IMPACT OF HEAVY METAL CONTAMINATION FROM COAL FLUE GAS ON MICROALGAE BIOFUEL AND BIOGAS PRODUCTION THROUGH MULTIPLE CONVERSION PATHWAYS

by

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ABSTRACT

Impact of Heavy Metal Contamination from Coal Flue Gas on Microalgae Biofuel and Biogas Production through Multiple Conversion Pathways

by

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Utah State University, 2016

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Department: Mechanical and Aerospace Engineering

Large scale biofuel production from microalgae is expected to be integrated with point source CO₂ sources, such as coal fired power plants. Flue gas (CO₂) integration represents a required nutrient source for accelerated growth while concurrently providing an environmental service. Heavy metals inherent in coal will ultimately be introduced into the culture system. The introduced heavy metals have the potential to bind to microalgae cells, impact growth due to toxicity, and negatively impact the quality of biofuel and other microalgal derived products.

Heavy metals As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sb, Se, Sn, V and Zn, commonly present in coal, were introduced to the microalgae growth medium at a concentration expected from a 7 day growth period using coal flue gas. Experimentation was conducted with Nannochloropsis salina cultivated in photobioreactors at a light intensity of 1000 μmol m⁻² s⁻¹. Heavy metals negatively impacted the growth with the average productivity being 0.54 ± 0.28 g L⁻¹ d⁻¹, corresponding to a decrease of 52% in biomass yield compared to control growths. Heavy metal analysis showed significant binding of the majority of the heavy metals to the biomass. A lipid content analysis found a decrease in lipid content from 38.8 ± 0.62% to 31.58 ± 0.50% (percent dry biomass).
Control and heavy metal contaminated biomass were processed into biofuel through one of two different in-situ transesterification techniques, either acid-catalyzed or supercritical methanol conversion. The acid-catalyzed conversion resulted in an average crude biofuel production decrease from 0.31 ± 0.03 grams biofuel/gram microalgae for the control algae to 0.28 ± 0.02 grams biofuel/gram microalgae for the heavy metal algae, representing a 9.7% reduction. Supercritical methanol conversion exhibited a similar trend corresponding to a 15.8% reduction. Compared to the control, the total production of biofuel from the contaminated system was decreased by 51% for the acid-catalyzed conversion and 55% for the supercritical methanol conversion. Heavy metal analyses were performed on the biofuel, lipid extracted algae, and other biofuel conversion byproducts. Biochemical methane potential testing was performed on the lipid extracted algae to determine the effect of heavy metals on the generation of biogas. The effects of heavy metals in combination with the effects of acid catalyzed transesterification were found to have a positive effect on the amount of methane produced with an average productivity of 105.89 mL g-COD⁻¹ from the heavy metals contaminated LEA compared to the control microalgae biomass which produced 53.25 mL g-COD⁻¹.
Impact of Heavy Metal Contamination from Coal Flue Gas on Microalgae Biofuel and Biogas Production through Multiple Conversion Pathways

Derek E. Hess

Microalgae has a great potential to help alleviate the world’s current dependence on fossil fuels. Microalgae is a single celled organism that produces lipids as a food storage device much like humans and animals store fat. These lipids are a kind of oil that when harvested can be utilized as biofuel.

As the technology surrounding using microalgae as a source of biofuel has advanced key bottlenecks in the eventual large-scale production of microalgae based biofuel have been discovered. One of these bottlenecks is the need to cheaply supply carbon for microalgae growth. One idea that has emerged as a plausible solution is the utilization of exhaust from coal power plants which dump large amounts of CO$_2$ into the atmosphere every year. However, there are many contaminants contained within the exhaust that could have unknown effects on the microalgae and limit its potential use.

The goal of this work is to better understand the effects of combining a microalgae growth setup and coal power plant exhaust by growing microalgae in the presence of heavy metals found in coal exhaust and measuring the effects the contaminants have on microalgae growth, biofuel production and methane production.

Results show that heavy metals from flue gas have negative effects upon the growth of microalgae and the production of lipids. Heavy metals were found to have positive effects on lipid recovery and on the production of methane.
 ACKNOWLEDGMENTS

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CHAPTER 1
INTRODUCTION

1.1 Background / Literature Review

The world continues to search for more efficient and economic alternatives to fossil fuels due to increased concerns over global climate change, petroleum resource availability, and continually increasing global energy consumption. The area of biofuels is a promising alternative to fossil fuels. Crops such as microalgae, palm, soybeans, cottonseed, and sunflowers have been demonstrated as viable feedstocks for biofuel, with microalgae having inherent advantages. Benefits of microalgae, when compared to other feedstocks, include: higher lipid production per ground area, higher solar energy yield, year round cultivation, utilization of low quality and saline water, does not require agricultural land, and integration with various waste streams [1-3]. A promising avenue to improve sustainability of coal fired power plants is the utilization of the carbon dioxide in flue gas with microalgae cultivation systems. The impact of the integration of flue gas into the microalgae growth cycle has not been fully explored.

Previous studies have shown that integration of flue gas derived from coal with the microalgae growth cycle can be cost effective, however undesirable contaminants such as heavy metals can be introduced into the growth phase [1]. Few studies have assessed the effects of the integration of industrial flue gas with microalgae cultivation, yet the majority of the studies of the microalgae to biofuels process including: economic [4-6], lifecycle [7-9], and scalability [10, 11] assessments make a simplifying assumption of seamless integration. The core of the assumption includes no negative effects caused by the co-location and ignores the potential limitations on end products and co-products due to the introduction of contaminants including heavy metals. This could plausibly be an improper assumption seeing that microalgae is a well-known metal bioaccumulator [12, 13]. Internalized metals form metallic-compounds that can be stored in
different parts of the cell (cytoplasm, nuclei, chloroplast, mitochondria, vacuoles, and lipids) and these internalized metals represent potentially undesirable results for flue gas integration.

Downstream processing of the biomass for various products has the potential for absorbed heavy metals to contaminate end products, thus limiting the product uses [14, 15]. Studies using simulated and actual flue gas have been conducted, but fail to evaluate the end fate of heavy metals [16-20]. Downstream processing of the biomass after lipid extraction is proposed to improve the economic feasibility and environmental impact of the microalgae-to-biofuel process, but the impact of using heavy-metals-contaminated biomass in anaerobic digestion systems has not been studied.

1.2 Research Objectives

The overarching hypothesis for this research is “Flue gas from a coal fire power plant contains heavy metals that will be beneficial to the microalgae to biofuel and biogas process”. To test this hypothesis research was performed to directly assess the impact of 14 heavy metals (As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sb, Se, Sn, V and Zn), commonly present in coal flue gas, on biomass, lipid, biofuel and methane production and evaluate the end fate of heavy metals in the microalgae-to-biofuel process. Key questions to be answered are:

- What is the end fate of heavy metals introduced into the microalgae to biofuel and biogas system, i.e., where do the heavy metals accumulate?

- To what extent will heavy metal contamination effect microalgae growth, biofuel production and biogas production?

- To what extent will the end fate of heavy metal contamination be a function of biofuel conversion type, i.e., will using acid catalyzed transesterification vs. supercritical methanol transesterification effect the end fate of heavy metals?
Results from this work can be integrated with sustainability modeling to understand the impact of heavy metal contaminants on a large-scale through metrics such as global warming potential and economics.

1.3 References


7. Frank, E.D., et al., Life-cycle analysis of algal lipid fuels with the GREET model. 2011, Center for Transportation Research, Energy Systems Division, Argonne National Laboratory: Oak Ridge, TN.


CHAPTER 2
QUANTIFICATION OF THE EFFECTS OF FLUE GAS DERIVED HEAVY METALS ON MICROALGAE SYSTEMS AND END FATE OF HEAVY METALS

2.1 Abstract

Increasing demand for renewable fuels has researchers investigating the feasibility of alternative feedstocks, including microalgae. Inherent advantages of microalgae include high potential yield, use of non-arable land, and integration with waste streams. Large-scale production of biofuel from microalgae will require the integration of growth platforms with point source carbon dioxide such as coal derived flue gas. The introduction of this waste stream into the growth system will inevitably introduce trace heavy metals which have a high affinity to bind to microalgal cells, could be toxic to the cells, and if transferred to the microalgae could impact the end use of the derived products. heavy metals As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sb, Se, Sn, V and Zn were added to microalgal growth medium at a base concentration estimated to be representative of concentrations expected from 7 day growth periods where coal derived flue gas is used as the carbon source. *Nannochloropsis salina* was cultivated in photobioreactors at outdoor light levels, 984 µmol m⁻² s⁻¹, with results for biomass, lipid yield, and fatty acid profiles evaluated. Results show trace heavy metals negatively impacted growth and lipid yields with an average biomass productivity of 0.37 g L⁻¹ d⁻¹ ± 0.18, corresponding to a 67.5% decrease in biomass yield compared to control growths and a lipid decrease from 43.8 ± 1.6 to 29.8 ± 5.7 (% dry biomass). Heavy metals analysis performed using inductively coupled plasma mass spectrometry (ICP-MS) shows significant biomass sorption of the majority of the heavy metals.

2.2 Introduction

Global demand for energy is putting increased pressure on various resources including traditional fossil reserves while igniting interest in the development of substitute energy such as
non-traditional fossil reserves, alternative energy and biobased energy. Negative environmental impacts associated with the consumption of fossil fuels have further inspired the development and investigation of alternative energy resources. The US annually consumes approximately 100 quadrillion BTUs of energy with 80% being derived from fossil sources corresponding to 5 billion metric tons of carbon dioxide emissions, with approximately 30% of those emissions derived from the combustion of coal for electrical energy production [1,2]. Recent regulation on the reduction of carbon emissions from coal has further sparked the evaluation of the synergistic integration of microalgae production with coal based flue gas as the carbon source [3,4]. Further, microalgae represent a promising alternative biofuel feedstock with high productivity rates, year round cultivation, integration with various waste streams, and the use of low quality land and water [5]. The technical evaluation of microalgae based biofuel systems has been traditionally performed through techno-economics and life cycle assessment with the majority of these evaluations assuming the seamless integration of industrial carbon dioxide such as coal fired power plants [6-8]. There has been minimal work on the evaluation of the impact of coal derived flue gas on microalgae productivity.

Large-scale phototrophic cultivation of microalgae for the production of a biofuel feedstock in traditional growth platforms such as open raceway ponds or photobioreactors will require a concentrated carbon source to support high productivity rates. Various carbon sources, gaseous CO$_2$ and bicarbonate, have demonstrated to effectively support growth at these high productivity rates [9,10]. The integration of industrial CO$_2$ will include the introduction of potentially non-desirable components into the growth system such as sulfur and nitric oxide components as well as trace heavy metals such as Hg, Cd, Pb and As [11-13]. Trace heavy metal contaminants contained in coal for example are volatilized during combustion and are released into the atmosphere. Routing this stream through a microalgae cultivation system will introduce these
contaminants into the growth system [14-16]. These trace heavy metals contaminants are then susceptible to being sorbed into the biomass contaminating the end products and co-products, thus limiting product use [17,18]. Further, there exists the potential for these contaminants to negatively impact productivity [19-21]. Simulated flue gas studies focused on the effects of NOx and SOx have been conducted and show improved growth but fail to evaluate heavy metals found in real flue gases [22, 23]. Other studies have used actual flue gas for cultivation but again fail to evaluate trace heavy metals bioaccumulation [24, 25, 3, 4]. A significant effort has pursued the investigation of individual heavy metals with the majority of previous studies not representative of what would be expected from a flue gas system in terms of duration and concentration [26-31]. Napan et al. [32] investigated the impacts of inorganic contamination at concentrations that are representative of integration with coal based flue gas on the growth of a freshwater microalgae species. Results from this study showed an increase in productivity at inorganic contaminant concentrations expected with flue gas integration. However, inorganic contaminant concentrations near twice the expected baseline concentration detrimentally impacted the productivity. Application of these results to large-scale biofuel production systems are limited based on the species (Scenedesmus obliquus) being a low lipid algae, multi-week batch growth, and low light intensity. There exists a need to understand the impacts of a multi-inorganic contaminant system representative of conditions expected from flue gas integration on a high lipid yielding microalgae under conditions that are representative of large-scale outdoor cultivation.

