Inactivation of pathogens by a novel composting toilet: bench-scale and field-scale studies

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ABSTRACT

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Pathogen transmission is a major concern when implementing sanitation systems in resource poor areas. Multiple studies have shown that ecological sanitation systems are an effective means for improving health in areas where standard systems cannot reach disparate populations. Emerging designs of ecological sanitation systems provide needed access to hygiene but they still require confirmation of their effectiveness in reducing pathogen loads in the waste and transmission of disease. "The Earth Auger" is a novel composting toilet design that has incorporated critical elements to accelerate pathogen die off and has proven to be popular among recipients. A subset of active composting toilets were monitored for 2 months, taking samples at different points in the treatment line to determine the presence and quantity of total coliforms, *E.coli*, helminth ova and *Salmonella*. In addition, two scaled composting units were inoculated with *Ascaris suum*, *Salmonella enterica*, *poliovirus 1*, and *MS2* bacteriophage. Over a period of two months tested for *E.coli* and inoculated pathogens for die-off. Samples were used to determine pathogen die-off through the treatment line according to time, pH, temperature and moisture content. Multi-variable linear regression compared *E.coli* levels in the treatment line with community as a modifying variable, statistically significant reductions were observed *from* the bowl to the receiving tank (avg. $\log_{10}$ reduction: -1.27; 95% CI: -2.22 to -0.32), and from the bowl to into storage (avg. $\log_{10}$ reduction: -2.71; 95% CI: -3.55 to -1.87). There were no statistically significant differences seen between communities (p-value=0.566) or from the bowl to the end of the horizontal reaction tube (p-value=0.120). Additionally, sample sizes in study were too small to see statistically significant differences between communities and between different points along the treatment line. Helminths were found in some endpoints during field work, increasing in numbers through treatment line, but prevalence was low and die-off patterns could not be established. Tested environmental factors did not significantly contribute to indicator reduction. At the endpoints for this study, however, indicator levels in compost directly out of treatment system were still above acceptable values delineated by EPA biosolids class B and below WHO guidelines for excreta reuse in agriculture. *Salmonella* were not found in samples throughout the treatment line. Experimental composting units did not yield useful die-off results but suggested modest decreases in *Ascaris suum* ova and bacteriophages. Additional human subject participation would have greatly benefited bench-scale experiments and elicited more information about the system. Under the current deployment and usage practices, these composting toilets do not strongly reduce indicator organisms after initial treatment, which will limit the use of post-treatment compost and extend storage time.

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INTRODUCTION

Global Need for Sanitation

Worldwide 2.5 billion people lack access to clean, safe and dignified sanitation. This presents numerous problems as improper sanitation is associated with poverty, poor health, and environmental degradation. In the Millennium Development Goals, Goal 7: Ensure Environmental Sustainability, the United Nations proposed halving the number of people without access to sustainable water sources and improved sanitation by 2015. In contrast to meeting many of their other proposed goals, the sanitation deficit is still large and they missed the 2015 goal by 700 million people (WHO/UNICEF, 2015). While 2.1 billion people have gained access to improved sanitation from 1990, 2.4 billion people still lack sanitation as of 2015 making the challenge of improving sanitation globally is immense and critical to improving the health of people. When sanitation deficits are broken down we find that the 70% of people lacking improved sanitation facilities live in rural areas as opposed to urban areas (WHO/UNICEF, 2014).

Despite an 18% improvement in the least developed countries over a 25 year period, only 37% of people in these regions have access to improved sanitation (WHO/UNICEF, 2015). Areas with the highest need for improved sanitation are: Sub-Suharan Africa (30% sanitation coverage), Oceania (35% sanitation coverage) and Southern Asia (47% sanitation coverage) (WHO/UNICEF, 2015). The need for improved sanitation also carries into rural populations, where only half of rural dwellers have access to sanitation compared to 4 of 5 urban dwellers (WHO/UNICEF, 2014 2015). This presents a unique issue in that rural areas have disperse populations, making infrastructure initiatives more difficult and less impactful compared to urban areas.

Lack of sanitation has huge consequences from a public health and environmental perspective. Pathogens from improperly disposed excreta are readily transmitted by contamination of water, food, and other vehicles and are the main concern when dealing with wastewater, and human excreta. Diarrheal diseases are the second leading cause of death in children under five, killing about 760,000 children each year (WHO/UNICEF, 2013). Additionally, human fecal matter have high levels of nitrogen, phosphorous and biological oxygen demand which have detrimental effect on the environment by increasing the number of algae blooms and hypoxic aquatic environments (WHO, 2006). As the global population balloons, sanitation becomes critically important in protecting the environment and human health.

Meeting Global Need for Sanitation

Current global paradigms about the treatment of human excreta of "drop and cover" and "flush and forget" are unrealistic given the gravity of the sanitation issue and continuance of unsafe practices. Due to few resources and a low priority status, conventional wastewater conveyance and treatment are not legitimate solutions in meeting the global need for sanitation. Small- scale or residential level sanitation systems which are able to do on-site treatment are of increasing in importance when it comes to meeting the global need. Using the concept of ecological sanitation, where sanitized human excreta is treated as a resource, is a holistic, closed-loop approach towards sanitation that could be feasibly implemented into resource limited regions and rural areas. The goals behind ecological sanitation is to reduce hygienic concerns of pathogen transmission from feces, optimize the management of nutrients, reduce environmental degradation, and reduce water contamination (Langergraber & Muellegger, 2005). Ecological sanitation can be incorporated at large or small scale levels and include a variety of techniques.

Small-scale or on-site treatment of feces or feces combined with urine, are usually in the form of latrines, composting toilets, dry-composting toilets, and septic tanks (see Table 1 for comparisons). Latrines are the easiest of improved sanitation options to construct and implement but typically have the lowest rates of pathogen die-off and require a long storage period for pathogen reduction prior to agricultural application. Composting toilets can be easily implemented but do require more involvement on the user to correctly manage environmental conditions important to pathogen reduction. Dry composting toilets are typically urine diverting creating multiple waste streams and a slightly different approach to managing environmental conditions important to standard composting, as moisture is low. Both dry and standard composting have a wide range in application and can come in easy to use commercial set ups which are costly or can be implemented inexpensively in low resource settings with just a bucket and sawdust. Septic tanks can be used in with the ecological sanitation mindset with pumped sewage out of the tank going back into reuse after more conventional wastewater treatment. While septic tanks provide the most first world bathroom experience and ease to the user they are extremely expensive to install, treat waste, require a liquid conveyance and still require outside wastewater treatment. They are often not considered to be legitimate solutions to the global sanitation solution but do provide an opportunity to reutilize nutrients while sanitizing waste.

Goals of Sanitation and Small-Scale Sanitation Systems

From a hygienic perspective, sanitation systems are designed to reduce pathogen loads to a level which poses little to no risk based on the proposed usage. Large scale sanitation systems have strict guidelines for pathogen elimination and solid waste application according to local, national, and international regulations/guidelines. Regulations on small sanitation is complicated because they can be informal, difficult to monitor or highly variable in efficacy. Manufactured small sanitation systems, should however, be validated on their efficacy to reduce indicators/pathogens in both field testing and experimental conditions prior to commercialization.

The World Health Organization (WHO) has guidelines for the reuse of treated excreta and wastewater, aimed at helping determine pathogen/indicator reductions necessary to meet health based targets of disease. For the reuse of fecal matter in agriculture with minimal restrictions on usage the WHO expects there to be <1 helminth ova/g total solids and 6- $\log_{10}$ reduction of bacterial indicators (WHO, 2006). These values are designed to meet a proposed tolerable disease burden for waterborne disease of 10^{-6} Disability-Adjusted Life Years per person per year, with mild diarrhea having a case- fatality of 10^{-5} and annual disease risk of 1 in 1,000 (WHO, 1984). Countries can adjust these values to reflect feasible health targets, with developing countries raising health targets based on high disease incidence and low resources. Monitoring and system validation of small-scale systems can be more tedious and is often limited to visual monitoring and simultaneous system validation to determine exposures to fecal-borne diseases and contamination. The WHO has storage guidelines based on ambient temperatures and pH, which small-scale systems should follow if formal validation cannot be conducted or if the system does not meet criteria for pathogen reduction (WHO, 2006).

In the United States, the Environmental Protection Agency (US EPA) has enforcement power under the Clean Water Act and the federal biosolids rule contained in 40 CFR Part 503 to regulate discharges of pollutants into surface waters, and land application of treated sewage sludge (biosolids). Under these regulations, guidelines for wastewater and wastewater discharges have been developed to protect human health and prevent environmental degradation (EPA, 1999). The biosolids rule has specific pathogen and usage requirements for treated sewage sludge that is broken up into two categories: Class A and Class B. These two classifications have different pathogen/indicator requirements and usage requirements. Class A or "Exceptional Quality" biosolids has the most stringent pathogen requirements but can be applied and even sold by treatment facilities for a wide range of uses including agriculture for close human contact. It requires that biosolids have <1,000 MPN/g total solids fecal coliforms or <3 MPN/4 g total solids *Salmonella*, <1 PFU/ 4 g total solids enteric viruses, and <1 helminth ova/4 g total solids. Class B has only fecal coliform requirements of <2,000,000 MPN/g total solids fecal coliforms and is much more restricted in its usage and almost always requires a fallowing period before livestock or plants meant for human consumption can be harvested (EPA, 1999).

The NSF/ANSI 41- Non-liquid saturated treatment system standard is an international certification specific to non-liquid sanitation systems (NSF/ANSI, 2011). This certification is designed to set minimum standards for sanitation systems that do not use water or any other liquid to treat or store human fecal matter. The majority of these requirements fulfill design, manufacturing and performance criteria that a certified system would have. The only microbial quality requirements listed under performance criteria are that end products must be <300 MPN/g total solids fecal coliforms and there must not be unpleasant odors associated with the usage of system (NSF/ANSI, 2011).

Table 1: Comparative table of important Ecological Sanitation methods (Pit latrines, composing, dry-composting and septic tanks) where nutrient recovery, hygiene and reduction of environmental pollution are key to spirit of ecological sanitation.

Sanitation Type	Pros	Cons	Pathogen Reduction Capacity	Source
Pit latrines	• Easy to implement • Lowest cost • Little maintenance • Can be implemented with minimal skill and training	• Long storage requirement for pathogen reduction • Highly dependent on environmental conditions • Typically outside the home • Restricted to residential or small communities	• >6 months for indicators • Variable time for helminths but up to 2 years • Mehl et al- found helminth ova after 2 years treatment • Sherpa et al., found 2.31 $\log_{10}$ reduction in indicator bacteria	• Mehl et al., 20011 • Jensen et al., 2009 • Sherpa et al., 2009
Composting	• Wide range in application • Can be scaled to large communities • Utilization of microbial metabolic processes • Combined organic wastes • No water utilization	• Cost dependent on technology • Highly involved • Management of environmental parameters can be technical	• Highly dependent on managed environmental parameters • Normally between 6 weeks and >6 months	• Berendes et al., 2015 • Langergraber et al., 2005 • Cabanas-Vargas et al., 2014
Dry-composting	• waste separation • Increased desiccation • Utilization of microbial metabolic process for degradation • No water utilization	• Cost dependent on technology used • Highly involved • Management of environmental parameters can be technical	• Highly dependent on managed environmental parameters • Typically between 6 weeks and >6 months	• Berendes et al., 2015 • Langergraber et al., 2005

Septic tank and drain field	• Little involvement on daily basis by users • Can be implemented at community level with drainage fields • Highly contained system	• Extremely costly • No immediate return on excreted nutrients • Requires expensive upkeep • Requires large capacity wastewater treatment after emptying tank • Requires skilled implementers • Resource intensive • Requires liquid conveyance of waste	• Pathogen reduction not as important as system is completely contained and additional waste treatment is required off-site	• US EPA, 1999

Value of Process and Indicator Organisms

Using specific microorganisms as indicators of fecal contamination in the environment or in validating a sanitation system can help characterize environmental conditions that drive indicator reduction and to what extent potential pathogens are eliminated. Fecal coliforms, *Enterococci* and *E.coli* are the most widely used set of bacteria used to monitor fecal contamination, but their absence does not necessarily guarantee the absence of pathogens (Payment, 2011). Additionally, ubiquitous and environmentally persistent pathogens such as *Salmonella* spp., *Ascaris* ova and enteric viruses have been used to monitor the quality of treated wastes and biosolids prior to land application (EPA, 1999; WHO, 2006) . Indicator organisms are valuable when evaluating systems and can help validate a system through side-by-side evaluations of indictors and environmental factors predicted to be important to pathogen die-off. These studies do not directly predict the die-off of specific pathogens but can be broadly applied to determine the efficacy of a system.

In experimental studies, process organisms can act as proxies for a panel of similar microorganisms or directly predict die-off of the inoculated pathogen. Process organisms are usually spiked into the study material at known quantities and monitored through the experiment for survival, growth increases or die-off. These organisms are chosen based on specific characteristics and testable environmental conditions. For example, *Ascaris suum* is a process organism used ubiquitously in sanitation studies based on the high environmental resistance of helminth ova in human feces. Monitoring levels of inoculated ova in an experimental conditions will elicit information about conditions and system efficacy as they relate to helminth ova survival. Similar evaluations of helminth ova in the field rarely provide the same information about system efficacy due to variable infection rates in different regions. Both process and indicator organisms can act as surrogates for a wide range of pathogens as long as they are chosen appropriately. Choosing the right organism or panel of organisms can help validate that a system meets design requirements, monitor its operations while in use and provide verification monitoring that ensures pathogen reduction is occurring (WHO, 2006).

