

**A COMPREHENSIVE STUDY OF THE CHARACTERIZATION OF
PARTICULATE MATTER EMISSIONS FROM A DELMARVA BROILER
POULTRY OPERATION**

by

Shannon E. Carter

A dissertation submitted to the Faculty of the University of Delaware in partial
fulfillment of the requirements for the degree of Doctor of Philosophy in Plant and
Soil Sciences

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~Shannon Carter-Cahill

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ABSTRACT

Particulate matter (PM) emissions from agricultural practices, including those from animal feeding operations (AFO's) have become an increasingly important topic, and has generated considerable interest from local and state agencies, as well as, the local community over the past decade. Because of growth in population, and an increase in commercial and residential development within close proximity to these operations, which house a large number of animals in confinement, and because of a better understanding of the effects of exposure to airborne contaminants on health, this has lead to an increase in concerns and a demand for more research to be conducted on PM from AFO's.

Particulate matter generated within, and emitted from, AFO's can carry with it various components including metals and microorganisms that can negatively affect health. This research was conducted in order to verify if PM from a broiler poultry operation on Delmarva has the potential to become a health concern. The first step was to determine concentrations of two size segregated fractions of PM from indoor and outdoor sampling sites over four seasonal periods, early summer (ES), late summer (LS), Fall (F), and Winter (W). Both PM_{10} and $PM_{2.5}$ were collected because of their classification from the Environmental Protection Agency as having the ability to cause significant health effects with short-term exposure. Next, temporal and spatial characteristics were investigated to determine their effects on PM concentrations over the four seasonal periods. Following this, the chemical composition and morphology of PM_{10} and $PM_{2.5}$ generated from the broiler poultry

operation was investigated. Finally, further detailed information was obtained on arsenic speciation and oxidation state in PM to investigate toxicity. Arsenic use in the poultry industry has been occurring for a number of decades, and is most frequently administered in the organic form. However, studies have shown that these organo-arsenicals can quickly degrade into organic by-products, methylated arsenicals, and inorganic arsenic (III and V). Because oxidation state determines mobility and toxicity in humans, animals, and the environment this is a key reason to investigate it further in PM.

The results from this research indicate that the concentrations of both PM size segregated fractions that were sampled are within the regulatory guidelines of EPA and OSHA. Outdoor concentrations were mainly influenced by wind speed changes over the seasonal periods, and bird weight was the main management factor influencing indoor PM concentrations. In addition, upon performing chemical analysis on the PM using inductively coupled plasma mass spectrometry (ICP-MS), the arsenic concentrations found are not above background ambient arsenic levels for outdoor samples; however, total arsenic was found to be above those background concentrations in both indoor PM₁₀ and PM_{2.5} samples. Although the arsenic concentrations were found to be higher than background inside the poultry operation, they are currently within the regulated limits set by the Occupational Safety and Health Administration (OSHA) and the National Institute of Occupational Safety and Health (NIOSH). Other metal(loid)s such as copper, manganese, and zinc were also within regulatory limits in both indoor PM₁₀ and PM_{2.5} samples.

While the EPA has National Ambient Air Quality Standards set for PM₁₀ and PM_{2.5}, these regulations are not suitable when evaluating indoor occupational

concentrations from an animal feeding operation such as a broiler poultry operation. In addition, the EPA does not currently have standards set for arsenic in ambient or general air pollution. It is also questionable to use the current dust regulations set by the OSHA or NIOSH because they are generalized to two categories that are not easily translatable to the current PM₁₀ and PM_{2.5} size segregations accepted under the EPA. In addition, there is an assumption made that particles within their total suspended and respirable regulatory categories are “inert” or nuisance, which infers that particles under this classification would not lead to any significant health problems. This is not the case with PM generated from a broiler poultry operation, which can carry with it a number of contaminants that have been proven to cause various health disorders from exposure. These classifications also apply to inhalable arsenic standards and are also questionable when determining whether arsenic concentrations in PM from a poultry operation are permissible.

Arsenic oxidation state and speciation in PM₁₀ and PM_{2.5} was investigated using X-ray absorption spectroscopy (XAS) and X-ray fluorescence (XRF) spectroscopy. The results indicate that there is a mix of organic species present, as well as, oxidized As(V) and reduced As(III) in all samples analyzed. The main organic species found were in the form of Roxarsone, 4-hydroxy-3-aminophenylarsonic acid (HAPA), and dimethylarsinic acid (DMA(V)). This indicates that much of the organic form that was originally administered has degraded into more toxic by-products that are then becoming incorporated into airborne particulate matter.

Chapter 1

LITERATURE REVIEW: A BRIEF OVERVIEW OF PARTICULATE MATTER AND ARSENIC IN AGRICULTURE

1.1 Particulate Matter: A Brief Description

Particulate matter (PM) is particles that are formed from solid and/or liquid material and are either re-suspended, or form while in the atmosphere. They can consist as one unit of many molecules through intermolecular forces or can be a combination of two or more types of molecules held together by interparticle adhesive forces (Seinfeld and Pandis, 2006). The formation of PM occurs through a combination of various processes, both chemical and physical, and can be comprised of agglomerated materials such as crustal metals, trace elements, inorganic ions, and biological and carbonaceous components. These materials can vary depending on locale and source, whether natural or anthropogenic; because of this PM can be considerably complex and unstable. Particulate matter can occur naturally through the re-suspension of earthen materials such as mineral oxides, or through such anthropogenic processes as fuel combustion, or dust generated from construction sites or fields; these are referred to as primary particle sources. Other means of PM formation are through atmospheric oxidative processes between gases (ie ammonia, SO_x , NO_x); considered secondary sources (Seinfeld and Pandis, 2006; Koutrakis and Sioutas, 1996). Because of the many sources and components that make up particulate matter they can vary in size, shape and chemical composition.

The most extensively studied characteristic of PM currently is size. Size distribution of PM can vary from a few nanometers to well over tens of micrometers (Figure 1.1). The criteria by which size-segregation of particles is determined, and thus defined, is through its aerodynamic diameter, which is the diameter of a spherical particle with a density of pure water with the same settling velocity in air, at atmospheric pressure as the particle under investigation (Baron et al, 1999). According to the EPA, PM is size-segregated into PM_{10} and $PM_{2.5}$ and is based on their aerodynamic diameter and the location within the airway that they can penetrate; the fraction between these two end points is referred to as the “coarse fraction” (EPA, 2013a, 2012).

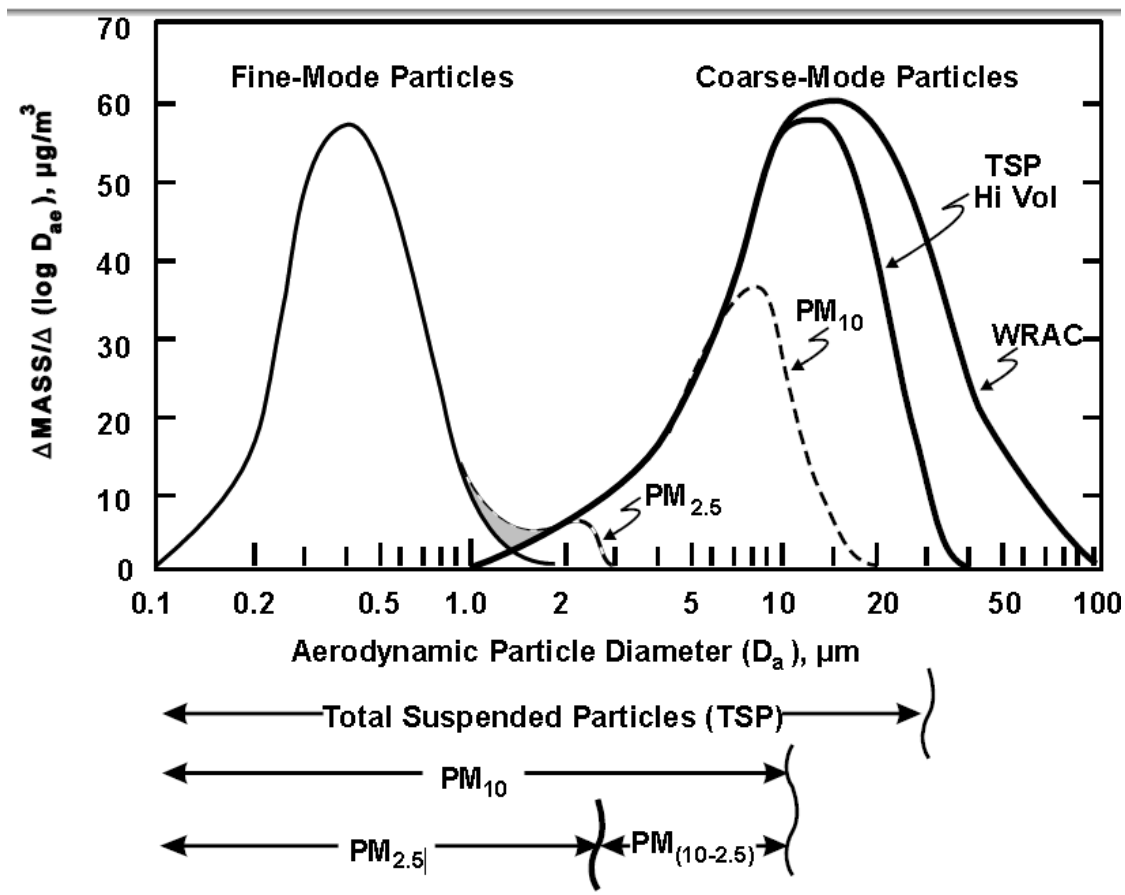


Figure 1.1: Particle size distribution scheme based on aerodynamic diameter. Included in the diagram are the distinctions between “fine” and “coarse” modes and the defined ranges for PM_{10} and $\text{PM}_{2.5}$. TSP = total suspended particulate and WRAC = wide range aerosol classifier (USEPA, 1996).

Currently the National Ambient Air Quality Standards (NAAQS) set by the Environmental Protection Agency (EPA) are $35 \mu\text{g}/\text{m}^3$ for $\text{PM}_{2.5}$ and $150 \mu\text{g}/\text{m}^3$ for PM_{10} in a 24hr period, respectively, both of which encompass primary and secondary standards. According to the EPA “primary” standards are those put in place for public health protection, and “secondary” standards are those in place for providing public welfare protection. In addition, The United States Department of Labor, Occupational

Safety and Health Administration (OSHA) also have standards set for particulate pollutants. Currently, they regulate total dust or total suspended particulates (TSP), which is a general reference to any airborne particle, and the respirable fraction, which consists of those particles less than $4\mu\text{m}$. The current standards are 15 mg/m^3 and 5 mg/m^3 , respectively; these are given as 8 hour time weighted averages (TWA's). However, neither of these regulations are suitable for determining exposure limits for those individuals working inside of a poultry operation; this is because EPA's limits are set for particles in an outdoor environment, whereas OSHA standards only apply to "inert" or nuisance dusts. The EPA's standards are based on a general assumption that all particulate pollution within the $\text{PM}_{2.5}$ or PM_{10} criterion are created equal, without recognizing the chemical composition. In addition, the EPA doesn't set standards based on occupational environment but instead is directed towards the general population, and bases its criteria on those who are at most risk like the elderly or young. On the other hand, even though OSHA considers workplace exposure, the terms "inert" and nuisance refer to particles that essentially have no harmful effect, these terms generalize substances associated with PM, and can be misleading because some irritants or contaminants can have long term effects on health. The regulation for these particles is within the TSP and respirable criteria. Also, OSHA categorizes particulate irritants not defined or identified by toxicological data as particles not otherwise regulated (PNOR); again, this category generalizes the pollutants and continues to be regulated by the same criteria set for TSP and respirable dust (OSHA, 1988).

This proposed work will be conducted in order to determine approximate re-suspended particle concentrations within a broiler poultry operation, and shed light

into their composition. The data provided by this research could help establish more suitable regulations for high volume occupational environments such as a poultry operation.

1.2 Health Effects Associated With Particulate Matter Exposure

1.2.1 Human and Environmental Health Concerns

Particle size has been extensively studied for the implications it has on human health. The size of the particle greatly determines how it behaves in air, and the deposition site within the respiratory and/or cardiovascular systems. Larger particles between 2.5 and 10 micrometers tend to deposit within the conducting airway while particles that are smaller than 5 micrometers deposit further in the airway and can end up deep within the lung tissue in the respiratory bronchioles (WHO, 2006; WHO, 2003; Wilson and Spengler, 1996). Fine particles, those less than 2.5 μm have been found to diffuse through respiratory tissues and can influence other systems within the body including the cardiovascular system.

Particles within the 10 to 2.5 μm size range, and below, have been widely studied for their toxicological and epidemiological effects (Simkhovich, et al. 2008; Valvanidis, et al. 2008; Pope and Dockery, 2006; Li et al., 2003; Pope et al., 2002; Samet, et al., 2000; Dejmek et al., 1999). A study done by Pope et al. (2002), found that increased lung cancer and cardiopulmonary mortality increased with increasing exposure to the fine fraction of PM or PM_{2.5}. In another study by Dejmek et al. (1999) determined that increased exposure to both PM₁₀ and PM_{2.5} resulted in an increase in preterm birth and intrauterine growth retardation (IUGR). These reports demonstrate correlations between particle size and corresponding health conditions.

Not only is size an important characteristic for determining health implications, but it can also be used to determine residence time in the atmosphere, and subsequent deposition in the terrestrial environment. Fine and ultrafine particles tend to have much higher residence times and can drift further than larger coarse particles. As particles drift they can change both physically and chemically; once deposited they can become incorporated into the terrestrial environment where further physical and chemical transformation can occur. An example of this was investigated in a study conducted by Pavlik and others (2011), which determined the adverse effects of trace elements associated with PM on lettuce. Upon treating the soil and plant leaf tissue with PM, they found that plant biomass (dry yield) decreased to 14.9 ± 4.8 g and 8.1 ± 1.6 g respectively, compared to the control which had a biomass of 17.8 ± 3.6 g. In addition, trace elements including arsenic (As), chromium (Cr) and Lead (Pb) had increased in the above-ground biomass as a result of the PM application to the soils, and to a lesser extent from applying it to the leaves directly. This showed significant correlation with the assimilation of carbon and nitrogen, which are used in plant metabolic processes. The decrease in nitrogen and subsequent decrease in amino acid concentrations resulted in reduced metabolic activities, such as biosynthesis of nucleic acids and the production of ATP (Pavlik et al., 2011).

Human health problems can develop as a result of acute or chronic exposures to PM, and can lead to problems such as respiratory infection, asthma and bronchitis to more severe health problems such as chronic respiratory infections like chronic obstructive pulmonary disease (COPD), heart disease and a range of various types of cancer (Pope and Dockery, 2006; Pope et al, 2004; Linaker et al, 2002; Radon et al, 2002; Donham et al, 2002; Zuskin et al, 1994; Donham, 1990). In many cases, size

and concentration aren't the only factors leading to long term health problems. Just as Pavlik et al (2011) had described regarding lettuce plants, the exposure to materials or irritants associated with PM can also contribute and influence the type of health condition that develops. Epidemiological and toxicological research has looked closer at the chemical and biological components associated with PM which could potentially contribute towards the development of health problems (Valvanidis et al, 2008; Simkhovich et al, 2008; Mar et al, 2005; Samet et al, 2000).

In small doses most metal(loid)s are essential for the body to function; however, they can become toxic when inhaled or ingested at high concentrations, over long duration of exposure, or based on their oxidation state. One study looked at how metals such as manganese, copper and nickel can contribute to bio-accumulation in the body and can possibly lead to toxicity and health problems (Kampa et al, 2008). A review by Gao and others (2005) also referenced a number of studies that have shown a link between metal(loid) exposure from inhalation of airborne PM and increased morbidity and mortality. There are currently 187 air pollutants regulated by the U.S. EPA's Office of Air Quality Planning and Standards under the Integrated Risk Information System (IRIS) and are defined by the Clean Air Act; some pollutants include As (not currently available), Mn ($0.05 \mu\text{g}/\text{m}^3$), Ni ($0.1\text{-}0.2 \mu\text{g}/\text{m}^3$), Zn (not currently available), Cu (not currently available), and Cr^{6+} ($0.008\text{-}0.1 \mu\text{g}/\text{m}^3$) (EPA, 2006; EPA, 2005; EPA, 2002; EPA, 1993; EPA, 1991). The values represent inhalation reference concentrations (RfC), which is an estimate of a daily inhalation exposure of the human population (including sensitive subgroups like the chronically ill and elderly) that likely will cause a minimal risk of harmful effects over a lifetime. In addition, there are current occupational safety regulations for such contaminants as

inorganic As (10 ug/m^3 and 2 ug/m^3 as permissible exposure limits (PEL)), Mn (5 mg/m^3 and 1 mg/m^3 PEL), Cu (1 mg/m^3 PEL), and Zn (as Zn oxide) (5 mg/m^3 and 15 mg/m^3 PEL for respirable and total dust, respectively) set forth by OSHA and NIOSH (ATSDR, 2012; ATSDR, 2005; ATSDR, 2004; OSHA, 1993).

Having knowledge of the concentration of PM, their size distribution, chemical composition and morphology can help determine the influence they will have on the environment and human health.

1.2.2 Avian Health Concerns

The environmental and air quality in a poultry facility can have a major effect on broiler poultry health. In systems with large numbers of animals in confinement, it is important that bird health remain optimal. It is also these confined conditions, along with a decrease in air quality that can lead to substantial losses from the increase in health issues and spread of disease.

The impacts of high concentrations of particulate matter and noxious gases can be detrimental both to bird health and economically. A number of studies have reported links between higher concentrations of these contaminants to an increase in susceptibility to disease and infection due to immunological issues (Banhazi et al, 2008; Al Homidan et al, 1998; Brown et al, 1997; Quarles and Caveny, 1979). Work cited in the review by Banhazi et al, 2008, suggests that upon deposition of PM an immune response is activated; however, instead of the immune activation helping, it has been found to hinder, and has been linked to reduced performance, size, and feed efficiency. In addition, the work reported by Harry in 1978 suggests that the major health concern from suspended particulate matter is the spread of disease via pathogenic microorganism association. The deposition of particles within the airway

of the avian respiratory system is fairly quick and can result in the onset of many types of upper respiratory diseases, which can spread rapidly in such confined conditions. The drive for economic efficiency is high in the poultry industry; therefore bird health is a primary focus of concern.

1.3 Particulate Matter in Agriculture

Agriculture has long been associated with the surrounding environment through the various activities and practices that are customary during production of crops, livestock and poultry farming. The generation of pollutants such as gases and particulate matter (PM) are inevitable. In the past, research has primarily focused on gas emissions and nutrient runoff from fertilization, and the ways in which these two important issues could be mitigated; however, little research about PM emissions coupled with detailed chemical and physical characterization of the PM was ever performed. It wasn't until the Clean Air Act was enacted in 1963 that more attention was given to the study of particulate pollution (Seinfeld and Pandis, 2006). Since then it has become clearer that PM can have a greater effect on the environment and human health than once believed, therefore much more research regarding PM emissions has been and continues to be performed.

In agriculture, PM can be made up of various elemental, biological and gaseous components, many of which can become hazardous if inhaled or ingested in high concentrations or over an extended period of exposure. The type of contaminants associated can also vary by the type of activity or practice being performed. The range of practices includes harvesting, ploughing and tilling to working daily on livestock or poultry farms. For instance, what is associated with particles from crop farming might be very different from those in a poultry or swine operation. In crop

farming there are generally more particles produced from diesel fuel exhausts from farming equipment, plant residue and earthen materials from re-suspended soils. The PM generated from animal feeding operations can contain a number of irritants and potentially hazardous materials, which animals, farmers and farm workers are exposed to, including various gases such as ammonia and methane, biological components like microorganisms and endotoxins, and metals and metalloids (ie arsenic, iron, zinc, manganese, copper and nickel), which are associated with litter, fecal material, feed and feed supplements (EPA, 2013c; Rylander et al, 2006; Ad hoc Committee on Air Emissions from Animal Feeding Operations; Committee on Animal Nutrition, National Research Council, 2003; Donham et al, 2002).

Because of an increase in population and development within close proximity to agricultural operations, which have changed to include more animals in confinement, this has become a major concern among communities regarding air, water and soil quality. Livestock and poultry operations generate a lot of attention because of the odors they produce, but within the past decade or so there has been increasing concerns about particulate emissions. In recent years a number of studies have looked at the emission levels of PM, biological aerosols and chemical information of PM from these operations (Wang-Li et al, 2013; Li et al, 2011; Cambria-Lopez et al, 2010; Vanderstraeten et al, 2008; Oppliger et al, 2008; Hartung et al, 2007; Roumeliotis et al, 2007; Patterson et al, 2005; O'Connor et al, 2005; Ritz et al, 2004). The study of O'Conner and others (2005) provided a thorough investigation of air transported arsenic and other metals such as copper and zinc in homes located near sources of agriculturally derived dust, and found that 37 of the 50 homes sampled for interior dust had arsenic concentrations that were above the

screening levels for industrial indoor workers set forth by the EPA, and also found that 37 of those homes had higher levels of As, Cu and Zn than were found in average soils in the area. Also, home dust levels of As, Cu and Zn were comparable to those found in the ambient PM_{2.5} samples and in broiler litter samples. In addition, the study revealed that the primary species of As in litter and house dusts were roxarsone, mono-methyl arsenic acid (MMA), As(III), As(V) and several other unidentified species. This study is consistent with other species specific investigations on poultry litter samples (Bednar et al, 2004; Arai et al., 2003, Garbarino et al., 2003, Rutherford, et al., 2003, Bednar, et al., 2003; Garbarino et al, 2001). The research suggests that there is significant reason to study the details of PM from agricultural operations, including those being emitted from livestock and poultry operations.

1.4 Poultry Industry On The Delmarva Peninsula

According to the USDA's "Poultry Production and Value Summary" for 2010, of the ~8.6 billion birds produced in the United States, almost 800 million birds were produced on the Delmarva peninsula (DE, MD, VA) alone. In 2013 that number decreased to almost 600 million birds, but as a whole the Delmarva Peninsula still ranks high on the list of broiler poultry producers in the United States (Delmarva Poultry Industry, 2013). In fact, according to the 2007 USDA's U.S. census of agriculture, Sussex County Delaware has ranked number 1 in broiler poultry production among any other U.S. county since 1944. The number of growers in 2013 on the Delmarva Peninsula was 1,538, with 4,620 actively operating houses and almost 13,500 poultry employees (DPI, 2013). The estimated wholesale value in 2013 of meat producing poultry on Delmarva exceeded 2.8 billion dollars (DPI, 2012). According to a new economic impact study compiled in 2012, the Delmarva Peninsula

contributed a total economic revenue of more than 4.5 billion dollars per year (DPI, 2012; Dunham and associates, 2012). Delaware's contribution alone from meat chickens to its overall cash farm income was approximately 66% (DPI, 2012). In addition, the poultry industry also contributes towards other agricultural businesses such as crops; most of the feed crops are grown locally (soybeans, wheat and corn), and it provides one out of every twelve jobs in the region.

1.5 Metal(loid) Use In The Poultry Industry

1.5.1 Arsenic

Organo-arsenicals have been utilized for a number of decades in the poultry industry (Silbergeld and Nachman, 2008); these drugs are primarily used to control coccidiosis (parasitic disease), and are used to help promote growth. The most common organo-arsenicals currently being used in the poultry industry are Roxarsone (3-nitro-4-hydroxyphenylarsonic acid) and Nitarsone (4-nitrophenylarsonic acid) (Figures 1.2a & 1.2b). The most recent estimation given by the USDA indicated that 88% of the approximately 9 billion broiler chickens produced for human consumption in the United States are receiving some form of organo-arsenical (Nachman, et al. 2012; USDA, 2011). Inputs of Roxarsone into feed can exceed upwards of ~2.2 million pounds (~1000 tons) per year (Walinga, 2006). The significance of supplementing these into feed is how toxic they can become once they are excreted. The litter can contain upwards to 48-50 mg/kg of organo-arsenicals, which can increase As litter levels by seven-fold (Bolan et al, 2010; Makris et al, 2008; Stolz, et al, 2007; Arai, et al, 2003; Garbarino, et al, 2003). The litter is generally used as fertilizer on crop land, and has traditionally been applied at a rate of 8.96-20.16 Mg

ha⁻¹ (~9-20 metric tonnes) (Arai, et al. 2003). Because these drugs eventually degrade from their organic form into methylated and inorganic species (As(III) and As(V)), which are much more toxic, it has become an increasing concern (Arai, et al., 2003; Garbarino, et al. 2003; Seiter (dissertation), 2009).

In recent years a number of studies have investigated the transport and transformation of these organoarsenicals, primarily Roxarsone, in poultry tissues, litter material, soil and in groundwater, as well as, through biological degradation (Nachman, et al., 2013; Kazi, et al, 2013; USDA, 2011; Seiter(dissertation), 2007; Jackson, et al, 2006; Arai et al, 2003; Cortinas, et al, 2006; Stolz, et al, 2007; USGS, 2004; Rutherford, et al, 2003). The process of organoarsenical transformation in soils, litter and groundwater can occur under aerobic or anaerobic conditions. According to the review by Mandal and Suzuki (2002), in soils, these compounds can become methylated under oxidizing conditions, forming MMA (monomethylarsinic acid), DMA (dimethylarsinic acid) and trimethylarsine oxide (TMAsO); alternatively under anaerobic conditions these can be reduced to volatile and oxidized methylarsines. Makris and others (2008) determined that suspensions of swine waste containing Roxarsone were being transformed under a microbially mediated process under anaerobic conditions, which lead to the formation of organoarsenical by-products and inorganic As(V); this result has also been found in studies on paper recycling sludge as well as in poultry litter samples (Cortinas et al., 2006; Arai et al. 2003).

Recently, questions regarding the use of Roxarsone and its subsequent accumulation in poultry tissue and human exposure have contributed towards the temporary suspension of Roxarsone, and have lead to legislation to ban the use of any organoarsenical use in poultry production in Maryland (New York Times, 2011;

Schmidt, 2013; Nachman et al., 2013). However, little has been studied regarding how transport and transformation of arsenic occurs in the air, and limited species specific data have been provided. Of the studies that have been done, many of them have focused on urban and industrial sources (Godelitsas et al., 2011; Tsopelas et al., 2008; Sanchez de la Campa et al., 2008; Sanchez-Rodas et al., 2007; O'Connor et al., 2005; Oliveira et al., 2005; Huggins et al. 2004; Utsunomiya et al. 2004; Farinha et al., 2004).

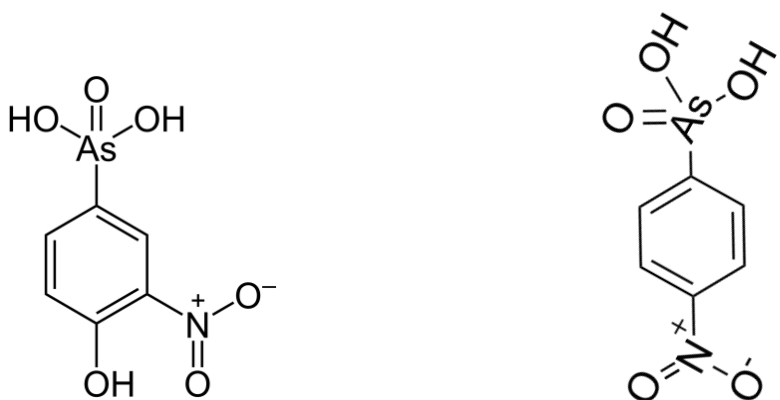


Figure 1.2: Roxarsone

Nitarosone

1.5.2 Other Metal(loid)s

Trace metals such as Zn, Mn, and Cu, are commonly used in the poultry industry and are supplemented in inorganic salts in the feed. These trace minerals are used primarily to support good poultry health, promote growth, and improve feed efficiency (avitech, 2002). They are essential for enzymatic activity and metabolic processes within the birds. When these minerals are broken down in the digestive system they first dissociate into free ions which then go through a complexation process with organic molecules where they are then absorbed by the intestines;

however, if there are more free ions than organic ligands available for complexation then those free ions are excreted (ur Rehman et al, 2012; avitech, 2002). When the ions are excreted they can then accumulate in litter material which can then become re-suspended in the air.

Here we seek to investigate how arsenic and other metal(loid)s are distributed and associated with particulate matter (PM) from a poultry operation, and to determine the dominating species of arsenic present. This will help determine the potential toxicity of airborne PM from a poultry operation and will provide insight into how this can contribute to the development of human, animal, and environmental problems.

1.6 Metal(loid) Toxicity

1.6.1 Arsenic

Arsenic can come in various forms or species, which include two main categories, organic and inorganic. In general, inorganic species tend to be much more toxic in the environment and can have a greater effect on human health than organic species (ATSDR, 2009; ATSDR, 2007). However, that is not to say that organic species aren't significant. In many cases organo-arsenicals can become degraded through chemical and biological processes making them more bioavailable, which can lead to environmental damage, contamination and the development of human health issues.

Inorganic forms are naturally occurring and can either be found as As (V), which is less mobile and toxic than the more reduced As (III), which is highly mobile in the environment and is much more toxic. Organic As occurs when arsenic is bound to carbon; the toxicity of these compounds comes from the type of disruptive activity

it may cause on plants and animals as a result of becoming transformed into more toxic methylated and inorganic forms.

The mechanisms by which As accumulates in plants and animals have been studied extensively. A number of studies show how inorganic species of As can effect such metabolic processes as phosphorylation and other enzymatic activity (Kitchen et al. 2008; Mandal and Suzuki, 2002). In general, the As has been found to compete with phosphorous in a wide variety of processes. An example of this is through the inhibition of phosphorylation that must occur in order to convert ADP to ATP for energy (NRC, 1977). Here inorganic As(V) is reduced to As(III) which can interfere with enzymatic activity through bonding to sulfhydryl and hydroxyl groups. Strong bonds with sulfur molecules create a chelating complex which prevents the continuation of enzymatic activity (Figure 1.3). In addition, As(V) can compete with phosphate, which has been implicated in the disruption of oxidative phosphorylation by creating an arsenate ester that causes non-enzymatic hydrolysis to occur and subsequent interruption of the conversion from ADP to ATP (Mandal and Suzuki, 2002). As a result, when such processes like this are affected, organisms typically succumb over time.

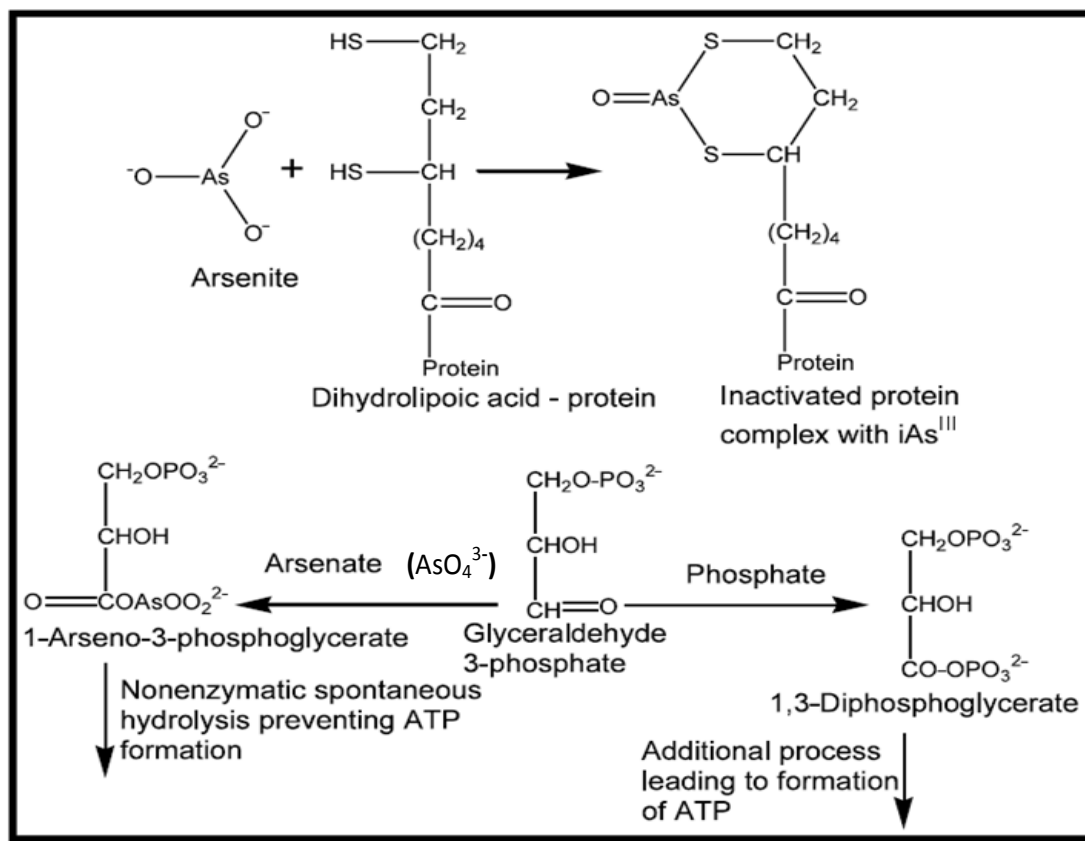


Figure 1.3: Schematic representation of reactions responsible for the disruption of enzymatic processes which lead to the formation of ATP (adapted from Mandal and Suzuki, 2002).

