Research Note

WOODCHIP PATHOGEN DECONTAMINATION WITH A BENEFICIAL MICROBIAL MIXTURE[1,2]

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Vegetative materials collected and reduced during a hurricane can be exposed to diverse contaminants that would make the materials a public hazard. Tree debris, animal manures, animal deaths and many others can be mitigated with the process of composting outlined in this study. Although other methods such as burning have been used, burning eliminates the opportunity to recycle the organics into agricultural systems, unless converted into biochar. What's more, burning is not permitted in some localities. The sudden huge volumes of organic debris after atmospheric phenomena, such as hurricanes and tropical storms, require the development of new methods of recycling organics.

During the 2017 Atlantic hurricane season, Irma and María left an estimated 6.5 million m^3 of waste and debris across Puerto Rico (Antonio Ríos, 2018, Autoridad de Desperdicios Sólidos, Personal communication). Approximately 70% of the volume was vegetative materials, which included leaves, branches, trunks, and whole trees, most of which were reduced with industrial grinders into woodchips. Grinding whole trees to woodchips significantly reduces biomass volume to approximately 10 to 20% of the original tree volume. Actual volume reduction depends on moisture content, material density, equipment, and chipping size. Further composting woodchips can reduce the chipped volume to approximately 30 to 40% of the original non-decomposed material. In general, composting whole trees residue can reduce the original volume by more than 90%. Hence, an adequate composting process can reduce large organic material debris after a hurricane, which can then be recycled onto farms. An estimated 1.82 million m^3 woodchips were produced and disposed of at sanitary landfills, farms and other locations after hurricanes Irma and María (Antonio Ríos, 2018, Autoridad de Desperdicios Sólidos, Puerto Rico, Personal communication). Little of the material was composted or underwent a controlled degradation process, which would have resulted in vast amounts of compost increasing organic matter in farmed soils.

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Hurricane winds topple trees that fall into flood waters containing human pathogens from sewage and flooded septic tanks. The composting of materials, including protocols to reduce human pathogens, is regulated in Puerto Rico through the "Regulation for the Design and Operation of Compost Installations" of the Environmental Quality Board [Junta de Calidad Ambiental (JCA), 2016)] under the Puerto Rico Department of Natural and Environmental Resources. The composting process aims at the stabilization of organic matter by reducing its volume and producing substrates that are beneficial to soil and plants and are safe for intended use. This is after assuring that the compost is free of or has reduced pathogens and is free of weedy seeds. Stable organic matter provides soil with a source of nutrients, increased water holding capacity and buffers soil pH (Chong and Dumas, 2012). Different methodologies that can be used to compost organic matter include passively or actively aerated compost piles as well as within-vessel composting or windrow composting. These methodologies can provide stable organic matter, yet the method used must be evaluated based on the amount of organic matter, quality, space, available equipment, time requirements, scope and purpose, and climate, among others. When vast amounts of homogeneous organic material, such as woodchips, are produced, windrow composting is preferred because it can handle large volumes.

