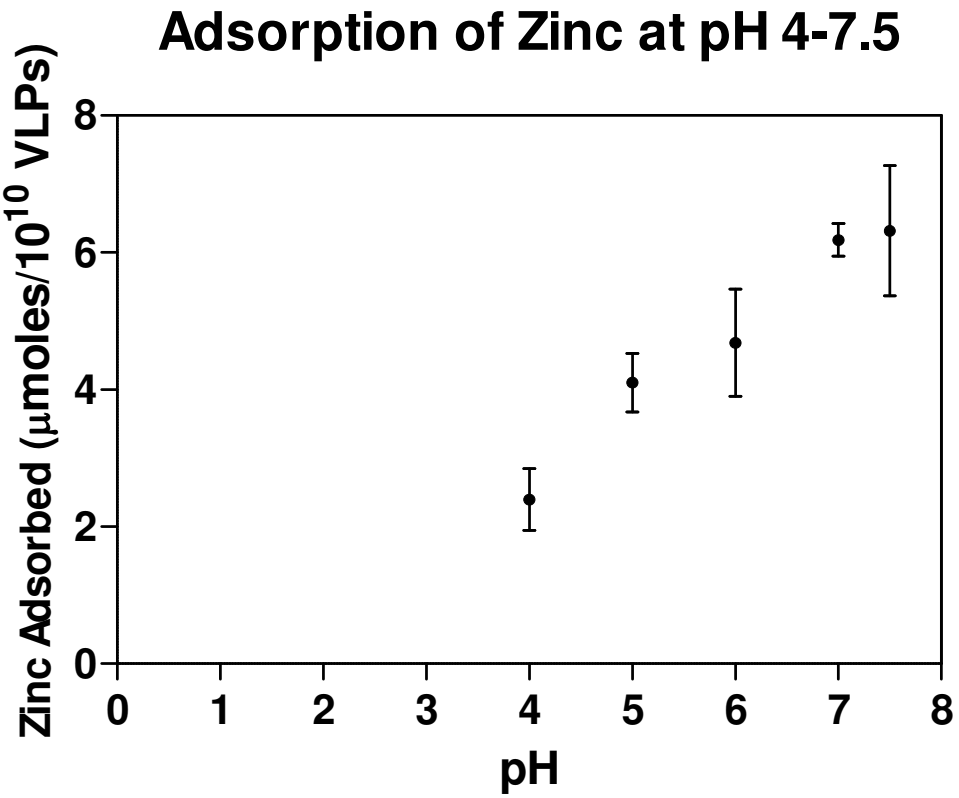

Adsorption of Zinc at pH 4-7.5
Zinc Adsorbed (μmoles/10^10 VLPs)
0
2
4
6
8
0
1
2
3
4
5
6
7
8
pH

Figure 5. (Table 1) Different treatments applied for the bacteriophage titer experiment. (Table 2) Number of plaque forming units (PFUs) enumerated from the bacteriophage titers at two different time points, t= 4 hours and t= 20 hours. The experiment was performed in triplicate.

Treatments	
A	Negative Control
B	150 µM Zn
C	150 µM Zn + 300 µM EDTA
D	150 µM NaCl

Table 1

	PFUs at -5 dilution (t=4)	PFUs at -5 dilution (t=20)
A1	43	22
A2	65	34
A3	70	38
B1	105	73
B2	124	86
B3	155	91
C1	51	43
C2	36	33
C3	45	44
D1	56	41
D2	66	45
D3	79	35

Table 2

Figure 6. Bar graph depicting the effects of various treatments on the infectivity of bacteriophage T4 through a series of phage titer experiments. The treatments consists of the negative control (No zinc), 150 µM Zinc, 150 µM Zinc + 300 µM EDTA and 150 µM NaCl respectively. The results show that the infectivity of phage T4 treated with 150 µM Zinc only was at least a magnitude higher compared to the other treatments in terms of PFUs/mL.