1.6.2 Other Metal(loid)s

Other metals including Zn, Cu, and Mn are essential for good health; however, these can also become toxic. When these metals are inhaled or ingested in high concentration they can lead to negative impacts on health including stomach issues, nausea, vomiting, skin irritation, metal fume fever, and neurological disorders (ATSDR, 2012; ATSDR, 2005; ATSDR, 2004).

1.7 Arsenic Speciation, Distribution, and Association with Other Metal(loid)s Using Synchrotron Microprobe Spectroscopy And Imaging

1.7.1 X-ray Absorption Spectroscopy: A Brief Description

X-ray absorption spectroscopy (XAS) is an element specific analytical technique that allows one to investigate the chemical properties of the target element within a complex material. This technique can give valuable information on the local coordination environment, and is used to elucidate atomic characteristics such as oxidation state, coordination number, and identity of next nearest neighbors (Sparks, 2003). It is used in a number of different areas of science, including: physics, chemistry, biology, biogeochemistry, and environmental and materials sciences (Newville, 2004). Because XAS is a noninvasive technique that requires little sample preparation, it can be used to investigate a variety of different sample types including non-crystalline and amorphous materials *in situ*.

When discussing XAS one should consider two distinct regions of the absorption spectrum, each able to provide invaluable information regarding local coordination environment. They are defined as the XANES (x-ray absorption near edge structure) and EXAFS (extended x-ray absorption fine structure) regions. The XANES region of the spectrum is primarily used for fingerprinting or to determine oxidation states of an element, whereas the EXAFS region gives more detailed atomic information, such as coordination number, bond distances and identity of next nearest neighbors (Sparks, 2003). Figure 1.4 depicts the two primary spectral regions that can be obtained from XAS analysis.

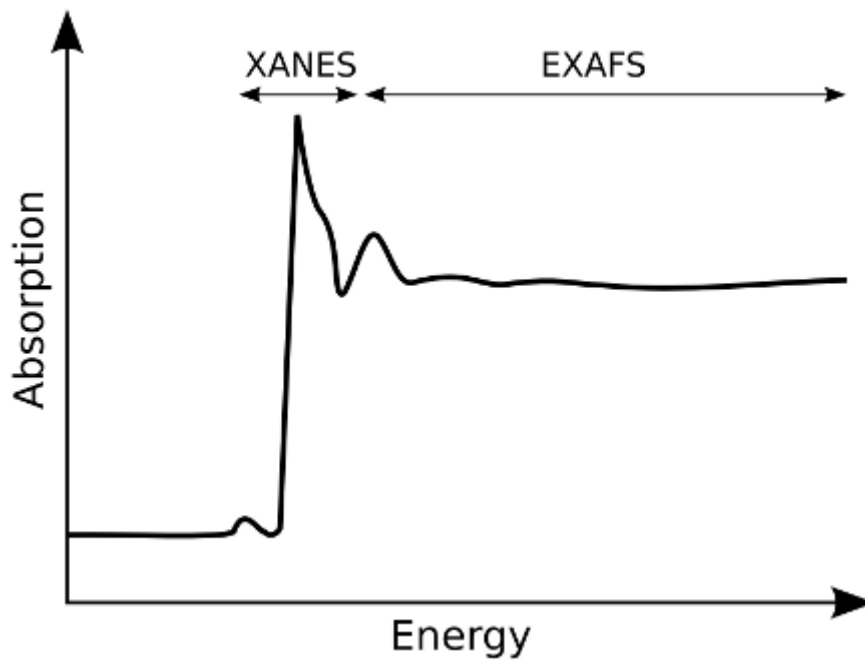


Figure 1.4: Absorption spectrum showing the two distinct regions of interest in a single XAS scan (University of Manchester Paleontology, 2014)

A basic synchrotron XAS experiment begins when a sample is exposed to a monochromatic beam of x-rays that can be tuned near the binding energy of the target element. Beam intensity is monitored before and after the sample to determine the proportion of x-rays absorbed at a particular energy. Each element has a characteristic binding energy, which results from the energy required to remove a core-level electron from the element. When the incident beam energy is tuned below the binding energy of the element, x-rays are not absorbed; however, x-rays are absorbed as the incident energy is tuned above the binding energy. Effectively, XAS measures the energy dependence of the X-ray absorption coefficient. (Grafe et al, 2014; Lanzirotti and Sutton, 2006).

Research conducted on natural systems, including soils, sediments, water and atmosphere have been extensively studied using a variety of XAS technique. Many of these studies have probed sorption mechanisms and metal interactions occurring in soils; many have been referenced in the review by Ginder-Vogel and Sparks, (2010). These studies are not limited to soils and sediments, but have also been performed to a lesser extent on atmospheric particulate matter, primarily from urban and industrial sources (Datta et al., 2012; Elzinga et al., 2011; Godelitsas et al., 2011; Fittschen et al., 2008; Majestic et al., 2007; Wang et al., 2007; Werner et al., 2007; Pattanaik et al., 2007; Ohta et al., 2006; Werner et al., 2006; Huggins et al., 2004; Galbreath, 2003; Ressler et al., 2000). A number of these studies have used XAS to analyze trace elements in ambient PM with a focus on various elements, such as Ni, Cr, Mn, Cu, Zn and Fe. For example, the study performed by Wang et al. (2007) examined a number of these metals from urban and agricultural dusts using XANES spectroscopy to determine chemical speciation; the focus being on Cr, Mn, Cu, and Zn. They found that samples of different particle size, PM_{2.5} and PM₁₀ collected in the Shanghai area, showed similarities in both oxidation state and speciation for the elements of interest. When comparing their urban samples to a standard reference material (SRM 1648), which is an urban PM standard, they also found similarities. Because of the variability in samples and sources it is important that appropriate reference materials be utilized when performing such techniques as XANES and EXAFS.

In a bulk XAS analysis, information is collected on the chemical species contained within several square millimeters of sample; this information can be helpful for understanding the dominant chemical species in the sample, but does not provide information about the minor species in the sample which might be the most reactive

components and hence control toxicity and bioavailability. This can be problematic when dealing with heterogeneous samples like poultry PM, litter material, soils and plants, which can have variability in chemical speciation, including intra-mixed species of organic and/or inorganic nature and multiple species, occur within a few microns (Majestic et al., 2007; Bertsch and Hunter, 2001). Since beam sizes for bulk XAS analysis can range from 1-10 mm, the resolution of the probe is coarser than the heterogeneity found in environmental samples, which largely consist of particles $<10 \times 10 \text{ } \mu\text{m}^2$ (Scheidegger et al, 2006). Micron-size x-ray beams can be used to explore the chemical and spatial heterogeneity in environmental samples.

1.7.2 Microprobe Techniques

The synchrotron microprobe can provide spatially resolved chemical information about trace elements in a heterogeneous sample. Scanning x-ray microprobe combines a number of analytical x-ray techniques (e.g., x-ray fluorescence spectroscopy, XAS, and x-ray diffraction) into a single microscope with spatial resolutions less than a micrometer. There are a number of benefits of coupling spectroscopic measurements with imaging techniques. For one, the microprobe offers the ability to determine localizations and elemental associations of trace elements with very little sample preparation, whereas many of the conventional methods require aggressive sample preparations or extreme sample environments such as vacuum (Lanzirotti, et al. 2010; Lanzirrotti and Sutton, 2006). Secondly, the benefits of high spatial resolution and high detection sensitivity aid in the ability to elucidate the heterogeneity in chemical state for trace elements in environmental samples. Using synchrotron based X-ray μ -fluorescence spectroscopy (μ -XRF) and imaging, one can elucidate elemental abundance and distribution in samples that are heterogeneous at

the micrometer or sub-micrometer scale. Coupling this technique with spatially resolved XAS spectroscopy, one can determine oxidation states, coordination numbers and identity of next nearest neighbors at select points of interest in a heterogeneous sample.

1.8 Arsenic In Agriculture

Arsenic is a naturally occurring element in the environment; in fact, in some locations around the world such as Bangladesh naturally occurring arsenic has been the leading cause of contamination issues in rice crops and in ground water sources (Ravenscroft, 2011; Seddique et al., 2008; Hossain, 2006). Although it can occur naturally it has also been anthropogenically introduced to the environment as well. Man-made arsenicals have been utilized in such processes as coal burning and smelting for many years. In agriculture, arsenicals have been used in various ways for controlling problems associated with crop farming and livestock and poultry production. These synthetic arsenicals have been used for decades in the form of herbicides and pesticides, and also to control disease in livestock and poultry (organic trade association, 2013).

Both forms of As, inorganic and organic, are found in agriculture, but the main products used are in the organic form. The most commonly found compounds used in agriculture today are monosodium methane-arsonate (MSMA) and 3-nitro-4-hydroxyphenylarsonic acid (Roxarsone). Of the dozens of different herbicides that were once used MSMA is the last remaining applicant approved for use in cotton crop farming; however, the EPA is looking at re-evaluating its use (EPA, 2009). Although in 2011 Roxarsone was voluntarily pulled from the market it is still not banned, and the use of other organo-arsenicals as coccidiostats, agents used to control the coccidian

intestinal disorder, are still being used today in livestock and poultry operations. Banning of, and suspending the use of organo-arsenicals stems from how they can become transformed in the environment and can lead to contamination of water, soils and air, and can lead to harmful effects on humans.

Researchers suggest, through a biotransformation pathway, organo-arsenicals can transform under both anaerobic and aerobic conditions, and yield both organic arsenic derivatives and inorganic species of arsenic (Makris et al. 2008; Stolz et al. 2007; Cortinas et al. 2006; O'Connor et al. 2005; Garbarino et al. 2003; Arai et al. 2003). Two studies in particular from Cortinas et al. (2006) and Makris et al. (2008) looked at these conditions. In both studies, Roxarsone was shown to be reduced initially into intermediate organic phases under anaerobic conditions with high solids content; however, in the study performed by Cortinas et al. (2006) they suggest that under aerobic conditions with low solids the Roxarsone appeared to remain unconverted, this was validated again in the work performed by Makris et al. (2008) on swine waste. Although this was seen under low solids conditions, in Makris et al. (2008), a sample containing higher solids content showed reduction of Roxarsone was fast. At around 8 days 100% of the Roxarsone had been transformed. The primary by-products of the transformed Roxarsone were consistently of organic nature (3-HPPA, HAPA) for samples under anaerobic conditions with both high and low solids contents. However, it appeared that samples containing higher solids contents also contained inorganic arsenic in the form of As(V) as a by-product, and after extended biodegradability periods, As(III) was also present (Makris et al. 2008; Cortinas et al. 2006). These studies suggest that Roxarsone, under some form of biological mediation, can be transformed into more toxic by-products, and this occurrence can be

found under both anaerobic and aerobic conditions with high enough solids content. Figure 1.5 shows the possible biological pathways for the biotransformation of Roxarsone (adapted from Momplaisir et al 2001; Holderbeke et al. 1999).

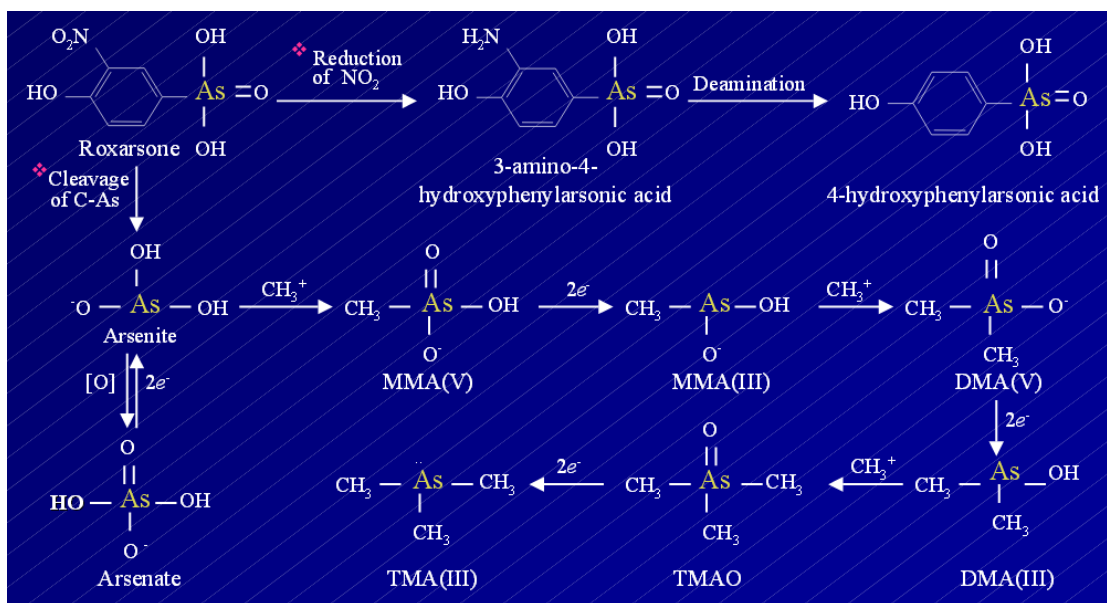


Figure 1.5: Possible biotransformation pathways suggested for Roxarsone (Momplaisir et al 2001; Holderbeke et al. 1999).

Although the degradability of Roxarsone has been studied in sludges, litter material, and in swine waste, to our knowledge there has not been any research performed on the by-products of Roxarsone in re-suspended particulate matter from an agricultural operation.

1.9 Arsenic Speciation In Particulate Matter

The investigation of toxic species of metals and metalloids such as arsenic and chromium in the atmosphere has become an increasing focus of research in the last

few decades. Arsenic has long been associated with such processes as mining, tanning and can be found in fertilizers and herbicides. Concerns have risen due to the levels of contamination that these man-made processes have been associated with, including airborne contamination. Arsenic occurring in the atmosphere can become a carcinogenic environmental contaminant and can contribute to the development of human health problems (Mandal and Suzuki, 2002; Kitchen and Wallace, 2008). The toxicity and mobility of As is species specific. Inorganic arsenic species have been studied extensively for their toxic properties and their ability to mobilize easily in the environment. Whereas, organic and methylated species of arsenic such as MMA (monomethylarsinic acid), DMA (dimethylarsinic acid), p-arsenilic acid and Roxarsone (3-nitro-4-hydroxyphenylarsonic acid) are said to be less toxic and mobile, these organic and methylated species are capable of transforming into more toxic inorganic forms (Mandal and Suzuki, 2002). Therefore, determining the species of arsenic in the atmosphere is significant and can help identify possible sources of contamination.

Arsenic in the atmosphere can be found associated with both gaseous and particulate phases (Mandal and Suzuki, 2002; Tsopelas et al. 2008), and are important for study due to the potential hazards they can cause to the environment and to human health. Few studies have investigated specific arsenic species occurring in atmospheric particulate matter (Lewis et al, 2012; Sanchez-Rodas et al. 2012; Godelitsas et al. 2011; Sanchez de la Campa et al. 2008; Tsopelas et al. 2008; Sanchez-Rodas et al. 2007; Oliveira et al. 2005; Huggins et al. 2004; Farinha et al. 2004). More commonly, research on arsenic in PM has focused on determining total elemental concentrations, which does not give information on the chemical speciation

which can determine toxicity (Niyobuhungiro et al. 2013; Chang et al. 2008; Karthikeyan et al. 2006; Utsunomiya et al. 2004; Wang et al. 1997).

Most studies suggest that the arsenic is associated with the finer fraction of PM in both urban and industrial samples (Sanchez-Rodas et al. 2012; Sanchez de la Campa et al. 2008; Tsopelas et al. 2008; Chang et al. 2008; Seinfeld and Pandis, 2006; Utsunomiya et al. 2004; Farinha et al. 2004). Table 1.1 shows various metals and metalloids that are associated with particulate matter, and the mode they are correlated with.

Table 1.1: Trace element concentrations and associated mode, F (fine) or C (coarse), in atmospheric particulate matter (adapted from Seinfeld and Pandis, 2006).

Element	Mode ^a	Concentration (ng m ⁻³)		
		Remote	Rural	Urban
Fe	F and C	0.6–4,200	55–14,500	130–13,800
Pb	F	0.01–65	2–1,700	30–90,000
Zn	F	0.03–450	10–400	15–8,000
Cd	F	0.01–1	0.4–1,000	0.2–7,000
As	F	0.01–2	1–28	2–2,500
V	F and C	0.01–15	3–100	1–1,500
Cu	F and C	0.03–15	3–300	3–5,000
Mn	F and C	0.01–15	4–100	4–500
Hg	—	0.01–1	0.05–160	1–500
Ni	F and C	0.01–60	1–80	1–300
Sb	F	0–1	0.5–7	0.5–150
Cr	F and C	0.01–10	1–50	2–150
Co	F and C	0–1	0.1–10	0.2–100
Se	F and C	0.01–0.2	0.01–30	0.2–30

^aF = fine mode; C = coarse mode.

Two studies performed by Sanchez-Rodas and others and Sanchez de la Campa and others focused on comparing the two most significant size fractions of PM (PM₁₀ and PM_{2.5}), and speciation of arsenic to determine which contains the most

arsenic and which contains more reduced, toxic As(III) (Sanchez-Rodas et al. 2012; Sanchez de la Campa et al. 2008; Sanchez-Rodas et al. 2007). In their findings, arsenic was always more closely associated with the finer fraction ($PM_{2.5}$) as compared to the coarse fraction (PM_{10}), at roughly around 85%. In addition, these studies were able to determine that As(V) and As(III) concentrations were more pronounced in the finer fraction at 81% and 71%, respectively. However, this does not negate the importance of the coarse fraction, which still contained significant levels of inorganic arsenic. It is also important to point out that these studies were performed on urban PM and were influenced by many environmental factors. Currently, there is very limited species specific research being performed on agriculturally derived PM.

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Chapter 2

THE CHEMICAL AND MORPHOLOGICAL CHARACTERIZATION OF PARTICULATE MATTER FROM A DELMARVA BROILER OPERATION

2.1 Abstract

Chemical and morphological characterization of particulate matter from a broiler poultry operation on Delmarva can provide essential information on the composition that will aide in understanding the potential health risks posed by individuals working in, and living near these facilities. In this study, time-integrated PM₁₀ and PM_{2.5} samples were collected inside and outside of a Delmarva poultry operation, and the elemental composition and morphology were investigated. The trace elements of most interest were As, Fe, Zn, Mn, Cu and P because of their use in the poultry industry and their effects on the environment. Arsenic was further highlighted in this research because of its potential toxicity and negative effects on health. Statistical analysis was implemented for examining variability in PM trace metal composition. Results show that season does not significantly affect metal(loid) concentrations in both PM₁₀ and PM_{2.5}, except in the late summer season where F, Zn, P, and As are higher in PM_{2.5}. Of the two locations sampled, indoor samples were mainly found to contain As levels above background. Of the 39 samples collected for each size fraction, 31% of PM₁₀ and 26% of PM_{2.5} samples from inside were above the background levels for As, and 0% of PM₁₀ and only 15% of PM_{2.5} samples from outside were above background.

Morphological and single-particle information was performed using SEM-EDX. Results reveal that larger particles within PM₁₀ tend to be agglomerated spheres, where spherical particles in PM_{2.5} tend to remain separated. Information obtained from EDX indicated that PM samples do have similar compositions and contain Cl, Ca, K, C, O, Na, Mg, P, S, which are commonly found in feed, fecal, and skin particles.

2.2 Introduction

As air pollution rates rise worldwide, so do the concerns regarding the implications this will have on the environment and human health. Over the past few decades, studies have focused on understanding how emissions (i.e. aerosols, particulates, etc.) from industrial, commercial (i.e. car exhaust), and, to a lesser extent, agricultural outlets are affecting the environment as well as human health (Gurjar et al, 2010). Gaining a detailed understanding of the chemical and morphological characterization of these emitted materials can help determine their toxicity; thereby, leading to a better understanding of the potential hazards that are present during exposure, and potentially leading to better policy and regulations.

Airborne particulate matter (PM) is defined as any solid and/or liquid material that has become re-suspended or generated from the upward movement of air (Sienfeld and Pandis, 2006, EPA, 2013a). Particulate matter can be constituted of many agglomerated materials from both primary and secondary sources. The materials can vary from crustal metals, trace elements, inorganic ions, and biological and carbonaceous components, and can come from both natural (volcanoes, erosion) and anthropogenic activity, such as the dust associated with animal feeding operations.

Because of the many source types and materials that make up PM, it can vary in complexity and stability.

Agriculturally derived particulate matter generated from livestock and poultry operations has become an increasing concern to farmers, workers and surrounding communities. Because of population growth and an increase in commercial and residential building within close proximity to these facilities, it is important to understand the characteristics of the particles being generated, which can have an effect on human health and the environment.

Presently there are four features of PM that are of most concern; these include PM concentration, size, chemical, and biological composition. Particle concentrations from animal feeding operations can vary depending on animal class, type of housing and ventilation, bedding and feed material, and environmental factors such as temperature and relative humidity, and can be relatively high both inside and outside of the facility (Jager, Msc dissertation, 2005). Another concern is the chemical and biological composition of poultry PM. Animal farming can generate particles that carry many components, including soil particles, bedding debris, fecal matter, litter material, feed, bacteria, fungi and viruses (EPA, 2013b; Grubb et al, 1965). In addition, these particles may also contain high levels of ash, nitrogen, calcium, iron, zinc, copper, arsenic, manganese, magnesium, and/or aluminum (Ellen, et al., 2000; Nakaue, et al., 1981). Many of these components have the potential to become hazardous and can lead to environmental contamination and to human health problems.

The materials associated with PM from these agricultural operations should be considered in order to understand the toxicity. PM from a poultry operation can be

made up of various elemental, biological, and gaseous components, many of which can become hazardous if inhaled or ingested at low and high concentrations over extended durations of exposure. For instance, organo-arsenicals, such as Roxarsone (ROX), have been utilized for decades in order to control coccidiosis, promote growth and improve pigmentation (Silbergeld and Nachman 2008). The significance of supplementing these into feed is how toxic they can become once they have been excreted. Research has shown that the majority of the 23-45 grams per ton of organo-arsenic that is fed to the birds is released almost exclusively in its original form (Sierra-Alvarez et al 2010; Ewall, 2007). The litter materials have been shown to contain between 35-50 mg/kg (ppm) of arsenic (Bolan et al 2010, Ewall, 2007; Garbarino et al 2003). However, other studies have looked at the biotransformation of the organo-arsenical roxarsone in poultry litter and determined that as a result of anaerobic microbial activity and chemical processes that the ROX can transform into substituent organo-arsenic species, as well as methylated and inorganic forms, which are more toxic (Sierra-Alvarez et al 2010; Seiter, 2009; Garbarino et al 2003; Rutherford et al 2003). In addition, other metals such as Zn, Cu, and Mn can also be a concern and can potentially become harmful if inhaled at high levels (ATSDR, 2012; ATSDR, 2005; ATSDR, 2004). These metals are primarily used in feed mineral supplements to improve feed efficiency and health, and to promote growth. However, they too can be excreted and can accumulate in the litter material, which can then become re-suspended into the air and can bind to particulate matter where it can then be inhaled.

Current regulations have been set mainly for inorganic As species, despite some research showing that even methylated and organic species can potentially affect

health (Gomez-Caminero et al 2001). The current workplace standards set for inorganic As are 10 $\mu\text{g}/\text{m}^3$ PEL from OSHA, 10 $\mu\text{g}/\text{m}^3$ TLV from the The American Conference of Governmental Industrial Hygienists (ACGIH) and 2 $\mu\text{g}/\text{m}^3$ set by the National Institute for Occupational Safety and Health (NIOSH) (OSHA, 2008; ACGIH, 2005; NIOSH, 2005). Other occupational standards set forth by OSHA and NIOSH are for Mn compounds (as Mn) (5 mg/m^3 and 1 mg/m^3 PEL), Cu compounds (as Cu) (1 mg/m^3 PEL), and Zn (as Zn oxide) (5 mg/m^3 and 15 mg/m^3 PEL for respirable and total dust, respectively) (ATSDR, 2012; ATSDR, 2005; ATSDR, 2004; OSHA, 1993).

Currently, there is a lack in environmental standards set by the EPA for arsenic, zinc, and copper in ambient air, despite the fact that EPA considers these under the Clean Air Act as hazardous air pollutants, and have been shown to increase mortality and cause serious illness after significant exposure (EPA, 2007). However, the EPA does currently have some regulations with regard to airborne pollutants including Mn (0.05 $\mu\text{g}/\text{m}^3$), Ni (0.1-0.2 $\mu\text{g}/\text{m}^3$), and Cr^{6+} (0.008-0.1 $\mu\text{g}/\text{m}^3$) (EPA, 2005; EPA, 1993). The values represent inhalation reference concentrations (RfC), which is an estimate of a daily inhalation exposure of the human population (including sensitive subgroups like the chronically ill and elderly) that likely will not cause risk of harmful effects over a lifetime.

Size is also a significant characteristic of PM; since it determines how a particle behaves in air, it has generally been used as a means of determining the location of deposition within the respiratory tract, and the potential development of human health problems. There are two primary categories of PM based on aerodynamic diameter; these are “coarse particles”, particles with an aerodynamic

diameter less than 10 μm and larger than 2.5 μm (PM_{10} - $\text{PM}_{2.5}$), and “fine particles” which are 2.5 μm and smaller ($\text{PM}_{2.5}$) (EPA 2013a). In addition, PM_{10} is typically characterized as particles that are 10 μm and below, and are significant in terms of their effects on the environment and human health. The deposition of these particles into the respiratory system depends solely on their size. For example, particles in the 10 μm and below fraction can travel throughout the respiratory system and can affect the nose, throat and enter into the lungs where it can then begin influencing the inflammatory response mechanisms within the body (Yatera et al, 2007; Soukup and Becker, 2001; Imrich et al. 2000; Li et al. 1997). Furthermore, fine and ultrafine particles, those less than 2.5 μm , can embed deep into the lung tissues affecting the bronchioles and alveolar sacs, and can pass through the gas exchange region entering other systems within the body, including the cardiovascular system (Brook et al. 2004; Samet et al. 2000; Holgate et al. 1999). Size can also be used to determine deposition in the environment. Larger particles tend to have much shorter drift periods than smaller, lighter particles, which can drift far from originating sources. Because these particles can contain many types of contaminants, and have the potential for long range drifting, it is important to investigate and understand the characteristics including, size, concentration, chemical composition, and morphology of PM from a broiler poultry operation. This information can then be used to determine their impacts to health, air and environmental quality.

To our knowledge there is a gap in cohesive data collected on PM from a broiler operation on Delmarva which encompasses chemical and morphological characterization for both PM_{10} and $\text{PM}_{2.5}$. Reported data on PM derived from poultry operations have been focused on single characteristics such as indoor PM levels,

emissions (PM and gases), chemical characterization (of PM_{2.5}), and biological characterization, and have not looked at a combination of both the chemical and morphological characterization of PM₁₀ and PM_{2.5} from indoor and outdoor sampling locations (Li et al. 2011; Yang et al. 2011; Roumeliotis et al. 2010; Oppliger et al. 2008; Fabri et al. 2007; Wang et al. 2007; Roumeliotis et al. 2007; Visser et al. 2006; Lim et al. 2003; Hinz and Linke, 1998).

2.2.1 Objectives and Focus

The objectives of this study are: 1) to identify and compare chemical composition of PM₁₀ and PM_{2.5} from both locations, 2) to utilize microscopic methods to investigate morphology (size, shape) of PM.

2.3 Experimental Methods And Materials

2.3.1 Sampling Site

PM_{2.5} and PM₁₀ samples were collected from the University of Delaware research poultry house. The UD poultry facility houses ~ 2500 birds per flock. This location was chosen due to ease of access. In all cases permission to gain access to perform research on a commercial poultry farm were denied. The University of Delaware's 36' x 44' broiler house is managed following typical industry specifications, and is suitable as a representative poultry operation for this research. The half curtain wall of the house is equipped with a Choretronics CT2 controller (Chore-time poultry production systems, Milford, IN), 2 Choretronics weigh scales, a Bintrac Pro Loadster 4.5 T bin weigh scale system, 2 radiant tube propane heaters, misters, attic vents, 2 x 30" exhaust fans, 2 x 18" stir fans, and 1 x 48" summer mixing fan. Electric, propane, feed, and water are monitored electronically. Birds are raised

on existing sawdust litter, (last change out was in November 2009), with a cake out or removal of crusted material, between flocks; new shavings are added for each flock. In addition to temperature and relative humidity measurements from the CT2 there are also 5 Hobo U12 and U23 (Onset, Cape Cod, MA) temperature and relative humidity sensors distributed throughout the house. An experimental machine vision camera system including surveillance camera and novel National Instruments based data collection software is used to collect images of bird activity. Figure 2.1 shows a schematic diagram of the structural layout of the sampling site.

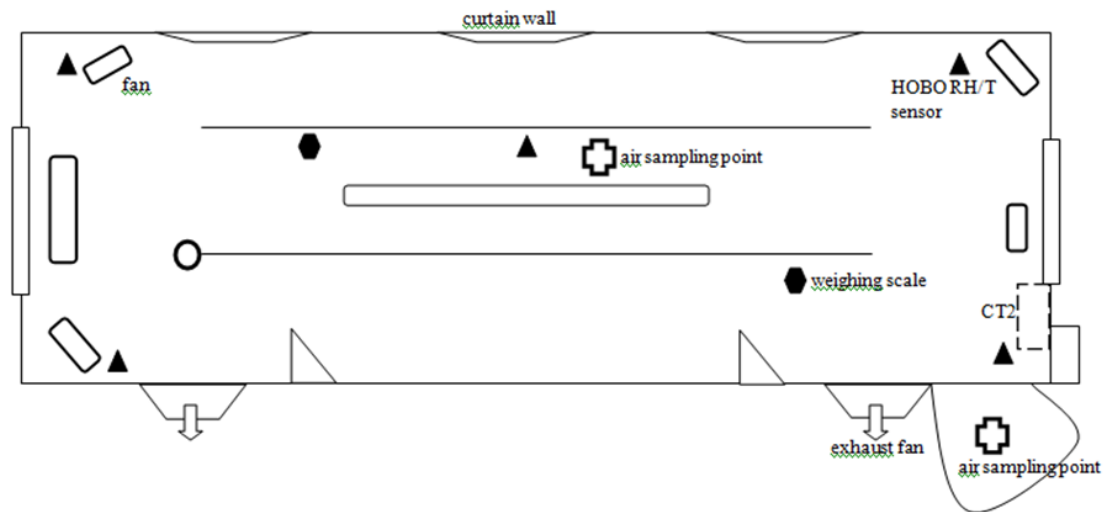


Figure 2.1: Schematic representation of sampling site.

2.3.2 Air Sampling

During the period between 6/2011 and 2/2012, particulate matter samples from a Delmarva poultry operation for four different seasonal periods were collected (Table 2.1).

Table 2.1: Sampling and growout cycle (in parentheses) dates and corresponding season when samples were taken.

