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Eureka!

The great mathematician Archimedes reportedly exclaimed "EUREKA! EUREKA!" (which roughly translates to "I HAVE FOUND IT, I HAVE FOUND IT") when the solution to a complex problem was revealed in his mind. As all scientists know, the *moment of discovery* is a cherished event; the prospect of discovery is the reason to get up in the morning, and it is what carries scientists through long nights of struggle and frustration. It is this Eureka! moment we wish to share with our students. "Eureka!" is therefore the perfect distillation of the spirit of science and an appropriate title for a journal whose goal is to provide a forum in which students can share their Eureka! moments.

To those who reviewed manuscripts for this publication;

Thank you for your time and effort on the behalf of our students. We know the density of your schedule and understand the sacrifice you have made to review our work. This sacrifice is greatly appreciated. The students participating in this research program are enthusiastic, and their work strives to reveal interesting and pertinent things about the world around us. Each manuscript published herein is the result of input from at least three faculty reviewers and the interpretation of this input by the student researchers. We have done our best to address the concerns expressed in each review, and your comments and suggestions have greatly improved the quality of our manuscripts. Please understand that final manuscripts are the result of the efforts of reviewers, students and mentors, and that not all suggestions may be incorporated, but were certainly considered. We will continue to recruit new students, and therefore hope that this is not the last time we call upon you to review such work.

We sincerely thank you for your help,

Cover art credits: Computer-generated image of a landscape from Minetest. Image produced and provided by Austin Smith (see pages 16-26).

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Impact of Canopy on Abundance of Ciprofloxacin Resistant-Bacteria Colonies in Soil

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Abstract

Antimicrobial resistance in bacteria presents a bleak danger in modern-day medical treatment as it can render current antimicrobial chemotherapies ineffective. Antimicrobial resistance is generally promoted in clinical and agricultural overuse. An increased understanding of the environmental factors that might foster these resistant strains is imperative to addressing this challenge. This study investigated the influence canopy (i.e. the presence of many trees forming a canopy above the ground) had on the abundance of ciprofloxacin-resistant bacteria in soil samples. The number and type of bacteria from soil samples from six canopy and non-canopy sampling sites, paired in close proximity to each other, growing on soil extract agar with and without ciprofloxacin was determined. This study revealed that non-canopy sites held a greater abundance of ciprofloxacin-resistant colonies and that the presence of ciprofloxacin possibly releases selective pressure on ciprofloxacin-resistant colonies. Several site-specific characteristics (e.g. sunlight, roots, and different fungal communities) may explain the differences seen between canopied and non-canopied sites. This preliminary study, while not conclusive, indicates that there are many environmental factors that might influence the rise of antimicrobial resistance in bacteria, and warrants further in depth study.

Introduction

Antimicrobials are relatively easily produced medicines that either kill or constrain the growth of infection-causing microorganisms. These chemotherapies have allowed accessible and effective methods of treatment against a plethora of medical disorders that would otherwise be considered terminal conditions. However, as antimicrobials have become a staple of medical care, their overuse has revealed antimicrobial resistance as a grave danger that may remove a vital tool from our arsenal against infectious diseases (Allen et al., 2010).

Antimicrobial resistance, when microbes become immune to the various medications used to kill or keep them from growing, spells the possibility of an era without conventional chemotherapies for infectious diseases. The overuse of antimicrobials can lead to contamination of surrounding ecosystems

with low concentrations of these drugs, creating selective pressures killing all bacteria except those with resistance (Allen et al., 2010). As a result, the surviving bacteria dominate the remaining genetic pool, and may be able to horizontally transfer resistance to the rest of the microbial population (Allen et al., 2010).

In the clinical scene, these resistances are promoted through the over-prescription of medication to patients, as well as their misuse (Allen et al., 2010). But to fully understand the extent and full impact of antimicrobial resistance, it is also important to identify ecosystem attributes promoting antimicrobial resistance in the environment.

Apart from the clinical scene, the prevalence of antimicrobial use in agriculture and antimicrobial seepage from other points of pollution lead to exposure, further increasing chances of microbial resistance in the environment (Tello et al., 2012). Interactions between antimicrobials and the environment can create selective pressures that promote the success of resistant bacteria. The danger of these bacteria can be further exacerbated by their spread to different populations through natural modes of transmission (e.g. a bird carrying seeds, or a gust of wind carrying pollen with resistant strains from one environment to another) (Allen et al., 2010).

Ciprofloxacin is a fluoroquinolone antimicrobial effective against a variety of disease-causing bacteria. The ciprofloxacin mode of action is by inhibiting bacterial enzymes (DNA gyrase and DNA topoisomerase) involved in DNA replication, rendering these enzymes ineffective, thereby keeping the bacteria from growing and eventually killing them (Fàbrega et al., 2009; Levine et al., 1998). Despite its classification of a last-resort antimicrobial, ciprofloxacin is still commonly used in clinical and agricultural situations, creating further opportunity for the development of resistance.

There are various mechanisms by which bacteria can become resistant to ciprofloxacin, including mutations in the genes encoding topoisomerase and DNA gyrase, protein products targeted by

ciprofloxacin, usually occurring in the "quinolone resistance-determining regions" (QRDR) of these genes (Vlieghe et al., 2012; Giles et al., 2004). Additionally, some bacteria have a quinolone resistance gene (QNR) which encodes a protein that protects DNA gyrase from the effects of ciprofloxacin (Conley et al., 2018; Li, 2005; Tran and Jacoby, 2002). Resistance can also arise through the expression of multi-drug efflux pumps that can keep ciprofloxacin from reaching its intracellular targets (Allen et al., 2010; Fàbrega et al., 2009; Li, 2005).

Another general example of a pathway to antimicrobial resistance can be seen in bacterial enzymes that degrade antibiotics (Allen et al., 2010). Examples of such enzymes conferring resistance to ciprofloxacin are CrpP (Chavez-Jacobo et al., 2018) and aac(6')-Ib-cr (Park et al., 2006). Finally, changes in cell membrane and cell wall structure that limit the intake of antimicrobials could possibly lead to antimicrobial resistance (Allen et al., 2010; Fàbrega et al., 2009). Genes that confer resistance to ciprofloxacin can be imported into naïve populations from resistant populations via horizontal transfer (Allen et al., 2010).

The overuse of ciprofloxacin and subsequent exposure of soil bacteria to low-levels of ciprofloxacin provides ample opportunity for the development and transfer of ciprofloxacin resistance. However, there could be different characteristics between environments that could promote ciprofloxacin resistance. Sites with canopy and without canopy each provide their own unique characteristics that can result in different challenges and opportunities for their microbial populations. The presence of canopy provides different levels of exposure to sunlight, rain and human input. The root system in canopy sites will also be physically and biologically different than in non-canopied sites, which tend to be dominated by smaller plants (Ali and Yan, 2017; Gilliam, 2007; Mason et al., 2011). The absence of large, canopy producing trees allows for more exposure to sunlight, more direct access to rainwater, and easier access to the ecosystem for humans. Canopy provides unique microclimates to

its ecosystem and is known to host intricate interactions within its populations (Nakamura et al., 2017). The resulting unique characteristics each pose possible selective pressures that could potentially spur mutations in, or allow for the transfer of ciprofloxacin-resistance genes to, the native population.

