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This is my abstract. The abstract page is a required component of the thesis/dissertation. The abstract should be a brief summary of the paper, stating only the problem, procedures used, and the most significant results and conclusions. Explanations and opinions are omitted. Remember to include the necessary information regarding any multimedia components included in the document. The abstract must be approved by your advisor/committee chair.

RESPONSE OF MERCURY METHYLATING BACTERIA TO THE DAN RIVER
COAL ASH SPILL WITH A SURVEY OF METAL TOLERANCE OF
MICROORGANISMS ASSOCIATED WITH COAL ASH

by

Ashley S. Williams

A Thesis Submitted to
the Faculty of The Graduate School at
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Approved by

Committee Chair

The dedication is often short. Longer statements are usually in the acknowledgements. The dedication is optional.

APPROVAL PAGE

This thesis written by Ashley S. Williams has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro.

Committee Chair _____
Parke Rublee

Committee Members _____
Anne Hershey

Martin Tsui

Date of Acceptance by Committee

Date of Final Oral Examination

ACKNOWLEDGMENTS

It is customary to recognize the assistance of the advisor and/or committee chair, all other members of the committee, and only those organizations and/or persons who actually aided the research. If financial support was provided to make the study possible, credit for such assistance should be given.

TABLE OF CONTENTS

	Page
LIST OF TABLES.....	vi
LIST OF FIGURES.....	vii
CHAPTER	
I. INTRODUCTION	1
II. RESPONSE OF MERCURY METHYLATING BACTERIA TO THE COAL ASH SPILL IN THE DAN RIVER	3
Abstract.....	3
Introduction	3
Methods.....	6
Results.....	8
Discussion	8
III. IDENTIFICATION AND CHARACTERIZATION OF HEAVY METAL TOLERANCE OF BACTERIA CULTURED FROM COAL ASH	9
Math.....	9
Chemistry 101: Symbols {-#}	10
Physics.....	11
Biology.....	11
IV. TABLES, GRAPHICS, REFERENCES, AND LABELS	12
Tables.....	12
Figures.....	14
Footnotes and Endnotes	18
Bibliographies	18
Anything else?	19

Conclusion.....	21
APPENDIX A. THE FIRST APPENDIX.....	22
APPENDIX B. THE SECOND APPENDIX, FOR FUN	24
Colophon.....	25
References	33

LIST OF TABLES

	Page
Table IV.1. Correlation of Inheritance Factors for Parents and Child	12

LIST OF FIGURES

	Page
Figure II.1. Map.....	7
Figure IV.1. UW logo	15
Figure IV.2. Mean Delays by Airline.....	17

CHAPTER I

INTRODUCTION

Coal is the second most used fuel for electricity generation in the United States. In 2017, approximately 30% of all electricity production was fueled by coal combustion (Administration, 2019). Although the percentage has fallen in recent years due to retirement of plants and increase in natural gas and other energy sources, coal remains a main fuel for electricity. During the process of coal combustion, coal combustion residuals, or coal ash, is produced.

Coal combustion residues (CCRs) include fly ash, bottom ash, and flue gas desulfurized gypsum. Fly ash is a fine powdery substance, comprised primarily of silica that moves up the exhaust system. It is produced during the combustion of finely ground coal and most is captured from the exhaust using electrostatics and scrubber systems (US EPA, 2014a). Bottom ash is formed during the combustion of pulverized coal in boilers. It ranges in size from fine sand to fine gravel and is grey to black in color. Bottom ash is too large to be carried up the exhaust system and is collected in an ash hopper (US EPA, 2014b). Flue gas desulfurized gypsum is not a direct product of coal combustion, but a product of the scrubber system to remove SO₂ emissions from exhaust (US EPA, 2014c).

Physical and chemical properties of coal ash are determined by the geographical location where the raw coal was mined, the type of boiler, and the operating conditions of the power plant (Jayaranjan et al., 2014). Fly ash is composed mainly of oxides such as SiO₂, Al₂O₃, Fe₂O₃, TiO₂, and CaO. All natural elements can be found in coal ash, trace elements include, As, Cd, Cr, Hg, Pb, Se, and Zn (Greely Jr.

et al., 2014; Jayaranjan et al., 2014; Shaheen et al., 2014). Coal bottom ash consists of silicate, carbonate, aluminate, ferrous materials and several of heavy metals and metalloids. Like fly ash, the chemical composition of the bottom ash is dependent on the source of the raw coal, boiler type, and the refinement process of the raw coal (Jayaranjan et al., 2014).

Once produced and collected, CCRs are transported to an impoundment pond or landfill. Impoundment ponds are constructed either lined or unlined; open to the atmosphere or capped. In an open lagoon, the waste settles to the bottom of the pond, leaving the shallow surface water free of waste. To prevent overflow of these ponds, this shallow water is pumped and directed to a waterway adjacent to the power plant. In the US, of the approximately 120 Mt of CCRs are produced annually, 54% is disposed of in landfills or surface impoundments (American Coal Ash Association, 2012). Leaching and impoundment failures allow the mobilization of CCRs including their associated heavy metals into the environment, where these metals may enter the food web directly or indirectly through microbially-mediated transformations (Cabral et al., 2016; Deonaraine et al., 2013; Otter et al., 2012).