Sampling Dates (growout cycle dates)	Season
June 15th – July 13th, 2011 (6/5/11-7/15/11)	Early summer
Aug. 10th – Sept. 9th, 2011 (8/5/11-9/11/11)	Late summer
Oct. 5th – Nov. 8th, 2011 (9/29/11-11/8/11)	Fall
Jan. 3rd – Feb. 9th, 2012 (1/10/12-2/13/12)	Winter

Prior to sampling, 25 mm and 37 mm Teflon filters were pre-weighed in a temperature and humidity controlled weighing room after equilibrating for ≥ 24 hours using a Mettler T5 microbalance with precision of ± 0.003 mg (Mettler-Toledo, Toledo, OH); this was performed at Johns Hopkins Bloomberg School of Public Health (JHU). A total of 2 samples per sampling location for each size fraction, PM_{10} and $PM_{2.5}$, were collected once a week over the 42 day growout cycle for each of the sampling seasons. In addition, feed samples were collected during a separate sampling period during a 56 day growout cycle; these samples represented the four types of feed that can be given during a growout cycle of that length.

The process of sampling took approximately two days total. This process included preparation of sampling units (ie, cleaning) and pre and post calibration of all airflow equipment, which was supplied by the Environmental Health Sciences department at JHU. The methods of sampling used to perform this research were the personal environmental monitor samplers (PEMS) (SKC, inc., Eighty Four, PA), which collected PM_{10} ; defined as particles from 10 μm and below, and the personal

micro-environmental aerosol speciation sampler (PMASS) (MSP corporation, Shoreview, MN) which collects fine particles, defined as particles $2.5\ \mu\text{m}$ and below. PM_{10} samples were collected using a $2.0\ \mu\text{m}$ pore size, 37mm Teflon filter with a PTFE support ring (Pall Life Sciences, Ann Arbor, MI), which requires a single pump at a target flow rate of 4L/min (Figure 2.2a and 2.2b).



Figure 2.2 PEM



PMASS

The PMASS includes a single size selective inlet with a cut size of $2.5\ \mu\text{m}$ at a target flow rate of 4L/min, and has two parallel sampling channels. A $3.0\ \mu\text{m}$ pore size, 25 mm Teflon filter with a PTFE support ring (Pall Life Sciences, Ann Arbor, MI) was placed in each channel of the PMASS. The target flow rate of 4L/min and is internally split to 2L/min through each channel, and calibrated individually. The flow rates were calibrated using a flow meter (Dry DC-Lite & DC-2, BIOS, Butler, NJ). A Side-by-side rotameter was used to determine the proportional flow through each filter within the PMASS. Samples were collected for 24, 8, and 6 hour periods, and were collected both inside (centrally located) and outside (adjacent from exhaust fan) the poultry facility. Blanks and duplicates were collected for quality control purposes. Upon

collecting the samples they were placed under nitrogen and shipped to JHU where they were equilibrated in a temperature and humidity controlled weighing room and then post weighed using the Mettler T5 microbalance to get the mass of the collected particulate matter. Teflon filters were chosen because they are non-hygroscopic, which allows for more precise measurements of mass difference by limiting the effect of humidity on the actual mass of the filter. In addition, these filters were used because they have 99.9% collection efficiency, and they are generally free of background trace metals.

2.3.3 Sampler Setup

The PMASS and PEM units were attached to a stand that elevated the samples to an approximate height of 4 feet (1.2 m) off the ground. The stand had been equipped with loops that allowed the units to hang facing various directions in the house. The inside setup was placed in the center of the poultry house where the units were equally exposed to the suspended material, and away from exhaust and mixing fans. The outside setup was located approximately 3 feet (~1.0 m) from the poultry house adjacent to an exterior exhaust fan (Figures 2.3).



Figure 2.3: Images of indoor (left) and outdoor (right) sampler setup.

The outside units were covered with a plastic bin to protect them from rain, and prevent any disruption or blockage of air flow. The sampling vacuum pumps, both inside and outside of the house, were placed in a covered box, which provided them with added protection.

2.3.4 Microwave Acid Dissolution and ICP-MS Analysis

A set of samples for both PM₁₀ and PM_{2.5}, including blanks and duplicates, were used to look at total elemental concentrations of As, Fe, Mn, Ni, Zn, Ca, K, Cu, Mg, Al, and P. A microwave-digestion and acid dissolution procedure adapted from Han et al (2012) for the Department of Environmental Health Sciences at Johns Hopkins Bloomberg School of Public Health were employed; this included the use of nitric acid (HNO₃) and optima grade hydrofluoric acid (HF) (Fisher Scientific, Columbia, MD) (Kulkarni et al., 2003). Samples were acid digested using a Mars5

Xpress microwave system (CEM, Matthews, NC). Prior to digestion, the polyolefin outer support ring was removed from the Teflon filters using a Lucite template, which matched the circumferences of the 25mm and 37mm filters. The filter membrane was transferred to a 7-mL Teflon digestion microwave vessel (CEM) where it was wetted with 100 μ L of ethanol, 160 μ L of ultrapure water (Millipore, Billerica, MA), and 1.35 mL of concentrated optima grade nitric acid (HNO_3) (Fisher Scientific, Columbia, MD). The sample was digested using a two-stage ramp-up to temperature method with a maximum temperature of 165 $^{\circ}\text{C}$ and a hold time of 30 min. Following the initial digestion period, 300 μ L of concentrated optima HNO_3 and 55 μ L of 28M optima grade HF acid (Fisher Scientific) were added and a second digestion was performed according to the same ramp-up method used in the previous step. Following the second digestion, the Teflon membrane was removed and the sample was diluted for metals analysis to 1mL. To each sample, 25 μ L of an internal standard, 50 mg/L Li, Ge, Sc, Tb, Bi, Y, In (CPI International, Santa Rosa, CA), was added to each sample to monitor for instrument drift over analysis time. For every batch of samples being analyzed, 3 samples of the National Institutes of Standards and Technologies (NIST) standard reference material 1648a Urban Particulate Matter (National Institutes of Standards and Technologies, Rockville, MD) and reagent blanks were digested and analyzed for quality control. Total metals analysis was performed using an Agilent 7500ce ICP-MS (Agilent Technologies, Santa Clara, CA). The analytical limit of detection (LOD), calculated as 3 times the standard deviation of the lowest detectable calibration standard (1 mg/L), was determined for each metal analyzed. For samples with values that were below the analytical LOD, which are ~ 0.0068 for As, 0.0033 for Mn, 0.0068 for Cu, and 0.0142 for Zn, $\frac{1}{2}$ LOD values were

substituted in calculations and were used in statistical analyses. The ICP-MS is equipped with an octipole reaction system (CRS), which reduces interferences, which could influence results.

Samples for microwave acid dissolution and ICP-MS for all seasons were collected throughout the period of a year ($N_{\text{total}}\text{PM}_{10}=46$; $N_{\text{total}}\text{PM}_{2.5}=46$; N includes blanks and duplicates). Feed samples were taken for each of the four types (starter, grower, finisher, withdraw), used during a separate 7 week sampling period which occurred during the spring season ($N=4$). Because of limitations with equipment during the regular sampling period, background sampling took place during a separate seasonal sampling period. The background samples were collected once a week over a five week period ($N_{\text{total}}\text{PM}_{10}=13$; $N_{\text{total}}\text{PM}_{2.5}=15$). The background samples were used to primarily determine ambient levels of arsenic.

2.3.5 Microscopy

Microscopic methods were used to look at structural characteristics of the PM (ie morphology, size) (Sielicki et al, 2011). Scanning electron microscopy (Hitachi S4700 Scanning Electron Microscope, Hitachi High-Technologies America, Inc., Clarksburg, MD), coupled with energy dispersive spectroscopy (SEM-EDX) performed at the Delaware Biotechnology Institute (DBI), Newark, DE, allowed semi-quantification of major elements present on the filtered samples to be elucidated, which was used as a secondary approach to the ICP-MS, and also provided a view of the morphology of the PM samples (size, shape). Samples were prepared initially by carbon coating a portion of the filtered material, and were mounted on carbon coated aluminum plates; this is done because of non-conductivity of the samples. The SEM operates similarly to x-ray fluorescence (XRF) and must be calibrated at an energy

level befitting of imaging and identifying elements of interest; in this case the samples were scanned at 15 to 20 keV, in order to excite any As atoms if present.

Also, transmission electron microscopy, coupled with electron energy loss spectroscopy and energy dispersive spectroscopy (STEM/EELS/EDS), performed at the High Resolution Microbeam Facility of the Integrated Imaging Center (HRMFIIC) at The Johns Hopkins University (JHU), was used to look at atomic composition of single particles, as well as give insight on valence state of metals associated with single particles. PM deposited on filters were scraped off with a scalpel into a reservoir of water and then pipetted into a 5 ml glass bottle. The suspension was placed in an ultrasonic bath for 3 mins. A lacey-carbon TEM grid (SPI, inc., West Chester, PA) was dipped into the suspension and allowed to dry. The samples were examined in a Philips CM 300 FEG transmission electron microscope (Philips Innovative Services, Netherlands) operating at 297 kV. Images were collected on a Gatan 1k x 1k CCD camera (Gatan, Inc., Warrendale, PA) using Digital Micrograph software. The energy-dispersive X-ray (EDX) spectra were collected using an Oxford light element detector (Oxford Instruments, Scotts Valley, CA) and an Emispec multichannel analyzer using ES Vision 4 software. Care was taken in analyzing individual particles to ensure that As was or was not present. The presence of Mg and Pb hindered the identification of As since the As L_{α} and As K_{α} X-ray lines overlap with the Mg K_{α} and Pb L_{α} 1 lines, respectively. When Mg and Pb were present, only the As K_{β} line is free from interference. This information was then used as a comparison with the data obtained from the microprobe beamline at the National Synchrotron Light Source (refer to Chapter 4).

Confocal microscopy (Zeiss 510 NLO multiphoton microscope, Carl Zeiss Microscopy, LLC., Thornwood, NY), performed at DBI, Newark, DE, was used to investigate the relationship between particle and microorganisms present. Through syto-13 staining one can detect nucleic acids associated with biological organisms present. These studies were conducted at the University of Delaware Bioimaging Facility (Delaware Biotechnology Institute, Newark, DE).

2.3.6 Statistical Analysis

Concentrations of metal(loid)s are reported as raw data. One-way ANOVA analysis and t-tests were performed using JMP statistical software (SAS Institute Inc., Cary, NC) to determine the significance of location and season on concentrations of metal(loid)s of interest (As, Cu, Mn, Zn, Fe and P) found in PM₁₀ and PM_{2.5}.

2.4 Results and Discussion

2.4.1 Total Trace Metal Composition

The following elements were identified using ICP-MS: As, Cu, Mn, Zn, Fe and P. These elements were chosen based on their prevalence in the environment and potential impacts on human health problems, as well as, their frequency of use in feed and supplements in the poultry industry. The results show that concentrations for the elements analyzed are higher, and significantly different, for indoor PM₁₀ samples compared with the outdoor PM₁₀ samples, and that concentrations are generally insignificant between locations for PM_{2.5} (Table 2.2) (Appendix A.1).

Table 2.2: P-values^a and t-ratio's of the statistical comparison of metal(loid) concentrations in PM₁₀ and PM_{2.5} for each sampling location.

Metal(loid)	IN vs OUT		t-values	
	PM ₁₀	PM _{2.5}	PM ₁₀	PM _{2.5}
As	< 0.0001 ^a	< 0.2187	-5.38	-0.79
Fe	< 0.0078 ^a	< 0.1019	-2.54	-1.29
Zn	< 0.0073 ^a	< 0.1328	-2.57	-1.13
Mn	< 0.0001 ^a	< 0.4388	-4.84	-0.16
Cu	< 0.0001 ^a	< 0.1067	-8.10	-1.27
P	< 0.0001 ^a	< 0.1572	-5.01	-1.02

^a A difference with p-value ≤ 0.05 is considered significant.

The 24 hour adjusted mean concentrations for Mn, Cu and As for indoors ranged from 0.0-0.15 $\mu\text{g}/\text{m}^3$ for PM_{2.5} and 0.0-0.5 $\mu\text{g}/\text{m}^3$ for PM₁₀. The concentrations for As were lower than the 10 $\mu\text{g}/\text{m}^3$ regulated workplace limits set by OSHA and 2 $\mu\text{g}/\text{m}^3$ from NIOSH. In addition, indoor concentrations for other metal(loid)s such as Mn and Cu were also found to be lower than the regulated workplace limits set by OSHA and NIOSH. Outdoor concentrations ranged from 0.0-0.26 $\mu\text{g}/\text{m}^3$ in PM_{2.5} and 0.0-0.03 $\mu\text{g}/\text{m}^3$ in PM₁₀. Mean manganese concentrations were above the EPA's regulated limit of 0.05 $\mu\text{g}/\text{m}^3$ for inhalable Mn compounds during the late summer season in outdoor PM_{2.5} samples at 0.26 $\mu\text{g}/\text{m}^3$. Currently, the EPA does not have regulated inhalation limits for As or Cu in air. Mean concentrations were highest overall in PM₁₀ and PM_{2.5} for Fe, Zn and P. The mean indoor concentrations ranged from 0.01-50.1 $\mu\text{g}/\text{m}^3$ in PM_{2.5} and 0.1-23.1 $\mu\text{g}/\text{m}^3$ in PM₁₀; outdoor concentrations ranged from 0.0-16.0 in PM_{2.5} and 0.0-1.2 in PM₁₀ (Figures 2.4 and 2.5). Zinc concentrations for both PM₁₀ and PM_{2.5} samples from inside were below the implemented exposure limits set by OSHA and NIOSH, and is not regulated by the

EPA in outdoor air. Tables 2.3 and 2.4 show a comparison of metal(loid) concentration ranges and the regulated exposure limits of OSHA, NIOSH, and EPA.

Table 2.3: Concentration ranges for As, Zn, Mn, and Cu in PM₁₀ for both indoor and outdoor sampling locations, and regulated exposure limits of OSHA, NIOSH, and EPA

Metal(loid)	PM ₁₀			Regulation		
	IN	OUT				
	Concentration Range (µg/m ³) ^a					
As	0.0-0.05	0.0-0.003		10 µg/m ³	2 µg/m ³	4.3 ug/m ^{3***}
Zn	0.1-0.7	0.0-0.1		5 mg/m ³ (respirable)*	5 mg/m ³ (respirable)*	N/A
				15 mg/m ³ (TSP)*	15 mg/m ³ (TSP)*	
Mn	0.2-0.5	0.0-0.1		5 mg/m ³	1 mg/m ³	0.05 µg/m ^{3c}
Cu	0.2-0.3	0.01-0.03		1 mg/m ³	1 mg/m3	N/A

^{*} standards are set for Zn (as Zn oxide)

^{**} ATSDR, 2004; ATSDR, 2005; ATSDR, 2012; OSHA, 2008; EPA, 2007

^{***} inhalation unit risk estimate

^a pre-background removal concentrations

^b permissible exposure limits

^c inhalation reference concentrations

Table 2.4: Concentration ranges for As, Zn, Mn, and Cu in PM_{2.5} for both indoor and outdoor sampling locations, and regulated exposure limits of OSHA, NIOSH, and EPA

Metal(loid)	PM _{2.5}		Regulation		
	IN	OUT			
	Concentration Range (µg/m ³) ^a		OSHA ^{b**}	NIOSH ^{b**}	EPA ^{**}
As	0.0-0.07	0.0-0.04	10 µg/m ³	2 µg/m ³	4.3 µg/m ^{3***}
Zn	0.1-1.13	0.0-0.72	5 mg/m ³ (respirable) [*]	5 mg/m ³ (respirable) [*]	N/A
			15 mg/m ³ (TSP) [*]	15 mg/m ³ (TSP) [*]	
Mn	0.02-0.15	0.01-0.26	5 mg/m ³	1 mg/m ³	0.05 µg/m ^{3c}
Cu	0.05-0.11	0.0-0.08	1 mg/m ³	1 mg/m ³	N/A

* standards are set for Zn (as Zn oxide)

** ATSDR, 2004; ATSDR, 2005; ATSDR, 2012; OSHA, 2008; EPA, 2007

*** inhalation unit risk estimate

^a pre-background removal concentrations

^b permissible exposure limits

^c inhalation reference concentrations

There was no significant difference between season and metal(loid) concentrations in PM₁₀ for both sampling locations; however, in PM_{2.5} samples metal(loid) concentrations during the late summer were significantly different when compared to other seasons for Fe, Zn, P and As at $\alpha = 0.1$ p-values ranged from 0.02-0.1. Average concentrations indicate that metal(loid)s are more closely associated with PM_{2.5}, except in Mn where values are slightly higher in indoor PM₁₀ samples. This is in agreement with previously documented results where many trace elements have been found to be more closely associated with the finer fraction (Sanchez-Rodas et al. 2012; Tsopelas et al. 2008; Cheng et al. 2008; Sanchez de la Campa et al. 2008; Seinfeld and Pandis, 2006; Utsunomiya et al. 2004; Farinha et al. 2004). The mean background levels of As, Mn, Cu, and Zn over the five week sampling period in PM₁₀ (N=13) were 0.014 µg/m³ SD of 0.03, 0.038 µg/m³ SD of 0.08, 0.008 µg/m³ SD of 0.14, and 1.04 µg/m³ SD of 3.50, respectively. In PM_{2.5} (N=15) the background levels were

0.006 $\mu\text{g}/\text{m}^3$ SD of 0.01, 0.042 $\mu\text{g}/\text{m}^3$ SD of 0.00, 0.042 $\mu\text{g}/\text{m}^3$ SD of 0.07, and 0.958 $\mu\text{g}/\text{m}^3$ SD of 2.24, respectively. Because many of the samples for background were determined to have concentrations below the limit of detection for ICP-MS, which is ~ 0.0068 for As, 0.0033 for Mn, 0.0068 for Cu, and 0.0142 for Zn, some values were calculated using $\frac{1}{2}$ LOD at 0.003, 0.002, 0.003, and 0.007, respectively. Upon applying background subtractions to the sample values established for mean arsenic in PM_{10} and $\text{PM}_{2.5}$ for both locations, it was found that 16 of the 39 or 41% of samples used in the calculations for $\text{PM}_{2.5}$ remained positive for As, and 12 out of 39 or 31% of samples were positive for As in PM_{10} . There were no positive values for As in outdoor PM_{10} upon background subtraction, and only 6 of the 16 samples that were positive for As in $\text{PM}_{2.5}$ were from outdoor samples. Other metals that had values above background levels in both PM_{10} and $\text{PM}_{2.5}$ included Mn with 59% of the total evaluated samples ($n=39$) in PM_{10} and 15% in $\text{PM}_{2.5}$ ($n=39$), Cu with 85% of the total ($n=39$) in PM_{10} and 34% in $\text{PM}_{2.5}$ ($n=39$), and to a lesser extent Zn with 5% of the total ($n=39$) in both PM_{10} and $\text{PM}_{2.5}$ ($n=39$). Blanks were analyzed using the same method for quality control, and were determined to be clean with regard to the elements of interest. Any impurities on the blank filters were subtracted out as background.

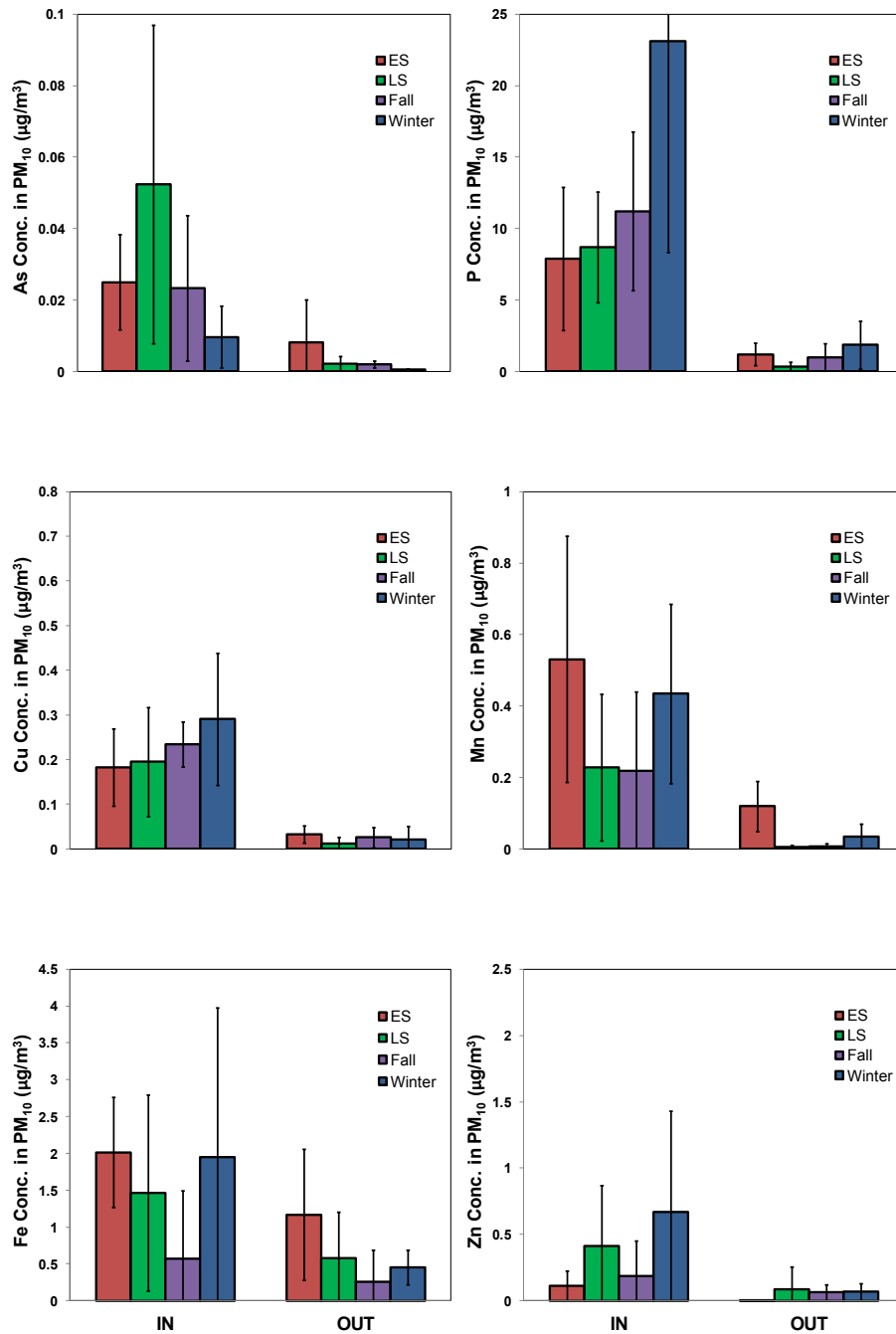


Figure 2.4: Concentrations of total metal(loid)s (As, P, Cu, Mn, Fe, Zn) in PM₁₀, indoor versus outdoor samples. *Note: Error bars represent standard deviations.*

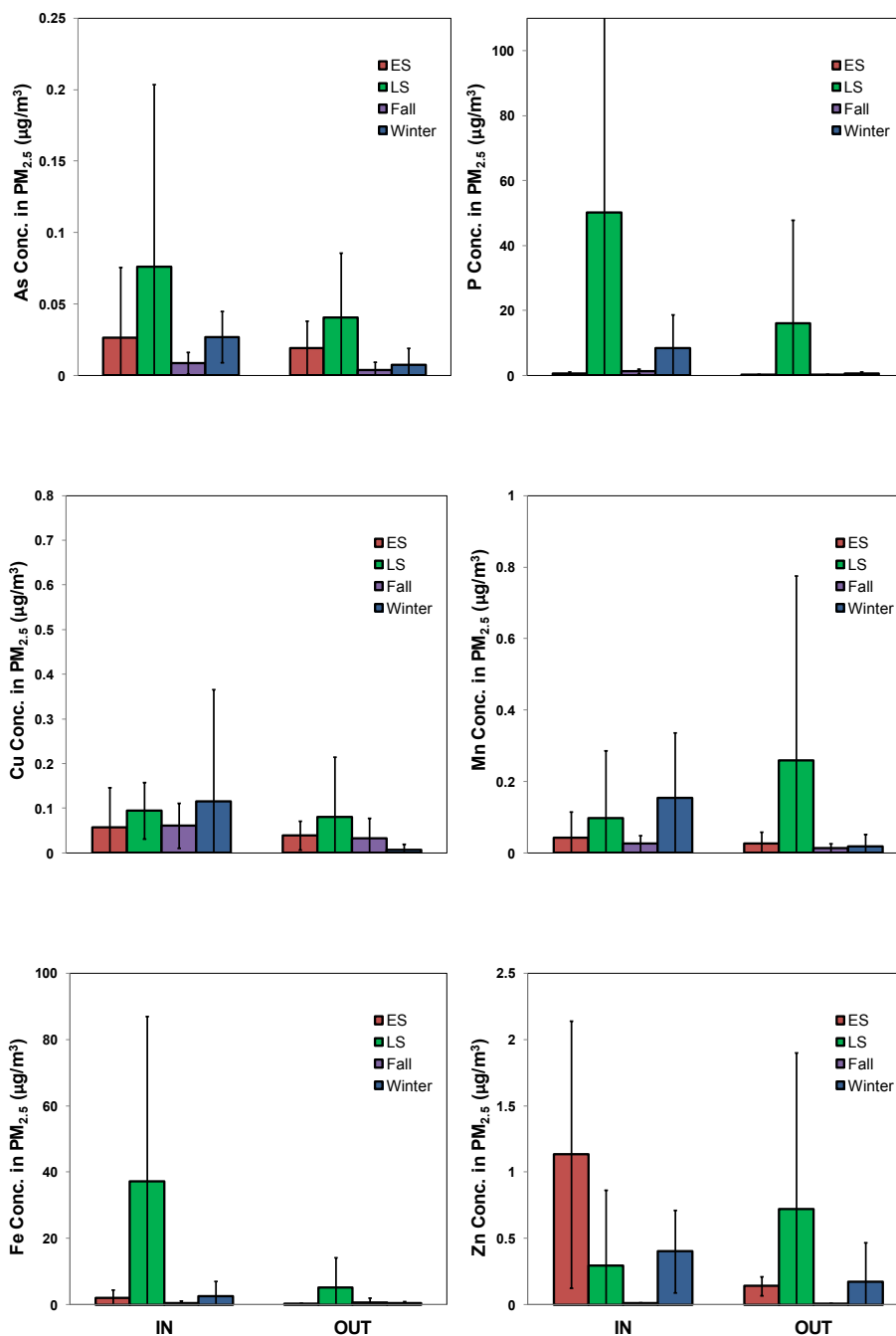


Figure 2.5: Concentrations of total metal(loid)s (As, P, Cu, Mn, Fe, Zn) in $PM_{2.5}$, indoor versus outdoor samples. *Note: Error bars represent standard deviations.*

Mass concentrations of As, Cu, Mn, Zn, Fe, and P were determined for four feed samples from a separate sampling period which occurred during the spring. All feed types were used during production including, starter, grower, finisher and withdraw (Table 2.5). Arsenic, P, and Fe concentrations were highest in starter and finisher feeds whereas Cu, Mn and Zn had higher concentrations in grower feed, and in feed sampled during the finisher/withdraw period (Table 2.5). The variation in concentrations for these elements is likely due to the changes in the content of the supplemental mineral mix added to the feed.

Table 2.5: Mass concentrations of trace elements in feed samples (units: $\mu\text{g/g}$).

	STARTER	GROWER	FINISHER	FINISHER/WITHDRAW
As	12.4	1.7	14.4	1.3
P	7317.2	5131.8	6759.2	5818.2
Cu	169.5	151.8	115.5	6.7
Mn	144.8	52.2	104.7	178.2
Fe	369.2	82.8	133.0	129.2
Zn	220.6	117.7	161.9	179.7

In addition, a major focus of the analysis was to determine the relationship between arsenic found in feed and in indoor PM_{10} and $\text{PM}_{2.5}$ samples. The results found in Figures 2.6 (A) and (B) shows that there is a consistent trend between the As found in feed and in $\text{PM}_{2.5}$ throughout the weeks where feed was sampled; whereas, in PM_{10} there is a pattern through week 3, then it appears to shift opposite by weeks 4

and 6. A number of internal factors, including bird activity, RH, ventilation rate and temperature can affect the composition of the PM being generated and what elements are associated with them. In addition, there can be an overlap in time between change out of feed (seen in gray). These factors are likely why the shift in As in PM₁₀ and As in feed is seen.

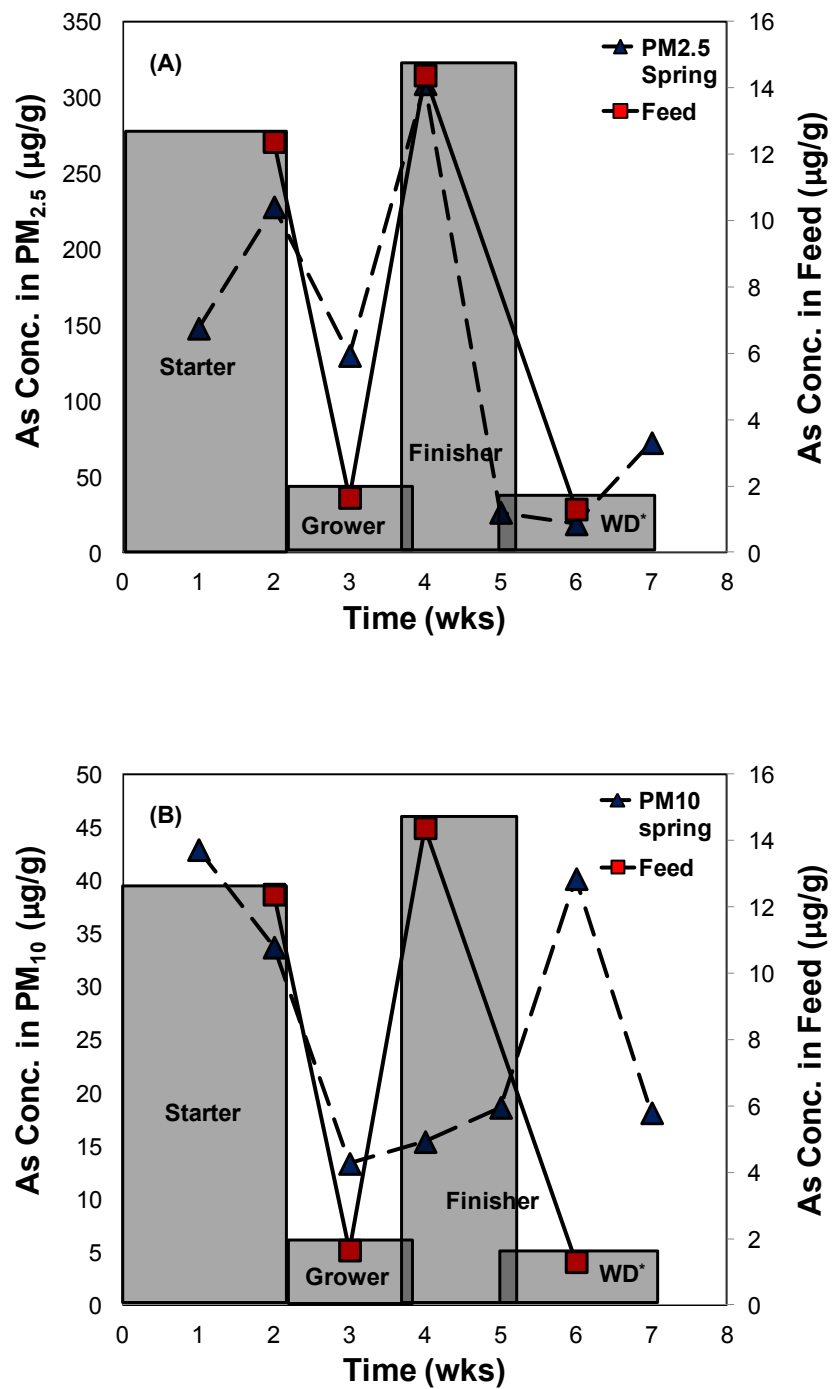


Figure 2.6: Arsenic concentrations of indoor PM_{2.5} (A) and PM₁₀ (B) versus As concentrations in feed recorded over a 7 week period. Areas in gray represent the relative length of time each feed type was given.
**Withdraw(WD)*

2.4.2 Microscopic Analysis of Particulate Matter

Morphological data was collected on feed particles ($N=2$), litter ($N=2$), and both PM_{10} ($N=3$) and $PM_{2.5}$ ($N=2$) from indoor and outdoor sampling locations using scanning electron microscopy (SEM). Multiple areas of interest were investigated for each sample ($N_{PM10}=56$, $N_{PM2.5}=25$, $N_{feed}=11$, $N_{litter}=6$). In addition, Basic chemical information was obtained using the SEM coupled with energy dispersive x-ray spectroscopy (EDX). Again multiple EDX spectra were collected for each area of interest for the samples investigated. Feed, litter and the indoor PM_{10} sample investigated had similar elemental composition according to the chemical spectra from EDX (Figures 2.7-2.9).

