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The Effectiveness of Vermiculture in Human Pathogen Reduction for USEPA Biosolids Stabilization

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A field experiment tested the feasibility of vermicomposting as a method for eliminating human pathogens to obtain United States Environmental Protection Agency (USEPA) Class A stabilization in domestic wastewater residuals (biosolids). The experimental site was at the City of Ocoee's Wastewater Treatment Facility in Ocoee, Florida, and Class B biosolids were used as the earthworm substrate. Two windrows of biosolids 6 m long were heavily inoculated with four human-pathogen indicators, fecal coliforms, *Salmonella spp*., enteric viruses and helminth ova. The test row was seeded with earthworms, *Eisenia fetida*. The quantity of *E. fetida* was calculated at a 1:1.5 wet weight earthworm biomass to biosolids ratio and the earthworms allowed time to consume the biosolids and stabilize the biosolids. The test indicated that all of the pathogen indicators in the test row were decreased more than in the control row within 144 hours. The test row samples showed a 6.4-log reduction in fecal coliforms compared with the control row, which only had a 1.6-log reduction. The test row samples showed an 8.6-log reduction in *Salmonella spp*., while the control row had a 4.9-log reduction. The test row samples showed a 4.6-log reduction in enteric viruses while the control only had a 1.8-log reduction. The test row samples had a 1.9-log reduction in helminth ova while the control row only had a 0.6-log reduction. Dr. Jim Smith, Senior Environmental Engineer and Pathogen Equivalency Commission (PEC) Chair, for the USEPA, indicated by personal communications, that a three- to four-fold reduction in indicator organisms would be sufficient to warrant serious consideration of vermicomposting as an effective stabilization methodology (Smith 1997). These results in conjunction with pilot project results strongly indicate that vermicomposting could be used as an alternative method for Class A biosolids stabilization. This was obtained statistically by vermicomposting.

Introduction

Of the 3500-4000 wastewater treatment facilities in 1997 in the State of Florida there were only nineteen Class AA Type I (> .5 MGD) and four Class A Type I wastewater treatment facilities. Therefore, the vast majority of these facilities generated a product below EPA Class A standards. In Florida, approximately 230,000 metric tons (mton) of biosolids were generated in 1995. Eight percent were burned or incinerated, 9 percent were distributed and marketed, 17 percent were landfilled, and 66 percent were land applied (FDEP 1998).

Within the last decade, implementation of the Florida Department of Health, Chapter 64-E-6, the subsequent USEPA 40 CFR, Part 503, the Florida Administrative Code 62-640, and other local codes has revolutionized biosolids processing in Florida (FDOH 1998; FDEP 1998). Previously, biosolids stabilization varied greatly and requirements for septage biosolids stabilization prior to the publication of the current rules and regulations were minimal at best. The only requirement was lime stabilization that could be satisfied by inducing a pH of 12 for a minimum duration of

2 hours for septic tank biosolids. This was accomplished by adding lime to a pump truck before picking up biosolids. Agitation of the biosolids and lime mixture while the truck traveled from point to point was considered sufficient. Adequate stabilization therefore depended on the integrity of the biosolids hauling company. No records indicating nitrogen loading rates or metals site life were compiled making it impossible to determine violations of the existing rule, Florida Administrative Code: 17-7 (FDEP 1984).

Public and privately-owned wastewater treatment facilities were required to stabilize their biosolids to a minimum Class C standard for land application, where most facilities used aerobic or anaerobic digestion. While record keeping was more organized than for septage stabilization, it was still insufficient. With the implementation of the new rules, these facilities are now required to stabilize to a Class B standard with Class C no longer satisfactory for land application. For most of the small facilities this was impossible to achieve without prohibitive retrofitting and expansion, as they usually generated Class C biosolids.

The obvious inherent environmental and health hazards of unstabilized human waste can be seen in the third world nations. Rampant diseases that have debilitating consequences are common for people living in these countries. Unstabilized or improperly stabilized biosolids are a real concern and the regulations regarding stabilization reflect this ongoing concern.

Hence, we investigated into the feasibility of using vermiculture as a pathogen stabilization method. Vermiculture is the practice of cultivating earthworms, such as *Eisenia fetida*, while transforming solid waste and organic biosolids into a beneficial agricultural soil amendment product. Vermiculture's potential as a beneficial human pathogen stabilization and management technique for biosolids has been addressed (Hartenstein *et al.* 1979; Neuhauser *et al.* 1988; Bogdanov 1998). Mitchell (1978) demonstrated that there was a marked reduction in populations of the pathogenic *Salmonella enteriditis*, *Escherichia coli* and other enterobacteriaceae during vermicomposting of aerobic sewage sludge by *Eisenia fetida* but these were only laboratory culture studies. It has not been addressed as a clear methodology for obtaining an USEPA Class A biosolids product in the field.

