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Estimation of Aerosolized Antibiotic Resistant Bacteria Concentrations from Wastewater Treatment Facilities Using Spatially-Based Dispersion Modeling

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DEDICATION

I dedicate this thesis to my wonderful parents, siblings, and friends who have supported my passion for public health. I thank my parents for their constant support and encouragement in my academic accomplishments and my pursuit of a career in environmental health. I thank my siblings for their endless patience and care throughout my academic endeavors. I would also like to thank my friends, new and life-long, that have been a strong support system over the past two years. I am incredibly grateful to all of these wonderful people that have encouraged me to pursue my passion for environmental health.

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ABSTRACT

Pathogenic bacteria can develop resistance to antibiotics to which they were previously sensitive, resulting in increased morbidity and mortality worldwide [1]. The increase in drug resistance in bacteria is an emerging public health concern. The activated sludge tanks of wastewater treatment plants are known sources of antibiotic resistant bacteria. These resistant bacteria can become aerosolized and disperse downwind. Using previously sampled aerosol concentrations and meteorological data, a Gaussian dispersion model was developed to estimate the concentration downwind from a selected wastewater treatment facility. The emission rate was calculated to be 7,941 CFU/m^2/sec ± 2,149 CFU/m^2/sec. This value was used in Arcview 10.1 to construct a visualization of the concentration of antibiotic resistant bacteria (ARB) downwind from the activated sludge tanks during specific meteorological conditions. This was accomplished through the identification of the activated sludge tanks as the source cells through the use of grid fishnet overlay. Two final layers displayed the dispersion of ARB downwind from activated sludge tanks. The concentrations were highest directly downwind from the activated sludge tanks. The demonstration of the ability of a GIS model in the visualization of bacteria dispersion signifies the potential use of spatial modeling in future environmental epidemiological studies and the use of spatial modeling to identify geographic areas of interest.

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LIST OF ABBREVIATIONS

ARB .... Antibiotic Resistant Bacteria

GIS .... Geographic Information Systems

MDR .... Multidrug Resistance

UTM... Universal Transverse Mercator

WWTP .... Wastewater Treatment Plan

CHAPTER 1

BACKGROUND

The rapid expansion of antibiotic resistant bacteria is seen as one of the major emerging public health issues in modern society. Pathogenic bacteria can develop resistance to antibiotics which previously were successful in combating infections caused by specific bacterial strains, resulting in increased morbidity and mortality worldwide [1]. For example, the World Health Organization estimates that there are nearly 630,000 cases of multi-drug resistant (MDR) *Mycobacterium tuberculosis* infection worldwide [1]. The methods used by bacteria to protect themselves from antibiotics and the means through which they attain resistance vary with four identified pathways. The bacteria can: 1) prevent the antibiotic from reaching the site of action, 2) prevent the antibiotic from entering the cell or increase the rate of excretion, 3) produce an alternative target for the antibiotic, typically enzymes, or 4) alter the site of action [2]. Microbes – especially pathogenic bacteria – can have resistance to antibiotics naturally and pass this genetic material to offspring through replication. Bacteria can also acquire genetic resistance through the exchange of genetic material from cell-to-cell interactions through horizontal gene transfer [3]. There are three known ways that bacteria can acquire antibiotic resistance through horizontal transfer. One such method is *conjugation*, occurring when bacteria are in very close proximity and directly pass genetic material. A second method, *transformation*, occurs when a bacterial cell takes up genetic material from the medium in which it is growing. *Transduction* is the third mechanism through which antibiotic resistance can be acquired by bacterial strains. Through this method a bacteriophage (e.g., a virus) carries the genetic material into the bacterium cell from a donor cell [4].

The overuse and misuse of antibiotics is an important factor in the increased prevalence and spread of antibiotic resistance [5, 6]. In 2010, 258 million courses of antibiotics, or 833 per 1000 persons in the US, were prescribed by health care providers with rates higher in southern regions of the United States [7]. A 2005 analysis of 14 estimates of prescription antibiotic use in North America indicated 57.4% compliance in antibiotic therapy use with a worldwide prescription compliance of 62.2% The 95% confidence interval for the North America range of 44-70.8%, demonstrates a misuse of antibiotics [8]. With high antibiotic use and misuse, trace amounts of antibiotics can be found in wastewater, natural waterways, and soil [9-11].

The wastewater treatment process has been investigated as a source of antiobtic resistance in bacteria. The wastewater treatment process begins with primary treatment when raw sewage, known as influent, enters the plant. The treatment process for a WWTP can vary, but often consists of several major processes. Primary treatment involves the removal of large debris and grit. The primary treatment process removes particles that cannot be decomposed later in secondary treatment. The secondary process utilizes bacteria and other microorganisms to degrade biological content. There are different approaches that can be utilized for this process, which will alter the dispersion of bacteria downwind. Those systems which utilized mechanical agitation of the influent generate larger amounts of bioaerosols than air diffuser aeration systems; the mechanical agitation generated between 450 and 4580 CFU/m3 of bacteria concentrations where the diffuser aeration systems generated only 22-57 CFU/m3 [12]. Oxygen is introduced into the activated sludge tank for the aerobic microbes to utilize for metabolic processes. The tertiary treatment is the disinfection treatment. This procedure may be a chemical treatment involving chlorination or sodium hypochlorine treatment. Alternatives for tertiary treatment may be UV radiation or ozonation treatment. Once treated, the treated water discharges into an environment, typical a waterway such as a river, stream or bay.

Wastewater influent and effluent contains a wide range of prescription drugs including antibiotics, diuretics, psychiatric, anti-inflammatory, and lipid regulating drugs [13]. The conditions of activated sludge tanks in a wastewater treatment plant (WWTP) are conducive to the propagation of antibiotic resistant bacteria [14]. The presence of antibiotics in wastewater and high microbial diversity and density has been linked to antibiotic resistance in bacteria in WWTP facilities [15]. In several studies, six main antibiotic classes were identified in wastewater. Antibiotic concentrations in the influent have been identified in concentrations from 61-64 μg/L and included β-lactam, sulfonamides, tetracycline, fluoroquinolone (ciprofloxacin), macrolide, and trimethoprim. Soluble concentrations existed in a range from less than or equal to 1.3 μg/L up to 3.4 μg/L in the effluent, relatively unaffected by the size of the wastewater treatment facility [11, 16]. The presence of an antibiotic in wastewater has been associated with the development of resistance to that antibiotic in bacteria [6]. In *Escherichia coli* strains tested from WWTPs, 40% were resistant to at least one antibiotic, with 31.8% acquiring resistance [17]. Zhang *et al*. (2009) investigated the resistance of *Acinetobacter* spp. isolates due to wastewater processes. Research by Zhang *et al*. displayed an increase in antibiotic resistance due to wastewater processes with 98% of isolates resistant to at least one antibiotic and 3.6% resistant to five or six antibiotics [18]. High prevalence of resistance was shown to exist against several of the leading antibiotic therapies. The portion of total heterotrophic bacteria resistant to penicillin, ampicillin, cephalothin, and chorolramphenicol were 63%, 47%, 55%, and 69%, respectively [19]. Li *et al.* (2013) found that 52% of the aerosolized bacteria from WWTP processes were smaller than 3.3 µm, with 65% of Gram-positive *Actinomycetes* bacteria within the respirable range [20]. The research indicates that the activated sludge tanks serve as a source of ARB, which can be potentially harmful to human health and the environment.

The concentrations of aerosolized bacteria and fungi differ with the stage of treatment within a WWTP with concentrations highest at the sewage inflow and aeration tanks [20-22]. The concentration of airborne bacteria downwind is dependent on the operation and processes of the WWTP [12, 23]. The effluent of bacteria has shown significant variation in bacteria density when comparing WWTP with different dimensions and filtering processes [24]. A study by Gibbs, *et al.* (2006) was conducted to study the concentration of (ARB) downwind from an animal feeding operation. The average concentration of ARB was 63 CFU/m^3 at a point 25 m upwind from the facility. The concentration of the ARB within the facility was the highest with average concentration of ARB equal to 18,132 CFU/m^3. The concentration of the ARB then decreased as the distance downwind from the facility increased. 150 meters from the facility was the furthest point sampled and resulted in ARB concentrations of 141 CFU/m^3. The percentage of bacteria found resistant to antibiotics was also higher than the upwind bacteria. [25]

Kaarakainen *et al.* (2011) investigated differences in bioaerosol concentrations from various bioaerosol emission sources. The research identified waste centers, sewage treatment plants, farming environments (swine), and manure spreading as sources of bioaerosols. For the WWTP, the concentrations of bacteria and fungi were inversely related to the distance from the wastewater facility. Viable bacteria concentrations were roughly four times higher within the plant than 50 or 200 meters downwind. The total concentration of bacteria within the plant was 4320 cells/m^3 that decreased to 392 cells/m^3 measured at 200 m from the facility. The concentration of bacteria within the plant was 1743 cells/ m^3 for Gram-negative bacteria. The concentrations of Gram-negative bacteria for a distance 50 and 200 m downwind were 385 cells/m^3 and 298 cells/ m^3, respectively. The concentrations of ARB above upwind levels show that communities closely downwind from ARB may be potentially exposed to ARB. [26]. The United States Environmental Protection Agency (EPA) does not have regulatory standards for aerosolized bacteria concentrations as it does with other air pollutants, thus determining a safe distance from bacteria point sources is a challenge. Research in the exposure and adverse health outcomes due to aerosolized bacteria concentrations is a critical step in the determination of health outcomes and establishing regulatory standards or recommended aerosolized bacteria concentrations.