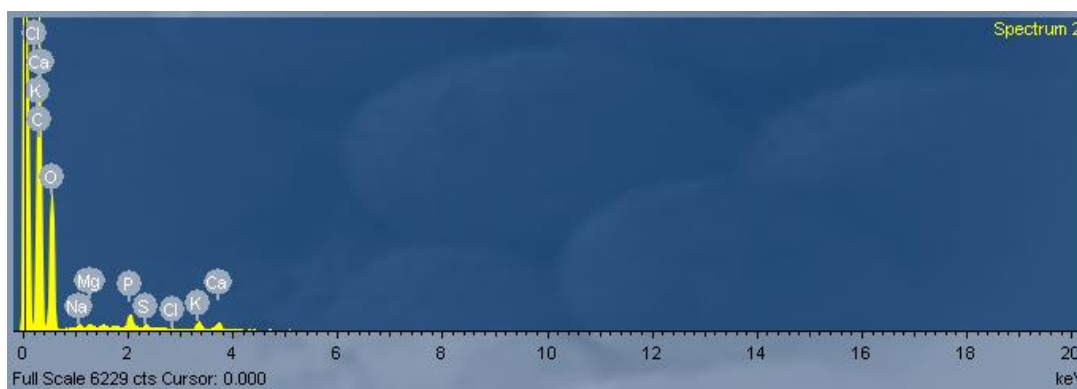
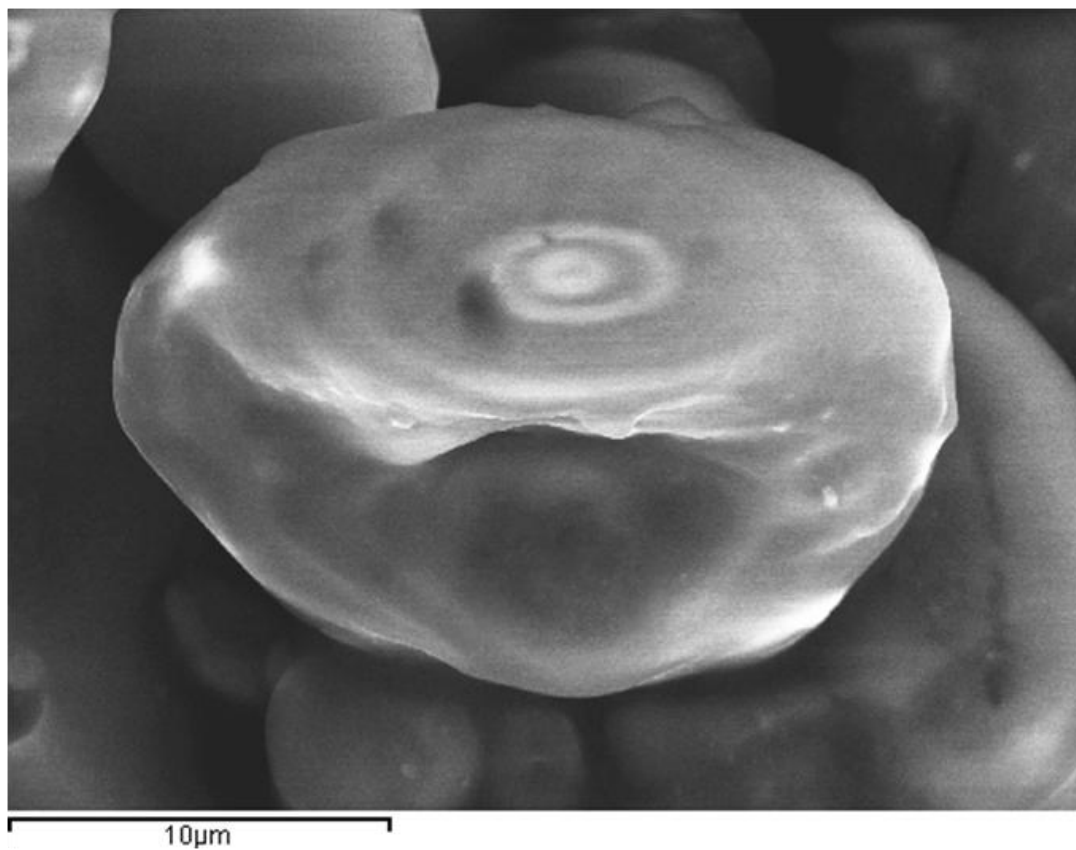


Figure 2.7: Scanning electron microscopy image of feed sample (top), and corresponding elemental spectra (Cl, Ca, K, C, O, Na, Mg, P, S) provided by energy dispersive x-ray analysis (EDX) (bottom). *Note: elemental analysis was performed but was unable to detect trace elements such as As, Fe, Mn, Zn and Cu.*

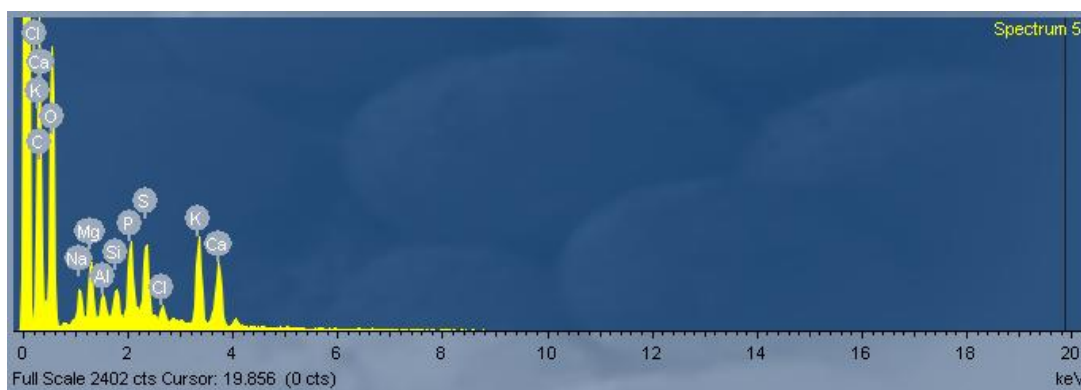
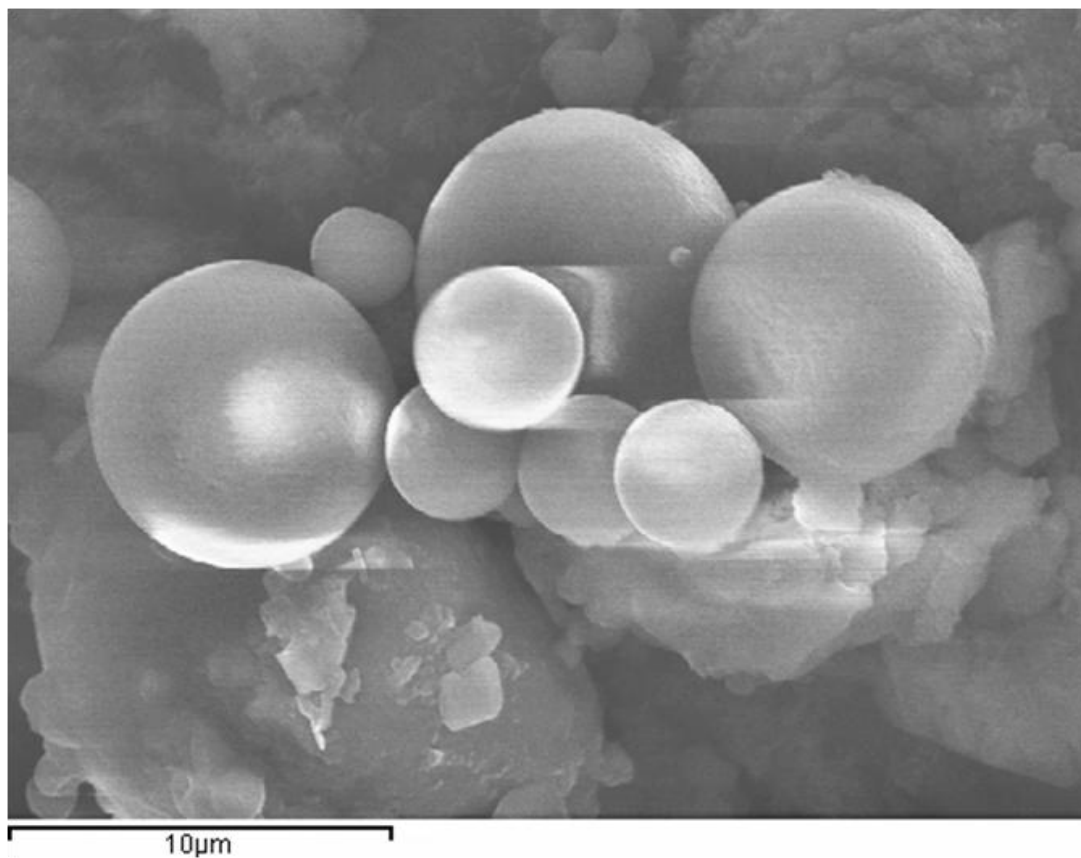


Figure 2.8: Scanning electron microscopy image of litter sample (top), and corresponding elemental spectra (Cl, Ca, K, O, C, Na, Mg, Al, Si, P, S) provided by energy dispersive x-ray analysis (EDX) (bottom).

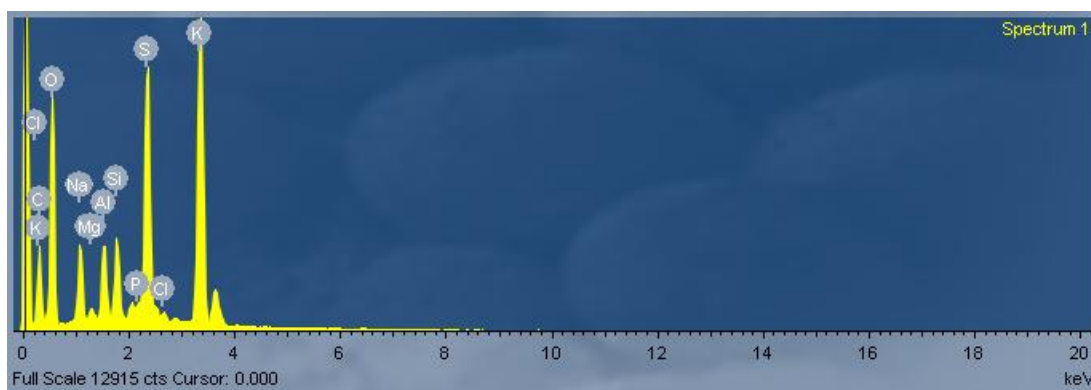
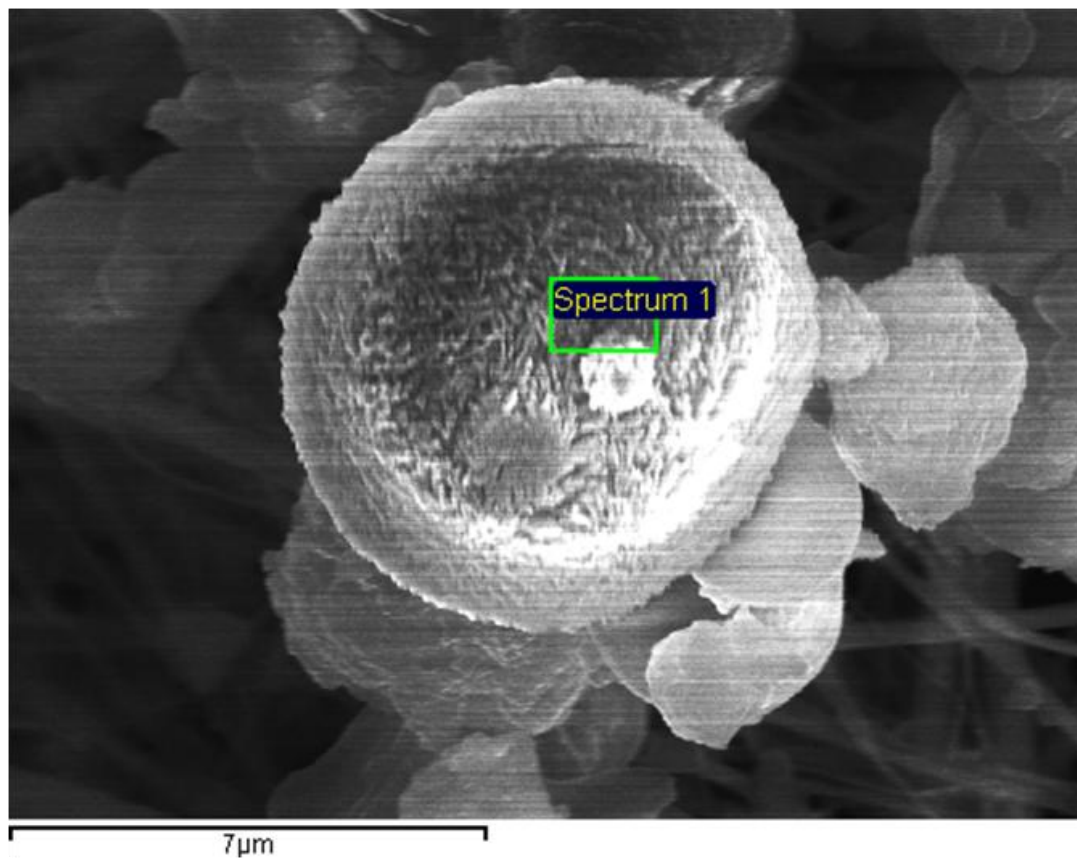


Figure 2.9: Scanning electron microscopy image of PM₁₀ sample (top), and corresponding elemental spectra (Cl, K, O, C, Na, Mg, Al, Si, P, S) provided by energy dispersive x-ray analysis (EDX) (bottom).

The major elements present from multiple scans performed on the samples are Cl, Ca, K, C, O, Na, Mg, P, S, which are commonly found in the internal environment of the poultry house, and are in feed supplements and in fecal and skin particles (Cambra-Lopez et al, 2010; Cambra-Lopez and Torres, 2008; Aarnink et al, 1999). The major difference between the feed spectra, and the litter and PM₁₀ sample, is the presence of Al and Si. These elements may be present in bedding material or as a result of external flow of air from open ventilation during the summer months when these samples were collected.

The morphological data indicated that the majority of PM₁₀ samples are made of agglomerated clusters of single particles, where the PM_{2.5} samples show more particles as single spheres (Figures 2.10A-D). This information may indicate that the PM₁₀ represented here are aged particles and may have been influenced by gases and other particles in the air before collection occurred, and are typically composed of fecal materials (Cambra-Lopez et al, 2011).

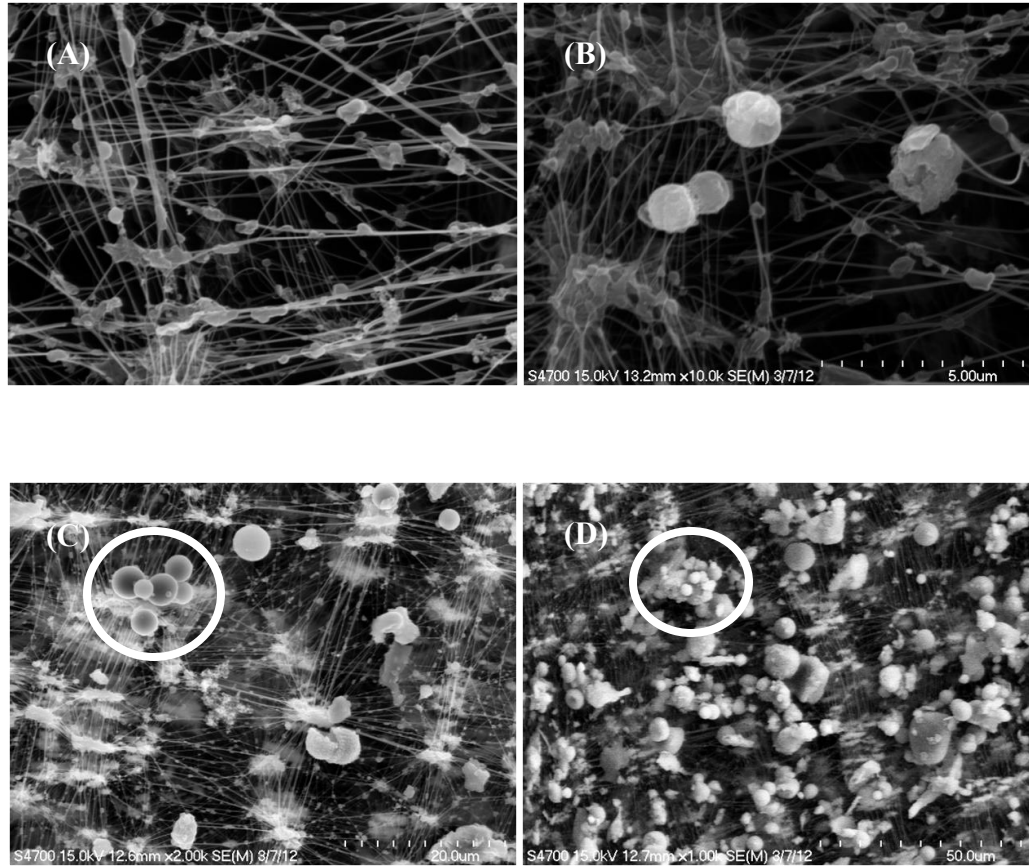


Figure 2.10: Scanning electron microscopy images of blank Teflon filter (A), outdoor PM_{2.5} (B), outdoor PM₁₀ (C) and indoor PM₁₀ (D). Images of PM₁₀ show the formation of agglomerated clusters for indoor and outdoor samples (highlighted in white). Note: the scales for the images are 5.0μm (A,B), 20.0μm (C) and 50.0μm(D).

These agglomerated formations have been identified in urban systems where combustion sources are nearby, and have also been seen in source apportionment studies on broiler poultry houses (Cambra-Lopez et al, 2011a; Cambra-Lopez et al, 2011b; Cambra-Lopez et al, 2010; Dye et al, 2000). In addition, particles were consistently represented as either spherical or flattened-platy type, which have been

associated with fecal and woody litter materials (Figure 2.10A-D) (Cambra-Lopez et al, 2011).

Confocal microscopy, with syto-13 staining, was used to identify the extent at which microorganisms are found to be associated with PM (Appendix A.2). The syto-13 staining showed that the prevalence of nucleic acids was much greater for indoor samples than for outdoor samples. In addition, the indoor PM₁₀ image shows nucleic acid residues encompassing clusters of particulate matter; however, the outdoor PM₁₀ image shows that these residues are independent of the particulates. A number of species of microorganisms are commonly found in poultry operations and have been known to cause health problems in both human and animals, including mycoplasma, staphylococcus, mycobacterium and various spore producing fungi (Just et al, 2011; Oppliger et al, 2008; Lee et al, 2006; Poultry disease Network, 2006). Because of time limitations more extensive investigation of the species of microorganisms and their physical and chemical interactions could not be performed on the sampled particulate matter. However, because microorganisms play a significant role in chemical transformation and in the development of human and animal health problems, it is an area that has had very little exploration and should be further investigated.

Samples used for TEM were mounted two ways to identify which method would be suitable for EELS analysis. The result revealed that microtoming the samples caused degassing of the particulates and thus was unsuccessful in determining any elemental information. However, when PM was removed from the filter and directly mounted onto a grid surface, samples were able to be analyzed successfully (Appendix A.3). The information obtained indicated that the majority of the material

is comprised of elements including, C, O, Mg, sometimes Al, Si, P, S, Cl, K, Ca and Fe. Some images show structures primarily comprised of Fe and O, and were needle-like in morphology which could indicate the presence of iron oxy(hydroxides) (i.e. goethite). Because of the limited number of samples that were able to be analyzed the TEM analyses are inconclusive. However, the information obtained from TEM analysis is in agreement with SEM-EDS analysis.

2.5 Conclusions

The detailed information obtained on morphology and chemical composition were carried out on particulate matter from a Delmarva broiler poultry operation using a modified HF dissolution method and ICP-MS, along with SEM. It has been shown that season does not significantly affect metal(loid) concentrations in both PM_{10} and $PM_{2.5}$, except in the late summer season where Fe, Zn, P, and As are higher in $PM_{2.5}$. In addition, metal(loid)s are more closely associated with $PM_{2.5}$, which is in agreement with previously documented results where trace metals were found to be associated with the fine particles (Sanchez-Rodas et al. 2012; Tsopelas et al. 2008; Cheng et al. 2008; Sanchez de la Campa et al. 2008; Seinfeld and Pandis, 2006; Utsunomiya et al. 2004; Farinha et al. 2004). When background ambient As was subtracted out from indoor and outdoor sample concentrations, ~60% of $PM_{2.5}$ samples and ~70% of PM_{10} samples were at background levels, indicating that the majority of As present in the samples is related to the background ambient levels present in the environment. The vast majority of the samples that remained above background levels of As were for indoor PM samples. When analysis was run on feed samples, the concentrations of As found in $PM_{2.5}$ over the 7 week sampling period were consistent to the trend of As concentrations found in the feed types during the same period of time they were being

given. However, As in PM₁₀ and feed were opposite during weeks 4, 5, and 6. This may be attributed to other influences, including management practices, bird activity, and overlap period in feed change out that would influence the presence of As in PM₁₀ during that period.

Morphological and single-particle information from SEM showed that larger particles within PM₁₀ tend to be agglomerated spheres, where spherical particles in PM_{2.5} tend to remain separated. The agglomeration of larger spherical particles may be indicative of aged particles that have been influenced by other atmospheric particles and gases and are generally found to be composed of mostly organic fecal material (Cambra-Lopez et al, 2011). In addition, elemental analysis performed on both PM fractions, feed, and litter were done in order to back up information obtained from ICP-MS. Because of limitations from EDX, trace elements like As, Zn and Fe were not identified in any of the samples. However, the information obtained did indicate that these samples do have similar compositions and contain Cl, Ca, K, C, O, Na, Mg, P, S, which are commonly found in feed and fecal and skin particles (Cambra-Lopez et al, 2010; Cambra-Lopez and Torres, 2008; Aarnink et al, 1999). The exception to this was in feed where Al and Si were also present.

In the current study it was demonstrated that elemental composition of PM from a broiler poultry operation on Delmarva are characteristically generated from feed and litter materials; the majority being associated with PM_{2.5}. The concentrations of As in outdoor PM samples taken at this research site show that the majority of As is within background ambient levels, and are not likely associated with poultry production practices.

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Chapter 3

TEMPORAL AND SPATIAL CHARACTERIZATION OF PARTICULATE MATTER FROM A DELMARVA BROILER OPERATION

3.1 Abstract

Agriculture has long been associated with the environment through the various activities and practices that are customary during production of crops, livestock and poultry farming. The generation of pollutants such as gases and particulate matter (PM) are inevitable. In the past, research has primarily focused on gas emissions and nutrient runoff from fertilization, and the ways in which to mitigate these important issues. However, little research has been documented on the characterization of the temporal and spatial variability of PM from broiler operations on the Delmarva Peninsula. Gaining a detailed understanding on the variability will allow for better policy and regulations on emissions from these operations, and will likely help determine the impacts it can have on the surrounding environment and on human and animal health.

The research has focused on understanding how environmental and operational factors affect PM_{10} and $PM_{2.5}$ concentrations on both temporal and spatial scales. Two types of integrated sampling devices, the PEM and PMASS, were used to collect size segregated filtered samples of PM_{10} and $PM_{2.5}$. These devices were chosen for sampling because of their ability to precisely collect size segregated PM. Also, because the PM was collected on a filtered surface this allowed for flexibility in doing multiple analyses.

The collection of indoor and outdoor environmental factors, such as temperature and relative humidity, were collected using data retrieved from the Delaware Environmental Observation System's (DEOS) weather station and HOBO data loggers. Bird weight was determined using scale measurements over the sampled time period.

Results from the collection of integrated samples of PM₁₀ and PM_{2.5} from indoor and outdoor locations of the broiler poultry operation indicate that concentrations of PM were 10 times higher over a 24 hr period inside than on the outside of the poultry house. Seasonal influence and bird weight were the main factors affecting concentrations of PM. Mean concentrations of PM₁₀ and PM_{2.5} were higher during the winter for samples collected inside the poultry facility, but were higher during the summer for those samples collected outside of the facility. It also appears that as bird weight increased so too did the concentrations of particulate matter generated within the facility.

3.2 Introduction

Because of their classification as a contributing source of atmospheric particulate matter (PM), animal feeding operations (AFO's) (NRC, 2003; NRC, 2002), in particular broiler poultry facilities have become more scrutinized by local, state and government agencies. Population growth and a rise in commercial and residential development within close proximity to these facilities, which have changed over the past few decades to include more animals in confinement, have facilitated this increase in concern.

Particulate matter is currently a criteria pollutant defined by the United States Environmental Protection Agency's (USEPA) National Ambient Air Quality

Standards (NAAQS). Concentration and size are the most important physical characteristics of PM, and generate the most concern because of their defined roles and influence in the development of human and animal health issues, and environmental impacts (van de Hooven et al, 2012; Pavlik et al. 2011; Simkhovich et al. 2008; Valvanidis et al. 2008; Pope and Dockery, 2006; Rylander et al, 2006; WHO, 2006; Pope et al, 2004; WHO, 2003; Pope et al, 2002; Samet et al, 2000; Dejmek et al. 1999).

Both PM₁₀ and PM_{2.5} are currently regulated under the National Ambient Air Quality Standards (NAAQS) (EPA, 2012), and have been classified as influential on human health. The current NAAQS regulations are 35 ug/m³ for PM_{2.5} and 150 ug/m³ for PM₁₀ in a 24hr period respectively. Because the EPA doesn't set standards based on occupational environment, and are instead directed towards the general population, and base its criteria on those who are at most risk like the elderly or young these regulations are not always suitable for comparison to those found within a poultry facility. Although OSHA considers workplace exposure, the same unsuitability can be said for the standards that are set when comparing them to indoor PM levels for a poultry operation. OSHA currently regulates particles within the total dust or total suspended particle (TSP) range, which is a reference for any airborne particle, as well as, the respirable fraction, which consists of those particles less than 4µm. These regulations only apply to "inert" or nuisance dusts; these terms refer to particles that essentially have no harmful effect and are not classified under Table Z of their airborne contaminant limits (OSHA, 1988). However, this can be misleading since PM within the 10-2.5 µm size range and below have been extensively studied and shown to contribute towards long term effects on health. The current standards are 15

mg/m³ and 5 mg/m³, respectively; these are given as 8 hour time weighted averages (TWA's). Neither of these regulations are suitable for determining exposure limits for those individuals working inside of a poultry operation; however, they are the most widely recognized and utilized regulations for comparison, and for determining whether a problem exists in the occupational environment in question.

In addition, the PM generated from litter, feed, skin, and feathers may carry potentially hazardous materials such as metals, microorganisms, and gaseous components, which can be emitted from these facilities and are not considered in the regulations set by either EPA or OSHA (EPA, 2013a; Arslan et al, 2012; Ad Hoc Committee on Air Emissions from Animal Feeding Operations; Committee on Animal Nutrition, National Research Council, 2003; Donham et al., 2002; OSHA, 1988; Grubb et al, 1965).

Although there has been significant research conducted on PM emissions and transport from poultry and livestock facilities (Roumeliotis et al. 2010; Vanderstraeten, et al. 2008; Oppliger et al. 2008; Hartung et al. 2007; Roumeliotis and Van Heyst, 2007; O'Connor et al. 2005; Ritz et al. 2004), there are still gaps in research that specifically address spatial and temporal variability of size selective PM₁₀ and PM_{2.5} concentrations generated and emitted from broiler poultry facilities (Wang-Li et al. 2013; Roumeliotis et al. 2010; Visser et al. 2006). In addition, because of the dynamic differences in many factors including, sampling methodology, housing systems, management and operational practices, and geographical location, published data is highly variable (Cambra-Lopez et al. 2010; Aarnink and Ellen, 2008; Redwine et al. 2002; Ellen et al. 2000; Hinz and Linke, 1998). The information gained from understanding the impacts these differences have on PM concentration

not only are valuable for understanding its impacts on the surrounding environment, but also on the health implications posed on individuals living near these facilities, and to the individuals working within them. In addition, better PM regulations that address the unique occupational environment of confined animal feeding operations (CAFO's), such as a broiler poultry operation, may need to be re-evaluated for better comparison and thus a more realistic evaluation of occupational risk.

3.2.1 Objectives and Focus

To our knowledge there is a gap in cohesive data collected on the spatial and temporal variability of size selective PM concentrations generated and emitted from a broiler operation on Delmarva. Therefore, this project aimed to address this gap through the following objectives:

1. Determine concentrations of PM_{10} and $PM_{2.5}$ generated within and emitted from an mechanically ventilated broiler poultry operation on Delmarva using a time-integrated sampling approach.
2. Investigate major factors that influence PM_{10} and $PM_{2.5}$ concentrations generated within and emitted from the facility. These factors include animal weight, ventilation, spatial and temporal (weekly, seasonal) variations.

3.3 Experimental Methods and Materials

3.3.1 Particulate Matter Sampling

Between 6/2011 and 2/2012, particulate matter samples were collected from a Delmarva poultry operation over four separate growout cycles, which corresponded to

four seasonal periods. Prior to sampling, 25 mm and 37 mm Teflon filters were pre-weighed in a temperature and humidity controlled weighing room after equilibrating for ≥ 24 hours using a Mettler T5 microbalance with precision of ± 0.003 mg (Mettler-Toledo, Toledo, OH); this was performed at Johns Hopkins Bloomberg School of Public Health (JHU). Samples were collected weekly during each cycle which ranged from 56 to 42 days. The process of sampling took approximately two days total. This process included preparation of sampling units (ie, cleaning) and pre and post calibration of all airflow equipment, which was supplied by the Environmental Health Sciences department at JHU. The methods of sampling used to perform this research were the personal environmental monitor samplers (PEMS), which collected PM_{10} (SKC, Inc., Eighty Four, PA); defined as particles from 10 microns and below, and the personal micro-environmental aerosol speciation sampler (PMASS) collects fine particulates, which is defined as particles 2.5 microns and below (MSP Corporation, Shoreview, MN). PEM samples (cut size of 10 μ m at 4L/min) were collected onto a 2.0 μ m pore size, 37mm Teflon filter with a PTFE support ring (Pall Life Sciences, Ann Arbor, MI), which required a single pump at a target flow rate of 4L/min. The PMASS includes a single size selective inlet with a cut size of 2.5 μ m at a target flow rate of 4L/min, and has two parallel sampling channels. A 3.0 μ m pore size, 25 mm Teflon filter with a PTFE support ring (Pall Life Sciences, Ann Arbor, MI) was placed in both channels of the PMASS. The target flow rate of 4L/min and is internally split to 2L/min through each channel, and calibrated individually. The flow rates were calibrated using a flow meter (Dry DC-Lite & DC-2, BIOS, Butler, NJ). A Side-by-side rotameter was used to determine the proportional flow through each filter within the PMASS. Samples were collected for 24, 8, and 6 hour periods, and were

collected both inside (centrally located) and outside (adjacent from exhaust fan) the poultry facility. Blanks and duplicates were collected for quality control purposes. Upon collecting the samples they were placed under nitrogen and shipped to JHU where they were equilibrated in a temperature and humidity controlled weighing room and then post weighed using the Mettler T5 microbalance to get the mass balance of the collected particulate matter. Teflon filters were chosen because they are non-hygroscopic, which allows for more precise measurements of mass difference and limits the effect of humidity on the actual mass of the PM. In addition, these filters were used because they have 99.9% collection efficiency. Ambient PM_{10} (N=15) and $PM_{2.5}$ (N=20) background samples were taken on a weekly basis over five weeks during a separate period from the seasonal sampling.