E. fetida (the red wiggler or manure worm) is commonly used in vermicomposting (Princine *et al.* 1980). Since earthworms are able to consume up to twice their weight of biosolids per day, a high biomass ratio of earthworms to biosolids makes it possible to quickly and economically convert the biosolids to earthworm casts (Hartenstein *et al.* 1979; Loehr *et al.* 1985; Huhta *et al.* 1988). The earthworm casts can then be marketed with little additional processing.

The Orange County Environmental Protection Division, in partnership with the American Earthworm Company, Mid Florida Mining and the City of Ocoee, Florida, initiated this vermiculture field experiment in March 1996 at the City of Ocoee's Wastewater Treatment Facility in Ocoee, Florida.

Initially, a preliminary pilot study was conducted to evaluate vermiculture's effectiveness with biosolids on a small scale. The pilot study demonstrated a noticeable reduction in the four human pathogen indicators, fecal coliforms, *Salmonella spp.*, enteric viruses and helminth ova in the biosolids. The next step in the implementation of this project was to begin a full-scale operation to define its operational feasibility. The USEPA issued a 2-year experimental permit in March 1997 with project oversight for the USEPA being performed by the Florida Department of Environmental Protection (FDEP). From the information gathered throughout the full-scale experiment, Standard Operating Procedures would be developed for the USEPA methodology.

For vermicomposting to be considered by the USEPA as an alternative methodology for Class A pathogen stabilization, the project needed to demonstrate a three- to four-fold reduction (defined as being divided into a specified number of parts) of pathogen indicators in the biosolids (Fold 1997). This could provide a suitable stabilization of the biosolids to ensure public health concerns with the vermicomposted product.

Materials and Methods

Pilot Project

A shelter was constructed at the City of Ocoee Wastewater Treatment Facility to house inoculated experimental plots. A chicken wire fence was installed to keep out animals. Dewatered biosolids (17 percent solids) were land applied into two windrows approximately 9 m long by 1.5 m wide by 46 cm deep, utilizing approximately 7.3 mtons of biosolids each. One row was designated the test row and the second row was designated the control row.

To obtain sufficient levels of all the pathogen indicators (fecal coliforms, *Salmonella spp.*, enteric virus and helminth ova) required for the study, biosolids from another municipal plant were incorporated. In addition, the biosolids had to be inoculated with enteric virus. The resulting material was applied on a bed of impermeable clay covered with filter sand for leachate recapture, if needed.

Three representative samples were analyzed from each row to establish a baseline for determining the initial concentrations of pathogen indicators. After 68 days, final samples were collected using the same methodology as the baseline sampling.

The test row was then seeded with *E. fetida* at approximately 1:1 earthworm biomass to biosolids ratio. Earthworm density was determined by amount of food that past research suggested *E. fetida* were capable of consuming in one day. Earthworms were provided approximately 3.6 mtons of biosolids for a 90-day feeding period.

A garden hose was used to irrigate the rows as needed, to provide the earthworms with optimal moisture. A pitchfork was used to turn the biosolids as needed for inspection of the earthworms.

Full-Scale Operation

Biosolids (15-20 percent solids) were land applied into two windrows approximately 6 m long by 1.5 m wide by 20 cm deep, utilizing approximately 1.4 mtons of biosolids each. One row was designated the test row and the second row was designated the control row. These two rows were inoculated with three of the four pathogen indicators, fecal coliforms, *Salmonella spp.* and enteric viruses.

The inoculation mixture was prepared as follows for the three pathogen portion of the project. First, 50 g of cake biosolids were mixed with approximately 50 ml of deionized water for each pathogen and the pathogen controls were blended into individual biosolids/water mixtures. Finally, each mixture was then divided into two one-liter bottles marked "control" and "test". Both rows were then inoculated with a minimum 105 inoculum of the three pathogen indicators (fecal coliforms, *Salmonella spp.* and enteric viruses). Three representative samples were analyzed from each row to establish a baseline for determining the initial concentrations of pathogen indicators. The biosolids test row was then seeded with *E. fetida* at a 1:1.5 earthworm biomass to biosolids ratio. This ratio represented the earthworm's feeding rate for a 24-hour peri-

od based on the pilot project. Earthworms were provided approximately 1361 kg of biosolids for a 14-day feeding period.

