EFFECTS OF COMPOSTING PROCESSES ON MICROBIAL INDICATORS

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ABSTRACT

Biosolids are a useful reusable and beneficial resource as they are rich in nutrients and some trace elements. However, their use can be limited by the presence of unacceptable levels of microbiological pathogens and organic pollutants.

In a recent study on the suitability of biosolids originating from a provincial wastewater treatment plant as a composting material, the presence and viability of several microbiological indicators was followed over the entire composting process and compared to the sludge before composting. The composting processes were found to be effective against the microbial indicator pathogens. If other pollutants are within the limits of the guidelines, the biosolids thus obtained, are safe for use by the general public for land application.

Free living nematodes are abundant in freshwater, saltwater and soils. These were found to be present despite undergoing the compositing process. This suggests that the latter had no impact on their survival. In contrast, microbial indicators were not detected at levels considered pathogenic according to the Guidelines for the Safe Application of Biosolids to Land in New Zealand indicating that the composting conditions used were effective in their elimination.

KEYWORDS

Biosolids, pathogens, microbial indicators, sewage sludge disposal, land application, composting processes

1 INTRODUCTION

Biosolids are sewage sludges co-composted with other materials like bark, green waste sand etc... The former being rich in nitrogen content and moisture, and the latter being high in organic content and good bulking quality, complement each other. This could result in a product that has high fertilizing and soil conditioning properties when applied to land. Sewage/ wastewater sludge can contain a wide variety of pathogens such as bacteria (*Escherichia coli*, *Salmonella spp.* and *Campylobacter spp.*), enteric viruses, parasites (helminthes/protozoa) and fungi. For the product to be considered useful, these pathogens need to be eliminated or reduced to an acceptable level under Section 15 of the Resource Management Act of 1991.

There are 320 municipal wastewater treatment plants in New Zealand (MFE, 2007). Sewage sludge from them can be disposed off in one of the following ways:

- sewer (ex thickener)
- landfill (on-site monofills and regional sanitary landfills)
- natural water
- forest, and
- permanently in lagoons.

Environmental concerns arising from direct disposal or incineration, land scarcity and various other reasons has prompted the need to look at a more suitable and useful way of disposing sludge.

Two different approaches have been adopted in the US and in Europe. The United States Environmental Protection Agency has adopted a risk assessment approach. This involves analyzing risks to plants, animals, humans and soil organisms for exposure to contaminants and forms the basis for the Standards for the Use and Disposal of Sewage Sludge (US EPA, 1993) commonly referred to as the Part 503 Rule. In contrast, the European approach (LOAEC) involves setting soil limits at the lowest observed adverse effects concentrations.

Australia has been preparing its own Guidelines for Sewage Systems: Biosolids, based loosely on the European approach..The *Guidelines for the Safe Application of Biosolids to Land in New Zealand* (*referred to as the Guidelines henceforth*) includes recommended soil limits based on the LOAEC approach and is similar to those adopted in Australia. (NZWWA, 2003)

According to the Guidelines (NZWWA, 2003), for the biosolid to be accepted as stabilisation grade A it must satisfy the conditions listed in *Table 1*.

Table 1: Pathogenic indicators, their MAVs, and sampling regime

Indicators	**Verification sampling**	**Routine sampling**
Escherichia coli	< 100 MPN/g dry weight	< 100 MPN/g dry weight
Campylobacter	< 1/25 g dry weight	N/A
Salmonella	< 1/25 g dry weight	N/A
Enteric viruses	<1 PFU/4g dry weight	N/A
Helminth ova	<1 /4g dry weight	N/A

MAV: Maximum Acceptable Values
MPN: Most Probable Number
PFU: Plaque Forming Unit
N/A: Not Applicable

This paper investigates the effects of co-composting on the populations of the abovementioned microbial indicators in biosolid samples obtained from a provincial wastewater treatment plant. Sludge was mixed with shredded greenwaste and then composted in an in-vessel system. Yanko *et al* (1988) reported in-vessel composting systems for sludge mixed with sawdust with a resultant annual production of 60,000 cubic yards. Their study involved 15 days of composting followed by 15 days of curing before distributing the resultant biosolids to homeowners, nurseries, landscapers and other users.

2 METHODS

2.1 SAMPLING

The *Guidelines* (NZWWA, 2003) states that depending on the objective of sampling, the biosolid product can be sampled for two reasons: verification or routine monitoring. For verification purposes the sampling regime should include greater than or equal to 15 evenly dispersed grab samples per month for a 3-month period with less than or equal to 3 failures. If greater than 3 failures then the 15 following consecutive grab samples must comply. Parameters to be monitored for verification purposes include *Escherichia coli*, *Salmonella*, *Campylobacter*, enteric viruses and helminth ova.