This study seeks to examine the impact of canopy on the abundance of ciprofloxacin-resistance microorganisms in the soil. Soil samples were taken from a variety of sources and soil types from the Scott Northern Wake Technical Community College campus, which was built on land that was mostly wooded but may have been used as a pasture area in the past (Wendell B. Goodwin, P.E.; Project Manager (Mechanical) Wake Technical Community College, personal communication). Due to the nature of the soil (i.e. potentially used as pastureland in the past), there may be higher concentrations of resistant bacteria. However the impact of canopy on the abundance of resistant strains could still be discernable. To examine the results of these differences, samples were taken from six different sites on the campus with half of the sites being wooded (i.e. canopied) and the other half from sites that lack covering expanses of larger flora (i.e. non-canopied). Preliminary data indicate that non-canopied soil samples actually harbor more antimicrobial resistant bacteria.

Materials and Methods

Soil samples were collected from six separate sites across the Scott Northern Wake Technical Community College campus (see Figure 1), three with extensive growth of trees (i.e. canopied) and with no trees (i.e. non-canopied). Sites were paired, so that one non-canopied sample site was located within approximately 20 feet of a canopied sample site, to minimize other potential differences between sites to the presence of trees. The site pairs were also selected to have near to no incline separating them so that rainwater run-off was not a source of cross-contamination between the bacteria ecosystems. Sites were chosen so that each pair was at least 300 feet from another pair.

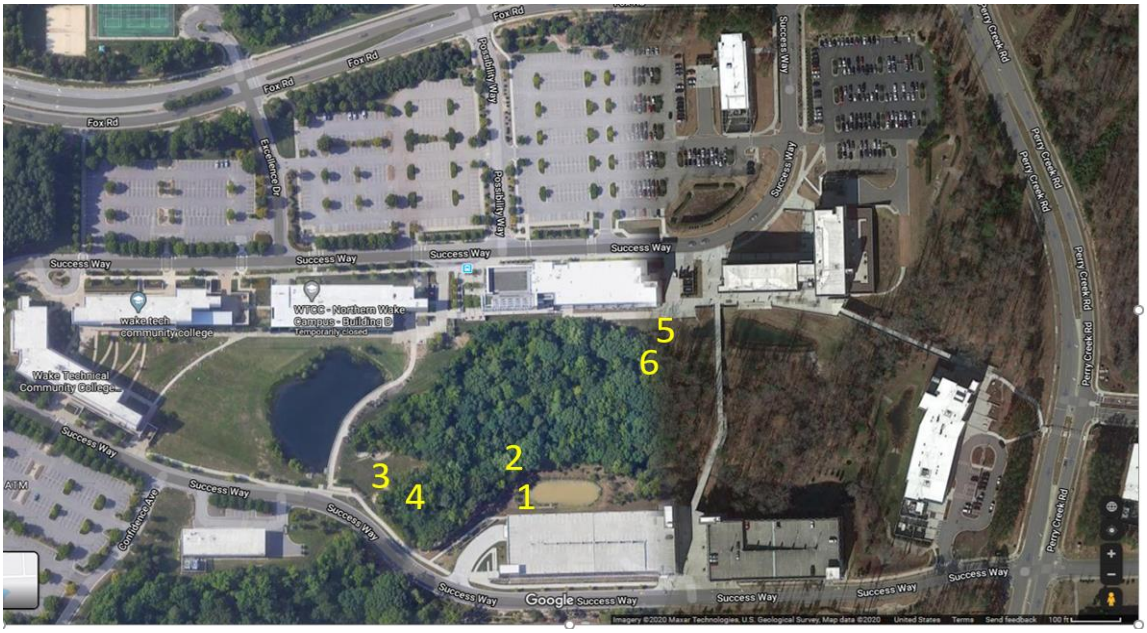

Figure 1: Map of Scott Northern Wake Campus. Numbers indicate sampling sites as described in Materials and Methods.

In this experiment, the sites were labeled one through six, with the even numbered sites being those that were canopied and the odd were those that were without canopy. Site one was near a parking complex, lake, and approximately 10 feet away from a sidewalk. It had firm, root-abundant soil covered by live grass and had a lux reading at the time of sampling to be approximately 8400 lx (see Table 1). A lux, abbreviated lx, is a measure of luminance and is equal to one lumen per square meter; lux measurements were taken with a smartphone application from soil level to provide context as to

Table 1: Descriptions of sampling sites used in the study

Site	1	2	3	4	5	6
Canopy or Non-Canopy	Non-Canopy	Canopy	Non-Canopy	Canopy	Non-Canopy	Canopy
lux	8400	5200	5600	4500	7250	7000
Soil Description	Firm-Rooty Soil	Loose Soil	Firm-Rooty Soil	Very Loose Soil	Rocky-Firm-Rooty Soil	Firm Soil
Special Attributes of Site	Side-Walk / Parking Complex / Lake	Substantial Dead Foliage	Side-Walk	Moss	Side-Walk / Building	Visible Tree Roots / Dead Foliage

exposure of each site to sunlight. Site two was within approximately 15 feet of site one. Site two was within the woods with soil covered under a considerable amount of dead foliage. The soil in site two was looser and darker in color with a lux reading of approximately 5200 lx. Site three was located approximately 20 feet from a busy pedestrian sidewalk and road. It had firm and root-abundant soil covered by a mix of dead and live grass. The lux reading for site three was approximately 5600 lx. Site four was 18 feet from site three. Site four was under canopy and had moss, loose topsoil, and a considerable population of insects. Site four had a lux reading of approximately 4500 lx. Site five was located approximately six feet from a busy sidewalk and building and was covered by abundant live grass and clover. The soil was rocky, firm, and with an abundance of roots. The lux reading for site five was approximately 7250 lx. Site six was located approximately 25 feet from site five and was sampled from near tree roots and covered by dead foliage with a lux reading of approximately 7000 lx.

At each site, a sterile metal spoon or spatula was used to excavate and move top soil into a single-use, sterile 50 ml plastic tube. Spoons or spatulas were only used once. All samples were taken within an hour of each other. Once all soil samples were collected, the samples were taken to the STEM lab at Scott Northern Wake Building H. Here, 10 g of each soil sample was aseptically transferred to an individual sterile, covered 100 ml beaker containing a sterile magnetic stir bar, to which was added 95 ml of sterile deionized water. Each sample suspension was mixed thoroughly through the use of a stir bar and hot plate stirrer. Once fully mixed, 1 ml of the suspension was aseptically transferred to 9 ml sterile, deionized water in a sterile, plastic tube. This new suspension was then vortexed to mix thoroughly. Subsequently, 1 ml of this dilution was then placed in another sterile, plastic tube containing 9 ml water and mixed thoroughly. These three dilutions were then used to inoculate solid agar media.

Agar media was created by taking 1 g of glucose, 0.5 g of dipotassium phosphate, 10% soil extract, and 15 g of agar at a pH of roughly 6.8 (recipe modified from HiMedia). The soil extract for this recipe was created by taking 50 g of soil (from a site entirely separate from the sampling sites) mixed into 250 ml of distilled water. After thorough mixing, the suspension was autoclaved for 20 minutes at 14 psi and 121°C. The solution was cooled while stirring and autoclaved a second time. The resultant was filtered through a 0.22 um filter, and 100 ml was mixed with 900 ml of an agar solution containing the other ingredients. Ciprofloxacin in 0.1 M nitric acid was added to half the media preparation to a final concentration of 10 μg/ml, while and the other half received only nitric acid. The media was then aseptically poured into individual, sterile plastic petri dishes.

One milliliter of each dilution from each site's sample was then used to aseptically inoculate three soil agar plates with ciprofloxacin and three soil agar plates without ciprofloxacin. Inoculated plates were incubated for 2 to 3 days at room temperature. Once the plates were incubated, the number of colonies on each plate were manually counted, corrected for dilution, and a count of Colony Forming Units (CFUs) per milliliter was reported for each site. For growth on medium without ciprofloxacin, an average CFU count could only be reported for site 4, as all other samples yielded too many colonies to count with dilutions one and two. Colonies with distinct macroscopic appearances were counted as individual macrotypes.