Composting Human Fecal Matter

Composting is a complex, aerobic, microbial process that relies on microbes to metabolize and stabilize organic matter in the composting material. During microbial respiration, organic matter is consumed by rapidly reproducing and metabolizing microbes until consumable nutrients are limited and bacterial populations collapse and are succeeded by more dominant populations (Nakasaki, Nag, & Karita, 2005; Schloss, Hay, Wilson, & Walker, 2003). The compost will change in nutrient and microbial composition making environmental conditions for pathogens more difficult, resulting in their decline. Environmental parameters are important to pathogen die-off when composting human fecal matter and will affect the efficacy and overall decline of pathogens with respect to composted human excreta.

Microbial respiration during the composting process can produce heat sufficient enough to have a sanitizing effect, eliminating pathogens (WHO, 2006). Temperatures reaching above 50˚C for a week or more is considered appropriate to fully sanitize a compost pile and reduce pathogens to a safe level (WHO, 2006). These higher temperatures, in addition to reducing pathogens, favor thermophilic microbes, allowing them to outcompete microbes of human origin and be replaced with fewer and more environmentally based microbes (Novinscak, Surette, Allain, & Filion, 2008)). Increases in temperature typically occur in the middle of a composting mass, where outside edges do not get fully exposed to the produced heat for sufficient time or at all (Germer, Boh, Schoeffler, & Amoah, 2010). These issues are typically remedied through insulated reaction chambers or mixing to fully ensure that temperature contact time has a sanitizing effect (Niwagaba, Nalubega, Vinneras, Sundberg, & Jonsson, 2009).

Human excrement is typically has between 53-92% water content (Nishimuta et al., 2006) which can greatly affect the productivity of microbes in compost. Aerobic decomposition in compost has an ideal range between 40-60% total solids (Redlinger, Graham, Corella-Barud, & Avitia, 2001). Compost with higher moisture content is at risk for anaerobic conditions with increased odors and compost with less moisture is associated with reductions in fecal coliforms but may leave un-decomposed organic matter or pathogens with regrowth potential (Redlinger et al., 2001). Hence, the balance between desiccation and sufficient moisture content for continued aerobic decompositions must be kept in check if organic matter and indicators/pathogens are to be reduced.

Increased exposure to the sun or ultra violet (UV) rays can help drive pathogen die-off by increasing temperatures and material desiccation (Redlinger et al.). Even degrees of solar exposure has been shown to increase the total reduction in composted fecal matter when tested in solar pit latrines (Redlinger et al., 2001). Mechanistically, direct UV exposure damages nucleic acids, killing sensitive pathogens and microbes. Solar exposure has even been recommended as a post treatment for stored pit latrine material prior to agricultural usage (Mehl et al., 2011).

Increasingly basic conditions can have a profound effect on microbial survival and has been demonstrated to have a threshold effect in composting fecal matter where high pH can help eliminate pathogens but slow the process of aerobic decomposition (Hill, Baldwin, & Vinneras, 2013). Aerobic decomposition seen in composting typically works within neutral pH ranges but with increasing pH pathogen die-off can be increased. McKinley et al., found that effective *Ascaris* inactivation was driven by a combined mix effects of both high pH and high ammonia concentration. These were mediated through the addition of ash, fresh urine and stored urine (McKinley, Parzen, & Mercado Guzman, 2012), with ash providing the needed alkaline conditions and increased toxicity to *Ascaris* ova.

The addition of a carbonaceous additive to composting material can help control the carbon: nitrogen ratio. These bulking agents adds carbon to the nitrogen rich feces and/or urine but can also help control odors, deter vectors, aid in desiccation and increase porosity for increased aerobic activity. The material of the bulking agent can also have a direct effect on pathogen die-off through antimicrobial properties or changes in pH (Niwagaba, Kulabako, Mugala, & Jonsson, 2009). In the dry composting toilet study done by Magri et al., they found that oyster shells were more effective at reducing pathogens as a bulking agent than ash or sawdust, and found that organic additives such as woodchips experienced reductions similar to storage alone. This study also found that quantity of the bulking agent was important and that too much addition of a bulking agent could create a “protective” layer resulting in almost no pathogen reduction or compost modification (Magri, Philippi, & Vinneras, 2013). Bulking agents are an important part of composting fecal matter but must the selection of the material and quantity used will affect the amount of time necessary for indicator and pathogen reduction.

Novel Composting Toilet

Novel technologies in sanitation are filling sanitation gaps and have great potential to bridge problems that traditional waste conveyance and treatment systems have. One such design, the Earth Auger designed by Critical Practices LLC, intends to improve sanitation options for rural and urban dwellers while providing an end-product (composted fecal matter) that can be used as a beneficial soil conditioner. This toilet is a urine-diverting dry/composting toilet with a mechanical “flush” component that mixes and aerates feces. This mechanical “flush” is connected to a larger spiral system inside of an eight inch horizontal tube, where mixing of and advancement of fecal matter and sawdust occurs. Mixed and partially decomposed fecal matter and sawdust are then deposited into a collection system that is changed out when it reaches capacity. Collection systems are usually dark colored catchment bags or plastic holding tanks. The Earth Auger can hold an estimated 100 “flushes” in the horizontal tube, and depending on the number of users and quantity of waste matter deposited, residence time varies. Sawdust or other dry organic matter is added to the system as a covering, odor reducer and compost amendment. By design, The Earth Auger has tried to utilize multiple composting components anticipated to accelerate pathogen die-off. The auger system, organic bulking materials and anticipated storage conditions are expected to aid the composting process by stabilizing organic waste and reducing bacterial and pathogen loads. These toilets have been and are continued to be installed as part of a pilot program into low-income Ecuadorian communities, where around 2 million people lack dignified sanitation as of 2012 (WHO/UNICEF, 2014).

Study of a Small-Scale Urine-Diverting Dry Toilet

Small scale sanitation systems are key in alleviating a global deficit for proper sanitation. They can be inexpensive to users and installed without significant infrastructural changes, making them instantly accessible to consumers. Validation of these systems for pathogen reduction is critical to ensure that the system reduces bacterial loads and pathogens, and minimizes pathogen transmission. The Earth Auger, provides a technology set that requires validation of pathogen reduction and monitoring of environmental conditions. To do this, field studies of deployed units in Ecuador were conducted where helminths, *E.coli* and *Salmonella* were monitoring along with pH, total solids and temperature. Additionally, scaled-composting systems were inoculated regularly with feces, sawdust, *Ascaris suum* ova, *Salmonella enterica* serovar LT2, MS2 Bacteriophage and poliovirus 1 for a total of two months. Inoculated pathogen levels, *E.coli* levels, pH, total solids and temperature were monitored for reductions after initial treatment. The purpose of this study was to determine the efficacy of pathogen reduction and to determine which environmental conditions are the primary drivers of pathogen die-off in The Earth Auger.

METHODS AND MATERIALS

Monitoring of deployed systems

Deployed toilets and communities

Approximately 80-90 toilets are installed in various parts of Ecuador. The majority of these deployed units are located in the provinces of Guayas, Manabi, and Santa Elena. Three primary communities were sampled from in the coastal area. Each had distinct features but are considered to be in the same climatic range and altitude. Community 1: was a group community located just outside the major city of Guayaquil amongst banana plantations and dense agricultural production. This community was more secluded and reliant on locally raised food sources and rain collection for water. It was noted by researchers that there were a number of pigs that were kept in close proximity to houses and large numbers of children were observed. Community 2: was a series of peri-urban slums outside the major city of Guayaquil. These were informal communities with direct ties to developing infrastructure such as in-house electricity, paved roads and bus routes. Community 3: were dispersedly located homes along a major interprovincial highway along the coast of Manabí and Santa Elena. These were within close proximity to popular beach areas but not located immediately next to beach properties. Cooler daytime temperatures and drier conditions were noted by researchers. Samplings were chosen on the basis of previously scheduled monthly visits of the hosting organization. Sampling of deployed composting toilets in Ecuador were conducted weekly from February 2nd until March 9th 2015. Previous samplings and one sampling in this time period was interrupted and processing of samples was incomplete.

Sample collection

During sampling approximately 100 grams of compost from various points strategically along the treatment line (bowl, horizontal reaction tube, receiving tank and storage containers). Samples were grab samples and if taken from a receiving tank or storage container were taken from the upper most layer to represent the “worst case-scenario.” Samples were collected in sterilized glass jars and kept on ice in a cooler for transport to the bacteriology lab at the Universidad Católica de San Guayaquil in Guayaquil, Ecuador. Other observations of the toilet’s actual operation, any observed handling behavior, waste storage conditions and unanticipated exposure risks were documented as possible according to WHO guidelines for small-scale systems (WHO, 2006).

pH, Total Solids and Temperature

Total solids were determined by taking 15-20 grams of well-mixed compost, drying overnight at 103-105˚C and determining final dry weight. Sample pH was determined by taking a 5 grams of dried compost sample and diluting it into 25 ml of distilled water, after 10 minutes pH paper was used to determine pH of sample. The temperature was measured at the time of sample collection in the horizontal reaction tube, receiving tanks and storage containers.

Indicator Organisms

Samples were processed for *Ascaris lumbricoides* and *Trichuris trichuria* ova, *E.coli* and *Salmonella*. Helminths were extracted using a method developed at The University of Arizona and modified for field settings accordingly. Helminth ova were extracted by taking 30 grams of representative sample, dividing it up into 50ml Falcon centrifuge tubes. Samples were washed using 1% 7X detergent, solids were settled using a modified handspun salad spinner and top liquid portion was discarded. This process was repeated 3 times. After this the addition of $MgSO_4$ solution (specific gravity= 1.24) allowed for density gradation and settling using a modified handspun salad spinner at constant speed. Liquid portion was screened using a #50 and #400 Tyler sieve. Contents were washed using deionized water and taken up via pipette into a final collection tube. Extract was viewed using a Sedgewick Rafter cell where *Ascaris lumbricoides* and *Trichuris trichuris* ova were identified and counted (Schmitz & Pepper, 2014).

To detect *Salmonella* spp., 30 grams of sample was homogenized and serially diluted with sterile deionized water into a Tryptic Soy enrichment broth (TSB) tubes and incubated at 36˚C overnight. Of the positive tubes, 30 ul of TSB were dropped in triplicate on to modified semisolid Rappaport-Vassiliadis (MSRV) medium, with 0.002% novobiocin, plates and incubated for 18 hours at 42˚C. Growth showing motility in the form of a halo were streaked for isolation on Xylose Lysine Deoxycholate (XLD) agar. Colonies showing Salmonella characteristics would be confirmed using lysine iron agar slants, triple sugar iron agar slants and *Salmonella* O antiserum Polyvalent Groups A-1 and Vi. Samples that were homogenized and serially diluted for *Salmonella* spp. were taken and used with Colilert®-18 and Quanti-tray®2000 from Idexx Laboratories, incubated at 36˚C for 18 hours and florescent wells were detected using UV light to determine the presence and quantity of *E.coli*.

Bench-Scale Study

Experimental studies were performed on two ¼-scale composting sanitation systems that are designed to model full-scale use under controlled conditions. Organisms that are less prevalent in populations but can be highly persistent in environmental conditions were used as process organisms. Feces and sawdust from a locally sourced sawmill were inoculated sporadically between 50-300 grams of feces each introduction and 20% (w/w) sawdust into one scaled system for 55 days and the other for 25 days. The auger in each was turned each day it had fecal matter in it regardless of feces introduction. Feces were collected from healthy, adults volunteers who were not taking medications related to gastrointestinal illnesses or conditions and were feeling well on the day of submission. Process pathogens and inoculation levels were: *Ascaris suum* ova (1000 ova per gram wet feces), *Salmonella enterica* serovar LT2 (10^6 CFU per grams of wet feces), MS2 bacteriophage (10^8 PFU per 10 gram wet feces), and *Poliovirus* 1 (10^6 per 10 grams wet feces). Additionally, naturally occurring *E.coli* in donated feces was used as an indicator organism.

Process organisms

Poliovirus and MS2 bacteriophage were extracted from the compost using a polyethylene glycol (PEG) precipitation method and cultured on Buffalo Green Monkey Kidney (BGMK) cells to provide plaque-forming units per total solids of waste. Viral extraction requires viral particle suspension and homogenization for a half hour in 3% beef extract, 0.05M glycine buffer followed by centrifugation (2,500 xG for 20 minutes at 4˚C). Supernatant was discarded and remaining solids were shaken overnight at 4˚C in a 9% PEG 8000 and 0.4M NaCl precipitate solution. Samples were centrifuged for 20 minutes at 10,000x g at 4˚C and the supernatant was discarded. This was followed by a decontamination step with the addition of MS-782 Vertrel® XF or chloroform at one-third the volume followed by agitation, centrifugation (4,500x g for

15min) and collection of the supernatant into phosphate buffered solution. Extracted material was inoculated in duplicate for four serial dilutions of 200 ul inoculum onto Buffalo Green Monkey Kidney cells in monolayer and identified as poliovirus by cytopathogenic effects. Extracted material was serially diluted and used in a double-agar layer method to determine the quantity of MS2 bacteriophage by plaque formation on a lawn of *E. coli* F*amp* (Adams, 1959). *E.coli* F*amp*, incubated at 36°C overnight in LB broth, used as a bacterial host for *MS2* bacteriophages.

Ascaris extraction methods were similar to field methods with hand-spinning of samples was replaced by centrifugation during washings were done at 3200x g for 15 minutes and centrifugation during for floatation step was 3400x g for 30 minutes. Extracted ova were then counted in well plates and incubated at 29˚C in 0.05M H_2SO_4. Morphological changes were monitored at 12 and 28 days incubation to determine viability and development (Schmitz & Pepper, 2014).