The helminth ova portion of the experimental project was conducted at a later date due to difficulty in acquiring the helminth ova eggs. Biosolids (15-20 percent solids) were land applied into two rows approximately 2.3 m long by 1.5 m wide by 23 cm deep. One row was designated as the test row and the other the control row. The helminth ova inoculation mixture was prepared as follows. Two containers were prepared by mixing 500 g of cake biosolids with approximately 500 ml of deionized water. Each container was then inoculated with the helminth ova. The control row container was inoculated with one million (84.1% viable) helminth ova (*Ascaris spp.*) eggs. The test row container was inoculated with one million (82.6% viable) helminth ova (*Ascaris spp.*) eggs. Dr. Robert S. Reimers' research team of Tulane University Medical Center conducted viability tests of the helminth ova to confirm initial concentrations (Little 1998). The biosolids test row was then seeded with *E. fetida* in a way similar to the other three pathogen tests. Florida peat, the substrate in which the earthworms were held, was spread across the test row, adding approximately 15 cm to the depth. Earthworms were provided approximately 531 kg of biosolids for feeding for a 7-day period.

Representative auger samples were collected by using a 46 cm PVC pipe with a 2 cm inside diameter. Augers were autoclaved for 30 minutes at 121°C after each sampling event for sterilization. The sampling regimen consisted of collecting composite samples 72 and 144 hours after inoculation from the test and control rows. These samples were analyzed for *Salmonella spp.* and enteric viruses. The helminth ova sampling was also conducted after 72 and 144 hour intervals. For fecal coliform analysis to comply with USEPA recommended sampling and testing, three series of composite samples were collected concurrently from the test and control rows. For each set one composite sample was collected for each of seven consecutive days and analyzed to obtain counts of the most probable number (MPN) of colonies. Samples of the pathogens were taken at these intervals to determine how quickly the earthworms were reducing the pathogens. This data would be used in establishing standard operating procedures (SOPs) in a future project.

Due to the extremely high numbers of pathogen indicators in the spiked project, the helminth ova analytical method was modified to obtain the most accurate readings.

A Student's t-test was used to compare the significance of the difference of the human pathogens and any decrease in the test and control rows.

Nonchlorinated water (less than 0.2 mg/L) was used to water the test and control rows as needed, to provide the earthworms with optimal moisture.

Due to the infectious nature of the experimental pathogens, intense precautionary measures were taken addressing safety concerns necessary to protect personnel and the environment. The City of Ocoee's Wastewater Treatment Facility is completely fenced in with restricted public access.

Results and Discussion

Pilot Project

The fecal coliform samples collected showed an initial baseline analysis for the test row of 0.093, 1.05 and 1.05 log most probable number/gram (log MPN/g) (Table 1). The initial baseline analysis for the control row was 1.05, 1.05 and 1.06 log MPN/g. The final test row sample results were -.16, -.00 and -.00 log MPN/g. The control samples were 0.047, 0.058 and 0.049 log MPN/g.

TABLE 1.
"Baseline and final samples of biosolids tested for four pathogen indicators from Orange County," Florida trial on stabilization of biosolids using vermiculture.

	Vermiculture Test Row Samples			Control Row Samples		
Pathogen indicator	NE1	C1	SE1	NE2	C2	SE2
Fecal coliform (CFU/g)[1,2]						
Baseline	301	314	313	313	326	329
Final	2.8	-0.0	-0.0	70	99	76
Fecal coliform (log MPN/g)[3]						
Baseline	0.093	1.05	1.05	1.05	1.05	1.06
Final	-.16	-.00	-.00	0.047	0.058	0.049
Salmonella sp. (cell/g)						
Baseline	7	3	4	9	2	6
Final	<1	<1	<1	1	<1	<1
Enteric virus[4]						
(Cytopathic effects)						
Baseline	neg	pos	pos	pos	neg	pos
Final	neg	neg	neg	neg	neg	neg
Helminth Ova (ova/4 g)						
Baseline	4	1	4	<1	2	1
Final	<1	<1	<1	<1	<1	<1

[1] Numbers for fecal coliform are the geometric mean of seven grab sample all taken on the same day.
[2] CFU/g = Colony Forming Units per gram
[3] MPN = Most Probable Number
[4] The cytopathic effects of enteric virus were measured as PFU/1 g as an indicator, rather than as PFU/4 g

The *Salmonella spp.* samples collected showed an initial baseline analysis for the test row of 7, 3, and 4 cells/gram (cell/g) (Table 1). The initial baseline analysis for the control row was 9, 2 and 6 cells/g. The final test row sample results were <1, <1 and <1 cells/g. The control sample results were 1, <1 and <1 cells/g.

The enteric virus samples collected showed an initial baseline analysis for the test row of negative, positive and positive, indicating the presence or absence of the cytopathic effects (Table 1). The initial baseline analysis for the control row was positive, negative and positive. The final test row sample results were negative, negative and negative. The control sample results were negative, negative and negative.

The helminth ova samples collected showed an initial baseline analysis for the test row of 4, 1 and 4 ova/4 grams (ova/4 g) (Table 1). The initial baseline analysis for the control row was <1, 2 and 1 ova/4 g. The final test row sample results were <1, <1 and <1 ova/4 g. The control sample results were <1, <1 and <1 ova/4 g.