In Puerto Rico, the composting rules are described in the "Regulation for the design and operation of compost installations" as amended 22 December 2016 by the Environmental Quality Board (Junta de Calidad Ambiental, 2016). The regulation divides organic residues into Type I and Type II material. Vegetative composting is designated as using Type I material, which requires the least regulation due to the low possibility of being a source of environmental contaminants such as nutrients or human pathogens. Animal manures and food residues, for example, require greater regulation as these are considered Type II materials and are subject to nutrient and human pathogen testing. In many states when windrows are used, temperatures at or above 55° C must be reached for at least 15 days during which time a minimum of five windrow turnings must be performed (EPA, 1994: Appendix B of 40 CFR Part 503) with a minimum of three days between turns (US Composting Council, USCC, 2013). Additionally, the USCC, an industry leader, states that for windrow composting of 15 days or more, at or above 55° C, turning does not have to be uninterrupted. Currently the Puerto Rico Department of Natural and Environmental Resources regulates composting of homogenous substrates, such as woodchips, and authorizes 48° C, a lower temperature than EPA requires, to further reduce pathogens when using the windrow method. The main reason for the lower temperature is that industrial homogenous Type I materials, such as woodchips are high in C and require a large N source to reach high temperatures; however, a large source of N would be impossible to obtain easily at the compost location. Similarly, coffee pulp, an industrial homogenous material, is high in N and would require large amounts of C amendment to reach high temperatures. Temperatures are the indicators that compost operators use to monitor the process and assure compliance with the reduction of pathogens and proper organic matter stabilization (Haug, 1993). These temperatures are set by method of composting and not by feedstock blend used in the compost. Beyond the USA jurisdiction, the evaluation for sludge treatments for pathogen reduction by the European Commission Directorate-General for Environment (2001) states that compost is an acceptable method for pathogen reduction and provides compliance protocols less stringent than standards in the USA and its territories. For example, windrow composting requires 55° C for four hours between each of three turnings followed by a maturation period. Puerto Rico regulations and the USA composting industry take note of the EPA rules for biosolid processing to reduce pathogens. Standard sampling organisms are fecal coliforms and *Salmonella* sp. Most probable number (MPN) for fecal coliform density should be <1000 MPN/g solids, and for *Salmonella* sp. <3 MPN/4 g total solids, both on a dry-weight basis (EPA, 1994; Part 503 Rule Subpart D).

Composted vegetative materials in Puerto Rico are not subject to pathogen testing, having shown that under normal conditions these materials do not contain human pathogens. Nonetheless, it is important that any compost method achieves certain temperatures and temperature durations, based on compost methodology, to further reduce weed seeds and to properly sanitize compost. Regardless of compost type, compost producers that want to be certified by the US Composting Council Seal of Testing Assurance (STA) program are mandated to test for pathogens. For a compost to be STA approved it must pass a fecal coliform test of <1000 MPN/g or a salmonella test of <3 MPN/4 g. The USCC STA follows the Test Method for the Examination of Composting and Compost (TMECC). The TMECC provides benchmark methods for the analysis of compost and was developed by a joint effort of the US Department of Agriculture and the Composting Council Research and Education Foundation.

The commercial microbial product, Effective microorganisms•1® (EM•1®)[5] is a mixture of facultative, anaerobic and aerobic microorganisms, such as lactic acid bacteria, yeast and photosynthetic bacteria. These microbes live in a liquid consortium mixture and provide an antioxidant effect on soils, plants, compost or wherever they are applied. Developed by Teruo Higa, EM-1® has been commercialized throughout the world and used effectively in feed supplementation (Laskowska et al., 2017), salt soil plant tolerance (Talaat, 2014 and 2019), aquaculture wastewater bioremediation (Lananan et al., 2014), compost use (Park, 2011; Fan et al., 2018), among other environmental and agricultural uses. According to Higa and Kanal (1996), EM-1® is a liquid concentrate containing more than 80 different varieties of microorganisms, some of which are mentioned previously, from 10 genera and five families that include anaerobic and aerobic species, which coexist in a liquid culture medium. The goal of this work was to demonstrate the use of microbial inoculants, EM•1®, to reduce the amount of available human pathogens, measured as fecal coliforms, in composted woodchips to acceptable levels for safe use and distribution. Our study could provide a method to reduce contamination of pathogens in agricultural animal manures.

On 8 May 2018, truckloads totaling approximately 3,822 m^3 of chipped wood material were transported from Punta Santiago, Humacao, to the University of Puerto Rico Agricultural Experiment Substation in Gurabo, following the devastation caused by Hurricane María on 20 September 2017. Approximately 1,400 m^3 were arranged on about 0.48 ha (1.19 acres) with a seven-pile configuration averaging 48 m long, 3 m wide and 1.37 m high, leaving about 3 m between piles for windrow composting equipment to transit. The piles were turned twice weekly with a PT-120 Midwest Bio-System Windrow Turner (Tampico, IL). Calcium was supplemented into the woodchip piles with calcium carbonate and gypsum at a rate of 61.8 kg/m^3 each. Measured compost temperatures averaged 52.4° C, higher than the regulatory requirement of 48° C for homogenous material to reduce pathogens.