The utilization of flue gas as a source for carbon dioxide in the cultivation phase of microalgae represents an environmentally favorable process in microalgae based biofuels. This study evaluates the impact of heavy metals found in coal based flue gas on the productivity of microalgae including determining the end fate. Experimental work was done to characterize the
impact on *Nannochloropsis salina* productivity and the end fate of contaminants introduced into the growth media. Growth media was spiked with 14 heavy metals, arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), antimony (Sb), selenium (Se), tin (Sn), vanadium (V), and zinc (Zn) at a baseline concentration (1X) that is expected from the integration of algal growth systems with coal flue gas. Multiple heavy metals concentrations were evaluated ranging from the control up to 10X. Higher concentrations would be representative of systems that integrate media recycling to reduce production costs or integration with systems with higher heavy metals concentrations. The experimental growths were performed in triplicate in photobioreactors (PBRs) illuminated at a light intensity representative of an outdoor system. The impact on biomass productivity, lipid yield, and the end fate of the trace heavy metals contaminants are quantified for various initial contamination concentrations. Results are used to illustrate the importance of understanding the potential impact of integrating microalgae cultivation systems with industrial flue gas for the microalgae-to-biofuels process.

### 2.3 Materials and Methods

Details of the cultivation system, integrated heavy metals concentrations, and analytics for determining biomass density, lipid content, and heavy metals concentrations in the biomass and spent media are presented.

#### 2.3.1 Cultivation System

##### 2.3.1.1 Inoculum Setup

This study used *Nannochloropsis salina* (UTEX 1776) microalgae obtained from The Culture Collection of Algae at the University of Texas at Austin. Initially the culture started in sterile petri-dishes containing solid nutrient rich medium and 3% (w/v) agar and was maintained in a 24 hour low light setup. The colonies were then transferred to baffled Erlenmeyer flasks containing
200 mL of nutrient rich medium detailed below and kept on an illuminated shaker table. The cultures were then transferred to a 1.1 L sterile PBR illuminated at 200 µmol m$^{-2}$ s$^{-1}$ on a 16/8 hours on/off duty cycle and maintained at 23°C ± 1°C by submersion in a water bath that was actively temperature controlled as seen in Figure 1. The culture was mixed with sparge air enriched with CO$_2$ maintained at 2.5 L min$^{-1}$ and 25 cc min$^{-1}$, respectively. Experimental medium was made consisting of NaCl (761.2 mM), CaCl$_2$·2H$_2$O (1.0 mM), KCl (6.4 mM), Na$_2$SiO$_3$·9H$_2$O (0.2 mM), MgSO$_4$·7H$_2$O (6.0 mM), KNO$_3$ (10.1 mM), KH$_2$PO$_4$ (0.5 mM), Ammonium Ferric Citrate (2.0*10$^{-2}$ mM), H$_3$BO$_3$ (1.5*10$^{-2}$ mM), Na$_2$MoO$_4$·2H$_2$O (5.0*10$^{-5}$ mM), MnCl$_2$·4H$_2$O (1.5*10$^{-3}$ mM), ZnSO$_4$·7H$_2$O (2.1*10$^{-4}$ mM), CuSO$_4$·5H$_2$O (8.0*10$^{-5}$ mM). Analytical grade reagents were used, and the medium was autoclaved at 120 ºC for 30 minutes. Sterile Biotin, Vitamin B12, and Thiamine solution were added after the autoclaved medium reached room temperature.

Figure 1. The inoculum growth system is comprised of a glass tank capable of holding twelve 1.1L PBRs and illuminated by a light bank.
2.3.1.2 Experimental Growth System

The experimental growth system consisted of 12 borosilicate glass tube reactors of 4.5 cm diameter and 80 cm length filled with 1.1 liters of microalgal culture as seen in Figure 2. The experimental system was maintained at 23°C through active temperature control and constantly illuminated at a 984 µmol m$^{-2}$ s$^{-1}$ with T5 fluorescent lights. Reactors were divided into four groups of three with a pH sensor monitoring one of the three reactors in each group for pH control. Sparge air was humidified and supplied through a glass capillary tube to each reactor at a rate of 0.5 L min$^{-1}$ with CO$_2$ supplied on demand at 10 cc min$^{-1}$ for the first three days and at 16.7 cc min$^{-1}$ for the remainder of the growth for each reactor group. The pH was maintained at 7.0 +/- 0.1 through injection of CO$_2$ based on pH feedback control. Reactors were capped with a silicon stopper with a port for the sparge air capillary tube, an exhaust port vented into a fume hood, a sampling port consisting of a second glass capillary tube, and a port for a pH probe. Prior to each inoculation, reactors were sterilized and decontaminated by acid wash using 10% HNO$_3$ overnight, followed by rinsing with deionized water (17.7 MΩ·cm resistivity) and autoclaving at 120 °C for 30 minutes.

2.3.1.3 Biomass Growth Measurement

PBRs were inoculated at a density of 1 gram of algae dry weight per liter of medium with a total volume of 1.1 liter per reactor. Daily growth was measured through optical density (OD) at 750 nm and correlated to total suspended solids (g L$^{-1}$) based on previous dry mass experimentation (R2=0.98). OD was measured daily from all reactors.

2.3.2 Heavy Metals Concentrations

Contaminant levels in flue gas is a function of the fuel with this work focused on the simulation of coal based flue gas. There is inherent variability in the contaminant levels in coal.
The microalgae cultivation system is comprised of a glass tank capable of holding twelve 1.1L PBRs. The glass tank sits upon a wooden platform that also supports four light banks for the illumination or the microalgae.

The concentrations used in this study are conservative and representative of highly contaminated coal [33]. The required CO₂ and corresponding heavy metals that would be delivered were calculated and include the following assumptions: 20% of coal is converted to ash, fly ash represents 80% of the total ash, 99% of the fly ash is removed by air pollution control devices (1% introduced into the cultivation system) [34], heavy metals are equally distributed in the fly ash, carbon content of coal is 60%, the carbon content of the algae is 50%, and the carbon dioxide uptake efficiency is 4% [10]. Based on these assumptions, the concentration of heavy metals expected to be sorbed into the media after one week of growth is referred to as the baseline case,
or 1X. The concentrations corresponding to the baseline scenario for the 14 heavy metals added to each reactor is presented in Table 1.

Table 1. Heavy metals concentrations found in coal and the baseline or 1X concentrations.

<table>
<thead>
<tr>
<th>Heavy Metals</th>
<th>Heavy metal concentration in fly ash (mg/kg)*</th>
<th>Salt source</th>
<th>Baseline (1X) concentration (mg·L⁻¹)</th>
</tr>
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<tr>
<td>As</td>
<td>391</td>
<td>NaAsO₂</td>
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</tr>
<tr>
<td>Cd</td>
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<td>CdCl₂</td>
<td>1.50E-02</td>
</tr>
<tr>
<td>Co</td>
<td>79</td>
<td>CoCl₂·6H₂O</td>
<td>1.56E-02</td>
</tr>
<tr>
<td>Cr</td>
<td>651</td>
<td>Na₂Cr₂O₇·2H₂O</td>
<td>1.29E-01</td>
</tr>
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<tr>
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<tr>
<td>V</td>
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<td>V₂O₅</td>
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</tr>
<tr>
<td>Zn</td>
<td>2200</td>
<td>ZnCl₂</td>
<td>4.36E-01</td>
</tr>
</tbody>
</table>

* [35]

The baseline contaminant concentrations are intended to be conservative and represent a worst case scenario without recycling medium. Experiments were conducted at 1, 2, 5, and 10 times the baseline concentration. Heavy metals were prepared individually in stock solutions and were sterile filtered through a 0.2 µm syringe filter.

2.3.3 Heavy Metals Analysis

Trace heavy metals analysis was performed based on the methods presented in Napan et al. [36]. Analysis with the exception of Hg was performed through Inductively Coupled Plasma
Mass Spectrometry (ICP-MS Agilent 7700x Series) in both the biomass and the medium after a 7 day growth. Hg levels were analyzed using Cold Vapor Atomic Absorption Spectrometry (CV-AAS, PerkinElmer Analyst 800). Biomass was separated from the medium samples through the use of centrifuge operated at 9,936 \( \times \) g for 5 minutes. Biomass or media samples were prepared for microwave digestion through the addition of 7 mL of analytical trace metal grade nitric acid and 3 mL of hydrogen peroxide to 50 mg of biomass sample or 10 mL of supernatant media to acid washed microwave reaction vessels. Samples were digested at a power of 1000 W and ramped from room temperature to 180°C in 15 min and then maintained at this temperature for 15 min. Samples were transferred to volumetric flasks, brought to a volume of 25 mL with deionized water (Type I), and transferred to a capped container, and preserved at 4°C until analysis. Quality control (QC) samples including laboratory reagent blanks (LRB), laboratory fortified blanks (LFB), and laboratory fortified matrix (LFM) were run concurrently with prepared samples. LRBs were blank samples. LFBs were blank samples spiked with known concentrations of analyte. LFM samples were made with a biomass or supernatant digested sample spiked with a known concentration of analyte. The results from the QC samples were used to determine the percent recovery and the percent difference. ICP-MS was used to quantify the metals and metalloids in the digested samples. On the day of sample analysis, calibration standards diluted in 28% trace metals grade nitric acid (70%) solution were prepared. The ICP-MS was operated at an RF power of 1500 W, a nebulizer gas flow rate (carrier and dilution) 1.1 L \( \text{min}^{-1} \), and a dwell time of 1 s. Results were used to calculate the QC standards (percent recovery, percent difference, and relative percent difference) and compared to the project data quality criteria.

The CV-AAS was operated with a carrier gas of Ar at 100 mL \( \text{min}^{-1} \), a cell temperature of 100°C, a sample volume of 500 \( \mu \text{L} \), a carrier of 3% HCl at 9.23 ml \( \text{min}^{-1} \), and a reductant of 10%
stannous chloride at 5.31 ml min⁻¹. In conjunction, an electrodeless discharge lamp was used and set at 185 mA, a wavelength of 253.7 nm, and a slit of 0.7 nm. Approximately 5 mL of digested sample was drawn into the instrument where Hg in the sample was reduced to elemental Hg gas that is purged from solution with the carrier gas and then detected by cold vapor atomic absorption technique. On the same day of analysis calibration standards were prepared using a concentrated Hg standard diluted in 28% trace metal grade nitric acid (70%) solution.

