

# Occurrence and Levels of Fecal Indicators and Pathogenic Bacteria in Market-Ready Recycled Organic Matter Composts

W. F. BRINTON, JR.,<sup>1</sup>\* P. STORMS,<sup>1</sup> AND T. C. BLEWETT<sup>2</sup>

<sup>1</sup>Woods End Laboratories, Inc., 290 Belgrade Road, Mt. Vernon, Maine 04352; and <sup>2</sup>Dow AgroSciences, LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268-1054, USA

MS 08-186: Received 17 April 2008/Accepted 13 September 2008

## ABSTRACT

Landfill diversion of organic wastes through composting is making compost products available for agricultural and horticultural crops. On certified organic farms, nonsludge green waste and manure composts are widely used because the use of these products removes harvest date restrictions imposed by the U.S. Department of Agriculture when raw manure is applied. We quantified several pathogens in point-of-sale composts from 94 nonsludge facilities processing 2.2 million m<sup>3</sup> year<sup>-1</sup> of recycled green waste. Only one compost contained *Salmonella* (1.8 most probable number [MPN]/4 g), 28% had fecal coliforms exceeding the Environmental Protection Agency 503 sludge hygiene limits (1,000 MPN g<sup>-1</sup>), and 6% had detectable *Escherichia coli* O157:H7. In 22 of 47 samples, very low levels of *Listeria* spp. were found. However, in one sample the *Listeria* level was very high, coinciding with the highest overall level of all pathogen indicators. Seventy percent of the compost samples were positive for *Clostridium perfringens*, but only 20% of the samples had levels >1,000 CFU/g. All samples were positive for fecal streptococci, and 47% had >1,000 MPN g<sup>-1</sup>. Statistical analyses conducted using documented site characteristics revealed that factors contributing to elevated pathogen levels were large facility size, large pile size, and immaturity of compost. Application of the California Compost Maturity Index distinguished compost products that had very low levels of *E. coli* from those with high levels. Products produced with windrow methods were of higher microbiological quality than were those produced with static pile methods, and point-of-sale bagged composts scored very high. These data indicate that compost that is hygienic by common standards can be produced, but more effort is required to improve hygiene consistency in relation to management practices.

The practice of composting has emerged as an important alternative for recycling waste organic matter instead of landfilling and incineration. Thus, increasing amounts of compost are available to the general public. Originally regarded as a fertility management tool for organic farming (19), composting was adapted by developed nations in the 1950s to reduce municipal solid waste (6, 13). Dramatic recent growth in composting has come about in Europe and North America in response to legislated recycling and landfill reduction mandates (11, 35). Because bulky components of waste such as woody yard trimmings, grass clippings, and food scraps occupy up to 45% of the waste stream volume (35), at least 22 states in the United States have imposed restrictions or bans on disposal of yard trimmings in landfills.

Compost is widely viewed both as contributing to a balanced ecology and as possessing no significant risk to society. Results of studies in the 1960s indicated thermal inactivation of numerous pathogens from the sustained biological heating of composting materials (42). The U.S. Environmental Protection Agency (EPA) evaluated pathogen reduction in composting sewage sludge and drafted the findings into regulation as the EPA 503 rule (39), now widely applied to a broad category of recycled organic matter (ROM) composting (8, 40).

The hygiene premise in the framework of the EPA 503 rule is that the level of fecal coliform bacteria can be an indicator of the presence of *Salmonella*, which is widely prevalent in sludge (39, 43) but is more costly to analyze. Regression analyses from 260 composted sludges across the United States indicated that pathogenic *Salmonella* was found in less than 20% of the samples when fecal coliform counts were below 1,000 most probable number (MPN) per gram (43). Because *Salmonella* levels were essentially 0 when fecal coliform counts were less than 47 MPN g<sup>-1</sup> (43), the EPA established 1,000 MPN g<sup>-1</sup> as the maximum limit for fecal coliform Class A biosolids compost; composts with levels exceeding 2 × 10<sup>6</sup> MPN/g were classified as Class B restricted application material (39).

The early work on compost bacteriology coincided with the very first reports of toxigenic *Escherichia coli*. The presence of *E. coli* is of concern because larger quantities and more types of ROM composts are reaching agricultural and especially consumer markets (33). Use of a *Salmonella* surrogate to evaluate hygiene compliance of nonsludge compost materials not known to contain *Salmonella* at the start is problematic. The availability of newer microbiological methods, evidence that direct *E. coli* monitoring may better safeguard public health than testing only for fecal coliforms (14), and the increased concern about *E. coli* from manures and green composts entering the food chain (9, 21, 32, 34) were the reasons for conducting this ROM study.

\* Author for correspondence. Tel: 207-293-2457, Ext 17; Fax: 207-293-2488; E-mail: wfbr17@woodsendlab.org.

## MATERIALS AND METHODS

**Market sampling.** We sampled 94 market-ready, nonsludge ROM composts produced in the states of Washington, Oregon, and California, where exemplary statutory landfill diversion programs are in place (8, 27, 40, 41). The sum of registered annual volumes of these facilities is approximately 2.2 million cubic meters.

The structure of the composting industry and the hygiene regulations affecting it differ somewhat among these states. In Washington, organic wastes are classified and regulated by feedstock type, and small-scale facilities processing less than 200 m<sup>3</sup> at a time are exempt from regulations. Large facilities that use yard waste and preconsumer feedstock and any facility that composts postconsumer food waste must perform one or more tests per year for fecal coliforms and *Salmonella* (40). In Oregon, the need for regulatory permitting is dictated only by feedstock type. Only nongreen facilities handling sludge, animal by-products, and municipal solid waste are regulated, and therefore bacterial tests such as we employed in this study are not required (27). In California, both *Salmonella* and fecal coliform test results must be documented annually (8).

**ROM sampling.** We developed a sampling plan within each region by consulting published databases for the location, registered volumes, and nature of source materials. We selected 39, 25, and 30 sampling locations for Washington, Oregon, and California, respectively. A log book of the sample process was kept throughout and included site descriptions, type of technology employed at the site, and types and volumes of source ingredients utilized in composting. A requirement in our study was that only market-ready composts were sampled, the status of which was confirmed with site operators. When sampling commercial bagged composts at retail locations, a minimum of two 15-kg bags were purchased, and an empty printed bag was retained for documentation of the origin and listed ingredients.