3.3.2 Sampler Setup

PMASS and PEM units were affixed to a stand that elevated the samples to an approximate height of 4 feet (1.2 m) off the ground. The stand had been equipped with loops that allowed the units to hang facing various directions in the house. The inside setup was placed in the center of the poultry house where the units were equally exposed to the suspended material, and away from exhaust and mixing fans.

The outside setup was located approximately 3 feet (~1.0 m) from the poultry house exterior side nearest the exhaust fans. The outside units were covered with a plastic bin to protect them from rain, and prevent any disruption or blockage of air flow. The sampling vacuum pumps, both inside and outside of the house, were placed in a covered box, which had provided them with added protection.

3.3.3 Sampling Location

PM_{2.5} and PM₁₀ samples were collected from the University of Delaware research poultry house. The UD poultry facility houses ~ 2500 birds per flock. This location was chosen due to ease of access. In all cases permission to gain access to perform research on a commercial poultry farm were denied. The University of Delaware's 36' x 44' broiler house is managed following typical industry specifications, and is suitable as a representative poultry operation for this research. The half curtain wall of the house is equipped with a Choretronics CT2 controller (Chore-time Poultry Production Systems, Milford, IN), 2 Choretronics weigh scales, a Bintrac Pro Loadster 4.5 T bin weigh scale system, 2 radiant tube propane heaters, misters, attic vents, 2 x 30" exhaust fans, 2 x 18" stir fans, and 1 x 48" summer mixing fan. Electric, propane, feed, and water are monitored electronically. Birds are raised on existing sawdust litter, (last change out was in November 2009), with a cake out or removal of crusted material, between flocks; new shavings are added for each flock.

3.3.4 Monitoring of Environmental Factors

Weather data was collected using the Delaware Environmental Observation System (DEOS), which has an extensive archive of meteorological data. The DEOS station is located approximately 500 ft from the poultry facility in an open field adjacent to the poultry facility where sampling took place. In-house environmental factors such as temperature and relative humidity were collected from measurements logged from the Choretronics CT2 controller system (Chore-time Poultry Production Systems, Milford, IN); there are also 5 Hobo U12 and U23 (Onset, Cape Cod, MA) temperature and relative humidity sensors distributed throughout the house. Bird weights were measured and logged from 2 choretronics weigh scales during each

sampling period. An experimental machine vision camera system including surveillance camera and novel National Instruments based data collection software is used to collect images of bird activity.

3.3.5 Statistical Analysis

Particulate matter concentrations were reported as raw data in this chapter. However, log transformations of data were required prior to statistical analyses. The following comparisons were made using ANOVA analysis with paired t-tests: PM concentration (PM₁₀, PM_{2.5}) vs location (JMP statistical software, SAS Institute, Inc., Cary, NC). In addition, multivariate analysis with F-tests was run to evaluate the relationship between season, week and location on PM concentrations. Bivariate analyses were used to identify relationships between meteorology factors (T, RH, precipitation, wind speed) and bird weight on PM concentration.

3.4 Results and Discussion

3.4.1 Indoor vs Outdoor PM Concentrations

The concentrations of indoor and outdoor PM₁₀ and PM_{2.5} were determined using mass difference, and then were adjusted to reflect approximate values for a 24 hr sampling period (eqtn 1).

$$\frac{PM \text{ mass } (\frac{\mu g}{m^3}) \times 1440 \text{ mins}}{1440 \text{ mins} - \text{integrated sampling time (mins)}} \quad (eqtn 1)$$

The concentrations for indoor PM₁₀ and PM_{2.5} samples, in many cases, are ~6-10X greater than outdoor samples from the same size fraction, and are similar when comparing PM_{2.5} to PM₁₀ shown in figures 3.1 and 3.2. The range of concentrations

for indoor PM₁₀ samples (N=38) are from 281 µg/m³ to 4721 µg/m³ [avg: 1307 µg/m³; standard deviation (SD): 1013 µg/m³], and 35 µg/m³ to 302 µg/m³ [avg: 123 µg/m³; SD: 76 µg/m³] for outdoor samples (N=38). The accepted units for the evaluation of particulate matter and air pollutant concentrations for indoor and outdoor air quality assessments are in µg (micrograms) per cubic meter (m³). These units are commonly used so that direct relationships can be made between sample concentrations and regulations issued by government agencies such as the EPA, OSHA, and NIOSH. This expression of units for the concentration of PM is based on the mass of contaminant per unit volume of atmospheric air at sea level (eqtn 2 and 3).

$$\frac{\text{Average Flow Rate (L/min)} \times \text{Elapsed Time (mins)}}{1000 \text{ L/m}^3} = \text{Total Volume} \quad (\text{eqtn 2})$$

The denominator represents the conversion factor used to convert liters to m³.

$$\frac{\text{Mass of contaminant (}\mu\text{g)}}{\text{Total Volume (m}^3\text{)}} = \text{PM concentration} \quad (\text{eqtn 3})$$

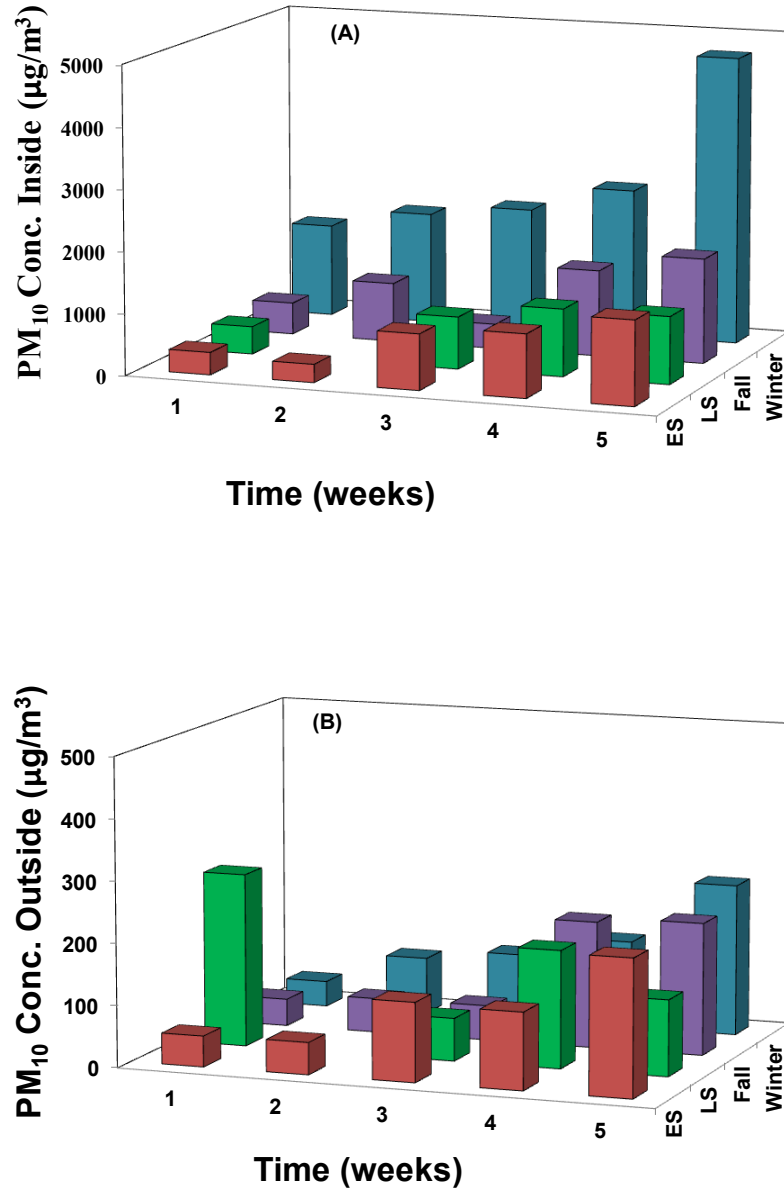


Figure 3.1: The distribution of concentration of particulate matter (PM₁₀) over time (weeks of grow-out cycle) inside (A) and outside (B) of broiler poultry house. *Note: Adjustment to concentrations have been made to reflect approximate values for a 24hr period, which is used for determining air quality standards set by the EPA NAAQS. Scale of y-axis is 10X higher for (A) than (B) due to significant changes in concentration inside versus outside.*

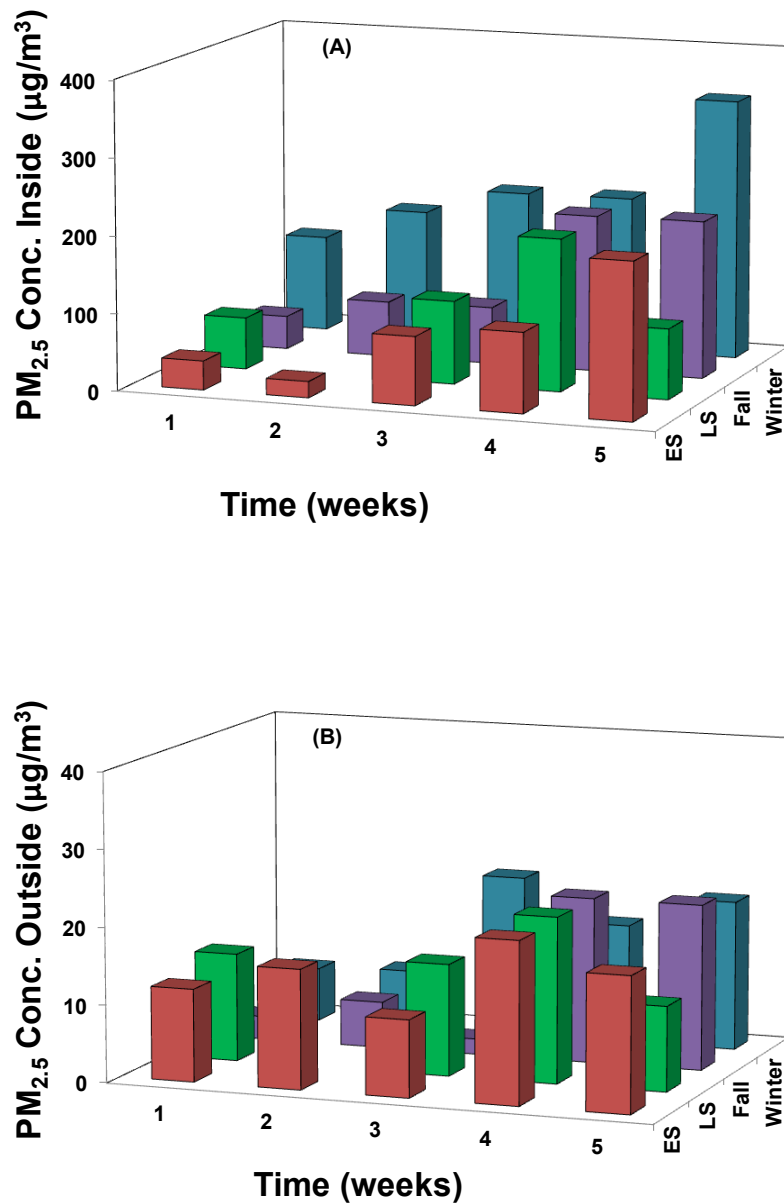


Figure 3.2: The distribution of concentration of particulate matter (PM_{2.5}) over time (weeks of grow-out cycle) inside(A) and outside(B) of broiler poultry house. *Note: Adjustment to concentrations have been made to reflect approximate values for a 24hr period, which is used for determining air quality standards set by the EPA NAAQS. Scale of y-axes are 10X higher for (A) than (B) due to significant changes in concentration inside vs outside.*

Concentrations for indoor PM_{2.5} (N=38) ranged from 20 µg/m³ to 365 µg/m³ [avg: 134 µg/m³; SD: 81 µg/m³], and outdoor samples (N=38) were 0 µg/m³ to 33 µg/m³ [avg: 14 µg/m³; SD: 6 µg/m³]. Mean and standard deviations for each season for indoor and outdoor PM₁₀ and PM_{2.5} samples are listed in Table 3.1.

Table 3.1: Mean and standard deviations for indoor and outdoor PM₁₀ (top) and PM_{2.5} (bottom) concentrations. Values in bold identify the seasons with the highest mean PM concentrations for indoor and outdoor PM₁₀ (top) and PM_{2.5} (bottom). *Note: there was no significant difference between seasons for outdoor PM_{2.5} concentrations $P=0.069$, $\alpha=0.050$.*

Season	PM ₁₀ Indoor (µ/m ³)			PM ₁₀ Outdoor (µ/m ³)		
	(n)	Mean	St dev	(n)	Mean	St dev
Early Summer	10	792	432	10	116	67
Late Summer	8	871	349	8	143	81
Fall	10	994	587	10	115	92
Winter	10	2484	1219	10	127	73

Season	PM _{2.5} Indoor (µ/m ³)			PM _{2.5} Outdoor (µ/m ³)		
	(n)	Mean	St dev	(n)	Mean	St dev
Early Summer	10	91	67	10	15	4
Late Summer	8	116	53	8	15	4
Fall	10	119	75	10	11	10
Winter	10	207	78	10	13	6

Values for duplicates that were greater than 10% different were not used in the calculations. Highest mean concentrations were found in the winter for indoor PM₁₀ and PM_{2.5}, and during the late summer for outdoor PM₁₀; outdoor PM_{2.5} concentrations were not found to be significant across the seasons ($p = 0.069$). The higher concentrations for PM₁₀ and PM_{2.5} can be attributed to their seasonal relationship, which corresponds to ventilation rate changes. During the winter season ventilation rates are generally lower than in the summer; this lessens the potential for PM to be emitted and confines it to the inside of the poultry facility. During the summer higher ventilation rates which creates more airflow, along with increased opening of the end barn doors for added ventilation is likely the reason for higher levels of outdoor PM₁₀ during the summer, in addition to the increased activity during the summer months on the farm (ie tilling, plowing, harvesting) which can generate re-suspended material. This seasonal observation is in agreement with the observations mentioned in literature (Li et al, 2011; Cambra-Lopez et al, 2010 (review); Redwine et al, 2002; Hinz and Linke, 1998). Indoor concentrations can also be influenced by internal temperature, bedding, building age and type, litter moisture and composition, animal activity, and gases such as ammonia (Cambra-Lopez et al, 2010 (review); Banhazi et al. 2008; Roumeliotis and Heyst, 2007; Kaliste et al, 2004). Outdoor concentrations may have also been influenced by outdoor environmental factors such as temperature, RH, wind speed, and precipitation.

When indoor and outdoor mean concentrations are corrected for ambient background levels across all seasons outdoor PM_{2.5} were reduced to 8 $\mu\text{g}/\text{m}^3$ for early summer (ES) and late summer (LS), 4 $\mu\text{g}/\text{m}^3$ for fall (F), and 6 $\mu\text{g}/\text{m}^3$ for winter (W); outdoor PM₁₀ were ~100 $\mu\text{g}/\text{m}^3$ for ES and F, 128 $\mu\text{g}/\text{m}^3$ for LS, and 112 $\mu\text{g}/\text{m}^3$ for W.

Indoor concentrations were as follows for $PM_{2.5}$, 76 $\mu\text{g}/\text{m}^3$ for ES, $\sim 100 \mu\text{g}/\text{m}^3$ for LS and F, and 192 $\mu\text{g}/\text{m}^3$ for W, and for PM_{10} were 777 $\mu\text{g}/\text{m}^3$ for ES, 856 $\mu\text{g}/\text{m}^3$ for LS, 979 $\mu\text{g}/\text{m}^3$ for F, and 2469 $\mu\text{g}/\text{m}^3$ for W. Mean background concentrations were calculated for the whole 5 week period and were 15 $\mu\text{g}/\text{m}^3$ with an SD of 7.0 $\mu\text{g}/\text{m}^3$ for PM_{10} and 7.0 $\mu\text{g}/\text{m}^3$ with an SD of 2.0 $\mu\text{g}/\text{m}^3$ for $PM_{2.5}$, respectively. When evaluating these concentrations the outdoor levels are within the regulation levels set by the EPA's NAAQS for PM_{10} and $PM_{2.5}$. If we compare the indoor concentrations to the limits set by OSHA then PM_{10} , which would fall under TSP, is below the TWA of 15 mg/m^3 , and $PM_{2.5}$, which falls under the respirable fraction, is also below the TWA of 5 mg/m^3 . Again, it is difficult to generalize the PM within the poultry operation and compare them to the classifications and regulations that OSHA has set because the PM is not inert.

In addition to season, weekly relationships were also evaluated and compared to mean PM values for each location (Appendix B). The results of the multivariate analysis indicated that there is a positive relationship between PM_{10} and $PM_{2.5}$ concentrations and week ($p < 0.0001$), which is not a surprise since dust levels have been previously found to increase with bird age and weight (Cambra-Lopez et al, 2010 (review); Aarnink and Ellen, 2008 (review); Roumeliotis and Heyst, 2007; Lacey et al, 2003).

Simple linear regression was applied to indicate the correlations between PM_{10} and $PM_{2.5}$ for each sampling location, between PM_{10} in versus PM_{10} out and the same for $PM_{2.5}$ (Figures 3.3 and 3.4(A) and(B)). The R^2 values for indoor and outdoor locations when comparing PM_{10} concentrations to $PM_{2.5}$ concentrations were 0.80 and 0.44, respectively; these values demonstrate that there is significant correlation

between the two size fractions that were sampled. When comparing each size fraction to location the R^2 values were 0.25 for PM_{10} in vs out and 0.20 for $PM_{2.5}$ in vs out, respectively. These values indicate that there is no significant correlation between the locations. To support this, ANOVA analysis and t-tests were conducted on indoor versus outdoor concentrations for PM_{10} and $PM_{2.5}$; results are shown below in figures 3.5 and 3.6.

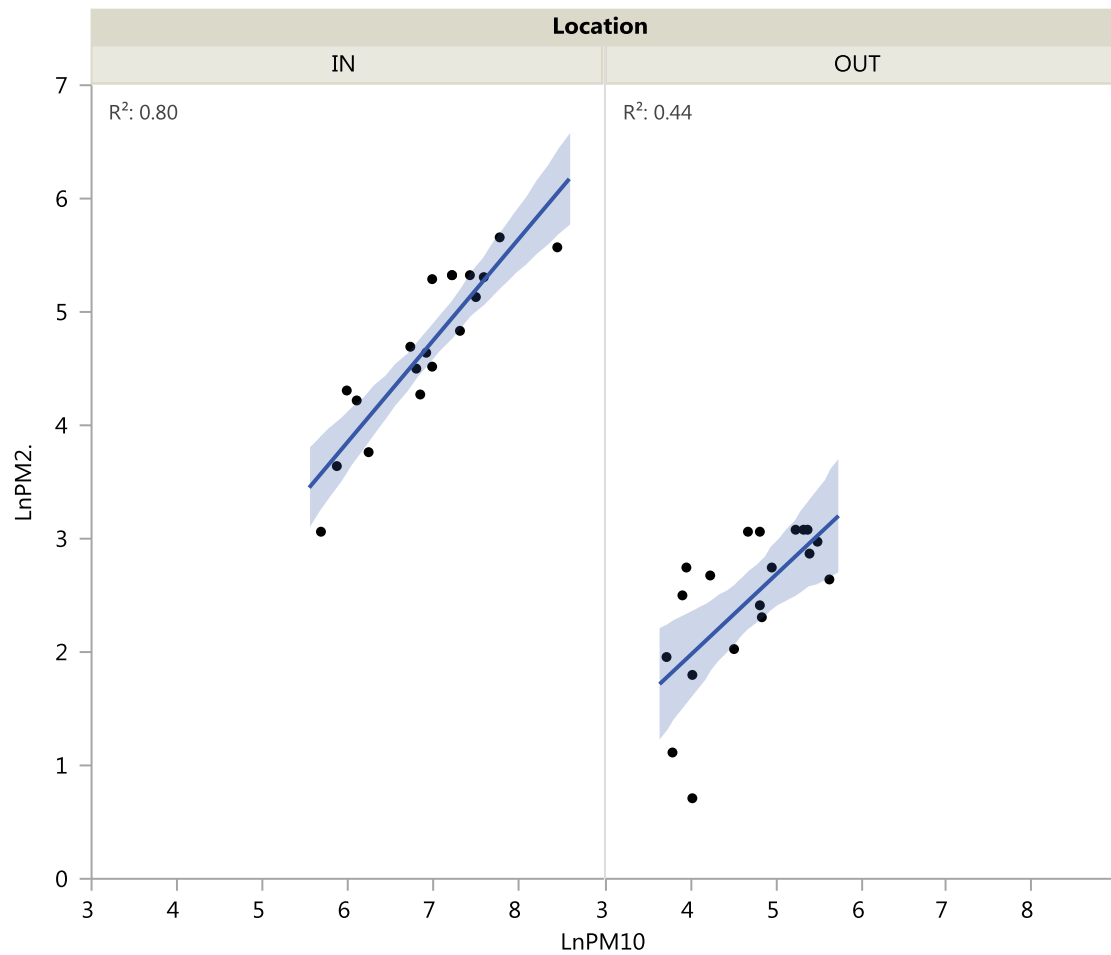


Figure 3.3: Linear regression plots of $PM_{2.5}$ vs PM_{10} for both sampling locations. The R^2 values are 0.80 ($p < 0.0001$) with a correlation of 0.90, and 0.44 ($p = 0.0019$) with a correlation of 0.66, respectively.

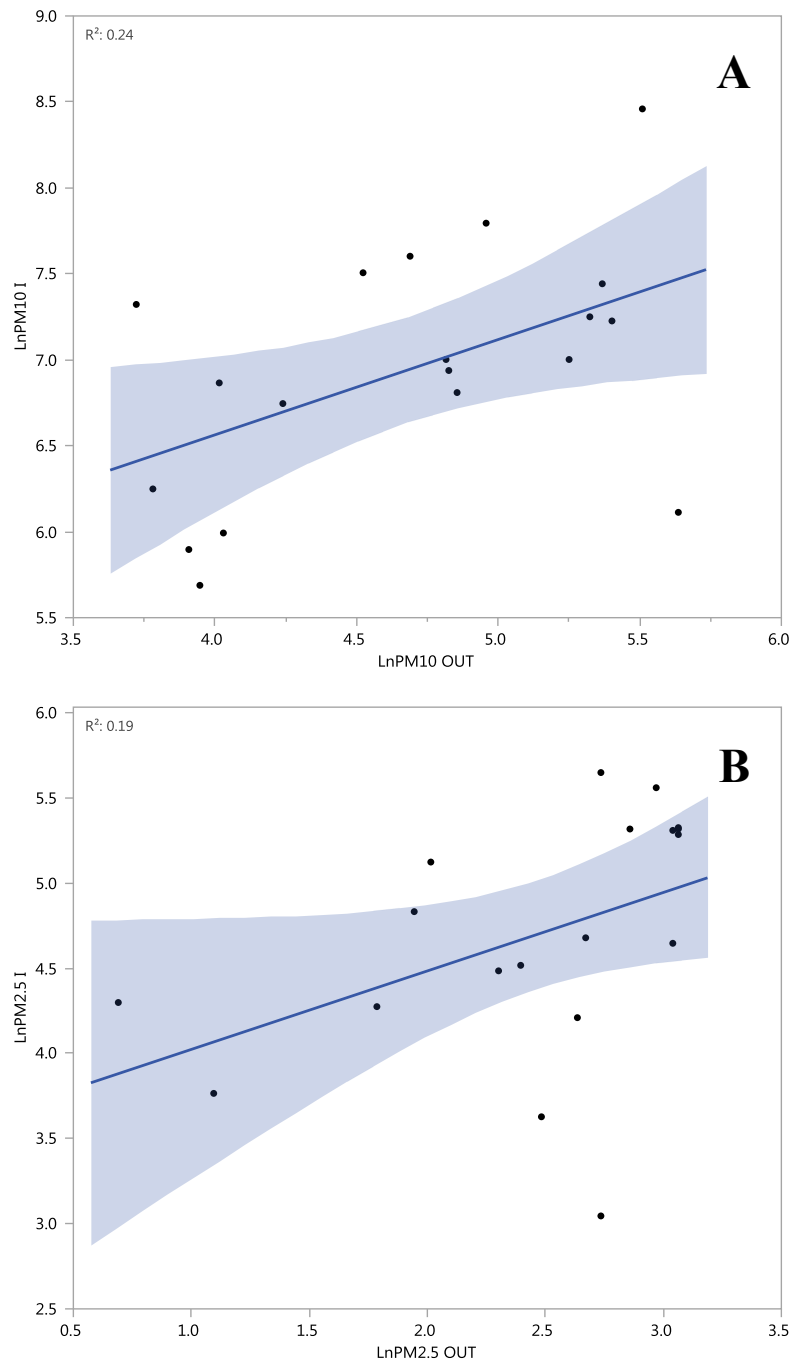


Figure 3.4: Linear regression plots showing correlations between PM_{10} vs location (A) and $\text{PM}_{2.5}$ vs location (B). The R^2 values were approximately 0.25 ($p=0.03$) with a correlation of 0.50, and 0.20 with a correlation of 0.44 ($p=0.06$), respectively.

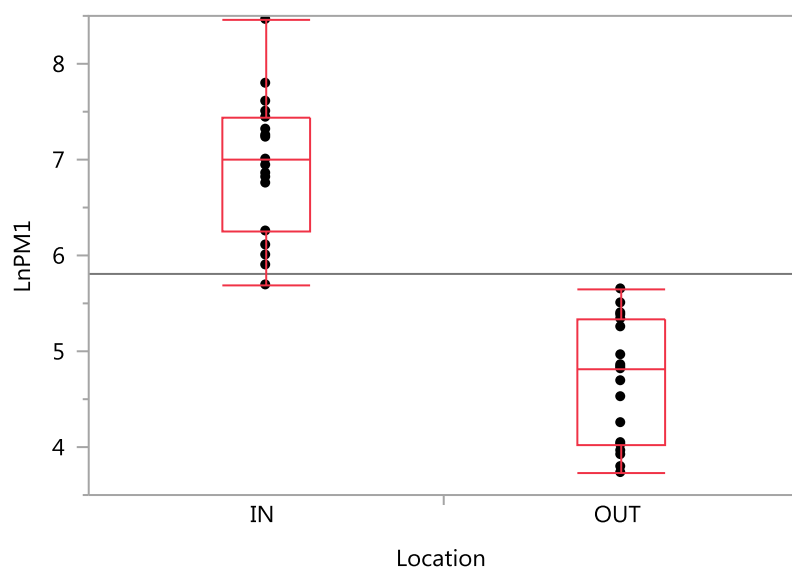


Figure 3.5: One-way ANOVA analysis of PM_{10} concentrations versus location. When comparing out versus in the t-ratio = -9.91 and $p < 0.0001$.

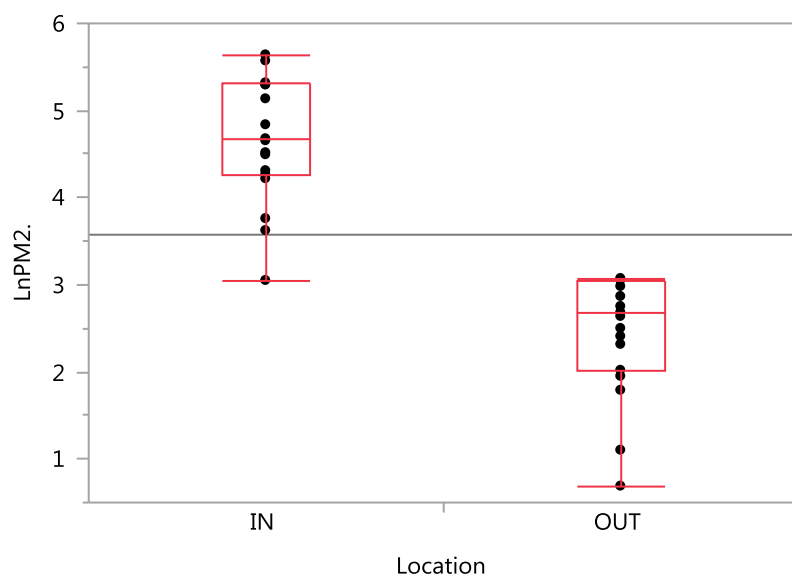


Figure 3.6: One-way ANOVA analysis of $\text{PM}_{2.5}$ concentrations versus location. When comparing out versus in the t-ratio = -10.29 and $p < 0.0001$.

3.4.2 Weather Data and Outdoor PM

The data in Table 3.2 shows average values for temperature, relative humidity (RH), wind speed and rainfall for each of the sampling dates during each season.

Table 3.2: Mean concentrations from each of the sampling dates for temperature (T), relative humidity (RH), wind speed (WS) and rainfall for each season. Note: \pm refers to standard deviation.

Season	Sampling Dates	Sampling Times	Week	T (°C)	RH(%)	Wind Speed (m/s)	Rainfall (mm)
Early Summer	6/15/11 to 6/16/11	2:16p-2:18p	1	22.0 \pm 3.8	60.1 \pm 16.5	1.5 \pm 0.8	0.0
	6/22/11 to 6/23/11	12:40p-12:50p	2	26.5 \pm 2.9	79.6 \pm 15.6	1.5 \pm 1.2	0.0
	6/29/11 to 6/30/11	9:02a-9:02a	3	22.8 \pm 4.0	61.4 \pm 14.3	1.6 \pm 0.9	0.0
	7/6/11 to 7/7/11	12:00p-12:07p	4	27.0 \pm 3.4	72.6 \pm 18.0	2.0 \pm 1.3	0.0
	7/13/11 to 7/14/11	10:56a-11:03a	5	24.3 \pm 4.2	57.2 \pm 8.7	1.8 \pm 0.8	0.0
Late Summer	8/10/11 to 8/11/11	10:29a-10:29a	1	24.3 \pm 3.8	62.0 \pm 15.6	1.6 \pm 1.1	0.0
	8/24/11 to 8/25/11	12:20p-1:00p	3	24.3 \pm 2.0	74.1 \pm 15.7	2.8 \pm 1.0	0.1 \pm 0.5
	8/31/11 to 9/1/11	10:30a-10:35a	4	22.6 \pm 4.4	71.1 \pm 21.4	0.7 \pm 0.6	0.0
	9/7/11 to 9/8/11	11:50a-12:05p	5	23.2 \pm 1.7	95.9 \pm 5.4	2.5 \pm 1.3	0.3 \pm 0.8
Fall	10/5/11 to 10/6/11	1:55p-2:00p	1	14.9 \pm 4.6	60.0 \pm 14.9	1.3 \pm 0.9	0.0
	10/12/11 to 10/13/11	12:45p-1:00p	2	16.3 \pm 0.7	96.2 \pm 5.6	3.2 \pm 1.1	0.2 \pm 0.6
	10/19/11 to 10/20/11	12:30p-12:30p	3	17.5 \pm 2.1	89.0 \pm 14.6	4.4 \pm 1.6	0.3 \pm 0.5
	10/26/11 to 10/27/11	12:35p-12:26p	4	14.6 \pm 1.5	87.2 \pm 12.0	1.7 \pm 1.1	0.1 \pm 0.3
	11/7/11 to 11/8/11	1:35p-2:00p	5	10.7 \pm 6.1	76.4 \pm 21.0	0.9 \pm 0.6	0.0
Winter	1/13/12 to 1/14/12	12:51p-12:50p	1	(-)1.2 \pm 1.6	55.4 \pm 5.8	3.4 \pm 1.0	0.0
	1/19/12 to 1/20/12	2:00p-2:05p	2	(-)0.348 \pm 1.6	57.3 \pm 13.5	2.3 \pm 1.3	0.0
	1/26/12 to 1/27/12	11:05a-11:40a	3	8.4 \pm 3.5	89.7 \pm 12.2	2.2 \pm 1.1	0.3 \pm 0.7
	2/2/12 to 2/3/12	11:50a-11:58a	4	4.0 \pm 3.0	62.0 \pm 6.7	2.6 \pm 1.1	0.0
	2/8/12 to 2/9/12	10:30a-10:15a	5	0.7 \pm 1.4	85.7 \pm 15.0	2.1 \pm 1.0	0.1 \pm 0.3

In figure 3.7, weather factors including mean temperatures, RH, wind speed and outdoor PM₁₀ and PM_{2.5} concentrations are compared. Upon performing Bivariate fits between outdoor PM₁₀ and PM_{2.5} with the weather factors, the results show that temperature and RH are insignificant with p-values at 0.89, 0.64 and 0.86, 0.88, respectively. However, wind speed was found to have the most significance on PM concentrations. Wind speed was determined to significantly influence outdoor PM₁₀

and $PM_{2.5}$ concentrations with p-values of 0.05 and 0.01, respectively. For each season rainfall data was also recorded; however, the level of precipitation ranged from 0 mm to just over 1 mm and did not show any clear evidence of significant influence on PM concentrations. Although rainfall amounts were low and results were inconclusive, it has been found that rainfall can greatly affect PM concentrations (Hinds, 1999).