For the purpose of this paper, samples were obtained from a provincial wastewater treatment plant site which is trialing composting methods for the safe application of sludge onto land. Since the project is still on-going, the exact site names are not disclosed to ensure client confidentiality.

2.2 INDICATORS TESTED

A comprehensive report published by the European Commission has listed a number of pathogens that could be found in sewage sludge (Carrington, 2001). However, many of these pathogens are highly sensitive to the heat generated by the composting process and die off easily. Hence it is only necessary to check the biosolids for

those indicators which are highly resistant to the process. These, if absent, should guarantee the elimination of the less resistant species. (Yanko, 1988; Carrington, 2001; Litterick, A, 2003; NZWWA, 2003).

The parasites that are generally monitored are *Salmonella*, enteric viruses, helminth ova and oocysts of protozoa. *Escherichia coli* is used as an indicator for the presence of bacterial pathogens found in sludge. *Salmonella* is a good indicator for other high risk bacterial pathogens. *Campylobacter* testing is included in New Zealand Guidelines since it is a highly prevalent pathogen of concern. Secondly both these pathogens i.e. *Salmonella* and *Campylobacter* have the potential to regrow during storage or after land application. Amongst enteric viruses, adenovirus is highly resistant to physical and chemical treatments and hence a good indicator for monitoring viral presence. The present study also included the testing of enteroviruses. Helminths in sewage sludge could include nematodes, cestodes and trematodes. Helminth ova, also being highly resistant to these treatments, are an important indicator and have to be monitored for their presence and viability. Protozoan oocysts like Giardia and Cryptosporidium are not required to be monitored as yet since current test methods are not yet sufficiently reliable to warrant setting standards for biosolids (NZWWA, 2003).

2.3 SAMPLE PREPARATION BASED ON INDICATORS

A summary of the sample preparation and testing methods used in the detection of the indicators listed earlier is given in *Table 2*. All the methods used are IANZ (International Accreditation New Zealand) accredited.

Table 2: Sample preparation and Testing Methods for the various pathogenic indicators

Indicators	**Sample preparation and testing methods**
Escherichia coli	Requisite weight of sample based on moisture content (to give < 100 MPN/g dry weight) was mixed with buffered peptone water and shaken for less than fifteen minutes and then dispensed into Lauryl Tryptose Broth and tested using Part 9221 MPN of APHA (2005)
Campylobacter	Requisite weight of sample based on moisture content (to give < 1/25 g dry weight) was mixed with buffered peptone water and then dispensed into Bolton enrichment broth tubes and proceeded with the 5-tube, 3 dilution series. Further selective isolation was done using mCCDA agar to obtain isolated colonies which if suspected to be *Campylobacter* were verified using Latex serotyping, oxidase and catalase tests followed by the confirmatory Gram staining and motility tests. (MIMM, 2004, Chapter 13.1; NZWWA, 2003)
Salmonella	Requisite weight of sample based on moisture content (to give < 1/25 g dry weight) was mixed with buffered peptone water and then dispensed and proceeded with the 5-tube, 3 dilution series. Further enrichment was done using Selenite Cystine Broth and Rapport Vassiliadis Soya Medium (R.V.S.) broth. Isolation was done on BGA/XLD agar and suspect colonies confirmed using Serotyping *Salmonella* latex agglutination kits. (MIMM, 2004 Chapter 13.2,,NZWWA, 2003)
Enteric viruses	Requisite weight of sample based on moisture content (to give < 1/4 g dry weight) and processed based on the method modified from the PhD thesis "Detection of human enteric viruses in the environment" by David. H. Green (1996), which involves virus isolation using Beef Extract followed by PEG. Concentrated samples were grown on 293 N3S - Human embryo Kidney cell lines and plaque-forming, suspect flask contents were confirmed by Adenovirus Direct Immunoflourescent Assay using adenovirus antibody kits.
Helminth ova	Requisite weight of sample based on moisture content (to give < 1/4 g dry weight) and proceeded based on the modified US/EPA method based on sodium nitrate flotation and concentration and further optional treatments with diethyl ether, acid-alcohol solutions

	to give a clear sample for microscopic examination (Simonart *et al*, 2003) For viability testing, the final prepared sample was incubated at 22°C for a minimum of 4 weeks and then examined under the microscope for any viable helminth ova.

Initially all the samples were tested for presumptive helminth ova based on microscopic examination with no viability studies carried out. However when some of the samples showed suspect helminth ova, which were difficult to identify or be confirmed for viability, the samples were cultured at 22°C for a minimum of 4 weeks and then examined microscopically.

