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Micro-Organisms of Human Health Importance in Growing Media

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Abstract

During the last decade, pressure from environmental lobbies to restrict the use of peat in the UK has led to an increase in the use of alternative materials such as timber wastes, green composts and composted bark. Epidemiological studies conducted in Australia in the late 1980s and early 1990s demonstrated a link between illness among elderly gardeners and the use of growing media based on composted bark, although the species from which bark is derived are very different in Australia compared to those in Western Europe. Pathological effects were attributed to elevated levels of human pathogens including species of *Legionella*, notably *L. longbeachae.* The legionella bacteria have also been detected in growing media in California, New Zealand and Japan.

In view of the detection of micro-organisms such as *Legionella* spp. noted above, media from the UK have been investigated for the presence and levels of human pathogens. The incidence and survival of micro-organisms that may cause problems to public health has formed part of studies into microbial activity in growing media at Nottingham Trent University from 1995 to the present. Investigations have included studies on *Escherichia coli*, *Salmonella typhimurium* and *Legionella* spp. in a range of peat-based and non-peat based media.

The presence of *Legionella* spp. has not been conclusively demonstrated in any media examined over the period of study. Coliforms have often been isolated from media, but at levels that constitute a very low risk to public health.

These organisms, and another pathogen, *Salmonella typhimurium*, were able to survive for a month or more in heat-sterilised, inoculated media. Patterns of survival differed in inoculated unsterilised media: in most cases populations of the bacteria declined. This decline was far more marked in composted materials than in peat-based media. The antagonistic properties of some composted materials to plant pathogens are well known, and it appears that this antagonism may also extend to micro-organisms that have the capacity to cause disease in human beings.

INTRODUCTION

All organic growing media contain micro-organisms. The species and number of these depends on several factors including the constituents of media, their associated moisture and nutrient content, pH, C:N ratio and types of carbon. Raw peat has a low microbial content, linked to its low pH and high lignin content (Kavanagh and Herlihy, 1975). Development of microbial numbers and activity in growing media containing peat is linked to liming of the substrate, and especially when peat is combined with other organic materials. The low microbial content of peat media extends to micro-organisms that have the ability to cause disease in human beings: human pathogens have rarely been detected in high concentrations in peat-based growing media.

Microbial activity is far more pronounced in many other organic materials used in the manufacture of growing media. This is particularly true of composted materials since the composting process itself is due to the degradative action of micro-organisms. Many studies (Dickinson and Carlile, 2004; Dickinson, 1995) have shown much higher levels of microbial activity in growing media based on composted materials. This microbial activity may in some circumstances be beneficial (Raviv, 2008) or detrimental in the case

of micro-organisms that may cause nitrogen immobilisation (Carlile, 1991) or those that attack plants.

The demonstration by Steele and his co-workers in Australia (Steele et al., 1990 a, b; Hughes and Steele, 1994) that composted bark, largely based on *Pinus radiata*, is a source of *Legionella* infections prompted safety concerns about the use of composted materials as growing media. *Legionella longbeachae* was the principal organism of concern, with infections by this species outnumbering infections by *L. pneumophila.* Both of these organisms may cause pneumonia-like symptoms. Indeed, growing media are now included in the risk assessment for pneumonia within Australia. Steel, Moore and Sangster (1990) found that 33 out of 45 samples of commercial potting mixes in Australia contained Legionellae. Studies of multiplication in these media did not show increases in numbers of Legionellae in the manufactured products but the bacteria were able to survive in media for 6–10 months at temperatures between 4 and 35°C.

Steel and his co-workers (Steele et al., 1990 b) examined two samples of peat-based growing media from Europe for Legionellae, and could not detect these. Studies carried out on composts in Poland also failed to discern any species of *Legionella* (Stojek and Dutkiewicz, 2002).

The pressure exerted by environmental and other lobbyists to reduce peat in growing media in the UK has led to an increase in use of alternative materials, including materials such as composted bark, composted timber wastes and latterly composted green materials derived from garden and household vegetable wastes. This paper reports some of the results obtained since 1995 as part of studies at Nottingham Trent University into microbial activity in growing media, and in particular the incidence and survival in media of micro-organisms with the potential to cause human disease.