Human pathogens sampling and analyses

After 65 days of windrow composting, we prepared a composite sample, with four subsamples per pile for an STA test. It was sent overnight in a cooler with ice gel to a commercial laboratory (Soiltest Farm Consultants, Inc, Moses Lake, WA) for pathogen analyses. The results showed a fecal coliform value of 1661 MPN/g, failing the STA

[5]Company or trade names in this publication are used only to provide specific information. Mention of a company or trade name does not constitute an endorsement by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

limit of <1000 MPN/g. Five days after STA results were received (25 days after the first sampling), a sample was collected and sent to a local laboratory (Eqlab, Environmental Quality Laboratories, Inc., Bayamón, PR) which showed positive results for fecal *Enterococcus* (4900 MPN/g) and *E. coli* (330 MPN/g). These results established a baseline for positive and over the limit fecal coliform levels, rendering the compost not suitable for distribution; hence experimental treatments with EM•1® were carried out to mitigate the contamination. The STA also provided chemical compost analysis, concentration of regulated metals, compost stability by CO_2 evolution and bio-assay for herbicide detection (Method: TMECC 05.05A).

Effective Microorganisms treatment

Approximately 371 m^3 of the contaminated material was rearranged in three windrow composting piles for treatment with Effective Microorganisms•1® (EM•1®) first generation solution (EMFG). The EMFG solution was prepared by incubating a 5% mother stock solution of Effective Microorganisms•1® (EM•1®) Microbial Inoculant (Teraganix, South Alto, TX), along with 5% molasses, and chlorine-free water at an average temperature of 37.7° C inside a 250 L high-density polyethylene tank with a tight-fitting lid and gas escape valve. To reach a pH of 3.5, the incubation process took approximately two weeks.

Two, three and seven days after the confirmation of fecal pathogens in the composted woodchip material by a local laboratory (Eqlab, Bayamón, PR), we sprayed the woodchip compost with a total of 10.6 L/m^3 at a 1:16 ratio of EMFG to chlorine-free well water. The piles were sprayed by turning them with a PT-120 Midwest Bio-System Windrow Turner that had a water trailer attached, whose tank of 1,514 L water was pumped through the nozzles of the PT120 with a high-pressure gasoline-engine water pump. The 1:16 solution was mixed in the tank by hooking the high-pressure water pump to the tank for five minutes prior to spraying the piles. A total of 3,937 L of solution was sprayed each day to all 371 m^3 of the woodchip compost. After two days of initial EMFG treatment, a solution of 1,200 mg/kg urea using chlorine free water was sprayed on all piles at a rate of 10.6 L/m^3 to decrease the high initial C:N ratio and increase temperature.

Temperature in windrow piles

Temperatures were measured every hour for 24 h from the beginning of the EMFG inoculation treatment using modified HOBO 2x External Temperature Data Loggers (Bourne, MA). Each temperature probe was set inside a stainless-steel bar to push the logger probe tip into the compost pile. The probes were set at half height pile, 45° angle, with a 45.7 cm depth at 2/3 the length of the compost piles. The average daily temperature from each thermocouple data logger was calculated and an average of the two probes reported.

Composts are soil amendments. Depending upon the initial feedstock, these can also provide nutrients to plants, increase soil organic matter and buffer the soils pH (Chong and Dumas, 2012). All composts have different biological, chemical, and physical properties that must be evaluated to determine their best use and rate of application. Results obtained before and after EMFG treatments provided general information for the possible use of this type of compost and how to reduce detrimental pathogens using a beneficial microbial mixture. Monitoring compost pile temperature is a tool to evaluate its maturity and quality.

