Issue No.7 Winter 2014

Putting waste to work

A CENTRE FOR INTEGRATED BIOWASTE RESEARCH PUBLICATION

Centre for Integrated Biowaste Research

Inside this issue

Update from the Programme Manager

Welcome to the winter 2014 CIBR newsletter.

It's been a very busy start to the year with what has become our annual CIBR workshop. A packed programme brought together members of the waste water industry together with researchers to discuss, understand and debate resource recovery and reuse of biowaste. Our keynote speaker, Tung Nygen, Product & Asset Manager at Sydney Water gave a well-received whistle stop tour of "Sydney Water's 25 years' experience in beneficial use of biosolids". The workshop was also the forum to welcome a new addition to the CIBR team – led by Louise Weaver from ESR, and with collaborators from NIWA, this group have been investigating virus removal in waste water. Louise and her team are a great addition to the capability of CIBR and we all look forward to working with them. The workshop was a great success and I'd like to thank everybody who helped with organization, especially Sarah Quaife, and thanks to all our presenters. If you have any ideas for topics for the workshop next year, please let us know.

Once again this year the CIBR workshop preceded the annual Land Treatment Collective Conference, we would like to take this opportunity to thank the NZLTC for their support of CIBR. At the conference this year seven scientists from the CIBR presented their research; and CIBR PhD student Dharini Paramashivam won the best junior presenter award – well done Dharini! Another person who needs a mention is Marie Dennis, not only did she organise the LTC conference in her role as the Technical Manager, but she also presented her research on Earthworm immune cells as indicators of toxicity, a highly technical topic which, she communicated excellently. (Read more on page 10).

This quarter we also welcome a new member to our Advisory Group – Associate Professor David Horne, a Professor in Soil Science at the Institute of Agriculture & Environment at Massey University. CIBR has worked with Dave for a number of years on the greywater project and we are really pleased to add his expertise to the Advisory group.

The 'Up-the-Pipe' solutions project team are continuing their work with a recent article published in Consumer Magazine "Germ warfare – Dishing the dirt on antibacterial soap" (Issue 544, March 2014). The team also recently took part in the Wellington Education Festival with Corinna School – read more about this fantastic day on page 3. We hope to continue this highly rewarding work and have submitted funding proposals – fingers crossed!

The team have all been working hard to publish the work of the last three years in peer reviewed journals – the scientists bread and butter. Look out for the work on "Assessing the impacts of chemical cocktails on the soil ecosystem" and "Can manuka (Leptospermum scoparium) antimicrobial properties be utilised in the remediation of pathogen contaminated land?" soon to be published in Soil Biology and Biochemistry, along with other papers submitted this quarter – thanks for all your hard work everybody.

I hope that you enjoy this quarter's newsletter and please let us know if you would like to publish an article – we'd love to hear from you.

Jacqui Horswell

UPDATE FROM THE SOCIAL AND CULTURAL TEAM:

Developments in Kaikōura and research presentations

Lisa Langer, Jamie Ataria, Jinny Baker, Alan Leckie and Joanna Goven

The Social and Cultural Team has been feeding news of the Kaikōura District Council's (KDC) acceptance of the Kaikōura case study report recommendations back to the community. They drafted a newspaper article which featured in the Kaikōura Star in mid-December 2013 and made a presentation to Te Korowai o Te Tai o Marokura (the Kaikōura Coastal Guardians) in March 2014, as a number of their members participated in our community engagement.

Jamie Ataria, Alan Leckie and Lisa Langer have had a number of positive meetings/discussions with KDC's CEO and new engineer. At present, the council are exploring the opportunity to spread the stockpiled biosolids onto an area of about 2 ha at the Wastewater Treatment Plant. Their aim is to explore planting this land with native species, such as mānuka, ngaio and karamu. Initial reaction of Te Rūnunga o Kaikōura Environmental Manager and Te Korowai was that they were supportive of the conceptual plan to use the stockpiled biosolids to rehabilitate local areas with native trees. However, they have not received a detailed plan to consider any area and have not evaluated the specific wastewater treatment site concept. This proposed process will take longer than the application of the biosolids to a plantation forest. Also there will be a significant cost to establish the native plants, particularly the cost of growing the seedlings for planting.

Although this rehabilitation option has not been evaluated and costed, it does demonstrate that the engagement process has equipped key individuals in the community to ask the necessary questions and understand the issues and impacts of solutions. It also demonstrates that community biowaste issues and other 'wicked problems' can be managed through collaborative partnerships. The Kaikōura community now has the skills to have further engagement and hence, improved science citizenship.

Jamie Ataria and Lisa Langer talk biosolids.

Social and cultural research has been showcased at the recent CIBR workshop and NZ Land Treatment Collective conference in Hamilton. Jamie Ataria and Jinny Baker gave an excellent workshop presentation on tapu to noa: Māori cultural views on biosolids management, drawing on interviews with the Mokai and Taupō communities. Lisa Langer presented the Life Cycle Assessment (LCA) of biowastes for rural communities, and outlined the LCA which was undertaken with input from the community as part of the Kaikōura case study approach, while promoting the use of LCA to determine impacts of biowaste solutions. Lisa Langer also presented a paper to the conference on the community decision-making process for biosolids reuse for Kaikōura which is leading to a community engagement framework.

In a leap towards new research, Jinny Baker has submitted a Community Environment Fund application to the Ministry for the Environment for a project entitled 'The Porirua Streamside Science Challenge': strengthening the capability of science, schools, iwi, local government and communities to tackle 'wicked' environmental problems. This project will bring collaboration with many partners including the New Zealand Council for Educational Research, Enviroschools, Te Rūnanga o Toa Rangatira, Victoria University of Wellington, and many other local groups and businesses.

CIBR involvement with the Wellington Festival of Education

By Sarah Quaife

On the 29th March 2014, the Wellington Festival of Education was held at TSB Bank Arena and Frank Kitts Park.

This Festival was as a celebration of the achievements of New Zealand's world recognised educational system, allowing schools, institutions and learning centres to showcase their contributions. As part of the festival, the Centre for Integrated Biowaste Research (CIBR) Greywater team at ESR, manned a booth to showcase the work with Corinna School – a local primary school from Waitangirua, Porirua. The students are undertaking 'real world' research for an issue of concern to their community: sustainable greywater re-use.