In Washington, we sampled bagged and bulk composts with a preponderance of bagged material widely available at nursery centers. In Oregon, composters employed large to very large compost pile methods, and therefore all samples were obtained from bulk processing facilities from 11 counties in the northeastern, western, and southwestern zones of the state. In California, we sampled bulk green-waste facilities in a relatively large geographical area, including northern, Central Valley, and southern sites. Some facilities accepted source-separated food scraps. All samples within each state were collected within a 3-week period, and the process for all states was completed by summer 2005.

Subsamples were taken from each bag of compost obtained from enterprises open to the general public; these subsamples were pooled to form a composite sample. Samples of bulk composts were taken directly from market-ready piles by forming composites of approximately 12 grab samples from a minimum depth of 30 to 80 cm deep to make a 10-kg sample. All blended samples were mixed in previously washed polystyrene containers, and subsamples were collected, double bagged, labeled, and placed on ice packs in a cooler to arrive within 24 h at the laboratory. Samples were stored at 5°C, and all analyses were begun within 16 h of arrival in the laboratory.

**Microbiological examination methods.** Initially, our study in Washington was focused only on fecal coliforms and *Salmonella* based on EPA protocols (39). In Oregon and California, we expanded the scope of the study to include *E. coli*, which is considered to be more exclusively fecal. We also analyzed fecal streptococci, which are resistant to die-off during composting, *Clos-*

*tridium perfringens*, which is an obligate fecal anaerobe and spore former, *Listeria* species, which are environmentally ubiquitous, and *E. coli* O157:H7. These bacteria were included because they often are discussed and considered as indicators of proper pathogen limits for sludge and composts (4, 10, 12, 21, 23, 26). *E. coli* O157:H7 was added because of its prevalence in manure (15–17) and the increasing concern for its potential presence on vegetable products (9, 21).

Methods for the enumeration of *Listeria*, *C. perfringens*, and *E. coli* O157:H7 were based largely on the *Bacteriological Analysis Manual* (BAM) (38) and methods reported for testing compost and manure (10, 22, 23, 28).

**Compost sample preparation.** For analyzing fecal coliforms, *E. coli*, *Salmonella*, *Listeria*, and *Clostridium*, we started with 30 g of well-mixed ROM sample, added 270 ml of phosphate-buffered water (PBW) (1), stomached the mixture (Stomacher 400, Seward, Worthington, UK) for 90 s at 200 rpm in strainer stomacher bags, and added 11 ml of this mixture to 99 ml of PBW to obtain a 1:100 dilution, which was further serially diluted to 10<sup>-7</sup> units. All analyses were based on the MPN method except those for *Clostridium* (plate counts) and *E. coli* O157:H7. In ROM samples that were positive for *E. coli* O157:H7, this organism was enumerated by the MPN method. Several samples were retested after various periods of refrigeration as part of an effort to clarify unusual results. New samples from selected composts also were obtained at the site and retested. Retesting routinely included analysis for fecal coliforms and *E. coli* and on occasion also *E. coli* O157:H7.

**Fecal coliforms and *E. coli*.** For Washington samples, fecal coliforms were enumerated according to standard method (SM) 9221E (1). For Oregon and California samples, fecal coliform enumeration employed the recently updated EPA method 1680 (36) modified to include *E. coli*. This method is an elaborated SM 9221E+F five-tube MPN method starting with 30 g of sample (22 g for SM 9221) and inoculating 1 g of sample into each of the first row of tubes (2× lauryl tryptose broth, Difco, Becton Dickinson, Sparks, MD). The MPN was calculated using tables provided in EPA 1680 and the BAM (38). The minimum detection limit for EPA 1680 is 0.18 MPN/g corrected for solids, and the limit for SM 9221E is 1.8 MPN/g corrected for solids.

***Salmonella.*** *Salmonella* cells were initially enumerated by SM 9260D (1), but the method was updated to EPA 1682 (37) for all subsequent samples. The initial method is a three-dilution five-tube MPN method with a minimum detection limit of 0.18 MPN/g corrected for total solids. The EPA 1682 method differs significantly from the previously described method for the first 48 h. Enrichment cultures are grown in three rows of five tubes of tryptic soy broth (TSB; Difco, Becton Dickinson) for 24 h at 37°C: 20 ml of the stomached sample (see above) was placed into each of five tubes of 10 ml of 3× TSB, 10 ml of the stomached sample was placed into each of five tubes of 10 ml of 3× TSB, and 1 ml of the stomached sample was placed into each of five tubes of 10 ml of 1× TSB. Modified semisolid Rappaport Vassiliadis agar (Difco, Becton Dickinson) plates are inoculated with the TSB cultures the next day (one tube per plate, six 30-μl drops from the tube distributed on the plate) and incubated for 16 to 18 h at 42°C. The MPN per 4 g was calculated using tables provided in EPA 1682 for solid samples, and the minimum detection limit was 0.065 MPN/g corrected for solids.

**Fecal streptococci.** SM 9230B (1) was followed, and plates containing gray colonies that blackened the agar were considered presumptive fecal streptococci. Confirmation of some colonies

was conducted at middle dilutions in brain heart infusion broth with 6.5% NaCl (Northeast Laboratories, Waterville, ME) incubated at 46°C for 24 h. The detection limit for this procedure was 1.8 MPN/g corrected for total solids.

**Listeria.** We used a modification of the BAM enumeration method for *Listeria monocytogenes* (30). A three-tube seven-dilution MPN analysis was begun in universal preenrichment broth at 30°C for 48 h, and 0.1 ml from each turbid tube was inoculated into *Listeria* enrichment broth (LEB), which was incubated for 24 h at 30°C. All turbid LEB tubes were streaked to modified Oxford agar (Fluka, Milwaukee, WI) and incubated for 48 h at 35.5°C. Flat to slightly concave gray colonies blackening the agar were assumed to be *Listeria*. The detection limit for this procedure was 3 MPN/g corrected for total solids.

