

Research Perspective of the Land Application of Biosolids

| START | END | PRESENTER | ACTIVITY |
|--------------|--------------|-------------------|--|
| 12:30 | 12:40 | Mary Papachristos | Opening and Welcome |
| 12:40 | 12:55 | Nancy Penney | Summary of the Water Corporations current and proposed research program. |
| 12:55 | 13:25 | Brad Clark | Persistent Organic Pollutants in Australian Biosolids |
| 13:25 | 14:10 | Stephen Smith | The significance of organic contaminants in biosolids recycled to agricultural land |
| 14:10 | 14:40 | Karen Schwarz | Inactivation of Pathogenic Contaminants in Land-Applied Biosolids: Research Progress |
| 14:40 | 15:00 | | Afternoon tea |
| 15:00 | 15:40 | Stephen Smith | Pathogen survival in biosolids-amended agricultural soil |
| 15:40 | 16:00 | Ian Dadour | Biosolids: Fly Dreaming, People Nightmare |
| 16:00 | 16:20 | David Collins | Nutrient and pH value of lime amended biosolids used for agriculture |
| 16:20 | 16:40 | Deb Pritchard | Phosphorus bioavailability from land applied biosolids in south-western Australia |
| 16:40 | 16:50 | Kevin Ho | Differentiation between biosolids from human or animal origin by DNA fingerprinting: a pilot study |
| 16:50 | 17:00 | Mary Papachristos | Close |

KEYNOTE SPEAKER SUMMARY

Dr Stephen R Smith

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Dr Stephen R. Smith is a Reader in Environmental Biochemistry in the Department of Civil and Environmental Engineering at Imperial College London. He has almost 20 years of experience investigating the treatment and agronomic properties of sludges and organic wastes recycled to land and their environmental impact, including nutrients, potentially toxic elements, organic contaminants and pathogens. Prior to joining Imperial College in 1997 he was a senior soil scientist at WRc (Water Research Centre) responsible for research programmes on the agricultural recycling of sewage sludge. He has a broad base of experience of the agronomic behaviour of organic wastes in relation to nutrient value and was the Principal Investigator (PI) of a major programme of work funded by EPSRC and in collaboration with the Environment Agency and Water Industry on agricultural utilisation of biosolids products. Currently he is PI on a follow up project supported by Defra and the UK Water Utilities on the influence of soil ecological interactions on pathogen survival in sludge-treated agricultural soil. Other work is quantifying the agronomic benefit of industrial and treated biowastes in a programme of field experimentation. He was co-investigator on a programme of research for the Food Standards Agency on the environmental impact of nonylphenol in sludge-treated agricultural soil.

Dr Smith has worked overseas on the problems of sewage sludge disposal and recycling on farmland in warm climates, notably in Egypt where he was a consultant to WRc on the Cairo Sludge Disposal Study funded by the European Investment Bank and sponsored by the Cairo Wastewater Organisation. He was recently the coordinator of a British Council Link Project with Alexandria University, Egypt, on alleviating environmental impacts of industrial and urban wastewater and biosolids in the Middle East and North African countries. He is PI for a study on composting putrescible household waste in the UK, recently chairing a national meeting on the subject. Another ongoing project, based in Cyprus, is developing biodrying technologies for production of high calorific value and consistent auxiliary fuels from biodegradable municipal and other wastes for use in the cement industry and other industrial applications. He has provided expert witness evidence on behalf of the Environment Agency and local authorities in the UK in a number of criminal proceedings on hazardous and biodegradable waste management practices on land. He is co-author of a number of major scientific reviews for UK Government and the European Commission on contaminant behaviour and impacts on the environment.

Dr Smith has written over 150 scientific papers, reports and publications for Government and Industry on recycling biosolids in agriculture including a standard reference text book on the subject published by CABI International. He lectures to undergraduate and postgraduate courses on environmental microbiology, and solid waste and sludge treatment and management. Dr Smith is a member of the Wastewater Management Panel of the Chartered Institution of Water and Environmental Management (CIWEM), the

Chartered Institution of Wastes Management (CIWM), the Expert Group on Risk Assessment of Sewage Sludge of the European Branch of the International Life Sciences Institute (ILSI), the Expert Advisory Group of the Sustainable Organic Resources Partnership, and he is registered as a Chartered Environmentalist (CEnv).



Dr Smith will deliver two presentations entitled, “The significance of organic contaminants in biosolids recycled to agricultural land” and “Pathogen survival in biosolids-amended agricultural soil.”

WATER CORPORATIONS BIOSOLIDS RESEARCH

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The Water Corporations three main metropolitan wastewater treatment plants produced approximately 86 000 wet tonne of lime amended biosolids and biosolids cake in 2005/2006, of which 57 000 wet tonne (or 73%) was applied to agricultural land as fertiliser for the production of broad acre crops.

The current biosolids management program commenced in 1997 and was founded on over 30 years of international and national research. The Water Corporation understands that although this valuable research provided the foundation for the beneficial use of biosolids in Western Australia, it noted that it may not always be comparable for Western Australian conditions.

The water corporation has invested more than \$2.5M in biosolids research over the past 8 years all of which has been conducted independently by academic institutions and research organisations.

Much of the research done for the Water Corporation is watched closely and sought after by other water utilities both nationally and internationally.

A composite list of biosolids Research and Development project to date are summarised below.

- Management of fly breeding in biosolids – several projects
- Phosphorous availability of land applied biosolids
- Assessment of nutrient and pH value of lime amended biosolids – several projects
- The fate of human pathogens following the land application of biosolids in agriculture
- Pathogen presence on grains at harvest following the application of biosolids
- Development of a method for identifying viable Helminths ova
- Development of DNA fingerprinting for biosolids
- Assessment of the suitability and benefits of biosolids applied to pine plantations including groundwater and soil monitoring - several projects
- Die off and risks assessment for pathogens following (new and aged) biosolids application to pine plantation
- Risk assessment for organic contaminants in biosolids
- Impact of metals and nutrients on sustainable agriculture following land applied biosolids
- Benefits and issues of pelletised biosolids
- Investigation to determine a suitable liner for biosolids stockpiles
- Value and benefits of alum sludges and biosolids

Internal Investigations

- Pathogen survival in soils following an application of biosolids
- Establish a baseline for dioxin concentrations in Perth's biosolids
- Establish a baseline for nonyl phenols concentrations in Perth's biosolids

PERSISTENT ORGANIC POLLUTANTS IN AUSTRALIAN BIOSOLIDS

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Introduction

Research into organic chemical contamination of biosolids has been identified as a high priority for the Australian water industry at workshops organised by the Wastewater Program of the Cooperative Research Centre for Water Quality and Treatment (2003), the National Biosolids Research Program (2003) and Biosolids Speciality Conferences (2004, 2005). Therefore a project entitled "Risk Assessment of Pathogens and Organic Chemicals in Biosolids" has been developed to explore this issue. A major aspect of the "organic chemical" research is monitoring the movement and persistence of target organic compounds over a period of time in working farms.