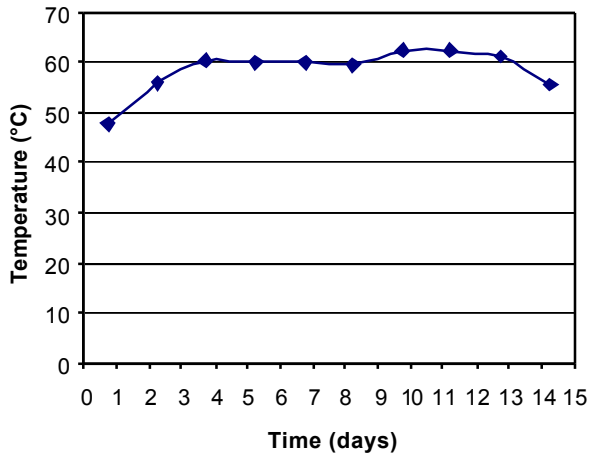
3 RESULTS AND DISCUSSION

3.1 COMPOSTING TEMPERATURES

During co-composting, the combination of heat generated and the time taken for the whole composting process kills or deactivates the pathogens, and within a certain number of days a 95-100% pathogen die-off is observed.

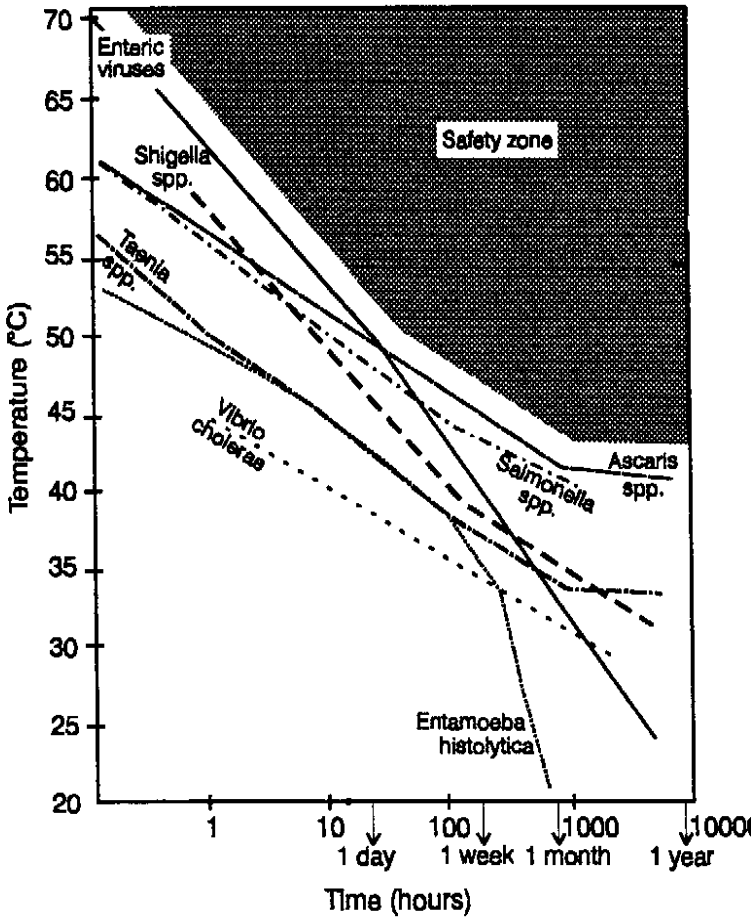
Figure 1 represents the temperature conditions during the composting process, as provided by the compost operator.

Figure 1: Typical temperature progression (pers.comm.)



The graph is in agreement with the Guidelines (NZWWA, 2003) recommended temperature of ≥ 55°C for ≥ 3 days for in-vessel composting. A number of researchers have reported time/temperature relationships for the inactivation of pathogens (Carrington, 2001; US EPA, 1999; Gallizzi, 2003). In essence, the survival of micro-organisms is a function of the local temperature, the specific pathogen and any antagonistic conditions. As shown in *Figure 2*, Strauch *et al* (in Carrington, 2001) collated a single graph indicating a 'safety zone' where the resultant sludge could be made pathogen-free at temperatures to the left of the zone. However, it must be noted that these data were based on pure culture studies while in reality, warming of the sludge, inhibitory factors, sludge thickness, different sensitivities of micro-organisms in the sludge environment and various other factors can affect the actual time/temperature relationships to a great extent.

Figure 2: Safety zones for different microbial indicators (by Strauch et al in Carrington, 2001)



3.1.1 INITIAL SLUDGE SAMPLE RESULTS

Initial testing of the sludge for the different microbial indicators showed the presence of *Escherichia coli*, enteroviruses, *Salmonella spp.* and suspected helminth eggs. *Campylobacter spp.* and adenoviruses were absent. *Table 3* shows the initial numbers of these indicators.