CHAPTER II

RESPONSE OF MERCURY METHYLATING BACTERIA TO THE COAL ASH SPILL IN THE DAN RIVER

Abstract

Introduction

On February, 2, 2014, two storm water drainage pipes located under a coal ash impoundment pond at the Duke Energy Dan River Steam Station near Eden, NC collapsed, releasing approximately 28,000 cubic yards of coal ash and about 27 million gallons of untreated ash wastewater into the Dan River (Dennis Lemly, 2015). Following the spill, water and sediment was sampled from the river and Kerr Reservoir downstream of the spill to determine water quality and human health concerns. Test results show no constituents to be at levels exceeding safe limits in the water column (US EPA, 2014d). Duke Energy dredged ash deposits at two locations along the river, but likely over 90% of the ash remains buried in river sediments or has been deposited into Kerr Lake (NC DEQ, 2014). While the test results are encouraging for immediate water quality, the long-term concern is the effect of mobilization of coal ash constituents into the riverine food webs.

One constituent of particular concern during leaching and/or impoundment failure is mercury. Mercury, a known neurotoxin and potential endocrine disruptor has a high affinity for sulfhydryl groups in proteins where destabilization leads to decreased enzymatic activity and reduced overall fitness (Driscoll et al., 2013; Ehrlich and Newman, 2008). In submerged anoxic sediments under certain conditions, inorganic mercury (Hg^{2+}) can be converted into MeHg by microbial metabolism (Dash

and Das, 2014; Schaefer et al., 2011). Methylmercury (MeHg) bioaccumulates and biomagnifies in the river food webs, posing a health risk to local residents who consume fish. (Dash and Das, 2014; Otter et al., 2012; Rowe, 2014). The total available amount of MeHg within an ecosystem is controlled by multiple microbial and abiotic processes that reduce availability of Hg^{2+} or degradation of MeHg. Hg^{2+} can be volatilized as Hg^0 through photoreduction or by bacteria with the merA gene (Boyd and Barkay, 2012). Additionally, MeHg can be demethylated into Hg^{2+} by sunlight (Tsui et al., 2013) or microbes with the merB gene (Bizily et al., 1999).

Microorganisms have developed various mechanisms to mitigate effects of high concentrations of heavy metal toxins. These include reduction of the metal to a less toxic form, metal complexation, efflux pumps via an energy-dependent membrane transporter, and extracellular sequestration (Binkley and Simpson, 2003; Poulain and Barkay, 2013). MeHg is produced in anaerobic conditions predominately by sulfate reducing bacteria (SRB) iron reducing bacteria (IRB) and methanogens (Liu et al., 2014). Coal ash may provide the necessary substrates such as sulfates to stimulate the microbial methylation of Hg (Deonaraine et al., 2013).

Two genes are required for methylation of Hg, hgcA and hgcB. As Hg^{2+} enters the cell, a methylated-HgcA protein transfers a CH_3 group to Hg^{2+} within the cytosol. HgcB protein is then required to recycle the methylated-HgcA protein (Poulain and Barkay, 2013). The hgcAB sequence is conserved across multiple genera and therefore could be utilized as a molecular biomarker for suspected contaminated sites with real-time quantitative PCR (Christensen et al., 2016; Dash and Das, 2014; Lima de Silva et al., 2012; Parks et al., 2009). Liu et al. (2014) found that the hgcA abundance and the concentration of MeHg in rice paddy soil near the Wanshan Hg mining area was positively correlated (Liu et al., 2014). This finding suggests that microbes may be

contributing to the MeHg in the sampled soils. They also found high genetic diversity within the microbial community and that environmental factors such as total Hg, SO₄, NH₄, and organic matter influenced the community structure. After phylogenetic analysis, the representative taxa in the community consisted of Deltaproteobacteria, Firmicutes, Chloroflexi, Euryarchaeota, and two novel taxa (Liu et al., 2014).

In 2008 a dike failure at the Tennessee Valley Authority Kingston Fossil Plant coal ash pond in Harriman, Tennessee, released an estimated 5.4 million cubic yards of ash into the surrounding community and rivers (Ruhl et al., 2010). The release ruptured a natural gas line, disrupted power and transportation, destroyed three homes, and resulted in the evacuation of nearby neighborhoods. The impoundment pond has since been rebuilt and reinforced to resist natural disasters such as earthquakes (TVA, 2011). In sediment samples collected downstream following the spill, total mercury concentrations were three to four times greater than sediments upstream of the spill. MeHg was also slightly higher than upstream (Deonarine et al., 2013).

The coal ash spill into the Dan River has mobilized heavy metals into the environment. The extent of long-term effects of methylated mercury into the food chain in the river is unknown. Mercury, along with other coal ash constituents may stimulate mercury-methylating microorganisms in anaerobic sediments. The goal of this study is to determine the overall microbial community response and specifically *hgcA* abundance as a result of the Dan River coal ash spill.