2.3.4 Lipid Analysis

Quantification of microalgal lipids was determined using gas chromatography (GC, Agilent Technologies 7890A). Samples were prepared based on the methods of Wahlen et al. [37]. Acid-catalyzed transesterification was used to convert lipids to fatty acid methyl esters (FAMEs). This process involved adding 2 mL of methanol containing 2% concentrated sulfuric acid into a glass vial with 100 mg of freeze-dried microalgae and digesting at 80 °C for 6 hours. Two mL of chloroform was then added to the digested sample and transferred into a clean test tube. An additional 2 mL of chloroform was used to rinse the sample vial and transfer any residual material. A small amount of water was added to ensure phase separation, and then the samples were centrifuged. The top layer consisting of H₂O and methanol was removed, water was again added, and the samples were centrifuged again. The bottom layer consisting of chloroform and FAME was then removed and placed in a clean volumetric test tube. A volume of 3 ml of chloroform was used to recover any FAME left behind. Chloroform was then added to the volumetric test tube for a final volume of 10 mL, and the sample was vortexed. A sample of 100 µL was drawn from the volumetric test tube and was added to a GC vial along with 900 µL of chloroform. The samples were then analyzed with a GC. The GC column used was a Restek Stabilwax-DA (30m X 0.32mm ID X 0.25 µm film) with a polyethylene glycol stationary phase. The inlet temperature was 250°C. The oven temperature was initially held at 100°C for 1 min
and ramped at 10°C min⁻¹ up to 235°C and held for 10 min, with the total run time being 24.5 min. Standards using methylmyristate, methylpalmitoleate, and methyloleate (Nu-Chek Prep, Inc.) were prepared in a chloroform matrix and run concurrently with the prepared samples.

2.3.5 Statistical Analysis

The experimental growth system consisted of 12 reactors with 6 reactors run as control and 6 run with trace heavy metal contamination. Some batches consisted of variations in the number of controls and trace heavy metals contaminated PBR based on biomass production needs for further testing. All growth experimental results presented represents a minimum of triplicate. Statistical processing of the data for comparison to control was done using a two-tailed, two-sample equal variance Student t-test with a 95% confidence interval. Data presented is a mean with +/- one standard deviation. The Taylor Series Method for uncertainty propagation was used for calculated results.

2.4 Results and Discussion:

Results are divided into three sections, 1) productivity results from the baseline (1X) heavy metals concentration experiments, 2) end fate of the heavy metals (biomass, spent medium, or environment), and 3) evaluation of increased contamination (2X, 5X, and 10X) on productivity.

2.4.1 Impact of Heavy Metals on Microalgae Productivity

The effect of heavy metals on microalgae productivity was determined and compared to a control. Five, 1 week long batch growths consisting of a total of 30 control growth replicates and 30 heavy metals contaminated growth replicates were run. A typical growth from the 1X contamination level and a control is presented in Figure 3A. The growth data presented is from one batch consisting of a total of 12 PBRs (6 control and 6 contaminated). Growth from all 5 batches is compiled and presented in Figure 3B. The heavy metals are shown to negatively
impact the biomass productivity of the system starting on day 2 (Student’s t-test, P < 0.01). The contaminated cultures were found to have a 67.5% ± 16.3 reduction in biomass productivity with the control and contaminated cultures averaging 1.13 g L⁻¹ d⁻¹ ± 0.12 and 0.37 g L⁻¹ d⁻¹ ± 0.18, respectively. The growth within the same batch were very repeatable as shown by the small standard deviations as seen in Figure 3A. Results between batches were less repeatable as shown by the larger standard deviations in Figure 3B. However, a comparison of the control to contaminated batches on an individual basis show similar results to Figure 3A. Individual batch data is presented in Appendix A.

The impact of the heavy metals on lipid production and profile was evaluated for the 5 batches presented, Figure 4. In this study, N. Salina predominantly produced fatty acids with a chain length of 16 which is typical for this strain [10]. The addition of heavy metals resulted in a 31.9% ± 13.2 decrease in lipid content at harvest after the 7 day growth period, from a total lipid content of 43.8% ± 1.6 for the control to 29.8% ± 5.7 for the contaminated replicates. The combination of the effects of contaminants on the biomass productivity and lipid content show that there is an overall decrease in lipid productivity of 77.9% ± 12.1 from 0.49 g L⁻¹ d⁻¹ ± 0.06 for the control replicates to 0.11 g L⁻¹ d⁻¹ ± 0.06 for the trace heavy metals contaminated replicates harvested after 7 days. This result highlights the importance of understanding the implications of integrating industrial flue gas with microalgae production systems. The majority of techno-economic and life cycle modeling efforts assume a seamless integration of industrial flue gas as the carbon source [38-41]. Results from this study show that heavy metals from flue gas have a significant impact on the final productivity of the cultivation system. Growth results show heavy metals from flue gas integration could dramatically impact the production of the system, thus negatively impacting the economics or environmental impact due to productivity being the functional unit.
Figure 3. Results from growth studies: A) Representative results from a typical individual batch for control and heavy metals contaminated cultures (Results for other batches presented in Appendix A). Error bars represent one standard deviation from 6 replicates. B) Combined growth results from 5 batches with each batch including 12 reactors with 6 control and 6 heavy metals contaminated reactors. Error bars represent one standard deviation from 30 control reactors and 30 trace heavy metals contaminant reactors.

Figure 4. Lipid content in the control and heavy metals contaminated biomass from 5 batches. Error bars represent one standard deviation from 3 replicates for the heavy metals contaminated reactors with single measurements performed on the control reactors.
The algal lipid profile has been shown to be impacted by heavy metals and therefore, is expected to affect FAME [42,43]. The fatty acid profile for the control and trace heavy metals contaminated biomass were similar with minimal changes (Table 2). The profiles were predominantly composed of C16:0 and C16:1 for both the control and heavy metals contaminated biomass with the sum of C16:0 and C16:1 representing 68.6% and 61.2%, respectively, of the total lipids. The heavy metals contaminated biomass showed a slight decrease in omega-3 eicosapentenoic acid (C20:5) from 3.4% to 2.7% (Student’s t-test, P < 0.01). Compared to previous studies, this is a relatively low C20:5 content with Volkman et al. [44] reporting 16.1%. The difference is attributed to the time of harvest with this study harvesting in the late log phase of growth which has been shown to impact the C20:5 concentration as shown by Volkman et al. [44]. There was no statistical difference for C18:0 and C18:2. However, C18:1 decreased from 6.6% to 4.5% (Student’s t-test, P < 0.01).

Table 2. Fatty acid profile comparison between control and heavy metals contaminated microalgae. Results are the average from 1 control replicate from batches 1-5 (n=5) and 2 contaminated replicates from batches 1-5 (n=10). Reported error represents one standard deviation

<table>
<thead>
<tr>
<th>Major Fatty Acid Profiles (carbon chain length: number of unsaturations)</th>
<th>% of total fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. Salina</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>16:0</td>
<td>39.6 ± 0.5</td>
</tr>
<tr>
<td>16:1</td>
<td>29.0 ± 0.6</td>
</tr>
<tr>
<td>18:0</td>
<td>3.0 ± 0.2</td>
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<td>18:1</td>
<td>6.7 ± 0.2</td>
</tr>
<tr>
<td>18:2</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>20:4</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>20:5</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td>Contaminated</td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
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<td>33.2 ± 2.7</td>
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<td>16:1</td>
<td>28.0 ± 2.9</td>
</tr>
<tr>
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<td>3.2 ± 0.8</td>
</tr>
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<tr>
<td>18:2</td>
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</tr>
<tr>
<td>20:4</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>20:5</td>
<td>2.8 ± 0.3</td>
</tr>
</tbody>
</table>

2.4.2 Heavy Metals Distribution:

Trace heavy metals added to the media at the beginning of the 7-day growth cycle are assumed to separate into three areas during cultivation. These areas were determined to be the (1) harvested microalgae biomass, (2) medium, (3) and lost to the environment. Previous
experimental work showed minimal sorption to experimental equipment. Losses to the environment were determined by subtracting the biomass and medium trace heavy metal values at day 7 from the heavy metal amounts initially added. The results from the trace heavy metal contaminants analysis of the biomass at the end of the growth period is presented in Figure 5 for 10 of the 14 contaminants tested. The fate of the heavy metal contaminants was found to be either predominately in the biomass for 6 of the contaminants (As, Cd, Co, Cu, Ni and Pb), lost to the environment for 2 contaminants (Hg and V) or a combination of both as seen in the last 2 contaminants (Cr and Sb). The large error bars seen here are due to the large variability within the 4 different batches. The variability on an individual batch is small as illustrated in Appendix B.

Results for three (Se, Sn and Zn) of the heavy metal contaminants are not presented due to detection limits and failure to meet quality control metrics during analysis. Specifically, Se and Sn are calculated to be approaching the detection limit of the ICP-MS. A total of 12 elements (As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sb, V and Zn) were fully recoverable after digestion as shown by the percent recovery of the LFB being close to 100%, indicating no losses, no gains and no cross-contamination of analytes during digestion. Calibration blanks for all the analytes were also below the method reporting limit. Matrix effects were assessed by analyzing LFM samples and obtaining the percent recovery. Matrix effects were also observed during the analysis of the digested supernatant and were addressed by a 3:1 dilution ratio making sure the dilution did not compromise the detection limit of the instrument. Zn did not pass quality control as the recoveries were higher than what was initially introduced into the system.

Results show that trace heavy metals introduced into the growth system were sorbed by the biomass with some remaining in the spent medium. The sorbed contaminants could limit the end use of the biomass (National Research Council. 2006.). It is expected in fuel recovery that the
Figure 5. End fate of heavy metals allocated between biomass, medium, and lost to the environment. Results are the average of 4 batches with error bars being one standard deviation (n=9, excluding Cd where n=6).

Trace contaminants will remain with the spent biomass. The transfer of the contaminants to the fuel would be undesirable based on potential health effects associated with the emissions produced. Economic viability of the microalgae to biofuels process typically requires the spent biomass or non-lipid fraction to be utilized as a high value product such as animal feed. High concentrations of heavy metals in the spent biomass could result in limitations in use. The contaminants that remain in the spent media could limit media recycle due to the potential to build up of contaminants to high concentrations which could be detrimental to growth. [21] shows, with a freshwater species, increasing heavy metals concentrations to 5X negatively impacts productivity with limited biosorption of As, Ni and Zn compared to growths at lower concentrations. The end use and transfer of heavy metals to products needs to be further explored for the microalgae to biofuels system.
2.4.3 Variable Heavy Metals Concentrations

Higher heavy metals concentrations were evaluated to understand the impacts of increasing contamination levels that might be seen with: media recycle or integration with systems that have higher contaminant levels in the flue gas, microalgae production systems with lower carbon utilization efficiency, or cultivation systems with higher productivity requiring higher flue gas loading. Experimental growth studies were performed with a control (0X) and at heavy metals concentrations of 1X, 2X, 5X, and 10X. Growth results from triplicate reactors at each concentration over the 7 day batch are presented in Figure 6. Results show the introduction of heavy metals at increasing concentrations continually have negative impacts on growth. The growth systems at 1X showed a lag phase from day one to day three where the growth was stunted compared to the control. After three days, the 1X concentration mirrored the growth pattern of the control growth system. Statistical difference started at day one (Student’s t-test, P < 0.05). Heavy metals contaminated growth systems at concentrations of 2X, 5X, and 10X all showed no growth or a decrease in biomass.