The size of particulate matter, including bioaerosols, greatly affects the deposition of bacteria in the respiratory system [27]. Particle sizes smaller than 10 µm are inhalable particles that can potentially penetrate deeper into the lungs. The ultrafine particles, ranging in size form 0.1 µm – 1.0 µm can remain airborne for long periods of time and can penetrate all levels of the respiratory system. The entrance and deposition of pollutants into the respiratory system are dependent on particle characteristics, individual physiology and environmental surroundings. Particle diffusivity, solubility, reactivity, and size, the airflow characteristics in the respiratory system, and the physiology of the respiratory and the vascular system are all contributing factors to particle exposure and biological dose. [28].

Importantly, time of day, solar radiation, relative humidity, and temperature are factors that impact the concentrations and viability of aerosolized bacteria. Increased solar radiation resulted in a decrease in atmospheric bacteria concentrations, however the sensitivity of the bacteria to solar radiation was impacted by the time of day the bacteria were sampled [21, 29, 30]. Increased temperature, especially temperatures above 24˚C, decreases viable, aerosolized bacterial concentrations. [31-33]. Relative humidity has yielded different results, which have been attributed to differences in experimental conditions and differences in species [32]. Generally bacterial concentrations and humidity have a negative correlation [34, 35]. The differences in concentration may be attributable to the idea that higher humidity favors the viability of bacteria whereas lower relative humidity is favorable for spore formation[35]. The dissemination process of the bacteria into a bioaerosol will also affect the bacteria’s viability due to humidity. Wet dissemination occurs when particles (e.g., bacteria) are aerosolized from a liquid such as the aerosolization of particles from an aeration tank or human mucus. When the bacteria *Pasteurella tularensis* is aerosolized from a liquid origin, it has the lowest viability in air at 50-55% humidity. In this same study, particles disseminated from dry origins have the lowest survival at 75% humidity for the same species of bacteria [36]. In addition to their effect on dispersion, the meteorological factors impact the survivability of bacterial cells.

Bacterial survival was found to be greatest at high humidity (70-80%) and low temperatures, nearly 12˚C. The size of the aerosolized droplet was also considered. Survival was reduced with a reduction in droplet size, which may be attributed to reduced evaporation [37]. Brooks *et al.* (2005) developed a transport model that predicted the concentrations of virus and bacteria downwind during the application of biosolids. A regression model was constructed and determined that, of the meteorological factors, temperature and wind speed were significant to the dispersion of bacteria, but humidity was not statistically significant. The final regression model for the scenario was $y = -0.0022x = 0.1849$ with y equal to the concentration of aerosolized coliphage dependent on distance X (meters). The model was then used to assess risk of infection to exposure to these viruses and bacteria. Brooks (2005) indicated that the dose to organism ratio is impacted by the number of organisms/m^3, breathing rate of the individual, and the exposure duration (hrs). For residents, the overall concentration and risk of infection was lower due to increased distance from the pollutant source. [38]

The dispersion of ARB particles downwind are a concern for human and environmental exposure. Resistant bacteria has been found in soil, water, and sediment[39-41]. The presence of antibiotics and corresponding resistant bacteria in the environment is problematic due to bacteria's ability to replicate and take up genetic material from the surrounding environment. The survivability of bacteria after aerosol dispersion could result in the bacteria replication in a host environment due to the protective effects of humidity [37]. Even with cell death, genetic material coding for resistance can be released into the environment. This genetic material can be taken up by bacteria through horizontal transfer mechanisms in originally non-resistant bacteria.

Occupational health and safety research of health outcomes of WWTP employees has shown workers at a wastewater facility experience adverse health impacts with causes still unknown. Employees have reported airway and gastrointestinal inflammation, nose and throat irritation, headaches, diarrhea and fatigue [42, 43]. A study of the risk of infection among WWTP employees has shown that Hepatitis A antibodies are found in greater prevalence among WWTP employees than the control groups [44-46]. A study conducted to investigate mortality and cancer incidence for wastewater treatment facility employees found that despite differences in overall mortality, WWTP employees did have statistically higher odds for mortality from stomach and prostate cancer[47, 48]. A literature review by Thorn and Kerekes (2001) further illustrates potential health outcomes due to employment at a WWTP where exposure to ARB are higher than surrounding areas, found that there is a need for greater research on the causality of these adverse health outcomes [49].

With occupational health outcomes including respiratory, gastrointestinal, and other symptoms, the exposure of bioaerosols from WWTP have the potential to impact communities downwind from the activated sludge tank source. Further research should be conducted to find the causality of these health outcomes, the potential exposure from inhalation, and risk levels of communities near WWTPs.

CHAPTER 2

GOALS AND OBJECTIVES

Given that research indicates adverse health effects for WWTP workers where ARB concentrations are high and that many ARB are found within the size range to travel a substantial distance from the source, further research into the downwind concentration and potential exposures to residents and surrounding environments is necessary. The presence of ARB in bioaerosols emanating from WWTP activated sludge tanks translates into concentrations of resistant bacteria within and downwind of a facility [26]. The goal of this research is to estimate the concentration of ARB in the air downwind of a WWTP and to visualize its distribution using Geographic Information Systems (GIS). Utilizing the Gaussian dispersion model, this research will estimate the concentration of particles carrying ARB emitted from the facility's activated sludge tanks at downwind and crosswind locations. This research contributes to efforts intended to provide data on potential downwind concentrations of ARB from a facility that could expose plant employees and community residents to drug-resistant bacteria leading to possible adverse health impacts, but also work towards future efforts to decrease exposures to persons located downwind of plant activated sludge tanks.

The goal of this research is to apply a spatially-based dispersion model for estimating potential exposure to ARB downwind from a WWTP. The objectives are to 1) assemble a spreadsheet for estimating emission rates of aerosols containing ARB using previously measured concentrations at several locations near the activated sludge tanks;. 2) integrate emission data and meteorological data into ArcGIS 10.1 © as raster layers in order to implement the dispersion model; and 3) construct spatially–based dispersion GIS layers through the use of emission information, meteorological data, raster calculations, and Gaussian dispersion equations.

CHAPTER 3

METHODS

3.1. ARB Concentrations and Meteorological Parameters for Model

To measure the presence and existence of ARB from the secondary treatment of wastewater treatment facilities, previous research was completed to monitor and analyze aerosolized bacteria particles due to the secondary treatment process at WWTP in the Southeastern region of the United States. On August 5, 2010 both meteorological data and air samples were collected concurrently. The aerosol samples were collected for sixteen minutes using a Sartorius (Goettingen, Germany) MD8 Airscan sampler with a sampling rate of 7.5 m^3/hr. Three sets of data were taken simultaneously at locations surrounding the aeration tanks; a total of 9 samples were collected. Two samplers were positioned downwind from the activated sludge tanks while one sampler was positioned directly upwind from the activated sludge tanks. The depiction, as illustrated by McKenzie (2011), of the locations of samplers and summarized meteorological data is featured in Figure 3.1. The sampler DW-SL represents the ground level sampler is nearest to the ground at an approximate height of 0.2 meters. The breathing zone sampler, represented by DW-BZ below, was positioned directly above DW-SL at a height of approximately 1.524 meters. The upwind sampler (UW) is directly above six of the activated sludge tanks as shown in Figure 3.1. The meteorological data was collected simultaneously with the ARB concentration sampling. The measurements were taken using a Kestrel® (Boothwyn, PA) Pocket Weather Tracker with the wind direction recorded from a local weather station nearly 6.4 kilometers away. After the air samples were cultured in growth media containing several levels of eight major antibiotics, the 16S rRNA gene was extracted and amplified from each bacterial isolate for classification. The complete methodology for this work can be found in McKenzie (2011) [50].