Objective and hypothesis:

Determine spatial distribution of mercury-methylating taxa as a result of the coal ash spill using qPCR. I hypothesize that there will be increased abundance of the *hgcA* genes and therefore, mercury methylating taxa downstream of spill site due to stimulation of sulfate and iron reducing bacteria and methanogens by coal ash

constituents present in the sediment.

Methods

Study Sites and Sediment Collection

The Dan River is a 344 km river that rises in Patrick Co. Virginia and crosses into North Carolina in Stokes County. It flows across the border between NC and VA several times before flowing into the Kerr Reservoir on the Roanoke River which then flows to the Atlantic Ocean at the Albemarle Sound in North Carolina. This study encompasses sites upstream of the spill site in Eden, NC and downstream to Milton, NC, a 103 km section of the Dan.

To characterize the extent of the coal ash spill impact on the microbial community, samples were collected at three upstream reference sites, one site parallel to the ash ponds but upstream of the spill (leaching site), and five downstream sites including near two sites that were dredged for remediation, one at Town Creek, near the spill site and one near Abreu-Grogan Park, Danville, Va., and depositional sites that were not dredged near Danville (Figure II.1).

```
knitr::include_graphics(path = "figure/map.png")
```

Each site was accessed by motorboat, where sediment from the riverbank and channel was collected. Riverbank sediment cores were collected in triplicate using a piston-style coring device. Channel samples were collected using a small dredge. Sediment cores were segmented by depth and mixed according to one of three sampling schemes to reduce the total number of samples to be assayed. 0.25 cm³ samples were preserved in CTAB (cetyltrimethylammonium bromide) for DNA extraction. Channel sediment was mixed thoroughly then 0.25 cm³ was preserved in CTAB.

Sampling Scheme:

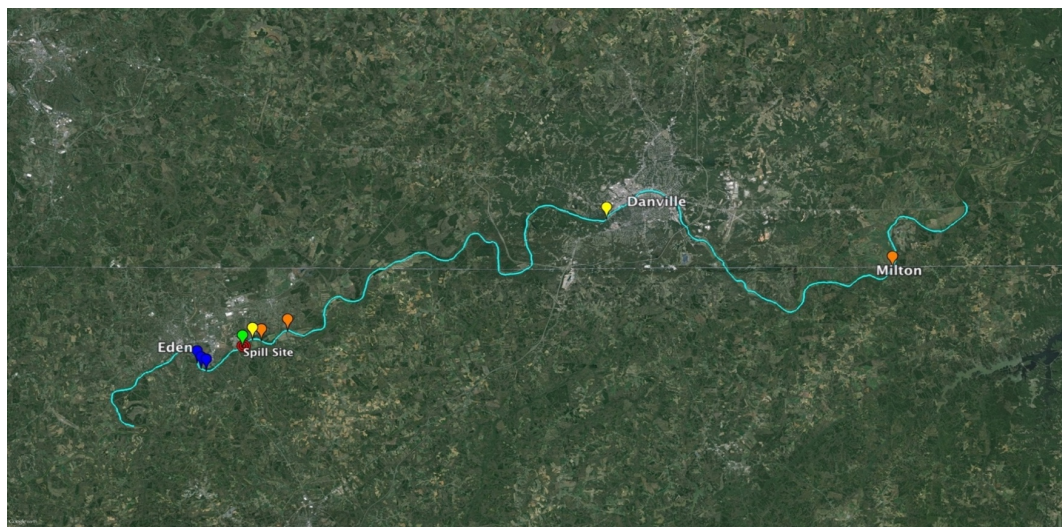


Figure II.1. Map

- A. Sediment cores extracted in triplicate, each 0-8 cm and 8-16 cm segments sampled
- B. Triplicate sediment cores pooled at 0-2 cm, 10-12 cm, 15-17 cm
- C. Triplicate sediment cores pooled 0-8 cm and 8-16 cm segments sampled.

DNA extraction and qPCR

A CTAB extraction was performed using standard protocol for each sample (Stewart and Via 1993). The DNA extracted was quantified and subsequently diluted to a standard concentration of 5 ng/L and stored in a 4C refrigerator. Extracted DNA quantity and purity were determined from 2 μ l subsamples of each extraction using Thermo Scientific Nanodrop Spectrophotometer based on the 260/280 wavelength ratio.

An Applied Biosystems StepOne[™] real-time PCR System was utilized to detect, amplify, and quantify target DNA gene proxies and representative taxa to meet the Objective. Primers were chosen from the literature based on targeting a

general metabolic category. These include generic primers to the *hgcA* gene, a sulfate reducing gene, *dsr*, the 16S rDNA of iron reducing bacteria, and methanogens (Geets et al. 2008, Schaefer et al. 2013, Wagner et al.1998, Wright and Primm 2003, Table X). This approach will define the microbial community abundance encompassed within each sample. Each reaction contained the following: 10 μ L of Power Sybr® Green PCR master mix, 1 μ L of forward primer, 1 μ L of reverse primer, 8 μ L of sterile deionized water, and 1 μ L of extracted DNA. Three negative control reactions, samples repeated in triplicate, and positive standard controls serially diluted in triplicate, were ran in each 48 well plate. The relative abundance of targets were computed by the StepOne software using the generated standard curve. The melt curve was examined to manually ensure that none of the amplifications were due to a false positive. Statistical analyses including one and two-way ANOVA and regression analyses were performed to determine if a significant difference exists between amounts of targeted DNA collected from sites upstream versus downstream of spill site and within depths of core samples.