Results

Physical Attributes of Sites

As described in table 1, the non-canopied sites 1 and 3 were very similar, with firm soil that had an abundance of small roots (rooty soil). Both sites were close to areas of high human activity (e.g. side-walks, parking complexes). Site 1 had the highest lux reading of all sites and was close to a large lake. The canopied sites 2 and 4 were similar to each other, with looser soil and the absence of roots. These

sites had lower corresponding lux values than their corresponding non-canopied sites, in fact site 4 had the lowest lux reading of all sites. Site 4 also had a considerable amount of moss, which was not typical of any other site. Compared to the other sites, sites 5 and 6 were unique. Both site 5 (non-canopied) and site 6 (canopied) had high lux values. In fact, canopied site 6 had a higher lux value than non-canopied site 3. Both sites 5 and 6 had roots and firm soil, although the roots at site 6 were larger tree roots while site 5 had the traditional rooty soil of small dense roots. There were more rocks in the soil at site 5 spacing the smaller roots. Like sites 1 and 3, site 5 was near areas of human activity. Site 6 was similar to site 2 in the presence of dead and decomposing foliage. Each site, regardless of the existence of canopy, sustained a thriving environment of insects, healthy grass, other flora, and other biotic factors. Therefore, although there were similarities, there were also significant differences between the sites that could promote differences in microbial communities. Key common physical differences in these non-canopy sites to their complementary canopy site are that non-canopy sites tend to have more exposure to sunlight (higher lux), more human interaction (located near a sidewalk/building), and rooty soil consistency (Table 1).

Overall Abundance and Diversity

Colonies were abundant on all countable inoculated media, with and without ciprofloxacin, indicating healthy and diverse microbial populations at all sites (Table 2). On average, fewer colonies appeared on plates inoculated with samples from canopied sites than non-canopied sites. The presence of ciprofloxacin reduced the average total bacterial abundance, with the exception of site 3. Site 1 had the highest microbial abundance. Site 6 had the lowest total bacterial abundance, while sites 2 and 5 had similar low levels of ciprofloxacin-resistant colonies.

Table 2: Distribution and abundance of bacterial macrotypes at study sampling sites (C - denotes growth on media without ciprofloxacin; C+ denotes growth on media with ciprofloxacin)

	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6	
Macrotype	(C-)	(C+)	(C-)	(C+)	(C-)	(C+)	(C-)	(C+)	(C-)	(C+)	(C-)	(C+)
A	2000	250	300	0	0	5	0	0	300	0	0	0
B	1200	95	0	0	300	170	50	0	0	0	0	0
C	1400	200	1000	0	2700	1140	10	60	600	0	400	0
D	4200	1200	400	195	1400	1650	345	70	1400	105	300	130
E	8300	1350	700	45	300	4500	230	145	1500	30	400	285
F	0	5	300	0	0	0	0	0	0	0	0	0
G	0	10	0	10	0	0	5	105	0	50	0	0
Total Abundance	17100	3110	2700	250	4700	7465	640	380	3800	185	1100	415

Prevalence of Macrotypes

Although there were abundant colonies on all plates, the diversity of the microbial community at all sites appeared to be low, with a total of seven macrotypes observed between the six sites. Macrotype A was noted as a small, brown, and partially yellow bacterial colony. Macrotype B was noted as a large, white, and clear bacterial colony. Macrotype C was noted as a large, white, and opaque bacterial colony. Macrotype D was noted as a small, white, and clear bacterial colony. Macrotype E was noted as a small, white, and opaque bacterial colony. Macrotype F was noted as a purple bacterial colony, and Macrotype G was noted as a filamentous bacterial colony.

Macrotype A appeared in a majority of sites (sites 1, 2, 3, and 5), but was only abundant at site 1 (Table 2). Macrotype B was found in abundance at site 1, and in lower numbers at sites 3 and 4. Macrotypes C, D and E were the most abundant and prevalent, appearing at all sites. Macrotype G was present at very low abundances and only in the presence of ciprofloxacin in sites 1, 2, 5, but also in the absence of ciprofloxacin at site 5. Macrotype F was the rarest of macrotypes, appearing only at site 1

and 2 at low abundance. Resistant strains of macrotypes A, B, and C are more prevalent in non-canopy sites than their canopy counterparts, except for site pair 5 and 6 where resistant strains were absent on both sites.

All seven macrotypes were found at Site 1 and all but macrotype B were found at Site 2 (table 2). Three sites had five different macrotypes, site 3 (macrotypes A, B, C, D and E), site 4 (macrotypes B, C, D, E, and G) and site 5 (macrotypes A, C, D, E, and G). Site 6 had the lowest diversity with only three macrotypes (C, D, and E).

As a general trend, there were similar or fewer ciprofloxacin-resistant colonies than ciprofloxacin-susceptible colonies of these macrotypes. Site 3 sees an exception to this as it's the only site that has more colonies on plates with ciprofloxacin than without. Macrotype C is seen following a general trend where it is nearly always found at reduced amounts on ciprofloxacin containing media. However, macrotype G, when present, was always found at higher abundances on ciprofloxacin containing media. Macrotypes D and E each see a similar decrease as Macrotype C when in the presence of ciprofloxacin, except at site 3 where D and E see an increase in the presence of ciprofloxacin.

Macrotypes B and F were not abundant in any sample, and found at only sites 1, 3, and 4 (macrotype B) and 1 and 2 (macrotype F). It must be recognized that the site with the lowest diversity is also the site with the lowest total abundance, so other macrotypes may be present but at such low abundances to preclude detection. It appears, however, the sites share a common set of abundant macrotypes, and are characterized by a low overall diversity

Conclusions

Although this study saw healthy microbial populations at each site, more bacteria were seen at non-canopy sites, both generally and ciprofloxacin-resistant. These differences in abundances could be due to physical characteristics of studied non-canopy and canopy sites. Some macrotypes varied in prevalence in the presence of ciprofloxacin.

This study discovered healthy and thriving microbial populations at each sampling site with a general trend of less bacterial abundance in canopied sites than non-canopied sites, regardless of the presence of ciprofloxacin. A possible explanation for this difference rises from non-canopy site soil having more exposure to sunlight than canopy sites. Exposure to UV light can lead to deleterious changes to DNA, but can also lead to gain-of-function mutations, providing a competitive edge for microbes harboring them. Therefore, sunlight could promote microbial abundance. Sunlight could also be a selective pressure that favors the manifestation of certain antimicrobial-resistant bacteria or even act as an origin for mutations leading to antimicrobial resistance. For example, resistant strains of macrotypes A, B, and C are present in non-canopy samples, but are not consistently present in canopy sites. Additionally, the similarity in lux at sites 5 and 6 could also possibly explain the absence of resistant colonies at both sites.

However, it also appears that the presence of roots may have a positive effect on bacterial abundance and ciprofloxacin resistance. In this study, non-canopy sites (sites 1,3, and 5) tended to have more dense smaller root systems packing the soil. Roots would provide an excellent environment for bacterial growth, as a potential source of organic material and water, and of surface area on which to grow. However, roots are also known to harbor multiple fungal species that can compete for these resources (Field and Pressel, 2018). Fungi are also known to produce antibiotics to antagonize bacterial growth. This might also provide a major selective pressure favoring antimicrobial-resistant bacteria in

these environments. This could possibly explain the break from the average trend of more ciprofloxacin resistant colonies present on non-canopy sites we see from sites 5 and 6. It is noted in table 1 that site 5 is unique from the other two non-canopy sites as site 5 has significant smaller rocks in alongside its rooty soil. These smaller rocks in the soil could possibly be spacing out the roots, decreasing the total surface area of the contact of roots to the bare soil. This rocky aspect of the soil could possibly negate the possible selective pressure effect of rooty soil, potentially explaining site 5 and its similarity in scale of ciprofloxacin resistant colonies seen to that of the less rooty soil of the canopy sites.