The term 'organic chemicals' covers a broad range of compounds – all of which need to be evaluated for their potential risk to humans, the environment, and soil quality. This category includes industrial chemicals, general household products, fire retardants, personal care products, surfactants and pharmaceuticals, amongst others. However, the focus of this research is a subset of the organic contaminants – the persistent organic pollutants (POPs). The characteristics of POPs are environmental persistence, long-range atmospheric transport, bioaccumulation, and biological activity. Therefore the compounds of interest are 'dioxins', polybrominated diphenyls (PBDEs), organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs). Most states of Australia independently regulate biosolids land application for a small number of OCPs (DDT, DDD, DDE, dieldrin, aldrin, lindane, chlordane, HCB, and heptachlor) and PCBs. No other organic chemical residues are required to be monitored.

Current Work

1. Field Trial – Monitoring of the persistence and movement of organic chemicals (PBDEs, OCPs and PCBs) in a field trial established in Moora, WA 2006. The field trial is designed with a biosolids treatment a fertilizer control. Samples are taken at three soil profile depths (0-10cm, 10 – 20cm and 20-30cm) at the time of 0, 0.5, 1, 3 and 6 months. Samples are currently being analysed as part of this work.
2. Australian 'dioxins' and PBDEs in biosolids survey – 14 samples have been collected throughout Australia, one urban and one rural from each state (including the Northern Territory). All samples will be analysed at the National Measurement Institute in Sydney for *dioxins* and PBDEs.
3. OCPs and PCB levels in freshly produced biosolids – Biosolids must be monitored for OCPs and PCBs. Therefore biosolids producers are being approached to supply this data. Currently we have data from Qld, Vic, NSW and WA biosolids. It is hoped to results will be provided by all of the major Australian biosolids producers.

Results

Dioxins

The word "*dioxins*" is used as an umbrella term to describe the 29 most toxic congeners (7 polychlorinated dibenzo-*p*-dioxins (PCDDs), 10 polychlorinated dibenzofurans (PCDFs)) and has recently been extended to cover the 12 co-planar "dioxin-like" polychlorinated

biphenyls (PCBs). PCCD/Fs are formed as unintentional by-products of industrial processes that involve chlorine such as waste incineration, chemical and pesticide manufacturing, smelting and pulp and some paper bleaching (if elemental chlorine is used in the process). Results are reported for three waste water treatment plants (WWTP) within Western Australia taken over a period of years (between 2002 and 2006). The samples were analysed at the National Measurement Institute in Sydney, Australia. A total of 20 samples were analysed for *dioxins* – 2002 (n=6), 2003 (n=6), 2005 (n=6) and 2006 (n=2).

While many countries regulate the amount of *dioxins* permissible in biosolids for land application, Australia does not (NRMCC 2004). The Victorian EPA has proposed an “investigation limit” of 50 pg TEQ/g dry weight (d.w.) for the land application of biosolids (EPA Victoria 2004). All samples had *dioxin* levels below this proposed limit, with the majority lying within the range of 4.2 to 17 pg TEQ/g d.w and generally well within the background soil *dioxin* concentration (0.05-23 pg TEQ/g). The contamination of WA biosolids with *dioxins* is low and therefore the risks of biosolids land application from *dioxins* are low.

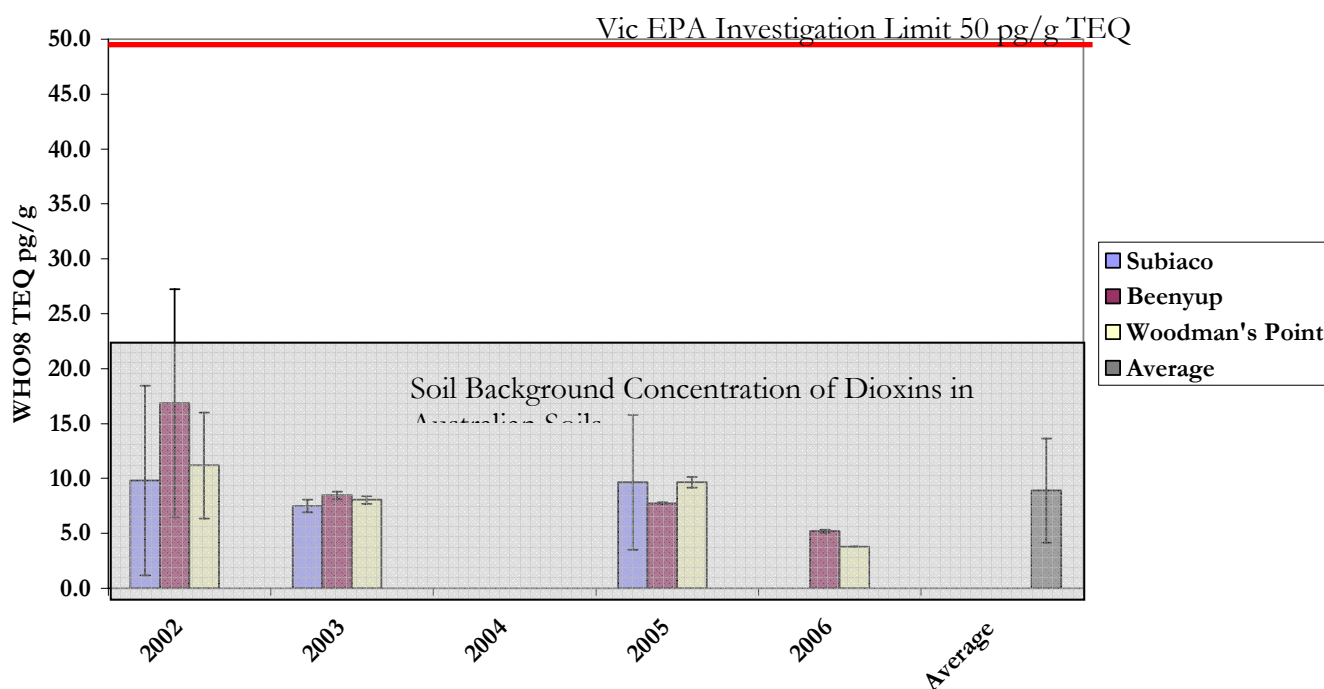


Figure 1: Concentration of *dioxins* pg TEQ/g dry weight in Western Australian biosolids collected from three Wastewater Treatment Plants. The error is expressed as one standard deviation. The shaded grey area is the reported background concentration range of *dioxins* in Australian soils (DEH 2005).