Different soils from around the Porirua region have been planted and irrigated with greywater twice a day. The students will monitor their plants for any changes in appearance, taking note of leaf colour, leaf number, stem height and appearance. They will also collect the water (leachate) that comes out of the bottom of their plant pots to see what it contains. At the end of the experiment the soil and leachate will be subjected to chemical analysis at the ESR laboratories to try to answer some of the student's questions about the impacts of greywater on environmental health. For example; does it improve the soil or does it damage the soil? Where do the contaminants go? Do they stay in the soil or flow through the soil? For New Zealand soils, we just don't know. The students at Corinna will be applying science knowledge to address real-world challenges ("wicked problems") which they will then present to key stakeholders such as the Kapiti Coast District Council. The project builds on a smaller project, looking into the effect of greywater application on plant growth, begun in collaboration with students from Tirohanga School in the Waikato Region.

Members of ESR, students of Corinna School and their teacher; Caro Begg, were present to not only present their work but also to actively complete a part of the project while on-site, with festival go-ers looking on and relaying queries as to the purpose of the activity and the aims of the project.

Overall, the day was a huge success, with many interested parties talking with the students and the team; including, foreign dignitaries, members of the press, and various education groups and members. An article was published in the Dominion Post relating to the work and sparking interest within the local community.

Participation in this event is an example of how collaborative partnerships are extremely beneficial to extending science within the community, engaging students and, by association, the general public, both locally and further afield, in a crucial area of science development and growth. Further work with student bodies is vital to increase growth in research areas and gain vital input for not only the current generation, but generations to come.

CIBR presence: ESR team members at the Festival booth. From left: Sarah Quaife and Jacqui Horswell.

Students from Corinna School, after collecting a soil sample under guidance from CIBR's Jacqui Horswell.

Corinna students getting down and dirty, preparing their soil samples for planting at the Festival booth.

Co-joint booth between ESR and Corinna School, showcasing display boards and photographs from the work to date.

Australian & New Zealand Biosolids Partnership

The Australia and New Zealand Biosolids Partnership (ANZBP) has, to some degree, moved on from the technical research focus that has existed over the past few years and is increasing its efforts in the policy and advocacy space.

The advisory committee of the ANZBP has acknowledged that a vast library of research exists in the biosolids space and that there are a number of skilled researchers in Australia and New Zealand able to address the technical challenges. The focus of the ANZBP of late has therefore been the development of two key member products:

1. A media response kit & protocol of response: a reference tool that ANZBP members can consult to inform the process and possible content of responding to media coverage or enquiries regarding biosolids matters. The tool will also inform “who” in the industry is best placed to lead any communications.

2. A code of practice for biosolids managers: a resource for biosolids managers across the areas of production, transport and spreading which will provide principles that a biosolids manager may employ to inform their decision making process. The document does not set out specific treatment and management requirements, but seeks to provide a common basis to promote avoidance of management practices that allow something to go wrong, rather than seeking to mitigate concerns.

In response to member queries, CIBR recently developed a resource for the ANZBP members exploring potential pathogens that may be in wastewater and sludge. The document summarises the vast volumes of literature in this field into a number of easy to read tables, which also considers the risks/hazards and pathogens of primary concern for both Australia and New Zealand. The document also includes information on survival and fate of pathogens in soil and vegetation; and pathways for exposure to waste-born biosolids – many thanks to Robina Ang and Jacqui Horswell at ESR for their great work on this product.

The ANZBP has a representative on the Standards Australia “mirror committee” for the International Standard currently being developed on Sludge Management. In order to ensure the Standard holds relevance for Australia and New Zealand, the outputs of the working groups (which consist primarily of representatives from European organisations) will be reviewed and commented upon by this mirror committee. While Standards New Zealand has not developed a similar mirror committee, the views of New Zealand will be considered via ANZBP. If you have an interest in this field, or any questions on the process, please contact the ANZ BP Program Manager.

The ANZBP is a subscription based program, formed in 2007 by the water sector, to place the beneficial use of biosolids on a sustainable footing across Australia and New Zealand. The ANZBP resides with the Australian Water Association (AWA) which has taken responsibility for implementing the program, and its agreed business plan. ANZBP Membership enquiries are welcome and can be directed to the Project Manager at admin@biosolids.com.au, additional information regarding the ANZBP can be found on the website www.biosolids.com.au.

UPDATE FROM THE SOIL SCIENCE TEAM:

Long-term effect of repeated biosolids application on soil chemical properties

By Jianming Xue

Fig.1 Soil samples of 4 layers (0–25, 25–50, 50–75 and 75–100 cm) were collected from the long-term biosolids research trial at Rabbit Island in Nelson.

The CIBR soils team collected soil samples of four layers (0–25, 25–50, 50–75 and 75–100 cm) from the long-term biosolids research trial at Rabbit Island in Nov 2013 and has now completed the analyses of soil chemical properties. The results showed that land application of biosolids significantly improved soil fertility of a radiata pine plantation at Rabbit Island.

Biosolids from the Nelson regional wastewater treatment plant have been applied to a 1000-ha *Pinus radiata* plantation at Rabbit Island since 1996. A research trial was established on the site in 1997 to investigate the long-term effects of biosolids application on soil and groundwater quality, tree nutrition and growth. Biosolids have been applied to the trial site every three years (1997, 2000, 2003, 2006, 2009 and 2012, respectively) at three application rates: 0 (Control), 300 (Standard) and 600 kg N/ha (High). Tree nutrition status and growth are monitored annually, soil properties every three years and groundwater quality quarterly. The latest soil sampling at the Rabbit Island biosolids research trial was completed in late November 2013 (Fig. 1). Here we update our recent findings on the beneficial effects of repeated biosolids application on soil properties.