Table 3.1 Measured Meteorological Data on August 5, 2010 from McKenzie (2011)

	Mean	Minimum	Maximum
Temperature	32.5˚C	31.5 ˚C	33.5 ˚C
Relative Humidity	55.30%	53.80%	57.20%
Wind (SSW)	3.31 m/s	2.15 m/s	4.96 m/s

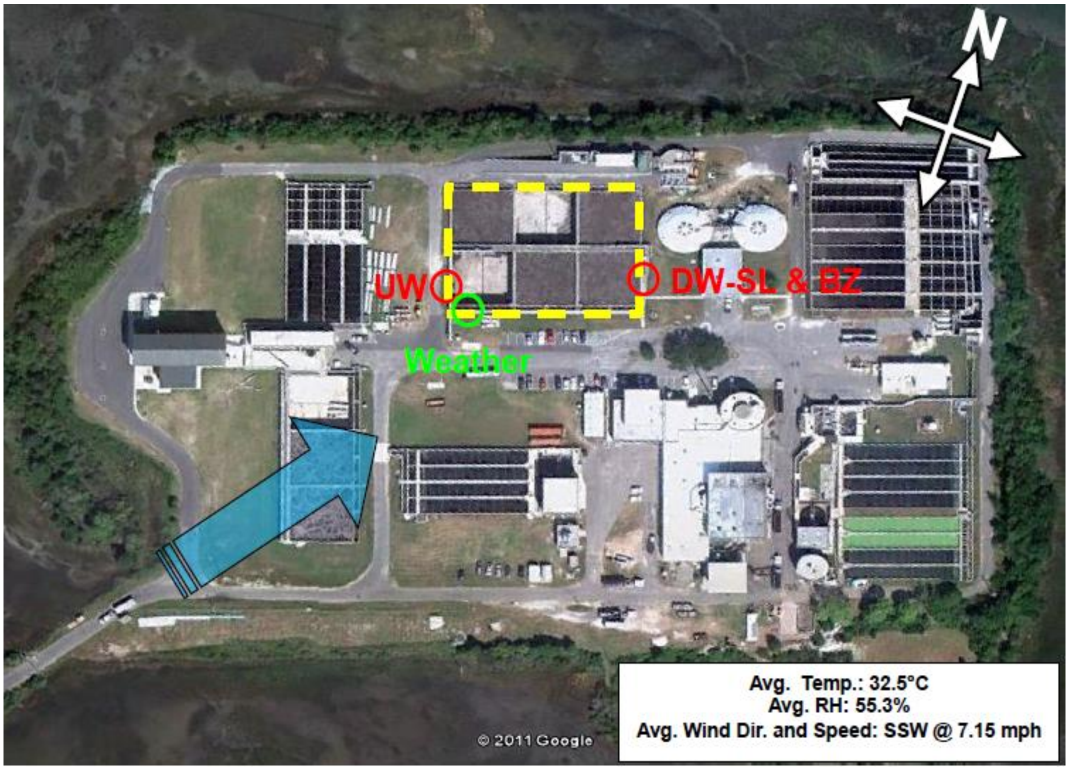

Figure 3.1 Sampler locations and summarized meteorological data (McKenzie 2011)

McKenzie's work displayed an increase in concentration of ARB at the downwind sample sites, as summarized in Table 3.2. For six of the eight antibiotics tested, resistance was higher in the downwind samples than in the upwind samples. Two family/genera of bacteria were resistant to multiple antibiotics in the upwind samples. The downwind sample sites had five genera that displayed resistance to multiple drugs. Gram–negative bacteria comprised of 84% of the total ARB population in the upwind sample found in Table 3.2, while the percentage of Gram-negative bacteria in the downwind sample decreased to 65 % and 66% of the downwind ARB concentrations. McKenzie also found that though the particle count did not significantly vary, the concentration of ARB was higher at the downwind locations than the upwind locations, suggesting the aerosolized particle composition was altered by the emission from the activated sludge tanks.

Table 3.2 Concentrations of aerosolized ARB at WWTP facility (McKenzie 2011)

Sampler	Mean (CFU/m³)	Minimum(CFU/m³)	Maximum(CFU/m³)
Upwind (UW)	2,275	683	3,308
Downwind (DW-SL)	4,480	3,728	5,933
Downwind (DW-BZ)	5,145	1,890	10,238

The data from McKenzie (2011) was utilized to develop a visualization of the downwind concentration of aerosolized ARB from the activated sludge tanks of a WWTP. The measurement of aerosolized ARB in addition to the concurrent measurement of meteorological data enables the development of a spatially–based dispersion model that estimates the concentration of ARB at given downwind distances using the commonly referenced Gaussian dispersion model.

3.2 The Gaussian Dispersion Model

The transport of a particulate in the atmosphere is dependent upon the size, shape, and density of the particle, as well as physical source characteristics and meteorological parameters. The Gaussian dispersion model is one of the mathematical models that can be used to estimate the concentration of pollutants downwind from a point source. The model is based on the concept of normal distribution of the particles downwind from the plume. The size of the particles sampled by McKenzie (2011), including the ARB, are less than 20 µm, allowing for the assumption that the particles behave very much like a gas-phase contaminant dispersed in air [51]. Modeling the concentration downwind from a continuous area source, such as an activated sludge tank from a WWTP, results in the equation given in Equation 3.1:

Equation 3.1: Gaussian dispersion equation for contaminants emitted at ground level (H=0) from Wark (1998):

$$C(x, y, z) = \frac{Q}{2\pi\mu\sigma_y\sigma_z} * \exp\left[\frac{-(y-y_0)^2}{2\sigma_y^2}\right] * \exp\left[\frac{-(z-z_0)^2}{2\sigma_z^2}\right]$$

Where:
C = concentration of pollutant at point (x, y, z) in units CFU/m³
$Q =$ emission rate of the source (mass/time)
$\mu =$ average wind speed
x = coordinate position of point of interest downwind
y = distance of point of interest perpendicular to plume direction
$y_0 =$ location on plume where point of interest is perpendicular
z = distance of point of interest in vertical direction
$z_0 =$location on plume where vertical point of interest is perpendicular
$\sigma_y =$ horizontal dispersion coefficient
$\sigma_z =$ vertical dispersion coefficient

For the Gaussian dispersion equation, y_0 and z_0 are descriptive of the centerline of the plume. The vertical and horizontal dispersion coefficients are a function of downwind distance from the source and atmospheric stability as shown below.

$$\sigma_y = cx^d$$
$$\sigma_z = ax^b$$

The equations above are based on the Pasquill-Giffords curves which model horizontal and vertical dispersion as a function of the downwind distance from the source for a rural environment at a given stability category. Stability categories were presented in 1961 and have since become commonplace in dispersion models. There are six stability categories (A through F) where the A stability category is the most unstable condition. The final stability category, F, is moderately stable. The stability category during air sampling was B determined from the time-of-day, solar radiation, cloud cover and surface wind speed for a rural environment. . The rural distribution curves model dispersion through relatively open land, which describes the land upwind from and immediately downwind from the WWTP [51, 52]. The assumptions made in the application of the Gaussian dispersion model as made in other Gaussian dispersion modeling [51, 53]

1. The emission of ARB from the activated sludge tanks is constant and continuous for each operating activated sludge tank
2. The meteorological measurement are constant through the application field
3. The particles will behave in the air in which they are immersed due to low settling velocity because of their size less than 20μm.
4. The wind direction is perpendicular to the field

The modeling of pollutant concentrations using Gaussian dispersion is only applicable in the lower one hundred meters of the atmosphere. Two heights were selected for modeling downwind dispersion. The first height, 1.524 meters (5 ft), was selected due to the relative height of the sampler by McKenzie (2011) and is also an estimate of the contact zone for adults. With the average height of females and males measuring 1.621 m and 1.759 m, respectively; the average height of the population is 1.69 meters (or 5 ft 6 in) [54]. The nasopharyngeal contact region is estimated to be 0.166 m (6.5 inches) lower resulting in a height of 1.524 m. The second height, 0.2 meters was the known height of the DW-SL sampler and allows for comparison in height difference downwind from the pollutant source.

3.3. Estimation of Emission Rate of ARB

Using the mean concentration of ARB for each of the three samplers found by McKenzie (2011), the emission rate of ARB was estimated using Microsoft Excel spreadsheets and the Gaussian Dispersion equation. A coordinate system was positioned onto the WWTP facility using Google Earth™ and the dimensions of the activated sludge tanks and surrounding structures were measured using the Google Earth™ “ruler” tool. The square activated sludge tanks were divided both horizontally and vertically to form 100 cells per activated sludge tank. The distance from the samplers to the center of each smaller grid cell determined by trigonometric calculations, which allowed for the application of the Gaussian equation to all 600 smaller grid cells, as in Kumar and Bhat (2008) The distance from the center of the cell to the sampler was found through Euclidean distance calculations as shown in Equation 3.2. The resulting distance (D) was then utilized to find the downwind distance (X) along the plume center line to the point of intersection of a perpendicular line that passes through the sampling point of interest., This calculation is shown in Equation 3.3.

Equation 3.2 Calculation of Euclidean Distance (meters)

$$D_j = \sqrt{[(C_{xj} - S_x)^2 + (C_{yj} - S_y)^2]}$$

Where C_x = X coordinate position of center of cell j
C_y = Y coordinate position of center of cell j
S_x = X coordinate position of sampler
S_y =Y coordinate position of sampler

Equation 3.3 Calculation of Downwind distance X

$$X = D_j \cos(\theta)$$

Where D_j = Euclidean distance (m) between the cell j and the sampler
θ = angle (radians) between the wind line and the sampler

In order to estimate the emission rate of ARB from the activated sludge tanks, Excel was utilized to calculate a hypothetical aerosolized concentration, using a hypothetical emission rate of 1 CFU/m²/sec, the meteorological data, and the Gaussian Dispersion Equation above. The area of each cell was 4.7844 m² resulting in an emission rate of 4.7844 CFU/sec. This assumption allows for the calculation of each cell's contribution to the hypothetical concentration measurement. Once the hypothetical concentrations were calculated for both the downwind Breathing Zone (DW-BZ) and near ground (DW-SL) sampler, they were utilized to find the proportional contribution of each cell to the sampled ARB concentration using the concentrations found in McKenzie (2011). Once this was achieved, the Gaussian dispersion equation was then utilized to back calculate the actual emission rate (Q) per square meter. The emission is assumed to be constant for each of the activated sludge tanks. This assumption is made through the observation of uniformity across the operating activated sludge tanks. The activated sludge tanks are assumed to experience the same meteorological conditions.