The project was scheduled to last for 90 days. However, it was terminated earlier than expected (68 days) on the advice of the contracted vermiculturist. It was believed that the earthworms were beginning to fast from lack of food. They did in effect eat up to 1.5 times their body weight each day.

The installed leachate recapture system proved to be unnecessary since leachate was not produced during the course of the experiment. In addition, there were no unusual occurrences.

Final analysis indicated a significant reduction of fecal coliforms. All samples from the test row were negative for *E. coli*, *Salmonella spp.*, enteric virus and helminth ova. Reductions of pathogens were observed in the control row. This reduction could have been attributed to the natural die-off of the organisms. However, the reductions in the test row were greater, which can be attributed to the vermicomposting process.

Full-Scale Operation

The fecal coliform samples collected showed an initial baseline analysis for the test row of 9×10^9, 9×10^9 and 8×10^9 most probable number/1 gram (MPN/1 g). The initial baseline analysis for the control row was 9×10^9, 7×10^9 and 9×10^9 MPN/1 g. Af-

ter 24 hours, the test row samples averaged a 1.9-log reduction, which equates to a six-fold reduction or 98.65% (percent is based on actual pathogen counts and not logarithmic numbers) (Figures 1 & 2). The control row samples averaged a 0.1-log reduction (less than one-fold or 20.00%). After 72 hours, the test row samples averaged a 5.3-log reduction (seventeen-fold or 99.99%). The control row samples averaged a 0.5-log reduction (up to one-fold or 71.60%). After 144 hours, the test row samples averaged a 6.4-log reduction (twenty-one-fold or 100.00%). The control row samples averaged a 1.6-log reduction (five-fold or 97.44%). After 336 hours (end of the third set), reductions continued in both rows until the test row achieved a 6.7-log reduction (twenty-two fold or 100.00%) and the control row samples showed an averaged 3.2-log reduction (ten-

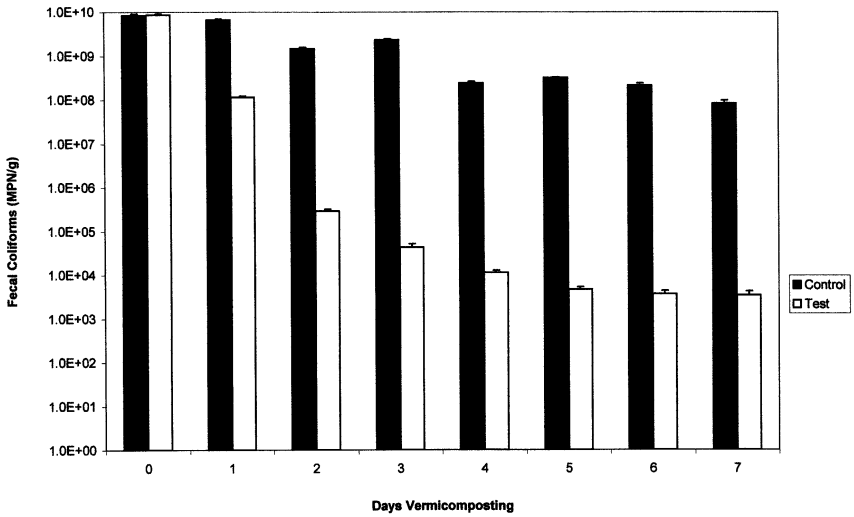

Figure 1. Fecal coliform average decrease (shown in logarithmic scale) in the control and test during 7 days of vermicomposting. Vertical T bars represent standard errors.

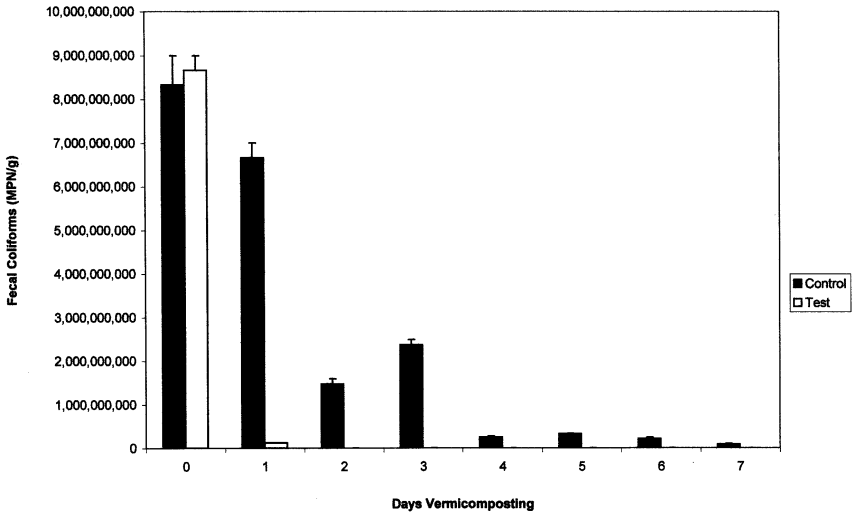

Figure 2. Fecal coliform average decrease (shown in linear scale) in the control and test during 7 days of vermicomposting. Vertical T bars represent standard errors.