In the top layer (0–25 cm), significantly higher total C, N and Olsen P but lower pH and exchangeable Ca were found for both the Standard and High treatments. In the subsoil layer (25–50 cm), the High treatment significantly increased total C, N and Olsen P while both Standard and High treatments reduced soil pH. Biosolids application had no significant effect on exchangeable cations, base saturation and CEC in this layer. In the layer of 50–75 cm, High treatment significantly increased total C, N and Olsen P while both Standard and High treatments had no significant effect on pH, exchangeable cations, base saturation and CEC. In the layer of 75–100 cm, both Standard and High treatments significantly increased total N, but had no significant effect on other soil chemical properties. The results indicate that the biosolids application, especially the High treatment, not only resulted in accumulation of total C, N and Olsen-P in the topsoil but also caused some movement of these nutrients down the soil profile. The accumulation of biosolids-derived C, N and P in this poor soil should be beneficial for the growth of radiata pine. However, the movement of N and P down the soil profile indicates the risk of groundwater contamination. The lower pH in both the

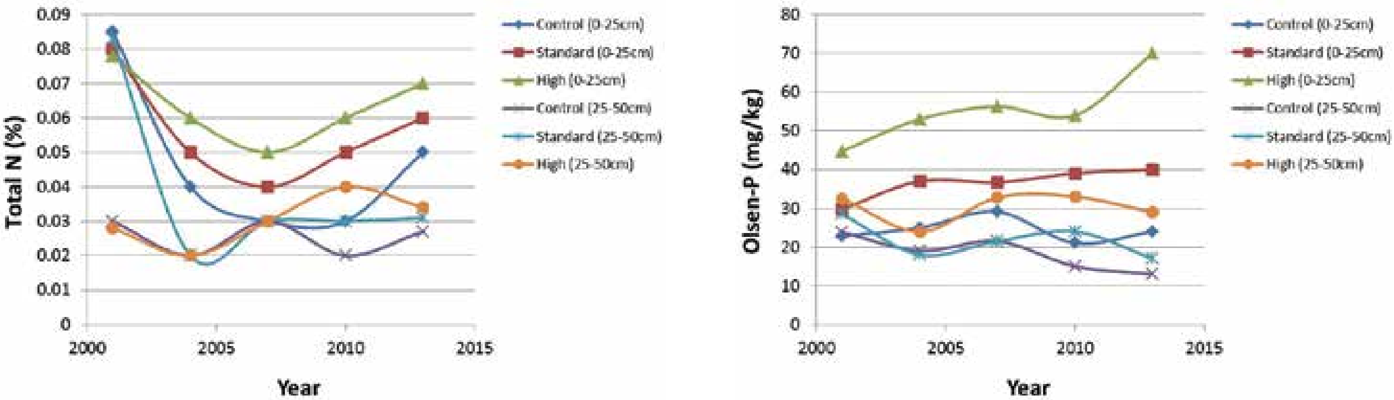

Fig. 2 Effect of repeated biosolids application on soil total N (Fig. 2a, left) and soil Olsen-P (Fig. 2b, right) over the years.

Standard and High treatments could be a result of the nitrification of biosolids-derived N.

Repeated biosolids application generally increased soil total C (data not shown), N (Fig. 2a) and Olsen-P (Fig. 2b) over the years, especially in the top soil layer (0-25 cm). Soil organic matter increases have often been shown to relate strongly to improvements in plant productivity on marginal lands.

We will report the fate and transport of biosolids-derived heavy metals in the litter layer on the forest floor and in mineral soil layers in the next issue of the newsletter.

A LIST OF NEW OUTPUTS

Conference paper and workshop presentation

Xue, J.M.; Kimberley, M; Thiart, J.; Wilks, P. 2014 Long-term effect of biosolids application on groundwater quality at Rabbit Island. *In:* Proceedings for the 2014 NZ Land Treatment Collective Annual Conference in Hamilton, New Zealand on 26-28 March. Pp 100-117.

Xue, J.M.; Kimberley, M; Wilks, P. 2014 Long-term biosolids application effect on soil chemical properties, tree nutrition and growth of radiata pine. *In:* Proceedings for the 2014 NZ Land Treatment Collective Annual Conference in Hamilton, New Zealand on 26-28 March. Pp 163-168.

Gielen, G J H, **Xue, J.M.**, Taylor, S C, Heaphy, M J. 2014 Microbial tolerance to triclosan after long-term biosolids application in Rabbit Island soils. *In:* Proceedings for the 2014 NZ Land Treatment Collective Annual Conference in Hamilton, New Zealand on 26-28 March. Pp 93-100.

Xue, J.M.; Kimberley, M. 2014 Increasing forest productivity and economic return through beneficial use of biosolids. A presentation for Biosolids Workshop in Hamilton on 25 March 2014.

SPECIAL INTEREST ARTICLE: "GREYWATER-WISE":

Assessing the risk of greywater application on home-grown vegetable gardens.

By Sarah Quaife

Greywater can account for 75% of wastewater produced by a domestic household. In water short areas, such as the Kapiti Coast, greywater reuse for irrigation is promoted.

The Kapiti Coast District Council (KCDC) has included water saving measures such as rainwater collection and greywater reuse as requirements for new developments in their District Plan. Although greywater reuse by sub-surface irrigation is recommended, this may still pose a risk to public health, and reports of greywater irrigation of vegetable gardens are common. While greywater is considered relatively clean and safe compared to blackwater, it may still carry pathogens including *Escherichia coli (E.coli), Campylobacteria, Listeria* and viruses.

To investigate the risks of contamination of food crops with pathogens originating from greywater, the "Greywater-wise" research program, lead by Dr. Alma Siggins (CIBR, ESR), has established a field trial in Otaki, Kapiti Coast, in association with Lowe Environmental Impact (LEI).

Three seed beds were constructed on a rural domestic property. Each bed is physically divided in two by timber and polythene plastic to prevent cross-contamination. Half of each bed is sub-surface irrigated with greywater from the property, while the other half of each serves as a control and is irrigated with freshwater from the local reticulated supply. During dry weather, all beds were occasionally supplementary irrigated with fresh water via a hosepipe. All six sections were planted with an identical range of selected vegetables: beetroot (root vegetable, grown underground, consumed cooked), radish (root vegetable, grown underground but consumption of leaves is also common, consumed raw or cooked), lettuce (leaf vegetable, grown above ground, consumed raw), and celery (stalk vegetable, grown above ground, consumed raw or cooked).

After approximately six weeks of growth, or when the plants are mature, uptake and internalisation of pathogens will be measured by testing the crops for the indicator organism *E. coli.* Analysis will be conducted on the leaves of the plants, and the root or bulb of the plant where relevant. Results of the greywater irrigated plants will be compared to those from the control plants. Leachate and soil samples will also be investigated for presence of *E. coli* and viruses.

This is one of the first research trials to investigate uptake of pathogens originating from greywater. Our results will be used to assist in developing a guideline for greywater reuse, to ensure the safety of homeowners carrying out such a practice.