Temperature in windrow piles

Average daily temperature in windrow piles varied during the first weeks as the compost piles were treated and mixed using the windrow turner PT-120 machinery.

Average daily temperature in windrow piles increased to 50.8° C for one pile and to 49.2° C for two averaged piles on days 39 and 37, respectively (Figure 1). Thereafter, temperatures decreased to ca. 42° C after 85 days. Paired t-test showed that temperatures in these two sampling piles were similar.

Effective Microorganisms Treatment

Prior to EMFG treatments, pathogens on compost piles showed fecal coliforms at a density of 1661 MPN/g, above the permitted level of <1000 MPN/g (Table 1). Pathogen confirmation tests for specific fecal *Enterococcus* and *E. coli* were performed prior to effective microorganism treatments, confirming the positive results. As mentioned, EPA, Part 503 Rule Subpart D (1994) used either fecal coliforms or *Salmonella* sp. as a pathogen testing standard, since it would be impossible to test for all possible pathogens. Testing before and after EMFG treatments at a local laboratory (Eqlab) and STA test confirmation showed reduced values of pathogens (Table 1), which denote the efficacy of EMFG to reduce human pathogens from contaminated woodchips.

The following characterization assesses the possible uses of treated organic matter in agriculture. Woodchip compost after EMFG treatment showed a reduced organic carbon (OC) content from 32.1% to 10.1% and an increase in pH from 7.2 to 7.7 (Table 2). The compost used in this study (Type I feedstocks) had low N, P and K nutrients, as expected. Total N was 0.25%, P_2O_5 averaged 0.075% and K_2O averaged 0.125%. Due to compost amendments of calcium carbonate and gypsum, Ca levels were between 3.1 and 7.3%. Compost C:N ratio before and after EMFG treatments were 128:1 and 40:1, respectively. The higher the C:N ratio of a compost, the greater the probability that N will be sequestered from the soil and growing plants. It is recommended that compost with high C:N be used as a mulch rather than plowed

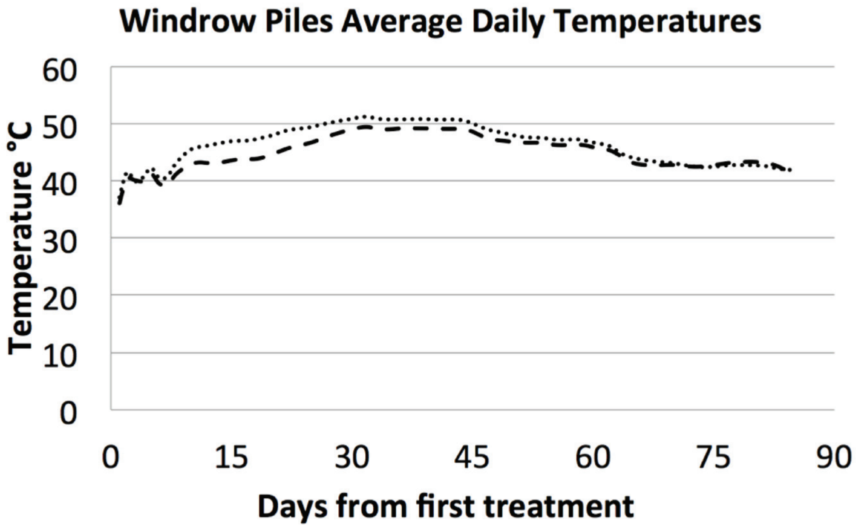

FIGURE 1. Woodchips average daily temperatures represent the average of hourly measurements every 24 h. A dotted line represents the two-probe average by one data logger of one pile and a dashed line, the average of four probes by two loggers of two piles.