MATERIALS AND METHODS

The media used in this study included freshly-made commercial peat-based and peat-free media, and some of the constituents of the latter. The constituents included composted samples of spruce bark and paper waste (CSBPW); composted pine bark (CPB) and composted timber wastes (CTW). Each of the materials had been composted for at least six months: further details of media and their constituents, as well as physical and chemical properties are given in Dickinson and Carlile (1995). The sterilised media used in these studies were autoclaved at 121°C for one hour.

Representative samples of media or their constituents were taken and were mixed in a 1:4 (vol:vol) ratio with one-quarter strength sterile Ringers solution. Samples were placed in an orbital shaker at 200 rpm at 20°C for 1h. Samples were then centrifuged at 2000g for 15 min.

For total viable counts, samples of supernatants were diluted in quarter-strength Ringers solution to 10^{-7} and 20 µl aliquots plated on to tryptone-soya agar. Bacteria developing on plates were counted after 24h, and data converted to colony forming units (cfu) per ml of growing medium, results being expressed as log cfu per ml of medium. This standard technique was used with different agars to isolate and quantify species of interest. For isolation of coliforms, violet-red bile agar was used; for *Salmonella typhimurium*, xylose-lactose-deoxycholate (XLD) agar was used; and for *Legionella,* a base medium – buffered charcoal yeast extract (BCYE) was employed, to which *Legionella* growth supplements were added. All reagents and media were from Oxoid Basingstoke, Hampshire UK.

Survival studies involved inoculation of media samples with selected microorganisms. Known concentrations of bacteria at around 10^{8} cfu (colony-forming units) per ml of broth culture were added aseptically to samples of media in a Category 2 microbiology laboratory. Aliquots of 0.4 ml were added to 5 ml medium in sterile Universal bottles. These were then incubated at 25°C, and sampled as described above at weekly intervals. Five replicate plates were prepared for each dilution.

RESULTS

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media samples with selected around 10^8 cfu (colony-forming samples of media in a Category 2 dded to 5 ml medium in sterile nd sampled as described above at each dilution.

RESULTS

Total viable counts showed greater numbers of bacteria in peat-free growing media, and their constituents (Fig. 1). Concentrations in composted spruce bark and paper waste, composted pine bark and the peat-free medium itself exceeded 10^8 cfu per ml of medium, with those in composted timber waste somewhat less, and the total viable count in peat-based media being three orders of magnitude lower. A similar trend was noticed with coliforms, with higher numbers in the constituents of peat-free media, and the medium itself with very low numbers in the peat medium. Coliform concentrations formed only a small part of the total bacterial count. A significant feature of this work was the failure to detect *Legionella* species in any of the growing media investigated.

Sterilised peat-free and peat-based media were able to support the growth of *E. coli, S. typhimurium* and *L. pneumophila* when these organisms were deliberately introduced into these media (Figs. 2, 3 and 6). Indeed the numbers of colony-forming units remained fairly stable over the period of incubation. No significant increases in numbers were seen during the period of incubation. However, in inoculated unsterilised media, numbers of *E. coli* and *S. typhimurium* declined substantially, although those of Legionella did not (Figs. 4, 5 and 6). Figs. 4 and 5 show that the decline in numbers of *E. coli* and *S. typhimurium* was much greater in unsterilised peat-free media than in peat-based media.

DISCUSSION

The total viable counts for bacteria in the peat-based and peat-free growing media are as expected. Peat-free media, here derived from composted materials, may be expected to contain higher numbers of micro-organisms than peat-based media, and indeed the results correspond well with the data of Dickinson and Carlile (2004), who reported microbial activity by measurement of dehydrogenase activity within the media used in these studies. This trend also extended to coliform counts with more being detected in peat-free than in peat-based media. The levels of coliforms present in both media were low, especially so in peat, and would present a very low risk to human health, especially if compared to risk assessments for coliforms in food (Forsythe, 2005). Higher levels of coliforms were detected in some of the raw materials used for the production of peat-free media, but again not at levels to cause concern to public health.