Phage Titer Post Exposure to Various Treatments

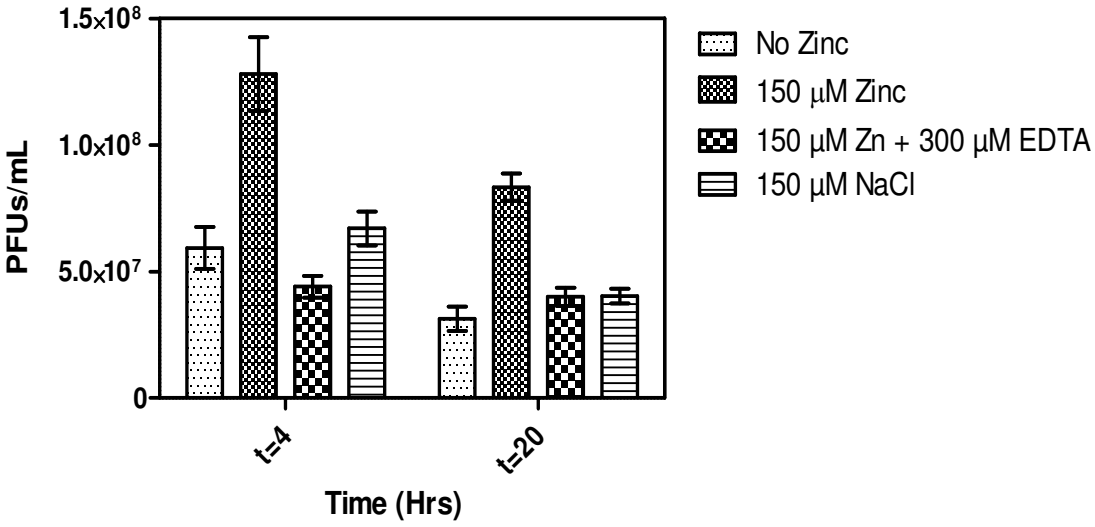


Figure A-1. Graph portraying a phage decay bacteriophage titer experiment over a 48 hour time period. Bacteriophage titers were conducted at 6 time points, which are t= 0 hr, 4 hrs, 8 hrs, 19 hrs, 24 hrs and 48 hrs respectively. The results show that there was no significant phage decay within a 24 hour time period.

