SALMONELLA PREVALENCE DURING SEWAGE SLUDGE TREATMENT

Tania Mondal[1], Duncan Rouch[1], Nerida Thurbon[1], Vennessa Fleming[1], Stephen R. Smith[2], Margaret Deighton[1]

1. School of Applied Sciences, RMIT University, Bundoora, Australia.
2. Department of Civil & Environmental Engineering, Imperial College London, London SW7 2AZ, UK.

ABSTRACT

This study aimed to enumerate and serotype isolates of *Salmonella* spp. recovered from biosolids. For this purpose, samples were collected from a large wastewater treatment plant, in Victoria, Australia. Mesophilic anaerobic digester (MAD) output, drying pan and stockpile samples were collected and analyzed by the membrane filtration technique. *Salmonella* spp. was recognised as black colonies on Xylose Lysine Desoxycholate agar and confirmed using biochemical and serological tests. From the examination of a large number of samples over a full year period, *Salmonella* spp. was found only in MAD samples. The serotype distribution did not match the distribution from human cases.

INTRODUCTION

Sewage treatment is a process of removing contaminants from wastewater, both industrial and domestic, using physical, chemical and biological processes. The safe disposal of treated sewage sludge (biosolids) is a global environmental concern. Application to agricultural land is generally believed to be the most economical and useful sludge disposal method (Metcalf and Eddy, 2003). Sewage sludge is composed of inorganic and organic compounds, macronutrients, a wide range of micronutrients, non-essential trace metals, organic micro pollutants and microorganisms (Kulling, 2001). Microorganisms are present in large numbers in material entering sewage treatment plant effluents. Potential pathogens commonly found in wastewater include *Salmonella* spp., *Shigella* spp. various pathotypes of *Escherichia coli, Giardia lamblia* and enteric viruses. *Taenia* spp., *Ascaris lumbricoides* and hookworm eggs may also be present in raw sewage. Although the numbers of potential pathogens are greatly reduced during the treatment of sewage sludge, biosolids originating from wastewater treatment plants could still contain human pathogens, including *Salmonella* spp. that may present a health hazard to the general public.

Salmonella spp. are among of the most important pathogens involved in human food-borne illness (Oliveira et al., 2003). The majority of human salmonellosis cases are caused by the consumption of contaminated egg, poultry, pork, beef and milk products (Geimba et al., 2004). *Salmonella* spp. cause a serious health problem in developing countries through a wide range of human diseases such as enteric fever, gastroenteritis and bacteremia (Banavandi et al., 2005). The infective dose of *Salmonella* spp. is generally in the order of 10^7 to 10^8 bacteria, but can be as low as 15 to 20 cells, depending upon age and health of the host (FDA, 2003).

Typically, the different stages for wastewater treatment include grit removal, primary sedimentation and biological treatment (activated sludge process). In metropolitan wastewater treatment plants in Victoria, Australia, these processes are followed by mesophilic anaerobic digestion of the sludge, air drying in pans and stockpiling. Biosolids are valuable nutrient sources for plants, however, before they are released for unrestricted use in agriculture, it is essential that treatment processes reduce pathogen loads to levels that do not impact on health or the environment (EPA,2004).

Previous work from our laboratory (2007) has shown that indicators (coliphage and *Escherichia coli*) drop to undetectable levels after about nine months in drying pans (Rouch et al.,2009), but, in that study no *Salmonella* spp. were detected in a snapshot of samples collected from drying pans and stockpiles. The output from anaerobic digester was also analysed for *Salmonella* spp. in that study. We wished to expand the previous study and examine a larger number of samples for the presence of *Salmonella* spp. over a full year period.

The aims of the present study are as follows:

1. To enumerate *Salmonella* spp. in a range of biosolids samples over a full year.
2. To determine the serotypes of any isolates.

This work, in analyzing the presence of *Salmonella* spp. during wastewater treatments, is planned to inform improvements in treatment processes and safe recycling of biosolids.

EXPERIMENTAL

Sample collection

Biosolids samples were collected from a large wastewater treatment plant in the Melbourne metropolitan area, Victoria, Australia. This plant uses the process of anaerobic digestion followed by air-drying in open pans and stockpiling (uncovered) for a period of at least three years. Sampling was conducted at 4 to 8 weekly intervals from March 2009 to June 2010 (total of 12 occasions). Over that period, the output from the pump of the mesophilic anaerobic digester (MAD output), two air-drying pans and two stockpiles were sampled. At each sampling, the following were collected: triplicate MAD samples, triplicate composite samples from air-drying pans and composite samples from stockpiles at 0.4-0.6 m depth.