**C. perfringens.** We derived a compost method from various sources, including the BAM and other reported methods (20, 38). We spread the original sample dilution in PBW onto Shahidi-Ferguson-*perfringens*-cycloserine agar with egg yolk plates (Northeast Laboratories, Winslow, ME) to achieve dilutions of  $10^{-1}$  to  $10^{-7}$  units. Anaerobic incubation was conducted at 36°C for 24 h in an anaerobic chamber (Remel, Lenexa, KS) with anaerobic indicators (Oxoid, Basingstoke, UK) and oxygen reduced by AnaeroPack-Anaero (Mitsubishi Gas Chemical Co., New York, NY). Colonies were then counted, and 10 colonies most typical of *C. perfringens* were inoculated into 10 degassed thioglycollate broth tubes (Northeast Laboratories) and incubated for 24 h at 36°C. One milliliter from each tube positive for turbidity and gram-positive rods was inoculated into an iron milk tube (38) and incubated for 6 to 12 h at 46°C. The percentage of iron milk tubes exhibiting dramatic frothing was applied to the original plate counts, giving the CFU per gram of total solids.

**E. coli O157:H7.** This organism is not commonly tested for in composts, but we developed a method based on available reports (21, 24) and the BAM (38). All samples were initially tested in duplicate for the presence of *E. coli* O157:H7. When tests results were positive, we used a three-tube six-dilution MPN method. The process steps were (i) 24 h of enrichment culture in modified TSB (TSB with bile salts and without novobiocin; Difco, Becton Dickinson) with cefixime (Dynal Biotech, Oslo, Norway) and vancomycin (Sigma) at 37°C; (ii) magnetic separation of *E. coli* O157:H7 from the broth using anti-*E. coli* O157:H7 Dynabeads (Dynal Biotech); (iii) streaking of the beads onto two agars: Chromagar O157 (Dynal Biotech) and sorbitol MacConkey agar with cefixime and potassium tellurite (Dynal); and (iv) incubation for 24 h at 37°C. Suspect colonies were streaked to Trypticase soy agar (Difco, Becton Dickinson) with yeast extract, incubated overnight, and then examined to establish purity. Single colonies were checked for indole with Kovac's reagent and tested with the Oxoid Dry Spot O157 agglutination test. Detection limits for *E. coli* O157:H7 differed depending on the procedure and solids content. For the 25-g enrichment bags, samples were considered negative at <1 MPN/25 g fresh weight. Those considered positive and were set up for MPN processing 3 days later but then were negative at that stage were <3 MPN/g, corrected for solids.

**Compost physical and chemical properties.** General physical and chemical properties of the compost were examined as follows: total solids by 70°C convection oven drying, total nitrogen by combustion (29), salinity by conductivity (1), soluble ammonium and nitrate by ion-selective electrode (29), volatile fatty acids by distillation (1), and respiration (stability) by the Solvita procedure (41). The CO<sub>2</sub> respiration test was interpreted according to Washington State Department of Transportation guidelines (41).

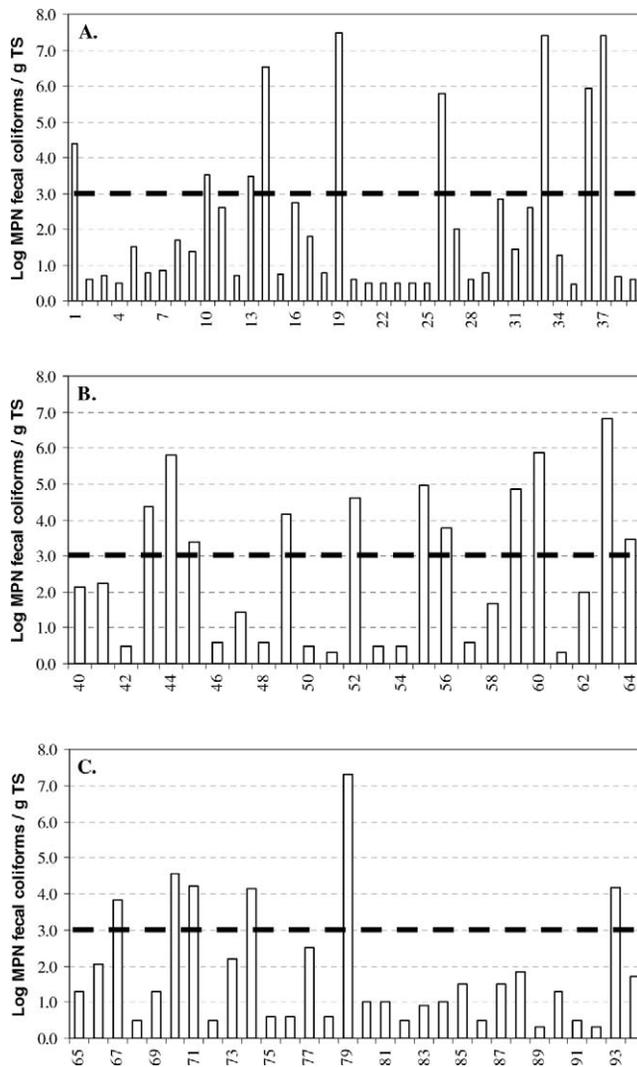


FIGURE 1. Fecal coliform levels (log MPN per gram) in ROM composts from Washington (A), Oregon (B), and California (C). Dotted lines indicate the U.S. EPA limit of 1,000 MPN/g.

The California Maturity Index protocol was conducted according to the guidelines of the California Integrated Waste Management Board (7). This protocol requires that a compost sample not exceed a C:N test ratio of 25:1 and that it pass two additional tests from a list of parameters associated with finished compost.

**Statistical analysis.** When performing analysis of bacterial data, any values that were less than the minimum level of detection were first converted to a non-zero format as +1 or by dividing the minimum level of detection by 2. To obtain means and standard deviations, actual MPN data were used, for which the log-transformed result is reported. For conducting the analysis of variance (ANOVA), regression analysis, and *t* tests, all data were log transformed before performing comparisons. The two-way ANOVA, regression analysis, and *t* tests were performed using Minitab (version 13, Minitab Inc., State College, PA).