Salmonella species and *E.coli* were determined the using the same methods in experimental units as deployed units.

Ethical Research Permission and Import Permit Statement

An ethics review has been conducted and approved by the Bill and Melinda Gates Foundation for the work Fundación In Terrís and Critical Practices LLC will conduct (ID#: OPP1033341) (APPENDIX A). Internal Review Board approval from the University of Washington, Human Subjects Division was approved on August 28th, 2015 and approved for modifications on February 17th, 2015 (ID#: 47742) (APPENDIX B). Additional permission was granted for on-site experiments by University of Washington, Environmental Health and Safety office through a Biological Use Authorization form (BUA #: 0374-006-001) (APPENDIX C) Permission to import samples for viral processing was approved March 12th, 2015 from the Center for Disease Control (ID#: 201503054) (APPENDIX D). A work contract between Fundación in Terrís and Universidad Católica de San Guayaquil was arranged for the study period (APPENDIX E).

RESULTS

Deployed Units

Incremental decreases in *E.coli* indicator levels were seen through the treatment line (Table 2) with storage containers having the lowest levels of indicators. In Figure 1, initial *E.coli* levels in the bowl were an average $5.87\text{x}10^8$ MPN/g solids, with a median of $6.25\text{x}10^8$ MPN/g solids (range: $3.39\text{x}10^7 – 9.23\text{x}10^9$ MPN/g solids). Levels at the end of the horizontal tube were an average $1.26\text{x}10^8$ MPN/g solids, with a median of $7.19\text{x}10^7$ MPN/g solids (range: $2.84\text{x}10^6$- $2.23\text{x}10^9$ MPN/g solids). Receiving tanks had a mean of $3.27\text{x}10^7$ MPN/g solids, with a median of $3.66\text{x}10^7$ MPN/g solids (range: $4.36\text{x}10^5$- $8.41\text{x}10^8$ MPN/g solids). Storage containers had a mean of $1.3\text{x}10^6$ MPN/g solids (range: $3.93\text{x}10^4$- $5.70\text{x}10^7$ MPN/g solids). Multi-variable linear regression compared *E.coli* levels in the treatment line with community as a modifying variable, statistically significant reductions were observed from the bowl to the receiving tank (avg. $\log_{10}$ reduction: -1.27; 95% CI: -2.22 to -0.32), and from the bowl to into storage (avg. $\log_{10}$ reduction: -2.71; 95% CI: -3.55 to -1.87) (Table 2). There were no statistically significant differences seen between communities (p-value=0.566) or from the bowl to the end of the horizontal reaction tube (p-value=0.120) (Table 2).

Collected storage samples ranged in age from freshly harvested (input as 0.01 days) to 250 days. No clear die-off trends were distinguished when compared with number of days in storage (Figure 2). Six storage samples between 1-100 days in age, were observed to have acceptable and declining levels of *E.coli* but several older samples had surprisingly high indicator levels for their age (Figure 2). A number of different types of storage containers (bags, buckets, permeable and impermeable) and practices (shaded, sun, covered) were observed, which could explain the lack of a clear die-off trend under storage conditions.

Ascaris ova were observed in one out of three communities in three unique toilets with a repeated measure in one toilet (Figure 3). *Trichuria* ova were observed in multiple samples from the same toilet in co-occurrence with *Ascaris* ova. *Trichuria* ova did not overwhelmingly contribute to the total helminth count but were included in the total helminth counts (Figure 3). Helminth ova were more numerous in the receiving tanks and at the end of the horizontal tube than the bowl (Figure 3).

Twelve systems from two communities were tested for *Salmonella*, observed presumptive colonies were all atypical lactose fermenters. Colonies typical of *Salmonella* are non-lactose fermenting but colonies on presumptive media were atypical. Biochemical tests were all negative for presumptive *Salmonella* colonies.

Physio-chemical parameters on Indicator Reduction

None of the physio-chemical parameters tested were correlated with decreases in E.*coli* levels (Figure 4, *Figure 5*, *Figure 6*). Compost pH for all samples were within a neutral range of 4.5-7.0 and did not change with age or through the treatment process (*Figure 5*). Temperature taken at the time of sampling, did not deviate from ambient temperatures for the region (*Figure 6*). Moisture content, measured as total solids, were unrelated to *E.coli* levels but did experience decreasing levels of moisture through the treatment process and with age (Figure 4).

Experimental units

The two composters were sampled at 30 days, 44 days and 54 days post initial inputs of feces, pathogens and sawdust from November 23rd, 2014- February 17th, 2015. The physical conditions of the two composting units were in mild winter conditions and temperatures in units were consistent with both the ambient air temperature and ambient floor temperature of the housing unit provided (Figure 7). Winter conditions provided protective temperatures with a combined chamber temperature range of -1.2° C to 21°C preventing microbial respiration and temperature evolution in system chambers (Figure 7). In samplings and initial values, pH remained neutral (pH=7.3 and 7.9) (Table 2). Total solids were in range for aerobic decomposition (Redlinger et al., 2001) and changes were not consistently seen (Table 3).

Ascaris suum ova experienced decreases in recovered numbers and in percent viability suggesting toxicity to ova after inoculation (Table 3). *MS2* bacteriophage experienced 2.68 $\log_{10}$ reduction in the first 30 days of initial treatment and an additional 1.18 $\log_{10}$ reduction after 54 days for a total reduction of 3.86 $\log_{10}$ reduction (Table 3) (Compost unit 1). *Poliovirus 1* levels were inconsistent and erratic, with the 54 day sampling having a larger of PFU/g solids than experimental initial values and the 30 day sampling. *E.coli* indicator levels remained relatively constant (range: $>5.66\text{x}10^8$ to $1.64\text{x}10^8$ MPN/g solids).

Table 2: Multi-variable linear regression shows the $\log_{10}$-reduction in *E.coli* levels along the treatment line and between communities. Using an alpha level of 0.05 showed that there were statistically significant decreases in E.coli between the bowl and receiving tanks with an average 1.27 $\log_{10}$ decrease (95% CI: -2.22 to -0.32) and between the bowl and storage containers with an average 2.71 $\log_{10}$ decrease (95% CI: -3.55 to -1.87). There was not a statistically significant difference between communities or in *E.coli* levels in from the bowl to the end of the horizontal reaction tube.

	Coefficient	95% Confidence Interval	P-value	Significant (α=0.05)
Intercept (Bowl)	8.60	7.75 to 9.44	3.70E-23	**
End of tube	-0.67	-1.52 to 0.181	0.120	
Receiving tank	-1.27	-2.22 to -0.32	9.95E-03	**
Storage Container	-2.71	-3.55 to -1.87	7.69E-08	**
Community	0.11	-0.27 to 0.49	0.566	

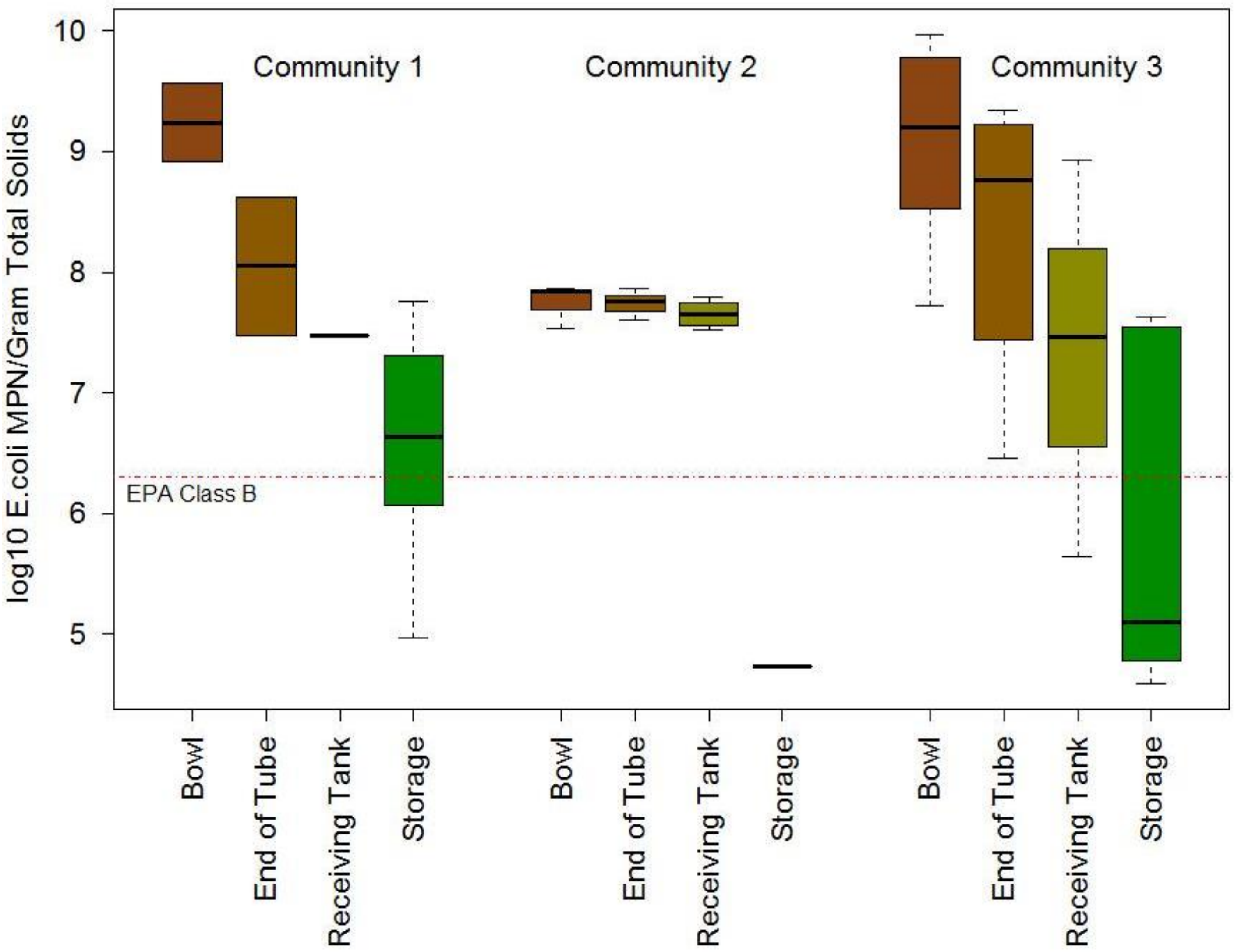


Figure 1: E.coli MPN/ gram of solids displayed by community and location along the treatment line. Mean *E.coli* levels in the bowl were 5.87×10^8 MPN/g solids, with a median of 6.25×10^8 MPN/g solids (range: $3.39 \times 10^7 – 9.23 \times 10^9$ MPN/g solids). Mean levels at the end of the horizontal tube were 1.26×10^8 MPN/g solids, with a median of 7.19×10^7 MPN/g solids (range: 2.84×10^6-2.23×10^9 MPN/g solids). Receiving tanks had a mean of 3.27×10^7 MPN/g solids, with a median of 3.66×10^7 MPN/g solids (range: 4.36×10^5- 8.41×10^8 MPN/g solids). Storage containers had a mean of 1.3×10^6 MPN/g solids (range: 3.93×10^4- 5.70×10^7 MPN/g solids).