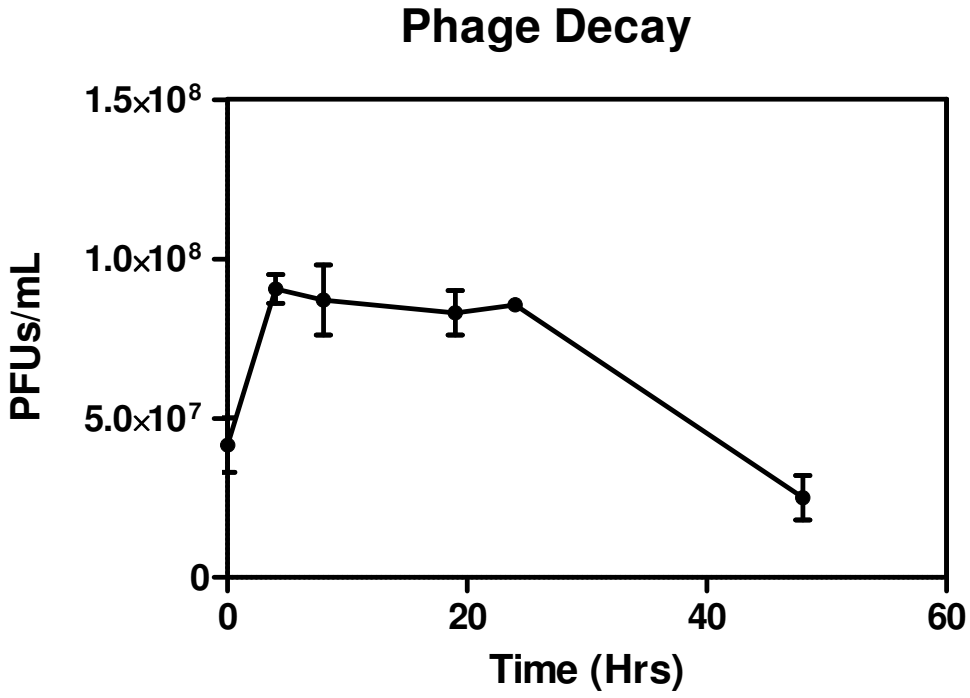

Phage Decay
1.5x10^8
1.0x10^8
5.0x10^7
0
PFUs/mL
0
20
40
60
Time (Hrs)

Figure A-2. Bar graph for pH adsorption experiment showing the concentrations of zinc for filtered and unfiltered samples ranging pH 4 to pH 9. The same amount of Zn was added into each experimental tube, where approximately 300 μM of $ZnCl_2$ was added. The results show that the amount of Zn from the unfiltered samples was similar throughout pH 4-7. However, there was a huge drop in the amount of Zn from the unfiltered samples the moment it reaches basic pH, which are pH 8 & 9. This demonstrates a significant occurrence of zinc hydroxide precipitation at basic pH.

Effects of pH on Filtered and Unfiltered Zinc Samples

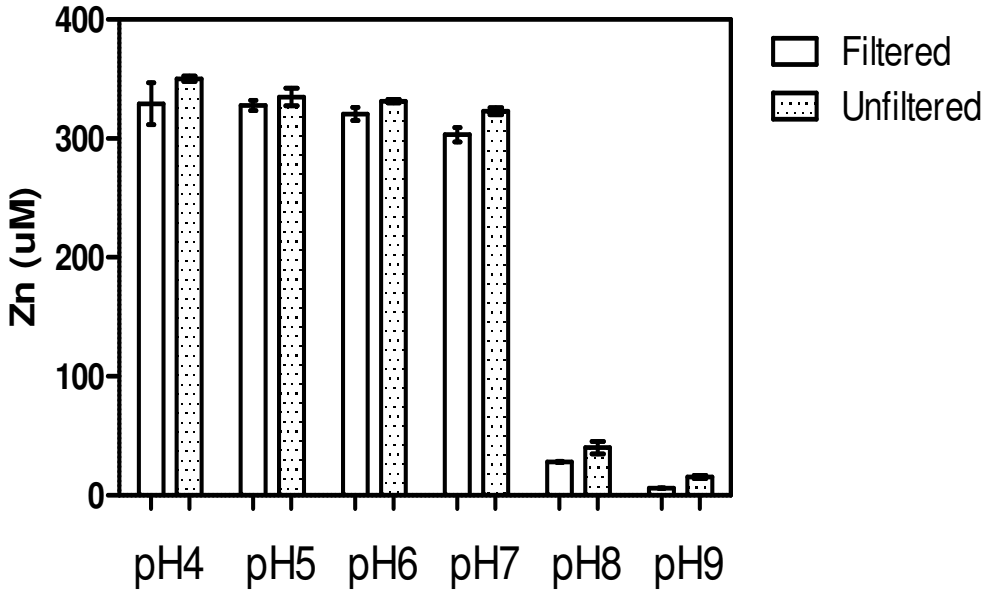


Figure A-3. Geochemical models on the effect of pH on zinc speciation. Figure (i) demonstrates zinc speciation in PIPPS buffer, Figure (ii) in PIPES buffer and Figure (iii) in water. All three Bjerrum plots depict that the zinc remains in the form Zn^{2+} from acidic pH until a pH of 7.5. The increase of zinc hydroxide and zinc carbonate and sharp fall in Zn^{2+} beyond the pH of 7.5 demonstrates that precipitation occurs at basic pH. (The Bjerrum plots was generated using PhreePlot with the help of Weberlab doctoral student, Jason Nolan.)