3.4 Development of the spatially –based dispersion model

Geographic Information System (GIS) is a computer based system used to aid in the collection, maintenance, storage, analysis and output, and distribution of spatial data and information [55]. Here, ArcGIS 10.1, allowed the implementation of a spatially-based dispersion model. ESRI ArcView's was selected for this modeling due to its capability to display complex spatial and temporal data in an easily understood geographic format [56]. The use of GIS spans many research fields from the social sciences, public health, and geography – continually expanding to new research fields and increasing use for city and emergency management[57]. The use of spatial and temporal modeling to display the transport and dispersion of aerosolized pollutants, along with population characteristics, enables the visualization of pollutant exposure and potential health outcomes [58]. The use of GIS in air pollution modeling can minimize the need to run hundreds of equations through the use of grid–based modeling [59]. GIS has many benefits that enable its widespread application and use in city planning [60]. GIS has been utilized to study the dispersion of NO_2, particulate matter, and other pollutants [61, 62].

Due to the continuous nature of the dispersion of particles, raster models were used to display the Gaussian dispersion of ARB. Features are represented as a matrix of cells rather than the specific points defined by coordinates as in vector data [63]. A fishnet grid with 30 m × 30 m overlay was placed over the WWTP and the surrounding 7 km using Universal Tranverse Mercator (UTM) coordinate system for spatial referencing and distance calculations. The size was selected to balance the short field application of the Gaussian dispersion model, the data available in the GIS storage, and the potential loss of data with too large of cell size [63]. The fishnet overlay allows for attributes of each cell including wind velocity, wind direction, and emission (for the source cells) to be added to the attribute table. Using the spatial analyst tool in ArcMap, the *Feature to Raster* was utilized to convert each feature in the attribute table to a raster.

The thirteen cells of the fishnet overlay that contained the operating activated sludge tanks were identified and the corresponding area was measured for each cell using the "ruler" tool. Each of these cells was identified as an ARB pollutant source. The emission for each identified cell was then calculated by multiplying the emission per square meter by the area of the activated sludge tank contained in the cell. A series of layers were constructed for each source cell that resulted in the raster calculation of the Gaussian dispersion equation. The layers were constructed for the breathing zone height and the ground level height corresponding to DW-BZ and DW-SL, respectively. The distance for each cell was the Euclidean distance from the center of the source cell to the center of the downwind cell as discussed above. Once the Euclidean distance and direction layers were constructed, the direction was reclassified in order to find the angle from the source cell to the downwind cell in radians and rid the layer of the upwind data. The reclassified angle was then used to find downwind distance X, using the trigonometric function mentioned in Equation 3.3. This downwind distance layer was then used for all of the subsequent layers.

For each of the source cells the following layers were constructed in raster format.

1. Emission source cell
2. Euclidean distance
3. Euclidean direction
4. Angle (radians) from cell center to source center
5. Downwind distance X (meters)
6. Downwind distance X (kilometers)

7. $TH = 0.01745\ (18.3330 - (1.8906 \ln X))$ when X is in kilometers
8. $\sigma_y = 465.11628 * X * \tan(TH)$
9. $\sigma_z = 90.673 * X^{0.93198}$
10. Full Gaussian equation at two distinct heights for each source cell
11. Full Gaussian equation at two distinct heights for all source cells

The equations for σ_y and *TH* are utilized in the Industrial Source Complex (ISC3) dispersion models developed under the guidance of the US Environmental Protection Agency. The equations are used to fit the Pasquill-Gifford curves mentioned above for rural models [51]. Once the layers were constructed for each of the thirteen source cells, the final concentration layers were added together to create one layer that described the concentration of all operation activated sludge tanks at the specific cell downwind at the breathing zone height. This was repeated for the near ground level height and the concentrations were compared

CHAPTER 4

RESULTS AND DISCUSSION

The use of the data provided by McKenzie (2011) supported the development of a spatially-based dispersion model using ESRI ArcGIS®. The estimation of the emission was the first step in the development of the dispersion model. The Excel spreadsheets were used to estimate the contribution of each of the 600 smaller grid cells to the bacterial concentrations at sampler locations. As found in McKenzie (2011), the cells closer to the samplers contributed a greater proportion of the ARB than cells farther away. The back-calculated emission rates for both the sampled ground level and the breathing level mean were averaged resulting in a mean emission rate of 7941 CFU/m²/sec (SD = 2149 CFU/m²/sec); this emission rate was multiplied by the source cell area resulting in the emission rate that varied depending on the area of the activated sludge tank contained in the raster grid cell. The same process was repeated with the minimum and maximum concentrations sampled as recorded in Table 3.2 yielding emission rates of 2930 (SD =1821 CFU/m²/sec) and 13739 CFU/m²/sec (SD =6162 CFU/m²/sec), respectively. Kumar and Bhat (2008) utilized Microsoft Excel spreadsheets to develop a dispersion model for predicting downwind concentration in unstable meteorological conditions during the application of biosolids to agricultural land. Kumar and Bhat sampled particle concentrations for several days, and a modified Gaussian dispersion model was developed. Showing that during high winds and unstable conditions, the modeling was more accurate in estimating concentrations [53]

The wind direction as recorded by McKenzie (2011) had a wind direction of 203 degrees clockwise from north i.e. coming from the south-southwest direction. This was used in GIS to find both the distance and direction to source cell as shown in appendix. The reclassification of angles in radians resulted in the layer also found in the included appendix. The distance downwind was found with the assumption that the wind line was perpendicular to grid cells at a given point downwind and the Pythagorean Theorem. Using trigonometric calculations and the reclassified angles, the distance downwind was calculated and displayed in Figure 4.1.

The full Gaussian dispersion model was calculated through the use of the raster calculation tool. Once the downwind distance was found, the succeeding values of the Gaussian dispersion model could be calculated. The raster layers displaying TH,σ_y, σ_z can be found in the included in the reference maps in Appendix A. These layers were all utilized in the calculation of the full dispersion model. The component of the Gaussian dispersion model that accounts for horizontal dispersion is shown in Figure 4.2. As a part of Equation 3.1, this portion of the Gaussian model shows plume shape from one emission source.

