PATHOGEN SURVIVAL PATTERNS IN WASTE-DERIVED COMPOSTS DESTINED FOR LAND RESTORATION

J. WILLIAMSON*, J. HOLMBERG, M. NASON, S. TANDY, R. JONES, J. HEALEY AND D.L. JONES

School of Agricultural and Forest Sciences, University of Wales, Bangor, Gwynedd, LL57 2UW, Wales.

SUMMARY: Organic wastes (deinking paper fibre, digested sewage cake and green waste) with or without mineral slate fines were co-composted in combination and tested for human pathogens to assess the suitability of the end-product for land restoration purposes. Composting was undertaken using an in-vessel system in which feedstocks were encapsulated in low density polyethylene bags and received controlled forced aeration over a two-month period, followed by one month maturation in the bag. *Escherichia coli* and *Salmonella* species were enumerated by drop plate, using compost-buffer serial dilutions onto Salmonella Chromogenic Medium (Oxoid Ltd., UK). The temperature reached during composting and the duration of the thermophilic and maturation phases were factors contributing to the reduction in pathogen colony-forming units (CFUs). Overall, composted green waste contained the highest CFUs of pathogens, especially *Salmonella* species, whilst composts containing digested sewage cake had lower pathogen CFUs.

1. INTRODUCTION

Europe produces around 2000 million tonnes of waste per year, growing by around 10% per year. The European Landfill Directive (1999/31/EC) seeks new strategies to enable greater recycling of wastes. Composting provides an environmentally acceptable means of diverting wastes from landfill and recycling of organic matter back to land: it is a rapidly expanding area for technology transfer because it offers practical solutions to the problem of continued increases in European waste production. By adding value to wastes through composting, the product becomes a valuable resource for tackling land degradation and addresses many of the concerns highlighted in the EU Thematic Strategy for Soil Protection such as soil fertility, microbial biodiversity, erosion, water infiltration and carbon sequestration. However, the composting of biodegradable waste within Europe is still conservative in nature and has yet to realise its full potential. Green waste is now routinely composted, often in bulk by Local Authority contractors, but there is a reluctance to make use of other common, high volume wastes e.g. digested sewage cake ('biosolids'), even though it is routinely spread to land in its uncomposted form. Unlike green waste, for example, biosolids generally contains too much water and has a low C:N ratio (<10) for optimum composting and consequently, there may be a difficulty in reaching the

thermophilic operating temperature necessary for pathogen control (Qiao & Ho, 1997). In this paper, we quantify the potential for increasing the human pathogen load in compost by including biosolids in feedstock mix, compared with routinely composted green waste.
Pietronave et al. (2004) found that indigenous microflora of matured compost played an important role in pathogen (*E. coli, S. arizonae*) suppression. Sidhu et al. (2001) reported that the potential for *S. typhimurium* regrowth in compost increased with ageing (two weeks compared with two years). However, both studies relied on inoculating pathogenic strains into compost, which may potentially affect the outcome since inoculants are far less likely to survive than indigenous populations. Zaleski et al. (2005) found that it was rainfall and external sources of faecal matter that gave rise to an increase in salmonellae, not the regrowth of salmonellae indigenous to the biosolids. It is possible that this could also happen in other stored composts and not just biosolids. The addition of mineral fines to compost feedstocks may enhance the composting process by stimulating biological activity (O'Brien et al., 1999). However, there appears to be little work done on how composting different feedstock components and proportions affect indigenous pathogenic loading. Our work describes such a comparative study, where in-vessel composting was used under conditions of controlled aeration and temperature, to test whether co-composting mineral additions with organic wastes affected the thermophilic phase 'pathogen kill' and whether this influenced regrowth during maturation.