Polybrominated Diphenyls

PBDEs are a prominent member of the brominated flame retardants (BFRs) that are widely used in consumer products including textiles, polyurethane foams and polymers used in electronic equipment such as computers and home entertainment systems. PBDEs are a candidate to be listed as POPs with the United Nations Environment Program (UNEP) as they have many of the necessary characteristics for inclusion on this list.

Biosolids samples were collected from three wastewater treatment plants (WWTP) in Western Australia and analyzed for PBDEs. There are currently no equivalent biosolids

guidelines for PBDE residues in Australia or overseas. The dominant species of PBDE present in sludge is BDE-209 (or Deca-BDE). The other major species are BDE-47, -99, -100, -153, -154. The concentration of BDE-209 in Western Australian biosolids ranged from 210-820 ng/g d.w. Compared to reported international studies the concentration of PBDEs is higher than European biosolids (de Boer, Horst et al. 2000; de Wit 2002) and lower than U.S.A. biosolids (Hale, Guardia et al. 2001). More work is required in order to understand the environmental risks associated with the land application of biosolids with this level of PBDE contamination.

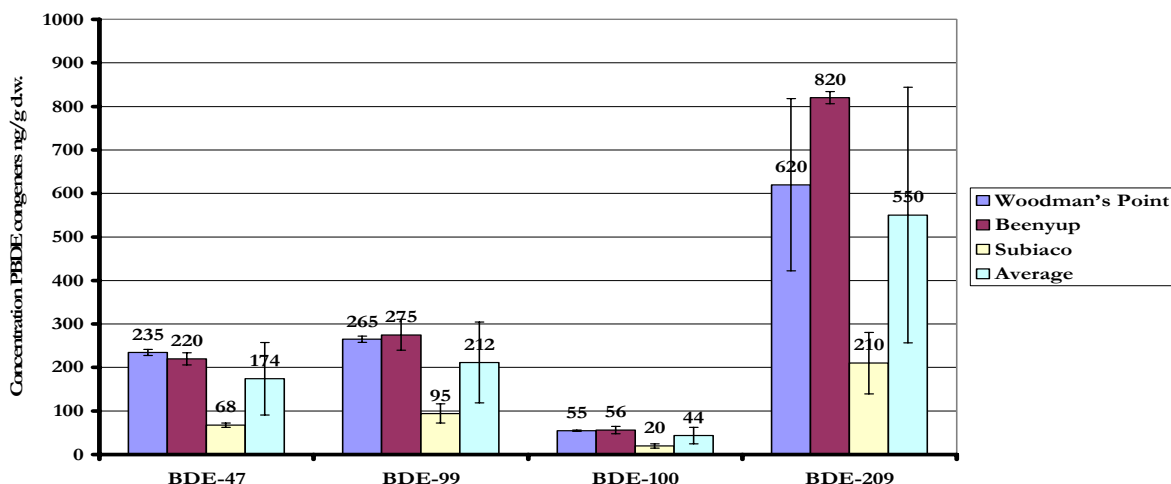


Figure 2: The average concentration of the dominant PBDE congeners in Western Australian Biosolids from Woodman's Point, Beenyup and Subiaco Wastewater Treatment Plants in 2005. Error reported is one standard deviation.

Organochlorine Pesticides and Polychlorinated Biphenyls

WWTPs were approached and asked to provide the routine analytical chemical data for OCPs and PCBs in their biosolids. The aim of this study is to consider freshly generated biosolids and not biosolids stockpiles that have residual historical contamination. The residue levels of 368 biosolids samples were obtained. The samples were from NSW (104 samples), Qld (218) and WA (46) and span the years 2004 (71), 2005 (178) and 2006 (119). As part of this study, 368 biosolids samples have been analysed for aldrin, BHC (not lindane) chlordane, DDT/DDD/DDE, dieldrin, HCB, heptachlor, lindane and PCBs.

There were three main pesticide residues present in Australian biosolids – dieldrin (av. conc. 0.07 mg/kg, present in 77% of samples), DDE (av. conc. 0.07 mg/kg, present in 21 % of samples) and chlordane (0.06 mg/kg, present in 20% of samples). Many of the organochlorine pesticides were not present or below the detection limit in the biosolids samples. These compounds are aldrin, DDD, DDT HCB and lindane. Where a breakdown of the 'total DDT' contribution was reported; in all cases the sole contribution was DDE. Heptachlor and PCBs were infrequently detected (av. of 2% and 4%) and were concentrated in particular WWTP biosolids, indicating localised contamination rather than widespread environmental pollution.

Each state has unique biosolids guidelines released by the respective Environmental Protection Agencies (NSW EPA 2000; WA DEP 2002; EPA 2002; EPA Victoria 2004; SA EPA 2006) that utilises a contaminant grading system (most often two tiered). The most stringent contaminant grading limit is imposed by Qld, NSW and WA that have adopted the 0.02 mg/kg pesticide residue in biosolids (C1). In all cases where pesticide residues were detected, the concentration was higher than C1. In no cases was the concentration of the

pesticide residue higher than the highest contaminant gradings in any state and therefore none of samples analysed as part of this study be prohibited from land application.

These findings are similar to internal studies that reported the absence of all the past-use organochlorine pesticides and polychlorinated biphenyls (Bright and Healey 2003; Northcott 2005) International trends also suggest that the concentration of these chemicals that are banned or restricted in use will decrease in biosolids with time.

Table 1: Average Concentration (mg/kg) of Regulated Organic Compounds in Biosolids

| Compound | 2004 | | | 2005 | | | 2006 | | | Average | | |
|-------------------|------|------|-------|------|------|-------|------|------|-------|---------|----|-------|
| | Av. | SD | % Det | Av. | SD | % Det | Av. | SD | % Det | Av. | SD | % Det |
| Aldrin | ND | ND | 0 | ND | ND | 0 | ND | ND | 0 | ND | | 0 |
| Chlordane | 0.08 | 0.07 | 11 | 0.08 | 0.07 | 24 | 0.03 | 0.01 | 19 | 0.06 | | 20 |
| Dieldrin | 0.04 | 0.06 | 58 | 0.06 | 0.04 | 84 | 0.09 | 0.09 | 80 | 0.07 | | 77 |
| DDE | 0.14 | 0.10 | 15 | 0.03 | 0.02 | 28 | 0.04 | 0.03 | 15 | 0.07 | | 21 |
| DDD | ND | ND | 0 | ND | ND | 0 | ND | ND | 0 | ND | | 0 |
| DDT | ND | ND | 0 | ND | ND | 0 | ND | ND | 0 | ND | | 0 |
| Total DDT | 0.14 | 0.10 | 15 | 0.03 | 0.02 | 28 | 0.04 | 0.03 | 15 | 0.07 | | 21 |
| Heptachlor | ND | ND | 0 | 0.04 | 0.01 | 1 | 0.03 | 0.01 | 5 | 0.03 | | 2 |
| HCB | ND | ND | 0 | ND | ND | 0 | ND | ND | 0 | ND | | 0 |
| Lindane | ND | ND | 0 | ND | ND | 1 | ND | ND | 5 | ND | | 2 |
| PCBs | 0.31 | 0.10 | 10 | 0.07 | 0.05 | 2 | 0.10 | 0.01 | 5 | 0.16 | | 4 |

Note: This concentration is the average of samples that were above the detection limit. This does not include samples where the residue concentration was below the detection limit or half the detection limit. Please consider this to be the highest possible average when contaminants are present.