## RESULTS

Analysis of ROM composts produced a very wide range of fecal coliform results for all regions (Fig. 1), with standard deviations slightly exceeding the mean for all areas (Table 1). For Washington ROM, 46% of samples were at or below the quantifiable level for fecal coliforms, and

TABLE 1. Levels of pathogen and pathogen indicators in ROM composts for three states and the criteria for the standards

Measure	Fecal coliforms (log MPN/g)	<i>E. coli</i> (log MPN/g)	Fecal streptococci (log MPN/g)	<i>Listeria</i> (log MPN/g)	<i>C. perfringens</i> (log CFU/g)	<i>Salmonella</i> (MPN/4 g)	<i>E. coli</i> O157 (pass-fail) <sup>a</sup>
Washington (n = 39)							
Minimum	0.46					<0.3	
Maximum	7.46					1.8	
Mean	6.34					0.2	
SD	6.86					0.2	
% above standard	23.1%					3%	
Oregon (n = 25)							
Minimum	0.30	0.30	0.48	0.48	1.04	<0.30	15
Maximum	6.82	6.82	7.45	4.60	3.95	<1.60	1
Mean	5.53	5.45	6.29	3.37	3.03	<0.90	
SD	6.12	6.12	6.83	3.99	3.37	NA	
% above standard	44.0%	36.0%		11.8%	11.8%	0	6.7%
California (n = 30)							
Minimum	0.30	0.00	0.00	0.60	0.78	<0.30	28
Maximum	7.30	7.30	7.36	2.63	4.88	<0.60	2
Mean	5.83	5.83	6.08	1.97	3.76	<0.40	
SD	6.56	6.56	6.65	2.23	4.27	NA	
% above standard	20.0%	16.7%		16.7%	18.8%	0	6.7%
Standard							
EPA 503	1,000 MPN/g	NA	NA	NA	NA	3 MPN/4 g	NA
European Union	NA	100 MPN/g <sup>b</sup>	NA	0/g <sup>c</sup>	0/g <sup>d</sup>	0/25 g	0/sample <sup>c</sup>

<sup>a</sup> Pass-fail, minimum and maximum values are the number of samples in which *E. coli* O157 was not and was detected, respectively.

<sup>b</sup> European Union standard for total *Enterobacteriaceae* for all categories of composts (12).

<sup>c</sup> Österreichisches Normungsinstitut standard for gardens and turf (28).

<sup>d</sup> European Union standard for category 3 composts with manure or fish waste.

23% exceeded the EPA 503 limit of 1,000 MPN/g (Table 1). Only one sample was positive for *Salmonella* at 1.8 MPN/4 g. Analysis included comparison of bacteria concentrations with various sample groupings using documented facility and product traits (Fig. 2). One group consisted of products listing manure as an ingredient. Composts listing manure were generally lower in fecal coliforms than were those not listing manure; however, the difference was not significant. Comparison between bulk and bagged compost products indicated bulk products had significantly higher fecal concentrations ( $P < 0.001$ ) (Fig. 2A), with 55% of samples in this group exceeding the EPA limit. Within the subgroup of bulk product samples, the documented inclusion or absence of manure did not significantly influence the fecal coliform results, and both subgroups had similarly high fecal coliform levels, with 50% exceeding the EPA limit. We compared other analytical data for the bagged group versus the bulk group, and the bagged group averaged higher  $\text{NO}_3^-$  (520 versus 128 mg  $\text{kg}^{-1}$ ;  $P < 0.05$ ) and higher stability based on a Washington State required field maturity test (6.0 versus 5.4 Solvita units;  $P < 0.05$ ).

For Oregon ROM, we observed a similarly wide variation in fecal coliform levels; 44% of samples exceeded the EPA 503 fecal coliform limit, and 36% had no detectable fecal coliforms (Fig. 1 and Table 1). No samples contained measurable *Salmonella*. We compared fecal coliform results against documented characteristics of the compost ingredients and methodology. Listing of manure as an in-

redient in the composts did not significantly affect the concentration of fecal coliform. Composting technologies had a measurable impact on compost hygiene levels; windrowing methods produced composts with the lowest fecal coliform counts and significantly lower *E. coli* concentrations than did other methods (Fig. 2B). No samples taken at Oregon facilities that employed windrow technology exceeded the EPA 503 limit. In contrast to the very large ROM piles we observed in Oregon (Fig. 3A and 3B), windrowing methods make the ROM accessible for mechanical mixing of outer and inner layers by turning machines (Fig. 3C) and reportedly lead to improved elimination of pathogens (32, 34).

For California ROM, the range of fecal coliforms was similar to that in both Washington and Oregon, with only 20% of samples exceeding the EPA 503 limit (Fig. 1). One facility produced compost with a very high fecal coliform level, and this facility was in a noted vegetable production area (Table 1). No *Salmonella* was detected in any sample. An apparent relationship existed between pathogen level and both the facilities annual volume and the type of windrow technology. Only 7% of the facilities handling less than 45,000 metric tons per annum (mta) exceeded the EPA fecal coliform standard, whereas 31% of samples from large facilities (>45,000 mta) exceeded the limit. These differences in fecal coliform levels were not significant, but differences were significant for *E. coli* ( $P < 0.02$ ), fecal streptococci ( $P < 0.01$ ), and the sum of all pathogens not in-