Table 3: Initial results for the sludge samples tested

Matrix	*Units*	WTP Sludge	WWTP Sludge	WWTP Sludge	WWTP Sludge	Other sources#
Sample Date		*09/03/2009*	*10/02/2009*	*20/04/2009*	*02/06/2009*	*Per g wet weight*
Helminth	*Per 4g**	9	2	2	4	$10^2 – 10^3$ (*Ascaris*)
Helminth cultured	*Per 4g**	Not done	Not done	Not done	< 0.98	10-10^2 (*Toxocora*) 5 (*Taenia*)
Enterovirus	*Per 4g**	303	498.6	<1.0	190	$10^2 – 10^4$
Adenovirus	*Per 4g**	<1.0	<2.0	<1.0	<1.0	*Not provided*
Campylobacter	*Per 25g**	<1.1.25	<1.25	<1.5	<1.375	*Not provided*
Salmonella	*Per 25g**	10	2.25	<1.5	7.5	$10^2 – 10^3$
Escherichia coli	*Per g**	2.4 x 10^5	9.2 x 10^5	> 9 x 10^5	13 x 10^6	10^6

**All laboratory results are based on dry weight;*
(Carrington, 2001)

The quantity and species of pathogens in sewage sludge can vary considerably both with time and location depending upon local circumstances and the current health of the local population. *Table 4* clearly proves this

point. The microbial density of the sludge indicates that a 10^4 reduction of *E.coli*, 10^2 reduction of *Salmonella* and a lesser reduction of the other indicators present can achieve a biosolid of grade A as per the Guidelines (NZWWA, 2003).

3.1.2 COMPOSTED SAMPLE RESULTS

Figures 3 and 4 show the changes in the different microbial indicators over the 8-month period. *Escherichia coli* has been excluded from *Figure 3* and is plotted separately in *Figure 4* since its numbers are too high to go on the same graphs as others. Also, wherever the numbers have been less than the minimum detection limit, they have been changed to zero to make it feasible to plot on the graph. *Table 4* summarises the actual results for the different microbial indicators during the period of study. *Figures 3 & 4* and *Table 4* clearly indicate that there is a significant drop in the indicators which were found to be initially present in the sludge sample i.e. *Salmonella*, helminth ova, enterovirus and *E.coli*. The peaks observed in both the graphs are for the sludge samples. They are all below the MAV for each of them according to the Guidelines (NZWWA, 2003). Each of these are discussed separately in the next few sections.

Figure 3: Changes in biota for the full period (excluding Escherichia coli)