TABLE 2.
Mean numbers of fecal coliform in the control and test during 7 days of vermicomposting.

Day Sample Taken	Sample Results (MPN/1 g) Control	Test	
Day 0	$8.33\ 10^9 \pm 6.67 \times 10^8$	$8.67 \times 10^8 \pm 3.33 \times 10^8$	NS
Day 1	$6.67 \times 10^9 \pm 3.33 \times 10^8$	$1.16 \times 10^8 \pm 6.67 \times 10^6$	***
Day 2	$1.46 \times 10^9 \pm 1.20 \times 10^8$	$2.90 \times 10^5 \pm 3.21 \times 10^4$	***
Day 3	$2.37 \times 10^9 \pm 1.20 \times 10^8$	$4.33 \times 10^4 \pm 8.82 \times 10^3$	***
Day 4	$2.47 \times 10^8 \pm 1.86 \times 10^7$	$1.13 \times 10^4 \pm 1.45 \times 10^3$	***
Day 5	$3.23 \times 10^8 \pm 1.20 \times 10^7$	$4.66 \times 10^3 \pm 6.67 \times 10^2$	***
Day 6	$2.13 \times 10^8 \pm 2.60 \times 10^7$	$3.66 \times 10^3 \pm 6.67 \times 10^2$	***
Day 7	$8.33 \times 10^7 \pm 1.45 \times 10^7$	$3.40 \times 10^3 \pm 8.08 \times 10^2$	**

The results are ± SE (n=3); NS = Not significant, ** = $p < 0.01$, *** = $p < 0.001$

fold or 99.00%). As shown in Table 2 the initial number of fecal coliform was not significant between the control and test. Afterwards in the 1st through 7th days of vermicomposting, mean number of fecal coliform in the test was significantly lower than that in the control.

The initial baseline analysis for *Salmonella spp.* in the test row was 4.2×10^9, 5.0×10^9 and 4.7×10^9 cells/25 ml. The initial baseline analysis for the

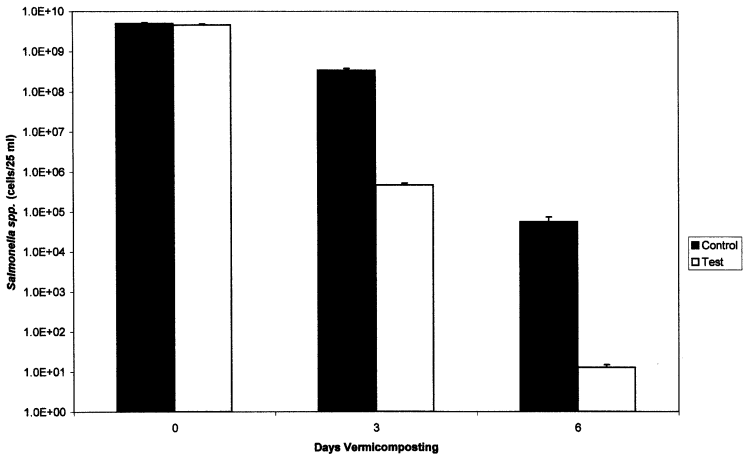

Figure 3. *Salmonella spp.* average decrease (shown in logarithmic scale) in the control and test during 6 days of vermicomposting. Vertical T bars represent standard errors.

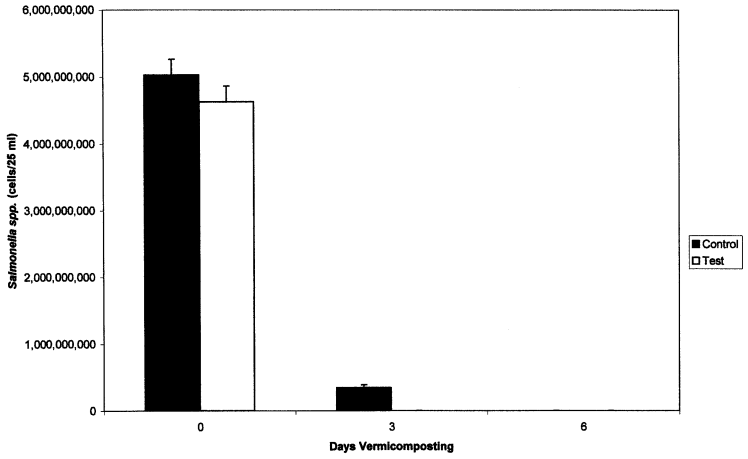

Figure 4. *Salmonella spp.* average decrease (shown in linear scale) in the control and test during 6 days of vermicomposting. Vertical T bars represent standard errors.