Statistical Analysis

Results

Discussion

CHAPTER III

IDENTIFICATION AND CHARACTERIZATION OF HEAVY METAL TOLERANCE OF BACTERIA CULTURED FROM COAL ASH

Math

T_EX is the best way to typeset mathematics. Donald Knuth designed T_EX when he got frustrated at how long it was taking the typesetters to finish his book, which contained a lot of mathematics. One nice feature of *R Markdown* is its ability to read LaTeX code directly.

If you are doing a thesis that will involve lots of math, you will want to read the following section which has been commented out. If you're not going to use math, skip over or delete this next commented section.

MATH and PHYSICS majors: Uncomment the following section ->

$$\sum_{j=1}^n (\delta\theta_j)^2 \leq \frac{\beta_i^2}{\delta_i^2 + \rho_i^2} \left[2\rho_i^2 + \frac{\delta_i^2 \beta_i^2}{\delta_i^2 + \rho_i^2} \right] \equiv \omega_i^2$$

From Informational Dynamics, we have the following (Dave Braden):

After n such encounters the posterior density for θ is

$$\pi(\theta|X_1 < y_1, \dots, X_n < y_n) \propto \pi(\theta) \prod_{i=1}^n \int_{-\infty}^{y_i} \exp\left(-\frac{(x-\theta)^2}{2\sigma^2}\right) dx$$

Another equation:

Lapidus and Pindar, Numerical Solution of Partial Differential Equations in Science and Engineering. Page 54

$$\int_t \left\{ \sum_{j=1}^3 T_j \left(\frac{d\phi_j}{dt} + k\phi_j \right) - kT_e \right\} w_i(t) dt = 0, \quad i = 1, 2, 3.$$

L&P Galerkin method weighting functions. Page 55

$$\sum_{j=1}^3 T_j \int_0^1 \left\{ \frac{d\phi_j}{dt} + k\phi_j \right\} \phi_i dt = \int_0^1 k T_e \phi_i dt, \quad i = 1, 2, 3$$

Another L&P (p145)

$$\int_{-1}^1 \int_{-1}^1 \int_{-1}^1 f(\xi, \eta, \zeta) = \sum_{k=1}^n \sum_{j=1}^n \sum_{i=1}^n w_i w_j w_k f(\xi, \eta, \zeta).$$

Another L&P (p126)

$$\int_{A_e} (\cdot) dx dy = \int_{-1}^1 \int_{-1}^1 (\cdot) \det[J] d\xi d\eta.$$

->

Chemistry 101: Symbols {-#}

Chemical formulas will look best if they are not italicized. Get around math mode's automatic italicizing in LaTeX by using the argument `$\mathrm{formula here}$` , with your formula inside the curly brackets. (Notice the use of the backticks here which enclose text that acts as code.)

So, $\text{Fe}_2^{2+}\text{Cr}_2\text{O}_4$ is written `$\mathrm{Fe_2^{2+}Cr_2O_4}$` .

Exponent or Superscript: O^-

Subscript: CH_4

To stack numbers or letters as in Fe_2^{2+} , the subscript is defined first, and then the superscript is defined.

Bullet: $\text{CuCl} \bullet 7\text{H}_2\text{O}$

Delta: Δ

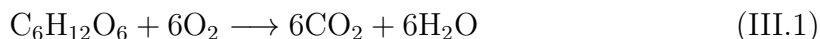
Reaction Arrows: \longrightarrow or $\xrightarrow{\text{solution}}$

Resonance Arrows: \leftrightarrow

Reversible Reaction Arrows: \rightleftharpoons

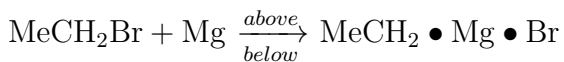
Typesetting reactions

You may wish to put your reaction in an equation environment, which means that LaTeX will place the reaction where it fits and will number the equations for you.



We can reference this combustion of glucose reaction via Equation (III.1).

Other examples of reactions



Physics

Many of the symbols you will need can be found on the math page <http://web.reed.edu/cis/help/latex/math.html> and the Comprehensive LaTeX Symbol Guide (<http://mirror.utexas.edu/ctan/info/symbols/comprehensive/symbols-letter.pdf>).

Biology

You will probably find the resources at <http://www.lecb.ncifcrf.gov/~toms/latex.html> helpful, particularly the links to bst files for various journals. You may also be interested in TeXShade for nucleotide typesetting (<http://homepages.uni-tuebingen.de/beitz/txe.html>). Be sure to read the proceeding chapter on graphics and tables.