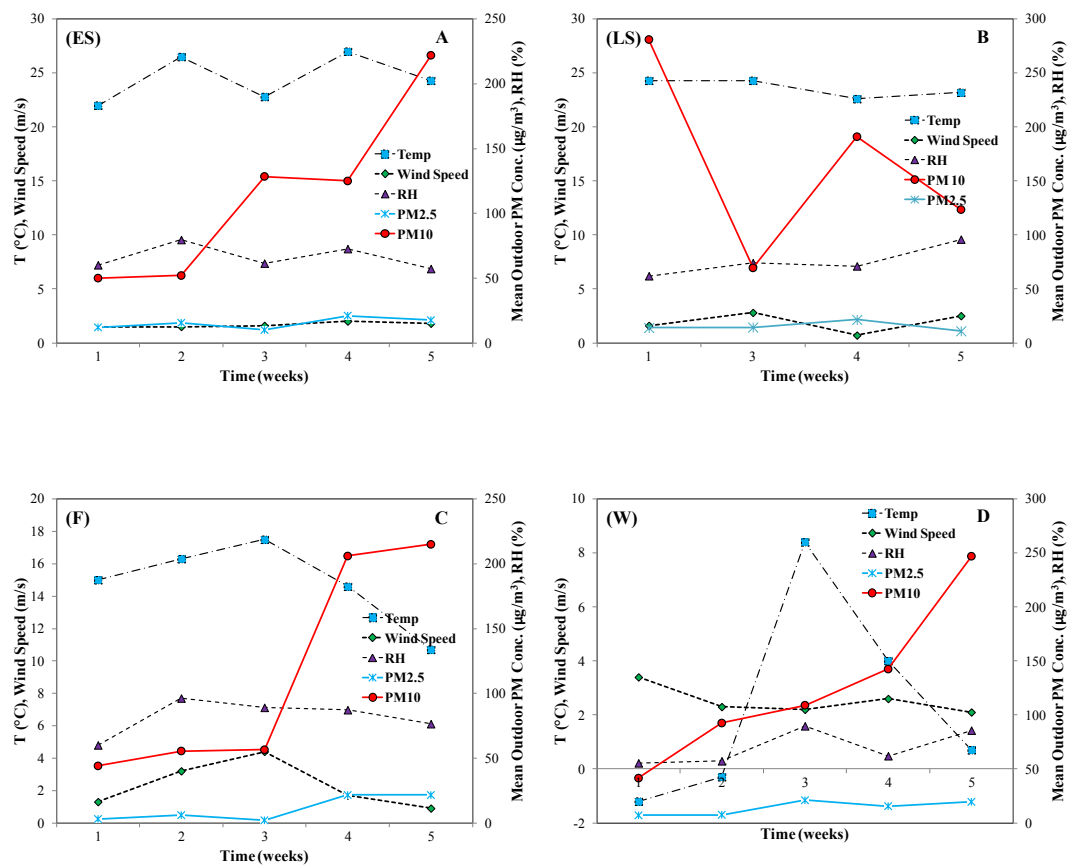


Figure 3.7: Meteorological factors including mean temperatures, RH, wind speed and outdoor PM_{10} and $PM_{2.5}$ concentrations for early summer (A), late summer (B), Fall (C) and Winter (D).

The influences of meteorological factors on PM concentration have been studied previously on both urban and agricultural sites, but primarily focused on PM_{2.5} (Bhaskar et al 2010; EPA, 2010; Visser et al, 2006). The study by Visser et al (2006) studied PM_{2.5} which was collected and monitored from 3 external distances and from within a broiler poultry operation. The meteorological data reported didn't provide conclusive evidence of the influence that factors such as temperature, RH and wind speed had on PM concentrations; however, this was likely due to the distance between the weather monitoring station and the poultry operation. In this study the weather station is within close enough proximity to the poultry facility that the influences of these factors can be identified. In other studies done on urban sites the meteorological and PM trends that are seen are consistent with what has been mentioned and recorded in this study (Bhaskar et al, 2010; EPA, 2010; Hinds, 1999).

3.4.3 In-House Environmental Factors and Indoor PM

In-house environmental factors such as temperature (T), relative humidity (RH) and bird weight (BW) were monitored for each of the periods of sampling for ES, LS, Fall and Winter (Table 3.3).

Table 3.3: Mean concentrations from each of the sampling dates for temperature (T), relative humidity (RH), bird weight (BW) for each season. Note: \pm refers to standard deviation.

Season	Sampling Dates	Sampling Times	Week	T (°C)	RH(%)	Bird Weight
Early Summer	6/15/11 to 6/16/11	2:15p-2:06p	1	30.8 \pm 0.91	52.2 \pm 3.3	0.24 \pm 0.03
	6/22/11 to 6/23/11	12:45p-1:00p	2	29.7 \pm 1.59	77.6 \pm 7.5	0.74 \pm 0.09
	6/29/11 to 6/29/11	9:13a-5:13p	3	28.2 \pm 0.56	58.9 \pm 4.0	1.68 \pm 0.09
	7/6/11 to 7/6/11	11:40a-7:30p	4	31.5 \pm 0.33	65.9 \pm 5.2	2.81 \pm 0.13
	7/13/11 to 7/13/11	11:06a-7:39p	5	30.2 \pm 0.39	65.7 \pm 3.7	3.99 \pm 0.06
Late Summer	8/10/11 to 8/11/11	10:35a-10:36a	1	31.1 \pm 0.42	56.2 \pm 2.8	0.21 \pm 0.02
	8/24/11 to 8/24/11	12:30p-7:50p	3	28.6 \pm 0.39	59.6 \pm 2.8	1.51 \pm 0.13
	8/31/11 to 8/31/11	10:37a-5:45p	4	29.4 \pm 0.67	48.8 \pm 3.6	2.66 \pm 0.08
	9/7/11 to 9/7/11	12:00p-7:40p	5	27.1 \pm 0.83	88.4 \pm 1.9	3.96 \pm 0.15
Fall	10/5/11 to 10/6/11	2:05p-2:05p	1	31.0 \pm 0.48	37.5 \pm 3.0	0.30 \pm 0.04
	10/12/11 to 10/12/11	12:35p-6:40p	2	28.8 \pm 0.43	57.2 \pm 2.5	0.92 \pm 0.08
	10/19/11 to 10/19/11	12:40p-6:20p	3	25.4 \pm 0.25	80.4 \pm 1.8	1.91 \pm 0.11
	10/26/11 to 10/26/11	12:45p-7:15p	4	23.7 \pm 0.16	71.6 \pm 1.7	3.17 \pm 0.16
	11/7/11 to 11/7/11	1:30p-6:25p	5	22.4 \pm 0.83	78.5 \pm 2.9	5.41 \pm 0.00
Winter	1/13/12 to 1/14/12	12:30p-12:45p	1	28.1 \pm 0.20	39.6 \pm 0.6	0.04 \pm 0.00
	1/20/12 to 1/20/12	7:15a-3:20p	2	26.2 \pm 0.60	38.8 \pm 0.5	0.60 \pm 0.11
	1/26/12 to 1/26/12	11:15a-6:40p	3	26.0 \pm 0.22	52.6 \pm 1.3	1.29 \pm 0.09
	2/2/12 to 2/2/12	11:45a-6:15p	4	24.2 \pm 0.27	56.8 \pm 1.3	2.37 \pm 0.09
	2/8/12 to 2/8/12	10:15a-4:20p	5	22.4 \pm 0.38	64.9 \pm 5.3	3.30 \pm 0.14

These factors were then compared to mean PM concentrations generated indoors for each sampled season over time (Figure 3.8).

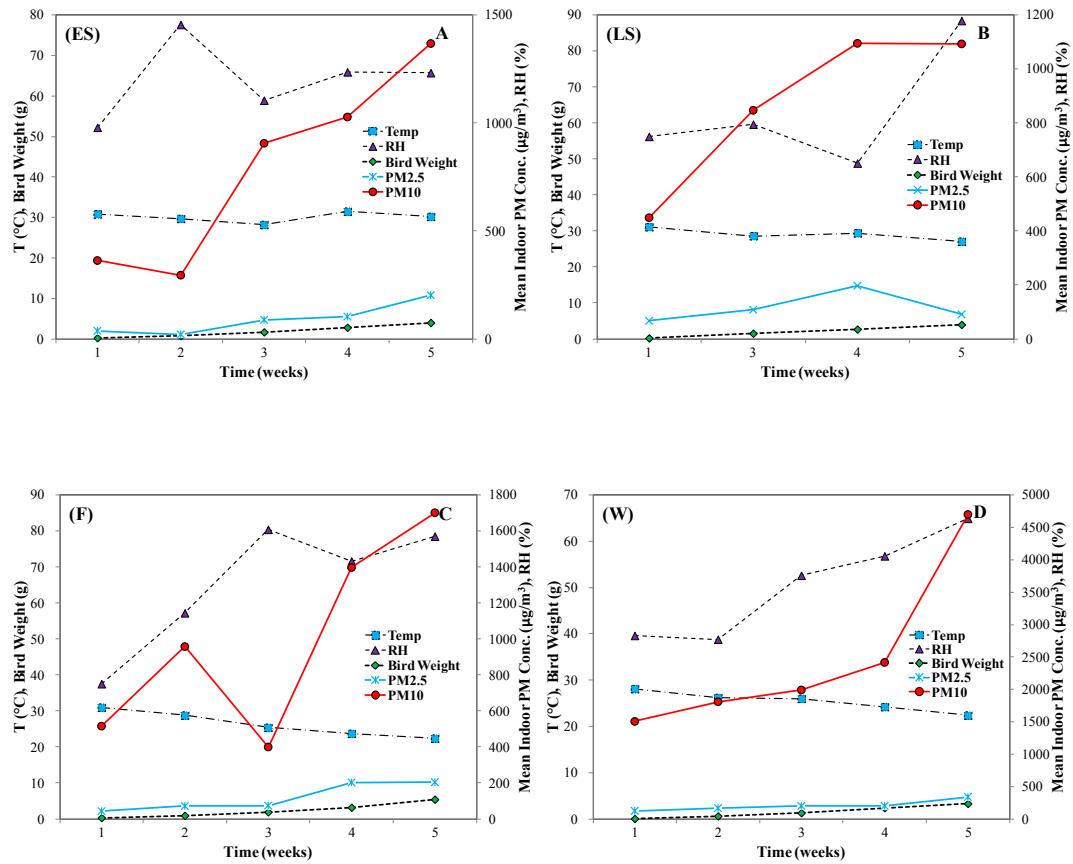


Figure 3.8: In-house factors including mean temperatures, RH, bird weight and indoor PM₁₀ and PM_{2.5} concentrations for early summer (A), late summer (B), Fall (C) and Winter (D).

The results of multiple regression and least squares fitting between PM₁₀ and PM_{2.5} concentrations and the various indoor environmental factors recorded for all seasonal sampling periods indicate that bird weight was the most influential ($p=0.0135$ and $p=0.0103$; $\alpha=0.05$) and that T and RH did not have much effect on PM concentrations (Figures 3.9-3.10). It has been documented that bird weight and age may have an effect on PM emissions and thus indoor concentrations as well (Roumeliotis and Van Heyst, 2007; Lacey et al, 2003).

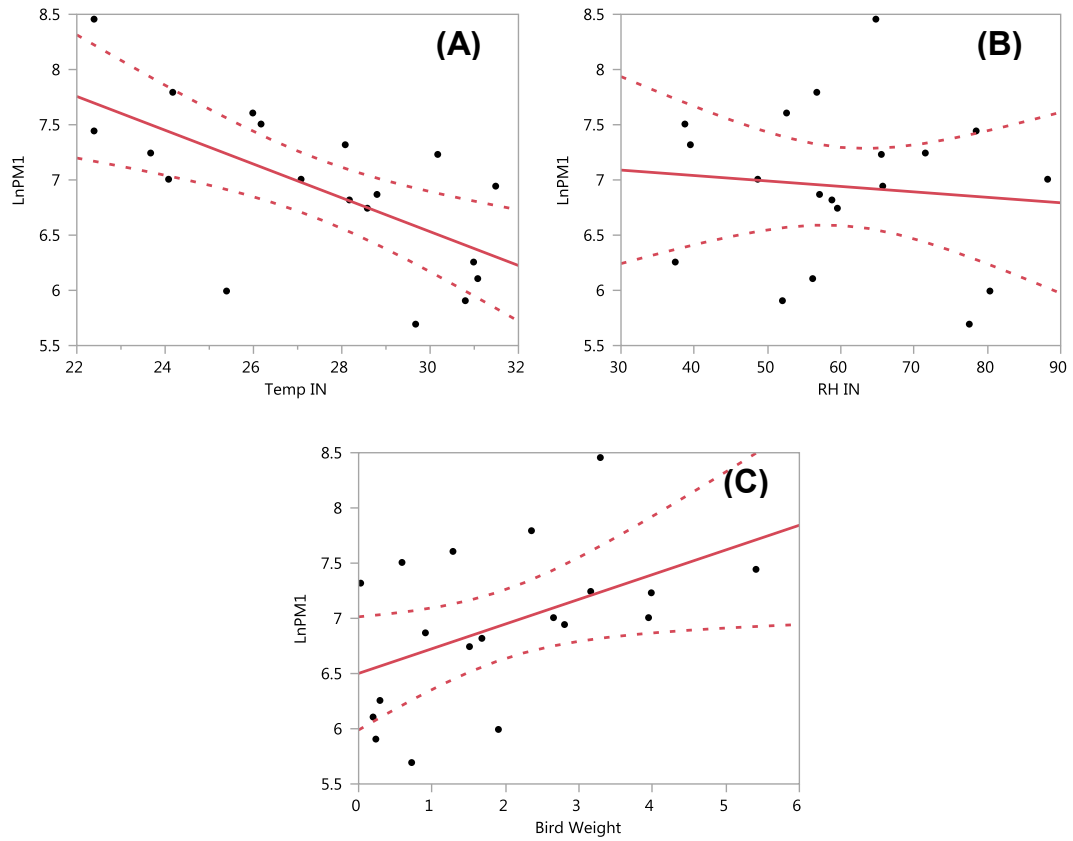


Figure 3.9: Linear least squares fitting of mean PM_{10} concentrations and in-house environmental factors such as temperature (A), relative humidity (B) and bird weight (C). *Dashed lines represent confidence of fit curves.*

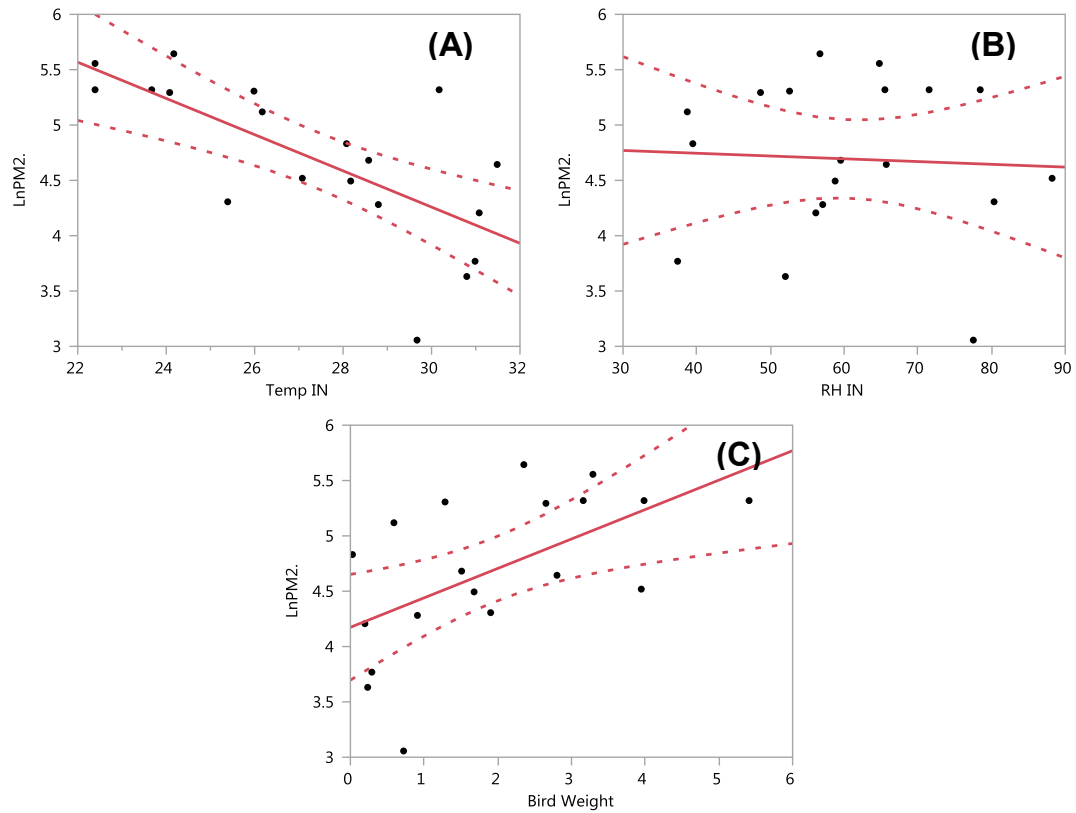


Figure 3.10: Linear least squares fitting of mean $\text{PM}_{2.5}$ concentrations and in-house environmental factors such as temperature (A), relative humidity (B) and bird weight (C). Dashed lines represent confidence of fit curves.

Table 3.4 shows a comparison of several other studies that have evaluated PM_{10} and $\text{PM}_{2.5}$ from poultry operations. Concentrations are highly variable from one study to another; however, this is likely due to a number of reasons including, the type of poultry operation, layer versus broiler, type of collection methodology, integrated versus real-time, and sampling durations. However, it is to the author's knowledge that the work in this study is the only one where integrated sampling methods were

used in a Delmarva broiler operation to evaluate the concentrations of indoor and outdoor PM₁₀ and PM_{2.5}.

Table 3.4: Measurement conditions and Particulate matter concentrations from various studies from poultry operations

Reference	Location	Type of operation	Measurement frequency	Sampling Type	PM ₁₀ (µg/m ³)	PM _{2.5} (µg/m ³)
Takai et al. 1998	Northern Europe	Laying	2 days total, 1 summer, 1 winter, 22 bldgs	integrated, gravimetric, personal IOM sampler and cyclonic samplers	3600 ^[a]	450 ^[b]
Lim et al. 2003	Indiana	Laying	24 hr means	Real-time, Tapered Element Oscillating Microbalance (TEOM)	518 ± 74	39 ± 8
Li et al. 2011	Iowa	Laying	17 mo./continuous	Real-time, Tapered Element Oscillating Microbalance (TEOM)	393	44
Visser et al. 2006	Georgia	Broiler	48 and 24 hr. integrated, 4 sites	Integrated, gravimetric measurement, Triplex Cyclone	N/A	60 ± 3.3
Hinz and Linke, 1998	Germany	Broiler	once a week over a 32 day cycle/2 hr avg day		N/A	1000 -14000 ^[b]
Jager, 2005	South Africa	Broiler	8 hr (TWA)		N/A	2420 ± 2130 ^[c]
Roumeliotis and Van Heyst, 2007	Canada	Broiler	3 production cycles/ each 49 days	Real-time, DustTrak aerosol particle counter	690	190
Carter et al. 2014 ^[d]	Delaware	Broiler	one 24hr period per week over 4 growout cycles/ each a 5 week period	Integrated, gravimetric, PEM and PMASS	1306 ± 850	126 ± 54

^a values represent inhalable dust (particles < 2.5 µm)

^b values represent respirable dust, which is defined as particles less than 4 µm

^c calculated as 8 hr time weighted average for an 8 hr work day

^d this study, unpublished values represent mean of means for four seasonal periods

3.5 Conclusions

In this study, the concentrations of indoor particulate matter exceeded outdoor PM concentrations by 10 times. The concentrations for indoor levels were found to exceed the current NAAQS regulations; however, these regulations aren't suitable for comparison to the concentrations that are within the poultry facility because of the limitations and rules governing the PM₁₀ and PM_{2.5} standards. In addition, although the limits do not exceed OSHA standards; they too generalize particles, and there are no precise standards for PM₁₀ and PM_{2.5}. Outdoor PM₁₀ and PM_{2.5} concentrations did not exceed the NAAQS standards. Season was found to be a contributing factor affecting PM concentrations. During the winter months both PM₁₀ and PM_{2.5} were elevated indoors, whereas outdoor concentrations were higher in the summer for PM₁₀ only.

Indoor and outdoor concentrations of PM₁₀ and PM_{2.5} were not correlated, indicating that they do not have a significant influence on each other. Because of this, further information was obtained on various environmental and operational factors that could potentially have an effect on PM concentrations. Of the various indoor and outdoor factors recorded in this study, wind speed was found to be the main factor affecting outdoor PM concentrations, where bird weight was found to be the main factor affecting indoor PM concentrations. This is in agreement with other studies, which have concluded that wind speed, along with precipitation can affect outdoor PM levels and bird weight and age can influence PM levels inside of a poultry operation (Bhaskar et al, 2010; Roumeliotis and Van Heyst, 2007; Lacey et al, 2003; Hinds, 1999).

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Chapter 4

DISTRIBUTION AND SPECIATION OF ARSENIC IN PARTICULATE MATTER FROM A DELMARVA BROILER OPERATION

4.1 Abstract

Determination of the forms of arsenic and the distribution of this and other metals present in particulate matter derived from broiler poultry operations can aid in understanding the toxicity, mobility, and bioavailability, and can then be used to assess the exposure of those working in this type of occupational environment. Therefore, this study was conducted to determine the distribution of metal(loid)s and speciation of arsenic in particulate matter (PM) from a Delmarva broiler poultry operation.

Samples were collected in two aerodynamically fractionated size ranges (PM₁₀ and PM_{2.5}) using integrated, impactor and cyclonic units deployed at two locations on a Delmarva broiler poultry operation. Total concentrations of As in the PM₁₀ fraction varied from 2 to 52 ng/m³, and in the PM_{2.5} fraction from 3-76 ng/m³ as measured by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) analysis. A duplicate set of samples were analyzed by synchrotron-based microfocused X-ray fluorescence imaging (μ -XRF) and X-ray absorption near-edge structure spectroscopy (μ -XANES), and were found to contain multiple organic and inorganic As species, including organic degradation products of Roxarsone. The As species present in the PM were similar to those observed in previously studied litter samples. Results from this study can be used to help assess the potential human health risks associated with exposure to airborne particles from poultry operations.

4.2 Introduction

Livestock and poultry facilities, especially confined animal feeding operations (CAFOs), are a major source of gaseous and particulate matter emissions. Because of the potential hazards associated with these emissions it has become an increasing concern among federal and state regulatory agencies, including the Environmental Protection Agency (EPA), the United States Department of Agriculture (USDA), the National Institute for Occupational Safety and Health (NIOSH), the Delaware Department of Natural Resources and Environmental Control (DNREC), and the Maryland general assembly (Nachman et al, 2013). As a result, air quality regulations, once limited to industry, are now being applied to agriculture (EPA 2001).

The health effects posed on animals as well as farmers and farm workers have been reported for almost two decades (Cambra-Lopez,*et al.*,2010, Linaker *et al.*, 2002, Radon *et al.*, 2002, Donham *et al.*, 2002, Zuskin *et al.*, 1994). Most of the reported symptoms correspond to respiratory and cardiovascular infections and diseases, and can include chronic bronchitis, coughing, sputum, and wheezing, as well as cardiovascular disease and cancer. The health problems that are incurred from exposure are not limited to the amount and size of particles being inhaled, but also to the type of materials that are associated with the particles.

Poultry house PM is generated by both the bird and bedding materials and contains pulverized fecal material, feed particles, feathers and epidermal fragments of skin, and microorganisms. The dust particles may also contain high levels of ash, nitrogen, calcium, iron, zinc, copper, arsenic, magnesium, and/or aluminum (Ellen, et al., 2000; Nakaue, et al., 1981). The toxicity of heavy metals is determined in large part by their speciation (i.e. forms, oxidation states). Arsenic is a metalloid that can become more toxic as its oxidation state changes to more reduced forms. It generally

exists in two oxidation states As(V), which is less mobile and less toxic, and As(III), which is more mobile and toxic, and is a causative agent in cancer formation.

Organo-arsenicals such as Roxarsone and p-arsenilic acid have been used in the poultry industry for decades. They are administered as feed additives, at a rate of approximately 1.7 to 2.2 million pounds (~1,000 tons) annually, to help with feed efficiency, growth promotion and as a preventative for coccidial intestinal infections (Sierra-Alvarez et al, 2010; Walinga, 2006; Chapman and Johnson, 2002). Litter management practices on Delmarva can include the re-use of litter over several growout cycles before a total cleanout is ever performed (Malone, 2006). In addition, the majority of the litter material is retained for use as fertilizers. Over this period, excreted organo-arsenicals have the ability to transform into more toxic forms. The bioaccumulation of organo-arsenicals have been previously investigated; with very little retained, they are found to be excreted almost exclusively in their organic form (Sierra-Alvarez et al. 2010). These organo-arsenicals have been found at high concentrations within the litter material, at around 14 to 54 mg/kg, and are capable of biotransformation into reduced organic forms, primarily 4-hydroxy-3-aminophenylarsonic acid (HAPA), methylated As dimethylarsinic acid and monomethylarsinic acid (DMA (V), MMA(V)) and inorganic forms (As(V), As(III)) (Seirra-Alvarez et al 2010; Seiter, 2009; Makris et al 2008; Cortinas et al 2006; Stolz et al 2007; Garbarino et al 2003; Jackson et al 2003; Jackson and Bertsch, 2001).

Although studies have been done to identify and quantify elements in PM from livestock facilities, such as swine and rabbits, there is limited information with respect to poultry PM (Adell et al 2012; Yang et al 2011; Cambra-Lopez et al 2010; Li et al 2009). What research has been performed on characterizing and quantifying elements

has primarily been done through mass spectroscopic analysis or by microscopic methods such as scanning electron microscopy coupled with energy dispersive x-ray spectroscopy. The limiting issues with these methods are that they can be destructive, and can contribute to changes in speciation, or they are used solely to determine total elemental concentrations, not species specific data. X-ray absorption spectroscopy (XAS) is a technique that allows one to investigate the atomic properties that are associated with an element(s) of interest. This technique can give valuable structural and chemical information, and is used to determine such properties as oxidation states and coordination numbers. The benefit of using this technique over others is that samples can be looked at under non-invasive conditions, which preserves the chemical integrity and eliminates issues of sample loss (Pattanaik et al 2007). Here we have used μ -XAS techniques to investigate the chemical speciation of arsenic, and to identify distribution of other metal(loid)s (Fe, Cu, Zn, Mn) associated with poultry PM (Corriveau et al, 2011; Walker et al, 2011).

From a human and environmental health perspective, it is critical to understand trace metal concentration, distribution, and speciation in both $PM_{2.5}$ and PM_{10} . Information obtained through molecular scale techniques such as synchrotron radiation-based spectroscopy can elucidate the speciation to help better understand the toxicity.

4.2.1 Objectives and Focus

The following were the objectives of this study:

- 1) Identify and semi-quantify individual chemical species of As in PM samples from two sampling sites on a Delmarva broiler operation, and

2) using μ -XRF imaging, to determine distribution and correlation of other metal(loid)s (Fe, Cu, Zn, Mn) in PM.

4.3 Experimental Methods And Materials

4.3.1 Particulate Matter Collection

During the period between 6/2011 and 2/2012, PM_{10} and $PM_{2.5}$ were collected during four separate 42 day growout “flock” cycles from a Delmarva broiler poultry operation, and were used for μ -XRF and μ -XAS analyses. Each flock cycle corresponded to a certain seasonal period throughout a year, including flock 3 (F3) early summer (ES), flock 4 (F4) late summer (LS), flock 5 (F5) fall (F), and flock 6 (F6) winter (W). Prior to sampling, 25 mm and 37 mm Teflon filters were pre-weighed in a temperature and humidity controlled weighing room after equilibrating for ≥ 24 hours using a Mettler T5 microbalance with precision of ± 0.003 mg (Mettler-Toledo, Toledo, OH); this was performed at Johns Hopkins Bloomberg School of Public Health (JHSPH). Samples were collected weekly over the 42 day growout cycle. Personal environmental monitor samplers (PEMS) (SKC, Inc., Eighty Four, PA) were used to collect PM_{10} ; defined as particles from 10 μ m and below, and the personal micro-environmental aerosol speciation sampler (PMASS) (MSP Corporation, Shoreview, MN) collected fine particulates, which is defined as particles 2.5 μ m and below. PEM samples (cut size of 10 μ m at 4L/min) were collected onto a 2.0 μ m pore size, 37mm Teflon filter with a PTFE support ring (Pall Life Sciences, Ann Arbor, MI), which required a single pump at a target flow rate of 4L/min. The PMASS includes a single size selective inlet with a cut size of 2.5 μ m at a target flow rate of 4L/min, and has two parallel sampling channels. A 3.0 μ m pore size, 25 mm

Teflon filter with a PTFE support ring (Pall Life Sciences, Ann Arbor, MI) was placed in both channels of the PMASS. The target flow rate of 4L/min is internally split to 2L/min through each channel, and calibrated individually. The flow rates were calibrated using a flow meter (Dry DC-Lite & DC-2, BIOS, Butler, NJ). A Side-by-side rotameter was used to determine the proportional flow through each filter within the PMASS. Samples were collected for 24, 8, and 6 hour periods, and were collected both inside (centrally located) and outside (adjacent from exhaust fan) the poultry facility.

4.3.2 Micro X-Ray Fluorescence Imaging Analysis

The μ -XRF analysis of PM₁₀ and PM_{2.5} samples were performed at beamline X27A of the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory, Upton, New York (BNL). The beamline is equipped with a Si(111) crystal monochromator, and the X-ray beam size of <10 μ m was achieved by focusing with Kirkpatrick-Baez mirrors (Ablett et al, 2006). Filters were directly mounted onto slide mounts carefully with kapton tape. It has been documented that adhesives can contribute to the alteration of As while being analyzed, therefore the kapton tape was not applied to the entire surface of the filter; this maintained the integrity of the samples and minimizes the possibility of beam-induced reduction from occurring (Seiter, 2009; Arai et al, 2003). The samples were then affixed to an automated x-y-z stage, which was positioned at 45° to the incident beam. Fluorescence signals were detected by either the Canberra 13 element Ge array (flocks 3(ES), 4(LS), 5(F)) or Vortex ME4 SDD array (flock 6 (W)) detectors. Elemental maps were collected at 12 KeV; with an average map size of 3.0 X 3.0 mm and pixel size of 0.01 mm. Elements

with K absorption edges below the incident energy were monitored; this included Ca, Fe, Cu, Zn, Mn and As.