![Figure 6. Growth results from various heavy metals concentrations with a direct comparison to the control. Error bars represent one standard deviation from 3 replicates](image)
FAME analysis was performed to determine the lipid content for the various levels of contaminated systems. The control batches yielded 43.8 ± 1.6% lipid content, as expected, while 1X, 2X, 5X, and 10X yielded 29.8 ± 5.7%, 23.1 ± 0.89%, 16.3 ± 1.14%, and 14.89 ± 0.43% lipid content, respectively. The overall lipid productivity for 2X, 5X, and 10X are effectively zero due to no biomass growth occurring at these higher levels of contamination.

The final biomass and FAME content at the end of the 7 day growth period was used to generate dose response plots, Figure 7. Results show for both biomass and lipid content increasing contaminants concentrations negatively impacts yields. Several heavy metals at low concentrations have been shown to induce stress in cultures that leads to improved lipid yields. Previous work by Napan et al. [21] showed a favorable zone in terms of both biomass and lipid yield for fresh water Scenedesmus obliquus when exposed to low concentrations of various heavy metals. However, there are other environmental factors such as nutrient deficiency, salinity and high light intensity that could increase stress in the growth system used in this study [45,46]. The light intensity used in this study was much higher than previous studies (in an effort to be more representative of outdoor cultures), which combined with the presence of heavy metals and nutrient deficient medium could all have led to elevated stress thus resulting in the negative productivity seen in this study compared to previous work.

2.5 Conclusions

This chapter evaluated the distribution of heavy metals from flue gas in an algal cultivation system and determined the effects that this distribution have over biomass and lipid yields. Some of the key conclusion of this chapter are as follows.

The 14 heavy metals studied here (As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sb, Se, Sn, V and Zn) were found to have a statistically negative effect on both the growth of the microalgae strain
Figure 7. Dose response plots for A) biomass productivity and B) lipid yield for multiple heavy metal concentration levels. Error bars represent one standard deviation from 3 replicates.

*Nannochloropsis Salina* and the production of lipids. The contaminated cultures were found to have a 67.5% ± 16.3 reduction in biomass productivity with the control and contaminated cultures averaging 1.13 g L⁻¹ d⁻¹ ± 0.12 and 0.37 g L⁻¹ d⁻¹ ± 0.18, respectively. A 31.9% ± 13.2 decrease in lipid content was also experienced at harvest after the 7 day growth period, from a total lipid content of 43.8% ± 1.6 for the control to 29.8% ± 5.7 for the contaminated replicates. The combination of the effects of contaminants on the biomass productivity and lipid content show that there is an overall decrease in lipid productivity of 77.9% ± 12.1 from 0.49 g L⁻¹ d⁻¹ ± 0.06 for the control replicates to 0.11 g L⁻¹ d⁻¹ ± 0.06 for the trace heavy metals contaminated replicates harvested after 7 days. This result highlights the importance of understanding the implications of integrating industrial flue gas with microalgae production systems.

The changes is the fatty acid profile for the control and trace heavy metals contaminated biomass were found to be minimal. The profiles were predominantly composed of C16:0 and C16:1 for both the control and heavy metals contaminated biomass with the sum of C16:0 and C16:1 representing 68.6% and 61.2%, respectively, of the total lipids.
Heavy metals introduced into the growth system were predominantly sorbed by the biomass and spent medium. The sorbed contaminants could limit the end use of the biomass, biofuel, and spent biomass. The transfer of the contaminants to the fuel would be undesirable based on potential health effects associated with the emissions produced. High concentrations of heavy metals in the spent biomass could result in limitations in use. The contaminants that remain in the spent media could limit media recycle due to the potential to build up of contaminants to high concentrations which could be detrimental to growth.

Effects of heavy metals at increasing contamination levels were found to incrementally decrease lipid production and growth. In measuring lipid production the control batches yielded 43.8 ± 1.6% lipid content, as expected, while 1X, 2X, 5X, and 10X yielded 29.8 ± 5.7%, 23.1 ± 0.89%, 16.3 ± 1.14%, and 14.89 ± 0.43% lipid content, respectively. The overall lipid productivity for 2X, 5X, and 10X are effectively zero due to no biomass growth occurring at these higher levels of contamination.

2.6 References


6. ANL, NREL, PNNL (2012) Renewable diesel from algal lipids: An integrated baseline for cost, emissions, and resource potential from a harmonized model. ANL/ESD/12-4,


CHAPTER 3
IMPACT OF FLUE GAS DERIVED HEAVY METAL CONTAMINANTS ON MICROALGAL
BIOFUEL AND BIOGAS PRODUCTION THROUGH
MULTIPLE CONVERSION PATHWAYS

3.1 Abstract

The benefits of relieving the world’s energy dependence on fossil fuels by finding promising alternatives has been a key area of research over the past few decades. In this search for economic and sustainable alternatives to fossil fuels, microalgae stands in a position of great potential due to its high productivity and ability to be integrated with waste streams (flue gas, wastewater) while not requiring agricultural land for cultivation. The integration of flue gas with microalgal cultivation represents a promising alternative to directly venting carbon dioxide and doubles as a nutrient for microalgal systems. The introduction of this waste stream into the growth system will inevitably introduce trace heavy metal contaminants which have a high affinity to bind to microalgal cells. These heavy metal contaminants could be toxic to the cells, and if transferred to the microalgal, could impact downstream processing and the end use of the derived products. Microalgal biomass (*Nannochloropsis salina*) grown in the presence of the heavy metal contaminants As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sb, Se, Sn, V and Zn and control biomass were processed into biofuel through one of two different in-situ transesterification techniques, acid-catalyzed or supercritical methanol transesterification. The acid-catalyzed transesterification resulted in an average crude biofuel production decrease from 0.31 ± 0.03 grams biofuel/gram microalgal for the control microalgal biomass to 0.28 ± 0.02 grams biofuel/gram microalgal for the heavy metal contaminated microalgal biomass, representing a 9.7% reduction. Supercritical methanol conversion exhibited a similar trend corresponding to a 15.8% reduction. Heavy metal analysis was performed using inductively coupled plasma mass spectrometry (ICP-MS) on the
biofuel, lipid extracted algae, and other biofuel conversion byproducts of both types of transesterification. The ICP-MS results indicated minimal heavy metal contamination was found in the biofuel resulting from acid catalyzed transesterification. Substantial heavy metal contamination was found in the biofuel resulting from supercritical methanol transesterification, and substantial heavy metal contamination was found in the lipid extracted algae resulting from both types of transesterification. Biochemical methane potential testing was performed on the lipid extracted algae, generated as a byproduct of the acid catalyzed transesterification, to determine the effect of heavy metals on the generation of biogas. A positive effect on the amount of methane produced was found with an average productivity of 105.89 mL g-COD\(^{-1}\) from the heavy metal contaminant LEA compared to the control microalgae biomass which produced 53.25 mL g-COD\(^{-1}\). Results show coal flue gas integration with algal production could have negative impacts on productivity and limit the end use of bio-based products.

### 3.2 Introduction

In response to increasing concern over global climate change, petroleum resource availability, and ever increasing energy consumption, society continues to search for more efficient, economic, and environmentally stable alternatives to fossil fuels. Due to these increasing concerns, microalgal based biofuels has become a topic of growing interest due to advantageous qualities such as high lipid production, year-round cultivation, does not require agricultural land, and integration with various waste streams\[1-3\]. A promising avenue for producing microalgae based biofuel and improving the sustainability of coal fired power plants is the integration of carbon dioxide in flue gas with microalgae cultivation systems.

To date, the effects of integration of industrial flue gas with microalgae cultivation on end products and co-products remains largely unknown due to the relatively few studies have been conducted. Despite this fact, the majority of the studies of the microalgae to biofuels process
including: economic [4-6], lifecycle [7-9], and scalability [10, 11] assessments make a
simplifying assumption of seamless integration of industrial flue gas with cultivation. This
assumption infers there are no negative effects caused by integration and ignores the potential
limitations the introduction of contaminants including heavy metals may have on end products
and co-products. This is plausibly an improper assumption considering that microalgae is a well-
known metal bioaccumulator [12, 13]. Internalized metals form metallic-compounds that can be
adsorbed by various regions of the microalgae cell (cytoplasm, nuclei, chloroplast, mitochondria,
vacuoles, and lipids). These internalized metals represent potentially undesirable effects from flue
gas integration. Microalgae biomass cultivated in such a system has the potential for absorbed
heavy metals to contaminate end products thus limiting the product uses [14, 15]. Studies using
simulated and actual flue gas have been conducted, but fail to evaluate the end fate of heavy
metal contaminants [16-20].

In many economic and life cycle assessments, the post processing of microalgae biomass
after lipid extraction through anaerobic digestion is proposed as a way to improve the economic
feasibility and environmental impact of the microalgae-to-biofuel process. In an effort to
correctly assess the environmental impact of large-scale biofuel production facilities a variety of
life cycle analyses have been performed [7, 8, 21-29]. However due to the immaturity of the
microalgae to biofuel and biogas production process, many of the individual steps and processes
are not fully defined. Including the effects that heavy metals in flue gas may have on the
performance of an anaerobic digester operated using LEA containing heavy metals [8, 23, 25, 26,
29]. Many life cycle assessments include simplifying assumptions about the anaerobic digestion
process itself and about the effects that LEA containing heavy metals may have on production
that have not been demonstrated experimentally.
Based on the current state of the microalgae to biofuel field there exists the need to understand the impact of heavy metals on the downstream processing of algal biomass. In this study the effects of the integration of flue gas into microalgal growth systems and the potential limitations the introduction of contaminants including heavy metals may have on end products and co-products are directly assessed for the microalgae to biofuel and anaerobic digestion processes. Biomass cultivated in the presence of trace heavy metal contaminants was converted to biofuel through acid catalyzed and supercritical methanol transesterification. Residual biomass was evaluated for methane production potential. Metals analysis was used to understand the end fate of the heavy metals contaminants and used to evaluate possible limitations to the end use of products and byproducts based on contamination levels. This study was conducted with the intent to provide vital information for the future improvement of life cycle, economic, and scalability assessments that will lead to the sustainability of the microalgae to biofuel process.

3.3 Materials and Methods

3.3.1 Biomass Generation Summary

Microalgae (*Nannochloropsis Salina*) was cultivated for 7 days in the presence of 14 heavy metals As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sb, Se, Sn, V and Zn, commonly present in coal. These 14 metals were added to microalgal growth medium at a concentration named 1X that was estimated to be representative of concentrations expected from 7 day growth periods where coal derived flue gas is used as the carbon source (See Table 3).

Growth experimentation was conducted with *Nannochloropsis salina* cultivated in 1.1L photobioreactors at a light intensity of 1000 µmol m$^{-2}$ s$^{-1}$. Heavy metals negatively impacted the growth with the average productivity being 0.54 ± 0.28 g L$^{-1}$ d$^{-1}$, corresponding to a decrease of 52% in biomass yield compared to control growths. After 7 days of growth biomass from both the control samples and the samples containing heavy metals were harvested.
Table 3. Heavy metal concentrations found in coal at the baseline or 1X concentrations.