Enumeration of *Salmonella*

Biosolids samples were stored at 4^{o}C and analyzed within 72 h for the presence of *Salmonella* spp. To count viable bacteria, biosolids were diluted 1:10 in Maximum Recovery Diluent (MRD, Oxoid) in sterile glass bottles that also contained 10 g sterile glass beads, then placed on an orbital shaker at 200 rpm for 4 min. Following a series of 10-fold dilutions in MRD, the viable counts were determined by the membrane filtration technique. Enumeration was performed on Rappaport-Vassiliadis Soya Peptone agar (RVSA), prepared by solidifying RSVB (Oxoid) with agar. Plates were incubated for 16-20 h at 41.5ºC. Filters were transferred to Xylose Lysine Desoxycholate (XLD, Oxoid) agars plates, which were incubated for 18-24 hrs at 37ºC. *Salmonella* spp. were recognised as black coloured colonies on XLD (The Microbiology of Drinking Water, 2002)

Confirmation of *Salmonella* spp.

Colonies resembling *Salmonella* spp. were subjected to the following tests: motility test, lysine decarboxylase activity, lysine deaminase activity, indole production, urea hydrolysis, api20E and 'O' antigen testing. Isolates resembling *Salmonella* spp. were sent to the Microbiological Diagnostic Unit, Public Health Laboratory, Department of Microbiology and Immunology, University of Melbourne for confirmation and serotype determination.

RESULTS AND DISCUSSION

Presence of *Salmonella* spp in drying pan and stockpile samples

Salmonella spp., at 10 CFU/g DS, were isolated from a sample collected in March 2010 from one pan aged about 7.5 months. The isolate was identified as the rare serotype, *Salmonella* Apapa No *Salmonella* spp. were isolated from any stockpile sample over the period of the study (limit of detection 10 *Salmonella* spp./g dry solids).

Presence of *Salmonella* spp. in MAD samples

The number of *Salmonella* spp. isolated from MAD samples fluctuated throughout the period of the study. The highest number of *Salmonella* spp., 9.43 x 10^2 CFU/g DS, occurred in the spring season (September). No *Salmonella* spp. were found in the autumn period (March), but low numbers were isolated over the summer and winter months (Figure 1). The greatest variety of serotypes was found in mid winter (July). The fluctuation in the number of *Salmonella* spp. in MAD samples was probably due to seasonal variation in the numbers of *Salmonella* spp. entering the treatment plant.

Serotyping of the isolates from MAD samples revealed a range of serotypes (Table 1). Only three isolates of 19 (16%) were serotype Typhimurium, compared with 45% from human infections in Australia in 2008 (Communicable Diseases Intelligence Annual Report 2008). In an analysis of the same MAD samples for *E. coli*, it was shown that number of *E. coli* was generally in the range of 10^5 to 10^6 CFU/g DS (Figure 2), but the numbers did not follow the same pattern of seasonal fluctuation as was the case for *Salmonella* spp.

CONCLUSION

Salmonella spp. were never isolated from stockpile samples and only isolated once in low numbers from a pan sample, ~7.5 months since completion of filling. This was thought to be due to post treatment contamination in treatment plant. These results indicate that the treatment of sewage sludge at this plant is effective in removing *Salmonella* spp. The results also suggest that there is seasonal variation in the presence of *Salmonella* spp. in material entering this treatment plant. The serotype distribution differed from the distribution among human cases, suggesting either the presence of animal serotypes in wastewater, human excretion of serotypes of low virulence or differences in ability of serotypes to survive in wastewater.

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AUTHOR'S CONTRIBUTION

TM performed membrane filtration and wrote the first draft of paper, NT and VF identified *Salmonella* spp. and edited the paper, MD and DR supervised TM and edited the paper.