Conclusion

The concentration of dioxins in Western Australian biosolids is low (within the background levels of soil) and is not likely to pose a threat to the environment or human health. Nationally the concentration of OCPs and PCBs is also low. Many of the regulated OCPs were not present at detectable concentrations in the 368 biosolids analysed in 2004 to 2006. When a residue was detected, it was never sufficiently high to prevent the land application. Based upon international studies it is expected that the concentrations of these compounds in biosolids will continue to decrease with time. The concentration of PBDEs in biosolids appears to be higher than those reported in Europe but lower than those reported in the USA. More research is required to understand the risk to humans, environment and soil quality from the land application of biosolids with this level of PBDE contamination.

References

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THE SIGNIFICANCE OF ORGANIC CONTAMINANTS IN BIOSOLIDS RECYCLED TO AGRICULTURAL LAND

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A large number of organic chemicals are used in industrial processes and domestically and consequently a wide range of compounds are potentially transferred to the urban wastewater (UWW) collection system. Attenuation occurs during wastewater treatment to varying degrees, principally by biological degradation, and the majority of the residual compounds partition to the sewage sludge due to their high affinity to sorb to organic matter. Further degradation may occur during sludge treatment, but this can be limited for some types of compound in anaerobic digestion processes. The compounds entering sludge may be generally listed as follows:

- Persistent compounds from incomplete combustion of fossil fuels that enter the UWW system through deposition onto paved surfaces via run-off (eg PAHs and PCDD/Fs);
- Persistent compounds that are associated impurities in wood preservatives such as creosote (PAHs) and pentachlorophenol (PCDD/Fs) and enter UWW in run-off;
- Controlled persistent compounds mobilised by volatisation from soil, deposition and transfer to UWW in run-off (eg PAHs, PCBs and PCDD/Fs);
- Persistent compounds generated by cooking food that are discharged from domestic sources (eg PAHs);
- Persistent compounds that are prohibited from use/manufacture, but domestic sources may exist and can transfer to UWW via run-off (eg chlorinated pesticides);
- Compounds discharged to sewer used directly in industrial processes or domestically including solvents, flame retardants or compounds that leach from plastics and surfaces during end-use and are carried in run-off (eg DEHP, PBDEs);
- Detergent residues (eg LAS, NPE);
- Pharmaceuticals, endogenous hormones and synthetic steroids.
- Compounds from the various above groups with endocrine disrupting potential

One of the primary concerns associated with the presence of organic contaminants in sludge is the potential for entry into the human foodchain from uptake into the edible parts of crop plants. However, despite the increasing amount of scientific investigation into the potential environmental consequences of organic contaminants applied to farmland in sewage sludge, there is no evidence for soil-crop transfer. This is explained due to the behaviour of organic contaminants in soil and the presence of only very small concentrations of some of the more potentially toxic compounds in sludge. Thus, organic compounds can be grouped into three broad categories according to their behaviour:

1. Volatile compounds which are quickly lost to the atmosphere from sludge and sludge-treated soil.
2. Compounds which are rapidly mineralised by microorganisms and have little or no persistence.

3. Persistent compounds which are strongly adsorbed onto the sludge and soil organic matrix.

Compounds exhibiting some solubility and potential for plant uptake are also susceptible to rapid degradation processes in soil, or are lost through volatilisation. Other more persistent compounds usually have very low solubilities, are present in very small concentrations, and are strongly adsorbed by the soil matrix in non-bioavailable forms. The principal concern and theoretical mechanism of entry of organic contaminants to the human foodchain from agricultural use is by surface spreading liquid sludge and intake by livestock grazing treated pasture resulting in the potential accumulation of lipophilic compounds in meat fat and milk. In practice, however, there has been no demonstrable link between sludge application and transfer of organics to animal tissues or milk by this route. This theoretical exposure pathway does not operate when surface spreading to pasture is not permitted, as is the case in the Western Australian Guidelines.

The potential impacts on the foodchain of persistent organic compounds in sludge, including: PAHs, PCDD/Fs or PCBs, has been a key concern for agricultural utilisation. However, international emission controls on the main point sources of these priority persistent compounds have significantly reduced their entry into the UWW collection system. Atmospheric deposition and environmental cycling are the main source of these compounds in sludge, and, consequently, the concentrations present in sludge generally represent background environmental values. Ongoing research has not identified any toxicological or ecotoxicological link with these compounds in sludge. Therefore, on balance, the importance of these contaminants has significantly diminished and there is no quantitative, scientific evidence to support the need for limits or controls on PAHs, PCBs or PCDD/Fs in sewage sludge.

Three other potentially important environmental end-points to be considered include: ecotoxicological impacts of major organic contaminants on soil functions, antibiotic resistance in soil microbial populations and endocrine disruption. The impacts of the surfactant, linear alkylbenzene sulphonate (LAS), a concern because it is one of the most abundant compounds present in anaerobically digested sludge, have received considerable attention. Recent investigations and risk assessments have revised the soil ecotoxicological limit value for LAS and conclude that this compound does not represent a hazard to soil quality at the concentrations and application rates in sludge and that limits in sludge are therefore unnecessary. The presence of trace amounts of antibiotics in sludge and livestock manures has been linked to the development of antibiotic resistant populations of bacteria in soil, and the possibility of the transfer of environmental sources of antibiotic resistance with potentially serious consequences for human health. The therapeutic use of antibiotics in intensive livestock production systems is the main source of potentially resistant populations in soil. Also, recent research has shown that, because there is a high maintenance cost associated with these resistance mechanisms, this characteristic is naturally attenuated when the selection pressure is removed following application due to degradation of the active substance in soil. A large range of organic compounds may exhibit the potential to cause endocrine disrupting effects. However, these compounds are influenced by the attenuation processes described above and, consequently, there is no pathway identified for the potential transfer to the human foodchain. Therefore, there is no risk of endocrine disruption through the human foodchain from agricultural utilisation of biosolids.