Price premiums for selected eco-friendly products as determined from a small study in central North Island

By Richard Yao, Lisa Langer, Grant Northcott and Alan Leckie

In 2010 New Zealanders bought more than $300 million worth of personal care and household cleaning products (Albertson 2013). Many consumers are not aware that some product ingredients are detrimental to the environment.

Some regular toothpastes contain 0.3% triclosan. Triclosan helps prevent gingivitis and improves oral health. However, once triclosan reaches waterways, it can be harmful to the environment. At very high concentrations, it can act as an endocrine disruptor and can hinder muscle movement in fish. At low concentrations, triclosan is toxic to algae which serve as food for phytoplankton that are subsequently eaten by fish. Therefore, if algae are killed or their population reduced, this will reduce the food supply for fish.

Most dishwashing liquids contain Sodium lauryl sulfate which can be moderately toxic for freshwater fish and other aquatic organisms. Other household products contain formaldehyde which is a recognised carcinogen and skin irritant; and parabens which are preservatives and antimicrobial chemicals. The Environmental Working Group in the United States provides guidance on how to select household products that are less harmful to the environment (www.ewg.org/guides/cleaners). In Canada, manufacturers are not required to warn consumers about the negative health and environmental impacts associated with chronic, or long-term, exposure to chemical ingredients in household cleaning products (Suzuki 2014). The situation in Canada holds true for New Zealand.

To test the impacts of providing environmental information on subsequent product use, the Centre for Integrated Biowaste Research (CIBR) scientists conducted a small study on fourteen participants of a hui in Mokai (north of Taupō) in August 2013. This preliminary study could help determine if New Zealand consumers would pay a premium for eco-friendly household products.

One month before the hui, supermarkets in Rotorua and websites were visited to determine the prices of two groups of products:

(1) environmentally certified products which claim to offer consumers health and/or environmental benefits; and

(2) conventional products which do not have eco-friendly labels.

Based on supermarket and online prices in July 2013, an eco-friendly, organic

toothpaste can retail for five times the price as a regular toothpaste while an eco-friendly dishwashing liquid can be 25% (or around a dollar) more expensive than a conventional equivalent (Figure 1). Although eco-friendly products are usually more expensive, some consumers purchase them because they experienced a reduction in health issues (e.g. disappearance of eczema and improvement of asthma) (NZTE 2013) or they would like to support sustainability and environmental initiatives (Colmar Brunton 2013).

Manufactured household cleaning or personal products which claim to be eco-friendly remain at the early stage of sales in the market but with a growing demand (Colmar Brunton 2013). We tested whether a small sample of people attending the hui in Mokai will be willing to pay more to shift

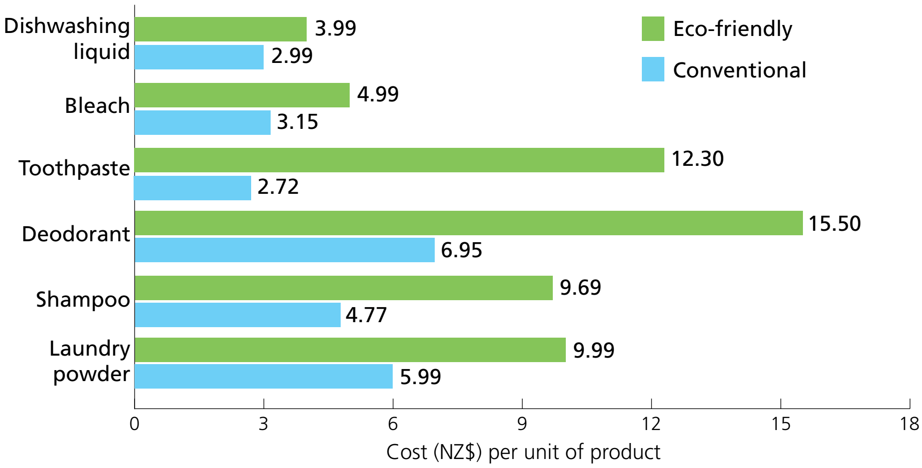

Figure 1: Prices of selected conventional and eco-products at Rotorua supermarkets and on-line, July 2013.

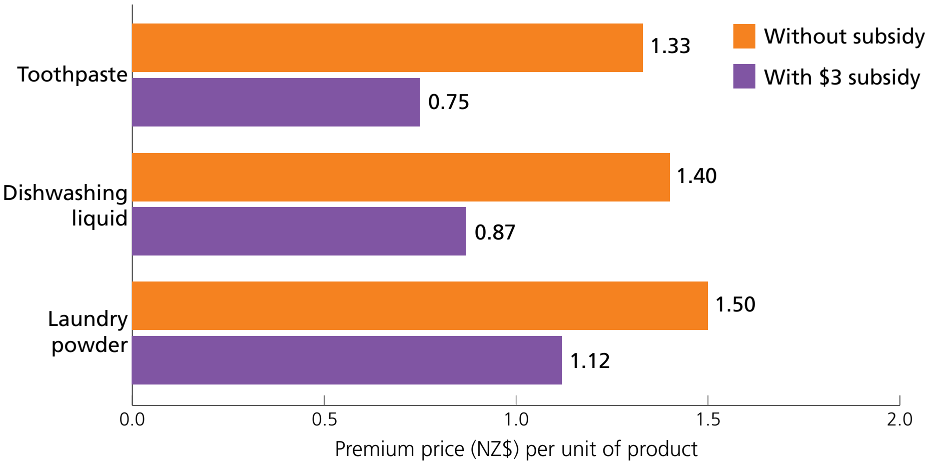

Figure 2: Price premium for the top three products with and without $3 subsidy

from using conventional products to eco-friendly products.

Mokai is a Māori rural community with a population of around 300. The Mokai area has social and economic ventures such as a marae, a pre-school, a primary school, livestock farms (sheep and cattle), geothermal power plant, commercial vegetable production and a milk powder processing plant.

Hui participants included members of the Turopaki Trust, parents of the local primary school and representatives from the Taupō District Council and Waikato Regional Council. The morning session of the hui included demonstrations of how to make a few home-made household products using ingredients that can be commonly found in households, for example; a lemon sugar hand scrub, foot care powder and coconut oil toothpaste.