control row was 5.4×10^9, 4.6×10^9 and 5.1×10^9 cells/25 ml. After 72 hours, the test row samples averaged a 4.0-log reduction (thirteen-fold or 99.99%) (Figures 3 & 4). The control row samples averaged a 1.2-log reduction (three-fold or 93.18%). After 144 hours, the test row samples averaged an 8.6-log reduction (twenty-eight fold or 100.00%). The control samples averaged a 4.9-log reduction (sixteen-fold or 99.99%). Table 3 shows that initial mean number of *Salmonella spp.* was not significant between the control and test. Afterwards in the 3rd and 6th days of vermicomposting, mean number of *Salmonella spp.* in the test was significantly lower than that in the control.

The initial baseline analysis for enteric viruses in the test row was 2.3×10^5, 1.5×10^5 and 2.1×10^5 plaque forming units/4 grams (PFU/4 g). The initial baseline analysis in the control row was 1.3×10^5, 2.2×10^5 and 1.7×10^5 PFU/4 g. After 72 hours, the test row samples averaged a 2.0-log reduction (six-fold or 98.92%) (Figures 5 & 6). The control row samples averaged a 0.3-log reduction (one-fold or 53.85%). After 144 hours, the test row samples averaged a 4.6-log reduction (fifteen-fold or 99.99%). The control row samples averaged a 1.8-log reduction (six-fold or 98.46%). Table 4 shows that initial mean number of the enteric virus was not significant between the control and test. Afterwards in the 3rd and 6th days of vermicomposting, mean number of enteric virus in the test was significantly lower than that in the control.

After 72 hours, the test row samples showed a 0.3-log reduction (less than one-fold or 47.54%) from an original inoculum of 8.26×10^5 helminth ova (*Ascaris spp.*) (Figures 7 & 8). The control row samples had a 0.0-log reduction (less than one-fold or 0.00%) from an original inoculum of 8.41×10^5 helminth ova. After 144 hours, the test row samples had a 1.9-log reduction (six-fold or 98.87%). The control row samples had a 0.6-log reduction (one-fold or 74.24%). As shown in Table 5 the initial number of helminth ova was not significant between the control and test. Afterwards in the 3rd till 6th days of vermicomposting, mean number of helminth ova in the test was significantly lower than that in the control.

TABLE 3.
Mean numbers of Salmonella spp. in the control and test during 6 days of vermicomposting

Day Sample Taken	Sample Results (cells/25 ml) Control	Test	
Day 0	$5.03 \times 10^9 \pm 2.33 \times 10^8$	$4.63 \times 10^9 \pm 2.33 \times 10^8$	NS
Day 3	$3.43 \times 10^8 \pm 4.37 \times 10^7$	$4.73 \times 10^5 \pm 4.91 \times 10^4$	***
Day 6	$5.66 \times 10^4 \pm 1.76 \times 10^4$	12.66 ± 2.19	**

The results are $\pm$SE (n=3); NS = Not significant, ** = $p < 0.01$, *** = $p < 0.001$

TABLE 4.
Mean numbers of enteric virus in the control and test during 6 days of vermicomposting

Day Sample Taken	Sample Results (PFU/4 g) Control	Test	
Day 0	$1.73 \times 10^5 \pm 2.60 \times 10^4$	$1.96 \times 10^5 \pm 2.40 \times 10^4$	NS
Day 3	$8.00 \times 10^4 \pm 2.08 \times 10^4$	$2.13 \times 10^3 \pm 1.45 \times 10^2$	**
Day 6	$2.66 \times 10^3 \pm 6.67 \times 10^2$	5.00 ± 2.88	**

The results are ± SE (n=3); NS = Not significant, ** = $p < 0.01$

TABLE 5.
Mean numbers of helminth ova in the control and test during 6 days of vermicomposting

Day Sample Taken	Sample Results (oval/4 grams dry wt.) Control	Test	
Day 0	$8.41 \times 10^5 \pm 0$	$8.26 \times 10^5 \pm 0$	NS
Day 3	$9.33 \times 10^5 \pm 8.82 \times 10^4$	$4.33 \times 10^5 \pm 1.45 \times 10^5$	**
Day 6	$2.16 \times 10^5 \pm 1.76 \times 10^4$	$9.33 \times 10^3 \pm 1.45 \times 10^3$	***

The results are ± SE (n=3); NS = Not significant, ** = $p < 0.01$, *** = $p < 0.001$