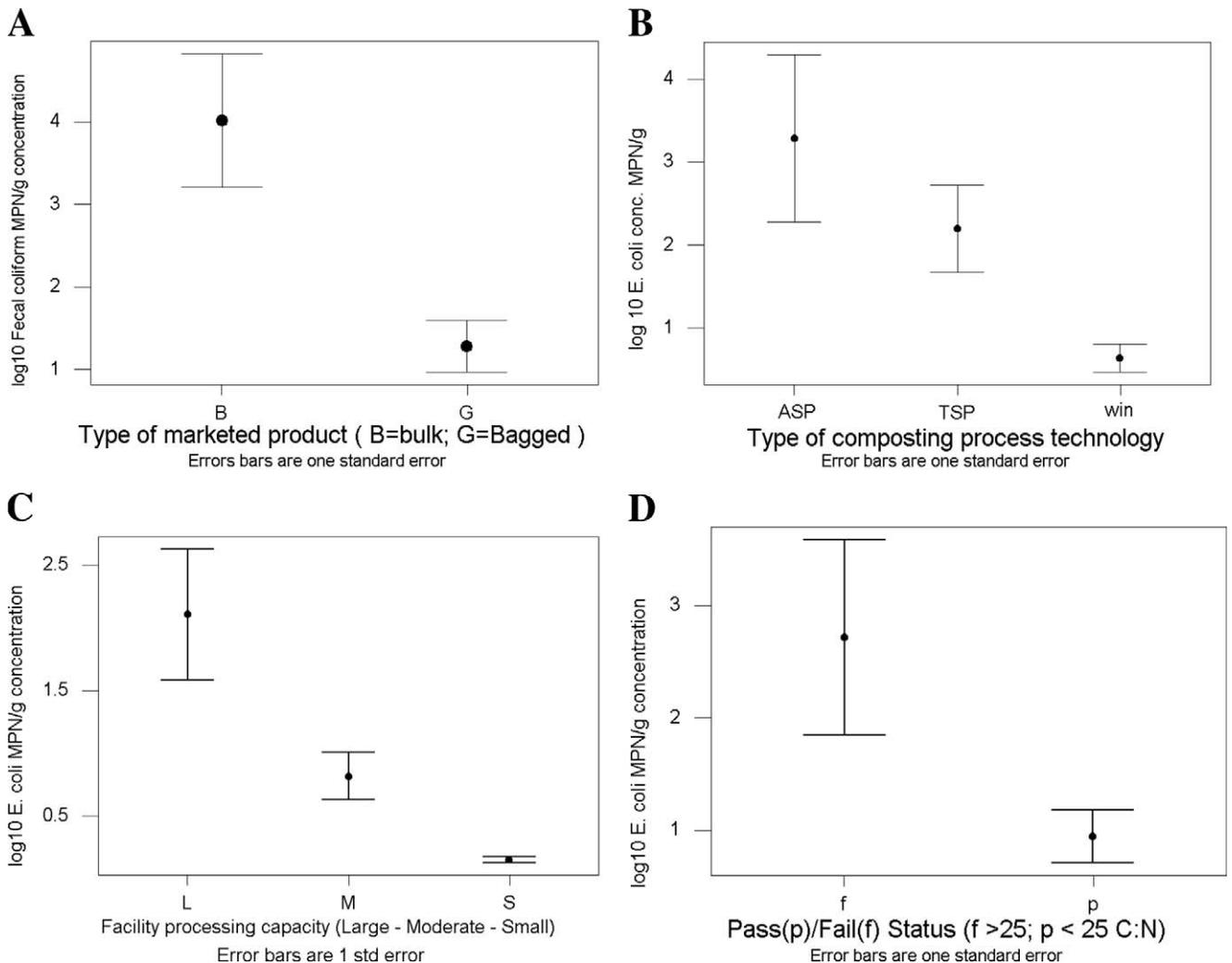


FIGURE 2. ANOVA of log-transformed MPN data for various traits for ROM composts. (A) Fecal coliform levels in relation to type of product in Washington. (B) *E. coli* content in relationship to ROM handling technology in Oregon. ASP, aerated static pile; TSP, turned static pile; win, windrow. (C) *E. coli* content in relation to size of California ROM compost facilities: large (>40,000 mta), moderate (12,000 to 40,000 mta), and small (<12,000 mta). (D) Relationship of *E. coli* levels to tested traits according to the California Maturity Index test protocol (7).

cluding fecal coliforms ( $P < 0.01$ ). Fewer composts exceeded the EPA limit when mechanically turned windrows were employed (8%) than when bucket-loader turning was used (17%). A significant difference in *E. coli* levels was observed based on groupings of facility size (Fig. 2C); small facilities (<12,000 mta) had very low pathogen counts (mean *E. coli* counts for four facilities processing <12,000 mta was 1.4 MPN/g).

We detected measurable *E. coli* O157:H7 in samples from three facilities. These facilities were in the large facility group and were situated within important vegetable growing regions. One of these three facilities also produced compost with one of the highest counts of *C. perfringens* ( $8 \times 10^4$  CFU/g). This obligate anaerobe is indicative of very wet, fecally contaminated conditions. We retained the sample with the highest *E. coli* O157 count for 3 weeks at 5°C, and it was still positive when retested. To confirm these results, we sampled compost from the same site 3 months later, taking the sample from a different batch of compost; the new sample was again positive for *E. coli*

O157. To investigate whether pathogen characteristics persisted over time at a facility, five facilities that produced composts with elevated fecal coliform counts were resampled in two regions within California (21 to 146 days after the first sampling). At three of the five facilities, these second samples also exceeded the fecal coliform limit.

## DISCUSSION

Composting is almost universally described as a sanitizing process, and green wastes are often viewed as free of pathogens (10, 21, 27, 34). The majority of 94 compost products we examined were hygienically clean as defined by current EPA standards. In all three regions, slightly more than a third of the compost samples had *E. coli* levels of less than 10 MPN/g, while a similar percentage of samples exceeded 1,000 MPN/g and therefore would not meet the EPA 503 requirement.

The potential impact on crop soils was estimated by equating registered volumes available from the California composting facilities with normal compost application