Finally, all sites within close proximity to human activity (non-canopy sites 1, 3 and 5) have greater total and ciprofloxacin-resistant bacterial abundances than the sites with less exposure to human activity (canopy sites 2, 4 and 6). Canopy sites tended to have protection from close human contact due their larger trees. Humans can directly impact microbial communities through direct, usually unintentional, inoculation with microbes associated with themselves. Humans can also alter microbial communities by depositing items containing exogenous microbes and/or nutrients. Each is a possible avenue to adding more bacteria and more selective pressures to the environment. Notable trends regarding the abundance and distribution of the seven macrotypes seem to correspond to site specific physical characteristics. When examining sites for differing characteristics, sites with increased exposure to sunlight, rooty soil, and increased human interaction (i.e non-canopy sites) saw an average rise in abundance of both nonresistant and resistant bacterial colonies.

At some sites, macrotypes (e.g. macrotype G) were seen to increase in abundance in the presence of ciprofloxacin. Macrotype C represents the opposite case of a colony type that generally decreases in abundance in the presence of ciprofloxacin. A possible explanation for this is that certain non-resistant macrotypes (macrotype C) might be better suited to live in the soil environment and thus, under normal circumstances of growing without exposure to the antimicrobial, can outcompete other

microbes. Macrotype G (and perhaps other macrotypes) is likely unable to compete with the more prominent non-resistant macrotypes under normal conditions and can only grow when ciprofloxacin is present to hinder and release the competition Site 3 is an extreme case of this, as the presence of ciprofloxacin media increase the abundance of colonies seen. When macrotype C decreased due to ciprofloxacin at site 3, an increase in macrotypes D and E are also seen. It is possible that when macrotype C is reduced due to ciprofloxacin, resistant strains of D and E are released to freely grow. This further highlights the danger of antimicrobial pollution, as unintended exposure to these antimicrobials would eliminate non-resistant colonies that would otherwise out-compete or suppress the growth of resistant colonies, perhaps dramatically altering the ecological dynamics of the affected site.

This experiment provided evidence that non-canopied sites harbor a greater abundance of ciprofloxacin-resistant bacteria in comparison to canopied sites. This could be due to the fact that wooded sites offer protection from mutation-causing sunlight and barriers of entry for possible mutation causing stimulus. The non-canopy sites lack this protection and are therefore more challenging environments. However, there appear to be other factors (e.g. presence of roots and proximity to human activity) that may also contribute to microbial abundance and ciprofloxacin-resistance. Other factors, such as the presence of the lake near site 1, and the abundance of decomposing material at sites 2 and 6, may also be contributing factors. These aspects deserve further testing. Determining trends of macrotypes exposes a limitation of this study of needing more precise separation of macrotypes for identification. While this preliminary experiment cannot discern the specific contribution of these environmental challenges to ciprofloxacin resistance, it does indicate that antimicrobial resistance develops more efficiently in more challenging environments.

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Terminal Velocity and Its Implications in Minetest

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Abstract

The open-source video game Minetest was analyzed for its validity regarding the physics of falling and terminal velocity. Suggestions were made about ways to increase the accuracy of the physical laws observed to create a fun and unique learning experience for introductory geology courses. Equations were used that factored in drag, gas density, and frontal area to find the point of terminal velocity along with the time and displacement associated with this point. All materials will accelerate at the same rate until the terminal velocity is reached. It was shown that heavier materials will take longer to reach terminal velocity thus they will accelerate for a longer period and have a greater displacement and impact velocity if enough time is allotted.

Introduction

Minetest is an open-source video game that allows modifications for game creation. Minetest is based on the well-known videogame counterpart, Minecraft. Much like Minecraft, there are large discrepancies between the physical laws of the real world and how these physical laws work. If Minetest were modified to better emulate reality and its natural laws, then it has the potential to be a fun and unique learning experience for introductory college geology courses.

In this project, the physics of materials falling in the real world was compared to how Minetest materials fall within the game. One aspect of the physics of falling is terminal velocity, which is the point when the velocity of a falling material is constant, and its acceleration is zero. All falling materials will accelerate at the same rate until the terminal velocity is reached. If falling materials have the same frontal area but different densities, the denser materials will accelerate for longer; thus their terminal velocities will be greater. This means, that if the materials fall for long enough that they reach their terminal velocities while still falling, then the densest material will impact the ground first with a greater velocity than that of less dense materials (GRC.NASA.gov, updated 2021).

Currently, the only material observed to fall with no underlying support in Minetest is sand. All the other available materials observed in Minetest did not obey the laws of physics expected in the real world. For example, if a material is laid down on a surface and the supporting structure is removed, the material will levitate and not fall. The goal of this project is to better illustrate in Minetest the physics associated with gravity and falling observed in the real world.

Methods

A variety of materials within Minetest were examined in order to improve the way materials respond to gravity within the game. Minetest version 5.4.1 was used along with the Mod, "Basic Materials and Items," created by VanessaE (accessed in game by doing a search). A Mod is an inputted modification available to a video game to alter certain aspects. The Mod used by VanessaE allowed all the materials to be readily available in the inventory at the start.

The size of materials is not specified in Minetest; therefore, dimensions from Minecraft were used in calculations instead. Materials in both games are at the same scale and visually look about the same size (when in comparison with a character, for instance), so this substitution was deemed suitable. Materials in Minecraft are all 1 m^3 in size (Minecraft Wiki, 2022). Therefore, by using the equation $D = \frac{m}{v}$ (density equals mass/volume) the density also equals the mass in this instance because the volume for all materials equals 1 m^3. If a certain material has a range of acceptable values listed for densities, the mean average value was recorded.

The following equations were used to find velocity, acceleration, displacement, time, and drag until the terminal velocity was reached:

Definition of variables:

V=velocity

V_i= initial velocity

Y= height

Y_i= initial height

a= acceleration

Δt= change in time

D= drag

Cd= drag coefficient

p= gas density

A= frontal area

$$V = a\Delta t + Vi$$

$$Y = Yi + Vi\Delta t + \frac{1}{2}a\Delta t^2$$

$V = a\Delta t + V_i$ (Equation 1, Solving for velocity)

$Y = Y_i + V_i\Delta t + \frac{1}{2}a\Delta t^2$ (Equation 2, Solving for height)

$D = \frac{CdpA}{2} \cdot V^2$ (Equation 3, Solving for drag)

$a = \frac{F}{m} = \frac{(w-D)}{m} = \frac{mg-CdpA \cdot V^2}{m} = g - (\frac{CdpA}{2m})V^2$ (Equation 4, Solving for acceleration)

As shown above, the equation for drag was substituted to solve the equation for acceleration. All the equations are related to each other and share some common variables. These equations were then solved in Microsoft Excel.

All the equations are cumulative; therefore, some assumptions were made to solve these equations for the Minetest materials, including a frontal area of 1 m^2, and a drag coefficient of 0.8. Materials of flint in Minetest resemble more of an elliptical shape with no specified frontal area. To simplify the results, calculations for all materials were made assuming every material occupies the same volume and has the same frontal area and drag coefficient. All falling is assumed to take place in the y-plane. Currently, it is assumed that no factors affect the x-plane such as wind, therefore it was assumed to be negligible. For simplification, the air density was assumed to be the same as the density of air at sea level and 15 °C. This equates to a value of 1.225 kg/m^3 (Engineering Toolbox, 2003).