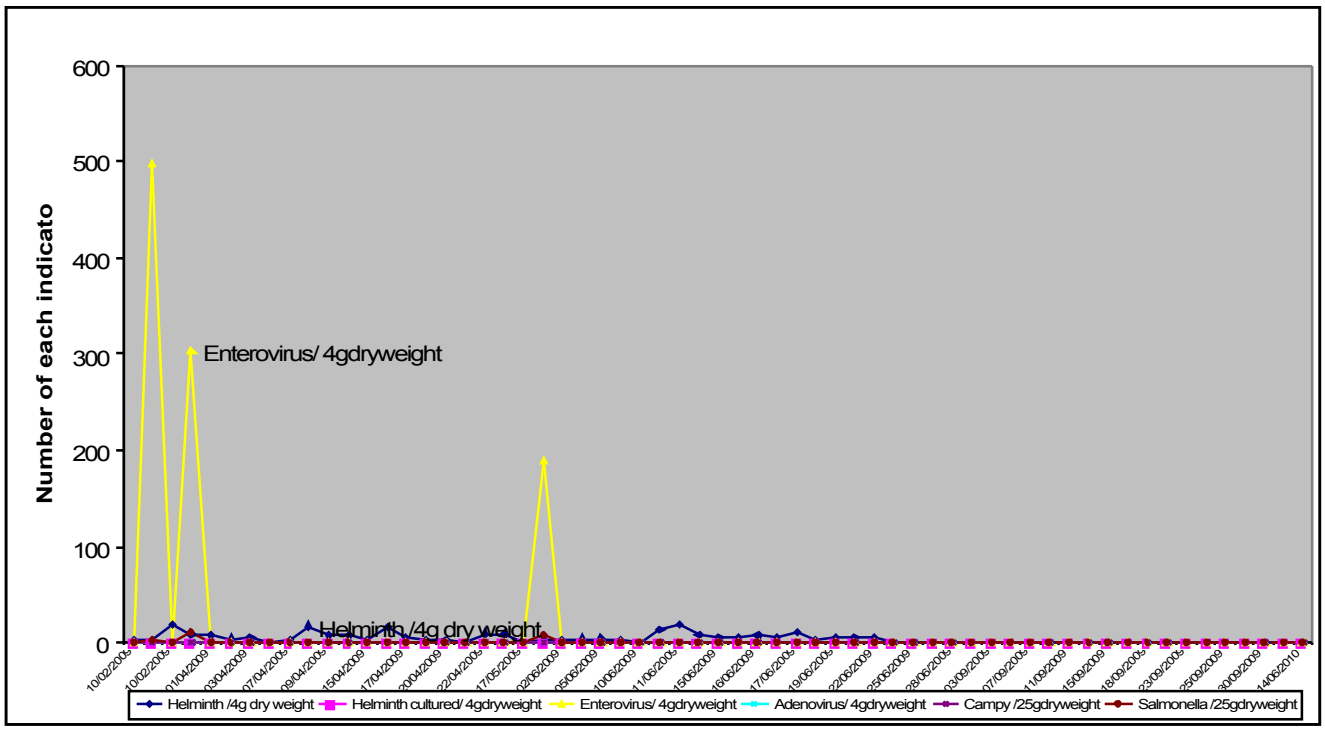


Figure 4: Changes in Escherichia coli over the period

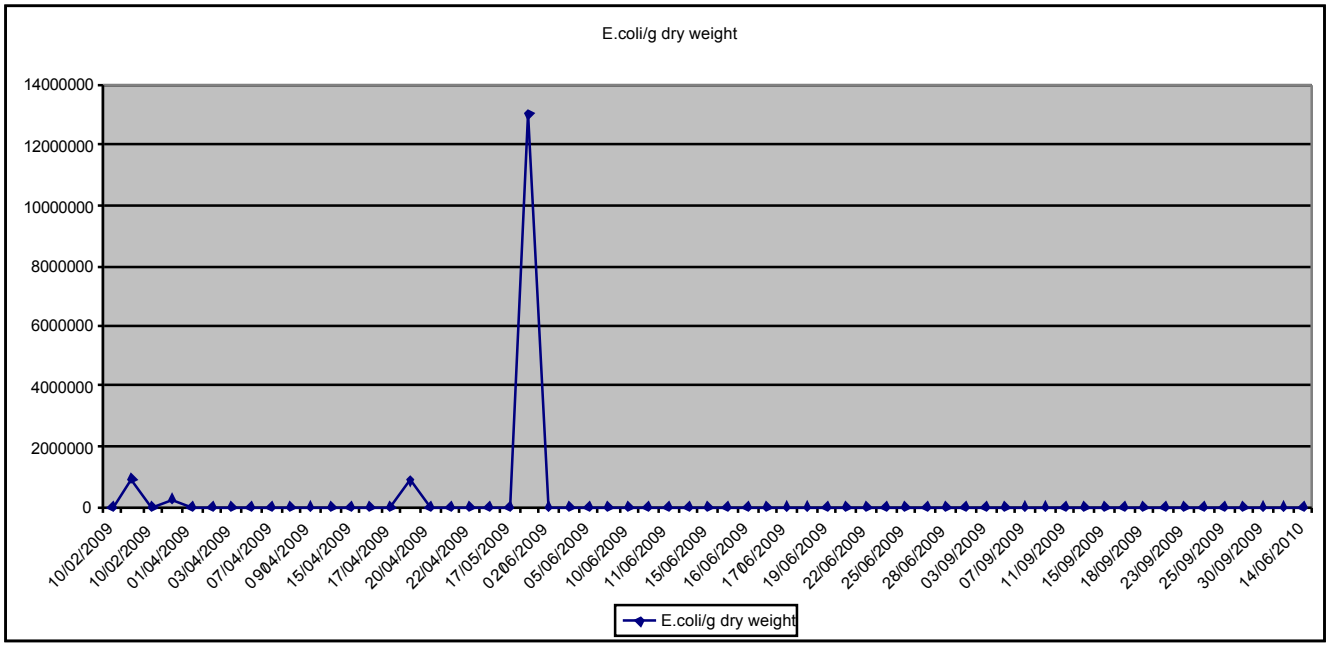


Table 4: Observed results for sample over the 8-month period of study

	Matrix	**Sample Date**	**Helminth per 4g**	**Helminth cultured per 4g**	**Enterovirus per 4g**	**Adenovirus per 4g**	***Campylobacter* per 25g**	***Salmonella* per 25g**	***Escherichia coli* per g**
Feb 2009	Beach Reserve Soil Site 2	10/02/2009	2	Not done	< 1.0	< 1.0	<0.5	<0.5	< 0.19
	Cemetery Soil Site 1	10/02/2009	20	Not done	< 1.0	< 1.0	<0.5	<0.5	< 0.25
	WWTP Sludge	10/02/2009	2	Not done	498.6	< 2.0	<1.25	2.25	920000
Mar 2009	WTP Sludge	09/03/2009	9	Not done	303	< 1.0	<1.125	10	240000
Apr 2009	Compost (15 samples)	01/04 to 24/04/2009	<0.82 to 18	Not done	<1.0	<1.0	<0.75 to <0.85	<75 to <0.85	<0.36 to 35
	WWTP Sludge	20/04/2009	2	Not done	< 1.0	< 1.0	<1.5	<1.5	> 900900.0
May 2009	Compost - Tank End	17/05/2009	< 0.8	Not done	Not done	Not done	Not done	Not done	Not done
June 2009	Compost (16 samples)	02/06 to 28/06/2009)	<0.75 to 20)	<0.55 to <0.9	<1.0	<1.0	<0.525 to <0.85	<0.525 to <0.85	<0.25 to <0.41
	Compost - Helminth Only	10/06/2009	<0.92	< 0.92	Not done	Not done	Not done	Not done	Not done
	Compost (Sample #2)	16/06/2009	6	< 0.81	Not done	Not done	Not done	Not done	Not done
	Compost Maturing Shed	22/06/2009	6	< 0.82	Not done	Not done	Not done	Not done	Not done
	Compost Maturing Shed	28/06/2009	<0.82	< 0.82	Not done	Not done	Not done	Not done	Not done