In the afternoon session of the hui, we conducted a 25-minute focus group exercise to test if any of the adult participants would pay a premium for eco-products. Fourteen participants joined the focus group, eight of which were male. Almost all participants were aged above 46 years old, with only one being less than 35 years old. Participants in the hui generally had strong concerns about the need to protect and preserve both the environment they lived in and the wider New Zealand environment. Therefore they were likely to be more receptive to shift from using conventional cleaning products to eco-friendly products than typical adult New Zealanders.

By presenting a list of six common household products, each participant was provided with three sticky dots and asked to use these to identify the top three household products each would consider switching from to a more environmentally friendly product.

The majority of the participants chose laundry powder, dishwashing liquid and toothpaste while bleach, deodorant and shampoo were the least likely products that would be switched.

We then asked each participant the amount they would be prepared to pay to switch to each of the top three products. We asked this question using two approaches. In the first approach, we handed each participant a $3 subsidy (6 pieces of 50-cent coins) which they allocated to the three products. In the second approach, the subsidy was removed and the participants told they needed to absorb the extra cost of switching to eco-friendly products.

Results showed that, for both approaches, a participant would spend the most money on switching to an eco-friendly laundry powder. For the approach with the $3 subsidy, thirteen out of fourteen participants allocated the subsidy to cover the switching costs. Laundry powder got the highest average premium price of $1.12, followed by dishwashing liquid at $0.87 and toothpaste at $0.75 (Figure 2). When the subsidy was removed, eleven out of fourteen participants reported they would be prepared to use their own money to cover the premium price. Interestingly, the eleven participants, on average, would pay higher than the situation with subsidy. Two main reasons reported by these participants as to why they would pay extra from their own pocket were “environmental concern” and “quantity of the products used”.

Participants were asked if they were already making or using household products out of natural ingredients in their homes. Just over half of the fourteen participants reported that they have already made and used homemade cleaning products such as baking soda, olive oil and vinegar for cleaning purposes within their homes.

We also asked participants that, out of those homemade products presented in the morning, which one would they try to create in their households. About half indicated that they would try to make their homemade toothpaste. On average, participants said they would be prepared to spend about 45 minutes per month to produce their own toothpaste. They also expressed interest in creating home-made hand cleaner, shoe deodoriser, hair rinse and bug spray.

Although this study was based on a very small sample of informed people, it indicated that increasing awareness or knowledge of alternatives that reduce their impact on the environment can encourage the use or creation of more eco-friendly household products. Since the sample size was small and participants came from a more focused segment of the population, results from the analysis should not be generalised for a wider population.

The dominant reason why the hui participants would pay more for eco-friendly products was their concern for the environment. The price premium found in this small study suggested that participants appreciated the efforts of product manufacturers in reducing the impact of their products on the environment. Some of the alternative less polluting chemicals used in eco-friendly products are quite similar to the chemicals they replace. Educating consumers about the numerous chemicals that can be present in household cleaning and personal care products is a critical issue, if they are to make informed decisions about what products they purchase.

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Updates from the ecotox team:

THE RESEARCH MANUSCRIPTS reporting the latest research outcomes from the programme are being submitted for publication in scientific journals. Marie Dennis (CIBR, Scion Research) and co-authors submitted the paper: "*In-vitro* sensitivity of coelomocyte cells from the earthworm *Eisenia fetida* to selected micro-contaminants," to Environmental Toxicology. Marie's research aimed to develop a new bioassay to evaluate the effects of contaminants on the immune system of earthworms. This is a key methodology in our efforts to characterise the potential effects of applying biowastes to soil on the critters likely to be exposed. This is why earthworms are a good model to study as they live in the soil and are likely to be directly in contact with micro-contaminants as they ingest soil particles. The assay described is lab based so that extracts of biosolids and soil can be used to mimic what happens in the field but under more controlled conditions. Marie was able to identify and purify immune cells (coelomocytes) and specifically; amoebocyte cells, of the earthworm *Eisenia fetida* that are involved in immune response activities. A reduced immune response can be associated with a higher susceptibility to diseases. Phil Scott is a PhD candidate at Griffith University who recently completed his research project looking at endocrine disruption in the Australian environment. Phil used both analytical and biological methodologies to estimate the level of those contaminants that can interfere with the normal functions of the endocrine system. Phil submitted the manuscript: "A national survey of trace organic contaminants in Australian rivers," to the Journal of Environmental Quality. Closer to home, Jason Strong, another CIBR PhD candidate with Waikato University is conducting a similar study across New Zealand, focussed on emerging contaminants. Jason is in the process of preparing manuscripts from the results of his research, co-supervised by Grant Northcott (CIBR, Northcott Consultants Ltd) and Louis Tremblay (CIBR, Cawthron Institute/University of Auckland).

THE WASTE MINIMISATION FUND "Up-the-pipe solutions" project is now completed. Jacqui Horswell and Louis Tremblay met with Heather Penny and Nigel Clark, both analysts with the Ministry for the Environment who were satisfied with the multiple outcomes from this multidisciplinary project which aimed at raising awareness around the consequences of our daily activities. The project attracted much attention from the media and some great general articles were produced, helping to promote aspects of the project. Reporter Fiona Terry wrote: "Good slop, bad slop," for the *North & South* January 2014 issue (p 72-75). This directly led to the more process focussed article: "Germ warfare – Dishing the dirt on antibacterial soaps," written by research writer Jessica Wilson and printed in the March 2014 issue of the *Consumer Magazine* (p 8-10). William Ray, science reporter with Radio New Zealand wrote an article for 'The Wireless,' on: "Antibiotic resistance: An evolutionary arms race," (http://thewireless.co.nz/themes/hauora/antibiotic-resistence-an-evolutionary-arms-race). This article presents links between the presence of antibiotic and anti-microbial residues in the environment and potential human health issues, mainly the increase in microbes that are resistance to mainstream drugs. The CIBR team is developing new projects to continue to grow and enhance this very important field of research on solution processes in order to reduce our pressure on the environment.

GRADUATE STUDENT PROFILE

Assay developed by CIBR team at Cawthron is being used in other research fields. Phoebe Argyle is an MSc candidate at the University of Auckland and Cawthron Institute. Her project "Toxicity assessment of marine dinoflagellates using echinoid fertilisation and fish embryo toxicity (FET) bioassays," aims at characterising the toxicity of algal natural toxins. Phoebe is using the zebrafish FET which has been developed as part of the Biowaste programme and she has developed a new toxicity test using the New Zealand sea urchin Evechinus chloriticus.