Despite the extensive range of organic chemicals that can be present in sewage sludge, the expanding experimental evidence base indicates that these are not a significant limitation to the agricultural use of sewage sludge. This view is based on a technical evaluation of the situation, which acknowledges that the presence of effective source

control measures and small concentrations of persistent contaminants in sludge, biodegradation and behaviour in soil, absence of crop uptake, and sludge application practices, minimise potential impacts of organics in sludge on soil quality, human health and the environment.

INACTIVATION OF PATHOGENIC CONTAMINANTS IN LAND-APPLIED BIOSOLIDS: RESEARCH PROGRESS

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Abstract

Research on the inactivation of enteric pathogens in biosolids applied to agricultural land in Western Australia began in 2005. This study was established as part of a national collaborative project to study the fate of organic contaminants, non-organochlorine pesticides, pharmaceuticals, personal care products and endocrine disruptors when introduced into the soil through the land-application of biosolids in agriculture. Preliminary work was carried out in 2004 with a field experiment at Toodyay and a glasshouse trial at CSIRO in Floreat, Perth to study inactivation rates of *E. coli*, *Enterococci* and bacteriophage over time.

The research into pathogenic contaminants in biosolids was carried out on three areas: laboratory, glasshouse and field. The glasshouse trial commenced 10 May 2006 to determine the different inactivation rates of *Salmonella typhimurium* and *E. coli* inoculated onto biosolids vs. nil biosolids treatments. Non-sterile biosolids (1%) were incorporated with soil from the Moora trial plot, spiked with cultured bacteria and approximately 3 to 4 grams distributed into each Microsep chamber. The chambers were placed at depth 0-10cm. Calingiri wheat (*Triticum aestivum*) was seeded into the pots and fertilised. Sampling was carried out at days 0, 5, 14, 19, 34, 54, 76 and 104.

On 10 September 2006, the wheat plants in the glasshouse were spiked with *Salmonella typhimurium*, *E. coli* and bacteriophage to determine inactivation rates on plant leaves and grain heads. Sampling was carried out at hours 0, 1, 2, 4, 6, 8, 26, 28, 53 and 64.

The field trial commenced 30 May 2006 to align with the experimental design used in the glasshouse. Non-sterile biosolids (20%) with Moora soil were inoculated with laboratory-cultured *Salmonella typhimurium*, *E. coli* and bacteriophage. Approximately 3 to 4 grams were distributed into each Microsep chamber and placed at depth 0-10 cm. Treatments were three plots spread to biosolids (10 dry t/ha) and three plots without biosolids. Calingiri wheat (*Triticum aestivum*) was sown and plots were managed according to standard practice. Sampling was carried out at days 0, 7, 13, 20, 34, 56, 69 and 83. At each sampling event, three chambers were removed for analysis as per the glasshouse experiment. This experiment determined the effect that the presence of biosolids may have on the decay of pathogenic organisms given conditions in the field, representing areas where biosolids are currently being spread. Field conditions such as rainfall, soil temperature, soil moisture, humidity and air temperatures were recorded using onsite weather station equipment.

An overview of the abovementioned experiments will be presented along with preliminary research findings. In summary, both the glasshouse and field experiments indicated a

more rapid decline of enteric pathogens where biosolids were present. Enteric pathogens inoculated onto wheat plants indicated a more rapid inactivation rate on grain heads as compared to plant leaves. This presentation will also illustrate some of the equipment and methodology used in the experiments.

PATHOGEN SURVIVAL IN BIOSOLIDS-AMENDED AGRICULTURAL SOIL

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The survival of enteric microorganisms during sewage sludge treatment was extensively researched during the 1980s in the UK and Europe and this work formed the basis of defined effective treatment processes for sludge for agricultural application. These measures were complemented by additional land use restrictions providing a multi-barrier approach to control the transmission of enteric disease to humans and farm livestock when sewage sludge is used in agriculture. However, subsequent health outbreaks and the increasing incidence of gastrointestinal infections in the human population, although unrelated to agricultural recycling, heightened concerns about the potential health impacts of environmental sources of enteric disease. The UK Retail Industry became particularly concerned whether the land spreading of sludge was sufficiently protective of human health and the foodchain. This followed a number of reports, including the 19th Report by the Royal Commission on Environmental Pollution on the Sustainable Use of Soil in 1996 and the House of Commons Environment, Transport and Regional Affairs Committee 2nd Report on Sewage Treatment and Disposal in 1998, that called into question the safety of this practice. Nevertheless, the absence of epidemiological evidence linking disease outbreaks in the human population or in farm livestock with the agricultural use of sewage sludge was recognised as a strong indication that the multi-barrier approach was effective and prevented the spread of infectious diseases when sludge is used on farmland. The UK Water and Retail Industries reached agreement on a further range of measures to secure confidence in the agricultural use of sludge, known as the Safe Sludge Matrix. This was supported by additional research funded by the Water Industry and Government to further underpin the scientific basis to agricultural recycling. This recent tranche of work included:

1. Development of methods for enumerating pathogens in sludge;
2. Survival investigations on enteric microorganisms during sludge treatment processes;
3. Microbiological risk assessment of pathogens in sludge;
4. Survival of enteric organisms in sludge-treated agricultural soil.

This paper presents the background to the development of the Safe Sludge Matrix and provides an overview of the key elements of the research programme undertaken in the UK on pathogens in sewage sludge. The natural decay of enteric microorganisms in soil following the application of sewage sludge represents the final barrier to the potential transmission of infectious disease. Therefore, understanding the mechanisms of pathogen inactivation in soil is critical to effectively manage the potential microbiological risk to human and animal health associated with the use of biosolids as soil amendments in agriculture. The remainder of the paper will therefore focus on current research to quantify the inactivation of enteric microorganisms in soil. Data from field and laboratory research will be presented on the survival of the indicator organism, *E. coli* and the important

gastrointestinal strain, *E. Coli* O157, in biosolids amended soil. This shows decay to the background population, or to below detectable limits, occurred in soil within 3 months after sludge application, irrespective of the environmental conditions, soil type or time of sludge spreading. This period is considerably shorter than the harvesting intervals stipulated in the Safe Sludge Matrix demonstrating that a large margin of safety is provided for the inactivation of pathogens to take place before potentially sensitive crops may be grown. The role of ecological interactions between added pathogens, microbial biomass and substrate resources and the indigenous soil microbial community is emerging as a critical factor influencing survival times of enteric bacteria in sludge-amended soil and recent data quantifying soil predator dynamics following sludge application will be presented.

BIOSOLIDS: FLY DREAMING, PEOPLE NIGHTMARE

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Each year the Water Corporation transports and land applies approximately 50,000 wet tonnes of dewatered biosolids cake and lime amended biosolids cake direct to agriculture.