The fact that *Legionella* species have not been detected within media used in the current studies is significant, given the problems that have been reported for bark-based growing media in Australia and elsewhere (Steel et al., 1990b). The latter authors as well as Stojek and Dutkiewicz (2002), also failed to isolate *Legionella* spp. from growing media of European origin.

Survival studies indicated the ability of *E. coli, S. typhimurium* and *L. pneumophilae* to persist in sterilised peat-free and peat-based media, but no clear evidence of multiplication in media was evident. Obviously the sterilised environment is rather artificial, and indeed chemical and physical characteristics of the media may have been altered during the autoclaving process, but the study proves a useful comparison for the results in unsterilised media. Here, populations of *E. coli* and *S. typhimurium* declined. The decline was more pronounced in peat-free media than in peat-based media and it is possible that the natural microflora of the media may be exerting an antagonistic or suppressive effect on these potential human pathogens. Many studies, such as those discussed by Raviv (2008), have shown the suppressive effects of media containing composted materials on plant pathogens. It seems likely from the current studies that such antagonism may also extend to organisms capable of causing disease in human beings.

However, the decline in numbers seen with *E. coli* and *S. typhimurium* was not evident with *L. pneumophilae* and this also concurs with the work of Steele, Moore and Sangster (1990) who found that *Legionella* bacteria inoculated into growing media could survive for several months. It would appear that legionellae, which live within amoebae, may be more resilient than free-living bacteria in growing media.

The studies reported in this paper and elsewhere indicate that the risk to human

health from potential human pathogens in growing media of European origin is currently low. Indeed, although currently the use of green compost as a constituent of growing media is increasing in the UK, studies on survival of *E. coli, S. typhimurium* and *S. enteritidis* during composting of green wastes showed that these bacteria did not survive for much longer than 30 min at 55°C (Noble et al., 2004), and may thus present a low risk to users of media containing green compost. However, it might be useful to confirm this apparent low risk through a survey of potential human pathogens in growing media containing green compost.

ACKNOWLEDGEMENTS

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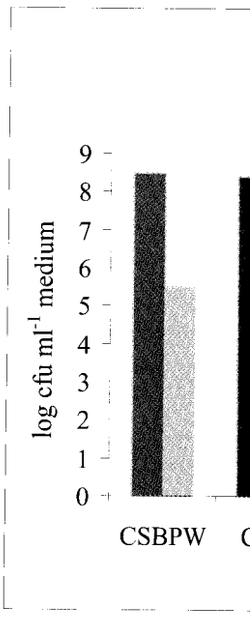
Figures



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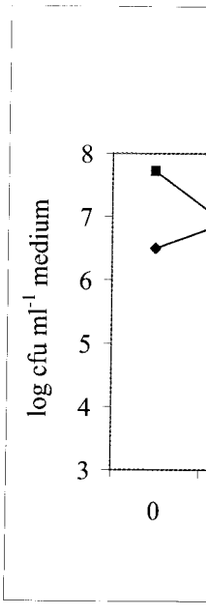

Fig. 2. Survival of *Escheri*
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Figures