The experiment demonstrates that the earthworms can reduce the USEPA pathogen indicators in as short a time as 144 hours. Since vermicomposting demonstrates such a great reduction in pathogen indicators in the spiked test, it shows that Class A stabilization equivalency is obtainable, as indicated in the pilot project. The reductions occurred greatly exceeded the required USEPA three- to four-fold reduction necessary for consideration of vermicomposting as a Class A stabilization method. The vermicomposting method is an inexpensive low-technological procedure for achieving results comparable to other more in-

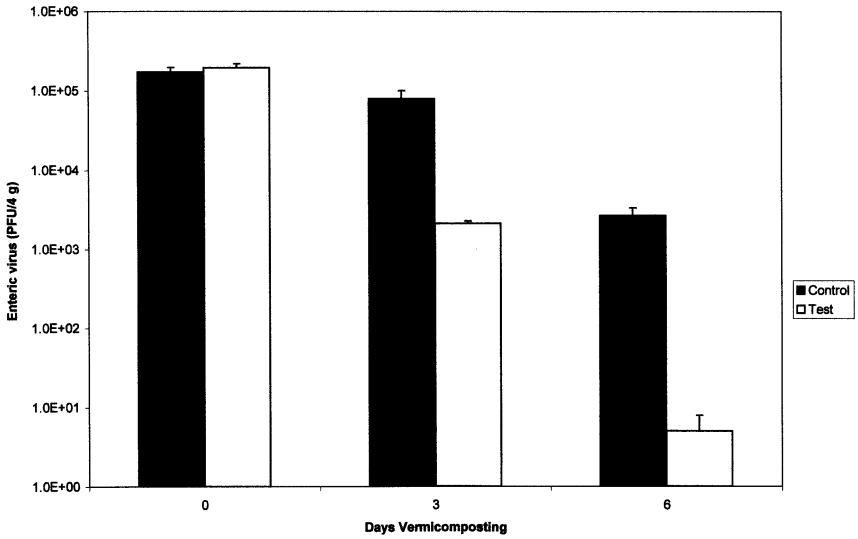

Figure 5. Enteric virus average decrease (shown in logarithmic scale) in the control and test during 6 days of vermicomposting. Vertical T bars represent standard errors.

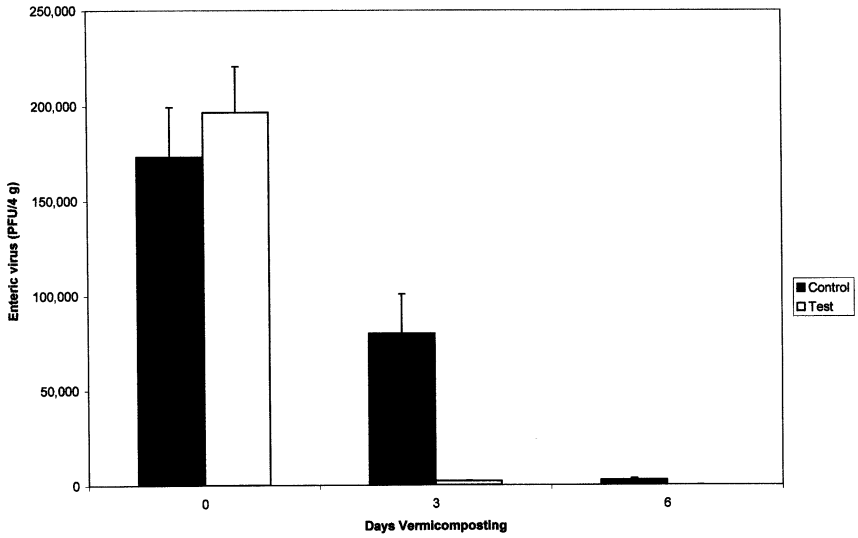

Figure 6. Enteric virus average decrease (shown in linear scale) in the control and test during 6 days of vermicomposting. Vertical T bars represent standard errors.

tensive and expensive USEPA biosolids stabilization methods. Also, a difference from other low-technology vermicomposting projects is the elimination of pre-composting. Until recently, this step was thought to be necessary to eliminate pathogens before adding earthworms. However, this project confirms that the earthworms greatly reduce the pathogens from the biosolids during vermicomposting making the pre-composting unnecessary. The use of earthworms to vermicompost biosolids exceeded even the initial experimental expectations for pathogen reductions.