Figure 1: Control sea urchin embryo.

Figure 2: Zebrafish embryo, 96 hr hours after exposure to a toxin extract.

Worm bioassays for determining toxicity

By Marie Dennis, Sean Taylor, Scion

A number of vermicomposting trials have been undertaken by CIBR to determine if vermicomposting is an effective means of further stabilising different types of biosolids and whether the presence of emerging contaminants has an effect on the vermicomposting process and health of earth worms. These trials have monitored pathogen reduction, C:N ratios, pH, volatile solids, nutrient levels and heavy metal and persistent organic pollutants (POP's) in the vermicompost along with worm mortality, cocoon production and adult survival.

Additional studies were also conducted to determine worm health and immune function following exposure to biosolid mixtures and contaminants.

Earthworms have been a recognised species in ecotoxicity studies for a number of years. They have developed cellular defense mechanisms which enable them to survive in what are often hostile environments. Their immune cells are able to recognise, engulf and excrete foreign matter and objects such as bacteria; however, this function can be compromised in the presence of contaminants.

The first line of defense for a worm, is its epidermis or body covering which acts as an antimicrobial layer. A digestive tract runs the length of the worm body (Figure 1) and a a tissue layer supports internal organs and a cavity called the coelomic cavity. It is within this cavity that the earthworm immune cells (coelomocytes) can be found.

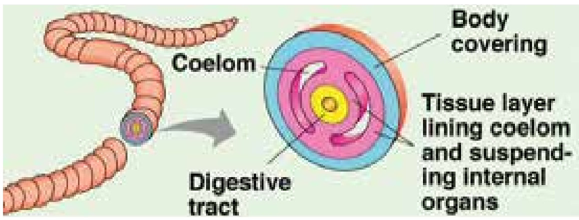

Fig 1. Cross section of earthworm body

We developed a non-invasive technique for extracting these immune cells from earth worms which requires exposure of worms to a 5% solution of ethanol – not enough to cause death but enough to anaesthetise and stress the worms, causing them to expel their immune cells. The worms are recovered, washed off and left to recuperate and the extruded cells spun down, washed and analysed by Flow Cytometry.

Three types of coelomocytes were differentiated and their identity confirmed by staining and microscopy. Eleocytes (Figure 2A) are thought to be responsible for cellular metabolism and nutrition while the two types of amoebocytes – hyaline (Figure 2B) and granular (Fibure 2C), are both responsible for encapsulation of foreign matter.

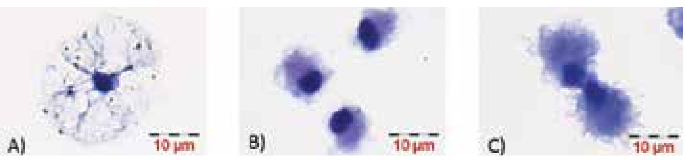

Fig 2. Leishman's Giemsa staining of flow cytometry sorted coelomocytes

The current thought is that hyaline amoebocytes are actually immature versions of the granular amoebocytes.

We investigated the differences in cell counts of total coelomocytes and their three types to determine if exposure to different biosolids and contaminants had an effect on vermicomposted worm immune cells. Four different vermicomposting trials were studied. The first trial involved vermicomposting of two very different biosolids – sourced from Kaikoura and Taupo. The Kaikoura biosolids had been stockpiled for a number of years whereas the Taupo biosolids had been collected fresh. Bulking agents were added to each biosolid as a carbon source and to assist aeration. These agents were locally available from the Taupo area and were aquatic lake week, broom, green house tomato prunings and pulp and paper process solids. Following a 20 week incubation and collection of samples for chemical and other analysis, 20 worms per treatment were collected and their immune cells extracted and counted.

There were significant differences in total coelomocytes counts between the two sites and also between amendments (Figure 3A).

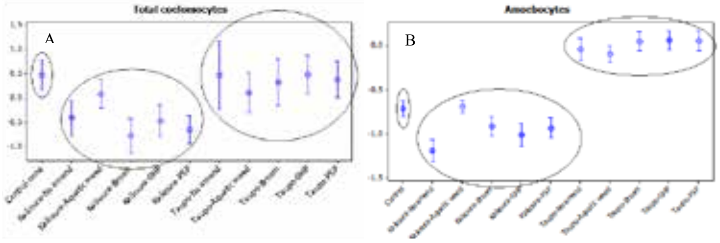

Fig 3. Total coelomocyte counts and amoebocyte counts in Kaikoura and Taupo biosolids treatments

Interestingly, the differences were most pronounced in the amoebocytes ('cleaners') with elevated numbers in the Taupo biosolids treatments and particularly compromised numbers in the Kaikoura biosolids without any amendments added (Figure 3B).

The next trial involved a small community's septic tank solids. These solids were dewatered to 25% and bulking materials of greenhouse tomato prunings and palm fibre were added. There were two treatments – one of 30% solids and the other 50% solids. The control in this trial had dairy shed solids substituted for the septic tank solids. This trial ran for 131 days after which the cells were harvested. The results from this trial were highly variable and the only significant differences were seen in the eleocyte populations, with the high septic tank treatment populations elevated compared to the control and low rate treatment.

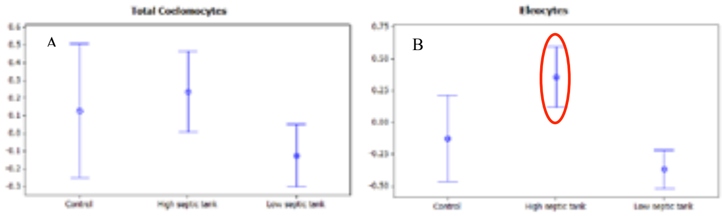

Fig 4. Total coelomocyte counts and amoebocyte counts in Septic tank biosolids treatments

The effect of adding emerging contaminants was then analysed and a compost comprising 70% sand, 20% kaolin clay, 10% spagnum peat and 1% dried horse manure for nutrition was made. Incremental doses of triclosan and Bisphenol A were then added. Significant differences in the total coelomocyte counts were seen in both bisphenol A and triclosan, at 280 mgkg-1 and 56 mgkg-1 respectively (Figure 5). Interestingly, at higher doses there was no effect, possibly indicating that there may have been an immune response at these levels and that at higher doses, the immune system had adjusted to the contaminants.