2. MATERIALS AND METHODS

2.1 In-vessel composting and feedstocks

Compost was produced using EcoPOD® in-vessel aerobic composting vessels (Ag-Bag International Ltd, Warrenton, OR, USA). Feedstock materials for composting included shredded green waste, tertiary treated digested sewage cake ('biosolids'), de-inking paper fibre and mineral slate fines. Slate fines consisted of 70 % sand, 11 % silt and 19 % clay, equivalent to a sandy loam texture. Wastes were mixed on a dry weight (DW) basis (sub-samples of the initial wastes were taken to estimate initial moisture content) as detailed in Table 1. Electrical conductivity and pH were determined by mixing 1 cm^3 field-moist compost with 2 cm^3 distilled water and measuring the resulting solution after 30 minutes with a conductivity meter (Jenway 4010, Jenway Ltd., Essex) and an Orion 410A pH electrode (Orion Research Inc., Massachusetts, USA). Total C and N in the composts were determined using a LECO 2000 combustion and non-dispersive infrared CHN analyser (Leco Corp., Missouri, USA). Wastes were weighed and thoroughly mixed using a vertical auger cattle feed mixer wagon (Biga Twin Eco, Peecon, Etten-Leur, The Netherlands) and loaded into a CT5 hydraulic ram (Ag-Bag International Ltd, Warrenton, OR, USA) that pushes the material into an extruding low density polyethylene EcoPOD® vessel (1.5 m diameter). At the same time as filling the EcoPOD®, a perforated plastic aeration pipe (76.2 mm diameter with 1.59 mm slits) was automatically inserted along the base of the vessel to provide forced aeration. The aeration regime was controlled by means of a timed fan (Ag-Bag International Ltd, Warrenton, OR, USA) running at a flow rate of approximately 140 dm^3 min^{-1} for two months. EcoPOD® composting vessels were filled with approximately 100 m^3 of feedstock materials. The composting study conformed to a randomised, fully replicated split pod design within the EcoPOD®s. Radio-linked temperature probes (Tinytag, Gemini Data Loggers UK Ltd., Chichester, Portsmouth) were inserted into each EcoPOD® section. Each 1 m probe recorded temperature both 10 cm below the surface, and at the centre (0.75 m) of the EcoPOD®. Temperature data were logged either at one hour or one minute intervals and displayed by means of a computer housed on-site within a portable office. Composting proceeded for 86 d with no aeration during the final month (maturation phase). Composts were sampled at 1, 4, 14, 32, 56 and 86 days after the start of composting by retrieving material from a depth of 10 – 20 cm into the core and sealing the pod afterwards.

Table 1. Compost feedstock composition and selected properties. Data are mean, n = 6. Standard errors (not shown) were < 5% for %C and pH and < 10% for %N and electrical conductivity (EC).

Composition	% C	% N	C:N	pH (H_2O)	EC mS cm^{-1}
GW: 100% green waste	19.5	1.12	17.5	8.4	1.21
GW+PP: 70% GW + 30% paper fibre (PP)	18.6	0.85	23.0	7.6	1.39
GW+BS: 80% GW + 20% biosolids (BS)	23.3	1.48	15.8	7.2	1.89
PP+BS: 40% PP + 60% BS	23.2	0.9	25.9	7.2	1.36
GW+PP+BS: 45% GW + 40% PP + 15% BS	21.8	0.85	25.6	7.5	0.59
GW+SF+BS: 45% GW + 40% slate fines + 15% BS	8.6	0.51	17.0	7.2	1.41

2.2 Pathogen enumeration

Enumeration of human pathogens was conducted on frozen compost samples, within four weeks of sampling. *Escherichia coli* and *Salmonella* sp. were chosen as indicators of human pathogens, in accordance with PAS 100 specification for composted materials (BSI, 2005). *E. coli* and *Salmonella* were enumerated by a drop plate method, onto Salmonella Chromogenic Medium (Oxoid Ltd., Hants, UK), which is a selective and differential agar base that allows the identification of *Salmonella* sp. from other organisms in the *Enterobacteriaceae*. The selectivity of the medium was further enhanced by the addition of Salmonella Selective Supplement (Oxoid Ltd., Hants, UK) which contains novobiocin and cefsulodin to inhibit growth of *Proteus* and Pseudomonads, respectively.

Five grams of compost were shaken in 40 cm^3 of buffer (Quarter Strength Ringers; Oxoid Ltd., Hants, UK) for 30 minutes and a dilution series prepared in the buffer. Three replicate suspensions were prepared and plated for each compost sample. Each agar plate was divided into four segments using a marker pen on the base and a 10 µl aliquot from each of four dilution tubes placed into one of each segment. Inverted plates were incubated at 37 ^oC for 24 h then counted and incubated for a further 24 h to confirm the smallest colonies. *Salmonella* sp. were identified by characteristic magenta coloured colonies with a raised, smooth morphology and *E. coli* were characterised as blue colonies with a raised, smooth morphology as specified by Oxoid Ltd. Both positive and negative controls were set-up using pure cultures of *E. coli* ATCC®, *Salmonella poona* NCTC and *Pseudomonas aeruginosa* ATCC®, and incubated simultaneously for quality control. The Salmonella Chromogenic Medium method was compared with the PAS 100 specified methods (BS EN 12824 for *Salmonella*; BS ISO 11866-3 for *E. coli*) on selected samples taken at 86 days.