CHAPTER IV

TABLES, GRAPHICS, REFERENCES, AND LABELS

Tables

By far the easiest way to present tables in your thesis is to store the contents of the table in a CSV or Excel file, then read that file in to your R Markdown document as a data frame. Then you can style the table with the `kable` function, or functions in the `kableExtra` package.

In addition to the tables that can be automatically generated from a data frame in **R** that you saw in [R Markdown Basics] using the `kable` function, you can also create tables using *pandoc*. (More information is available at <http://pandoc.org/README.html#tables>.) This might be useful if you don't have values specifically stored in **R**, but you'd like to display them in table form. Below is an example. Pay careful attention to the alignment in the table and hyphens to create the rows and columns. Generally I don't recommend this approach of typing the table directly into your R Markdown document.

Table IV.1. Correlation of Inheritance Factors for Parents and Child

Factors	Correlation between Parents & Child	Inherited
Education	-0.49	Yes
Socio-Economic Status	0.28	Slight
Income	0.08	No
Family Size	0.18	Slight
Occupational Prestige	0.21	Slight

We can also create a link to the table by doing the following: Table IV.1. If you go back to [Loading and exploring data] and look at the **kable** table, we can create a reference to this max delays table too: Table ???. The addition of the (`\#tab:inher`) option to the end of the table caption allows us to then make a reference to Table `\@ref{tab:label}`. Note that this reference could appear anywhere throughout the document after the table has appeared.

Figures

If your thesis has a lot of figures, *R Markdown* might behave better for you than that other word processor. One perk is that it will automatically number the figures accordingly in each chapter. You'll also be able to create a label for each figure, add a caption, and then reference the figure in a way similar to what we saw with tables earlier. If you label your figures, you can move the figures around and *R Markdown* will automatically adjust the numbering for you. No need for you to remember! So that you don't have to get too far into LaTeX to do this, a couple **R** functions have been created for you to assist. You'll see their use below.

In the **R** chunk below, we will load in a picture stored as `uw.png` in our main directory. We then give it the caption of "UW logo", the label of "uwlogo", and specify that this is a figure. Make note of the different **R** chunk options that are given in the R Markdown file (not shown in the knitted document).

```
knitr::include_graphics(path = "figure/uw.png")
```

Here is a reference to the UW logo: Figure IV.1. Note the use of the `fig:` code here. By naming the **R** chunk that contains the figure, we can then reference that figure later as done in the first sentence here. We can also specify the caption for the figure via the R chunk option `fig.cap`.



Figure IV.1. UW logo

Below we will investigate how to save the output of an **R** plot and label it in a way similar to that done above. Recall the `flights` dataset from Chapter ?? (Note that we've shown a different way to reference a section or chapter here.) We will next explore a bar graph with the mean flight departure delays by airline from Portland for 2014. Note also the use of the `scale` parameter which is discussed on the next page.

```
flights %>% group_by(carrier) %>%  
  summarize(mean_dep_delay = mean(dep_delay)) %>%  
  ggplot(aes(x = carrier, y = mean_dep_delay)) +  
  geom_bar(position = "identity", stat = "identity", fill = "red")
```

Here is a reference to this image: Figure IV.2.

A table linking these carrier codes to airline names is available at <https://github.com/ismayc/pnwflights14/blob/master/data/airlines.csv>.