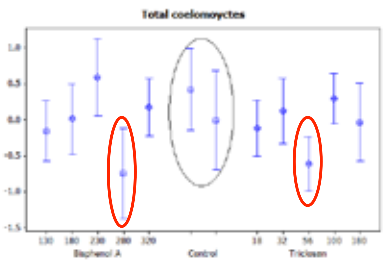

Fig 5. Total coelomocyte counts from worms exposed to contaminants

The last trial explored the addition of higher doses of contaminants, the introduction of carbamezepine and mixtures of the three. The same compost mixture as previous was prepared and contaminants added either as single contaminants or, 1:1 mixtures or, 1:1:1 mixtures. The trial was sacrificed after 28 days. There was concern in the trial results, in that the solvent control produced a highly significant effect.

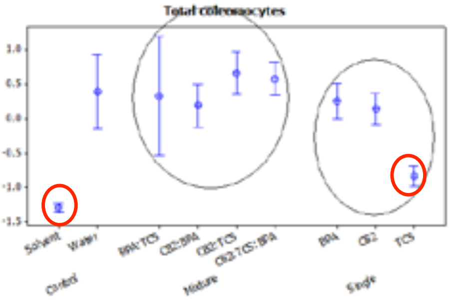

Fig 6. Total coelomocyte counts from worms exposed to single contaminants and mixtures

The method called for all contaminants to be dissolved in acetone as they are insoluble in water. Prior to worm addition to the compost mixtures, the contaminants and solvent control had been added to the compost then left for a period of time for all the solvent to evaporate. The results from the solvent control suggest that the evaporation time was insufficient for this treatment and we were seeing a toxic effect exerted on the worms due to the persistence of acetone in the compost. All the other treatments were not significantly different (apart from triclosan on its own) to the water control, thus supporting this theory. However, at 340 mg kg-1 triclosan showed a significant effect on total coelomocyte counts to a degree that suggests a toxic effect (Figure 6). Triclosan and bisphenol A also showed an effect on the eleocytes, both as single contaminants, and as a mixture of the two (Figure 7A).

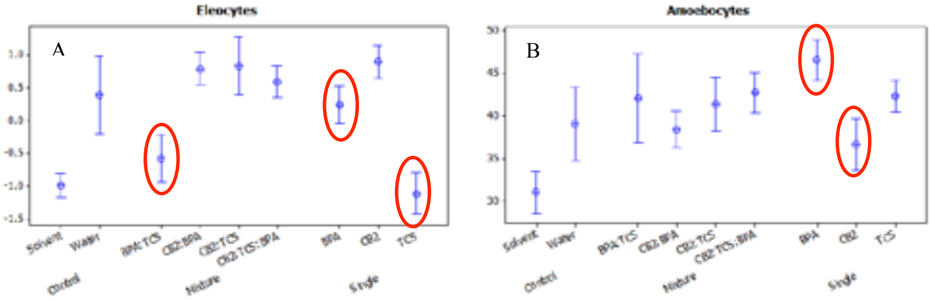

Fig 7. Eleocyte counts (A) and amoebocyte counts (B) from worms exposed to single contaminants and mixtures

Carbamazepine as a single contaminant showed a marked effect on amoebocytes and, interestingly, bisphenol A on its own (Figure 7B) showed an elevated count of amoebocytes. This was mirrored in the reduced counts of eleocytes, suggesting that perhaps the cellular nutrition and metabolism function is being compromised at the same time that the phagocytotic function is elevated.

While we know that toxicity is generally indicated by observations of disease and death, the investigation and monitoring of subtle changes in immune response may give early indications of sub-lethal effects and possible effects to the environment. Any down-regulation of immune cells in an organism is cause for concern.

By combining the results of the bioassay outlined here with standard vermicomposting assays for nutrients, worm health etc. we may be able to investigate the subtle effects that low doses of contaminants may be having on invertebrates prior to any toxic effects and, transfer this knowledge to potential effects to the environment and, ultimately, vertebrates.

Odour reduction in biosolids: Laboratory scale studies using sludge and biosolids from Western Australian wastewater treatment plants

By Yolanta Gruchlik (Curtin University, WA) and Nancy Penney (Water Corporation of WA)

Land application of biosolids is a viable and economical means of managing the residual materials from wastewater treatment, and is advantageous in terms of resource recycling. For example, biosolids produced from Western Australian (WA) wastewater treatment plants (WWTPs) are currently being used as organic humus and as fertiliser substitute on some agricultural properties, incorporated into commercial composts at licensed facilities and are also being trialled for mine site rehabilitation and forestry applications.

One of the main issues that can restrict land application programs is nuisance odours associated with biosolids. Considerable research has been carried out to understand the causes of odour formation in biosolids. For example, the Water Environment Research Foundation (WERF) conducted a multi-phase study, spanning several years, to better understand odours in anaerobically digested biosolids, as well as to develop management practices to minimize these odours. The research has shown that odour production in biosolids is a complex process influenced by many factors, including process variables within anaerobic digestion and dewatering processes, as well as the relationships between odours and concentration of odorants. Compounds that have been reported to be associated with odours from biosolids facilities include volatile organic sulphur compounds (VOSCs), such as methanethiol (MT), dimethyl sulphide (DMS), dimethyl disulphide (DMDS) and dimethyl trisulphide (DMTS), as well as inorganic sulphur compounds, such as hydrogen sulphide (H2S). Nitrogenous compounds, such as trimethylamine (TMA) and ammonia, as well as volatile fatty acids (VFA), can also be potential sources of odour. Odorous volatile aromatic compounds (OVACs), such as toluene, ethylbenzene, styrene, p-cresol, indole and skatole, have been identified in headspace samples from stored biosolids. Due to differences in biosolids characteristics, treatment processes and operating conditions associated with different wastewater treatment plants, the chosen odour reduction strategies need to be based on the site-specific conditions at each WWTP. In most cases, laboratory and/or pilot-scale trials would be required to find the most suitable odour reduction strategy.