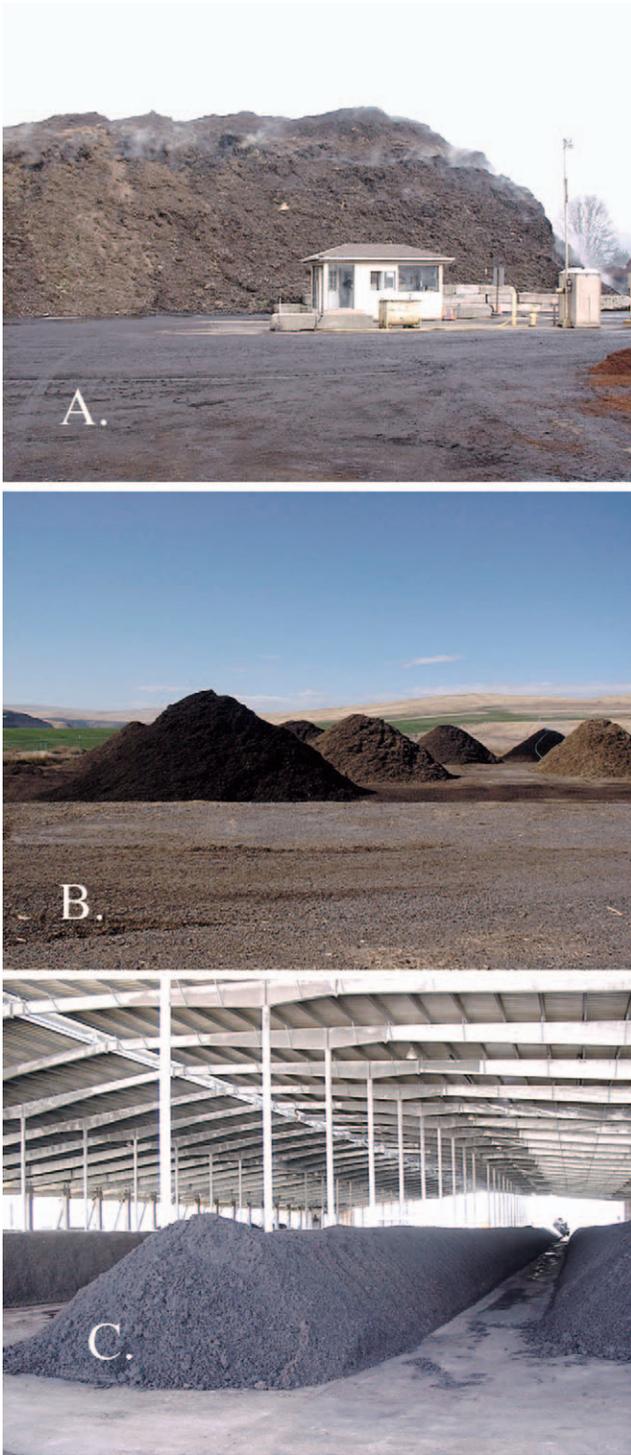


FIGURE 3. Examples of ROM compost facilities observed in study: (A) large bulk compost pile rarely turned, (B) static pile compost turned with excavator, and (C) windrow pile facility where piles are processed with mechanical turning machine.

rates. Compost with excessive fecal coliforms represented sufficient material to treat up to 35,000 hectares of farmland. In major fruit and vegetable producing states, the prevalence of composts with high pathogen levels offers the opportunity for pathogen contamination of these crops.

Fecal coliform tests per se do not give a complete indication of pathogenic organisms (14). Only one sample in this study was positive for *Salmonella* and thus this group

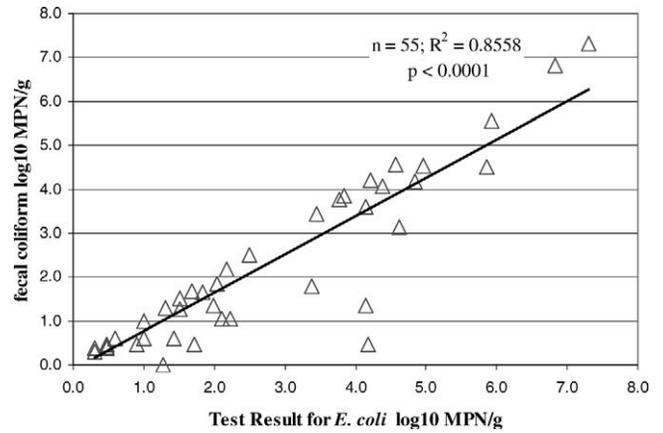


FIGURE 4. Relationship of *E. coli* counts and fecal coliform counts for Oregon and California

of samples was within the EPA acceptable threshold, whereas in Europe this single positive sample would cause the entire group to fail the *Salmonella* standard (10, 23, 28). An unexplored issue is the relevancy of *Salmonella* tests for green wastes. Under circumstances where green waste compost is intended to go back to food chain soils relatively quickly and where these composts may contain manures and food wastes, other useful indicator organisms and pathogens would include *E. coli*, *E. coli* O157:H7, fecal streptococci, *Listeria*, and *C. perfringens*. All these organisms have some niche that may allow them to resist the sanitizing actions of active composting, and all can be spread onto soils and crops (2, 5, 18, 25, 44). In addition to survival mechanisms, the opportunity for cross mixing of materials at compost sites and pathogen regrowth due to continued presence of sufficient substrate in immature compost would explain our findings (18). Böhnel and Lube (4) suggested that *Clostridium* presents a unique challenge because its spores survive thermophilic composting and therefore may accumulate as compost is successively applied.

Because of the interest in the relationship between fecal coliform and *E. coli* test results, we examined data from the 55 samples for which both tests were conducted. A highly significant correlation ( $r^2 = 0.85$ ) was found (Fig. 4). Fecal coliform results were also correlated with the sum of all other tested organisms ( $r^2 = 0.64$ ,  $P < 0.001$ ), indicating that the fecal coliform test is a useful hygiene indicator.

In the United States, procedures for reprocessing composts that may exceed a pathogen limit have not been defined but would presumably require continued composting or recomposting. The Österreichisches Normungsinstitut pass-fail compost standards for *E. coli* O157, *Salmonella*, *Campylobacter* sp., and *Listeria* sp. (28) impose end-use restrictions as a means to control composts that exceed specified bacterial limits. Recently updated European compost hygiene rules set strict limits for *Salmonella* and total *Enterobacteriaceae* (12). When either of these alternative schemes were used to rank our sample set, essentially the same result was obtained for percentage of compost samples that exceeded a hygiene standard limit (Table 1).

Meaningful differences were observed when compost

results were grouped by known characteristics of the products or facilities. This information could be used for improving compost site management. In Washington, bagged compost products were significantly lower in fecal coliforms than were nonbagged bulk products (Fig. 2A). Bagging operations are normally run indoors with more well-aged and stabilized material to avoid market issues such as odor and reheating. Because bulk compost products listed as not containing manure had higher fecal coliform counts than did other bulk composts (2.90 versus 1.76 log MPN,  $P \leq 0.05$ ), the presence or absence of manure content alone, as presently reported, is not a sufficient indication of bacteria levels. These results suggest that better standardization of end-product quality would improve hygiene. A useful distinction was observed when applying the voluntary California Maturity Index method for compost classification. Samples that did not meet the basic test requirement had a significantly higher *E. coli* content than did those that met the requirement ( $P < 0.01$ ) (Fig. 2D).

A difficulty associated with drawing conclusions about intrinsic factors in compost that influence or could predict pathogen levels is that variations in day-to-day operations over time must be known. Important variables include the practice of batching (more than one active compost blended at later points in the process), variation in ingredients, and equipment contact with materials at various stages in processing (31). Facility annual capacity and pile size, such as found in Oregon and California facilities, are factors that likely exert similar influence on pathogen levels indirectly through the mechanism of aeration and maturing of the product. Maturation of compost results in a reduction of available substrate for *E. coli* reproduction and regrowth (21, 44) but also lengthens the required processing time, which can have a negative economic impact (3).

Small facilities and windrowing methods for composting emerged as factors with the most positive impact on hygiene outcome. Windrowing technology scored well for compost hygiene in very early bacteriological studies (43); however, this technology requires more ground area (31) (Fig. 3C). All windrow samples tested did not attain equally high performance when compared between regions in our study, most likely because of the large within-technology type variations in hygiene performance reported for European compost facilities (26). In studies of manure spiked with *E. coli* O157 (32, 44), results indicated that the compost must be regularly turned for the pathogen to be eliminated; otherwise, the pathogen can remain viable for months. These local variations and the fact that facilities obtain different types of wastes over time are potentially large confounding factors.