The transporting and temporary stockpiling of dewatered biosolids cake has the potential to facilitate the translocation of different fly species between areas and contribute to an increase in overall fly populations.

As a consequence and to assist with compliance the Department of Health and Department of Environment and Conservation developed the Western Australian Guidelines for Direct Land Application of Biosolids and Biosolids Products (February 2002). These guidelines assist with avoiding recognized stable fly breeding in 3 shires, to cover biosolids cake when transporting to agricultural locations and minimize stockpiling periods.

Regulations such as these are useful but do not contribute to a sustainable control of fly breeding in biosolids cake. Over the last 4 years a series of experiments have been conducted to assist in developing methodologies to reduce the impact of fly breeding.

The first investigation concluded that fly breeding in biosolids cake can be effectively reduced by the placement of covers over the entire exposed surface of the biosolids stockpile. During storage of biosolids cake the presence of either a full plastic or shade cloth cover acts as an effective adult fly barrier limiting fly oviposition and emergence. Although both cover types are equally effective at reducing fly breeding, shade cloth covers appear to be the easiest to handle and the most cost-effective material.

The following investigation concluded that fly breeding in biosolids cake can be reduced by the addition of insecticide. Chemical control should only be considered as a short term measure because fly larvae may become resistant to the chemical during prolonged usage.

The third investigation noted that by producing drier biosolids cake (<40% moisture) fly breeding has been reduced to zero and although probably the best tool it did not conclude what happens when dry biosolids cake are exposed to a rain event while being stored.

The culmination of the above investigations led to a combined study to identify the factors facilitating fly breeding in stockpiles of biosolids cake. The constituents included, ambient temperature, NH₄ content, rainfall, moisture content, pH levels, the age of the biosolids cake. These parameters were used to assess the seasonality of fly breeding in biosolids cake and to establish the age (in weeks) after which biosolids cake is no longer an attractive resource for fly breeding. A further study underway is investigating the effects of relocating aged biosolids (> 10 weeks).

NUTRIENT AND PH VALUE OF LIME-AMENDED BIOSOLIDS USED FOR AGRICULTURE

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Abstract

Agricultural soils throughout much of Australia, and particularly Western Australia commonly suffer from nutrient deficiencies and/or soil acidity. Soil acidification is an increasing problem in broadacre agriculture in Western Australia, with an estimated 178,000 tonnes of lime applied to farming properties in 1994/95 (Moore *et al.* 1998). Plant growth can be reduced in acidic soils due to problems with aluminium toxicity, deficiencies of some nutrients and nodulation failure in legumes. The nutrient levels of soil can be improved by the addition of fertilisers, whereas the pH of acidic soils can be increased by the addition of products containing lime or dolomite.

A total of 9,300 tonne dry solids (t DS) of lime-amended biosolids (LAB) was produced from the Subiaco Wastewater Treatment Plant (WWTP) Perth during 2004/05. The use of lime-amended biosolids on acidic soils may be of benefit in neutralising soil acidity. Lime-amended biosolids are typically drier (35% TS) and lower in nutrients (except Ca) than dewatered biosolids cake (DBC) due to a dilution of nutrients resulting from the addition of the lime used to stabilise sludge. To date, no research has been conducted in Western Australia to investigate the neutralising value of LAB, monitor the plant response to LAB or establish the economic value of LAB in agriculture. If deemed unsuitable for agricultural land application, the potential landfill costs of LAB would escalate to almost \$2 m/pa (i.e. 34 000 wet tonne @ \$57/t) (Penney 2005).

The two-fold value of LAB as a source of nutrients for the growth and yield of wheat (*Triticum aestivum*) on an acidic soil in the central wheatbelt in agricultural Western Australia and for the neutralising value of the added lime was investigated as a pilot study over the 2005 season to assess the effect of LAB. Specifically, the investigation aimed to establish current LAB application rates to meet the required pH adjustment to sustain crop growth; establish maximum application rates to meet soil pH adjustment and nutrient requirement to sustain crop growth; and to establish the value of LAB from an agricultural perspective with the view of establishing a suitable monetary return.

Background

The LAB experiment was established on an acidic red/brown sandy loam at Wongamine, 90 km north east of Perth, Western Australia in 2005 and included four rates of LAB, four rates of lime, and one rate of dewatered biosolids cake (DBC).

The LAB has a typical analysis consisting of 35.4% DS, 3% total N, 1% total P, pH 13, with an expected neutralising value of 44.9%. Rates of LAB were determined by multiples of the nitrogen limited biosolids application rate (NLBAR) of 0, 0.5, 1 and 1.5xNLBAR using the following DEP *et al.* (2002) formula:

$$\text{NLBAR} = \text{Crop N required} / \text{plant available N}$$

Thus 1xNLBAR for the LAB: 64 kg/ha / 6,207 mg/kg = 10.3 t DS/ha.

Dewatered biosolids cake (DBC) was included in the study being the predominant biosolids product produced by Perth WWTPs in Western Australia, and thus provides for comparative research in terms of crop yield (Priestley 1997; Pritchard 2005; Pritchard & Collins 2005). The DBC was applied at 1xNLBAR = 7 t DS/ha, the standard rate used in Western Australia and was sourced from the Beenyup WWTP. Analysis of the Beenyup DBC was 20% t DS, 5% total N and 2.5% total P.

Lime was applied at 0, 2.3, 4.6 and 6.9 t/ha. Agricultural lime is commonly applied in Western Australia at rates between 1 and 2 t/ha, although rates in this experiment were higher to provide a direct comparison with the rates of LAB used in the study. The lime used in the experiment had a neutralising value of 96% and was <0.25 mm in size. The 1xNLBAR LAB (10.3 t DS/ha) would be expected to be equivalent in neutralising value to the 4.6 t/ha lime treatment. All lime treatments were supplied with a basal fertiliser dressing of 100 kg/ha urea and 100 kg/ha diammonium phosphate, to supply a total of 66 kg N/h and 18 kg P/ha.

At the rates of LAB used, soil pH (CaCl₂ 1:5) was increased significantly ($P<0.05$) from 4.4 in nil LAB to 7.2 in the 1.5xNLBAR LAB as measured in the 0-10 cm depth, eight months after application. Soil pH also increased in a study by Cooper (2005) following the use of lime-amended biosolids (N-Viro soil) in central New South Wales. The increases in soil pH after 8 months would be of significant benefit to farmers on acidic soils, even at 0.5xNLBAR LAB. From this preliminary investigation, the neutralising value of the lime in the LAB was similar to the predicted value of 44.9%. This value is consistent with that reported by Sloan and Basta (1995) for a 1:1 dewatered sewage sludge and cement kiln dust mixture, which is reduced to 28% if less kiln dust is added (Cooper 2005). In comparison, the 1xNLBAR DBC did not change soil pH. Soil pH in the depth below 10 cm needs to be measured to provide a more comprehensive indication as to overall soil chemical changes.