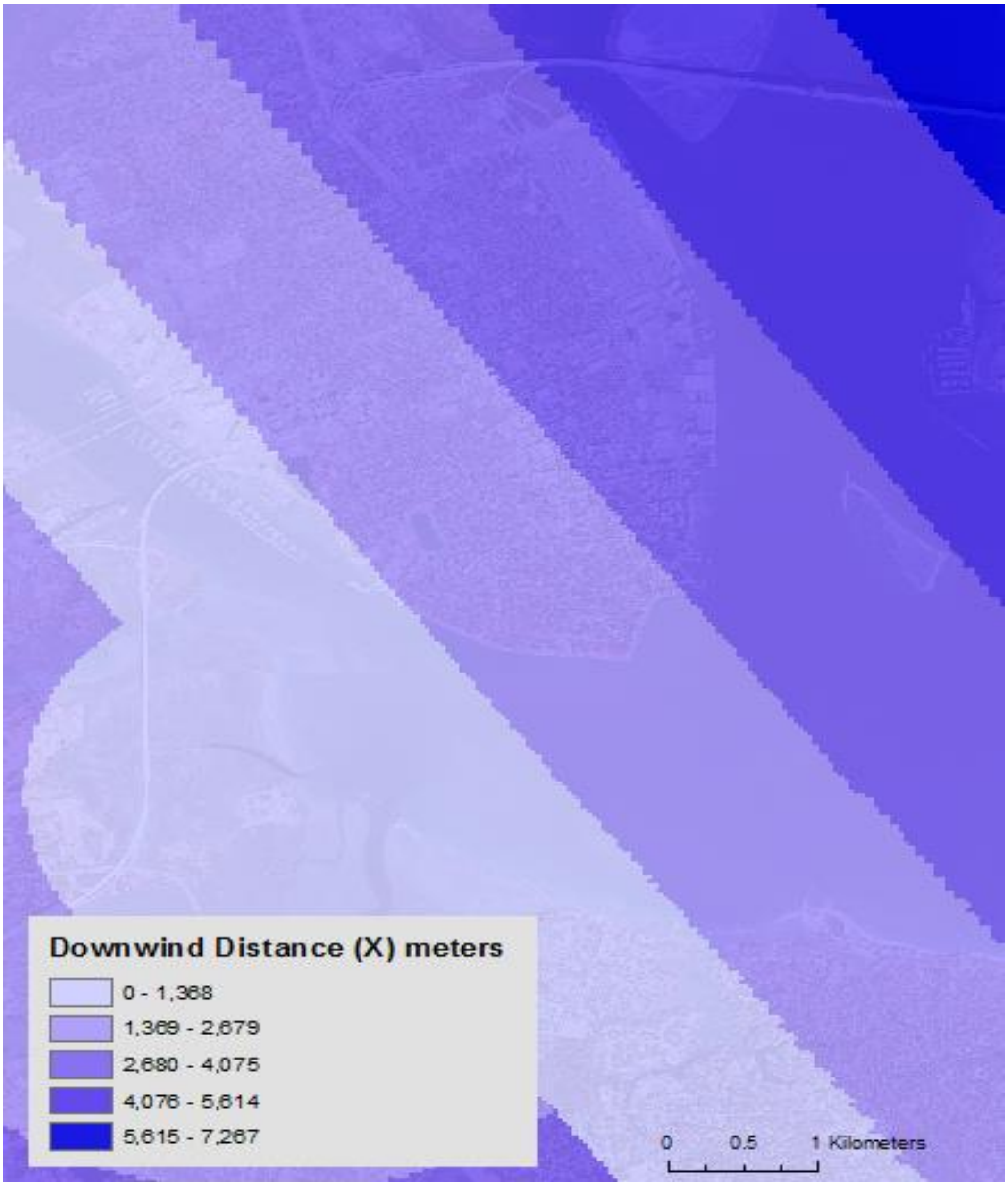

Figure 4.1 Downwind distance X (meters) for Gaussian dispersion model

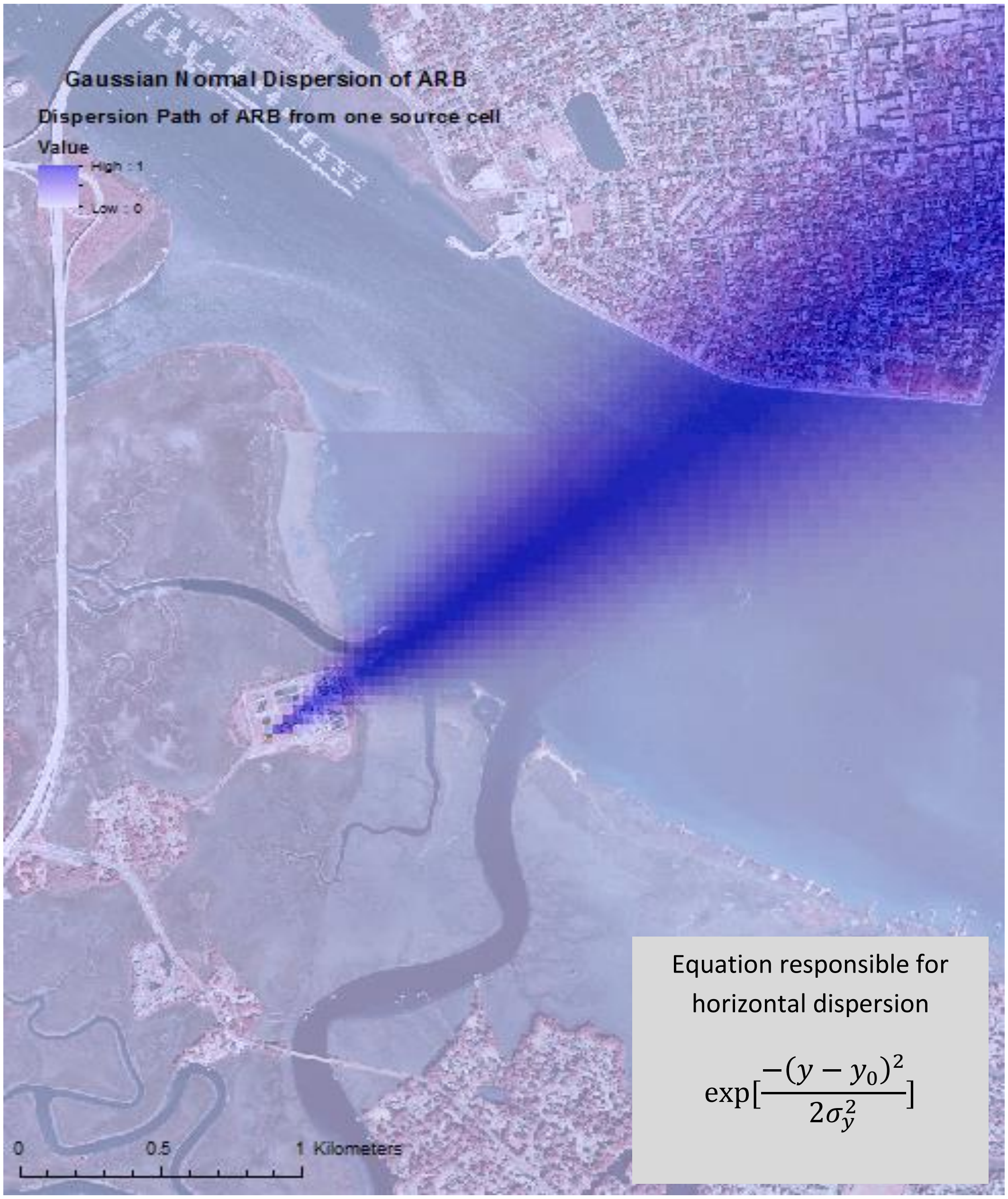

Figure 4.2 Horizontal dispersion of particles from one source cell

The full Gaussian dispersion model was applied, resulting in the estimated concentration of downwind ARB for one source cell at both the breathing zone height (1.524 m) and the ground level height (0.2 m), as shown in Figure 4.3 and Figure 4.4, respectively.

Figure 4.3 Concentration of ARB downwind from one source cell at 1.524 m

Figure 4.4 Concentration of ARB downwind from one source cell at 0.2 m

The models above demonstrate the inverse relationship between downwind distance and the concentration of ARB, and correspond with previous research [25, 26]. With increasing distance from the source cell, the concentration at both the ground and the breathing level heights decrease. The concentration in the WWTP modeled is higher than the concentrations found in other research. Brandi *et al*.(2000) measured the concentration of total viable bacterial concentration which showed no significant difference between ambient levels 50 and 100 meters downwind. This could be attributed to the difference in aerosol sampling methods, in addition to meteorological factors and secondary treatment methods [64, 65].

After repeating this process for all source cells, the layers were added together to estimate the concentration as a result of all operating activated sludge tanks. In total, over 250 layers were constructed to contribute to the information modeled in the final figures. The concentration modeled at the breathing zone height (1.524 m) is shown below as Figure 4.5 and the ground level height (0.2 m) is shown in Figure 4.6.