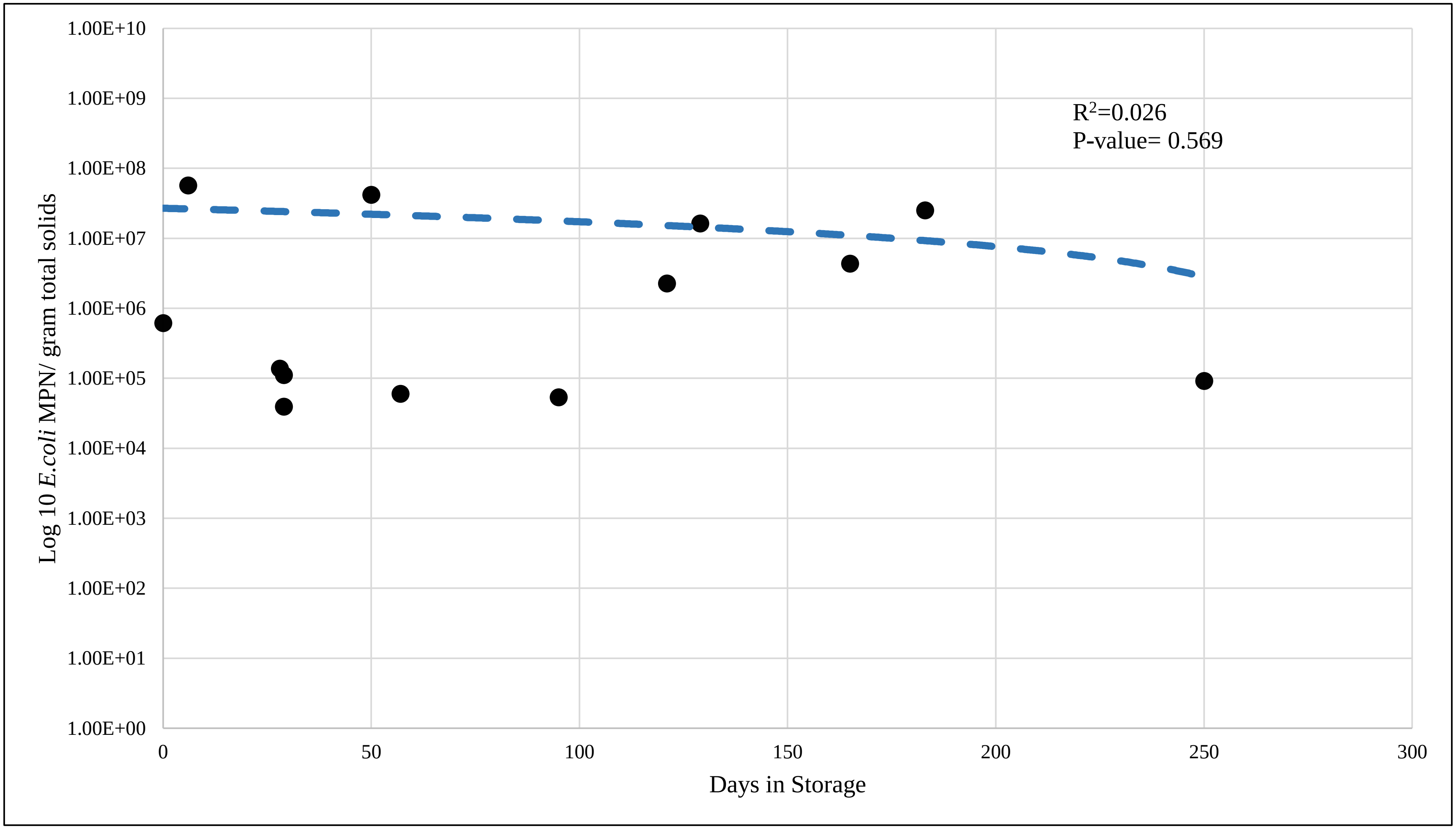
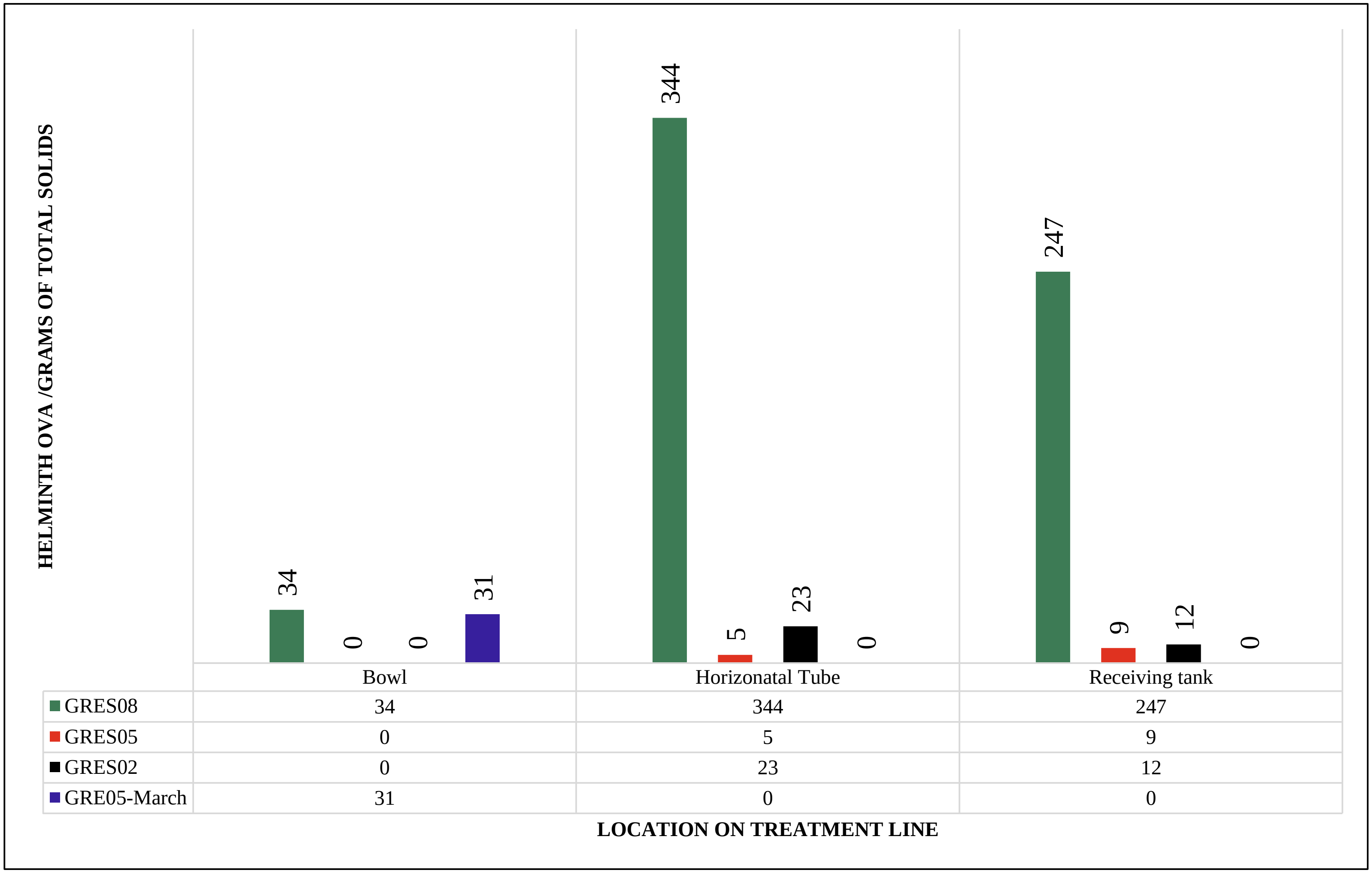


Figure 2: E.coli levels in stored containers by number of days since the last addition. Dotted line is a linear regression prediction line with a R-squared value of 0.026 and p-value of 0.596. These included all communities and storage container types.



	Bowl	Horizonatal Tube	Receiving tank
🟩 GRES08	34	344	247
🟥 GRES05	0	5	9
■ GRES02	0	23	12
🟪 GRE05-March	31	0	0

Figure 3: Helminth ova counted in different systems from Community 1. Helminth ova were quantified per gram total solids. *Trichuria* ova were found in co-occurrence with *Ascaris* in GRES08 in multiple locations through the treatment line. Helminth counts were more numerous at the end of the horizontal tube and receiving tank than the bowl.

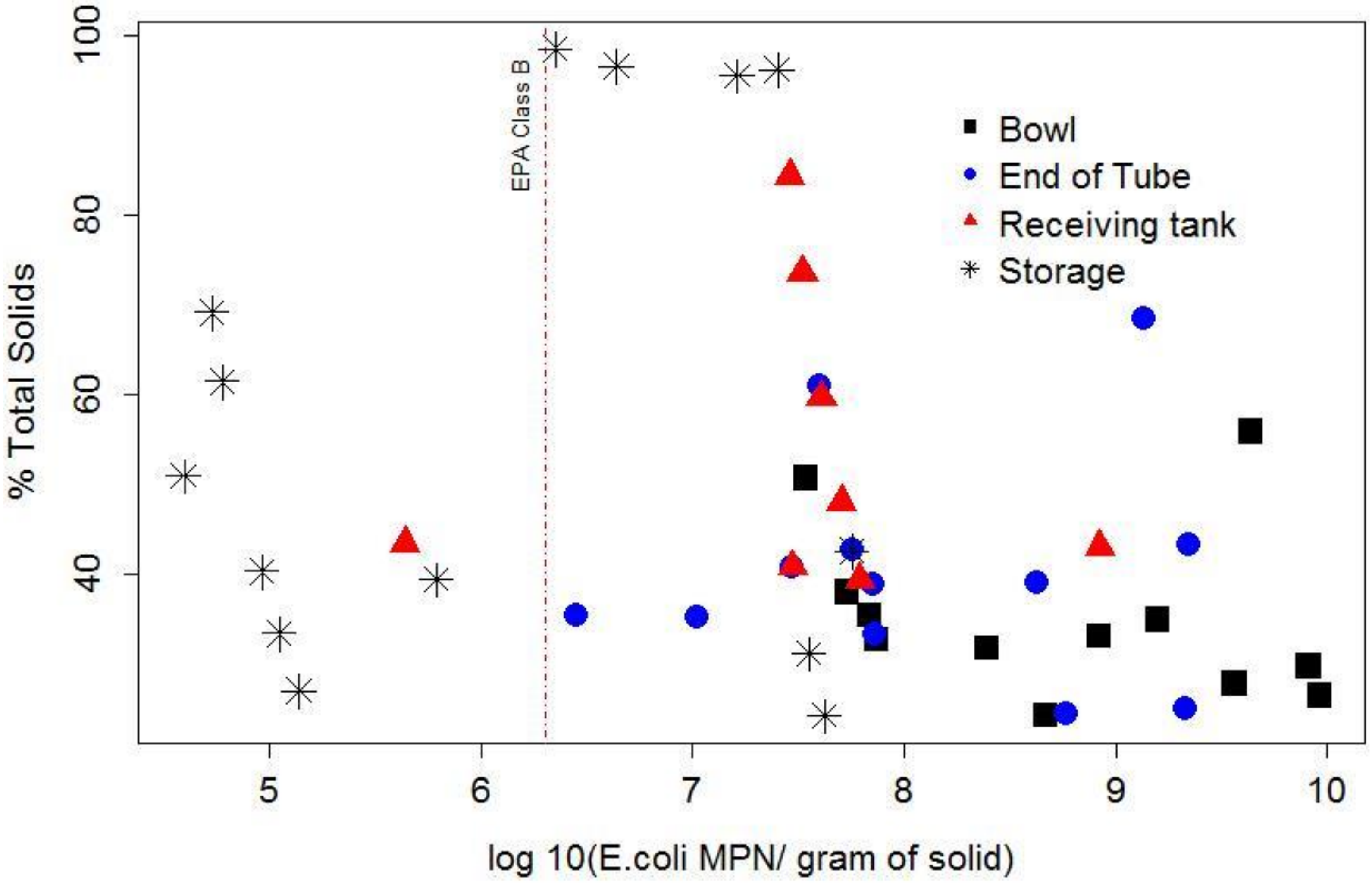


Figure 4: Total solids by percentage compared with measured E.coli levels by point on the treatment line (Bowl, End of tube, Receiving tank or storage).Storage containers and receiving tanks. Samples from later stages in the treatment had the highest solids content with earlier stages having the lowest solids content.

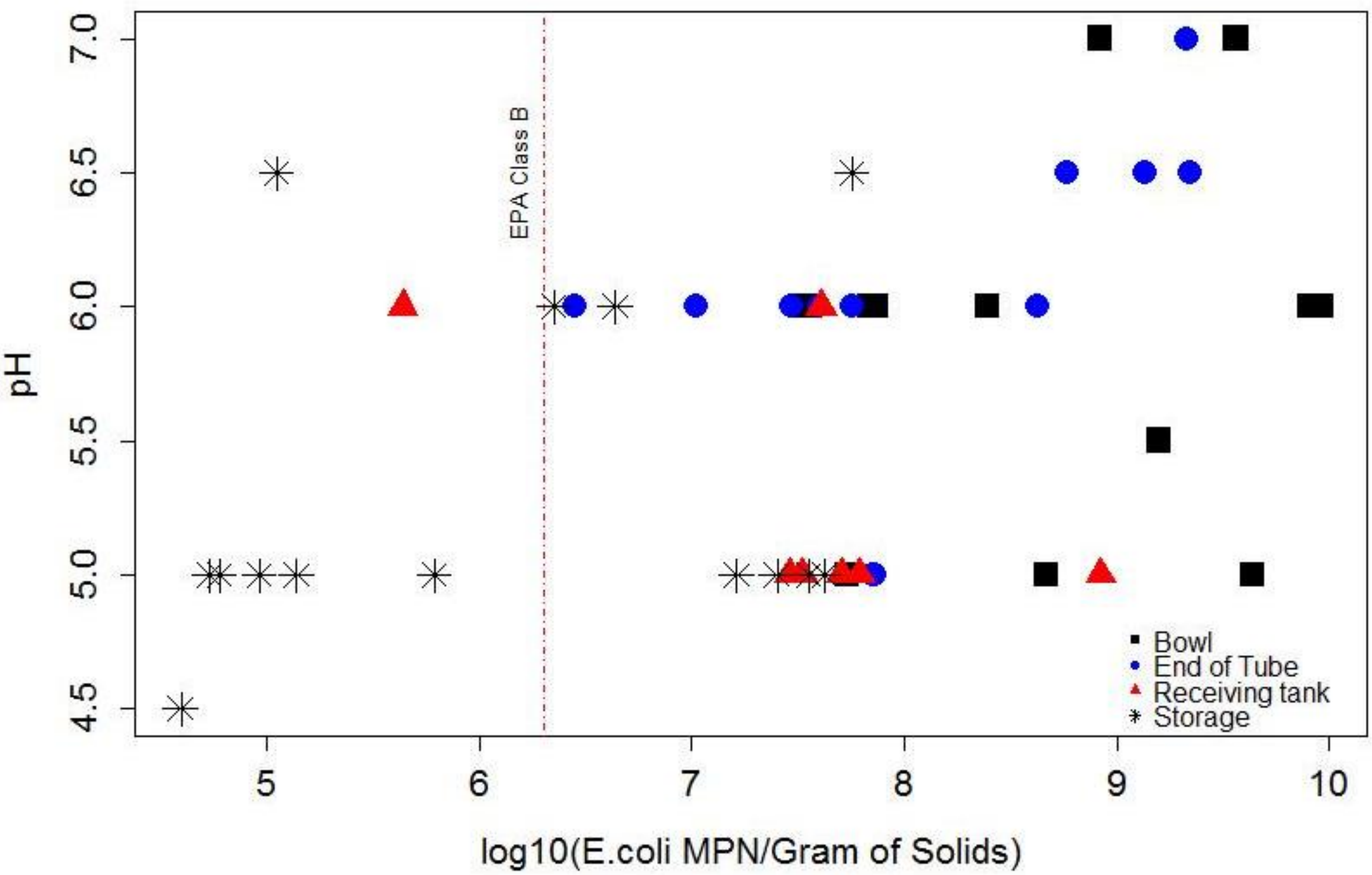


Figure 5: Measured pH levels compared with *E.coli* MPN by point in treatment line (Bowl, End of tube, Receiving tank or Storage).