The shoot growth of wheat at 8 weeks after sowing increased following the addition of any treatment containing a fertiliser source. Fertiliser included LAB, DBC or basal fertiliser (DAP) applied to the lime treatments, suggesting that nitrogen (N) and phosphorus (P) were primarily responsible. The concentrations of P in the shoot dry matter of wheat at 8 weeks were significantly higher ($P<0.05$) in treatments following the addition of DAP. Concentrations of N however, were slightly increased in treatments containing biosolids. The concentration of P in shoots of wheat at 8 weeks was higher in DBC (0.33%) than LAB (0.23%) at equivalent NLBAR, but would be explained by the higher initial P loadings in the DBC.

It should be mentioned that increasing rates of either lime or LAB significantly decreased concentrations of manganese (Mn) in the shoots of wheat, although concentrations remained above the critical values reported by Reuter & Robinson (1997). The likely explanation for this trend is related to the solubility of Mn, which declines with increasing pH (Moore *et al.* 1998) and also noted by Christie *et al.* (2001) in barley shoots grown in alkaline-stabilised biosolids produced using cement kiln dust. No other nutrient appeared to be affected by any of the treatments.

Wheat, the primary export crop in Australia, yields on average 1.62 t/ha (2000-03) (ABARE 2003). The grain yield of wheat at harvest in this experiment was increased significantly ($P<0.05$) by the additional of LAB from 1.23 t/ha in the nil treatment to 2.21 t/ha in the highest LAB rate. The highest rate of LAB had a large number of small or pinched grain, suggesting that the potential yield was greater than achieved. It is

uncertain as to what caused this decline, as soil moisture was not limiting at the end of the season. Plant symptoms were similar to that caused by the root disease Take-all (*Gaeumannomyces graminis var tritici*), which is more severe in soils which have been limed (Loughman *et al.* 2000), and is under further investigation.

Overall the grain yield of wheat in the LAB at 1xNLBAR (10.3 t DS/ha) was comparable with that obtained by DBC at 1xNLBAR (7 t DS/ha). Grain yield of wheat was reduced significantly ($P < 0.05$) at rates of LAB at 0.5xNLBAR indicating that adequate plant nutrition was not achieved. Therefore, from this preliminary study, the preferred LAB rate would be to apply at 1xNLBAR, as is currently used for DBC.

Grain yield of wheat was similar in all four rates of lime, indicating that soil pH was of marginal influence to grain yield in the first season of spreading. It is suggested that the neutralising value of the lime would have taken place slowly, and thus may not have been of great influence during the growing season. Given the large changes in pH over the duration of the experiment in LAB, crop yields in the second season are thus being monitored to establish any problems to plant growth and the environment.

The cost of lime in the LAB is valued at ~\$1.50/t and therefore if full cost recovery for the lime was implemented, the LAB at 1xNLBAR would cost the farmer ~\$15/ha. However, longer-term effects on soil pH would need to be monitored prior to an economic value being placed on the LAB. In addition, the use of LAB on non-acidic soil would not be recommended given the large increase in soil pH

Conclusion

The benefit of the LAB in the first season of application appeared to be from the nutrient value of the recycled nutrients (typically N and P) rather than that of the initial reduction in soil acidity. Further data is being collected over subsequent years to ensure that LAB does not impact adversely on crop production or cause environment harm, all of which may jeopardise the current market.

From this preliminary experiment, the most optimum rate of LAB to use to satisfy wheat growth and yield on the acidic soil used was at 1xNLBAR. This rate is higher than typically applied in Western Australia at present. Rates of LAB below this would need to be supplemented by additional inorganic fertiliser, whereas rates above this may cause excessive changes in soil pH due to the neutralising value of the lime.

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PHOSPHORUS BIOAVAILABILITY FROM LAND APPLIED BIOSOLIDS IN SOUTH-WESTERN AUSTRALIA

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Abstract

Research conducted in Australia to investigate the bioavailability of phosphorus (P) in anaerobically digested dewatered biosolids following agricultural land application in south-western Australia was completed in 2005 and is reported in this presentation. It is common for application rates of biosolids in agriculture to be determined by the crop nitrogen (N) requirement to reduce nitrate leaching from the root zone. However, the implementation of an N based biosolids nutrient management plan can lead to P being supplied in excess of that required for plant uptake. The Water Corporation initiated this study to ensure there was adequate data available to investigate the agronomic benefit of biosolid P in agriculture and to monitor P loadings to ensure there is minimum risk to the environment following the land-application of biosolids in Western Australia.

The resultant high loading rates of P are considered in the guidelines developed for the agricultural use of biosolids in Western Australia, but lack research data specific to local conditions and soil types. Regulatory changes throughout Australia and globally to protect the environment from wastewater pollution have created a need for more accountable and balanced nutrient data. Experiments presented in this thesis were undertaken to ascertain: the percentage relative effectiveness (RE) of biosolids as a source of plant available P compared with inorganic P fertiliser; loading rates to best supply P for optimum crop growth; P loading rates of risk to the environment; and the forms of P in local biosolids. Therefore, both the agronomic and environmental viewpoints were considered.

Anaerobically digested and dewatered biosolids produced from Beenyup Wastewater Treatment Plant, Perth with a mean total P content of 2.97% dry weight basis (db) were used in a series of glasshouse, field and laboratory experiments. The biosolids were sequentially fractionated to identify the forms of P present and likewise in soil samples after applying biosolids or monocalcium phosphate (MCP). The biosolid P was predominantly inorganic (92%), and hence the organic fraction (8%) available for mineralisation at all times would be extremely low. The most common forms of biosolid P were water-soluble P and exchangeable inorganic P (66%), followed by bicarbonate extractable P (19%) and the remaining P as inorganic forms associated with Fe, Al and Ca (14%). Following the application of biosolids to a lateritic soil, the Fe and Al soil fractions sorbed large amounts of P, not unlike the distribution of P following the addition of MCP. Further investigation would be required to trace the cycling of biosolid P in the various soil pools.

The growth response of wheat (*Triticum aestivum* L.) to increasing rates of biosolids and comparable rates of inorganic P as MCP, to a maximum of 150 mg P/kg soil was examined in the glasshouse. The percentage relative effectiveness (RE) of biosolids was calculated using fitted curve coefficients from the Mitscherlich equation: $y = a(1 - b \exp^{-cx})$ for dry matter (DM) production and P uptake. The initial effectiveness of biosolid P was comparable to that of MCP with the percentage RE of biosolids averaging 106% for DM production of wheat shoots and 118% for shoot P uptake at 33 days after sowing (DAS) over three consecutive crops. The percentage residual value (RV) declined at similar rates

for DM production in MCP and biosolids, decreasing to about 33% relative to freshly applied MCP in the second crop and to approximately 16% in the third crop.