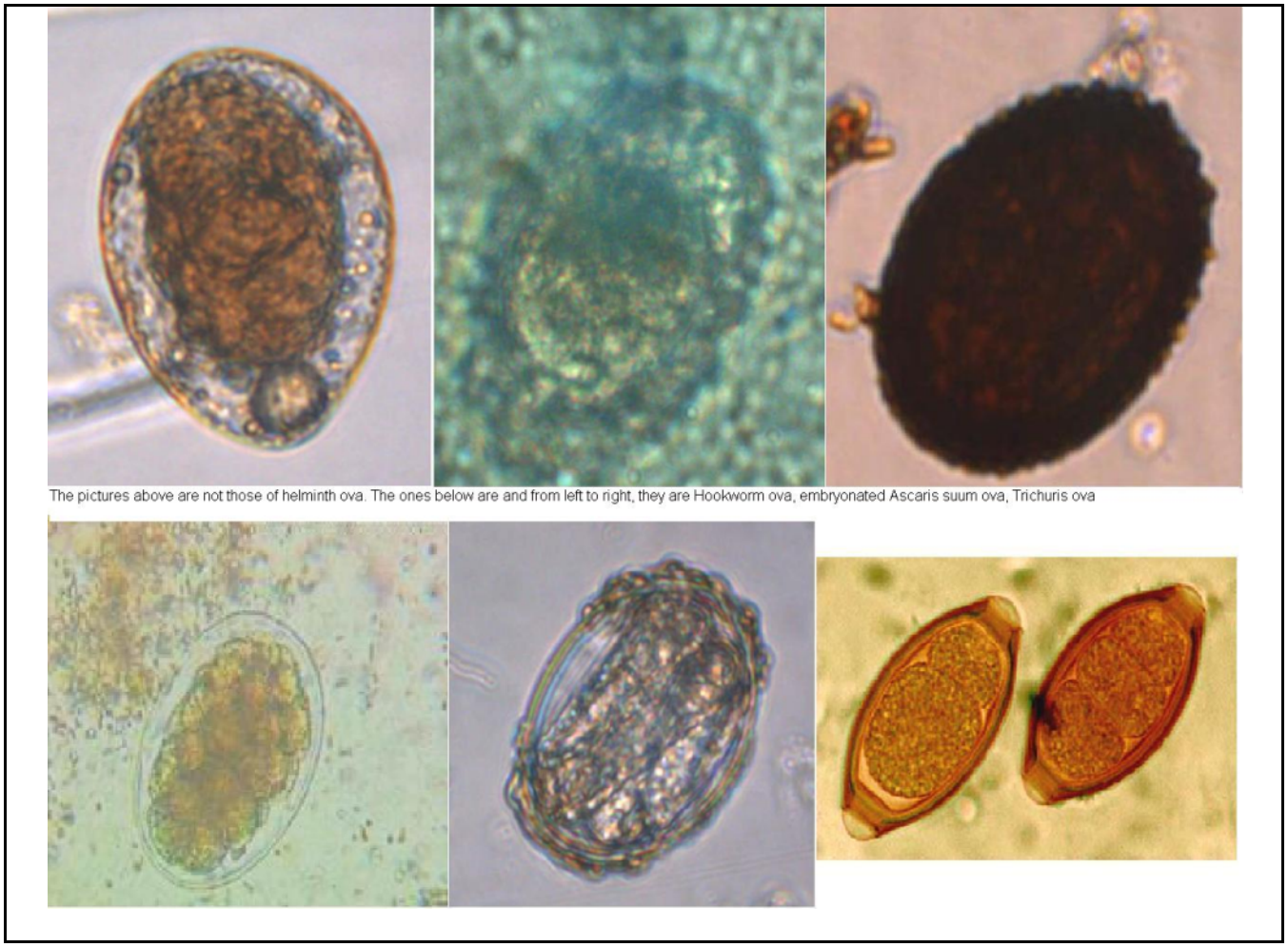
	Side Hatch-Helminth only	17/06/2009	6	< 0.85	Not done	Not done	Not done	Not done	Not done
	Side Hatch-Helminth only	19/06/2009	2	< 0.81	Not done	Not done	Not done	Not done	Not done
	WWTP Sludge	02/06/2009	4	< 0.98	190	< 1.0	<1.375	7.5	13000000
Sept 2009	Compost (15 samples)	03/09 to 30/09/2009	Not done	<0.17 to <0.38	<1.0	<1.0	<0.5 to <1.0	<0.475 to <1.0	<0.3 to 7.2
Jun 2010	Beach Reserve Soil Site 2	14/06/2010	Not done	Not done	< 1.0	Not done	<0.425	<0.425	1.1
	Cemetery Soil Site 1	14/06/2010	Not done	Not done	< 1.0	Not done	<0.65	<0.65	8.5