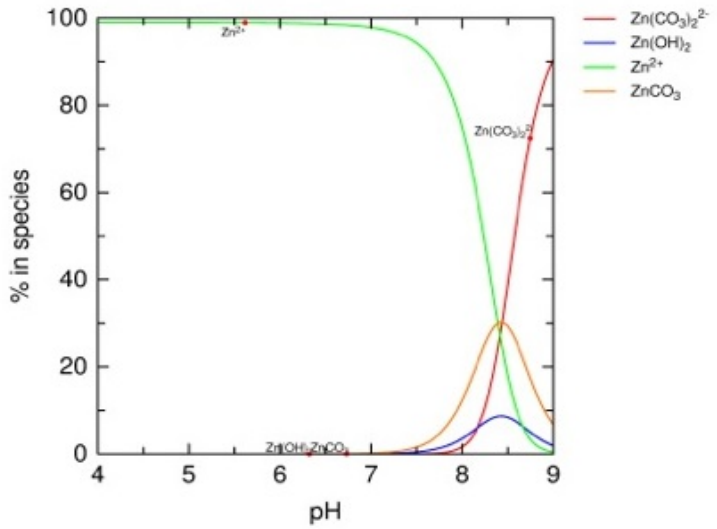


Figure (i)

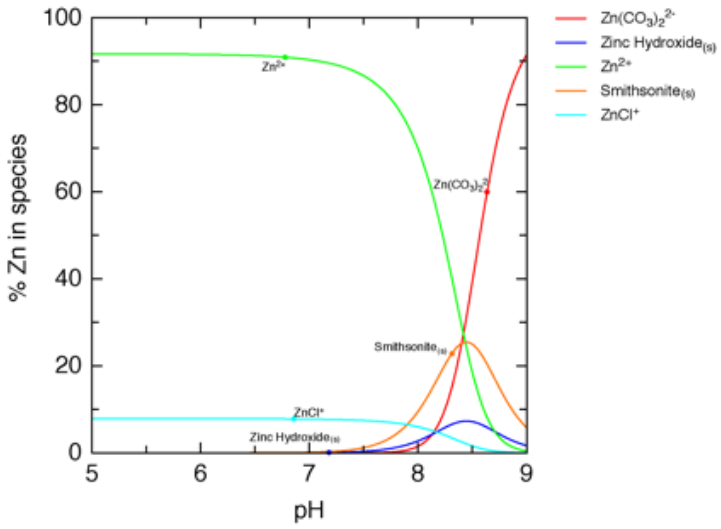


Figure (ii)

Figure A-3 (Continued). Figure (iii) demonstrates zinc speciation in water.