<table>
<thead>
<tr>
<th>Heavy Metal</th>
<th>Heavy Metal concentration in fly ash (mg/kg)*</th>
<th>Salt source</th>
<th>Baseline (1X) concentration (mg·L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>391</td>
<td>NaAsO₂</td>
<td>7.74E-02</td>
</tr>
<tr>
<td>Cd</td>
<td>76</td>
<td>CdCl₂</td>
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<td>Co</td>
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<td>CoCl₂·6H₂O</td>
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<td>Na₂Cr₂O₇·2H₂O</td>
<td>1.29E-01</td>
</tr>
<tr>
<td>Cu</td>
<td>655</td>
<td>CuCl₂·2H₂O</td>
<td>1.30E-01</td>
</tr>
<tr>
<td>Hg</td>
<td>49.5</td>
<td>HgCl₂</td>
<td>9.80E-03</td>
</tr>
<tr>
<td>Mn</td>
<td>750</td>
<td>MnCl₂·4H₂O</td>
<td>1.49E-01</td>
</tr>
<tr>
<td>Ni</td>
<td>1270</td>
<td>NiCl₂·6H₂O</td>
<td>2.51E-01</td>
</tr>
<tr>
<td>Pb</td>
<td>273</td>
<td>PbCl₂</td>
<td>5.41E-02</td>
</tr>
<tr>
<td>Sb</td>
<td>205</td>
<td>Sb₂O₃</td>
<td>4.06E-02</td>
</tr>
<tr>
<td>Se</td>
<td>49.5</td>
<td>Na₂SeO₃</td>
<td>9.80E-03</td>
</tr>
<tr>
<td>Sn</td>
<td>19</td>
<td>SnCl₂·2H₂O</td>
<td>3.76E-03</td>
</tr>
<tr>
<td>V</td>
<td>5015</td>
<td>V₂O₅</td>
<td>1.13E-01</td>
</tr>
<tr>
<td>Zn</td>
<td>2200</td>
<td>ZnCl₂</td>
<td>4.36E-01</td>
</tr>
</tbody>
</table>

*[30]

To determine the effects of the heavy metals on lipid content, a lipid content analysis was performed on both the control samples and samples containing heavy metals and found a decrease from 38.8 ± 0.62% to 31.58 ± 0.50% (percent dry biomass). A heavy metals analysis was performed on the samples containing heavy metals and showed significant binding of the majority of the heavy metals to the biomass (See Figure 8 and Table 4).

3.3.2 Acid Catalyzed Transesterification

A large number of the microalgal to biofuels conversion processes being studied currently or in the past utilize either dry or wet microalgal biomass as the feedstock for the conversion. In an attempt to characterize the effects of heavy metals on the products of both of these conversion pathways, the authors chose to convert the microalgae grown in the manner mentioned previously
Figure 8. End fate of heavy metals allocated between biomass, medium, and lost to the environment. Results are the average of 3 batches with error bars being one standard deviation (n=6, excluding Cd where n=4).

Table 4. Heavy metals concentrations found in biomass, media or to be lost to the environment. All results are the average of 3 batches. Concentration in biomass is in units of mg of metals per g of biomass, concentration in media is in units of mg of metals per L of media, and concentration lost to the environment is in mg of metals per L of growth experiment.

<table>
<thead>
<tr>
<th>Heavy Metal</th>
<th>concentration in biomass (mg/g)</th>
<th>concentration in media (mg/g)</th>
<th>concentration lost to environment (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>1.46E-02</td>
<td>1.22E-02</td>
<td>0.00</td>
</tr>
<tr>
<td>Cd</td>
<td>3.60E-03</td>
<td>8.33E-05</td>
<td>0.00</td>
</tr>
<tr>
<td>Co</td>
<td>3.29E-03</td>
<td>6.32E-04</td>
<td>0.00</td>
</tr>
<tr>
<td>Cr</td>
<td>1.78E-02</td>
<td>2.07E-02</td>
<td>2.39E-02</td>
</tr>
<tr>
<td>Cu</td>
<td>2.42E-02</td>
<td>1.16E-02</td>
<td>8.75E-03</td>
</tr>
<tr>
<td>Hg</td>
<td>8.34E-05</td>
<td>0.00</td>
<td>9.40E-03</td>
</tr>
<tr>
<td>Mn</td>
<td>6.02E-02</td>
<td>3.14E-02</td>
<td>0.00</td>
</tr>
<tr>
<td>Ni</td>
<td>4.62E-02</td>
<td>2.19E-02</td>
<td>1.02E-02</td>
</tr>
<tr>
<td>Pb</td>
<td>1.02E-02</td>
<td>3.14E-04</td>
<td>1.08E-02</td>
</tr>
<tr>
<td>Sb</td>
<td>4.97E-03</td>
<td>3.97E-03</td>
<td>1.73E-01</td>
</tr>
<tr>
<td>V</td>
<td>1.89E-03</td>
<td>7.70E-03</td>
<td>9.63E-02</td>
</tr>
<tr>
<td>Zn</td>
<td>2.08E-01</td>
<td>6.61E-03</td>
<td>0.00</td>
</tr>
</tbody>
</table>
into biofuel using acid catalyzed transesterification which utilizes a dry feedstock and supercritical methanol transesterification which utilizes a wet feedstock.

### 3.3.2.1 Experimental Setup

The conversion of microalgae to biofuel using acid catalyzed transesterification was performed based on the methods of [31] Six acid catalyzed transesterification runs were performed, 3 of which were performed using control microalgae and 3 using heavy metals contaminated microalgae. Conversions were performed in a 2 necked spherical 500 mL glass reactor heated on all sides by a temperature controlled electric mantel (See Figure 9). For all runs a 10:1 solvent to mass ratio was used in which 30 grams of freeze dried microalgae was added to 300 mL of solvent consisting of 98% methanol and 2% sulfuric acid. The mixture was heated to 62°C and stirred continually for 6 hours.

### 3.3.2.2 Products and Byproducts

After the transesterification process was complete the LEA was separated from the biofuel, and water/methanol mixture using an Erlenmeyer filtering funnel utilizing both course and fine filters (Whatman 1541-125 and 1542-125). A 1:1 solvent to water ratio (300 mL) and a 3:1 solvent to chloroform ratio (100 mL) were added to induce a phase separation. Phase separation was allowed to occur overnight. Biofuel and chloroform were separated from the methanol/water mixture using a separation funnel. The chloroform and biofuel were separated by evaporating and recovering the chloroform by heating the mixture to 62°C and then running the chloroform gas through a condenser. Samples were taken of each product and byproduct excluding chloroform so that a mass balance could be performed and the end fate of the heavy metals could be determined.
3.3.3 Supercritical Methanol Transesterification

3.3.3.1 Experimental Setup

Six supercritical methanol transesterification runs were performed, 3 of which were performed using control microalgae biomass and 3 using microalgae biomass containing heavy metals. Conversions were performed in a Parr 4575 500 mL reactor as seen in Figure 10. Control biomass and heavy metal contaminated biomass generated as mentioned previously was centrifuged to achieve more optimal conversion. Final concentrations of wet microalgae biomass were 22% solids for the heavy metal contaminated microalgae and 26.7% solids for the control microalgae. For all runs a 10:1 solvent to biomass ratio was used in which 10 grams of
Figure 10. The Parr 4575 500 mL reactor in which the supercritical methanol transesterification was performed.

Microalgae biomass corresponding to 37.5 g of wet control biomass or 45.1 g of wet metals contaminated biomass was added to 100 mL of the methanol solvent. The mixture was held at a temperature of 253 ± 2°C and stirred continually for 90 minutes.

3.3.3.2 Products and Byproducts

After the transesterification process was complete the LEA was separated from the biofuel, and water/methanol mixture using course and fine filters (Whatman 1541-125 and 1542-125). 200 mL of water, 100 mL of chloroform and 100 mL of methanol were added to induce a phase separation. Biofuel and chloroform were separated from the methanol/water mixture using a
separation funnel. The chloroform and biofuel were separated by evaporating and recovering the chloroform by heating the mixture to 62°C and then running the chloroform gas through a condenser.

3.3.4 BMP Testing

In many economic and lifecycle assessments, the post processing of the biomass after lipid extraction through anaerobic digestion is frequently proposed to improve the economic feasibility and environmental impact of the microalgae-to-biofuel process. However, the impact of using biomass containing heavy metals in anaerobic digestion systems remains largely unknown. Biochemical methane potential (BMP) tests were performed to further understand the effects that heavy metals introduced by flue gas have on the downstream processing of LEA into biogas through anaerobic digestion.

3.3.4.1 Biochemical Methane Production Potential

Biochemical methane potential tests were performed to determine the potential biogas production efficiency of an anaerobic digester system utilizing four unique feedstocks including: control microalgae and LEA and heavy metal contaminated microalgae and LEA. Both LEA samples were produced using the acid catalyzed transesterification conversion technique outlined previously. The viability of feedstocks for anaerobic digestion is commonly determined by the BMP assay [32]. The methods given in Owen et al. (1979) [32] were used in this experimentation.

The biochemical methane potential tests were conducted in 140 mL luer lock plastic syringes to achieve a controllable anaerobic environment. The tests involved the mixing of three ingredients, substrate, inoculum, and nutrient solution. Substrate is the biodegradable carbon source required for the production of biogas (microalgae, LEA, glucose). Inoculum is the strain(s) of anaerobic bacteria that produces biogas using nutrients found in the substrate and nutrient
solution. The inoculum used in this study was acquired from the City of Logan Wastewater Treatment Facility in Logan, Utah. The nutrient solution used in this study was made using the methods in Owen et al. (1979) [32]. The ingredients were added to clean 140 mL luer lock syringes each fitted with a two way valve to preserve the anaerobic setting and also allow sample acquisition. The amount of substrate added to each sample was normalized based on chemical oxygen demand (COD). COD tests of all substrate types were performed in triplicate. COD was determined using sealed digestion vials and employing the (Hach method 8000) and (Hach DR 5000) procedures. For each sample an amount of 0.05 g-COD L⁻¹ of substrate was added to each syringe corresponding to 0.023 g of control microalgae, 0.0365 g of control LEA, 0.0278 g of heavy metals contaminated microalgae and 0.0398 g of heavy metals contaminated LEA. This was followed by 25 mL of inoculum (22.7 g-COD L⁻¹) and 25 ml of nutrient solution. The mass of substrate used was chosen so that an appropriate quantity of biogas would be formed for measurement and test purposes.

Positive control syringes containing dextrose (D-glucose) and negative control syringes were also run in triplicate. Positive control syringes utilizing dextrose (0.050 g-COD L⁻¹) as the substrate were used to ensure the anaerobic bacteria within the inoculums were efficiently digesting the samples and producing biogas. Negative control syringes, which did not receive a substrate, were used to detect the production of biogas from the inoculum and nutrient solution. The methane produced by the negative control was called background methane. To account for background methane production, the average quantity of biogas produced by the negative control syringes was subtracted from the quantities of biogas produced by all other test samples.

All test syringes were placed in an orbital foam incubator set to 33 ± 3°C (See Figure 11). The volume of biogas produced was measured daily by recording the change in extension length of the syringe plunger using a digital caliper (measurement error of ± 0.02 mm contributing to
Figure 11. Styrofoam incubator in which the anaerobic digestion syringes were placed. Syringes were swirled inside the incubator by a shaker table.

<0.1% gas volume measurement error). The tests concluded when the change in the plunger extension of all samples was negligible over a 24 hour period. Biogas was sampled from the syringe through the 2 way valve and analyzed for methane content using gas spectrometry (GC, Agilent Technologies 7890A).