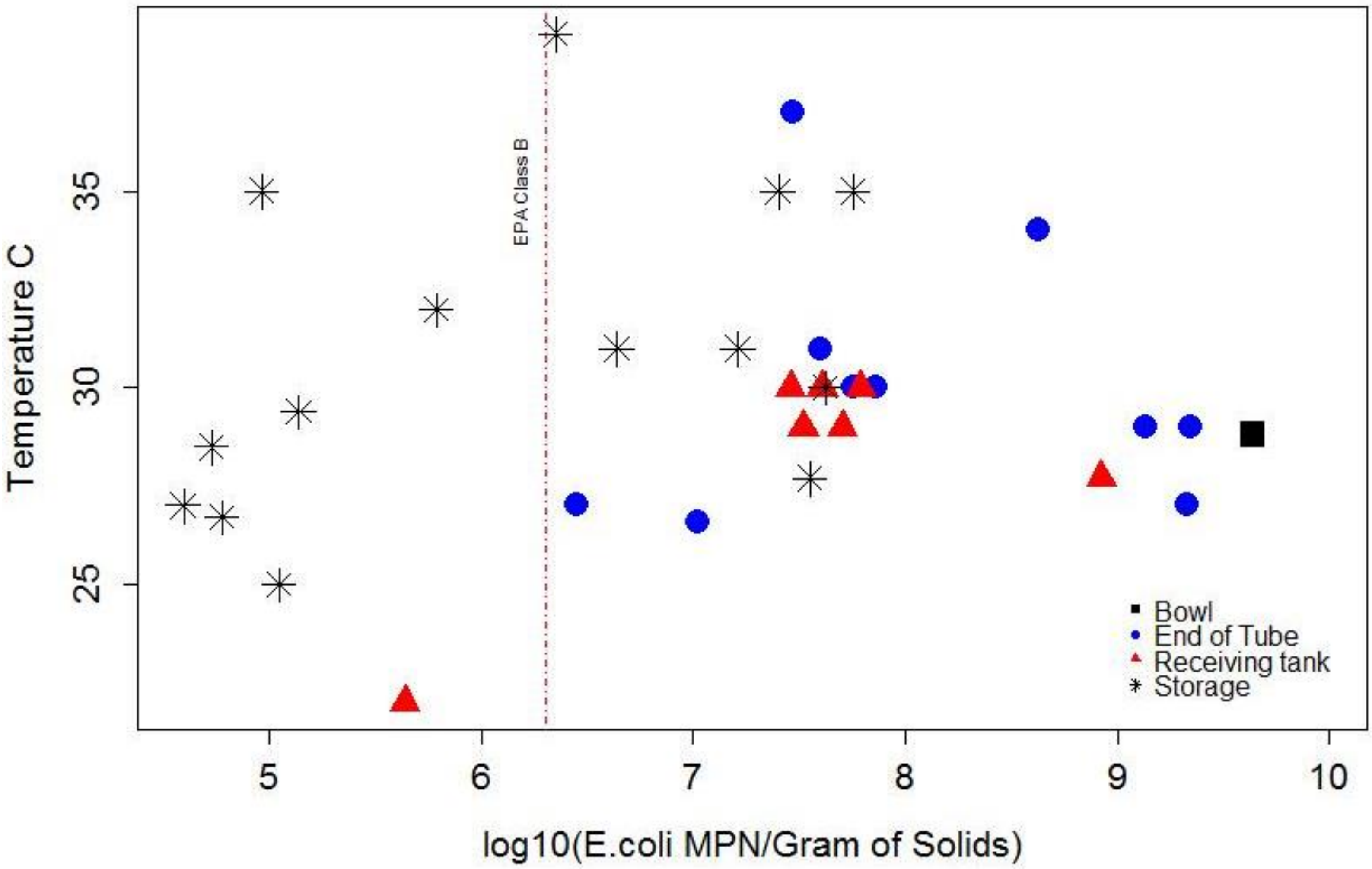


Figure 6: Temperature compared with *E.coli* MPN/gram of solids. Temperature was taken *in situ*. Temperatures taken were within ambient temperatures for Ecuadorian coastal region. Mean= 29.9°C, Median =30°C, Range: 22-39°C.

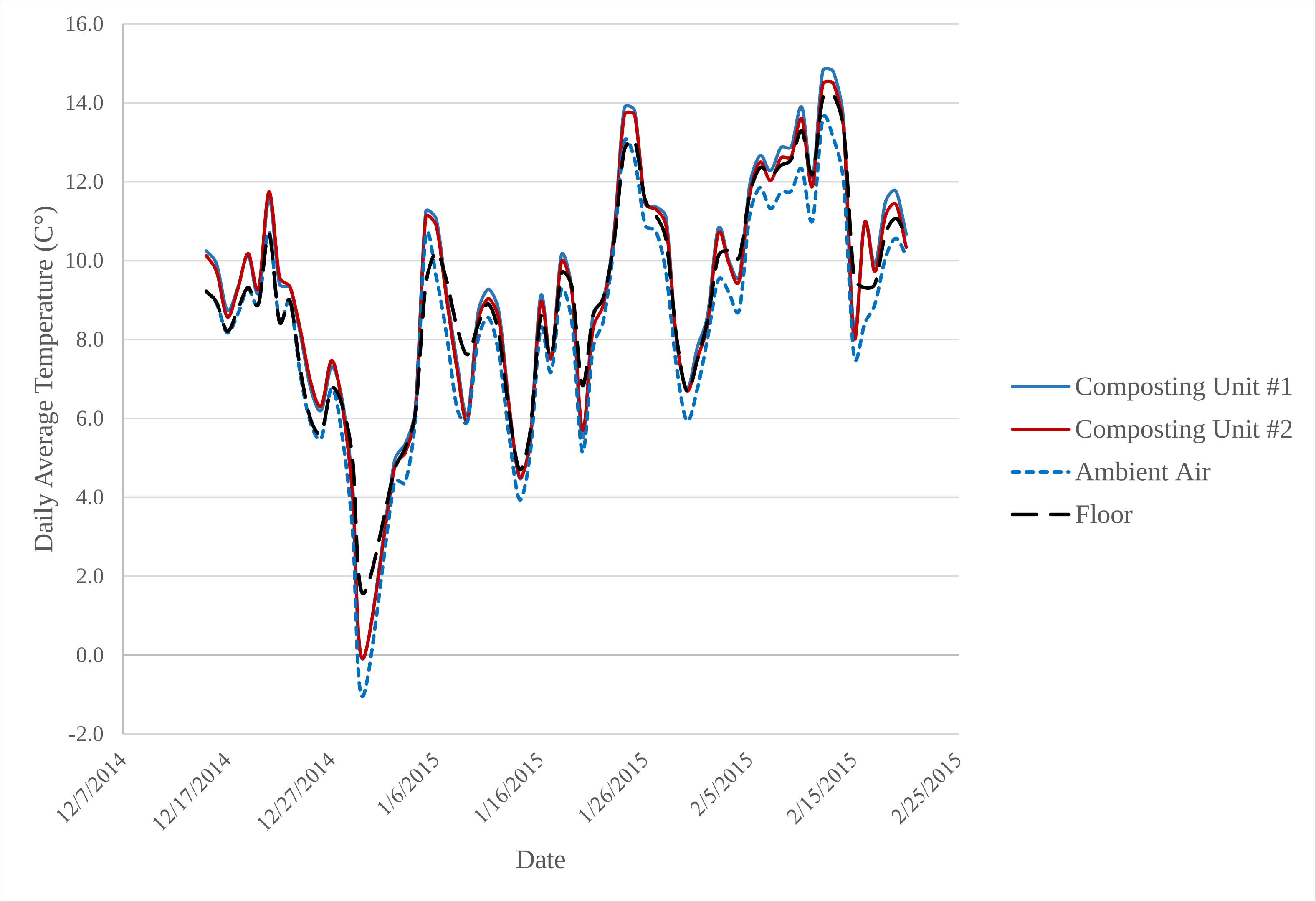


Figure 7: Daily average temperatures of the two scaled composting units' chambers (1 and 2) as well as the daily average temperatures of the ambient air and floor surrounding the composting units. Composting chamber temperatures corresponded with ambient and floor temperatures without demonstrating increases normally seen in aerobic decomposition. Temperatures were collected every 10 minutes and averaged based on date to find the daily average. Days which are missing data are 12/31/2014-1/1/2015 due to battery failure and holiday availability.

Table 3: Experimental results from experimental composting units. Samples taken were from the end of the horizontal tube, no storage conditions are represented here. Max resident time represents the time possible from activation of the composting unit until the time of sampling. Missing values (--) represent incomplete procedures.

Sampling	Max Resident Time	pH	Percent Total Solids	Viable *A. Suum* Ova/ g solids	% Viable Ova	*E.coli* (MPN/ g solids)	*Salmonella* (MPN/g solids)	*MS2* (PFU/g solids)	PV1 (PFU/g solids)
Initial Values	N/A	Neutral	*8-67%	† 671 ova/g	91.9%	Varied**	--	5.69x10^6	227
Unit 1:									
1st	30 days	--	43%	353	89.4%	>5.66x10^8	0	1.18x10^4	0
2nd	54 days	7.9	47%	56	27.2%	1.64x10^8	0	785	4379
Unit 2:									
1st	44 days	7.3	60%	--	--	4.03x10^8	0	683	--

* Nishimuta, Inoue et al. 2006 Solids content of feces taken from healthy individuals

*******E.coli* initial values would vary by individual and feces were not inoculated with *E.coli* cultures

† Initial value was calculated using stock concentration, experimental recovery values and control viability rates.

‡ *MS2* and *Poliovirus* 1 initial values were calculated using stock titers and experimental recovery values.

DISCUSSION

Evaluation and validation of small-scale sanitation systems are necessary to ensure that pathogens are being reduced and that systems can reduce pathogens and the possible risk of pathogen transmission (WHO, 2006). The Earth Auger, was unable to meet the least stringent recommended health based indicator/pathogen targets for fecal material use in agriculture, based on the 6-$\log_{10}$ indicator reductions and/or <1 helminth ova/gram solids (WHO) in almost all instances (Figure 1, Table 1 and Figure 3). Some, but not all, of the stored compost samples did meet the next less stringent US EPA class B biosolids requirements of <2,000,000 MPN *E.coli* for the restricted use of treated fecal material in agricultural or remediation settings (EPA, 1999).

While multi-linear regression (Table 2) showed that there were incremental decreases through the treatment line, this temporal relationship could not be distinguished in storage containers alone (Figure 2). Storage container types ranged in volume, aeration, material (plastic containers, cloth bags and impermeable tarpaulin bags), and were placed in a wide range of places such as under houses, hanging in trees, nested on top of houses or placed in the open; all of which would directly affect environmental conditions and subsequent indicator levels of the harvested product. The number of environments and observed storage conditions were so numerous that statistical significantly results would be impossible to observe given the 2.71 $\log_{10}$ average reduction (Table 2), which is small and below the recommended 6 $\log_{10}$ reduction required for reuse in agriculture by the WHO.

Helminth were observed in three different toilets, with one repeated measure, from the same community with increasing levels of ova in the horizontal tube and receiving tanks (Figure

3). Increasing levels along the treatment line could be affected by illness severity and individual *Ascaris* and *Trichuria* loads but is more likely to be explained by auger mechanism distributing and mixing end products. While further investigation on helminth die-off during storage conditions is necessary to establish the efficacy of the Earth Auger, these results have shown that helminth ova do survive initial treatment, arriving at potential manipulation points along the treatment line. The integration of strict management or the development of barrier controls to prevent exposure from partially treated waste is recommended.

When compared to other ecological sanitation studies, there are similarities seen in the literature when looking at pit latrines and other dehydrating toilets (Mehl et al., 2011; Sherpa et al., 2009). In Mehl et al., pit latrines in rural Panama viable helminths between 4-10 months of storage time. They recommended an increased storage time to up to 1 year, even given the tropical conditions of the region, and possible solar treatment before agricultural application (Mehl et al., 2011). Given the resistant nature of helminths and findings of helminths along the treatment line in this study, that storage treatment of 4-10 months is insufficient to eliminate the possibility of helminth infections even given tropical climates such as Panama or Ecuador. Indicator levels in a dehydrating toilet system in Nepal had reductions of 2.4 $\log_{10}$ in *E.coli* levels over 6 months (average= -0.4 $\log_{10}$ per month, 95% CI: -3.2 to -1.6 $\log_{10}$, $p<0.001$, n= 48). Indicator levels were not associated with physio-chemical factors tested (pH, moisture content, temperature) but were associated with storage time. The same study also found significant number of helminth ova (median count= 264 ova/cm^3, range= 26-896 ova/cm^3), with no sign of reduction with additional storage time (Sherpa et al., 2009). *E.coli* indicator reductions, in this study, were comparable to reduction seen in the Earth Auger and physio-chemical factors were not associated with these reductions as a result of the small indicator decreases.