The effectiveness of biosolid P was reduced significantly compared with inorganic P when applied to a field site 80 km east of Perth (520 mm annual rainfall). An infertile lateritic podsollic soil, consistent with the glasshouse experiment and representative of a soil type typically used for the agricultural application of biosolids in Western Australia was used. Increasing rates of biosolids and comparable rates of triple superphosphate (TSP), to a maximum of 145 kg P/ha were applied to determine a P response curve. The percentage RE was calculated for seasonal DM production, final grain yield and P uptake in wheat followed by lupin (*Lupinus angustifolius* L.) rotation for the 2001 and 2002 growing seasons, respectively. In the first year of wheat, the RE for P uptake in biosolids compared with top-dressed TSP ranged from 33% to 55% over the season and by grain harvest was 67%. In the second year, and following incorporation with the disc plough at seeding, the RE for P uptake by lupins in biosolids averaged 79% over the growing season compared with top-dressed TSP, and by grain harvest the RE was 60%. The residual value (RV) of lupins at harvest in biosolids compared with freshly applied TSP was 47%.

The non-uniform placement of biosolids (i.e. spatial heterogeneity) was primarily responsible for the decreased ability of plant roots to absorb P. The P was more effective where biosolids were finely dispersed throughout the soil, less so when roughly cultivated and least effective when placed on the soil surface without incorporation. The RE for grain harvest of wheat in the field decreased from 67% to 39% where biosolids were not incorporated (i.e. surface-applied). The RE could also be modified by factors such as soil moisture and N availability in the field, although it was possible to keep these variables constant in the glasshouse. Consequently, absolute values determined for the RE need to be treated judiciously.

Calculations showed that typical loading rates of biosolids required to satisfy agronomic P requirements of wheat in Western Australia in the first season could vary from 0 to 8.1 t DS/ha, depending on soil factors such as the P Retention Index (PRI) and bicarbonate available P value. Loading rates of biosolids were inadequate for optimum P uptake by wheat at 5 t DS/ha (i.e. 145 kg P/ha) based on the NLBAR on high P sorbing soils with a low fertiliser history (i.e. PRI >15, Colwell bicarbonate extractable P <15 mg P/kg). On soils of PRI <2 mL/g however, biosolids applied at identical loading rates would result in high concentrations of available P. Further work on sites not P deficient would be necessary to validate these findings on farmed soils with a regular history of P fertiliser. The sieving of soil samples used in the field experiment to remove stones and coarse organic matter prior to chemical analysis inadvertently discarded biosolids particles >2 mm, and thus their was little relationship between soil bicarbonate extractable P and P uptake by plants in the field.

The risk of P leaching in biosolids-amended soil was examined over a number of different soil types at comparable rates of P at 140 mg P/kg (as either biosolids or MCP) in a laboratory experiment. Given that biosolids are restricted on sites prone to water erosion, the study focussed on the movement of water-soluble P by leaching rather than by runoff of water-soluble P and particulate P. In general the percentage soluble reactive P recovered was lower in soils treated with biosolids than with MCP, as measured in leachate collected using a reverse soil leachate unit. This was particularly evident in acid washed sand with SRP measuring 14% for biosolids and 71% for MCP, respectively, although the differences were not as large in typical agricultural soils. Specific soil properties, such as the PRI, pH, organic carbon and reactive Fe content were negatively correlated to soluble reactive P in leachate and thus reduced the risk of P leaching in biosolids-amended soil. Conversely, the total P and bicarbonate extractable P status of the soils investigated were unreliable indicators as to the amount of P leached.

On the basis of the experiments conducted, soils in Western Australia were categorised according to their ability to minimise P enrichment and provide P necessary for crop growth at loading rates determined by the NLBAR. Biosolids applied at the NLBAR to soils of PRI >2mL/g with reactive Fe >200 mg/kg were unlikely to necessitate P loading restrictions. Although specific to anaerobically digested biosolids cake applied to Western Australian soils, the results will be of relevance to any industry involved in the land application of biosolids, to prevent P contamination in water bodies and to make better use of P in crop production.

Pritchard, D.L. 2005, *Phosphorus bioavailability from land-applied biosolids in southwestern Australia*. PhD Dissertation, Muresk Institute, Curtin University of Technology.

Full report via the internet accessible directly at

<http://adt.curtin.edu.au/theses/available/adt-WCU20060811.140234/>

DIFFERENTIATION BETWEEN BIOSOLIDS FROM HUMAN OR ANIMAL ORIGIN BY DNA FINGERPRINTING: A PILOT STUDY

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Abstract:

For many years, coliforms were used as the leading indicator of faecal pollution. However, the strategy presented with varying degrees of success and the complex issue of representation remains unresolved. Although methods exist for quantifying faecal pollution, the response times and accuracy can be improved. However, detection of host-specific molecular markers (by DNA techniques) does not require bacterial culture and has the potential to be more precise and rapid. It would also enable the targetting of bacteria that are potentially difficult to culture in the laboratory.

Recently, the *Bacteroides/Prevotella* group and the *Bifidobacteria spp*s are noncoliforms that have generated considerable interest as alternative faecal pollution indicators partly because of their abundance in faeces. They predominate the intestinal fauna in both human and animal digestive systems and significantly outnumber coliforms by some 10^2 to 10^3 -fold. Therefore, an opportunity is presented to develop a detection system with greater sensitivity based on these species. Several priming sequences on the 16S rRNA gene have been designed for the intra- and interspecies identification by DNA amplification (and variations from this technique) of both *Bacteroides/Prevotella spp* and *Bifidobacteria spp*. Furthermore, certain species residing in the human GI tract are absent from other animals adding testimony to host-specificity with these bacteria. Given the enormous reliance on the association between bacterial markers and either human or ruminant host, it can be argued that the focus on *Bacteroides/Prevotella spp* and *Bifidobacteria spp* 16S rRNA gene could improve the overall effectiveness of species differentiation in biosolids.

In this pilot study, we explore the utility of DNA fingerprinting for differentiating biosolids from human and animal origin. Preliminary investigations show that DNA can be isolated from the biosolid and faecal matrices. More significantly, the DNA isolates were amenable to PCR amplification with genus and species-specific primers to *Bacteroides/Prevotella* group and the *Bifidobacteria spp*s. However, follow-up research is required to determine the utility at the level of the host.

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