The Curtin Water Quality Research Centre (CWQRC), in collaboration with the Water Corporation of Western Australia (WCWA), has applied some of the methodologies developed by the WERF research team to determine the most suitable odour reduction strategy for biosolids produced from Western Australian WWTPs. In our Phase 1 study, we have:

- developed an analytical method, based on headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS SPME-GC-MS), for the identification and analysis of the odorous compounds in biosolids
- identified odour compounds in biosolids from Western Australian WWTPs

- investigated the following odour reduction options:
  - addition of aluminium sulphate (alum), polyaluminium chloride and ferric chloride to digested sludge prior to dewatering
  - addition of alum to dewatered cake
  - reduction of centrifuge speed

The sludge and biosolids for this study were obtained from a Perth metropolitan WWTP (WWTP 1), which is an activated sludge plant that uses sequencing batch reactors (SBR), egg-shaped mesophilic anaerobic digestion and centrifuge dewatering.

The main odour compounds identified in fresh biosolids were: DMS, DMDS and DMTS. Other compounds which were tentatively identified based on their mass spectra and/or library matches, but not confirmed with authentic analytical standards, included various long chain aliphatic hydrocarbons, terpenes, alkyl benzenes and other aromatic compounds. Biosolids that had been stored for a few months showed presence of indole and skatole and exhibited a faecal odour. This finding was consistent with literature reports that one of the major sources of odours during the first 1-2 weeks of biosolids storage is due to the production of VOSCs by microbial degradation of sulphur-containing amino acids, while compounds such as indole and skatole start to accumulate only after VOSCs have been depleted. HS SPME-GC-MS analysis of an aged (12 months old) biosolids sample which exhibited a strong earthy/musty odour showed that the bulk of the odorous compounds present in this sample were long chain hydrocarbons, various alkyl benzenes, terpenes, cyclic hydrocarbons and other aromatic compounds, consistent with the odour descriptors of earthy/musty. No VOSCs, indole or skatole were detected in the aged biosolids sample.

Among the odour reduction options that were investigated in the Phase I study, aluminium sulphate addition to liquid biosolids (mesophilic digested) prior to dewatering was found to be the most promising odour reduction measure, resulting in a 40% reduction of the peak concentration of total volatile organic sulphur compounds (TVOSC), relative to a control sample (Figure 1).

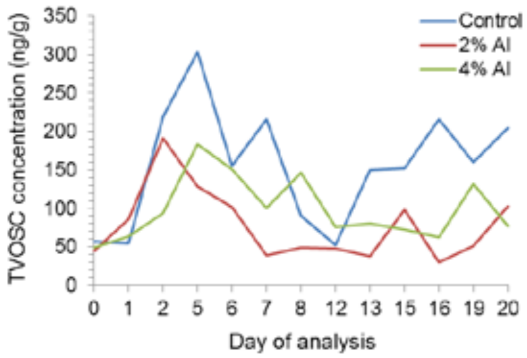

Figure 1. Effect of aluminium sulphate addition to digested sludge on TVOSC production. TVOSC was measured by HS SPME-GC-MS and represents the sum of DMS, DMDS and DMTS concentrations.

In the Phase II project, applicability of alum addition to wastewater sludge and biosolids produced from different treatment processes was evaluated as a means of odour reduction. In this study, we also included olfactometry measurements to complement the analytical HS SPME-GC-MS method, to obtain a more rigorous assessment of odour generated from the biosolids cake.

Four WWTPs were chosen for this study:

- WWTP 1 – SBR process and egg-shaped mesophilic anaerobic digestion and centrifuge dewatering (also used in Phase I).
- WWTP 2 – conventional aeration tank systems and conventional mesophilic anaerobic digestion and centrifuge dewatering.
- WWTPs 3 and 4 – oxidation ditch processes and centrifuge dewatering.

We found that alum addition was effective in reducing odours in biosolids from wastewater treatment plants that used mesophilic anaerobic digestion to process wastewater sludge (WWTPs 1 and 2). For example, addition of 4% Al to liquid biosolids from WWTP 1 prior to dewatering resulted in a 50% reduction in the overall odour concentration in the lab dewatered cake, relative to the control sample (Figure 2a). However, alum addition did not reduce odours in sludge from wastewater treatment plants which used oxidation ditch processes (Figure 2b). Comparison of peak odour concentrations for lab dewatered cakes from WWTPs 1, 2, 3 and 4 showed that cakes from WWTPs which use oxidation ditch processes (e.g. WWTP 4 (Figure 2b)) were much more odorous than anaerobically digested cakes (e.g. WWTP 1 (Figure 2a)).

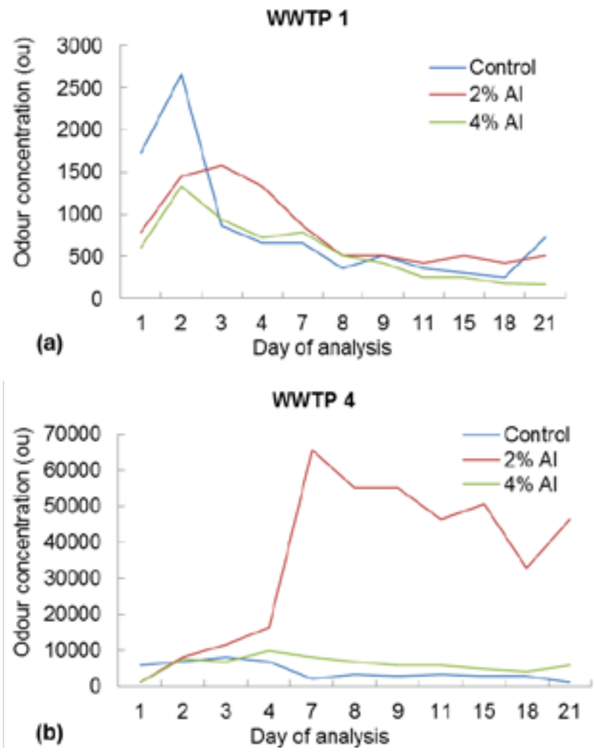

Figure 2. Comparison of odour concentration profiles for lab dewatered cakes from WWTP 1 (mesophilic anaerobic digestion) and WWTP 4 (oxidation ditch processes). Odour concentrations were determined by dynamic olfactometry. Each sample was treated with alum prior to dewatering.

Although alum addition to liquid biosolids from WWTPs 1 and 2 was shown to be promising as an odour reduction option for anaerobically digested biosolids, alternative odour reduction measures need to be investigated for processed sludge from WWTPs which use oxidation ditch processes. Possible options for further investigation include addition of potassium permanganate and/or calcium nitrate to processed sludge prior to dewatering.

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