Volunteer submissions were not numerous enough to continually dose experimental units with feces to produce results on treatment efficacy and pathogen die-off patterns. Of the results obtained environmental conditions tested were normal with regards to aerobic decomposition. Winter conditions meant that temperatures in chambers were protective of fecal bacteria but reductions in *A. suum* ova and *MS2* bacteriophage were observed (Table 2). Aggregation of viral particles can explain higher recoveries of *poliovirus 1* in samplings later in treatment (Table 2). Interestingly, *Ascaris suum* viability significantly decreased in subsequent samplings suggesting environmental toxicity. Overall, experimental units were unable to provide conclusive pathogen die-off reduction patterns needed to determine the efficacy of the treatment system but substantial evidence of the initial treatment from the bowl to receiving tanks and helminth survivability through initial treatments were elicited through field work.

The process involved in setting up this study provided unique set of challenges but it was successful in setting up field ready methods to sample sanitation systems in a developing country with a limited budget. These challenges could have directly affected the uncertainty of obtained results and certainly resulted in fewer samplings. One of the study's biggest limitation is that a temporal relationship between storage time and indicator reduction was unable to be established and lacked statistical power. In order to gain statistical power to make a meaningful conclusion on this relationship two things could happen: 1) magnitude of effect could increase; this would be a larger decrease in indicator levels over time as a result of a change in more effective treatment. 2) Increase the number of samples to increase the statistical power to see the true effect of indicator levels on time. Sample sizes required based on the paired samples of continuous data presented in Dell et al., would be approximately 132 pre-treatment and 132 post-treatment samples ($\log_{10}$ transformed SD bowl samples = 9.52) (observed effect size= 2.71 $\log_{10}$

reduction, see Table 2). Increasing the effect size to a 4.73 $\log_{10}$ reduction, which is the calculated reduction needed to meet WHO indicator reduction guidelines for the Earth Auger after reaching the end of the horizontal tube, would reduce the required sample size to 27 pre-treatment and 27 post-treatment samples.

Future lines of investigation would rely on the development of this new technology as it improves pathogen reduction capabilities. Additional monitoring of environmental conditions through the treatment line and during storage with higher precision methods and more frequency would be valuable and answer questions this study was unable to answer. Monitoring of deployed units for enterophages, mottle pepper viruses, enterococci or spore forming bacteria would help delineate pathogen die-off patterns using organisms with different survival characteristics. As this technology is installed into more climatically diverse places, pathogen die-off should be evaluated to determine geographical and climatic differences in pathogen reduction. Overall, this study demonstrated that the Earth Auger was unable to reduce indicators according to the US EPA regulations or WHO guidelines according to current treatment practices. While this study was unable to make a conclusion on the amount of storage time required to meet standards, future studies will be more powerful based on current effect sizes seen. Developing new and novel technologies is necessary in solving the global sanitation issue but efficacy of these systems should not be diminished in the name of improvement.

ACKNOWLEDGEMENTS

This study was funded by Critical Practices LLC (Cle Elum, WA), Fundación In Terrís (Guayaquil, Ecuador), the Boeing International Fellows program (Seattle, WA) and the Meschke Lab Group (Seattle, WA).

I want to thank the Universidad Católica de San Guayaquil of Guayaquil, Ecuador for the use of their laboratories during the study period and Fundación in Terrís for hosting and their in-country research support. I would especially like to extend a BIG thank you to the Meschke lab group, my family and boyfriend for their unwavering support and supervision.

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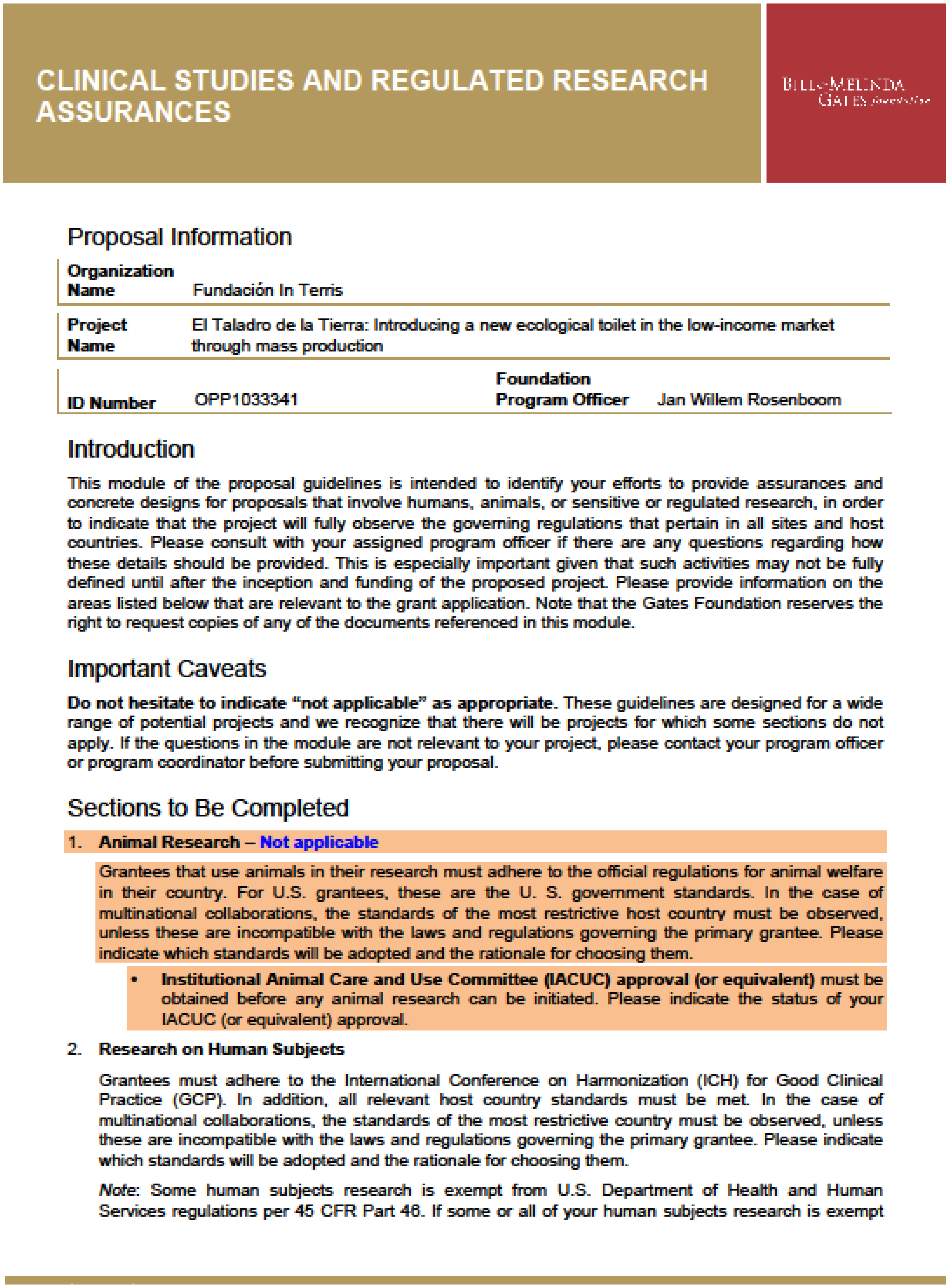
APPENDIX A



CLINICAL STUDIES AND REGULATED RESEARCH ASSURANCES

Proposal Information

Organization Name	Fundación In Terris		
Project Name	El Taladro de la Tierra: Introducing a new ecological toilet in the low-income market through mass production		
ID Number	OPP1033341	**Foundation Program Officer**	Jan Willem Rosenboom

Introduction

This module of the proposal guidelines is intended to identify your efforts to provide assurances and concrete designs for proposals that involve humans, animals, or sensitive or regulated research, in order to indicate that the project will fully observe the governing regulations that pertain in all sites and host countries. Please consult with your assigned program officer if there are any questions regarding how these details should be provided. This is especially important given that such activities may not be fully defined until after the inception and funding of the proposed project. Please provide information on the areas listed below that are relevant to the grant application. Note that the Gates Foundation reserves the right to request copies of any of the documents referenced in this module.

Important Caveats

Do not hesitate to indicate "not applicable" as appropriate. These guidelines are designed for a wide range of potential projects and we recognize that there will be projects for which some sections do not apply. If the questions in the module are not relevant to your project, please contact your program officer or program coordinator before submitting your proposal.

Sections to Be Completed

1. **Animal Research – Not applicable**

   Grantees that use animals in their research must adhere to the official regulations for animal welfare in their country. For U.S. grantees, these are the U. S. government standards. In the case of multinational collaborations, the standards of the most restrictive host country must be observed, unless these are incompatible with the laws and regulations governing the primary grantee. Please indicate which standards will be adopted and the rationale for choosing them.

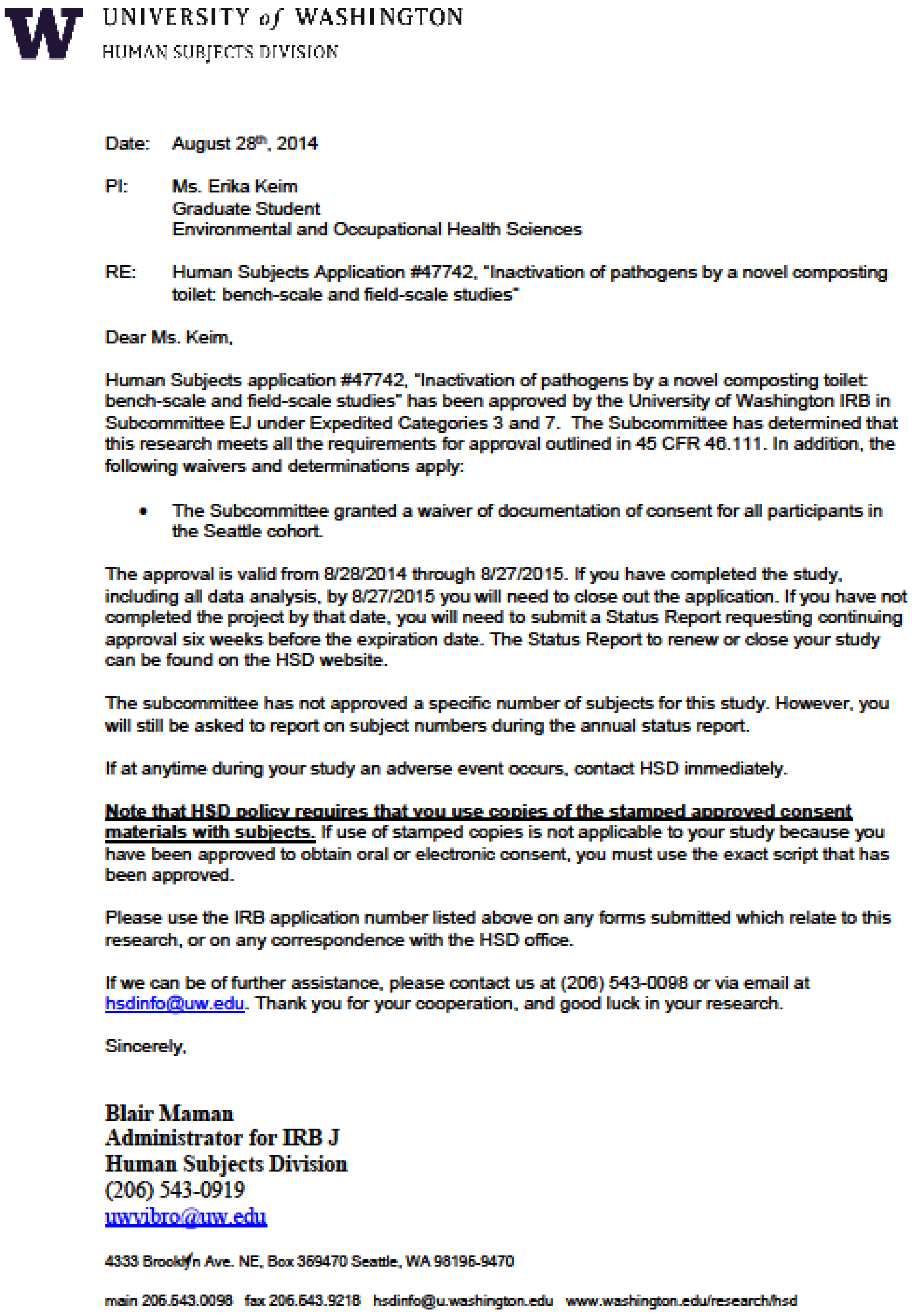
   - **Institutional Animal Care and Use Committee (IACUC) approval (or equivalent)** must be obtained before any animal research can be initiated. Please indicate the status of your IACUC (or equivalent) approval.

2. **Research on Human Subjects**

   Grantees must adhere to the International Conference on Harmonization (ICH) for Good Clinical Practice (GCP). In addition, all relevant host country standards must be met. In the case of multinational collaborations, the standards of the most restrictive country must be observed, unless these are incompatible with the laws and regulations governing the primary grantee. Please indicate which standards will be adopted and the rationale for choosing them.