3. RESULTS AND DISCUSSION

3.1 Temperature profiles during composting

The addition of mineral slate fines to green waste and biosolids, as a replacement for paper fibre, resulted in a significantly lower temperature maximum (63 ^oC *vs.* 83 ^oC; Figure 1a) and also increased the time taken to attain the maximum temperature by six days in one of the two replicated pods. In this respect, the compost would not meet PAS 100 critical limits (2 days at >60 ^oC or 1 hour at >70 ^oC; BSI, 2002: or 7 days at >65 ^oC; BSI, 2005). The green waste, biosolids and paper fibre mix did however meet these critical limits in both pods (Figure 1a). The addition of 40% by DW slate fines could be considered a high percentage; indeed, in a similar

a) b)

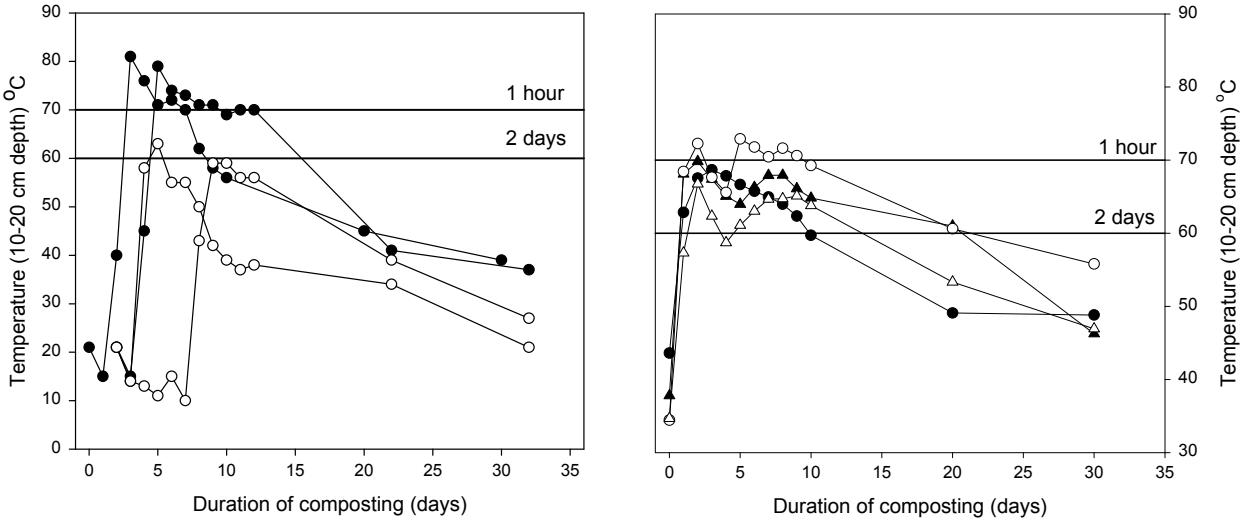

Figure 1. Temperature profiles during the thermophilic phase of composting: a) effect of mineral slate fines addition: green waste, biosolids and paper fibre mix (closed circles); green waste, biosolids and slate fines mix (open circles); b) effect of paper fibre or biosolids addition: green waste (closed circles); green waste and paper fibre mix (closed triangles); green waste and biosolids mix (open circles); paper fibre and biosolids mix (open triangles). Data are from individual pods in Figure 1a) and from the mean of four replicate pods in Figure 1b). The PAS 100 critical temperature limits for 1 hour at 70 ^{o}C or 2 days at 60 ^{o}C are shown for reference.

study by Sikora (2004) only a calculated 0.5 % (wet weight basis) was used. However, the 40% addition in our experiment was calculated as that required to dilute nutrients, in particular phosphate-P, necessary for the restoration of *Calluna vulgaris* heathland. The addition of paper fibre to green waste significantly increased the duration of the thermophilic phase above 60 ^{o}C from 10 to 18 days (Figure 1b) which is attributable to the paper fibre containing large amounts of readily biodegradable C which fuelled microbial activity. The addition of biosolids to green waste had exactly the same effect as paper fibre. The proportion of biosolids used in a mix affected both the maximum temperature attained and the duration of temperature above 60 ^{o}C, as illustrated in the contrast between the green waste and biosolids mix (80:20) and the paper fibre and biosolids mix (40:60) (Figure 1b), though both met PAS 100 critical limits. Green waste produced a more open structured mix which is likely to have enhanced aerobic microbial decomposition.