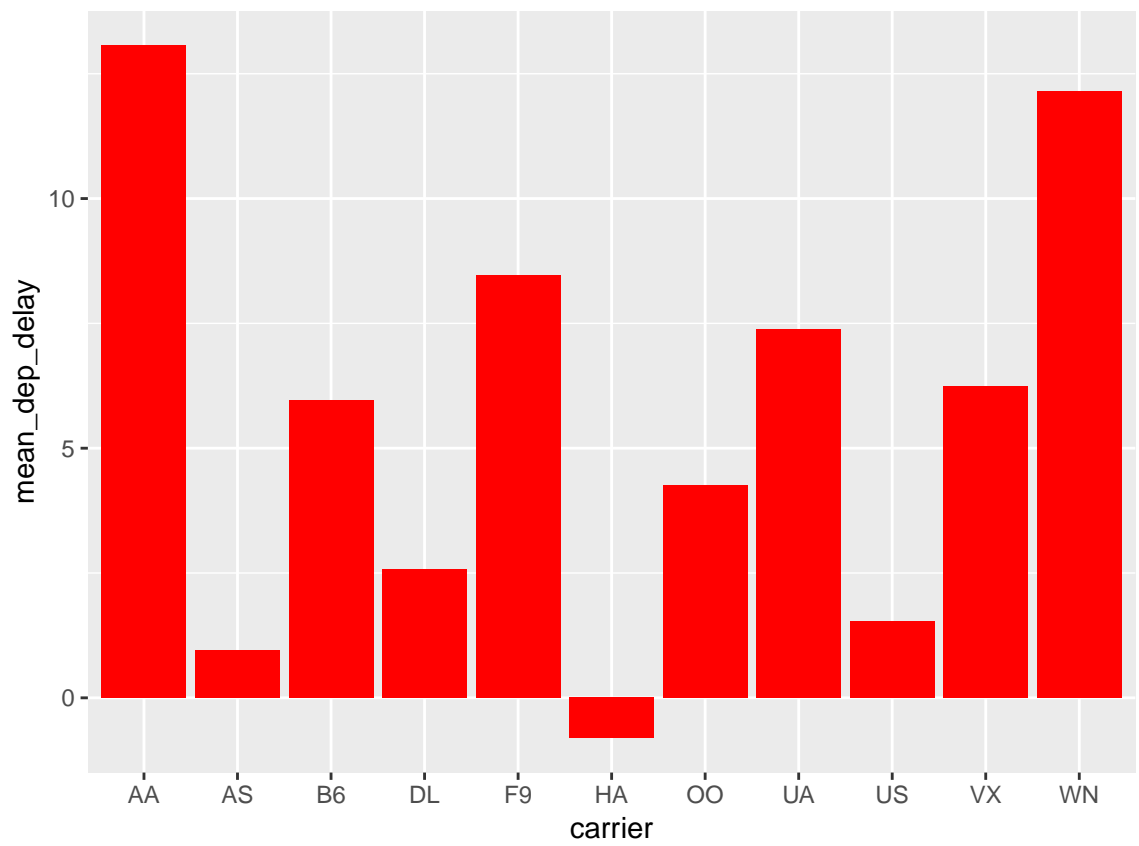


Figure IV.2. Mean Delays by Airline

Footnotes and Endnotes

You might want to footnote something.¹ The footnote will be in a smaller font and placed appropriately. Endnotes work in much the same way.

Bibliographies

Of course you will need to cite things, and you will probably accumulate an armful of sources. There are a variety of tools available for creating a bibliography database (stored with the .bib extension). In addition to BibTeX suggested below, you may want to consider using the free and easy-to-use tool called Zotero. Some Zotero documentation is at <http://libguides.reed.edu/citation/zotero>. In addition, a tutorial is available from Middlebury College at <http://sites.middlebury.edu/zoteromiddlebury/>.

R Markdown uses *pandoc* (<http://pandoc.org/>) to build its bibliographies. One nice caveat of this is that you won't have to do a second compile to load in references as standard LaTeX requires. To cite references in your thesis (after creating your bibliography database), place the reference name inside square brackets and precede it by the "at" symbol. For example, here's a reference to a book about worrying: (???). This `Molina1994` entry appears in a file called `thesis.bib` in the `bib` folder. This bibliography database file was created by a program called BibTeX. You can call this file something else if you like (look at the YAML header in the main .Rmd file) and, by default, is to placed in the `bib` folder.

For more information about BibTeX and bibliographies, see (<http://web.reed.edu/cis/help/latex/index.html>)². There are three pages on this topic: *bibtex* (which talks about using BibTeX, at <http://web.reed.edu/cis/help/latex/>

¹footnote text

²(???)

`bibtex.html`), *bibtexstyles* (about how to find and use the bibliography style that best suits your needs, at <http://web.reed.edu/cis/help/latex/bibtexstyles.html>) and *bibman* (which covers how to make and maintain a bibliography by hand, without BibTeX, at <http://web.reed.edu/cis/help/latex/bibman.html>). The last page will not be useful unless you have only a few sources.

If you look at the YAML header at the top of the main `.Rmd` file you can see that we can specify the style of the bibliography by referencing the appropriate `csl` file. You can download a variety of different style files at <https://www.zotero.org/styles>. Make sure to download the file into the `csl` folder.

Tips for Bibliographies

- Like with thesis formatting, the sooner you start compiling your bibliography for something as large as thesis, the better.
- The cite key (a citation's label) needs to be unique from the other entries.
- When you have more than one author or editor, you need to separate each author's name by the word "and" e.g. `Author = {Noble, Sam and Youngberg, Jessica},.`
- Bibliographies made using BibTeX (whether manually or using a manager) accept LaTeX markup, so you can italicize and add symbols as necessary.
- To force capitalization in an article title or where all lowercase is generally used, bracket the capital letter in curly braces.

Anything else?

If you'd like to see examples of other things in this template, please contact us (email bmarwick@uw.edu) with your suggestions. We love to see people using *R*

Markdown for their theses, and are happy to help.

CONCLUSION

If we don't want Conclusion to have a chapter number next to it, we can add the `{-}` attribute.

More info

And here's some other random info: the first paragraph after a chapter title or section head *shouldn't be* indented, because indents are to tell the reader that you're starting a new paragraph. Since that's obvious after a chapter or section title, proper typesetting doesn't add an indent there.

APPENDIX A

THE FIRST APPENDIX

This first appendix includes all of the R chunks of code that were hidden throughout the document (using the `include = FALSE` chunk tag) to help with readability and/or setup.