   Note: Some human subjects research is exempt from U.S. Department of Health and Human Services regulations per 45 CFR Part 46. If some or all of your human subjects research is exempt

APPENDIX B



UNIVERSITY of WASHINGTON
HUMAN SUBJECTS DIVISION

Date: August 28th, 2014

PI: Ms. Erika Keim
Graduate Student
Environmental and Occupational Health Sciences

RE: Human Subjects Application #47742, "Inactivation of pathogens by a novel composting toilet: bench-scale and field-scale studies"

Dear Ms. Keim,

Human Subjects application #47742, "Inactivation of pathogens by a novel composting toilet: bench-scale and field-scale studies" has been approved by the University of Washington IRB in Subcommittee EJ under Expedited Categories 3 and 7. The Subcommittee has determined that this research meets all the requirements for approval outlined in 45 CFR 46.111. In addition, the following waivers and determinations apply:

- The Subcommittee granted a waiver of documentation of consent for all participants in the Seattle cohort.

The approval is valid from 8/28/2014 through 8/27/2015. If you have completed the study, including all data analysis, by 8/27/2015 you will need to close out the application. If you have not completed the project by that date, you will need to submit a Status Report requesting continuing approval six weeks before the expiration date. The Status Report to renew or close your study can be found on the HSD website.

The subcommittee has not approved a specific number of subjects for this study. However, you will still be asked to report on subject numbers during the annual status report.

If at anytime during your study an adverse event occurs, contact HSD immediately.

Note that HSD policy requires that you use copies of the stamped approved consent materials with subjects. If use of stamped copies is not applicable to your study because you have been approved to obtain oral or electronic consent, you must use the exact script that has been approved.

Please use the IRB application number listed above on any forms submitted which relate to this research, or on any correspondence with the HSD office.

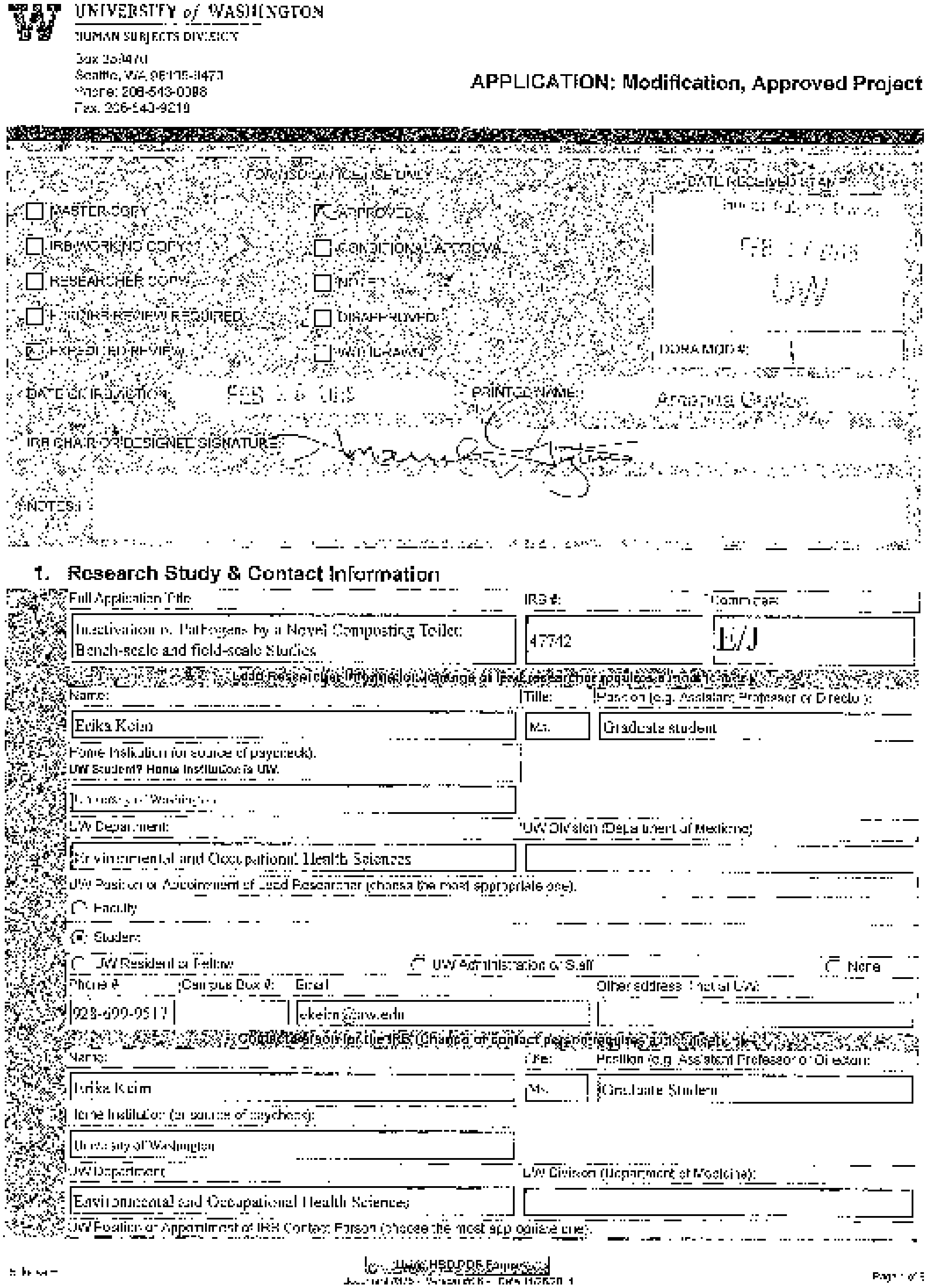
If we can be of further assistance, please contact us at (206) 543-0098 or via email at hsdinfo@uw.edu. Thank you for your cooperation, and good luck in your research.

Sincerely,

Blair Maman
Administrator for IRB J
Human Subjects Division
(206) 543-0919
uwvibro@uw.edu

4333 Brooklyn Ave. NE, Box 359470 Seattle, WA 98195-9470

main 206.543.0098 fax 206.543.9218 hsdinfo@u.washington.edu www.washington.edu/research/hsd



UNIVERSITY of WASHINGTON
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Fax: 206-543-9218

APPLICATION: Modification, Approved Project

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☐ FULL IRB REVIEW REQUIRED ☐ DISAPPROVED
☑ EXPEDITED REVIEW ☐ WITHDRAWN

DATE RECEIVED STAMP: Human Subjects Division FEB 17 [illegible] UW

DORA MOD #:

DATE OF IRB ACTION: FEB 26 [illegible]

PRINTED NAME: Amanda Gaylor

IRB CHAIR OR DESIGNEE SIGNATURE:

NOTES:

1. Research Study & Contact Information

Full Application Title: Inactivation of Pathogens by a Novel Composting Toilet: Bench-scale and field-scale Studies

IRB #: 47742

Committee: E/J

Lead Researcher (Change of lead researcher requires a modification)

Name: Erika Keim

Title: Ms.

Position (e.g. Assistant Professor or Director): Graduate student

Home Institution (or source of paycheck). UW Student? Home Institution is UW.: University of Washington

UW Department: Environmental and Occupational Health Sciences

UW Division (Department of Medicine):

UW Position or Appointment of Lead Researcher (choose the most appropriate one).

○ Faculty

● Student

○ UW Resident or Fellow ○ UW Administration or Staff ○ None

Phone #: 928-699-9517

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Email: ekeim@uw.edu

Other address if not at UW:

Contact Person for the IRB (Change of contact person requires a modification)

Name: Erika Keim

Title: Ms.

Position (e.g. Assistant Professor or Director): Graduate Student

Home Institution (or source of paycheck): University of Washington

UW Department: Environmental and Occupational Health Sciences

UW Division (Department of Medicine):

UW Position or Appointment of IRB Contact Person (choose the most appropriate one).

Using HSD PDF Forms

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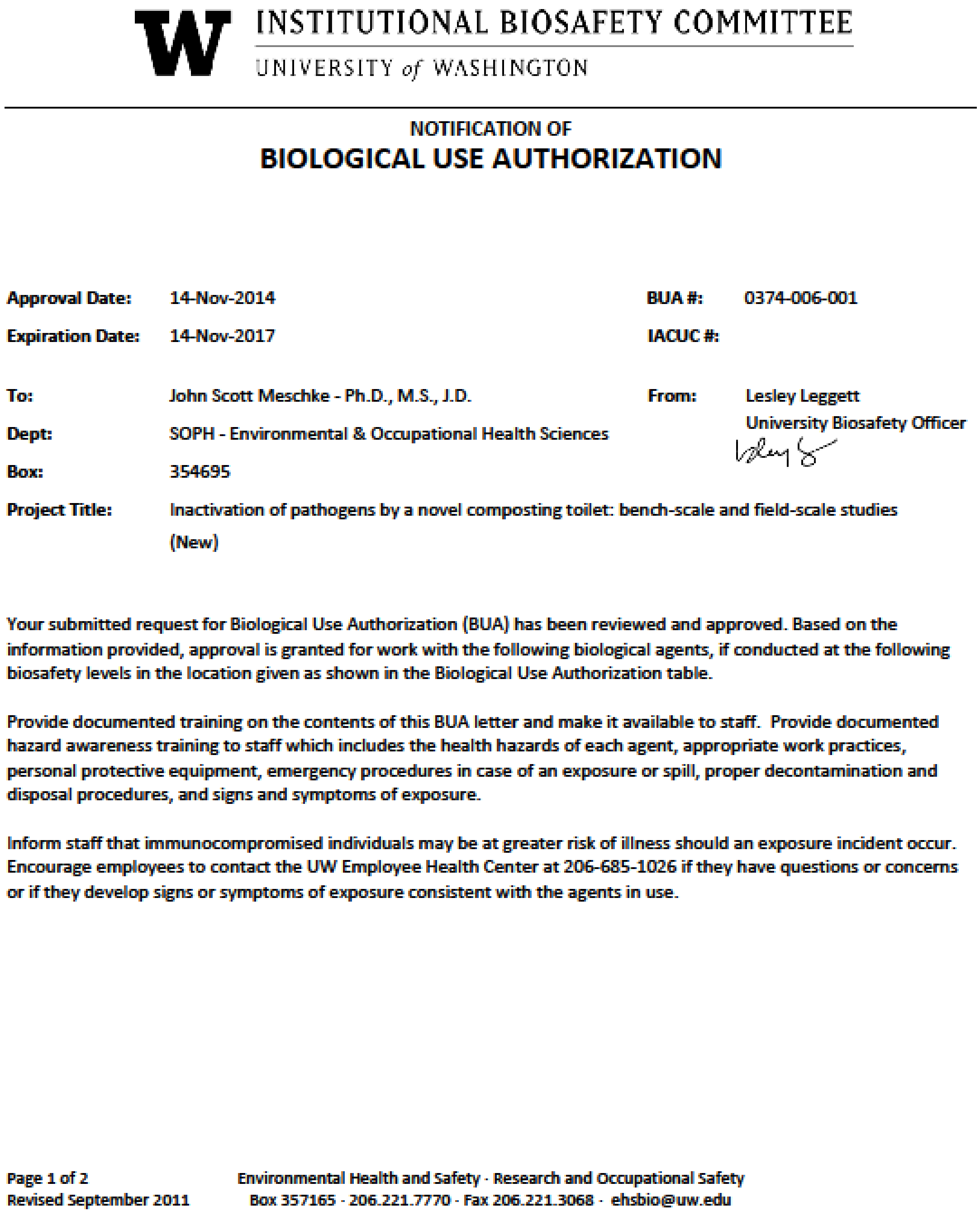
APPENDIX C



INSTITUTIONAL BIOSAFETY COMMITTEE
UNIVERSITY *of* WASHINGTON

NOTIFICATION OF
BIOLOGICAL USE AUTHORIZATION

Approval Date: 14-Nov-2014
BUA #: 0374-006-001
Expiration Date: 14-Nov-2017
IACUC #:

To: John Scott Meschke - Ph.D., M.S., J.D.
Dept: SOPH - Environmental & Occupational Health Sciences
Box: 354695
From: Lesley Leggett
University Biosafety Officer

Project Title: Inactivation of pathogens by a novel composting toilet: bench-scale and field-scale studies (New)

Your submitted request for Biological Use Authorization (BUA) has been reviewed and approved. Based on the information provided, approval is granted for work with the following biological agents, if conducted at the following biosafety levels in the location given as shown in the Biological Use Authorization table.

Provide documented training on the contents of this BUA letter and make it available to staff. Provide documented hazard awareness training to staff which includes the health hazards of each agent, appropriate work practices, personal protective equipment, emergency procedures in case of an exposure or spill, proper decontamination and disposal procedures, and signs and symptoms of exposure.

Inform staff that immunocompromised individuals may be at greater risk of illness should an exposure incident occur. Encourage employees to contact the UW Employee Health Center at 206-685-1026 if they have questions or concerns or if they develop signs or symptoms of exposure consistent with the agents in use.