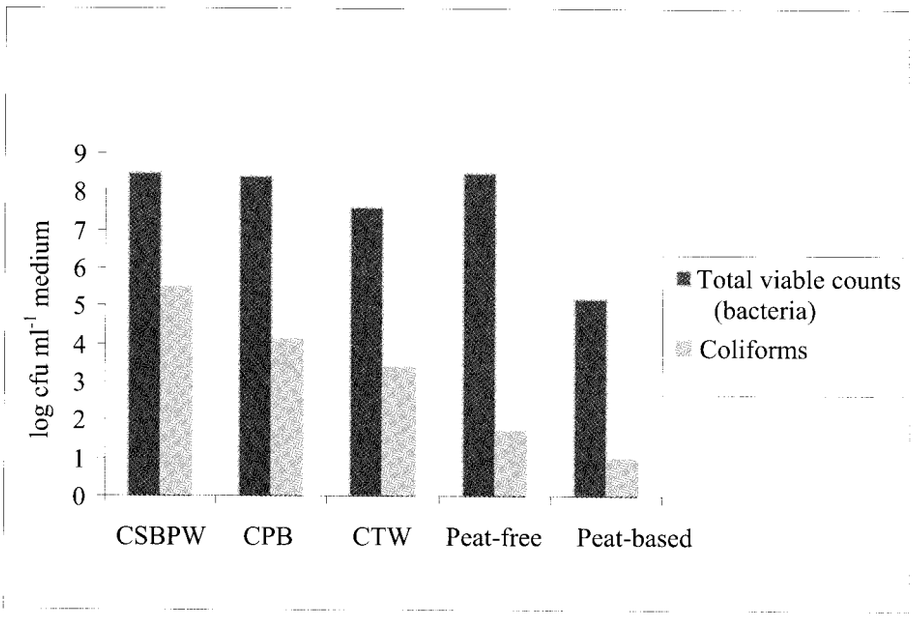


Fig. 1. Total viable counts (bacteria) and coliforms in growing media and their constituents. Details of media are given in the text.

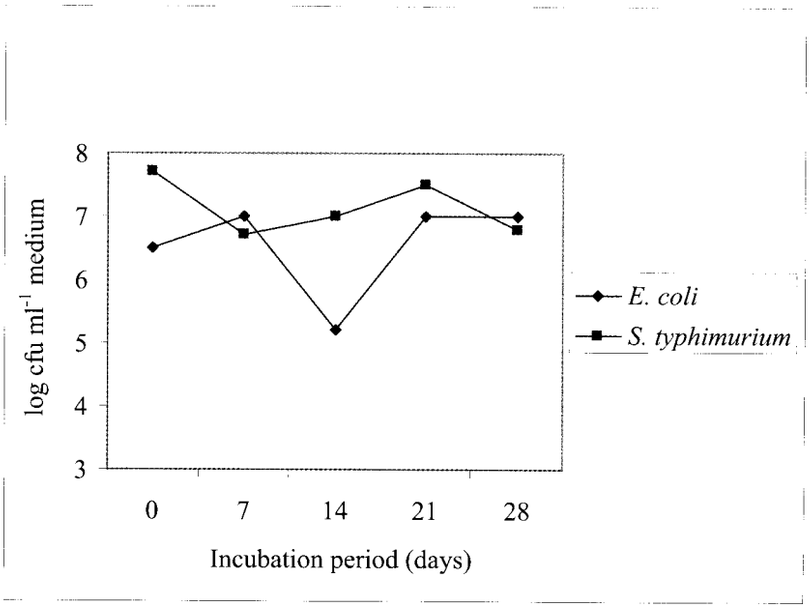


Fig. 2. Survival of *Escherichia coli* and *Salmonella typhimurium* in a sterilised peat-based medium.