3.2 Human pathogen numbers during composting

Neither the temperature nor dilution effects of adding mineral slate fines to the feedstock mix had a consistent effect on pathogen CFUs (Figure 2). The large difference in both *E. coli* and *Salmonella* sp. CFUs between the replicate pods in the mix containing slate fines at 4 days of composting was explained by the lag in one of the replicates attaining the thermophilic phase (Figure 1a) but the difference in CFUs was reduced and also reversed (Figure 2) following temperature rise. This suggests that not only the temperature maximum but also the length of time during which temperatures above 45 ^{o}C (considered the lower end of the thermophilic temperature range) were maintained are important for pathogen kill. After 86 days in-vessel, CFUs in both compost mixes exceeded the PAS 100 critical limits of 1000 CFU g^{-1} for *E. coli* and absence in 25 g^{-1} for *Salmonella* sp.. Pathogen CFUs in composts followed similar trends

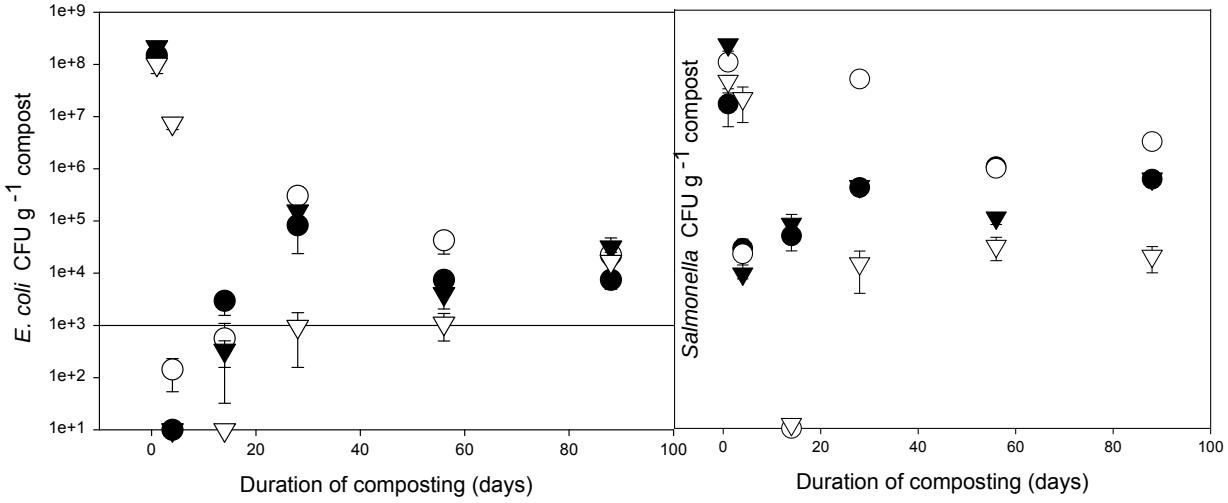

Figure 2. Effect of mineral slate fines addition on colony forming units (CFU) of human pathogen indicator species (*E. coli* and *Salmonella* sp.) during composting: green waste, biosolids and paper fibre mix (circles); green waste, biosolids and slate fines mix (triangles). Data are shown separately from the two individual pods (one open, the other closed symbols). The y-axis scale is the same on both panels.

over time regardless of feedstock composition. Following the onset of the thermophilic phase, there was a significant decline (at least 10^3) in CFUs of both pathogen species in all composts and *Salmonella* sp. were usually more prevalent than *E. coli* throughout composting (Figure 3). After the thermophilic phase (ca. 30 days), pathogen numbers quickly recovered but after three months the CFUs had subsided, again, to varying extents. No compost met the PAS 100 standard for *Salmonella* sp. whilst only green waste compost and paper fibre plus biosolids compost failed to meet the critical limit for *E. coli*. Overall, composted green waste contained the highest *Salmonella* sp. CFUs and the second highest *E. coli* per gram. The addition of biosolids to green