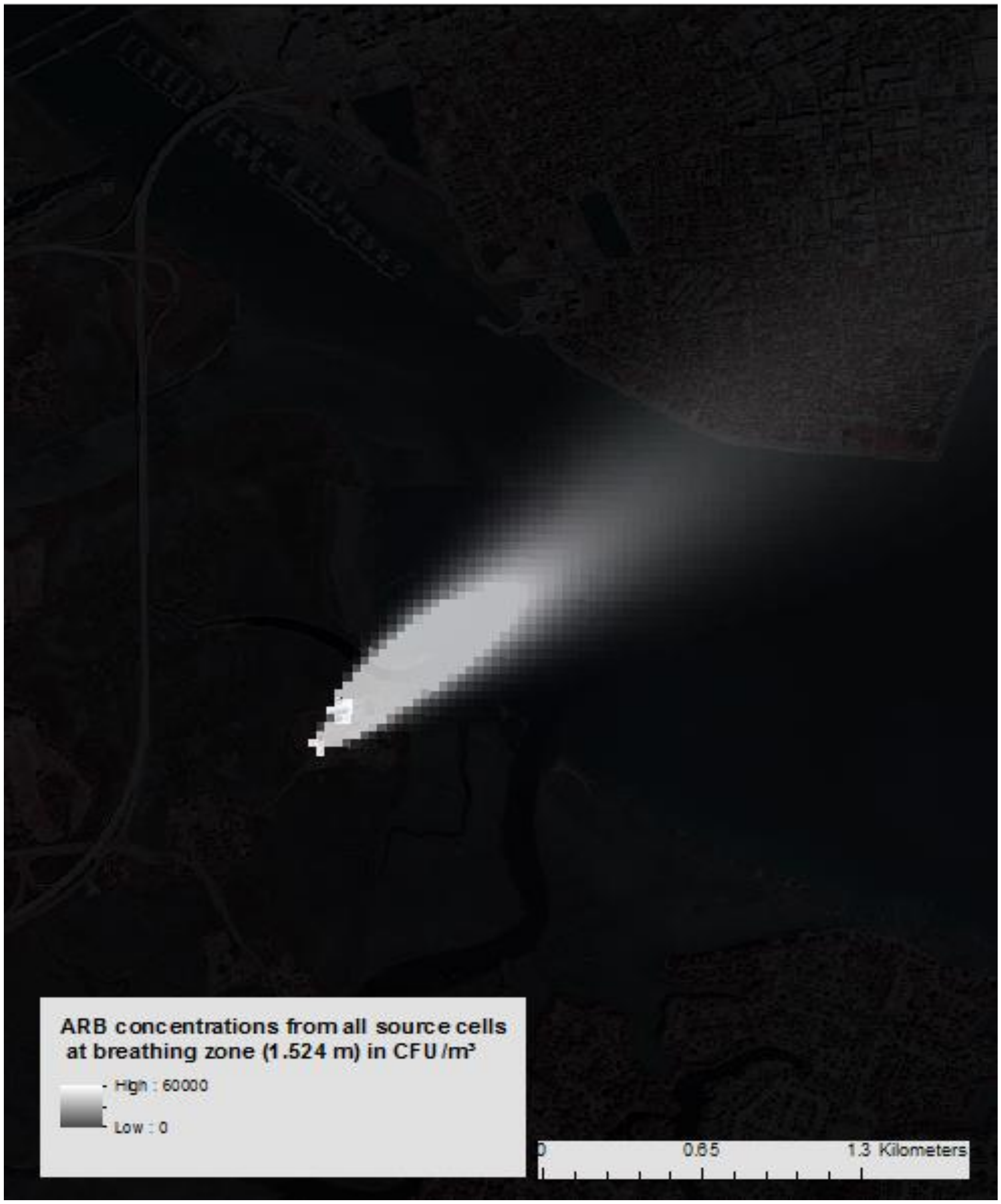

Figure 4.5 Concentration of ARB downwind from all source cells at 1.524 m

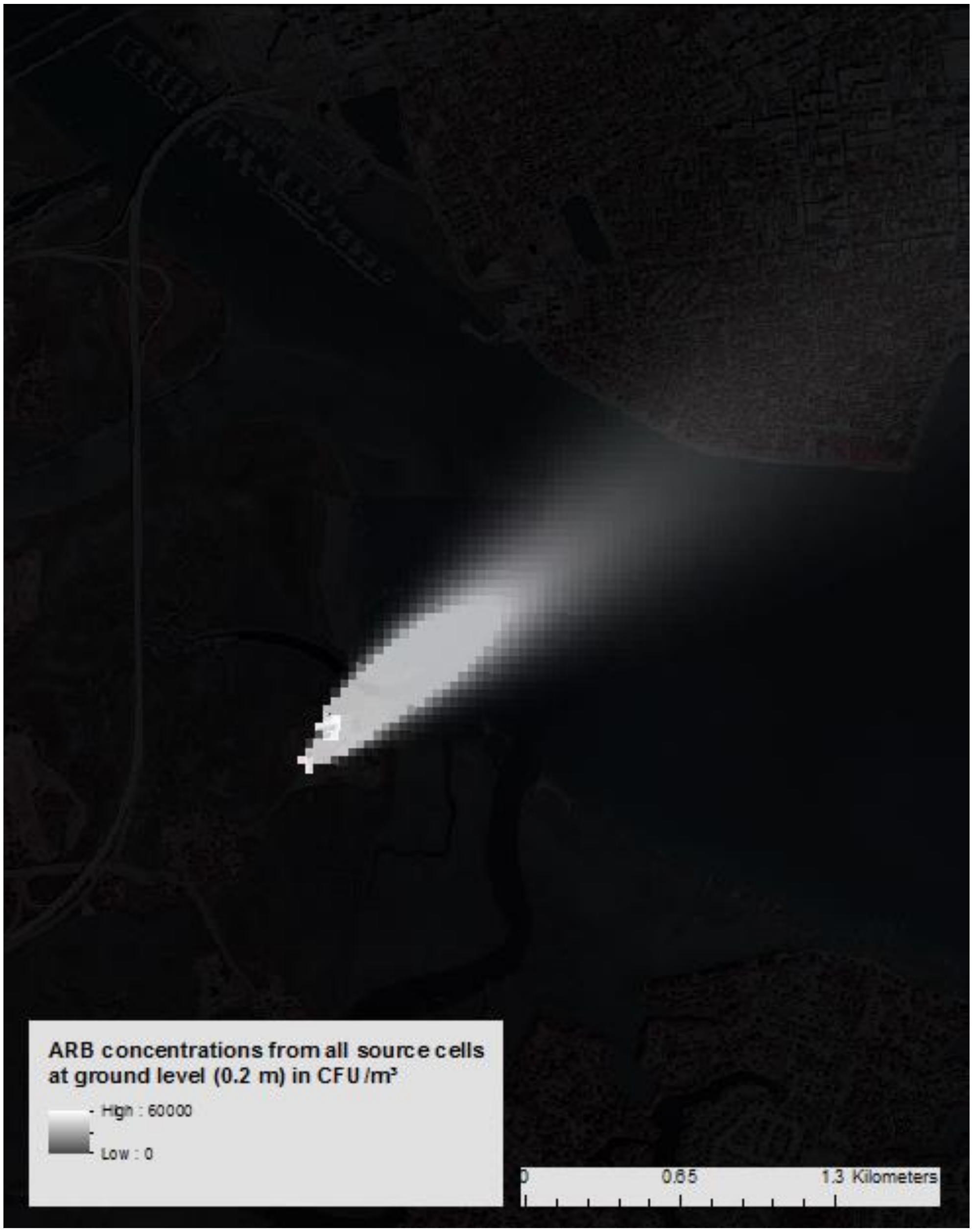

Figure 4.6 Concentration of ARB downwind from all source cells at 0.2 m

The above two final layers do not show a distinct plume shape downwind as the individual source cell do. This may be attributed to the high concentration of ARB directly above the activated sludge tanks and the addition of thirteen plume models.

The research by McKenzie (2011) does not provide a true upwind ambient concentration due to the placement of the sampler and the presence of an additional activated sludge tank slightly upwind of the sampler. Previous research by Gibbs (2006) can be thought of as the ambient concentration due to this lack of ambient upwind concentrations, though sampling measurements varied. ARB concentrations levels return to ambient levels nearly 1.3 kilometers away from the activated sludge tanks along the plume centerline. For the specific scenario modeled, the location of heightened ARB concentration exists over the Charleston Harbor. The potential exposure of ARB not only consists of the communities across the water, but also for those individuals spending time on the harbor for recreational or occupational purposes. Activities such as fishing, shrimping, sailing, where prolonged inhalation occurs downwind from the WWTP, may result in the exposure of those individuals to a higher concentration of ARB.

Outside of the overall plume, the concentration decreases substantially suggesting that the greatest concentrations are those directly downwind of the activated sludge tanks. The use of GIS in bacterial dispersion mapping is a useful tool in identifying hotspots where bacteria concentrations could have a negative impact on human and environmental health; the population exposed would change with changes in meteorological conditions. As stated previously, the United States Environmental Protection Agency does not have standards for overall bacteria concentrations nor ARB concentrations, thus more research should be conducted to establish regulatory standards of bacteria concentrations and establish a relatively safe distance downwind from ARB sources. The Republic of Korea established a maximum total bacterial bioaerosol concentration of 800 CFU/m^3 [66]. The United Kingdom has suggested airborne limits of 1000 CFU/m^3 for total bacteria concentration and 300 CFU/m^3 for Gram-negative bacteria[67].

Although there are many benefits to using GIS for particle dispersion, there are still some challenges in application and accuracy of modeling particle dispersion. Lin and Lin (2002) integrated a Gaussian dispersion model into GIS to model vehicle pollutant dispersion. The researchers utilized a grid system to estimate emissions. The model was found to overestimate the pollutant concentrations. In Lin and Lin's research, sampled pollutants were 20-50% of the modeled concentrations [68]. The concentrations modeled in this research are higher than other sampled ARB concentrations, suggesting a possible overestimation of the downwind concentrations [69]. Gulliver and Briggs (2005) note some of the challenges of modeling particle dispersion. Error is an issue when integrating dispersion equations into a GIS system. Though the error may not be larger than the simpler models, the additional time needed to integrate the model into spatial modeling software may not be necessary when there is no improvement in model error [70], which may not make the application for GIS modeling of ARB concentrations relevant for all scenarios. Another limitation of this spatially–based dispersion model is its application outside of the parameters used. Eslami *et al.* (2011) states that the integration of particle dispersion in GIS allows one specific scenario to be modeled at a time, which is true for this research. The given spatial model represents the concentration downwind solely for the given meteorological components. The estimation of ARB concentrations downwind model only the window of time where sampling occurred, indicating a need for additional scenarios or extrapolation of data, which may not give accurate results. The WWTP is placed in coastal region where wind velocity is frequently changing due to coastal influences. This change in wind direction and speed will alter the trajectory of the Gaussian plume resulting in a change of location and concentration of downwind concentrations. The population at risk for increased exposure will also vary with changes in wind direction and speed due to the shirt in plume direction. Though the Gaussian dispersion model and its modified versions are frequently used in particle pollutant dispersion, the model requires assumptions that often result in error. The wind velocity is assumed constant during the dispersion of the particle including the consistency of speed with variation in relative height; this may serve as a source of error [53]. This is particularly relevant for this research where the wind direction and speed may change frequently due to the facility's proximity to the coast. Additionally this model only models the dispersion of ARB without regard to other aerosolized particles. The interaction between ARB and other aerosolized particles may interact when aerosolized due to Brownian motion. This interaction is not considered as a component of the dispersion modeling, though it may impact dispersion and particle settling.