TABLE 1.—*Pathogens tested in woodchips before and after EMFG treatments of windrow composting piles.*

Pathogen Test Results				
Organic Material Received	Test	Results (MPN/g)	Rule Limits[1]	Method
Before EMFG Treatment				
01-Aug	Fecal coliform[2]	1661	<1000 MPN/g	TMECC 07.01B
13-Aug	Fecal *Enterococcus*[3]	4900	*	SM 9230 B
13-Aug	*E. coli*[3]	330	*	SM 9221 F
After EMFG treatment (August 15, 16 and 20, 2018 Treatment Days)				
25-Sep	Fecal coliform[3]	0.45	*	SM 9221 E
25-Sep	*E. coli*[3]	<0.18	*	SM 9221 F
12-Nov	Fecal coliform[2]	92	<1000 MPN/g	TMECC 07.01B

[1]EPA, Part 503 Rule Subpart D, 1994
[2]Soiltest Farm Consultants, Inc, Moses Lake, WA
[3]Eqlab, Environmental Quality Laboratories, Inc., Bayamón, PR.
*There is no specified ruling for these tests.

into the soil, because the soil and compost biota could relinquish N from plants. For example, percentages of N, P_2O_5 and K_2O in a compost from animal manures (Type II feedstock), like chicken manure, were 1.4%, 4.56% and 3.09%, respectively (unpublished results) and can be used to provide required nutrients to crops. Lower C:N ratios could be obtained with greater degradation of the wood lining content of the composted material, which appears to take place after the EMFG treatments. Additional minor elements tested in woodchip compost are reported in Table 2.

Hurricane winds, rain and high flowing water can mix not only pathogen-infected waters with tree debris but also many other contaminants, such as metals, plastics, concrete, rocks, soil and oils. Fallen trees need to be collected carefully to prevent contamination, and separated from debris that can cause equipment failure and potentially contaminate organic matter with heavy metals. Regulated metals in woodchip compost tested before and after EMFG treatments had values below EPA limits as stated in EPA 503 rule (Table 3). The findings previously mentioned indicate that the woodchips and the compost were not sources of regulated metals nor did heavy metals contaminate hurricane debris. Compost stability measured by the evolution of CO_2 was classified as very stable before and after EMFG treatments (Table 4).

In addition, compost piles can have herbicide residues (USCC, 2013), which can be detrimental to plant growth. A bioassay can determine the presence of herbicide residue. Woodchip compost before and after EMFG treatments showed 100% germination of cucumber seed bioassay, and vigor (seedling height, cotyledons and root length) above 98% when compared to vermiculture. Hence no herbicide residue is assumed to be present.

Warm temperatures or heat achieved otherwise and sustained play a major role in reducing pathogens. Temperature is a measurement used in the composting industry to monitor pathogen reduction and is the utmost regulating factor for the USCC, EPA and many states. However, studies have shown that market-ready composts (Brinton et al., 2009) still contain pathogens that could cause harm to consumers. Puerto Rico's Department of Natural and Environmental Resources

TABLE 2.—*Chemical analysis of woodchip compost before and after treatment with Effective Microorganisms•1 first generation solution on windrow piles.*

Parameter	Before EMFG	After treatment	Units	Method
Moisture	34	34	%	70° C for 12 h
Solids	66	66	%	70° C for 12 h
pH	7.2	7.7		1:5 compost:water ratio
E.C.	2.36	1.37	mS/cm	1:5 compost:water ratio
Organic Matter	39.3	21.8	%	TMECC 05.07A
Ash	60.7	78.2	%	550° C
Organic C	32.1	10.1	%	TMECC 04.01A
Total N	0.25	0.25	%	TMECC 04.02D
Ammonium-N	15.1	7.6	mg/kg	TMECC 05.02C
Nitrate-N	4.7	0.6	mg/kg	TMECC 04.02B
P_2O_5	0.07	0.08	%	TMECC 04.12B/04.14A
K_2O	0.14	0.11	%	TMECC 04.12B/04.14A
Potassium	0.12	0.1	%	TMECC 04.12B/04.14A
Calcium	3.1	7.3	%	TMECC 04.12B/04.14A
Magnesium	0.31	0.26	%	TMECC 04.12B/04.14A
Sodium	0.05	0.03	%	TMECC 04.12B/04.14A
Boron	11.1	44.3	mg/kg	TMECC 04.12B/04.14A
Chloride	370	150	mg/kg	TMECC 04.12D
Copper	26	25	mg/kg	TMECC 04.12B/04.14A
Iron	7985	7295	mg/kg	TMECC 04.12B/04.14A
Manganese	170	207	mg/kg	TMECC 04.12B/04.14A
Sulfate-S	5254	1750	mg/kg	TMECC 04.12D
Sulfur	1.37	0.83	%	TMECC 04.12B/04.14A
Zinc	54	46	mg/kg	TMECC 04.12B/04.14A