In the main Rmd file

```
# This chunk ensures that the spartanodown package is  
# installed and loaded. This spartanodown package includes  
# the template files for the thesis.  
if(!require(devtools))  
  install.packages("devtools", repos = "http://cran.rstudio.com")  
if(!require(spartanodown))  
  devtools::install_github("ashley-williams/spartanodown")  
library(spartanodown)
```

In Chapter IV:

```
# This chunk ensures that the huskydown package is  
# installed and loaded. This spartanodown package includes  
# the template files for the thesis and also two functions  
# used for labeling and referencing  
if(!require(devtools))  
  install.packages("devtools", repos = "http://cran.rstudio.com")  
if(!require(tidyverse))  
  install.packages("tidyverse", repos = "http://cran.rstudio.com")
```

```
if(!require(ggplot2))
  install.packages("ggplot2", repos = "http://cran.rstudio.com")
if(!require(bookdown))
  install.packages("bookdown", repos = "http://cran.rstudio.com")
if(!require(spartanodown)){
  library(devtools)
  devtools::install_github("ashley-williams/spartanodown")
}
library(spartanodown)
library(tidyverse)
library(bookdown)
```


APPENDIX B
THE SECOND APPENDIX, FOR FUN

COLOPHON

This document is set in EB Garamond, Source Code Pro and Lato. The body text is set at 11pt with *lmr*.

It was written in R Markdown and LaTeX, and rendered into PDF using spartanodown and bookdown.

This document was typeset using the XeTeX typesetting system, and the UNCG dissertation class created by Dan Yasaki. Under the hood, the UNCG dissertation LaTeX template is used to ensure that documents conform precisely to submission standards. Other elements of the document formatting source code have been taken from the Latex, Knitr, and RMarkdown templates for UC Berkeley's graduate thesis, and Dissertate: a LaTeX dissertation template to support the production and typesetting of a PhD dissertation at Harvard, Princeton, and NYU

The source files for this thesis, along with all the data files, have been organised into an R package, xxx, which is available at <https://github.com/xxx/xxx>. A hard copy of the thesis can be found in the University of North Carolina at Greensboro library.

This version of the thesis was generated on 2019-03-08 21:35:42. The repository is currently at this commit:

The computational environment that was used to generate this version is as follows:

```
- Session info -----  
setting  value  
version  R version 3.5.2 (2018-12-20)  
os       Windows 10 x64
```

```

system    x86_64, mingw32
ui        RTerm
language  (EN)
collate   English_United States.1252
ctype     English_United States.1252
tz        America/New_York
date      2019-03-08