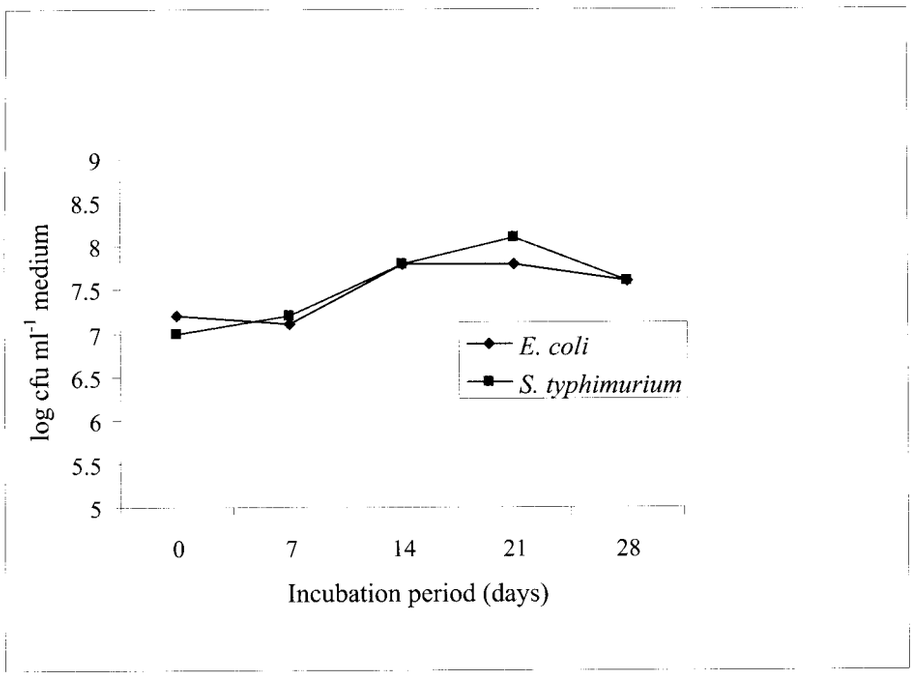


Fig. 3. Survival of *Escherichia coli* and *Salmonella typhimurium* in a sterilised peat-free medium.

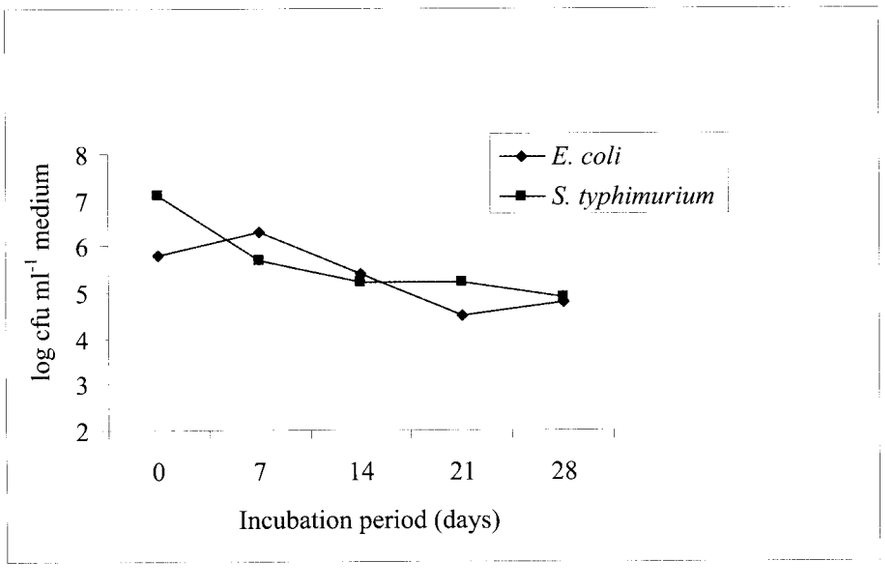


Fig. 4. Survival of *Escherichia coli* and *Salmonella typhimurium* in an unsterilised peat-based medium.

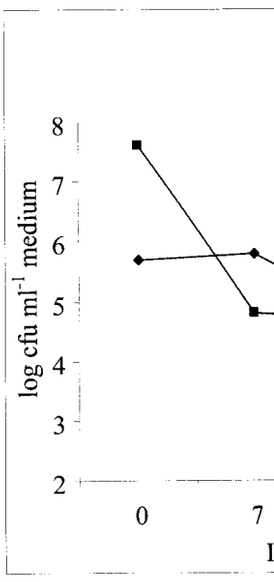
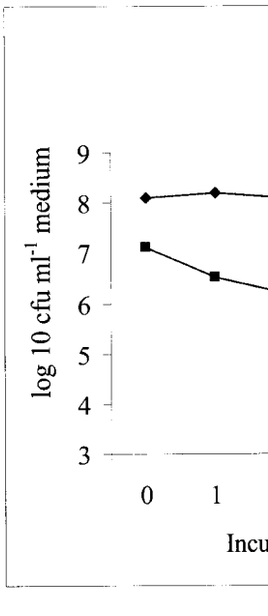


Fig. 5. Survival of *Escheric* free medium.



