DIE-OFF OF PATHOGENS AND ASSESSMENT OF RISKS FOLLOWING BIOSOLIDS APPLICATION IN PINE PLANTATIONS

THIS THESIS IS PRESENTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY OF MURDOCH UNIVERSITY

By

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I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institute.

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Papers and Presentations from this research

Book Chapters

1. Levitan, J., Nair, J., Ho, G., Penney, N. and Dumbrell, I. (2009). The potential health impacts and risks of utilising biosolids in a timber plantation**.** In *Technologies and Management for Sustainable Biosystems*, ed. Jaya Nair, Dr Christine Furedy, Chanakya Hoysala and Horst Doelle, 183-193. Nova Science Publishers

Journal Paper

1. Keeratirathawat, S., Nair, J. & Levitan, J. C. (in press). The antibacterial efficacy of pine oils on pathogens commonly found in biosolids applied to pine plantations. *International Journal of Environment and Waste Management*

Reviewed Conference Presentations

1. Levitan, J., Nair, J., Ho, G., Penney, N. and Dumbrell, I. (2008). *Die-off of pathogens and assessment of risks following biosolids application in pine forests.* AWA Biosolids Specialty IV Conference June 11th and 12th , 2008

2. Levitan, J., Nair, J. and Ho, G. (2009). *Biosolids and Pine Plantations – a carbon sink?*. Australian Climate Change Adaptation Research Network for Settlement and Infrastructure: 1st National Forum and Workshop for Postgraduate Researchers May 14th and 15th, 2009

3. Levitan, J., Nair, J., Ho, G., Penney, N. and Dumbrell, I. (2010). *Die-off of pathogens and assessment of risks following biosolids application in pine plantations.* AWA Biosolids Specialty V Conference June 2nd and 4th , 2010

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Abstract

In Western Australia over 80,000 tonnes per annum of biosolids are produced from the treatment of wastewater. The biosolids is being disposed in a variety of land-application processes as a soil amendment. However the presence of pathogenic organisms in the biosolids can result in a human health risk if individuals are exposed to the biosolids.

Application of these biosolids to pine plantations is a practice increasing worldwide due to the benefits of biosolids as a soil amendment. The regulations allow biosolids that may contain pathogenic organisms to be land-applied. In the case of pine plantations, the general public is not physically excluded from the area resulting in a situation arising whereby exposure of the biosolids to members of the general public can occur. This potential exposure results in a human health risk becoming present.

Instances of pathogen survival post-application of biosolids have been observed indicating that the risk to human health is certainly present. The main aim of this study was to assess the risk to human health from the pathogens found in biosolids land applied to the Myalup pine plantation in Western Australia. To achieve this aim the ability of the pathogens to survive post-application of biosolids was monitored and any instances of increased pathogen activity beyond one year post-application were established. The airborne pathogen risks through the formation of biosolids dust and the occurrence of plantation burns that may cause pathogens to become airborne in the smoke of a burn,

were investigated. *E. coli*, *Salmonella* spp. and *Clostridium perfringens* were the pathogen indicators selected for this study.

The results show that the pathogen levels in the land-applied biosolids pose a risk to members of the public and plantation workers via direct exposure for the first 3 months post-application. After 2 months *E. coli* was observed to have undergone significant die-off, *Salmonella* spp. was observed to be at undetectable levels after 3 months. However 11 months post-application in the following winter season, *Salmonella* spp. returned to high levels that would pose a human health risk. *Clostridium perfringens* remained at high levels throughout the 1 year monitoring period. During this initial monitoring period, a relationship between moisture content and pathogen populations was observed. *Salmonella* spp. indicated the strongest relationship with a return in its population from undetectable levels to high levels when an increase in moisture content was observed.

A mechanism that facilitates this increase in the populations of the pathogens has been referred to in the literature, but not described. An observation during the initial one-year intensive monitoring showed that as the moisture content of the biosolids reduced, the biosolids dried to form clumps. After testing, it was determined that these clumps were enabling weakened populations of the pathogens to survive the natural environmental factors that usually cause their die-off. After a rainfall event, the moisture content of the clumps increases allowing the weakened populations to re-colonise the biosolids. This clumping phenomenon was only observed to occur within the first year post-application.

Beyond one year post-application, instances of elevated pathogen activity were observed up to 1.5 years post-application. After this period of time had elapsed, all indicator pathogens were observed to be at low levels that are highly unlikely to cause a human health risk. The indication being that no health risk from biosolids pathogens exists beyond 1.5 years post-application.

An alternative exposure route identified is the airborne route through inhalation of the pathogens. Plantation burns are expected to occur within the life-cycle of a plantation. The possibility that pathogens could become airborne during a burn and be transported along with the smoke was investigated. No significant instances of pathogens being transported in the smoke were observed.

The formation of biosolids dust was an issue raised, and more specifically whether pathogens could become airborne with the dust. The ability of the pathogens to survive in biosolids dust was examined. The pathogens indigenous to the biosolids failed to survive to the point where moisture loss in the biosolids was significant enough to allow dust formation to occur. Laboratory cultures of the indicator pathogens were then inoculated into the samples and their ability to survive in biosolids dust conditions was observed. Significant die-off was observed within 3 days and after 10 days the pathogen levels were low. *Clostridium perfringens* was the exception as this pathogen was observed to survive within biosolids dust.

The combined results of this thesis and the literature indicate that the human health risks relating to airborne exposure are limited to an occupational risk only. The pathogen risks are only associated with the application of the

biosolids to the land and not with the formation of biosolids dust or with the smoke of plantation burn over land applied with biosolids.

The pathogen risk from direct exposure is present for all individuals who come into contact with the biosolids during the initial 3 months post-application and, due to pathogen re-growth or re-colonisation, the following winter season when moisture levels are increased. However this direct exposure risk is only present for 1.5 years-post-application after which the pathogens were observed to be of no health risk. In general the human health risk from the land-application of biosolids is low.

Additional research work needs to be conducted in relation to the clumping phenomenon. A full understanding of the process and why the biosolids forms clumps as it dries will aid in the development of strategies to prevent this action from occurring. Removing this action will greatly reduce the risk of pathogen re-growth and/or re-colonisation.

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List of Abbreviations

CFU	Colony Forming Units
DW	Dry Weight
FW	Fresh Weight
MPN	Most Probable Number
PFU	Plaque Forming Units
PPE	Personal Protective Equipment
QMRA	Quantitative Microbial Risk Assessment
TMTC	Too Many To Count
WA	Western Australia