```

```

- Packages -----
package      * version date      lib
assertthat   0.2.0   2017-04-11 [1]
backports    1.1.3   2018-12-14 [1]
bookdown     * 0.9     2018-12-21 [1]
broom        0.5.0   2018-07-17 [1]
callr        3.1.1   2018-12-21 [1]
cellranger   1.1.0   2016-07-27 [1]
cli          1.0.1   2018-09-25 [1]
colorspace   1.4-0   2019-01-13 [1]
crayon       1.3.4   2017-09-16 [1]
desc         1.2.0   2018-05-01 [1]
devtools     * 2.0.1   2018-10-26 [1]
digest       0.6.18  2018-10-10 [1]
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evaluate     0.13    2019-02-12 [1]
forcats     * 0.3.0   2018-02-19 [1]

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httr	1.4.0	2018-12-11	[1]
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yaml	2.2.0	2018-07-25	[1]

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[1] C:/Users/asw/Documents/R/win-library/3.5

[2] C:/Program Files/R/R-3.5.2/library

REFERENCES

- Administration, U.E.I. (2019). Electric Power Monthly with data for November 2018. 274.
- American Coal Ash Association (2012). 2012 Coal Combustion Product (CCP) Production and Use Survey Report (ACAA).
- Binkley, J., and Simpson, J.A. (2003). Heavy metals in wastewater treatment processes. In *Handbook of Water and Wastewater Microbiology*, D.D. Mara, and N.J. Horan, eds. (London ; San Diego: Academic Press), pp. 597–610.
- Bizily, S.P., Rugh, C.L., Summers, A.O., and Meagher, R.B. (1999). Phytoremediation of methylmercury pollution: merB expression in *Arabidopsis thaliana* confers resistance to organomercurials. *Proceedings of the National Academy of Sciences of the United States of America* *96*, 6808–6813.
- Boyd, E., and Barkay, T. (2012). The mercury resistance operon: From an origin in a geothermal environment to an efficient detoxification machine. *Frontiers in Microbiology* *3*, 349.
- Cabral, L., Yu, R.-Q., Crane, S., Giovanella, P., Barkay, T., and Camargo, F.A.O. (2016). Methylmercury degradation by *Pseudomonas putida* V1. *Ecotoxicology and Environmental Safety* *130*, 37–42.
- Christensen, G.A., Wymore, A.M., King, A.J., Podar, M., Hurt, R.A., Santillan, E.U., Soren, A., Brandt, C.C., Brown, S.D., Palumbo, A.V., et al. (2016). Development and Validation of Broad-Range Qualitative and Clade-Specific Quantitative Molecular Probes for Assessing Mercury Methylation in the Environment. *Applied and*

- Environmental Microbiology *82*, 6068–6078.
- Dash, H.R., and Das, S. (2014). Bioremediation Potential of Mercury by *Bacillus* Species Isolated from Marine Environment and Wastes of Steel Industry. *Bioremediation Journal* *18*, 204–212.
- Dennis Lemly, A. (2015). Damage cost of the Dan River coal ash spill. *Environmental Pollution* *197*, 55–61.
- Deonaraine, A., Bartov, G., Johnson, T.M., Ruhl, L., Vengosh, A., and Hsu-Kim, H. (2013). Environmental Impacts of the Tennessee Valley Authority Kingston Coal Ash Spill. 2. Effect of Coal Ash on Methylmercury in Historically Contaminated River Sediments. *Environmental Science & Technology* *47*, 2100–2108.
- Driscoll, C.T., Mason, R.P., Chan, H.M., Jacob, D.J., and Pirrone, N. (2013). Mercury as a Global Pollutant: Sources, Pathways, and Effects. *Environmental Science & Technology* *47*, 4967–4983.
- Ehrlich, H.L., and Newman, D.K. (2008). *Geomicrobiology*, Fifth Edition (CRC Press).
- Greely Jr., M.S., Elmore, L.R., McCracken, M.K., and Sherrard, R.M. (2014). Effects of Sediment Containing Coal Ash from the Kingston Ash Release on Embryonic Larval Development in the Fathead Minnow, *Pimephales promelas* (Rafinesque, 1820). *Bulletin of Environmental Contamination and Toxicology* *92*, 154–159.
- Jayaranjan, M.L.D., Hullebusch, E.D. van, and Annachhatre, A.P. (2014). Reuse options for coal fired power plant bottom ash and fly ash. *Reviews in Environmental*

Science and Bio/Technology 1–20.

Lima de Silva, A.A., de Carvalho, M.A.R., de Souza, S.A.L., Dias, P.M.T., da Silva Filho, R.G., de Meirelles Saramago, C.S., de Melo Bento, C.A., and Hofer, E. (2012). Heavy metal tolerance (Cr, Ag AND Hg) in bacteria isolated from sewage. *Brazilian Journal of Microbiology* 43, 1620–1631.

Liu, Y.-R., Yu, R.-Q., Zheng, Y.-M., and He, J.-Z. (2014). Analysis of the Microbial Community Structure by Monitoring an Hg Methylation Gene (*hgcA*) in Paddy Soils along an Hg Gradient. *Applied and Environmental Microbiology* 80, 2874–2879.

NC DEQ (2014). Rapid response timeline of events.

Otter, R.R., Bailey, F.C., Fortner, A.M., and Adams, S.M. (2012). Trophic status and metal bioaccumulation differences in multiple fish species exposed to coal ash-associated metals. *Ecotoxicology and Environmental Safety* 85, 30–36.

Parks, J.M., Guo, H., Momany, C., Liang, L., Miller, S.M., Summers, A.O., and Smith, J.C. (2009). Mechanism of Hg-C Protonolysis in the Organomercurial Lyase MerB. *Journal of the American Chemical Society* 131, 13278–13285.

Poulain, A.J., and Barkay, T. (2013). Cracking the Mercury Methylation Code. *Science* 339, 1280–1281.

Rowe, C.L. (2014). Bioaccumulation and effects of metals and trace elements from aquatic disposal of coal combustion residues: Recent advances and recommendations for further study. *Science of the Total Environment* 485/486, 490–496.

Ruhl, L., Vengosh, A., Dwyer, G.S., Hsu-Kim, H., and Deonarine, A. (2010). En-

- vironmental impacts of the coal ash spill in Kingston, Tennessee: An 18-month survey. *Environmental Science & Technology* *44*, 9272–9278.
- Schaefer, J.K., Rocks, S.S., Zheng, W., Liang, L., Gu, B., and Morel, F.M.M. (2011). Active transport, substrate specificity, and methylation of Hg(II) in anaerobic bacteria. *Proceedings of the National Academy of Sciences* *108*, 8714–8719.
- Shaheen, S.M., Hooda, P.S., and Tsadilas, C.D. (2014). Opportunities and challenges in the use of coal fly ash for soil improvements review. *Journal of Environmental Management* *145*, 249–267.
- Tsui, M.T.K., Blum, J.D., Finlay, J.C., Balogh, S.J., Kwon, S.Y., and Nollet, Y.H. (2013). Photodegradation of methylmercury in stream ecosystems. *Limnology and Oceanography* *58*, 13–22.
- TVA (2011). Fact Sheet Kingston Ash Recovery Project: December 7, 2011.
- US EPA (2014a). Fly Ash | Coal Combustion Products | US EPA (<http://www.epa.gov/osw/conservation/fly-ash>).
- US EPA (2014b). Bottom Ash | Coal Combustion Products | US EPA (<http://www.epa.gov/osw/conservation/bottom-ash>).
- US EPA (2014c). Flue Gas Desulfurization Material | Coal Combustion Products | US EPA (<http://www.epa.gov/osw/conservation/imr/ccps/fgd.htm>).
- US EPA (2014d). EPA’s Response to the Duke Energy Coal Ash Spill in Eden, NC (<http://www.epa.gov/region4/duke-energy/>).