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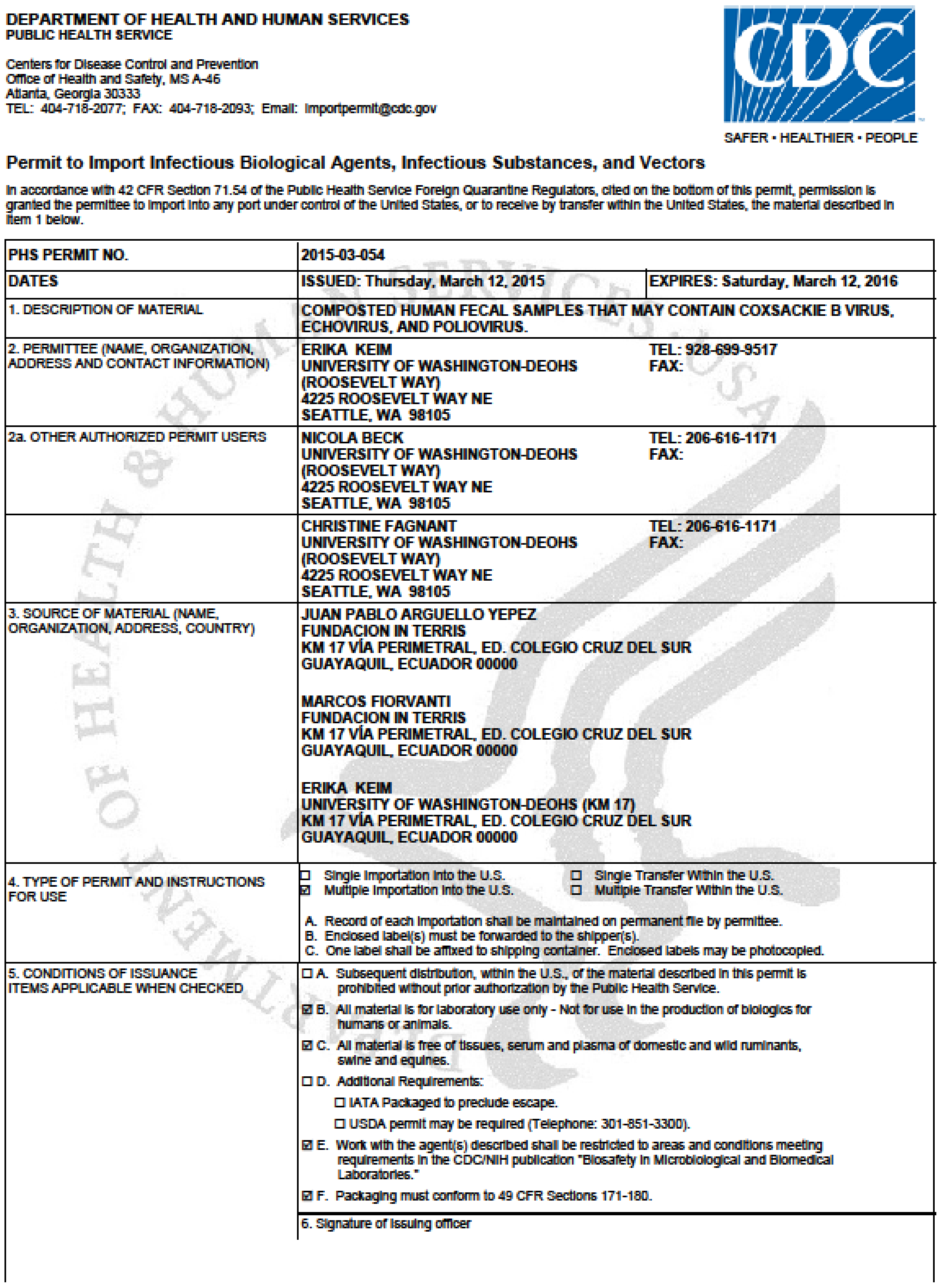
Revised September 2011

Environmental Health and Safety · Research and Occupational Safety
Box 357165 · 206.221.7770 · Fax 206.221.3068 · ehsbio@uw.edu

APPENDIX D



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

Centers for Disease Control and Prevention
Office of Health and Safety, MS A-46
Atlanta, Georgia 30333
TEL: 404-718-2077; FAX: 404-718-2093; Email: importpermit@cdc.gov

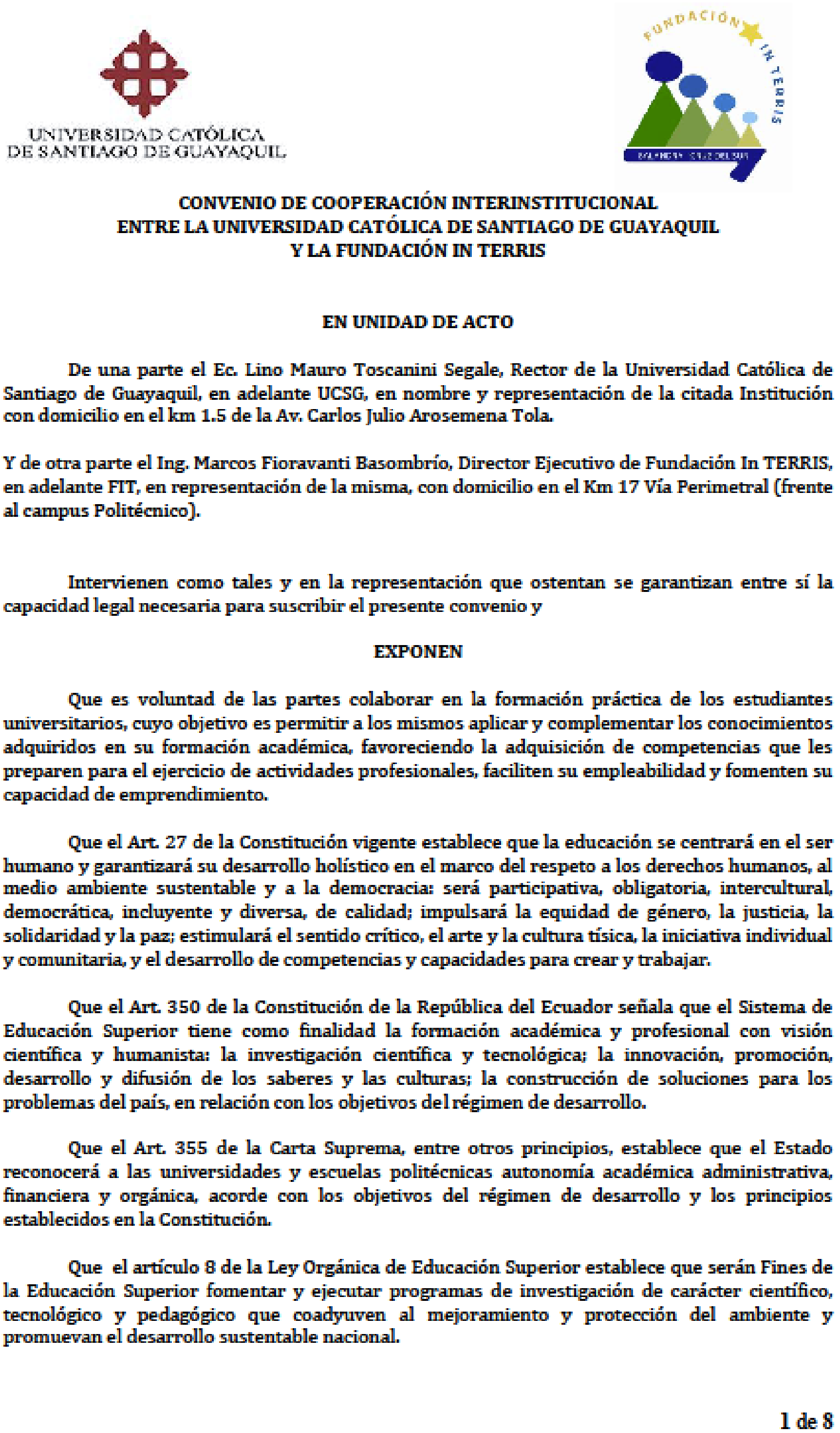
SAFER • HEALTHIER • PEOPLE

Permit to Import Infectious Biological Agents, Infectious Substances, and Vectors

In accordance with 42 CFR Section 71.54 of the Public Health Service Foreign Quarantine Regulators, cited on the bottom of this permit, permission is granted the permittee to import into any port under control of the United States, or to receive by transfer within the United States, the material described in item 1 below.

PHS PERMIT NO.	2015-03-054	
DATES	ISSUED: Thursday, March 12, 2015	EXPIRES: Saturday, March 12, 2016
1. DESCRIPTION OF MATERIAL	COMPOSTED HUMAN FECAL SAMPLES THAT MAY CONTAIN COXSACKIE B VIRUS, ECHOVIRUS, AND POLIOVIRUS.	
2. PERMITTEE (NAME, ORGANIZATION, ADDRESS AND CONTACT INFORMATION)	ERIKA KEIM UNIVERSITY OF WASHINGTON-DEOHS (ROOSEVELT WAY) 4225 ROOSEVELT WAY NE SEATTLE, WA 98105	TEL: 928-699-9517 FAX:
2a. OTHER AUTHORIZED PERMIT USERS	NICOLA BECK UNIVERSITY OF WASHINGTON-DEOHS (ROOSEVELT WAY) 4225 ROOSEVELT WAY NE SEATTLE, WA 98105	TEL: 206-616-1171 FAX:
	CHRISTINE FAGNANT UNIVERSITY OF WASHINGTON-DEOHS (ROOSEVELT WAY) 4225 ROOSEVELT WAY NE SEATTLE, WA 98105	TEL: 206-616-1171 FAX:
3. SOURCE OF MATERIAL (NAME, ORGANIZATION, ADDRESS, COUNTRY)	JUAN PABLO ARGUELLO YEPEZ FUNDACION IN TERRIS KM 17 VÍA PERIMETRAL, ED. COLEGIO CRUZ DEL SUR GUAYAQUIL, ECUADOR 00000 MARCOS FIORVANTI FUNDACION IN TERRIS KM 17 VÍA PERIMETRAL, ED. COLEGIO CRUZ DEL SUR GUAYAQUIL, ECUADOR 00000 ERIKA KEIM UNIVERSITY OF WASHINGTON-DEOHS (KM 17) KM 17 VÍA PERIMETRAL, ED. COLEGIO CRUZ DEL SUR GUAYAQUIL, ECUADOR 00000	
4. TYPE OF PERMIT AND INSTRUCTIONS FOR USE	☐ Single Importation into the U.S. ☑ Multiple Importation into the U.S. A. Record of each importation shall be maintained on permanent file by permittee. B. Enclosed label(s) must be forwarded to the shipper(s). C. One label shall be affixed to shipping container. Enclosed labels may be photocopied.	☐ Single Transfer Within the U.S. ☐ Multiple Transfer Within the U.S.
5. CONDITIONS OF ISSUANCE ITEMS APPLICABLE WHEN CHECKED	☐ A. Subsequent distribution, within the U.S., of the material described in this permit is prohibited without prior authorization by the Public Health Service. ☑ B. All material is for laboratory use only - Not for use in the production of biologics for humans or animals. ☑ C. All material is free of tissues, serum and plasma of domestic and wild ruminants, swine and equines. ☐ D. Additional Requirements: ☐ IATA Packaged to preclude escape. ☐ USDA permit may be required (Telephone: 301-851-3300). ☑ E. Work with the agent(s) described shall be restricted to areas and conditions meeting requirements in the CDC/NIH publication "Biosafety in Microbiological and Biomedical Laboratories." ☑ F. Packaging must conform to 49 CFR Sections 171-180.	
	6. Signature of issuing officer	

APPENDIX E



UNIVERSIDAD CATÓLICA
DE SANTIAGO DE GUAYAQUIL

FUNDACIÓN IN TERRIS

CONVENIO DE COOPERACIÓN INTERINSTITUCIONAL ENTRE LA UNIVERSIDAD CATÓLICA DE SANTIAGO DE GUAYAQUIL Y LA FUNDACIÓN IN TERRIS

EN UNIDAD DE ACTO

De una parte el Ec. Lino Mauro Toscanini Segale, Rector de la Universidad Católica de Santiago de Guayaquil, en adelante UCSG, en nombre y representación de la citada Institución con domicilio en el km 1.5 de la Av. Carlos Julio Arosemena Tola.

Y de otra parte el Ing. Marcos Fioravanti Basombrío, Director Ejecutivo de Fundación In TERRIS, en adelante FIT, en representación de la misma, con domicilio en el Km 17 Vía Perimetral (frente al campus Politécnico).

Intervienen como tales y en la representación que ostentan se garantizan entre sí la capacidad legal necesaria para suscribir el presente convenio y

EXPONEN

Que es voluntad de las partes colaborar en la formación práctica de los estudiantes universitarios, cuyo objetivo es permitir a los mismos aplicar y complementar los conocimientos adquiridos en su formación académica, favoreciendo la adquisición de competencias que les preparen para el ejercicio de actividades profesionales, faciliten su empleabilidad y fomenten su capacidad de emprendimiento.

Que el Art. 27 de la Constitución vigente establece que la educación se centrará en el ser humano y garantizará su desarrollo holístico en el marco del respeto a los derechos humanos, al medio ambiente sustentable y a la democracia: será participativa, obligatoria, intercultural, democrática, incluyente y diversa, de calidad; impulsará la equidad de género, la justicia, la solidaridad y la paz; estimulará el sentido crítico, el arte y la cultura tísica, la iniciativa individual y comunitaria, y el desarrollo de competencias y capacidades para crear y trabajar.

Que el Art. 350 de la Constitución de la República del Ecuador señala que el Sistema de Educación Superior tiene como finalidad la formación académica y profesional con visión científica y humanista: la investigación científica y tecnológica; la innovación, promoción, desarrollo y difusión de los saberes y las culturas; la construcción de soluciones para los problemas del país, en relación con los objetivos del régimen de desarrollo.

Que el Art. 355 de la Carta Suprema, entre otros principios, establece que el Estado reconocerá a las universidades y escuelas politécnicas autonomía académica administrativa, financiera y orgánica, acorde con los objetivos del régimen de desarrollo y los principios establecidos en la Constitución.

Que el artículo 8 de la Ley Orgánica de Educación Superior establece que serán Fines de la Educación Superior fomentar y ejecutar programas de investigación de carácter científico, tecnológico y pedagógico que coadyuven al mejoramiento y protección del ambiente y promuevan el desarrollo sustentable nacional.