4.3.3 Micro X-ray Absorption Near-Edge Structure Spectroscopy Analyses

Arsenic K edge μ -X-ray absorption near-edge structure spectra (XANES) were also collected at beamline X27A at NSLS, Upton, New York. Upon evaluation of the fluorescence maps, regions of interest (ROI's) were chosen for XANES analysis based off of concentrated areas of arsenic which were indicated by brightly colored regions (yellow, white) or "hotspots". XANES spectra were collected from 11757 to 12167 eV in fluorescence mode (Corriveau et al, 2011). Dwell times were 5.0, 0.5, 1.0 and 5.0 seconds with step sizes of 3.0, 10.0, 6.0 and 3.0 eV, respectively. Because of possible beam-induced redox, XANES spectra collection was limited to only one scan per spot (Seiter, 2009; Arai et al 2003). The beam was then shifted a couple of micrometers where another scan was performed; this would ensure reasonable and representative data was being collected.

Reference materials were mounted as described by Seiter (2009). Samples were prepared using mylar film and Ultralene, and were applied evenly in a single layer on the film. As described by Seiter, 2009, the mylar and petroleum jelly are used to minimize beam-induced redox. Spectra of reference compounds were collected for interpretive analysis of the XANES data at beamlines X11A, X11B and X26A. The reference compounds included Roxarsone (3-nitro-4-hydroxyphenylarsonic acid), HAPA (4-hydroxy-3-aminophenylarsonic acid), p-arsanilic acid, dimethylarsinic acid (DMA(V)), monomethylarsinic acid (MMA(V)), liquid As(V), NaH_2AsO_4 (Baker), CaHAsO_4 , realgar ($\alpha\text{-As}_4\text{S}_4$), As(III) oxide, As(III) Cysteine, and As(III) Methionine. The standards were chosen based on their common occurrence in litter, excreta and

feed material (Seiter, 2009; Stolz et al 2007; Arai et al 2003). These were used to aid in identifying the oxidation states of arsenic present in the PM samples (Seiter, 2009; Fittschen, *et al* 2008; Werner, *et al* 2007; Majestic, *et al* 2007; Wang, *et al* 2007; Ohta, *et al* 2006; Werner, *et al* 2006; Zatka, *et al* 2003; Arai et al 2003). Normalized derivative of the standards can be seen in Figure 4.4.

4.3.4 Data Analysis

Upon collection of the μ -XRF images, the data was analyzed using the X27A fly-scan plotter software. This software was also used for correlation plotting and to identify distribution of metals in the PM. XANES analysis was done using the X27A plot software. With this program, a collection of XANES scans can be converted into readable files by other analytical programs. Further interpretation of the XANES data was conducted using the Athena 0.9.18.2 (Ravel and Newville, 2005) and Sixpack v.60 (Webb, 2005) software packages. Alignment and background corrections of the raw data were performed in Athena in order to produce derivative normalized $x(\mu)$ E spectra. Standards were calibrated to 11874.5 eV using an inline As(V) standard. Least squares fitting was performed in Sixpack using the normalized (μ) E spectra, and were performed within an energy range of -20 below to +40 above the edge.

4.4 Results and Discussion

4.4.1 Micro X-ray Fluorescence Imaging

X-ray fluorescence imaging indicates that As is heterogeneously distributed throughout the PM samples, and are typically localized to concentrated regions or “hotspots” (i.e. locations where As was detected), which are indicated by areas of higher intensity or brighter colors. Figure 4.1 shows the maps of As distribution for

three indoor samples (F4 004-PM_{2.5} (LS), F6 016-PM₁₀ (W), F6 032-PM₁₀ (W)), and an outdoor PM₁₀ sample (F4 008-PM₁₀ (LS)). The circled regions are “hotspots” chosen for As XANES analysis.

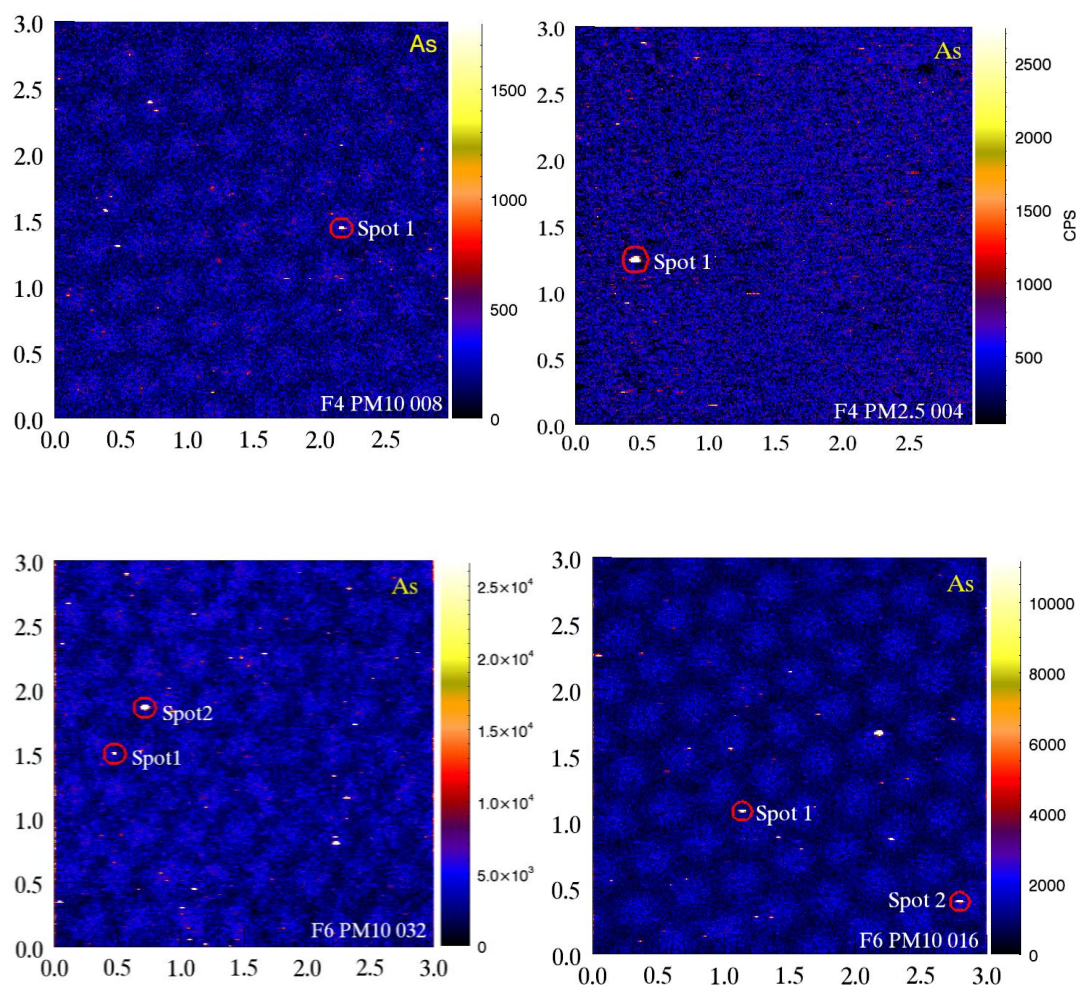


Figure 4.1: Synchrotron microfocused X-ray fluorescence maps (3.0 x 3.0 mm) of arsenic in PM₁₀ and PM_{2.5} for samples taken during late summer (top) and winter (bottom). The scale shows fluorescence counts for arsenic. Areas circled in red are “hotspots” where XANES analysis was performed.

The μ -XRF analyses also revealed that elevated “hotspots” of As are not associated with other trace elements. Lack of spatial association with Fe or other mineral elements is suggestive of organic As forms present. Also, localization of the trace metals and As prevents any direct associations, and are not prevalent in any of the PM samples. Figure 4.2 shows a tri-color map of As, Fe, and Mn for a sample taken during the winter season.

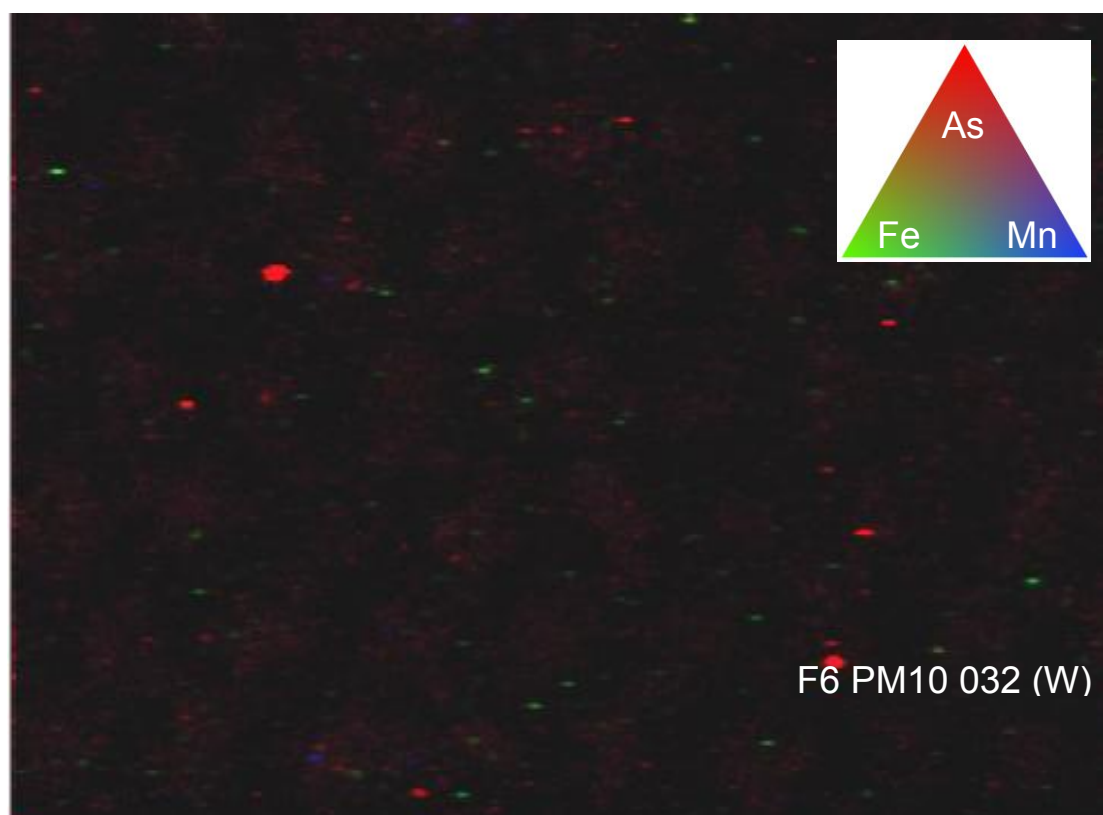


Figure 4.2: A 3.0 mm x 3.0 mm tri-color X-ray fluorescence image showing distribution of Fe, Mn and As in a PM₁₀ sample taken during the winter from the indoor sampling location.

In addition, the overall relationship between specific elements in a given map may be obtained by plotting element correlations of pixilated XRF data for that map. Using this approach, As and other metals such as Fe, Cu, and Mn data were plotted for each of the maps completed (Figure 4.3).

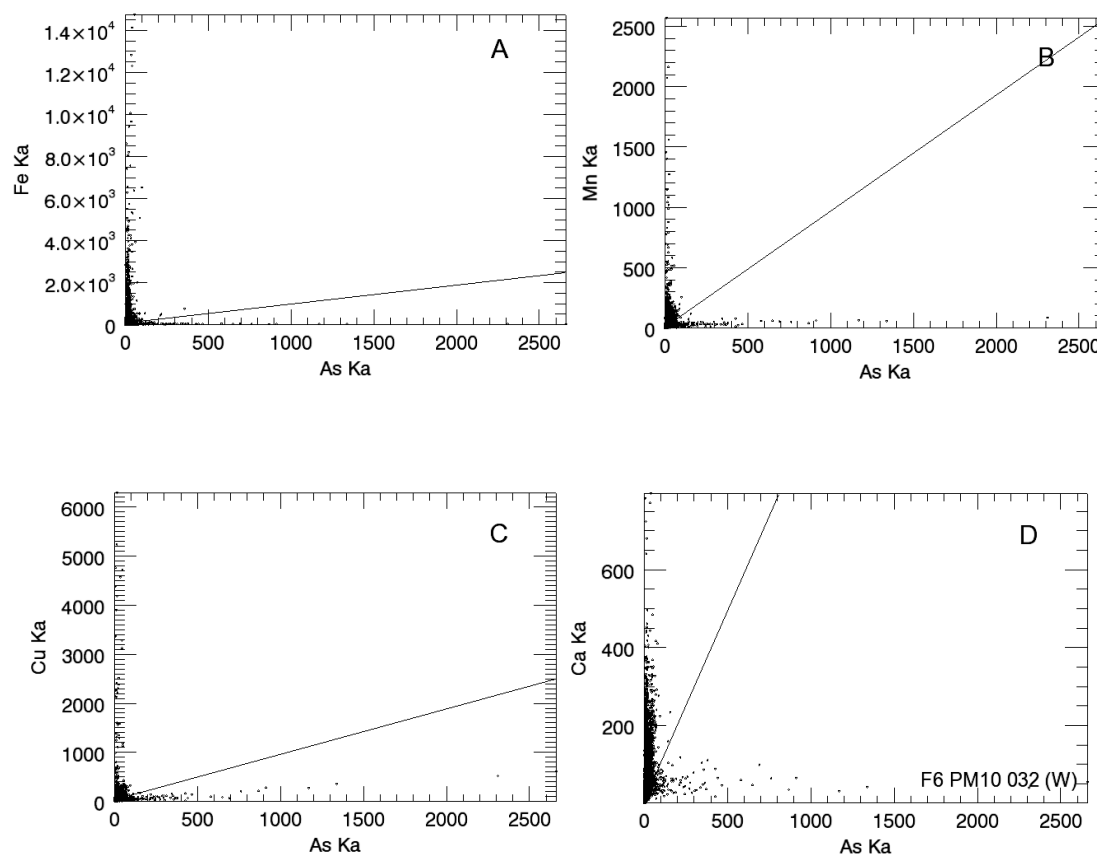


Figure 4.3: Raw count correlation plots between As and other elements for an indoor PM₁₀ sample collected during the winter season. Line represents a 1:1 relationship. Results indicate that in all of the PM samples analyzed there were no apparent relationships found, likely due to localization preventing any direct association.

The plots show that there are no apparent correlations between As and other metals present. This is in contrast to other studies which have indicated associations between As and divalent trace metals and oxides present in soils and in litter material and excreta samples (Chen et al, 2012; Seiter, 2009; Arai et al, 2003; Hansel et al, 2002). In Arai et al. (2003), correlations between As and Ca, Fe and Cu were present in poultry litter, and to a lesser extent As was also associated with Zn. In work done by Seiter (2009), similar correlations were found, in addition to strong correlations with Mn. However, in work done by Godelitsas et al (2011), who looked at urban particulate matter, it was found that As and Pb did not show strong associations to other heavy metals in the system.

XRD analysis at beamline X27A at NSLS showed no distinctive diffraction patterns for the particles; this is indicative of amorphous material, so no further XRD analysis was performed.

4.4.2 As μ -XANES Analyses

The μ -XANES analysis was performed on samples exhibiting high intensity regions or “hotspots” of As. Because the majority of PM_{2.5} samples (3 of 24) from both locations did not display “hotspots” and the overall concentrations of As were found to be at background levels, these were not included in further XANES or least squares fitting. In addition, outdoor PM₁₀ samples were also found to have concentrations of As at background levels, so they too were excluded in the XANES analysis. Some of the samples used for XANES analysis are seen in Figure 4.1 (others not shown). The “hotspots” (N=24, where N equals the number of spots) analyzed on indoor PM₁₀ samples exhibited wide whiteline peaks which are representative of

samples with mixed organic As species. Figure 4.4 shows derivative normalized $x(\mu)E$ XANES spectra of As in samples from ES, LS, Fall, and Winter.

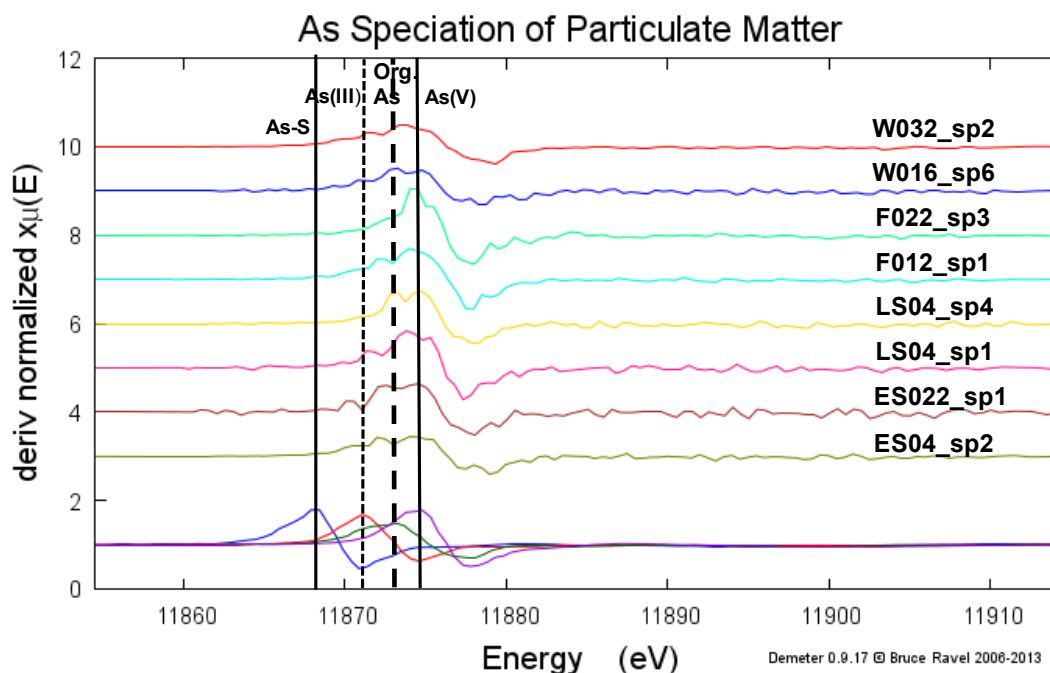


Figure 4.4: Derivative normalized $x(\mu)E$ XANES spectra of As in samples from ES, LS, Fall, and Winter. Vertical lines are at the absorption edge energy positions of As-S (~11868 eV), As(III) (~11871 eV), organic As species ($\sim 11873 \pm 0.8$ eV) and As(V) (~11874 eV), respectively.

Different species of arsenic will produce slightly different spectra, and have a characteristic absorption energy position associated with them; this can then be applied to determine specific species within unknown samples. To elucidate the mixed As species within this system, normalized $(\mu)E$ As XANES spectra were compared to a number of inorganic and organic reference spectra using least squares

fitting (LSF). This analysis will allocate an approximate percentage for each species that produces a best fit interpretation of the sample spectra based on reference spectra collected. Figure 4.5 shows the derivative normalized (μ)E XANES spectra for the standard references that were used for least squares fitting analysis. The monochromator was calibrated using As(V) containing topaz mineral; the whitenline was set to 11874 eV. The absorption edge energy position for As(V) is commonly found around 11874 eV, As(III) exhibited an energy position of ~ 11871 eV and organic species of As had a range of energy positions with many of them being $\sim 11873 \text{ eV} \pm 0.8 \text{ eV}$ (Arai et al 2003). In addition, an arsenic sulfide compound was also used (realgar), which has an absorption energy position at roughly 11868 eV. Sulfide compounds are commonly used in feed additives and thus have been postulated to form precipitates with As, and have been identified in LSF on litter and excreta materials (Seiter, 2009; Arai et al 2003). Lines have been plotted on the spectrum in Figures 4.4 and 4.5 to identify the energy positions of individual oxidation states of As (ie As(V), As(III)).

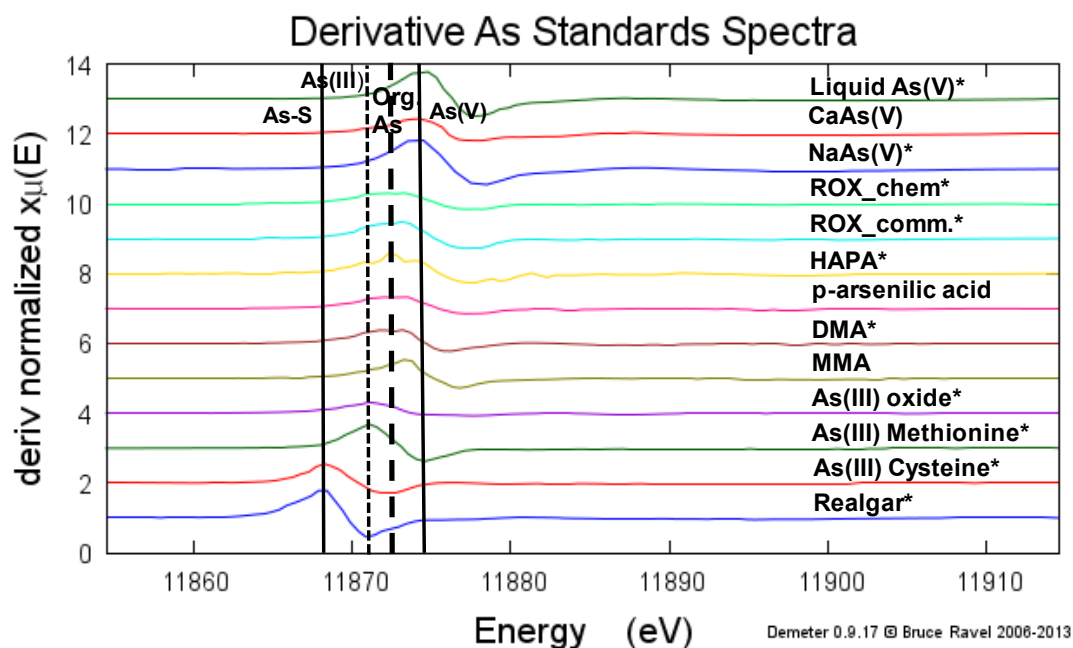


Figure 4.5: Derivative normalized $x(\mu)E$ XANES spectra of As reference standards used in least squares fitting. *standards that were most commonly identified from LSF in PM samples.

4.4.3 Least Squares Fitting

The result of LSF suggests that samples were mostly a mix of organic and inorganic As species. This is in agreement with other studies performed on litter material and excreta, where primary species identified by linear combination fitting were As(III), As(V), ROX, and degraded forms of organic compounds such as DMA, MMA, HAPA and p-arsenilic acid (Seiter, 2009; Garbarino et al 2001; Jackson and Bertsch 2001). Figure 4.6 shows mean percentages of As species found in each of the sampling seasons analyzed (ES, LS, F, and W).

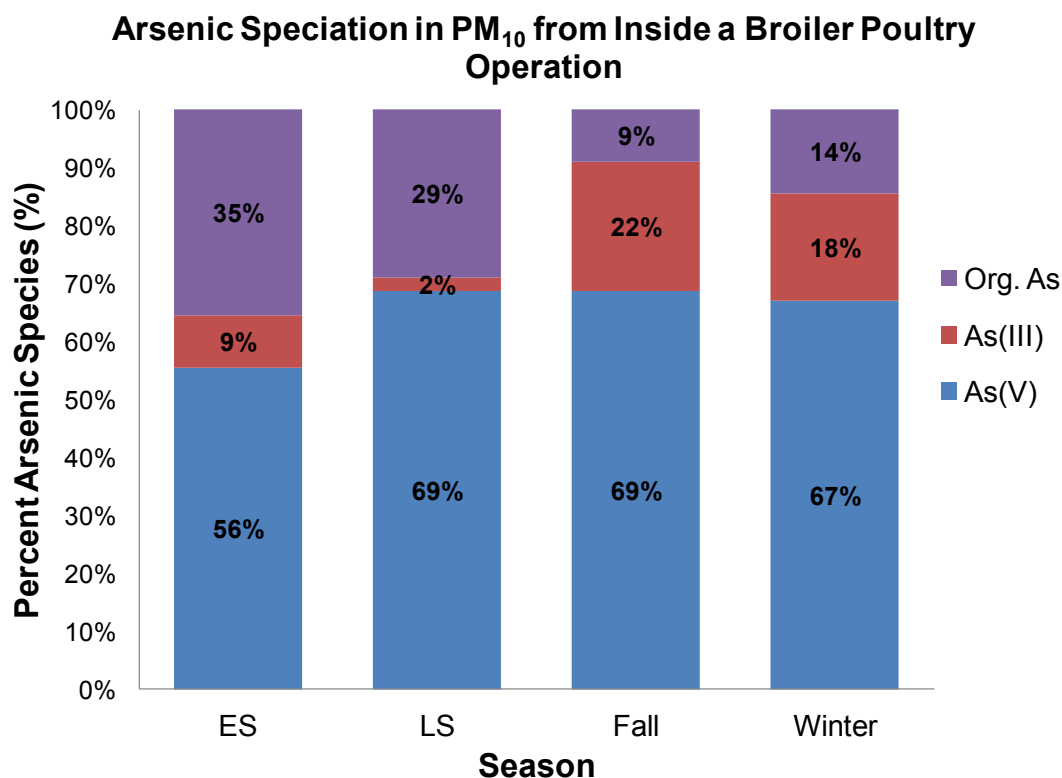


Figure 4.6: Percent of As species found from least squares fitting analysis for each of the seasonal periods. This data indicates a mix in arsenic species is present.

Because some inorganic As standards used (Na-As(V) salt, liquid As(V), CaHAsO₄ and As(III) oxide, As(III) Methionine) and organic As standards (ROX, DMA, MMA, HAPA, and p-arsenilic acid) had similar energy positions, for simpler interpretation these species were grouped together.

When considering the results for all the “hotspots” that were used in least squares fitting (Appendix C), it appears that the dominating oxidation state present on average was As(V) at 0.66 ± 0.15 (average \pm stdev for all seasons combined). In

addition, organic arsenic species constituted an average of 0.19 ± 0.17 ; the main organic species identified in the system were Roxarsone, HAPA, and DMA, which indicate that the ROX was being degraded. Results from previous studies have identified various species related to the degradation of ROX in a litter system (Sierra-Alvarez et al 2010; Garbarino et al 2001; Jackson and Bertsch 2001). The by-products of this degradation can include HAPA, p-arsenilic acid, DMA, and MMA, as well as, inorganic As. In many of the previous systems studied, including litter, the internal environment of the system was anaerobic which played a major role in the presence of more reduced inorganic As species, as well as As bound to sulfides. Here we see that most of the As has been converted to As(V), and an average of 0.13 ± 0.14 being in the more reduced As(III) oxidation state; this is likely due to changes occurring from initial anaerobic decomposition conditions to aerobic conditions as a result of being re-suspended in the air.

Extended EXAFS data could not be collected due to the high amount of noise produced in the extended region of the unknown samples.

4.5 Conclusions

When considering the internal environment of a poultry house and the time most agricultural workers spend working inside these facilities, it is important to understand the exact species that are present in the system. It is also important from an environmental standpoint as well. In this case, more inorganic As(V) was found in the PM samples than As(III) and organic As combined. Although inorganic As(V) is less mobile and toxic in the environment it is still considered hazardous due to its reactivity and its ability to reduce once it has deposited back to the terrestrial surface. In addition, As(V) can be detrimental to metabolic processes occurring within the

body. Studies have shown that As(V) can either directly affect oxidative phosphorylation processes, or can become more reduced and affect/hinder other metabolic processes (Kitchen et al 2008; Mandal and Suzuki, 2002).

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Appendix A

TRACE METAL CONCENTRATIONS IN PM VERSUS LOCATION, AND MICROSOPIC METHODS INVESTIGATION OF PM

A.1 Trace Metal Concentrations in PM_{10} and $PM_{2.5}$ and Their Relationship to Location

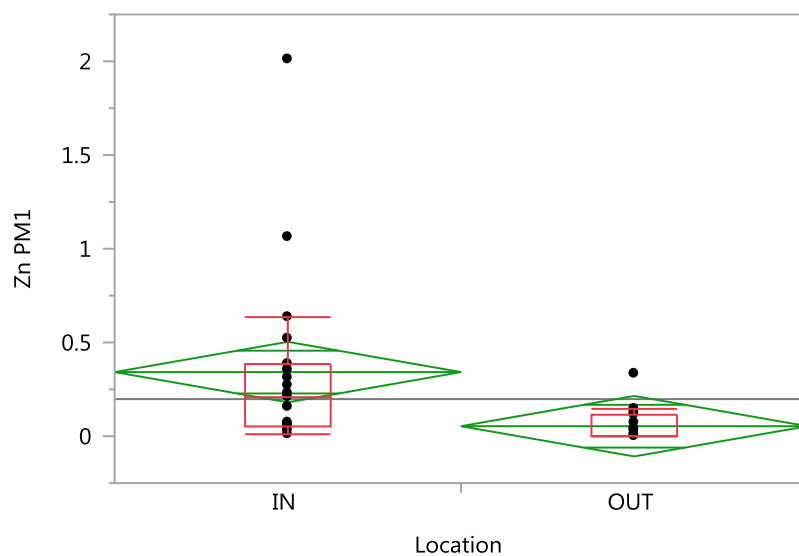


Figure A.1: Zinc concentrations in PM_{10} versus location. Green diamonds represent means.

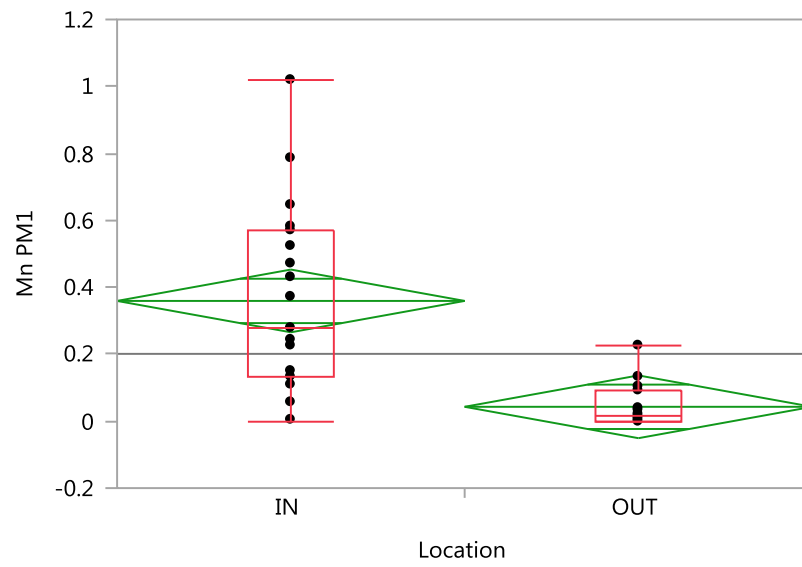


Figure A.2: Manganese concentrations in PM_{10} versus location. Green diamonds represent means.

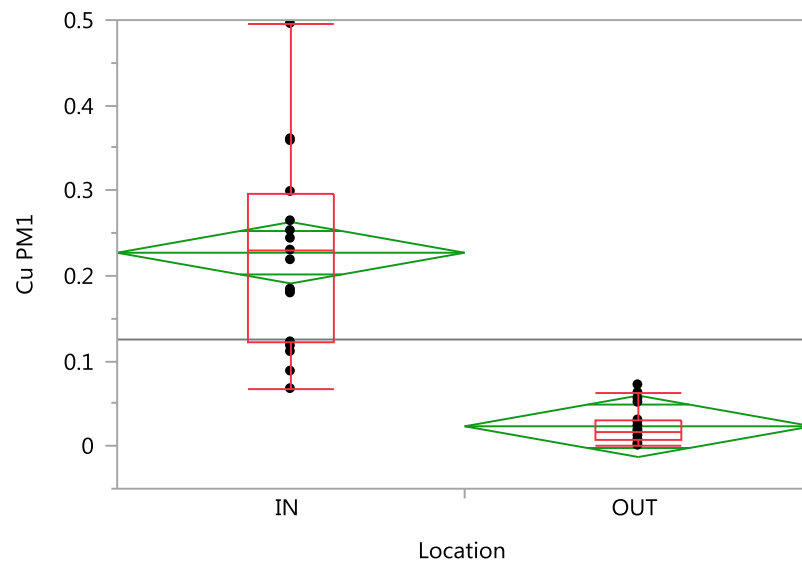


Figure A.3: Copper concentrations in PM_{10} versus location. Green diamonds represent means.