3.3.4.2 Theoretical Methane Yield

The theoretical methane yield was based on the assumed lipid (35%), protein (40%), and carbohydrate (20%) concentrations found in the whole microalgae and LEA. The theoretical methane yield was found using an adaptation of the formula from Buswell and Neave (1930) [33] which balances the conversion of organic material to CH₄ and CO₂ with H₂O under anaerobic conditions (See Equation 1)

\[
C_nH_aO_b + \frac{4n - a - 2b}{4}H_2O \rightarrow \frac{4n + a - 2b}{8}CH_4 + \frac{4n - a + 2b}{8}CO_2
\]  (1)
Equation 1 represents the theoretical methane production of a sample and may not correctly speculate the effects of ruptured cells and lipid removal that will be present in the LEA samples. The specific methane yields for carbohydrates ((C$_{6}$H$_{10}$O$_{5}$)$_{n}$), lipids (C$_{57}$H$_{10}$O$_{6}$), and proteins (C$_{6}$H$_{13.1}$O$_{1}$N$_{0.6}$) based on Equation 1 are 66, 312, and 314 ml g-VS$^{-1}$, respectively.

3.3.5 Trace Heavy Metal Analysis

Trace heavy metals analysis was performed based on the methods presented in Napan et al.[34].

Analysis was performed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS Agilent 7700x Series) for all products and byproducts of the acid catalyzed transesterification and supercritical transesterification processes; including the biofuel, lipid extracted algae (LEA), water/methanol mixture, and filters). First, all LEA and filters of both conversion types were freeze dried. Biofuel, LEA, water/methanol, and filter samples for both conversion types were digested at 105°C over a period of 3 days or until the solution was visibly clear by adding analytical trace metal grade nitric acid (70%) to either 100 mg LEA sample, 100 mg of filter sample, 100 μL of biofuel or 5 mL of water/methanol mixture. Throughout the digestion process volumes of 0.1 - 0.5 mL of nitric acid was added incrementally so that the final volume of the digested sample was approximately 2 mL. Samples were regularly vortexed throughout the process. After digestion was complete all samples were transferred into 10 mL Luer-Lok syringes and filtered through a 0.45 μm filter into a 25 mL volumetric flask. The volume of each flask was brought to 25 mL using deionized water (Type I). Each sample was mixed thoroughly before transferring 10 mL to a capped analysis container. All samples were preserved at 4°C until analysis. ICP-MS analysis was performed by the Utah Water Research Laboratory.
3.3.6 Lipid Analysis

Quantification of fatty acid methyl esters (FAMES) contained within the biofuel made through acid catalyzed and supercritical methanol transesterification was determined using gas chromatography (GC, Agilent Technologies 7890A). Samples were prepared based on the methods of Wahlen et al. [31] with regards to steps taken after the conversion of microalgal lipids to FAMES. Samples for both the acid catalyzed system and the supercritical methanol system were made with both the control and heavy metals contaminated biofuels. For the acid catalyzed transesterification samples 20 μL of biofuel and 980 μL of chloroform was added to a GC vial. For the supercritical methanol transesterification samples 50 μL of biofuel and 950 μL of chloroform was added to a GC vial. The samples were mixed thoroughly and then analyzed with a GC. The GC column used was a Restek Stabilwax-DA (30 m X 0.32 mm ID X 0.25 μm film) with a polyethylene glycol stationary phase. The inlet temperature was 250°C. The oven temperature was initially held at 100°C for 1 min and ramped at 10°C min\(^{-1}\) up to 235°C and held for 10 min, with the total run time being 24.5 min. Standards using methylmyristate, methylpalmitoleate, and methyloleate (Nu-Chek Prep, Inc.) were prepared in a chloroform matrix and run concurrently with the prepared samples.

3.3.7 Statistical Analysis

All experimental system results including results from acid catalyzed transesterification, supercritical methanol transesterification, biochemical methane potential testing, ICP-MS analysis and lipid analysis presented in this report represents a minimum of triplicate. Statistical processing of the data for comparison to control was done using a two-tailed, two-sample equal variance Student t-test with a 95% confidence interval. Data presented is a mean with +/- one standard deviation.
3.4 Results and Discussions

Results are divided into three sections, 1) the effect of heavy metals on acid catalyzed transesterification, 2) the effect of heavy metals on supercritical methanol transesterification, and 3) the impact of heavy metals on methane yields through biochemical methane potential testing.

3.4.1 Impact of Heavy Metals on Acid Catalyzed Transesterification

The effects of heavy metals on the production of microalgal biofuel through the process of acid catalyzed transesterification were determined and compared to a control. Six acid catalyzed conversions were performed; three conversions used microalgae grown in the presence of the heavy metals and three conversions used control microalgae. The effects of the heavy metals was quantified in three major areas, being changes to the fatty acid profile (See Table 5), and changes to production and recovery efficiency (See Table 6).

3.4.1.1 Production of Biofuel and Byproducts

After the conversion of the heavy metals contaminated and control microalgae samples into biofuel, the major fatty acid profiles for both the resultant heavy metals contaminated biofuel and Table 5. Fatty Acid profile after acid catalyzed transesterification. Fatty acid profile comparison between control and heavy metals contaminated biofuel samples (n=9). Reported error represents one standard deviation.

<table>
<thead>
<tr>
<th>Major Fatty Acid Profiles (carbon chain length: number of unsaturations)</th>
<th>% of total fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. Salina</td>
<td>14:0</td>
</tr>
<tr>
<td>Control</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>Contaminated</td>
<td>0.3 ± 0.3</td>
</tr>
</tbody>
</table>
Table 6. Production efficiency and lipid recovery efficiency of the acid catalyzed transesterification process. Reported error represents one standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Production efficiency</th>
<th>Recovery efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g-biofuel g-algae^{-1}</td>
<td>STDV</td>
</tr>
<tr>
<td>Control</td>
<td>0.31</td>
<td>0.03</td>
</tr>
<tr>
<td>Contaminated</td>
<td>0.28</td>
<td>0.02</td>
</tr>
</tbody>
</table>

control biofuel were found using gas spectrometry. The fatty acid chain lengths accounted for in this study are the 14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 20:4, and 20:5 chain lengths. The predominant fatty acid chain length produced in this study was a chain length of 16 which is typical for the *Nannochloropsis Salina* strain of microalgae [11]. The sum of C16:0 and C16:1 representing 58.6% and 59.2%, respectively, of the total lipids. It was discovered that for all carbon chain lengths excluding 16:1, 18:1, and 18:2 there was a decrease in the total percent of each fatty acid chain length. An increase of percentage was found for the 16:1 and 18:1 chain lengths and little to no change was apparent from 18:2. These results were found to be statistically significant only for the 18:0, 18:1, 18:2, 20:4, and 20:5 chain lengths. This indicated that the production of the predominant fatty acid chain lengths 16:0 and 16:1 remained statistically consistent from the control biofuel to the heavy metals contaminated biofuel.

The effects of heavy metals on the production of biofuel was quantified by measuring the changes in the production efficiency and the recovery efficiency of biofuel from the extraction system. Production efficiency is defined as the grams of biofuel produced per gram of microalgae used in the transesterification process. Recovery efficiency is defined as the total FAME recovered after acid catalyzed transesterification divided by the total FAME available within the microalgae before acid catalyzed conversion (i.e. if a sample of microalgae contains 1 mL of FAME and after acid catalyzed conversion 0.8 mL of FAME was recovered then the recovery
efficiency is 80%). In this study it was found that the recovery efficiency increased from 80% for the control biofuel to 89% for the biofuel containing heavy metals. The author speculates cell degradation to be the reason for the increase in recovery efficiency associated with the heavy metals contaminated biofuel samples. As microalgae was grown in the presence heavy metals the individual microalgae cells experiences stunted growth and degraded of cell health due to the extra stress the heavy metals exhibited on the microalgae[34, 35]. Rupture of the microalgal cell wall is one of the most energy intensive processes of the microalgae to biofuel conversion and the degradation of the health and strength of the cell wall would lead to easier cell rupture and therefore easier FAME extraction and an increase in recovery efficiency.

3.4.1.2 Heavy Metal Analysis

The end fate of the heavy metals was determined by performing a mass balance of all of the ingredients and products and byproducts of the acid catalyzed transesterification. The inputs of the system included heavy metal contaminated microalgae, methanol, chloroform, and water the outputs of the conversion were biofuel, chloroform, LEA, and a mixture of water and methanol. Chloroform was not measured for heavy metals based on the assumption that it would not contain heavy metals after being separated from the other products by means of evaporation. The two filters (Whatman 1541-125 and 1542-125) used to separate the LEA from the other products were also analyzed due to the reasonable assumption that some heavy metals were trapped in the filters. The mass balance was performed using ICP-MS to measure the amounts of heavy metals contained within each of the products and byproducts of the biofuel conversion and comparing those contamination levels with the known amounts of heavy metals that were present in the microalgae initially.

The final distribution of the heavy metals after acid catalyzed transesterification between the 5 possible products and byproducts was determined using ICP-MS (See Figure 12 and Table 7).
Figure 12. End fate of heavy metals allocated between biofuel, LEA, methanol/water, filters and lost to the environment. Results are the average of 3 acid catalyzed conversion batches run in triplicate (n=9, excluding biofuel, the methanol/water mixture, and filters where n=8, n=7, and n=15).

Results for the distribution of Hg were not determined due to the low initial concentration of Hg in the contaminated microalgae and the high probability of Hg volatilizing during the transesterification process [36, 37] The majority of the heavy metals were determined to be in the LEA, methanol/water mixture or lost to the environment. Minimal contamination of the filters and produced biofuel was experienced.

The experimental procedure outlined in the methods section was used to ensure minimal contamination in the growth studies. Error in laboratory measurements (e.g. contaminant salts initially added, microalgae mass in the system, mass of microalgae analyzed in ICP-MS) have the ability to introduce error, although this is expected to be low. In addition, the ICP-MS results are accurate to +/- 10% at the 95% confidence level. Other plausible sources of heavy metal losses include volatilization [36, 37] and sorption of the heavy metals to acid catalyzed
transesterification glassware including the reactor, condensers, filtering funnel, Erlenmeyer flask, and separatory funnel [38-41].

Table 7. Distribution of heavy metals across the 5 possible pathways (biofuel, LEA, methanol/water, filters and lost to the environment). Results are shown as a percentage of the heavy metal concentration present in the microalgae sample before acid catalyzed conversion.