It is important to note that all the densities listed were dependent on the sources. For example, the only information in the game for bronze is the name "Bronze Block". Bronze is an alloy; however, the game does not specify all the metals in its makeup or composition. Therefore, it is assumed that the mixture is copper and tin. Sandstone, cobblestone, and most other sedimentary rocks can come in a variety of densities depending on the mineral composition of the clasts and cement. When applicable, the dry bulk density was used.

Two additional columns were used in Microsoft Excel to solve for the percent change in velocity and total displacement. Mathematically, the acceleration and percent change in velocity will only approach zero, but never actually reach it. Therefore, it was assumed that terminal velocity is reached when the first instance of a less than 0.01 percent change in velocity is reported at 0.1 second time intervals.

Results

The following rocks and minerals were found in the game and their densities were determined. For consistency, those density values were converted from g/cm^3 to kg/m^3.

Table 3: Density of materials in Minetest

Rock or Mineral	**Density (kg/m^3)**	**Source**
Coal	1250	(Alden, 2020)
Gravel	1680	(Engineering Toolbox, 2010)
Sandstone	2300	(Vitz, 2021)
Obsidian	2550	(World of Stones USA, 2019)
Flint	2600	(Engineering Toolbox, 2009)
Cobblestone	2800	(Xiamen, accessed July 6, 2022)
Diamond	3510	(Hyperphysics, accessed July 6, 2022)
Tin	7310	(Nuclear-Power.com, 2021)
Bronze	7702	(AmesWeb, accessed July 6, 2022)
Iron	7874	(Nuclear-Power.com, 2021)
Cooper	8960	(Royal Society of Chemistry, 2022)
Gold	19300	(Nuclear-Power.com, 2021)

In addition to the rocks and minerals found in the game, the densities of the following common rocks in the real world that are not currently found in Minetest were also determined:

Table 4: Density of additional materials not found in Minetest

Rock or Mineral	**Density (kg/m3)**	**Source**
Rhyolite	2525	(GPG, 2017)
Gneiss	2795	(GPG, 2017)
Granite	2650	(Alden, 2020)
Quartzite	2700	(Alden, 2020)
Marble	2750	(Smithson, 1971)
Slate	2800	(GPG, 2017)
Basalt	3000	(GPG, 2017)

Table 5: Comparison of Terminal Velocities, Time to Reach Terminal and Displacement till Terminal

Name:	Terminal Velocity	Time to reach Terminal	Displacement till
Coal	157.57	50.00	6151.32
Gravel	182.56	56.60	8017.15
Sandstone	213.43	64.50	10606.34
Rhyolite	223.57	67.00	11513.63
Obsidian	224.67	67.30	11620.66
Flint	226.84	67.80	11812.93
Granite	229.01	68.40	12028.86
Quartzite	231.14	68.90	12222.87
Marble	233.25	69.40	12417.66
Gneiss	235.15	69.90	12605.39
Slate, Cobblestone	235.36	70.00	12636.76
Basalt	243.57	72.00	13427.85
Diamond	263.32	76.80	15425.52
Tin	278.88	103.80	29452.07
Bronze	388.80	106.00	30818.52
Iron	393.07	106.90	31397.73
Cooper	419.02	112.70	35168.26
Gold	611.89	153.30	68318.19

Below are graphs summarizing the data recorded, comparison of the terminal velocity, time to reach terminal velocity, and displacement until terminal velocity.

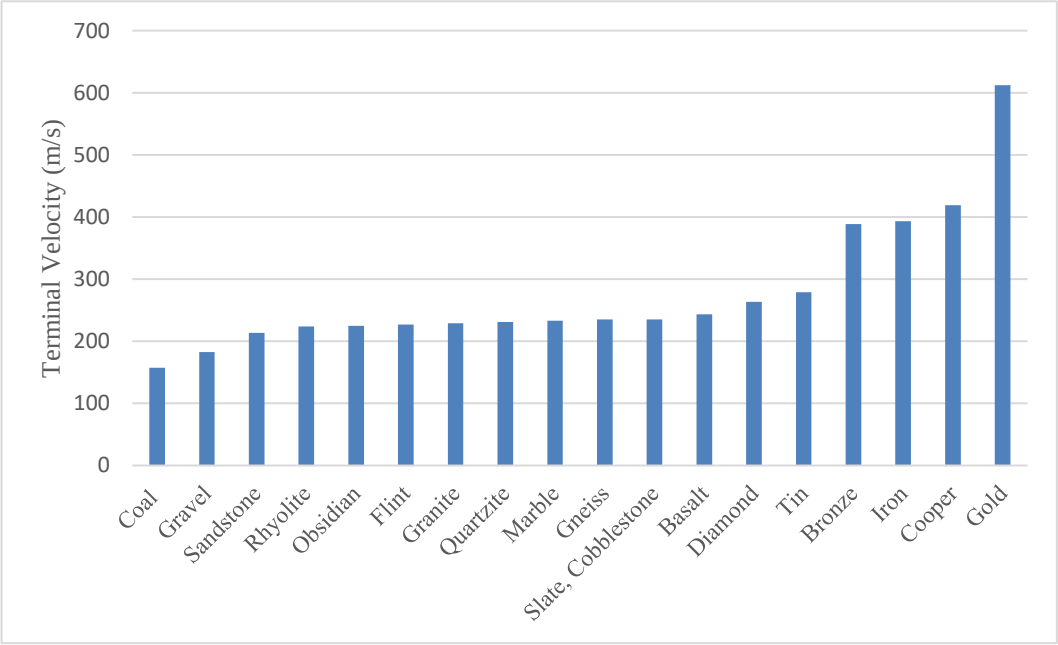

Figure 2: Comparison of the termi nal velocities of various materials as calculated as described in methods