(**All results are based on dry weight*)
Results shaded in light grey are results for sludge and expected to be outside MAVs
Results shaded in dark grey are out of MAVs for a biosolid to be accepted as Grade A

3.1.3 CHANGES IN HELMINTH OVA DURING THE TEST PERIOD

Of all the pathogen groups mentioned in this paper, representatives of helminth ova are the most resistant to high temperatures for a longer period of time. It can therefore be assumed, that if all helminth ova in the compost are dead or deactivated, all other pathogens have also been removed. In the present study, samples from February to May 2009 were tested for presumptive helminth ova only (*Table 4*). The processed samples were examined microscopically but were not incubated further to check for viability. These showed the presence of some suspect helminth ova which could not be confirmed as viable and pathogenic. Many of these ova seemed to be those of hookworms, and have been reported to be sensitive to compost temperatures (100% removal after 24 hours at temperatures above 35°C, Obeng, L.A; and Wright, F.W, 1987). Hookworms are also difficult to identify due to their close similarity to other non-helminth ova or other artifacts. *Figure 5* gives a brief idea of how some helminth ova can appear similar to some non-helminth ova or artifacts

Figure 5: Some helminth ova and non-helminth ova or artifacts



With these difficulties in mind, the samples from June 2009 onwards were cultured at 22°C for a minimum of 4 weeks to determine the viability of the suspect helminth ova. Microscopic examination of these samples showed that none of these samples contained viable ova (*Figure 3*). This is in close agreement with observations made by other researchers (Gallizzi, 2003). Helminth ova, mainly *Ascaris* and *Trichuris*, have been found in composting facilities (Yanko, 1988; Gallizzi, 2003). Many of the *Trichuris* ova observed by these authors have been of non-human origin and different in size than the human parasite, *Trichuris* trichiura ova. They also did not find any viable *Ascaris* after incubation, as is concurrent with the present study. The WHO Guidelines for field irrigation with wastewater asks for an average of <1 *Ascaris*, *Trichuris* or hookworm eggs, in the final product. (Gallizzi, 2003).

Ascaris species is one of the most resistant of the helminth ova and a good indicator for the presence/absence of other viable helminth ova and pathogenic indicators. Through an extended literature review, Faecham et al (1983) came up with the following derived equation for the time in hours (t) required attaining no viable organisms at different temperatures (T) for *Ascaris* ova:

$t = 177^{*}\ 10^{-0.1922(T-45)}$ (Gallizzi, 2003) (1)

Based on this equation, theoretically, inactivation of all *Ascaris* eggs could be achieved if the temperature of the compost heaps exceeded 45°C for at least 5 days, 44°C for at least 8 days, 43°C for at least 12 days, 42°C for at least 19 days and so on. However, in reality there are a number of factors which could affect this time-temperature relationship including the temperature profile throughout the compost, turning frequency of the heap, number of helminth eggs originally present in the sludge, position of these eggs etc.

A study on the effect of various factors in co-composting on helminth eggs (Gallizzi, 2003) was carried out by a group in Ghana. They concluded that faecal sludge which was highly contaminated with helminth eggs, mainly *Ascaris* and *Trichuris*, when co-composted resulted in a highly safe product where the helminth ova were inactivated. Their report showed that the temperature of the heaps ranged from 43.5°C to a maximum of 58°C. The consistent higher temperature in the present study and the initial lower count of helminth ova in the sludge indicates that any helminth ova would be expected to be inactivated or killed during the composting process, as was observed. Based on our findings and reported data, it can be safely inferred that the initial samples, whose viability was not done, would not have contained any viable helminth ova at the temperature of the compost.

It must be noted that getting a representative sample, loss of ova during processing in the laboratory, similarity of ova to other artifacts and getting a clear sample without particles attached to or masking the ova, are some of the difficulties in detecting and identifying the parasitic forms.