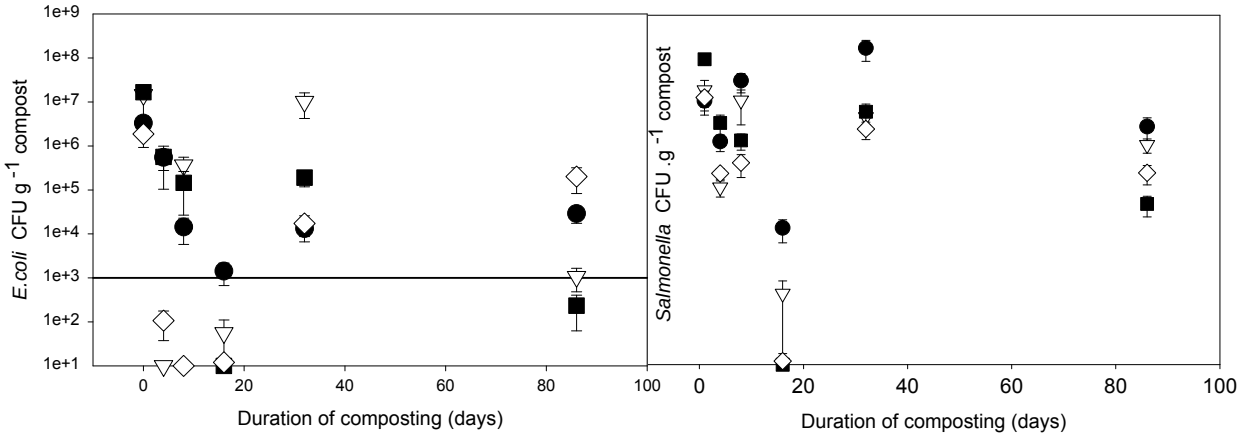

Figure 3. Effect of biosolids addition on colony forming units of human pathogen indicator species (*E. coli* and *Salmonella* sp.) during composting: green waste (closed circle); green waste and paper fibre mix (open triangle); green waste and biosolids mix (closed square); paper fibre and biosolids mix (open diamond). Data are mean ± SE, n = 4. The y-axis scale is the same on both panels.

waste feedstock did not increase the human pathogen loading and actually reduced both *Salmonella* sp. and *E. coli* CFUs (Figure 3). The Salmonella Chromogenic Medium (SCM) method gave significantly higher *Salmonella* sp. CFUs than the PAS 100 specified method (BS EN 12824), with the latter finding *Salmonella* sp. absent in all compost mixes. The SCM method gave slightly higher *E. coli* CFUs than the PAS 100 specified method (BS ISO 11866-3) but the ranking with compost type was similar, with green waste failing (250,000 CFUs g^{-1}) the critical limit as in the SCM method, whilst the mixes of green waste and paper fibre, green waste and biosolids, paper fibre and biosolids all passed (data not shown). One likely explanation for the higher human pathogen content in 100% green waste compost, compared with green waste composted with paper fibre or biosolids, was the shorter duration over which the temperature of the green waste compost was maintained at >60 ^{o}C.

4. CONCLUSIONS

This study has increased our knowledge on how composting different feedstock components and proportions affect indigenous human pathogen occurrence. Tertiary treated digested sewage cake (biosolids) can be included as a component of compost without prejudicing the capacity of the mix to reach the requisite thermophilic operating temperature and without adding to the human pathogen load in compost, compared with the composted green waste. Green waste is a variable product and more work is needed to determine how pathogen content may vary with the product. The substantial addition of mineral slate fines to organic feedstocks lowered the temperature maximum and in one instance delayed the onset of the thermophilic phase; however, there was no difference in the pathogen load after three months of composting, compared with feedstocks containing only organic wastes. All composts containing biosolids met the critical limits for human pathogen content when tested using PAS 100 specified methods. The Salmonella Chromogenic Medium method was more sensitive for *Salmonella* sp. than the industry standard.

ACKNOWLEDGEMENTS

This work was funded by the European Commission *Life*-Environment programme.

REFERENCES

British Standards Institution (2005) and (2002) PAS 100, London, UK.

O'Brien , T.A., Barker, A.V. and Campe, J. (1999) Container production of tomato with food by-products compost and mineral fines. *Journal of Plant Nutrition*, **22**, 445-457.

Qiao L. and Ho, G. (1997) The effects of clay amendment on composting of digested sludge. *Water Research* **31**, 1056-1064.

Pietronave, S., Fracchia, L., Rinaldi, M. and Martinotti, M.G. (2004) Influence of biotic and abiotic factors on human pathogens in a finished compost. *Water Research* **38**, 1963-1970.

Sidhu, J., Gibbs, R.A., Ho, G.E. and Unkovich, I. (2001) The role of indigenous microorganisms in suppression of Salmonella regrowth in composted biosolids. *Water Research* **35**, 913-920.

Sikora, L.J. (2004) Effects of basaltic mineral fines on composting. *Waste Management* **24**, 139-142.

Zaleski, K.J., Josephson, K.L., Gerba, C.P. and Pepper, I.L. (2005) Potential regrowth and recolonisation of salmonellae and indicators in biosolids and biosolid-amended soil. *Applied and Environmental Microbiology* **71**(7), 3701-3708.