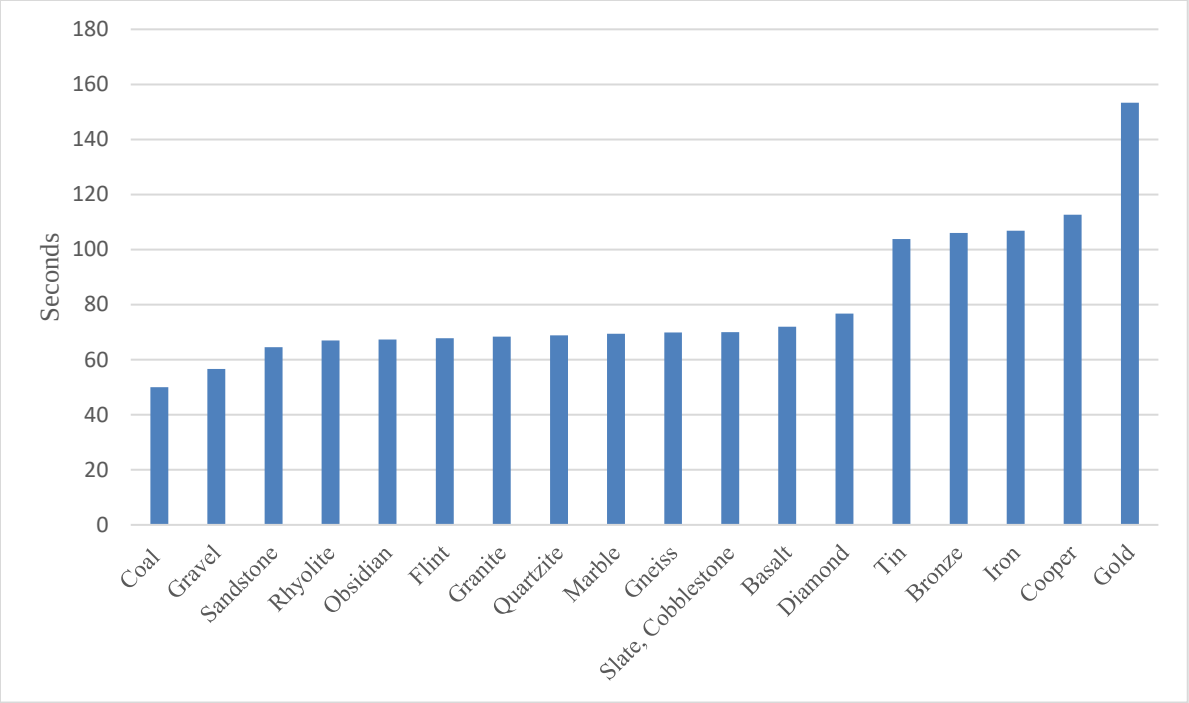

Figure 2: Comparison of the time to reach terminal velocities of various materials as calculated as described in methods.

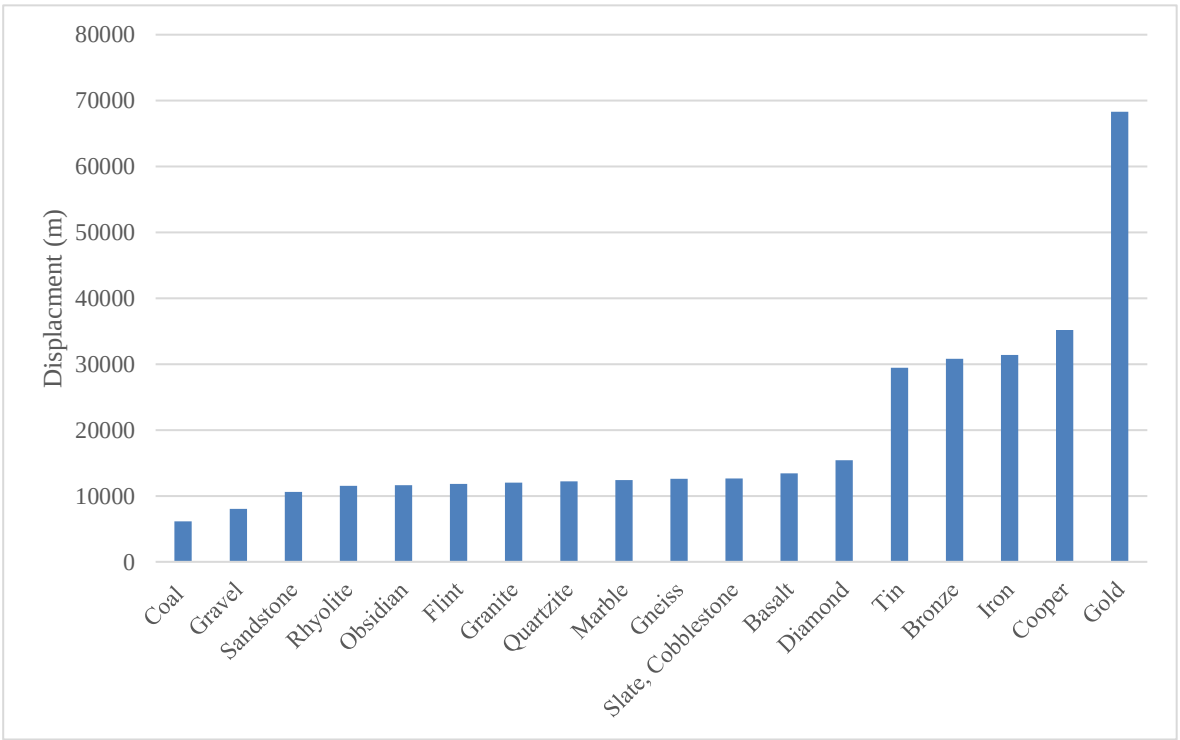

Figure 3: Comparison of the displacement until terminal velocities of various materials as calculated as described in methods.

From coal to diamond and from tin to copper as show in in chart one, most materials deviated in small increments and therefore had relatively similar terminal velocities. However, gold was the outlier and had the greatest density and terminal velocity.

Conclusions

There were negligible discrepancies with the velocity and acceleration of all materials leading up to terminal velocity. These were likely due to rounding and assumptions about the cutoff point used to determine the terminal velocity.

The boundaries in Minetest are reportedly 30912 to 30927 meters in every plane (X, Y, and Z). Therefore, the maximum height from sea level a material could hypothetically fall in the bounds of this universe is 30,927 meters and 61,839 meters from the very top to bottom bounds (Minetest Wiki, updated 2019). The only material that would not reach a terminal velocity in the confines of the in-game universe is gold. According to the calculations performed earlier, it would take 68318.19 meters before the terminal velocity of gold can be observed. Every other material, however, will be able to reach terminal velocity before reaching the boundaries.

The data collected on terminal velocity and the physics associated with falling have numerous possible applications in Minetest. Using the displacement until terminal velocity and the time until terminal velocity data, it is possible to the show the rate of falling in a more realistic way. As stated before, all the materials will fall at the same rate until the terminal velocity is reached. The heavier materials will accelerate for longer; thus, if enough displacement is allotted, then the heavier material can be observed to impact the ground first.

If someone were to make the modifications to incorporate the physics of falling and terminal velocity into Minetest, they can be as precise as desired. The velocity and acceleration were also

calculated at every 0.1 second interval. However, for simplicity, it may be acceptable to only illustrate the displacement or time until terminal velocity. For example, coal was calculated to have a displacement until the terminal velocity of 6,151.32 meters and a time of 50.00 seconds, whereas gravel had a displacement of 8,017.15 meters and 56.60 seconds. Therefore, coal and gravel should fall at the same rate until 6,151.32 meters or 50.00 seconds. At this point, the speed of coal will remain constant, and gravel will continue to accelerate faster than the coal until its terminal velocity reaches a displacement of 8,017.15 meters or 56.60 seconds.

Because the displacements and times to reach terminal velocities are so large, it may be more practical to scale the calculated values down to something more easily observable. For instance, instead of watching coal fall 6151.32 meters for 50.00 seconds, perhaps this could be factored down by 10. Then it would only take 5 seconds to observe the coal reach terminal velocity and only for 6151.13 meters. The scaled values are still large numbers, and a bigger divisible factor may be required as the terminal velocities increase. The scaling does affect the accuracy of the data and should be stated if this change is made. The most important thing to illustrate is the shared falling rates of each material until terminal velocity and how this relates to the density of each.

Further research could be conducted to investigate reasons why certain rocks and minerals are heavier and denser than others. Background knowledge on the chemical and physical compositions of the various materials could help in understanding the discrepancies. The findings could then be used to relate to this study and the correlation of density and terminal velocity found.

The findings of this study showed how an increase in density correlates to a greater terminal velocity. This conclusion can be made because of the standardized frontal area of materials in Minetest and our ability to analyze in-game movement in only the y-plane. The data reflects how the materials with greater density will accelerate for longer until the terminal velocity is reached.

Once incorporated into the Minetest software, this data will assist to better replicate the physics of falling materials as observed in real-life. Students will be able to observe the relationship of materials and their falling rates in real time. With mathmatical reasoning they will be able to understand the correlation of density and the terminal velocity. They should gain a better understanding in how factors such as drag, gas density and frontal area affect the terminal velocity. All of this will be achieved while keeping the students interest in the online software game of Minetest.