<table>
<thead>
<tr>
<th></th>
<th>Biofuel</th>
<th>LEA</th>
<th>Methanol / Water</th>
<th>Filters</th>
<th>Losses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percent (%)</td>
<td>Percent (%)</td>
<td>Percent (%)</td>
<td>Percent (%)</td>
<td>Percent (%)</td>
</tr>
<tr>
<td>As</td>
<td>1.76 ± 0.43</td>
<td>13.52 ± 0.73</td>
<td>43.25 ±3.28</td>
<td>0.88 ± 0.35</td>
<td>40.59 ± 3.43</td>
</tr>
<tr>
<td>Cd</td>
<td>0.04 ± 0.02</td>
<td>49.50 ± 6.71</td>
<td>47.93 ± 4.12</td>
<td>1.39 ± 0.62</td>
<td>1.15 ± 4.42</td>
</tr>
<tr>
<td>Co</td>
<td>0.33 ± 0.13</td>
<td>12.36 ± 2.23</td>
<td>74.98 ± 5.62</td>
<td>0.91 ± 0.47</td>
<td>11.40 ± 5.02</td>
</tr>
<tr>
<td>Cr</td>
<td>2.44 ± 0.86</td>
<td>9.75 ± 0.55</td>
<td>26.62 ± 2.23</td>
<td>1.19 ± 0.42</td>
<td>60.01 ± 3.02</td>
</tr>
<tr>
<td>Cu</td>
<td>1.41 ± 0.23</td>
<td>18.26 ± 0.48</td>
<td>46.29 ± 4.03</td>
<td>1.12 ± 0.40</td>
<td>32.91 ± 3.74</td>
</tr>
<tr>
<td>Mn</td>
<td>0.15 ± 0.04</td>
<td>15.52 ± 2.57</td>
<td>98.36 ± 10.45</td>
<td>1.02 ± 0.54</td>
<td>0.00 ±0.00</td>
</tr>
<tr>
<td>Ni</td>
<td>0.78 ± 0.19</td>
<td>8.40 ± 1.55</td>
<td>50.98 ± 3.77</td>
<td>0.59 ± 0.31</td>
<td>39.24 ± 3.70</td>
</tr>
<tr>
<td>Pb</td>
<td>0.14 ± 0.04</td>
<td>34.89 ± 4.76</td>
<td>29.92 ± 4.02</td>
<td>2.01 ± 1.03</td>
<td>33.04 ± 7.43</td>
</tr>
<tr>
<td>Sb</td>
<td>1.16 ± 0.075</td>
<td>4.20 ± 0.26</td>
<td>11.05 ± 0.66</td>
<td>1.25 ± 0.56</td>
<td>82.35 ± 0.95</td>
</tr>
<tr>
<td>Se</td>
<td>2.16 ± 0.60</td>
<td>26.02 ± 2.03</td>
<td>6.23 ± 0.49</td>
<td>1.21 ± 0.48</td>
<td>64.39 ± 1.94</td>
</tr>
<tr>
<td>Sn</td>
<td>5.50 ± 1.77</td>
<td>2.14 ± 0.98</td>
<td>2.72 ± 2.55</td>
<td>0.77 ± 0.73</td>
<td>88.86 ± 3.12</td>
</tr>
<tr>
<td>V</td>
<td>0.00 ±0.04</td>
<td>5.56 ± 0.34</td>
<td>8.80 ± 0.38</td>
<td>0.37 ± 0.13</td>
<td>85.38 ± 0.43</td>
</tr>
<tr>
<td>Zn</td>
<td>103.71 ± 34.34</td>
<td>124.50 ± 7.41</td>
<td>182.79 ± 12.78</td>
<td>20.65 ± 8.81</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

3.4.2 Impact of Heavy Metals on Supercritical Methanol Transesterification

The effects of heavy metals on the production of microalgal biofuel through the process of supercritical methanol transesterification were determined and compared to a control. Six supercritical methanol conversions were performed; three conversions used microalgae grown in the presence of the heavy metals and three conversions used control microalgae. The effects of the heavy metals was quantified in three major areas, being changes to the fatty acid profile (See Table 8), and changes to production and recovery efficiency (See Table 9).
Table 8. Fatty Acid profile after supercritical methanol transesterification. Fatty acid profile comparison between control and heavy metals contaminated biofuel samples (n=9). Reported error represents one standard deviation.

<table>
<thead>
<tr>
<th>Major Fatty Acid Profiles (carbon chain length: number of unsaturations)</th>
<th>% of total fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. Salina</td>
<td>14:0</td>
</tr>
<tr>
<td>Control</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>Contaminated</td>
<td>0.7 ± 0.3</td>
</tr>
</tbody>
</table>

Table 9. Production efficiency and lipid recovery efficiency of the supercritical methanol transesterification process. Reported error represents one standard deviation.

<table>
<thead>
<tr>
<th>Production efficiency</th>
<th>Recovery efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>g-biofuel g-algae⁻¹</td>
<td>% Recovery</td>
</tr>
<tr>
<td>N. Salina</td>
<td>STDV</td>
</tr>
<tr>
<td>Control</td>
<td>0.38</td>
</tr>
<tr>
<td>Contaminated</td>
<td>0.32</td>
</tr>
</tbody>
</table>

3.4.2.1 Production of Biofuel and Byproducts

After the conversion of the heavy metals contaminated and control microalgae samples into biofuel, the major fatty acid profiles for both the resultant heavy metals contaminated biofuel and control biofuel were found using gas spectrometry. Fatty acid chain lengths 14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 20:4, and 20:5 were accounted for. The predominant fatty acid chain length produced in this study was 16, which is typical for the *Nannochloropsis Salina* strain of microalgae [11]. The sum of C16:0 and C16:1 representing 51.9% and 56.4%, respectively, of the total lipids. It was discovered that for all carbon chain lengths excluding 14:0, 18:1, and 20:4 there was an increase in the total percent of each fatty acid chain length. A decrease of percentage was found for the 18:1 chain length and little to no change was apparent from 14:0, and 20:4.
These results were found to be statistically significant only for 18:1, 18:2, 20:4, and 20:5. Seeing that the changes in the predominant fatty acid chain lengths 16:0 and 16:1 were not statistically significant indicates that comparing the control biofuel to the heavy metals contaminated biofuel the production of 16:0 and 16:1 remained statistically consistent.

The effects of heavy metals on the production of biofuel was quantified by measuring the changes in the production and recovery efficiency of biofuel from the extraction system. Production efficiency is defined as the grams of biofuel produced per gram of microalgae used in the transesterification process. Recovery efficiency is defined as the total FAME recovered after supercritical methanol transesterification divided by the total FAME available within the microalgae before supercritical methanol conversion (i.e. if a sample of microalgae contains 1 mL of FAME and after supercritical methanol conversion 0.8 mL of FAME was recovered then the recovery efficiency is 80%). In this study it was found that the recovery efficiency increased from 98% for the control biofuel to 100% for the biofuel containing heavy metals. The author again speculates cell degradation to be the reason for the increase in recovery efficiency associated with the heavy metals contaminated biofuel samples.

3.4.2.2 Heavy Metal Analysis

The end fate of the heavy metals was determined by performing a mass balance of the ingredients, products and byproducts of the supercritical methanol transesterification. The inputs of the system included heavy metals contaminated microalgae, methanol, chloroform, and water the outputs of the conversion were biofuel, chloroform, LEA, and a mixture of water and methanol. Chloroform was not measured for heavy metals due to the assumption that it would not contain heavy metals because it was separated from the other products by means of evaporation. The two filters (Whatman 1541-125 and 1542-125) used to separate the LEA from the other products were also analyzed assuming it to be probable for some heavy metals to be trapped in
the filters. The mass balance was performed using ICP-MS to measure the amounts of heavy metals contained within each of the products and byproducts of the biofuel conversion and comparing those contamination levels with the known amounts of heavy metals that were present in the microalgae initially.

The final distribution of the heavy metals after supercritical methanol transesterification between the 5 possible products and byproducts was determined using ICP-MS (See Figure 13 and Table 10). Results for the distribution of Hg were not determined due to the low initial concentration of Hg in the contaminated microalgae and the high probability of Hg volatilizing during the transesterification process [36, 37]. The majority of the heavy metals were determined to be in the LEA or lost to the environment. Unlike the acid catalyzed transesterification case, minimal amounts heavy metals were found to be in the methanol/water mixture. Substantial heavy metals contamination in the biofuel were experienced for Co, Cu, and Ni. All other metals exhibited minimal biofuel contamination.

The experimental procedure outlined in the methods was used to ensure minimal contamination in the growth studies. Error in laboratory measurements (e.g. contaminant salts initially added, microalgae mass in the system, mass of microalgae analyzed in ICP-MS) have the ability to introduce error, although this is expected to be low. In addition, the ICP-MS results are accurate to +/- 10% at the 95% confidence level. Other plausible sources heavy metal losses include volatilization [36, 37] and sorption of the heavy metals to acid catalyzed transesterification glassware including the reactor, condensers, filtering funnel, Erlenmeyer flask, and separatory funnel [38, 41].
Figure 13. End fate of heavy metals allocated between biofuel, LEA, methanol/water, filters and lost to the environment. Results are the average of 3 supercritical methanol conversion batches run in triplicate (n=9, excluding Filters where n=18).

Table 10. Distribution of heavy metals across the 5 possible pathways including biofuel, LEA, methanol/water, filters and lost to the environment. Results are shown as a percentage of the heavy metals concentration present in the microalgae sample before supercritical methanol conversion.

<table>
<thead>
<tr>
<th></th>
<th>Biofuel</th>
<th>LEA</th>
<th>Methanol / Water</th>
<th>Filters</th>
<th>Losses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percent (%)</td>
<td>Percent (%)</td>
<td>Percent (%)</td>
<td>Percent (%)</td>
<td>Percent (%)</td>
</tr>
<tr>
<td>As</td>
<td>5.30 ± 1.98</td>
<td>0.11 ± 0.10</td>
<td>47.40 ± 3.25</td>
<td>0.48 ± 0.10</td>
<td>46.70 ± 3.04</td>
</tr>
<tr>
<td>Cd</td>
<td>1.87 ± 1.07</td>
<td>32.55 ± 14.98</td>
<td>0.14 ± 0.29</td>
<td>24.35 ± 12.46</td>
<td>41.08 ± 8.91</td>
</tr>
<tr>
<td>Co</td>
<td>63.66 ± 2.68</td>
<td>6.20 ± 1.49</td>
<td>1.25 ± 0.33</td>
<td>5.36 ± 2.07</td>
<td>25.53 ± 2.34</td>
</tr>
<tr>
<td>Cr</td>
<td>0.40 ± 0.13</td>
<td>14.14 ± 6.63</td>
<td>0.31 ± 0.07</td>
<td>11.00 ± 5.76</td>
<td>74.14 ± 4.99</td>
</tr>
<tr>
<td>Cu</td>
<td>55.74 ± 3.83</td>
<td>3.40 ± 1.61</td>
<td>0.71 ± 0.20</td>
<td>3.37 ± 1.26</td>
<td>36.77 ± 2.40</td>
</tr>
<tr>
<td>Mn</td>
<td>0.57 ± 0.15</td>
<td>31.73 ± 15.84</td>
<td>0.31 ± 0.24</td>
<td>26.48 ± 14.75</td>
<td>40.92 ± 12.41</td>
</tr>
<tr>
<td>Ni</td>
<td>50.34 ± 2.90</td>
<td>6.44 ± 2.70</td>
<td>1.50 ± 0.18</td>
<td>5.40 ± 1.84</td>
<td>36.32 ± 2.45</td>
</tr>
<tr>
<td>Pb</td>
<td>0.72 ± 0.15</td>
<td>22.24 ± 8.94</td>
<td>0.17 ± 0.17</td>
<td>18.09 ± 9.36</td>
<td>58.78 ± 5.73</td>
</tr>
<tr>
<td>Sb</td>
<td>5.67 ± 0.91</td>
<td>1.08 ± 0.05</td>
<td>1.12 ± 0.18</td>
<td>1.09 ± 0.04</td>
<td>91.05 ± 0.90</td>
</tr>
<tr>
<td>Se</td>
<td>8.24 ± 0.92</td>
<td>0.12 ± 0.47</td>
<td>6.00 ± 0.15</td>
<td>0.16 ± 0.41</td>
<td>85.47 ± 0.54</td>
</tr>
<tr>
<td>Sn</td>
<td>1.68 ± 0.29</td>
<td>5.55 ± 2.89</td>
<td>0.91 ± 0.48</td>
<td>5.29 ± 3.98</td>
<td>86.56 ± 3.53</td>
</tr>
<tr>
<td>V</td>
<td>0.02 ± 0.14</td>
<td>3.92 ± 1.85</td>
<td>0.27 ± 0.08</td>
<td>3.31 ± 1.75</td>
<td>92.47 ± 1.28</td>
</tr>
<tr>
<td>Zn</td>
<td>234.10 ± 45.86</td>
<td>11.23 ± 2.78</td>
<td>25.31 ± 40.23</td>
<td>33.98 ± 6.64</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>
3.4.3 BMP Testing

To be able to perform the BMP testing with equivalent COD samples, the COD of the heavy metals contaminated microalgae, control microalgae, heavy metals contaminated LEA, and control LEA samples were measured. The COD for the heavy metals contaminated and control whole microalgae were measured to be $1.80 \pm 0.04 \text{ g-COD g-}^{-1}\text{-microalgae}$ and $1.93 \pm 0.04 \text{ g-COD g-}^{-1}\text{-microalgae}$ while the COD for the heavy metals contaminated and control LEA were measured to be $1.26 \pm 0.05 \text{ g-COD g-}^{-1}\text{-microalgae}$ and $1.37 \pm 0.09 \text{ g-COD g-}^{-1}\text{-microalgae}$.