Another important issue is the implication for sanitary organic farming practices (9, 21, 34). The U.S. Department of Agriculture National Organic Program established safety margins similar to those of the EPA 503 rule that consider the C:N ratio, length of composting time, and temperature for distinguishing compost from raw manure; raw manure requires 120 days between application and harvest for leafy vegetables (9, 32, 34). A large number of compost samples we examined possessed fecal bacteria levels that exceeded

the EPA 503 rule, and 6% exceeded the  $2 \times 10^6$  MPN/g level of an EPA Class B restricted application sludge. Such fecal bacteria levels could be viewed as similar to those of raw noncomposted manure. These results and the absence of statistical correlation between manure content and fecal coliform counts suggest that regulation of compost based on known presence of manure, as is done for organic food production facilities, may not adequately prevent crop contamination.

With the growth of the composting industry, there should be additional research to more closely examine critical processing factors that influence pathogen levels in finished compost. As modes of compost usage spread, there is increased concern that pathogens from compost could enter the food chain (21, 26). Additional work is recommended to determine the potential hazard of contaminating produce, agriculturalists, and gardeners through use of high-pathogen composts, which are frequently applied at very high rates (3, 33). Because a large percentage of compost processors in this study were making product with a very low pathogen content, this level of hygiene is achievable. Progress is needed on two fronts: a better understanding of what constitutes relevant hygiene assessment for ROM compost and a more detailed description of best management practices needed to routinely achieve composting hygiene goals.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the field survey and site sampling efforts of Jennifer Hart, Art Krenzler, and Marc Buchanan.

## REFERENCES

1. American Public Health Association. 1995. Standard methods for the examination of water and wastewater (SM). 1995. American Public Health Association, Washington, DC.
2. Anderson, G., S. J. Kenney, P. Millner, L. R. Beuchat, and P. L. Williams. 2006. Shedding of foodborne pathogens by *Caenorhabditis elegans* in compost-amended and unamended soil. *Int. J. Food Microbiol.* 23:146–153.
3. Blewett, C., D. Roberts, and W. Brinton. 2005. Phytotoxicity factors and herbicide contamination in relation to compost quality management practices. *Renew. Agric. Food Syst.* 20(2):67–72.
4. Böhnelt, H., and K. Lube. 2000. *Clostridium botulinum* and bio-compost. A contribution to the analysis of potential health hazards caused by biowaste recycling. *J. Vet. Med. B* 47:785–795.
5. Brandl, M. T., B. M. Rosenthal, A. F. Haxo, and S. G. Berk. 2005. Enhanced survival of *Salmonella enterica* in vesicles released by a soilborne *Tetrahymena* species. *Appl. Environ. Microbiol.* 71:2244–2249.
6. Breidenbach, A. W. 1971. Composting of municipal solid wastes in the United States. SW-47r. U.S. Environmental Protection Agency, Washington, DC.
7. California Integrated Waste Management Board. 2001. Compost maturity index. Available at: <http://www.epa.gov/compost/pubs.htm>. Accessed 12 April 2008.
8. California Integrated Waste Management Board. 2008. California Code of Regulations, title 14, division 7, chap. 3.1. Composting operations regulatory requirements, section 17868.3(b). Pathogen reduction. California Integrated Waste Management Board, Sacramento, CA.
9. Chen, M. C. 2005. Organic fruits and vegetables: potential health benefits and risks. *Nutr. Noteworthy* 7:1. Available at: <http://repositories.cdlib.org/uclabiolchem/nutritionnoteworthy/>. Accessed 19 March 2008.