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Prevalence of Ciprofloxacin-Degrading Enzymes in Bacteria Collected from Aquatic Environments in the Raleigh, N.C. Area

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Abstract

Because of the overuse of antimicrobials in agriculture and medicine, many forms of resistance have arisen, including the expression of enzymes that can degrade antibiotics, rending them ineffective. These mechanisms of resistance can sometimes be transferred to other bacteria, increasing the prevalence of these enzymes in the environment. Ciprofloxacin (cipro) is an important "drug-of-last-resort" antimicrobial whose effectiveness is threatened by bacterial resistance. This study sought to isolate bacteria expressing cipro-degrading enzymes from water samples collected from a suburban lake in Cary, N.C, as well as a pond located on the Scott Northern Wake Technical Community College campus. Both sample sites were once surrounded by farmland. Samples were tested for resistant bacteria by inoculating cipro-containing agar plates. Resistant colonies were analyzed for degrading enzyme activity using a modified Hodge test. Fifteen samples were collected from September 2021 to April 2022 resulting in 467 resistant colonies. None of the resistant colonies were found to contain cipro-degrading enzymes, verifying that they are uncommon. Further sampling with a larger sample size and more locations are needed to identify bacteria with a degrading enzyme.

Introduction

In 2019, the Center for Disease Control and Prevention (CDC) reported 2.8 million antimicrobial resistant infections in the United States alone, with 35,000 resulting deaths. This can be compared to the 2013 report that states at least 2 million people had antimicrobial resistant infections, with 23,000 resulting in death (AR Threats Report, 2021).

Cipro was first introduced in 1987 as a second-generation fluoroquinolone that inhibits DNA gyrase and topoisomerase, enzymes that are essential for DNA replication in a bacterium. Cipro is considered a broad-spectrum antimicrobial that is often used as a last resort, as it is still effective against many types of Gram-positive and Gram-negative bacteria (Fabrega et al., 2009).

The rise of antimicrobial resistance can be attributed to the abuse and overuse of these drugs, particularly in the preventative use in food animals and over-prescription in clinical settings. Despite the

call to stop the use of antimicrobials in agriculture to promote growth and disease prevention, as well as encouraging physicians to diagnose properly before prescribing antimicrobials, these practices continue to contribute to antimicrobial resistance (Antibiotic Resistance, 2020). The use of antimicrobials in agriculture exposes bacteria to sublethal doses and select the resistant species. By administering antibiotics on a large scale, microorganisms that are not susceptible to them will survive and reproduce without competition, causing exponential increase in the prevalence of those antimicrobial resistant strains (Fabrega and Madurga, 2016).

Antimicrobial resistance has many forms, for example there are enzymes that degrade cipro, like the cr variant of aac(6')-lb. This enzyme causes reduced cipro-susceptibility by promoting its acetylation by other enzymes, changing its chemical composition (Park et al., 2006). Bacteria with cipro-degrading enzymes can directly transfer these mutations to other bacteria through horizontal gene transfer. Such resistance genes can also indirectly grant resistance to other bacterial species by diminishing or eliminating cipro in their environment. Additionally, cipro-degrading enzymes are likely encoded by a single gene, which eases transfer between bacteria compared to multi-gene systems.

With the increasing number of morbidities associated with antimicrobial resistance and new ways that bacteria are becoming resistant, it is important to examine the prevalence of resistance factors in the environment. There is a deficiency of studies that characterize cipro-degrading enzymes, especially studies seeking these bacterial enzymes in the environment, which may be due to their rarity. Because cipro resistance threatens its utility, there is a need for more research to understand the scope of the issue. This study examined the prevalence of cipro-degrading enzymes within Kildaire Farms Pond, Cary NC (Kildaire Pond) and the pond behind building H at Wake Technical Community College's Scott Northern Wake Campus (WTCC Pond). The resistant species found on trypticase soy agar (TSA) plus cipro (10 µg/ml concentration) inoculated with the environmental sample were then tested for

degradation using a modified Hodge test (MHT.) Cipro-resistance is prevalent in these locations; however, no species were found to degrade cipro during the sampling period.

Materials and Methods

Collection of water samples

Kildaire Pond is located in a largely suburban area with many shopping centers and medical plazas directly surrounding it (Figure 1). Before this area was densely inhabited, it was largely farmland. The sampling site was once a part of Kildaire Farm (1920s-1970s), which had up to 200 head of beef cattle, 30,000 chickens, and eventually dairy cattle as well (Van Scoyoc, 2020). The subdivision of Kildaire pond includes a clubhouse with many nearby walking trails, which are frequented by people and their pets. There is a specific walking trail that leads to a dock on the southeastern part of the lake, where water samples were acquired using a sterile specimen cup that was placed in a cooler on ice packs, while transported to Scott Northern Wake Technical Community College's START lab. The WTCC Pond is in northeast Raleigh, also in a suburban area that is rapidly developing (Figure 2). Samples were taken on the water's edge by walking down a drainage bank and placed on ice packs in a cooler for transport.

Isolating resistant bacteria

Upon reaching the lab, tryptic soy agar (TSA) plus cipro (10 µg/ml concentration) agar plates were aseptically inoculated with 100 µl of an environmental sample. The samples were spread carefully over the surface of the agar on each plate using a sterilized stainless-steel spreader. After about 36 hours of growth in a room temperature incubator, the samples were refrigerated until they were able to be analyzed. The characteristics of the resulting colonies, such as color, shape, and texture, were recorded and the abundance of each type of colony was then counted.

Figure 3: Panel A is a view of Kildaire Pond from the shore near the sampling area, while panel B is a satellite view of the dock where samples were taken (circled in red on the southeast side of the pond).

Figure 4: Panel A is a view of the pond behind Building H on the Scott Northern Wake Technical Community College campus from which samples were collected, while panel B is a satellite view of the same pond (circled in red).

Determining the presence of cipro-degrading enzymes

To determine if cipro-degrading enzymes were present in each isolate, they were each subjected to a MHT (Amjad, 2011). A susceptible species of bacteria is needed to determine if there is a degrading enzyme present in the bacteria from the environmental samples. *Escherichia coli* was initially used as the susceptible species due to its large ZOI around the cipro disk. After concluding that the ZOI was too large to accurately determine results from the MHT, *Serratia marcescens* was chosen due to its smaller, clearer ZOI when exposed to cipro. Lab stocks of *E. coli* and *S. marcescens* were subcultured to ensure access to fresh bacteria.

In this study, the MHT consists of spreading an even layer, or lawn, of susceptible species of bacteria exposed to a cipro susceptibility testing disk (5 µg/disk) placed at the center of the plate (Figure 3). The potentially degrading isolates are then inoculated in a line from the disk to the edge to the plate to determine if they alter the zone of inhibition (ZOI) of the susceptible species and therefore contain the degrading enzyme. At most three different bacterial species were inoculated in equidistant lines from each other. MHT plates were placed at room temperature for about 48 hours and then analyzed. If the susceptible species had a clear ZOI that was symmetrical all the way around, this indicated that none of the isolates possessed cipro-degrading enzymes. If the lawn growth encroached closer to the disk adjacent to the isolate, this indicated that the particular isolate may degrade cipro.