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Table 1: Serotypes of Salmonella isolates from MAD samples and pan samples

Date of sampling	Month number*	MAD	PAN
20/07/2009	7	*Salmonella* Newport *Salmonella* Senftenberg *Salmonella* Abony *Salmonella* Virchow 8 *Salmonella* Abony *Salmonella* Aberdeen *Salmonella* Typhimurium RDNC *Salmonella* Enteritidis RDNC *Salmonella* Virchow 34a *Salmonella* Birkenhead	
05/10/2009	10	*Salmonella* Adelaide	
09/11/2009	11	*Salmonella* Typhimurium 170	
14/12/2009	12	*Salmonella* Birkenhead, *Salmonella* subsp. 1 ser 6, 8:eh.	
22/3/2010	15	*Salmonella* Albany	*Salmonella* Apapa
07/6/2010	18	*Salmonella* Typhimurium 135 *Salmonella* Rissen *Salmonella* subsp 1 ser rough:z:1,6 *Salmonella* Infantis	

MAD= Mesophilic Anaerobic Digestion

* Month 1 = January 2009

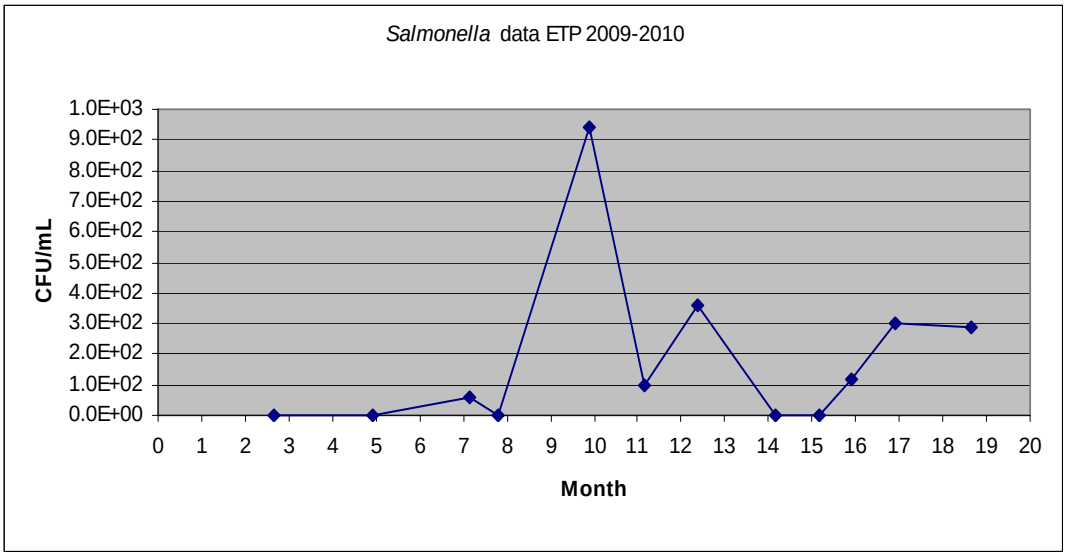

Figure1: Presence of Salmonella spp. in Anaerobic Digester sample

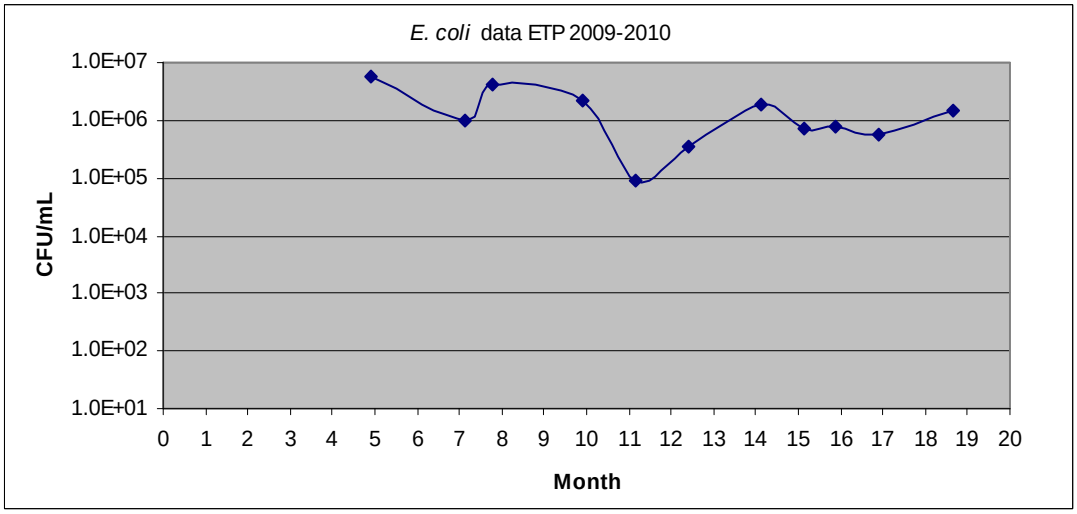

Figure 2: Presence of Escherichia coli in Anaerobic Digester sample

* Month 1 = January 2009