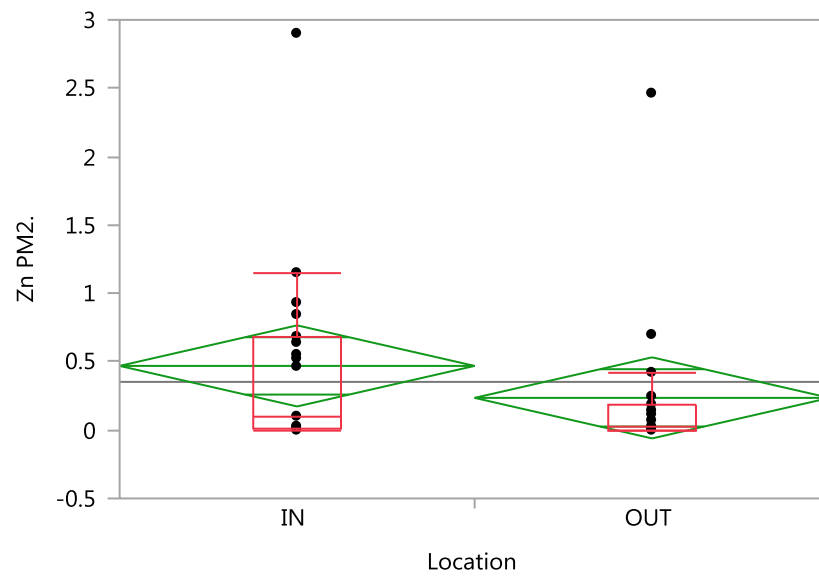


Figure A.4: Zinc concentrations in PM_{2.5} versus location. Green diamonds represent means.

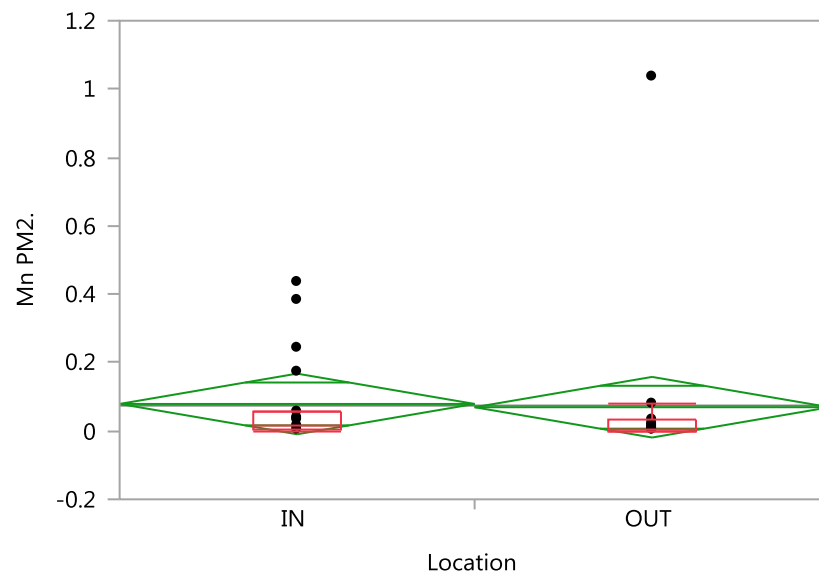


Figure A.5: Manganese concentrations in PM_{2.5} versus location. Green diamonds represent means.

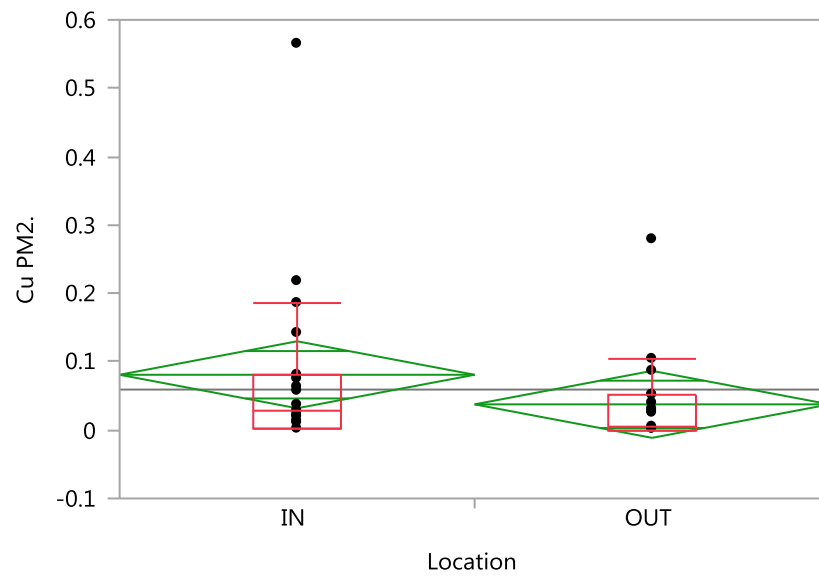


Figure A.6: Copper concentrations in PM_{2.5} versus location. Green diamonds represent means.

A.2 Confocal Microscopy

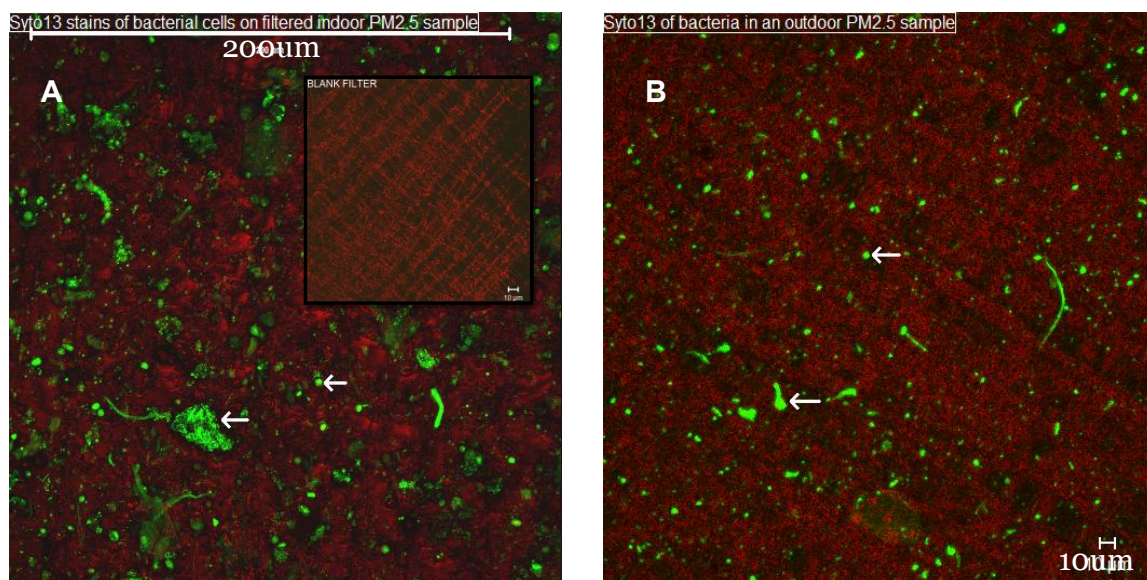


Figure A.7: Confocal microscopy images of an indoor PM_{2.5} sample (A) and an outdoor PM_{2.5} sample (B) which show the association of microorganisms with PM. *Note: inlaid image in (A) shows a blank sample for comparison.*

A.3 Transmission Electron Microscopy

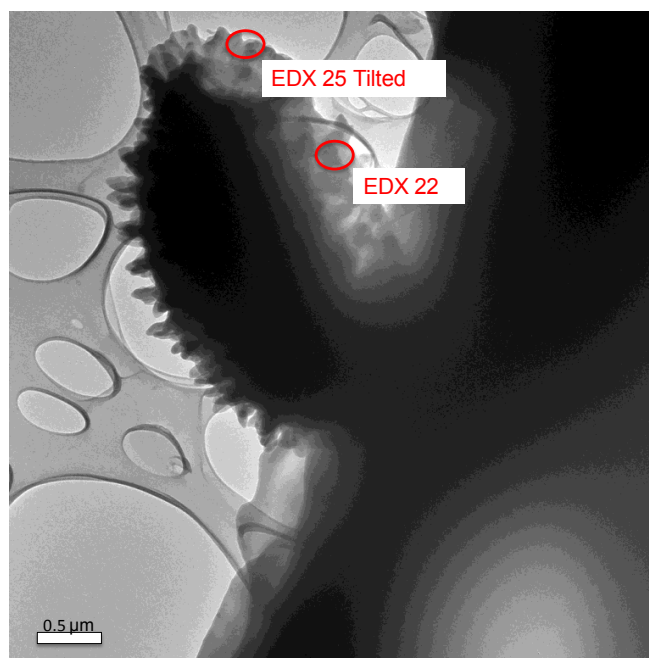


Figure A.8: TEM image of an indoor PM₁₀ sample used for elemental analysis.

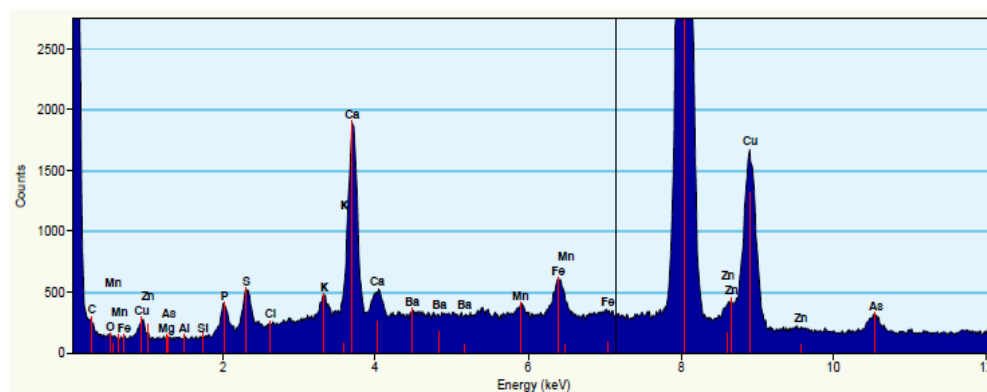


Figure A.9: Energy dispersive spectrograph (EDX) showing the elemental construct of the red circled location labeled EDX 22 in figure A.2.1 (above). Here the EDX shows the presence of Zn, Cu, Mn, S, and As. *Note: the presence of As La1/La2 could not be determined here because of the presence of Mg Ka1, making it difficult to conclude that As is present without a strong Ka1 peak.*

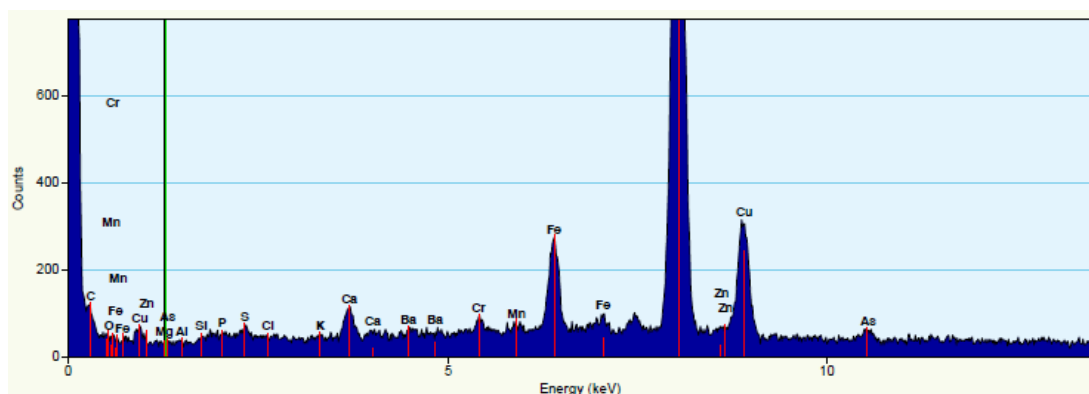


Figure A.10: Energy dispersive spectrograph (EDX) showing the elemental construct of the red circled location labeled EDX 25 in figure A.2.1 (above). *Note: this location was analyzed while sample was in a tilted position to try and counteract the effect of electrons being absorbed by other structures present; however, this only improved background slightly.*

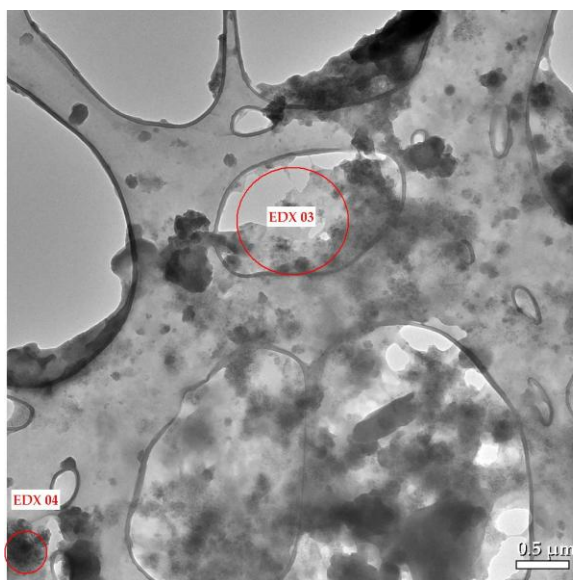


Figure A.11: TEM image from a second indoor PM₁₀ sample used for elemental analysis.

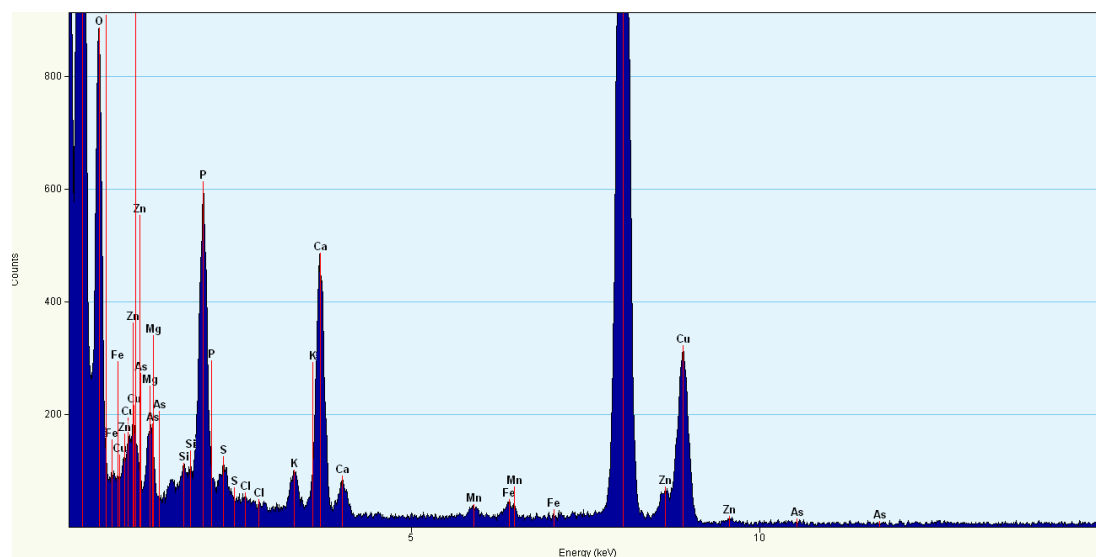


Figure A.12: Energy dispersive spectrograph (EDX) showing the elemental construct of the red circled location labeled EDX 03 in figure A.2.4 (above). Here the EDX shows the presence of Zn, Cu, Mn, Si, and As, similarly found in the EDX analysis of figure A.2.1. *Note: the presence of As La1/La2 could not be determined here because of the presence of Mg Ka1, making it difficult to conclude that As is present without a strong Ka1 peak.*

Appendix B

RELATIONSHIP BETWEEN PM CONCENTRATION AND WEEK

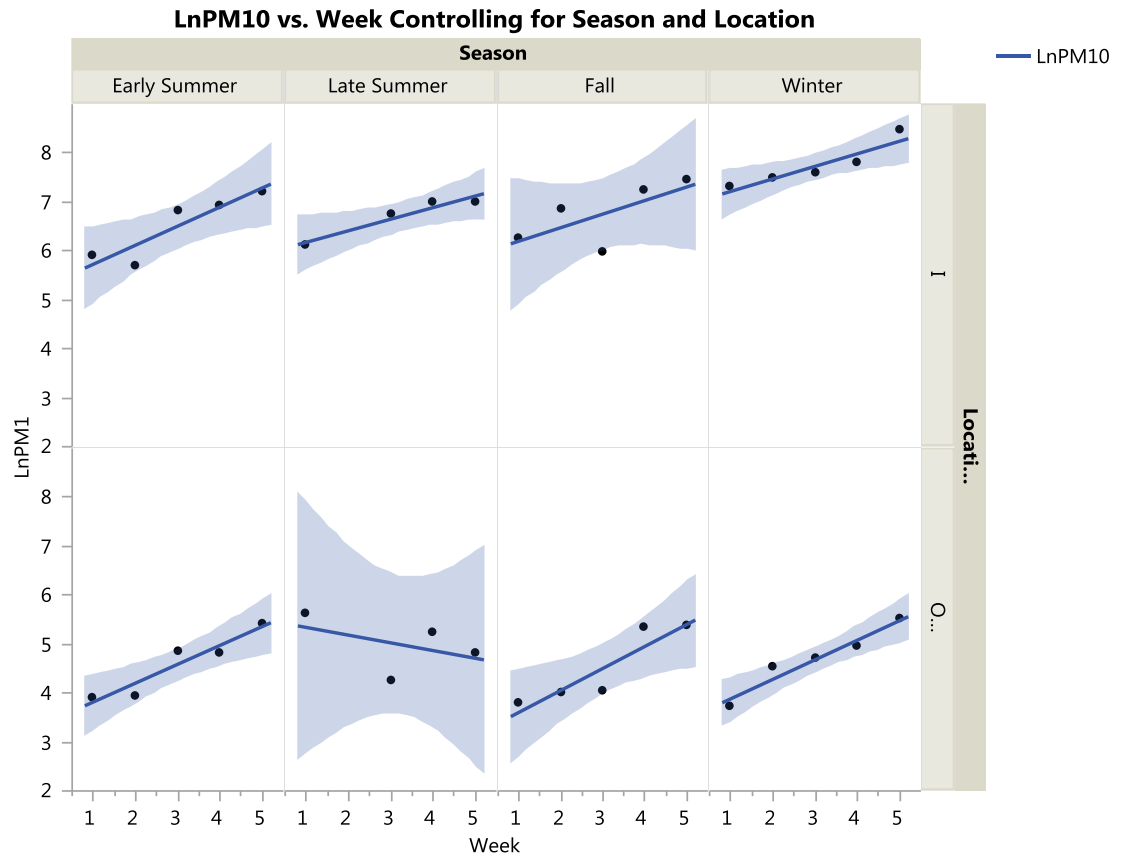


Figure B.1 Relationship between PM_{10} concentration and week, $P < 0.0001$ when least squares fitting analysis was performed for season, location, and week.

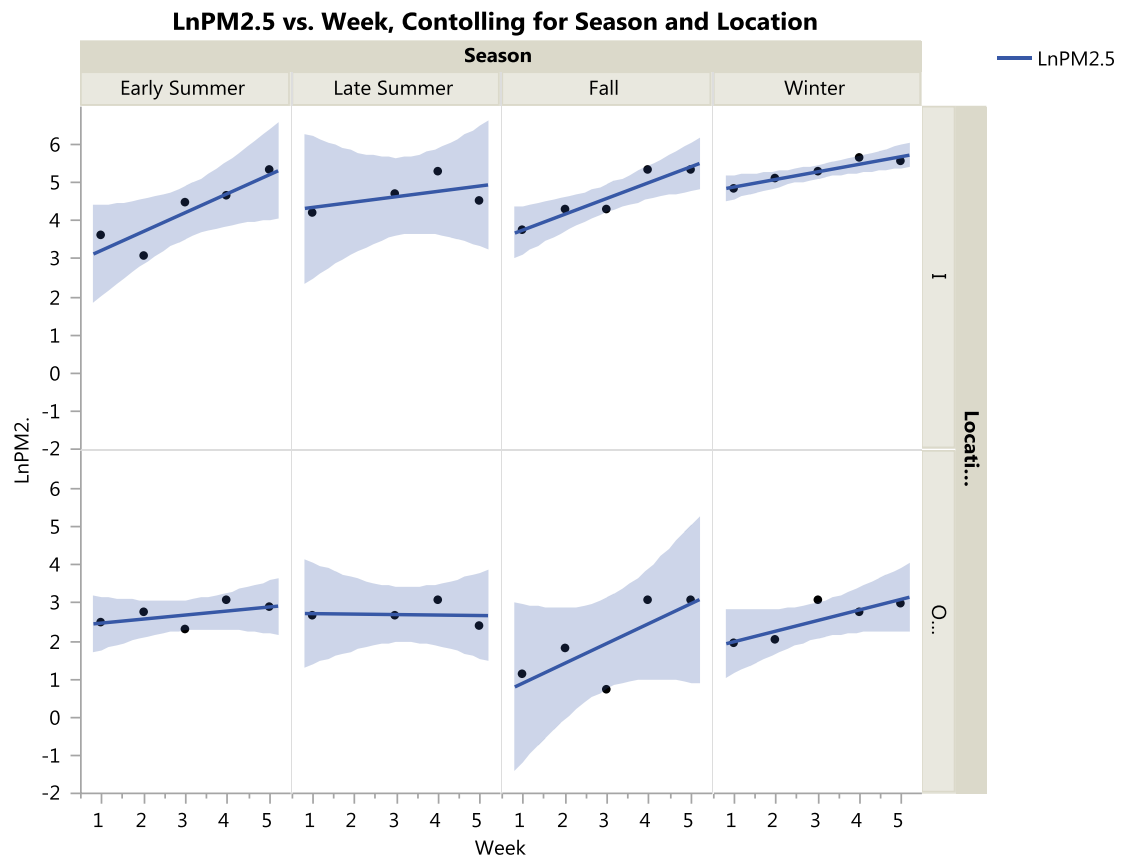


Figure B.2 Relationship between $PM_{2.5}$ concentration and week, $P < 0.0001$ when least squares fitting analysis was performed for season, location, and week.

Appendix C

ARSENIC SPECIATION AND LEAST SQUARES INTERPRETATION FOR EACH SEASON

C.1 Percent As for each “hotspot” analyzed using least squares fitting.

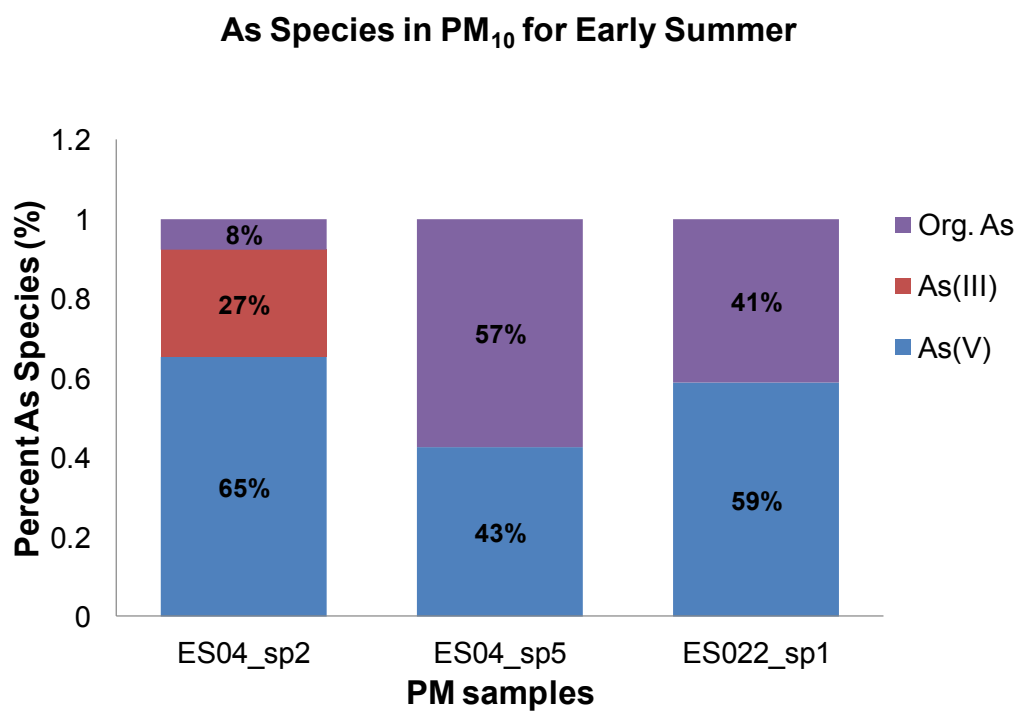


Figure C.1: Percent As for each “hotspot” analyzed using least squares fitting during the early summer sampling period.

As Species in PM₁₀ for Late Summer

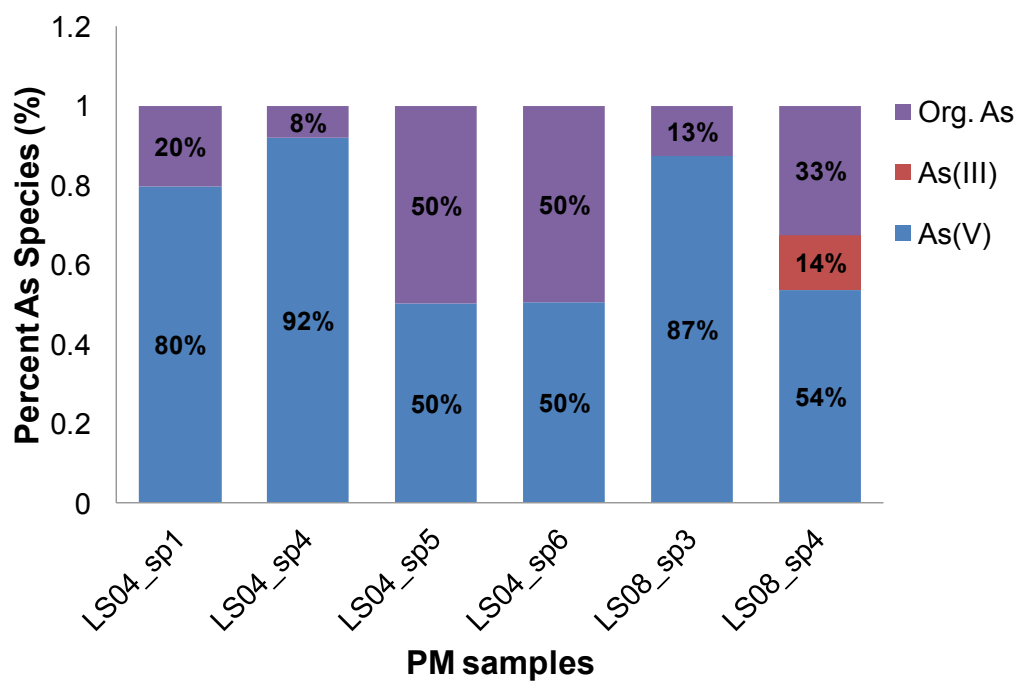


Figure C.2: Percent As for each “hotspot” analyzed using least squares fitting during the late summer sampling period.

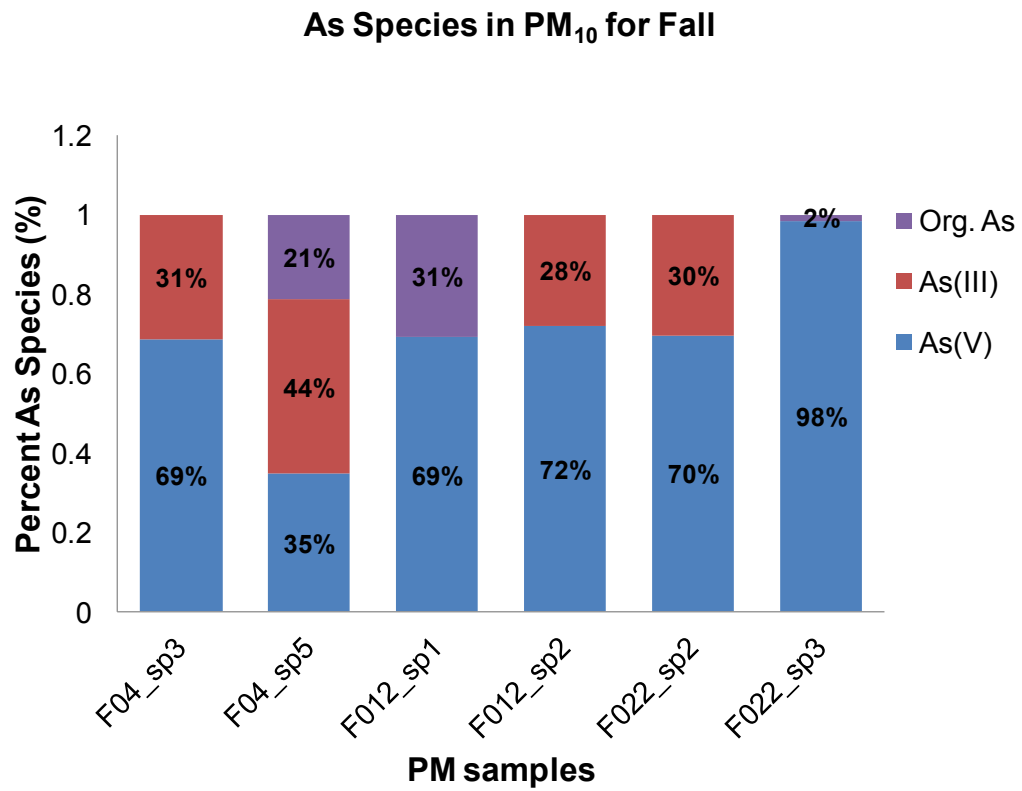


Figure C.3: Percent As for each “hotspot” analyzed using least squares fitting for the fall sampling period.

As Species in PM₁₀ for Winter

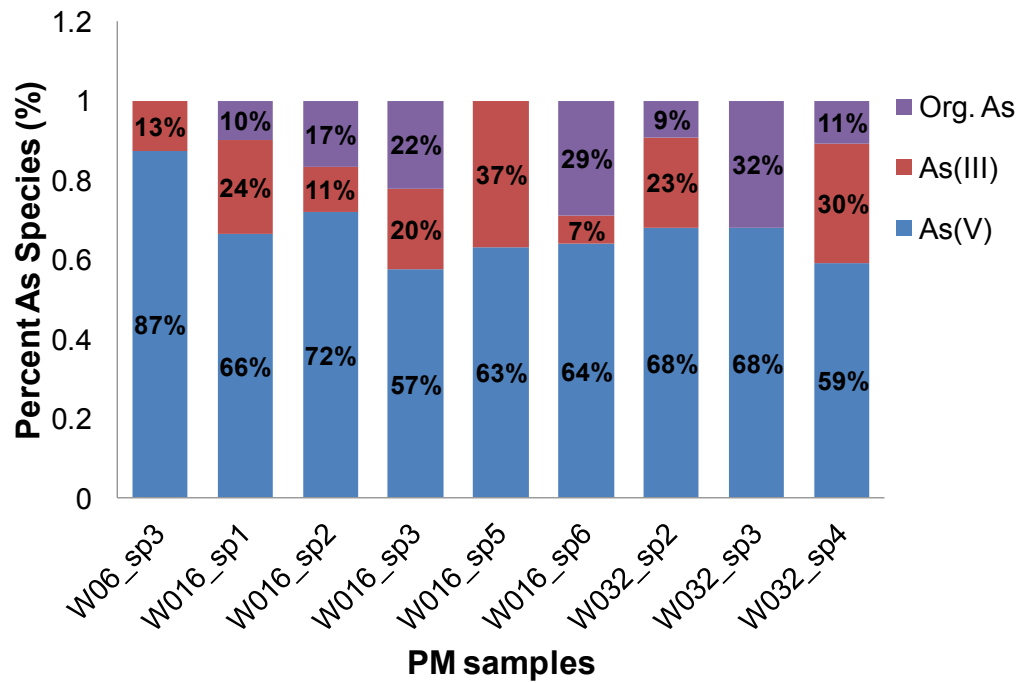


Figure C.4: Percent As for each “hotspot” analyzed using least squares fitting for the winter sampling period.