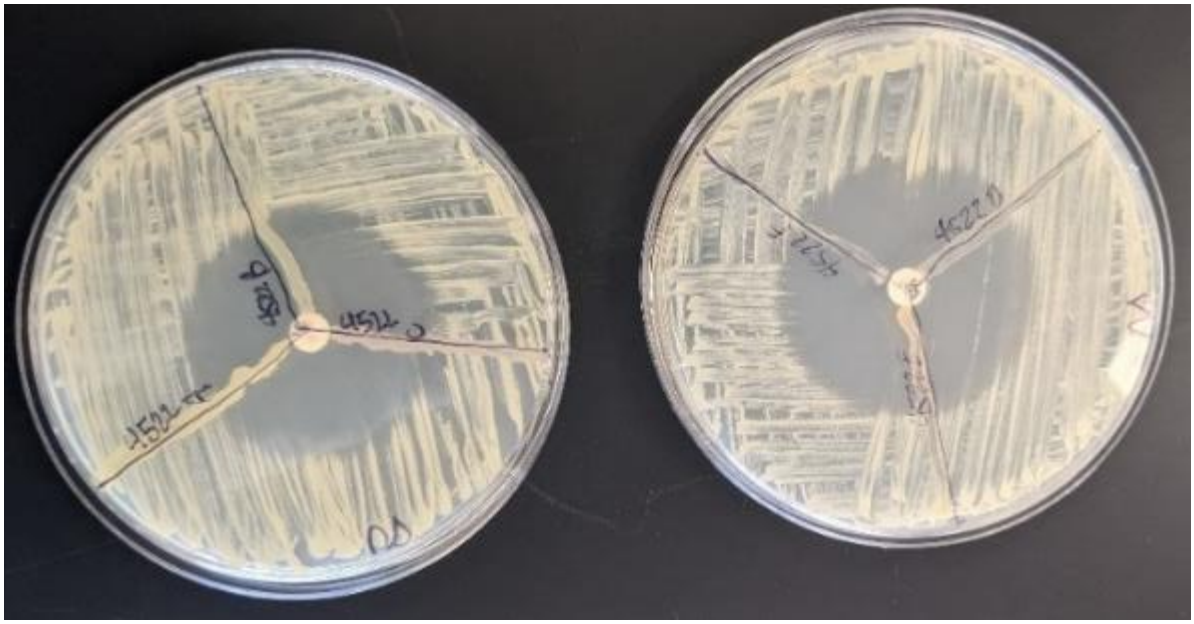

Figure 5: A representative modified Hodge Test performed on isolates. The lawn consists of bacterial species that is susceptible to cipro. Each line of bacteria extending from the disk to the outer edge of the plate is an isolate of different species from the environmental samples. These ZOIs are symmetrical, with no encroachment of the susceptible species toward the disk, indicating the absence of cipro-degrading enzymes.

Results

There was a consistent prevalence of cipro-resistance at each site. Kildaire Pond had an average of about 220 resistant colony forming units (CFUs) per mL of water sample over 11 sampling events from September, 2021 to April, 2022 (Figure 4). WTCC Pond had an average of 1020 resistant CFUs per mL of water sample over 5 sampling events all occurring in April, 2022. One sample from WTCC Pond was an outlier that greatly increased the average; it was sampled on April 19, 2022 and had a resistance CFU count of 4670 per mL. This sample greatly increased the average. Although cipro-resistant bacteria appeared to be abundant in both sampling sites, no bacteria harboring cipro-degrading species were detected. Of the 748 colonies, 71 individual species from both ponds were detected and isolated to undergo MHT. It was common to see bacteria that inhibited the growth of *E. coli* outside of the ZOI on the MHT. This effect was determined to be unrelated to the current study, as bacteria that inhibit the growth of other species are not the target of this study.

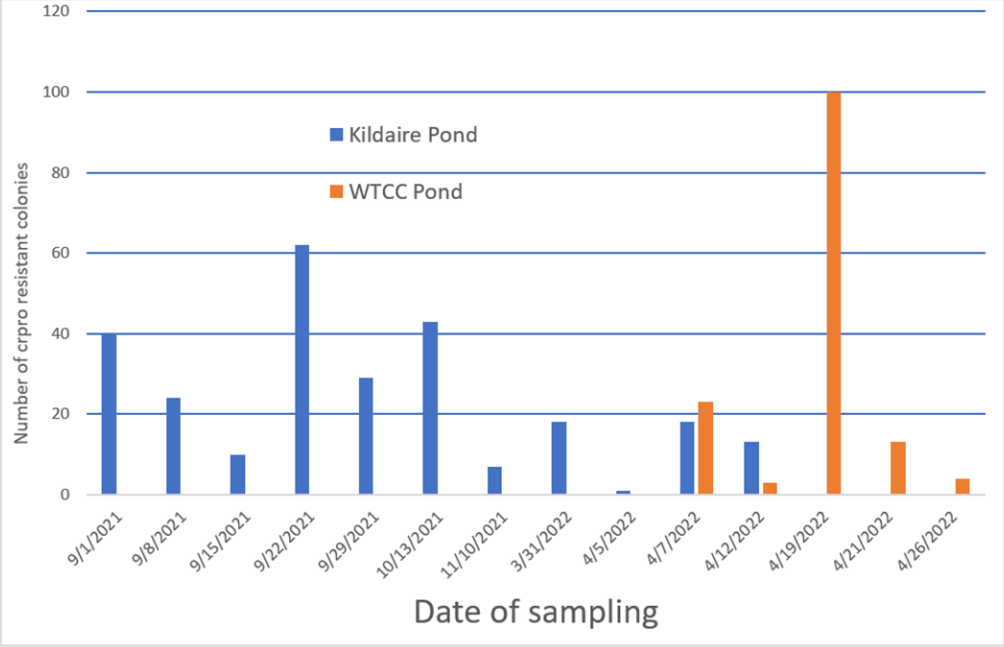

Figure 6: Graph showing the number of cipro-resistant colonies for each sampling event in each pond. Note the actual number of colonies for the WTCC sample taken on April 19, 2022 is 4670 CFU/ml. The Y-axis was truncated to allow results from the other sampling dates to be clearly seen.

Conclusions

The prevalence of cipro resistance in both WTCC Pond and Kildaire Pond is high, however, the presence of bacteria with cipro-degrading enzymes appears to be rare. Because cipro is an antimicrobial of last resort, if humans or pets were to become infected with a cipro-resistant bacterial species, treatment would be more challenging. On a larger scale, antimicrobial resistance is a growing issue, especially in the last 30 years, as antimicrobial use increased in human and livestock populations, while development of new antimicrobials has been cut by more than half. The number of antimicrobial drug application approvals has dropped from 19 between 1980-1984 to 6 in 2010-2014 (Ventola, 2015). The data suggests that this will continue if we do not change the way antimicrobials are often overprescribed and used for food animal growth and disease prevention, all while there is a lack of development of new antimicrobials (Antibiotic, 2020).

Although the amount of resistance found in Kildaire Pond and WTCC Pond is concerning, it is unsurprising considering the history of farming. Resistance will continue to be a growing problem if we do not curb the use of antimicrobials to cases that are medically necessary and properly diagnosed. Because these environments can serve as resistance reservoirs for clinically relevant bacterial species, the long-term resilience of cipro-resistance in environments which were exposed to agriculturally-related antimicrobials is worth more intensive, longitudinal study.

The results of this research support the prediction that cipro-degrading enzymes are rare. None of the bacterial isolates tested positive for degrading-enzymes, although a total of 748 cipro resistant colonies (with 71 individual species that were isolated and underwent MHT) were detected. Further research with a larger sampling size and more sampling locations is necessary to uncover cipro-degrading bacteria in this area. Although these degrading enzymes are rare, they may be more easily transferred between bacterial species and be more rapidly spread in the environment due to the high

transferability of enzymes encoded by a single gene, as well as their ability to allow other bacteria to thrive by destroying cipro in their environment.

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