Some of the samples in the current study showed the presence of some free living nematodes after the incubation period. The fact that helminth eggs need a host to grow into a full adult, confirms that these nematodes are not helminth and also that the suspect eggs seen earlier could be of these free-living nematodes. These being non-parasitic are not of any concern for this study.

3.1.4 CHANGES IN *E.coli* DURING THE TEST PERIOD

The initial counts for *E.coli* in the sludge was relatively very high as compared to all the other indicators as could be expected in a sewage sludge sample (*Figure 4 and Table 4*). With composting their levels dropped by 5 logs or more to counts lesser than the Maximum Acceptable Values according to the Guidelines (NZWWA, 2003). Larney *et al* (2003) reported more than 99.9% elimination of E. coli in the first 7 days of windrow composting with temperatures ranging from 33.5 to 41.5 ºC (92 to 107°F). Other papers have reported reduction of *E.coli* during composting (Arthurson,V, 2008; Yanko, 1988). The composted product in the present study has been applied on two sites. Though these sites do show some presence of *E.coli*,, these are well within the acceptable value of < 100 MPN per g and in accordance with the Guidelines (NZWWA, 2003). Other regulating authorities like US EPA(1999) NSW EPA(1997) have set a much higher limit of <1,000 MPN/g for *E.coli.* USEPA does so because this limit relates to a lower number of S*almonella* and hence the need to monitor only one of these two indicator pathogens. New Zealand Guidelines (NZWWA, 2003) have a much lower limit and require that both the pathogens be monitored. *E.coli* is the only pathogen indicator that needs to be monitored in routine sampling..

3.1.5 CHANGES IN OTHER BIOTA DURING THE TEST PERIOD

With regards to the other microbial indicators including *Salmonella*, *Campylobacter* and the enteric viruses (Enterovirus and Adenovirus), the observations made for them are similar. Some of the sludge samples showed presence of Enteroviruses and *Salmonella*.

Studies in Denmark (Carrington, 2001) have suggested that faecal streptococci (enterococci) are more resistant than E. coli and most pathogenic viruses and bacteria. Hence, if E.coli is inactivated then we can expect enteroviruses to be absent too. Yanko (1988) did not find any virus of concern in their compost samples too.

Salmonellae are representative of the vegetative enteric pathogenic bacteria; those which are most likely to be present in high numbers in sludge originating from faecal material. It has been reported (Carrington, 2001) that *Salmonella* have a relatively lesser survival times than *Ascaris* ova in sludge applied to soil surface. Yanko (1988) detected *Salmonella* more frequently than the others. Although Salmonella did die off in the compost, they found that it regrew later. This is usually a problem with free-living pathogenic bacteria. In the present study since no Salmonella was detected in the composted product (concurrent with Mena *et al*, 2003) there should be no cause for concern. However monitoring the product for regrowth or recontamination may be necessary.

Adenoviruses and *Campylobacter* were absent in the initial sludge samples and the composted samples and hence not of concern in this study. Survival times for these indicators have been reported to be as follows (*Table 5)* and they are within the safety zone shown in *Figure 2*.

Table 5: Survival times for certain pathogen indicators

Pathogens	**Survival time (days) in sludge at 20-30°C**
Viruses (Enterovirus):	< 100 but usually < 20
Salmonella spp.:	< 60 but usually < 30

Obeng, L.A. and Wright, F.W. (1987)

4 CONCLUSIONS

The present study has shown that co-composting of sewage sludge from the provincial wastewater treatment plant with shredded greenwaste in an in-vessel system has been effective in reducing the indicator pathogens in the sludge viz. *E.coli*, S*almonella*, enterovirus and suspect helminth ova to levels below the maximum acceptable as per the Guidelines (NZWWA, 2003). The fact that no adenovirus or *Campylobacter* could be detected in any of the sludge samples is an added advantage in the composting process. The composted product was applied onto two sites and these have also shown no indication of harmful levels for any of the pathogenic indicators. Since the helminth samples from June onwards showed no signs of viable, pathogenic ova, it can be safely inferred that the earlier samples from April would also not have any detectable, viable ova. All in all, if the biosolid meets the required standards for other contaminants it can be safely used for land application after attaining the resource consent.

Since previous reports from other authors have mentioned regrowth or recontamination of some of the free-living pathogen indicators like *E.coli* and *Salmonella*, consistent monitoring of the composted product may be necessary to check that they are under control. This can happen if there is contamination from various sources.

Compost stability is an important characteristic which if ensured can reduce the potential for recolonisation of the material by human pathogens such as Salmonella spp. This is usually ensured by the end of an actively managed composting phase. Compost, which is mature, is also stable and will not decompose to give undesirable products with lesser oxygen or nitrogen content or presence of phytotoxic compounds. Compost maturity is defined as the condition where compost poses no adverse effects on plants or wherever it is applied.

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