10. Christensen, K. K., M. Carlsbaek, and E. Kron. 2002. Strategies for evaluating the sanitary quality of composting. *J. Appl. Microbiol.* 92:1143–1158.
11. Commission of the European Communities. 1993. Landfill Directive 1999/31/EC. *Off. J. Eur. Communities* L182(16/07/1999):1–19. Available at: <http://eur-lex.europa.eu/en/index.htm>. Accessed 19 March 2008.
12. Commission of the European Communities. 2002. Regulation (EC) no. 1774/2002 of the European Parliament and of the Council 3 October 2002 laying down health rules concerning animal byproducts not intended for human consumption. *Off. J. Eur. Communities* L273. Available at: [http://eur-lex.europa.eu/RECH\\_naturel.do](http://eur-lex.europa.eu/RECH_naturel.do). Verified 19 March 2008.
13. Deportes, I., J.-L. Benoit-Guyod, D. Zmirou, and M.-C. Bouvier. 1998. Microbial disinfection capacity of municipal solid waste (MSW) composting. *J. Appl. Microbiol.* 85:238–246.
14. Doyle, M. P., and M. C. Erickson. 2006. Closing the door on the fecal coliform assay. *Microbe* 1:162–163.
15. Duffy, G. 2003. Verocytotoxic *Escherichia coli* in animal faeces, manures and slurries. *J. Appl. Microbiol.* 94:94S–103S.
16. Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koochmaria, and W. W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci. USA* 97:2999–3003.
17. Faith, N. G., J. A. Shere, R. Brosch, K. W. Arnold, S. E. Ansay, M. S. Lee, J. B. Luchansky, and C. W. Kasper. 1996. Prevalence and clonal nature of *Escherichia coli* O157:H7 on dairy farms in Wisconsin. *Appl. Environ. Microbiol.* 62:1519–1525.
18. Farrell, J. P. 1992. Fecal pathogen control during composting. In H. A. J. Hoitink and H. M. Keener (ed.), *Science and engineering of composting: design, environmental, microbiological and utilization aspects*. Proceedings of an International Composting Research Symposium, 27 to 29 March 1992. Renaissance Publications, Worthington, OH.
19. Heckman, J. 2006. A history of organic farming: transitions from Sir Albert Howard's *War in the Soil* to USDA National Organic Program. *Renew. Agric. Food Syst.* 21:143–150.
20. Hill, R., W. L. Straube, A. C. Palmisano, S. L. Gibson, and R. R. Colwell. 1996. Distribution of sewage indicated by *Clostridium perfringens* at a deep-water disposal site after cessation of sewage disposal. *Appl. Environ. Microbiol.* 62:1741–1746.
21. Ingram, D. T., and P. D. Millner. 2007. Factors affecting compost tea as a potential source of *Escherichia coli* and *Salmonella* on fresh produce. *J. Food Prot.* 70:828–834.
22. Jiang, X., J. Morgan, and M. P. Doyle. 2002. Fate of *Escherichia coli* O157:H7 in manure-amended soil. *Appl. Environ. Microbiol.* 68:2605–2609.
23. Kehres, B., and A. Pohle (ed.). 1998. *Methodenbuch zur Analyse von Kompost*. Bundesgütegemeinschaft Kompost e.V., Köln, Germany.
24. Lahti, E., O. Ruoho, L. Rantala, M. Hanninen, and T. Honkanen-Buzalski. 2003. Longitudinal study of *Escherichia coli* O157 in a cattle finishing unit. *Appl. Environ. Microbiol.* 69:554–561.
25. Lung, A. J., C. M. Lin, J. M. Kim, M. R. Marshall, R. Nordstedt, N. P. Thompson, and C. I. Wei. 2001. Destruction of *Escherichia coli* O157:H7 and *Salmonella* Enteritidis in cow manure composting. *J. Food Prot.* 64:1309–1314.
26. Martens, J. 2005. Indicator methods to evaluate the hygienic performance of industrial scale operating biowaste composting plants. *Waste Manag.* 25:435–444.
27. Oregon Secretary of State, Department of Environmental Quality. 2008. Oregon Administrative Rule, chaps. 340-093-50 and 340-096-0020. Available at: [http://www.sos.state.or.us/archives/rules/OARS\\_300/OAR\\_340/340\\_093.html](http://www.sos.state.or.us/archives/rules/OARS_300/OAR_340/340_093.html). Accessed 19 March 2008.
28. Österreichisches Normungsinstitut (Austrian Standards Institute). 2005. *Kompost—Hygieneanforderungen—E. coli, Salmonella sp., Listeria sp., Campylobacter sp.* Probenahme, Untersuchungsmethoden, Ergebnisdarstellung. S 2204. Ed. 2005-01-01. Österreichisches Normungsinstitut, Vienna. Available at: <http://www.on-norm.at/publish/home.htm>. Accessed 19 March 2008.
29. Peters, J. (ed.). 2003. *Recommended methods of manure analysis*. Publ. A3769. University of Wisconsin. Available at: <http://learningstore.uwex.edu/pdf/A3769.pdf>. Accessed 19 March 2008.
30. Robinson, J., P. J. Naylor, and E. I. Stentiford. 1992. Development of a method for the isolation of *Listeria monocytogenes* from compost. *Acta Hort.* (ISHS) 302:369–376. Available at: [http://www.actahort.org/books/302/302\\_36.htm](http://www.actahort.org/books/302/302_36.htm). Accessed 13 March 2008.
31. Rynk, R. F., M. van de Kamp, G. B. Willson, M. E. Singley, T. L. Richard, J. L. Kolega, F. Gouin, L. Laliberty, D. Kay, D. W. Murphy, H. A. Hoitink, and W. F. Brinton. 1992. *On-farm composting handbook*, NRAES-54. Northeast Regional Agricultural Engineering Service, Ithaca, NY.
32. Shepherd, M. W., Jr., P. Liang, X. Jiang, M. P. Doyle, and M. C. Erickson. 2007. Fate of *Escherichia coli* O157:H7 during on-farm dairy manure-based composting. *J. Food Prot.* 70:2708–2716.
33. Stoffella, P. J., and B. A. Kahn. 2002. *Compost utilization in horticultural cropping systems*. Lewis Publishers, Boca Raton, FL.
34. U.S. Department of Agriculture, Agricultural Marketing Service. 2000. National Organic Program. 7 CFR Part 205. U.S. Department of Agriculture, Agricultural Marketing Service, Washington, DC.
35. U.S. Environmental Protection Agency. 1999. Organic materials management strategies. EPA 530-R-99-016. Available at: <http://www.epa.gov/epaoswer/non-hw/compost/omms.pdf>. Accessed 19 March 2008.
36. U.S. Environmental Protection Agency. 2005. Method 1680: fecal coliforms in sewage sludge (biosolids) by multiple tube fermentation using lauryl tryptose broth (LTB) and EC medium. EPA-821-R-06-012. Available at: <http://www.epa.gov/waterscience/methods/method/index.html>. Accessed 28 March 2008.
37. U.S. Environmental Protection Agency. 2006. Method 1682: *Salmonella* in sewage sludge (biosolids) by modified semisolid Rappaport-Vassiliadis (MSRV) medium. EPA-821-R06-014. Available at: <http://www.epa.gov/waterscience/methods/method/index.html>. Accessed 28 March 2008.
38. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition. 2006. *Bacteriological analysis manual online*, chaps. 4a, 10, and 16. Available at: <http://www.cfsan.fda.gov/~ebam>. Accessed 28 March 2008.
39. U.S. Government Printing Office. 1998. *Electronic Code of Federal Regulations*. Title 40, part 503. Standards for the use or disposal of sewage sludge. Available at: [http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&tpl=/ecfrbrowse/Title40/40cfr503\\_main\\_02.tpl](http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&tpl=/ecfrbrowse/Title40/40cfr503_main_02.tpl). Accessed 19 March 2008.
40. Washington State. 2003. *Administrative Code 173-350-220 Statutory Authority*, chap. 70.95 RCW. 03-03-043, order 99-24, section 173-350-220. King County Government, Seattle, WA.
41. Washington State Department of Transportation. 2007. *Compost use guidelines*, section 9-14. Erosion control and roadside planting. Available at: <http://www.wsdot.wa.gov/eesc/design/projectdev/gsp/gspspdf/14ap9.pdf>. Accessed 14 March 2008.
42. Wiley, B. B., and S. C. Westerberg. 1969. Survival of human pathogens in composted sewage. *Appl. Microbiol.* 18:994–1001.
43. Yanko, W. A. 1988. Occurrence of pathogens in distribution and marketing municipal sludges. EPA/600/S1-87/014. U.S. Environmental Protection Agency, Washington, DC.
44. Zaleski, K. J., K. L. Josephson, C. P. Gerba, and I. L. Pepper. 2005. Survival, growth and regrowth of enteric indicator and pathogenic bacteria in compost, soil, and land applied biosolids. *J. Residuals Sci. Technol.* 2:49–63.