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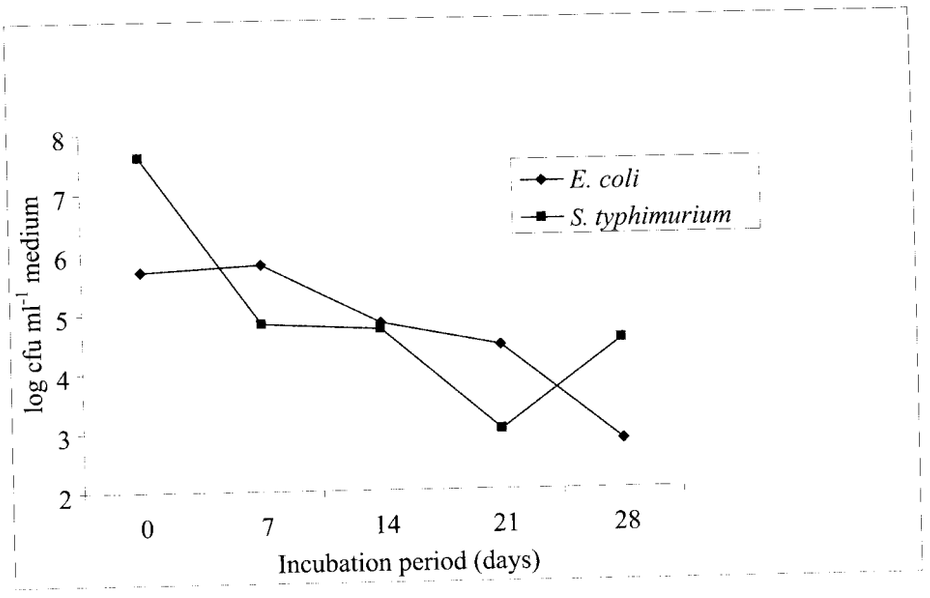

Fig. 5. Survival of *Escherichia coli* and *Salmonella typhimurium* in an unsterilised peat-free medium.

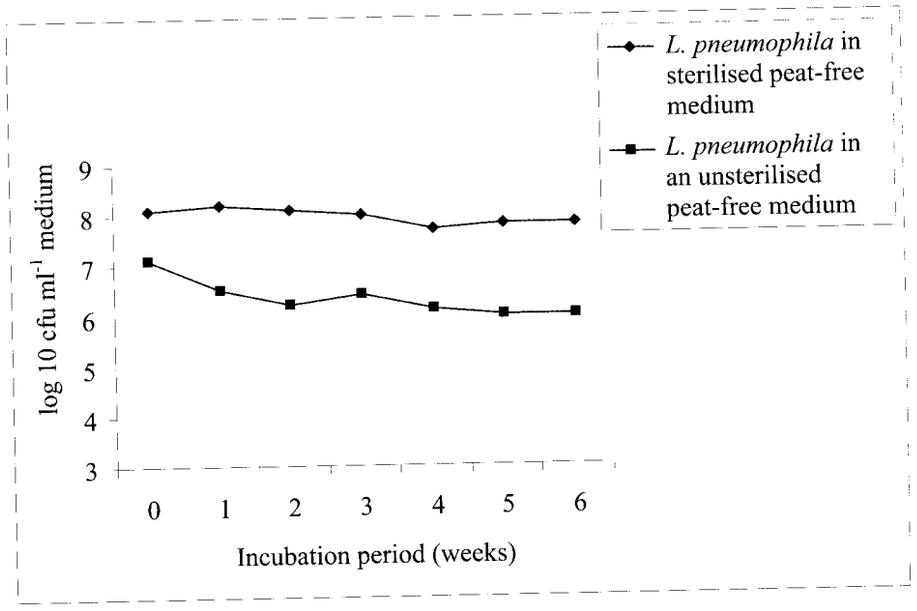

Fig. 6. Survival of *Legionella pneumophila* in sterilised and unsterilised samples of peat-free growing medium.