The visualization of dispersion from the activated sludge tank is an important step in the identification of ARB transport and exposure research. GIS in air quality, including this research, can result in the recognition of elevated risk individuals and the identification of hot spots which can improve risk management practices [71]. This has been shown in other GIS based dispersion research. Liao *et al.* (2006) applied GIS for exposure modeling. The use of GIS in Gaussian dispersion modeling was improved through the use of the geocoding capabilities of spatially based modeling, which allowed for the estimation of pollutant exposure at a specific point. Liao's research also noted that the use of modeling with GIS does not often account for the individualization of neighborhoods. Taking advantage of the geocoding abilities of GIS can mitigate this component of dispersion modeling [72].

This research shows potential for future investigations in particle dispersion and spatial modeling. There are several steps that could be taken to advance this research. One improvement would be the increase of sampling from sites downwind and upwind from the activated sludge tanks. This would allow for customization of the model and offer greater insight to ambient concentrations of ARB. In addition to increasing the number of samples taken and the locations, the sampling of ARB during numerous meteorological conditions would also allow the for spatial modeling during various scenarios and the comparison of meteorological impacts on downwind ARB concentrations.

This research could be a vital first step in understanding various sources of ARB exposure and the contribution of each ARB source to overall population exposure. The investigation of source apportionment of ARB in a community would allow the ability decrease emission and dispersion from the sources are most responsible for the concentration levels. The ability to integrate the Gaussian dispersion model into GIS modeling for the modeling of air pollutants shows potential for future studies in health outcomes, health disparities, and other epidemiology research

CHAPTER 5

CONCLUSIONS

With the rise in antibiotic resistance emerging as a major public health concern, the need for understanding the dispersion of ARB from known sources is essential. With the use of spatial modeling tools, the dispersion of pollutant sources can be visualized through an easily understood format. The research of McKenzie (2011) showed that activated sludge tanks of WWTP are a source of multi-drug resistant bacteria. Using the sampled concentrations and measured meteorological data from McKenzie's work, a Gaussian dispersion model was developed using GIS technologies. The input of the Gaussian dispersion model into GIS was successful through the use of grid overlay and raster layers. The process to construct a final concentration layer that modeled the full Gaussian dispersion model resulted in the ability to find the estimated concentration at any given point downwind within the viewing window when using GIS tools. This research is consistent with previous studies that show that the decrease of pollutant concentration with increasing distance from the pollutant source. To improve this model, more samples, both downwind and upwind, should be taken over a longer period of time to increase applicability to other sites. With over 250 layers, needed for the completion of this research, this may not be suitable for application for all city planning uses, but shows potential for use in environmental epidemiology research.

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APPENDIX A- REFERENCE MAPS

Figure A.1 Euclidean Distance

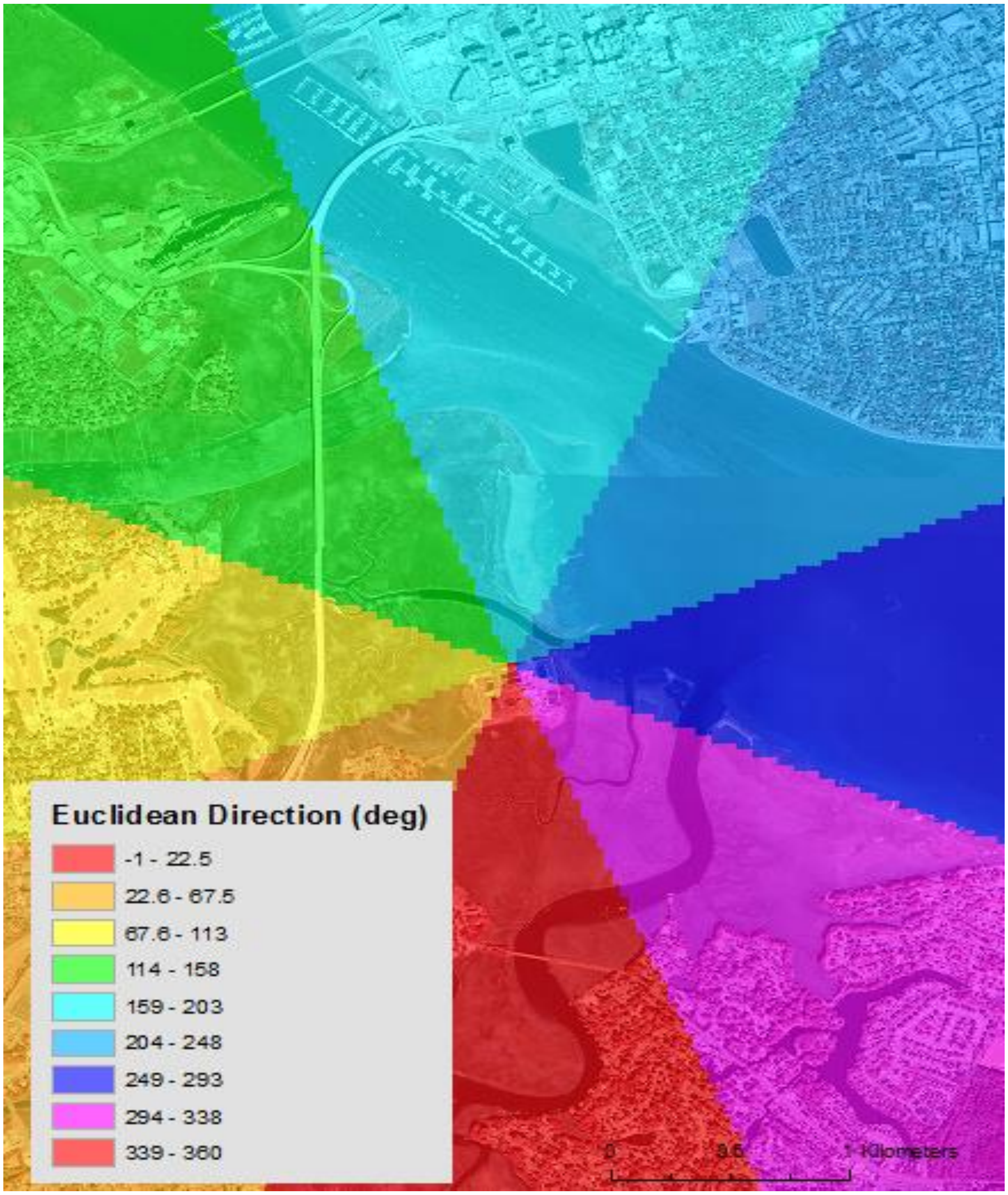

Figure A.2 Euclidean Direction

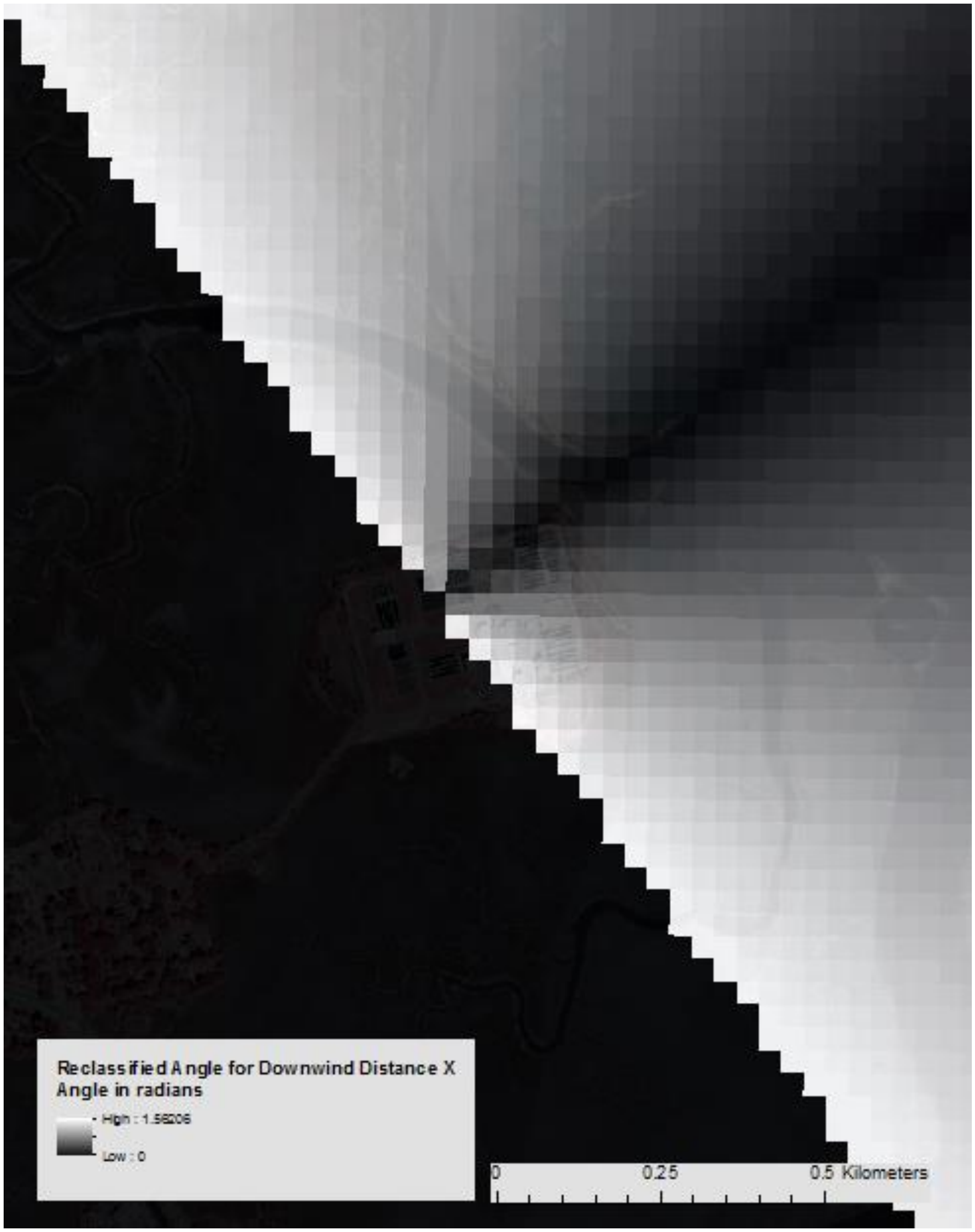

Figure A.3 Reclassified Angle to Find Downwind X (in radians)