3.4.3.1 Methane Production Results.

Methane production over the 25 day test period for the heavy metals contaminated microalgae, control microalgae, heavy metals contaminated LEA, and control LEA samples are presented in Figure 14. A positive control sample utilizing glucose as the carbon source was used to verify that the anaerobic bacteria contained within the inoculum was effectively producing biogas. A negative control was also used to determine that there was almost no background methane production. See Table 11 for total biogas results as well as the theoretical methane yield for the microalgae and LEA cases. Using gas spectrometry, the methane percentage for the heavy metals contaminated microalgae, control microalgae, heavy metals contaminated LEA, and control LEA samples was measured to be $36.28\% \pm 1.04\%$, $37.47\% \pm 1.12\%$, $49.15\% \pm 1.84\%$, and $12.25\% \pm 3.54\%$ respectively.

As shown in Table 11 heavy metals alone have very little effect on production of methane from microalgae as is shown by the similar methane production shown by the heavy metals contaminated microalgae and control microalgae samples. It was also found that for the control case the microalgae produced 2.5 times more methane than the LEA as has been seen in studies by other authors [42]. Another observation was that the combination of heavy metals and the
effects of the acid catalyzed conversion caused the production of methane to increase dramatically to over 5 times the production of the control LEA and almost twice the production of the heavy metals contaminated microalgae. This result could be expected as heavy metals commonly degrade the health of a microalgae cell. This degradation of the cell’s health would increase the efficiency at which the acid catalyzed transesterification would disrupt the microalgae cells and cause lipids, proteins, carbohydrates etc. to be more available for lipid harvesting and conversion into methane. This effect was seen elsewhere in this study when lipid recovery was increased by the presence of heavy metals in the growth phase of the microalgae cells.

Figure 14. Methane production from heavy metals contaminated microalgae, control microalgae, heavy metals contaminated LEA, and control LEA samples. All samples were COD equivalent and were run in triplicate. (M) refers to heavy metals contaminated samples (C) refers to control samples.
Table 11. Biogas production for heavy metals contaminated microalgae, control microalgae, heavy metals contaminated LEA, and control LEA samples, compared to calculated theoretical yields. The standard deviation is shown in parenthesis.

<table>
<thead>
<tr>
<th></th>
<th>Total Biogas (σ)</th>
<th>Methane (σ)</th>
<th>Theoretical Methane Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microalgae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>142.13 (4.16)</td>
<td>53.07 (2.12)</td>
<td>331.9</td>
</tr>
<tr>
<td>Heavy Metals</td>
<td>149.21 (2.40)</td>
<td>53.96 (2.06)</td>
<td>401.17</td>
</tr>
<tr>
<td>LEA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>49.96 (1.54)</td>
<td>6.17 (2.74)</td>
<td>322.24</td>
</tr>
<tr>
<td>Heavy Metals</td>
<td>215.46 (3.28)</td>
<td>108.70 (5.20)</td>
<td>351.37</td>
</tr>
</tbody>
</table>

3.5 Conclusions

This chapter evaluated the distribution of heavy metals from microalgae grown in the presence of heavy metals found in flue gas and determined the effects that this distribution have over biofuel production through acid catalyzed transesterification and supercritical methanol transesterification and methane production through biochemical methane potential testing. Some of the key conclusion of this chapter are as follows.

Heavy metal effects on the fatty acid profile of the control and trace heavy metals contaminated biofuel were determined for both the acid catalyzed and supercritical methanol transesterification conversion types. For the acid catalyzed case the control and contaminated profiles were found to be similar with minimal changes. The production of the predominant fatty acid chain lengths 16:0 and 16:1 for the control and heavy metals contaminated biofuel were found to not be statistically different their sums representing 58.6% and 59.2%, respectively, of the total lipids. Similarly the fatty acid profile for the control and trace heavy metals contaminated biofuel from supercritical methanol transesterification were found to be similar with minimal changes. The production of the predominant fatty acid chain lengths 16:0 and 16:1 for the control and heavy metals contaminated biofuel were found to not be statistically different their sums representing 51.9% and 56.4%, respectively, of the total lipids.
Heavy metal effects on conversion were determined by measuring changes in biofuel production and recovery efficiency. The acid catalyzed transesterification performed resulted in an average crude biofuel production decrease from $0.31 \pm 0.03$ grams biofuel/gram microalgae for the control microalgae biomass to $0.28 \pm 0.02$ grams biofuel/gram microalgae for the heavy metal contaminated microalgae biomass, representing a 9.7% reduction. Supercritical methanol conversion exhibited a similar trend corresponding to a 15.8% reduction. It was found that the recovery efficiency of the acid catalyzed biofuel increased from 80% for the control biofuel to 89% for the heavy metals contaminated biofuels. Supercritical methanol conversion exhibited a similar trend with recovery efficiency increasing from 98% for the control biofuel to 100% for the heavy metals contaminated biofuels. The author speculates cell degradation to be the reason for the increase in recovery efficiency associated with the heavy metals contaminated biofuel samples.

To determine the ending distribution of the heavy metals after transesterification a mass balance was performed on the system and it was found that following acid catalyzed transesterification the majority of the heavy metals were determined to be in the LEA, methanol/water mixture or lost to the environment. Minimal contamination of the filters and produced biofuel was experienced. Similarly, following supercritical methanol transesterification the majority of the heavy metals were determined to be in the LEA or lost to the environment. Minimal contamination of the filters was experienced though substantial contamination of Co, Cu, and Ni was experienced by the produced biofuel.

The effects of heavy metals on the production of biogas were also determined. The effects of heavy metals in combination with the effects of acid catalyzed transesterification were found to have a positive effect on the amount of methane produced with an average productivity of 105.89
mL g-COD$^{-1}$ from the heavy metals contaminated LEA compared to the control microalgae biomass which produced 53.25 mL g-COD$^{-1}$.

3.6 References


4.1 Conclusions

Large scale biofuel production from microalgae is expected to be integrated with point source CO₂ sources, such as coal fired power plants. Flue gas (CO₂) integration represents a required nutrient source for accelerated growth while concurrently providing an environmental service though contaminants such as heavy metals will be introduced to the microalgae system. This study aimed to directly assess the impact of 14 heavy metals (As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sb, Se, Sn, V and Zn), commonly present in coal flue gas, on biomass, lipid, biofuel and methane production and evaluate the end fate of heavy metals in the microalgae-to-biofuel process. After testing the effects of these 14 heavy metals on a microalgae growth system followed by 2 different microalgae to biofuel conversion processes (acid catalyzed transesterification and supercritical methanol transesterification) and BMP testing of the LEA byproduct, the following conclusions can be reached.

Heavy metals were found to negatively affect the production and lipid yield of the microalgae. On average contaminated biomass cultures were found to have a 67.5% ± 16.3 reduction in biomass productivity with the control and contaminated cultures averaging 1.13 g L⁻¹ d⁻¹ ± 0.12 and 0.37 g L⁻¹ d⁻¹ ± 0.18, respectively. The addition of heavy metal contaminants into the microalgae growth phase resulted in an average 31.9% ± 13.2% decrease in lipid content at harvest after the 7 day growth period. Combining the effects of heavy metal contaminants on biomass productivity and lipid content, there is an overall decrease in lipid productivity of 77.9% ± 12.1 from 0.49 g L⁻¹ d⁻¹ ± 0.06 for the control replicates to 0.11 g L⁻¹ d⁻¹ ± 0.06 for the heavy metal contaminated replicates harvested after 7 days.
Due to the addition of heavy metals the recovery efficiency of acid catalyzed biofuel increased from 80% for the control biofuel to 89% for the heavy metal contaminated biofuel. Recovery efficiency of supercritical methanol biofuel also increased from 98% for the control biofuel to 100% for the heavy metals contaminated biofuels. The author speculates cell degradation to be the reason for the increase in recovery efficiency associated with the heavy metals contaminated biofuel samples.

Heavy metal contaminants in combination with the effects of acid catalyzed transesterification increased the amount of methane produced through BMP testing to an average productivity of 105.89 mL g-COD\(^{-1}\) from the heavy metal contaminated LEA compared to the Control microalgae biomass which produced 53.25 mL g-COD\(^{-1}\).

Integration of the microalgal growth system with coal fire power plant flue gas was found to cause heavy metal contamination of biomass, media, biofuel, LEA and potentially other byproducts. Heavy metal contaminants introduced into the growth system were predominantly sorbed by the biomass and spent medium. Following acid catalyzed transesterification the majority of the heavy metals were determined to be in the LEA, methanol/water mixture or lost to the environment. Minimal contamination of the filters and produced biofuel was experienced. Following supercritical methanol transesterification the majority of the heavy metals were determined to be in the LEA or lost to the environment. Minimal contamination of the filters was experienced though substantial contamination of Co, Cu, and Ni was experienced by the produced biofuel.

4.2 **Recommended Future Research**

- The experiments of this study should be repeated using different growth setups such as raceways ponds to better understand comparison effects between different growth systems.
• Modeling of the effects found in this work should be performed to advance the accuracy of current TEA and LCA models concerning the microalgae to biofuel process.

• Other contaminants contained within flue gas should be studied to understand other potential product restrictions, such as use for the production of high and low value products such as chemicals and animal feed.
Appendix A. Growth Results for 5 Individual Batches

Individual growth results for the 5 batches run are presented in the following figures.

Figure A1. Growth Curve for Batch 1

Figure A2. Growth Curve for Batch 2
Figure A3. Growth Curve for Batch 3

Figure A4. Growth Curve for Batch 4
Figure A5. Growth Curve for Batch 5
Appendix B. Heavy Metal (ICP-MS) Analysis Results for Individual Batches

The following figures detail the IPCMS results for each batch tested.

Figure B1. Heavy Metal Contamination levels Batch 1

Figure B2. Heavy Metal Contamination levels Batch 2
Figure B3. Heavy Metal Contamination levels Batch 3

Figure B4. Heavy Metal Contamination levels Batch 4