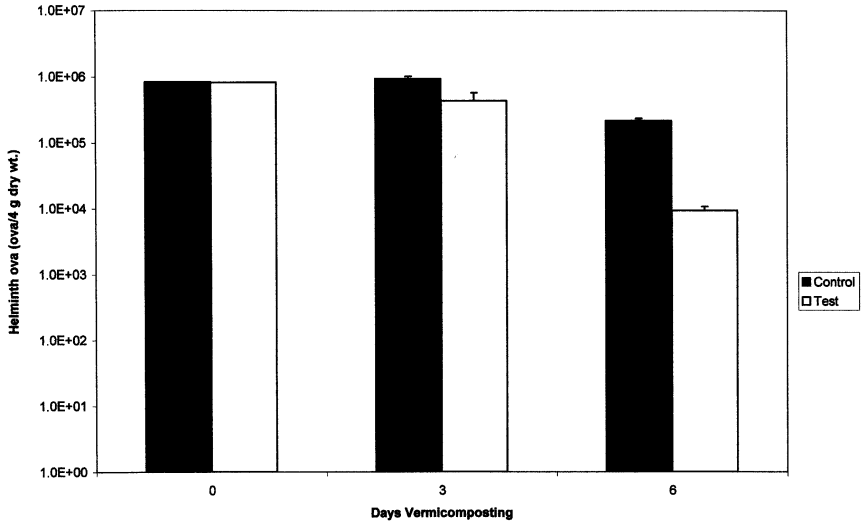

Figure 7. Helminth ova average decrease (shown in logarithmic scale) in the control and test during 6 days of vermicomposting. Vertical T bars represent standard errors.

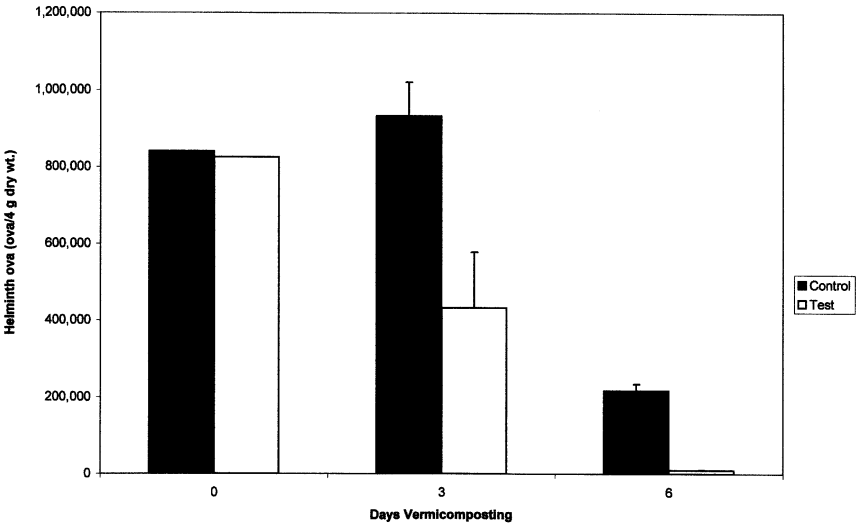

Figure 8. Helminth ova average decrease (shown in linear scale) in the control and test during 6 days of vermicomposting. Vertical T bars represent standard errors.

This project also showed that there were reductions of pathogens in the control row. This was attributed to the natural die-off of the organisms after inoculation. One of the requirements of an indicator organism is that it does not live long in the environment so that its presence is indicative of human contamination. However, the reductions in the test row were greater and quicker than in the control row due to the vermicomposting stabilization.

Observations were made which potentially affected the results. The introduction of earthworms to the helminth ova test row may have been more appropriate without the inclusion of Florida peat (substrate in which the earthworms were held). Due to the addition of the peat, the pathogen reduction times were probably slightly elevated compared to the previous test with the other three-pathogen indicators without peat. Slower reductions may have occurred because the earthworms already had a food source in the peat. Observations indicated the earthworms remained in the peat and did not migrate immediately to the biosolids thereby potentially increasing the stabilization time.

The helminth ova graph appears to indicate an increase in concentrations in the control row from the initial time to Day 3 (Figure 8). But, this may be an anomaly. The original helminth ova were counted and adjusted for viability. Representative samples, extrapolated for the amount of biosolids used in the control row, can indicate greater numbers of organisms. There was not an actual increase in the overall number of helminth ova (*Ascaris spp.*) eggs.

Conclusions

Based on experimental analyses from both the pilot and the full-scale operation, vermiculture can be used effectively as an USEPA process to treat pathogens and potentially produce Class A biosolids. Biosolids can be seeded with *E. fetida* by calculating a consumption rate of 1.5 times their biomass every 24 hours proportionally with the percentage of earthworm biomass to biosolids at a 1:7 ratio weekly. Additional biosolids should not be incorporated for a minimum of 144 hours to maximize pathogen reduction. Earthworms should not be harvested before this time.

Kg biosolids ÷ 7 = Biosolids per day ÷ 1.5 (consumptive rate of earthworms) = Kg of earthworms required for stabilization

This ratio is a baseline guide for quantity of earthworms and amount of biosolids to be stabilized as described. These population levels may not be practically sustainable. Therefore, due to earthworm population sustainability and fluctuation, the stabilization time will need to be proportionally adjusted until a stabilization equilibrium is obtained and maintained as confirmed by sampling analysis.

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