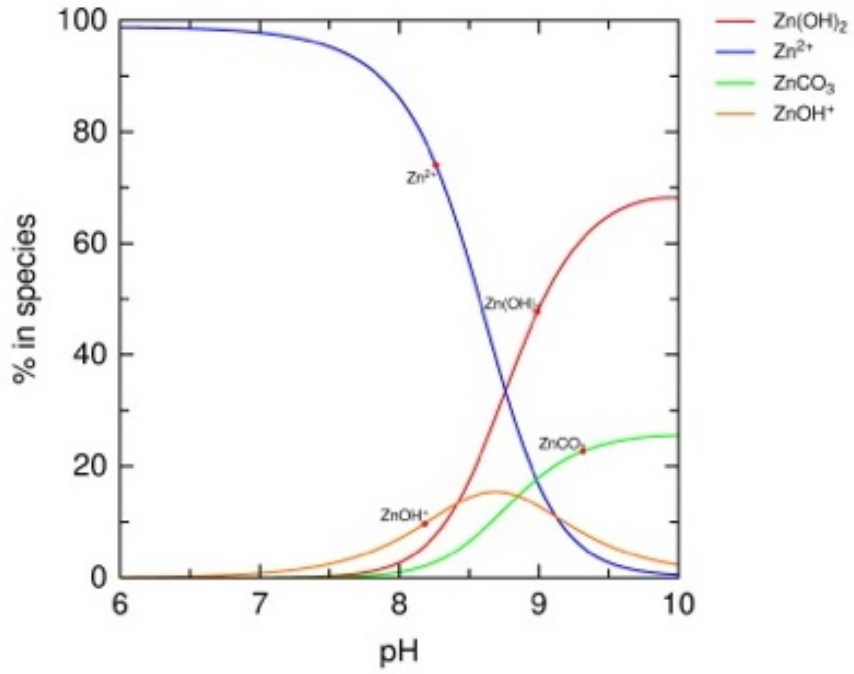
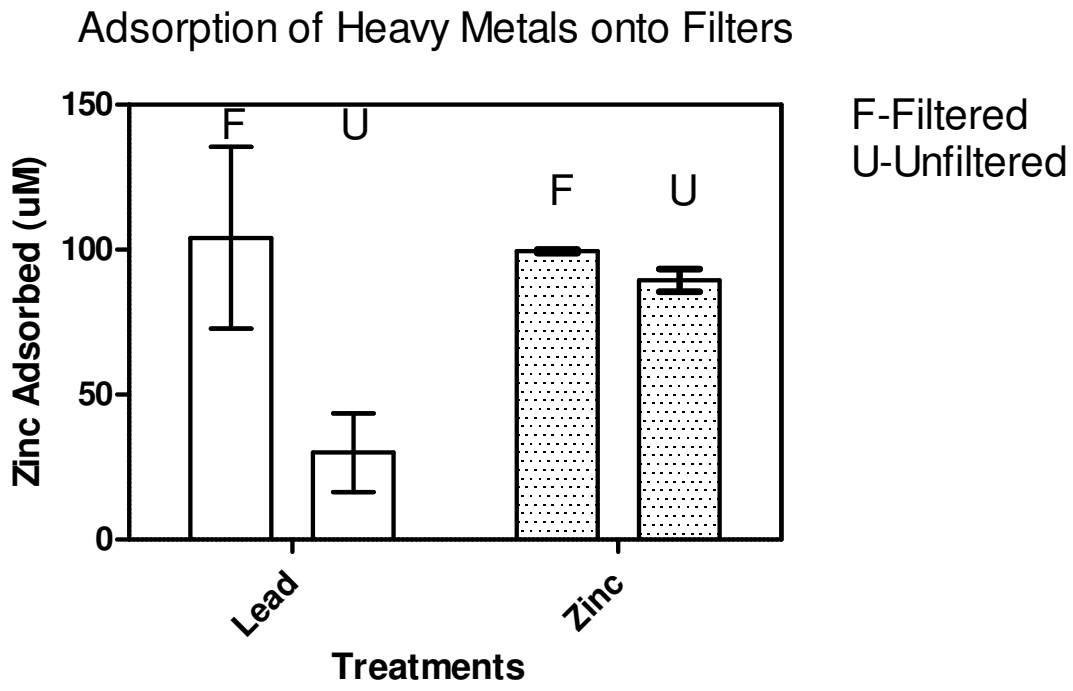

100
80
60
40
20
0
% in species
6
7
8
9
10
pH
Zn(OH)2
Zn2+
ZnCO3
ZnOH+
Zn2+
Zn(OH)2
ZnCO3
ZnOH+

Figure (iii)

Figure A-4. The bar graph shows the adsorption of heavy metals onto the 0.025 μm mixed cellulose ester filter membranes. Lead adsorbs significantly onto the filters as shown by the huge disparity between the filtered and unfiltered samples. On the other hand, zinc was shown to not have any significant adsorption onto the filter membranes.


Adsorption of Heavy Metals onto Filters
Zinc Adsorbed (uM)
150
100
50
0
F
U
F
U
Lead
Zinc
Treatments
F-Filtered
U-Unfiltered