regulation for homogenous material such as woodchips states that temperatures of ≥48° C are acceptable to further reduce pathogens. The composted material prior to treatment achieved temperatures greater than 48° C; however, temperatures at that time were not high enough to control pathogens, which resulted in fecal coliforms of 1661 MPN/g, above permitted limits. During the mitigation process no pile reached temperatures higher than ≥ 55° C, the regulatory EPA and USCC limit to further reduce pathogens; however, one pile reached temperatures above 48° C for 30 days (Figure 1, dotted line) and the averaged two piles, above 48° C for 18 days (Figure 1, dashed line).

No treatment can completely assure that organic matter is properly stabilized and completely sanitized, especially after hurricanes; however, in this study we have shown that an EMFG can be used to mitigate fecal coliforms. Additional studies must focus on the use of other EM•1® generations, concentrations and time of applications to further reduce cost and ease implementation. Our findings show that although the temperature composting process might have reduced pathogens, it did not meet regulatory expectations, requiring beneficial microbial mixtures to reduce pathogens to acceptable levels.

Current testing methodologies such as USCC-STA must be used in testing other contaminants such as oils and fuels to further assess the potential use of organic debris after a hurricane.

TABLE 3.—*EPA-regulated metals in woodchip compost in windrow piles before and after Effective Microorganisms•1 first generation solution treatment.*

	Before EMFG Dry Wt. (mg/kg)	After Treatment Dry Wt. (mg/kg)	EPA limit (mg/kg)	Method
Arsenic	17	17.1	41	TMECC 04.12B/04.14A
Cadmium	0.05	0.2	39	TMECC 04.12B/04.14A
Chromium	25.8	21	-[1]	TMECC 04.12B/04.14A
Cobalt	4.4	4.4	1200	TMECC 04.12B/04.14A
Copper	25.8	25.4	1500	TMECC 04.12B/04.14A
Lead	7.7	0.02	300	TMECC 04.12B/04.14A
Mercury	<MDL	3.1	17	EPA 7473
Molybdenum	3.9	5.6	-[2]	TMECC 04.12B/04.14A
Nickel	4.6	4.4	420	TMECC 04.12B/04.14A
Selenium	<MDL	<MDL	36	TMECC 04.12B/04.14A
Zinc	53.6	45.7	2800	TMECC 04.12B/04.14A

[1]Prior to 30 September 1995, EPA chromium levels were 1200 mg/kg and were removed from the Part 503 Numeric Criteria, Table in 503 Rule.

[2]A 25 February 1994 Federal Register Notice deleted the EPA level value, 18 mg/kg, for Molybdenum (Biosolids Management Handbook).

TABLE 4.—*Average stability measurements of CO_2 evolution of woodchip compost before and after Effective Microorganisms•1 first generation solution treatments.*

	Before EMFG	After treatment	Units	Normal Range	Method
CO_2 Evolution	0.1	0.3	mg CO_2-C/g OM/day	1 to 7	TMECC 05.08
	0.1	0.1	mg CO_2-C/g TS/day	0.5 to 5	TMECC 05.08
Stability Rating		Very Stable			

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