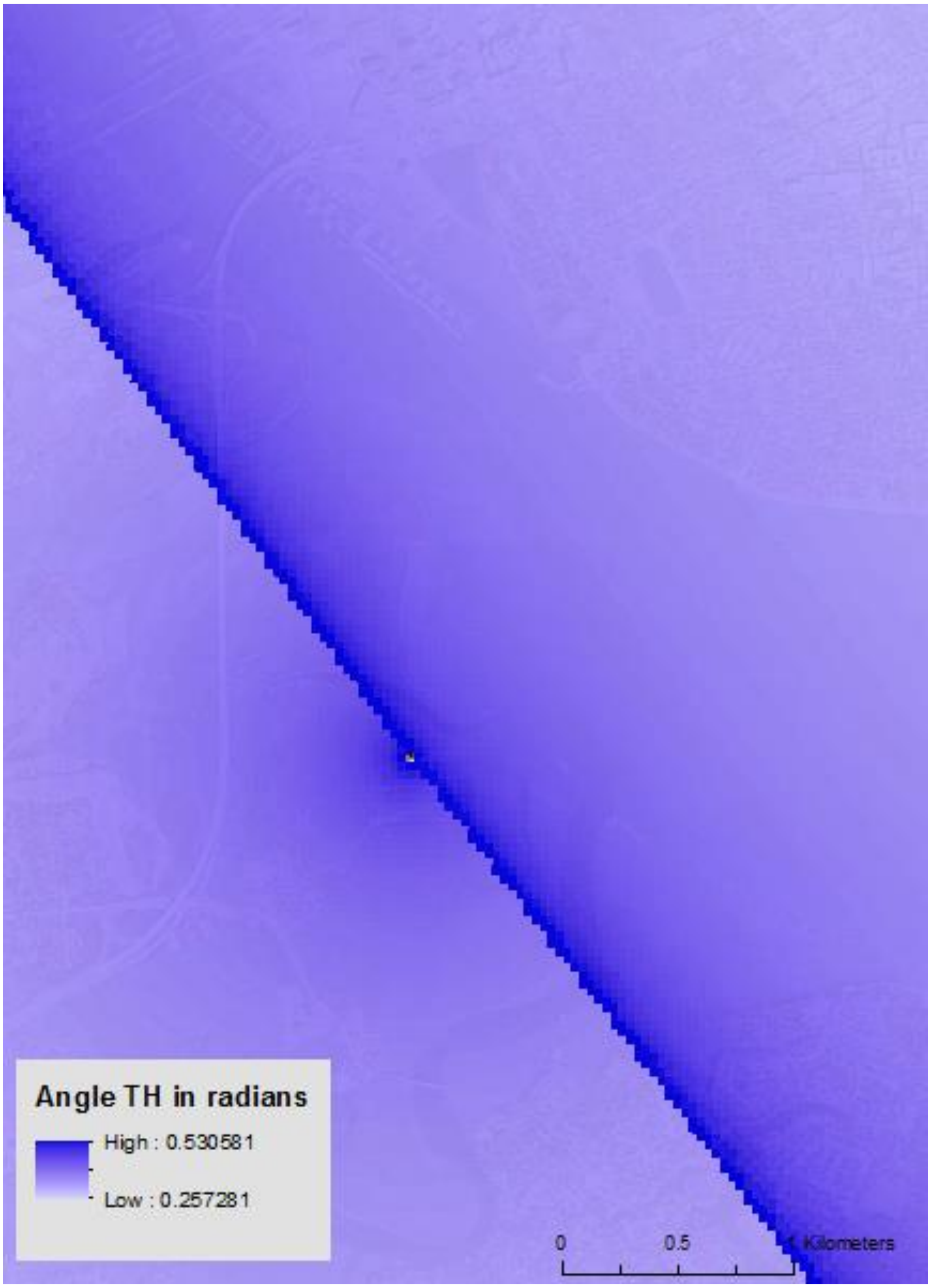

Figure A.4 Angle TH (in radians)

Figure A.5 Horizontal Dispersion Coefficient (σ_y)

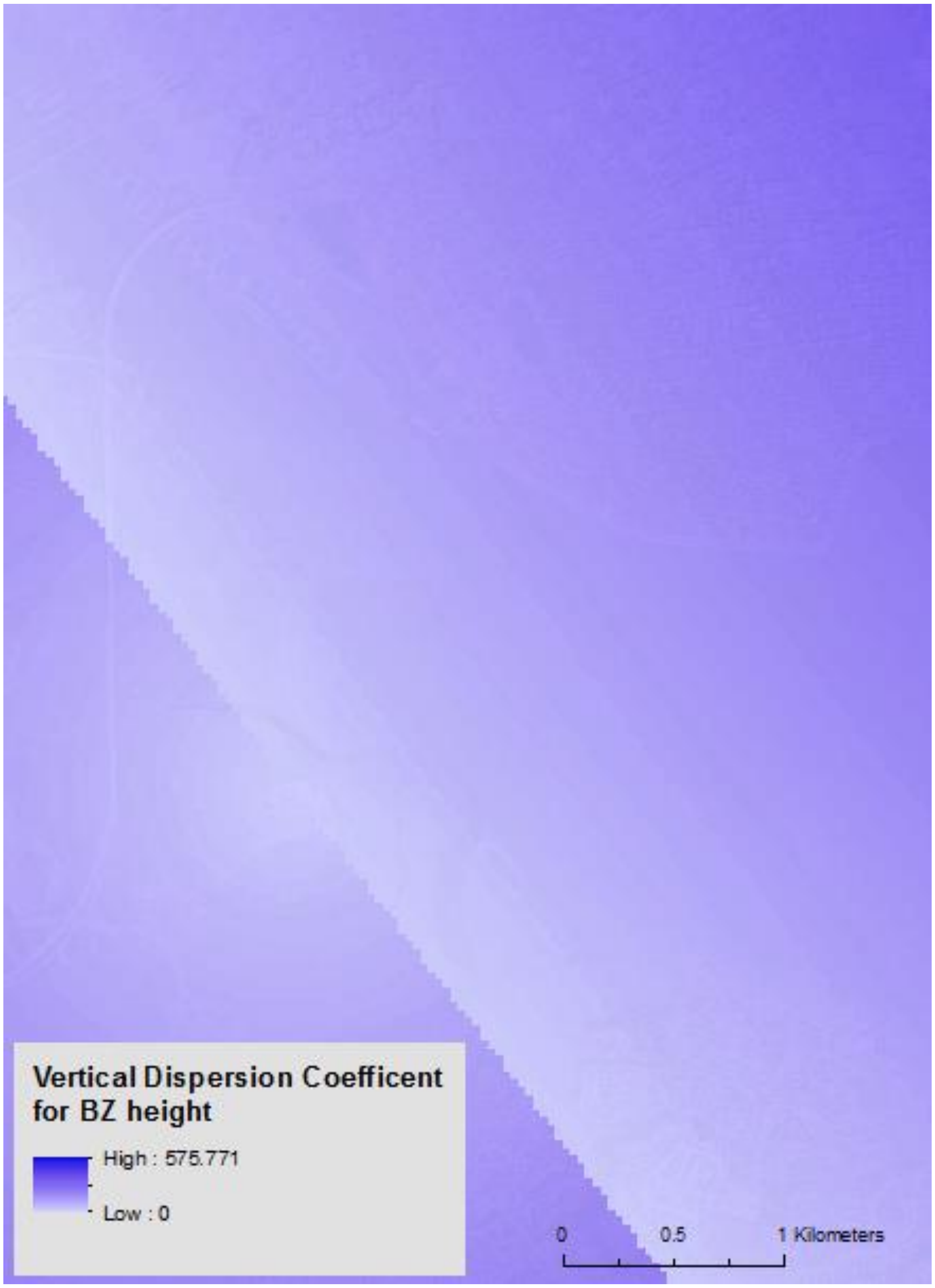

Figure A.6 Vertical Dispersion Coefficient (σ_z)