Remediation of Waterways in Agricultural Settings using Organic Mulch

Ch'nee Akuhata

Second Year Lincoln University Student

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Effluent Remediation of Waterways in Agricultural Settings with Organic Mulch

Ch'nee Akuhata

Otago Summer Student Internship

Indigenous Agroecology Research Group

Te Putahi Farm

Supervisors: Jamie Ataria and Olivier Champeau

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Abstract

Agriculture is becoming an increasingly important income for New Zealand's economy but it is also becoming a problem for landcare. Water systems among agricultural settings are degrading in quality due to pastoral (management) and the increase in livestock. With grazing livestock depositing pathogens in their faeces, surface runoff is one of the most destructive ways of contaminants reaching waterways. There are limited options available for the protection of waterways against contaminants that do not lower the standards of the agricultural settings, are affordable and easy to maintain as well as an effective worthwhile treatment. Manuka is a well known medicinal plant with antimicrobial properties contained within its oils. This information provided the basis for a potential remediation of waterways against pathogens such as *Escherichia coli (E..coli*)with the use of organic mulch biowalls. This paper reports upon an experiment using manuka biowalls to provide a medium that captures contaminants after events like surface runoff to minimise water degradation. In a preliminary laboratory experiment manuka had an inhibitory effect on *E.coli* growth rate down to 0.02% plant dilution factor. This showed there was a strong antimicrobial affinity present in manuka and would be a good source for a biowall used in agricultural settings.

Introduction

The contamination of waterways with pathogens from farmyards and agricultural settings can pose a significant threat to aquatic ecosystems and drinking water resources. However the risk for the aquatic community or for human health can often be substantially reduced by appropriate measures (Kreuger J & Nilsson E 2001). Pathogens in livestock wastes are of most concern as the rumen and digestive tract of agricultural livestock is host to a rich diversity of microflora and therefore can also act as a reservoir for pathogenic micro-organisms (Rasmussen et al. 1993). Fecal derived microbes that end up on the grasslands are then held on the soil surface and amongst its porous structure (Oliver et al. 2005). In this paper, the current knowledge on mitigation strategies to reduce pathogen inputs into waterways and their effectiveness when applied in practice is reviewed.

The paper will also review a current experiment to determine conditions that decrease potential adverse effects of farming effluent on water quality with mulch (from different species/from manuka *Leptospermum scoparium* and/or moss genus *Sphagnum*). Farm effluent will be used or an *E.coli* strand to assess the remediation of mulch. Members of the Indigenous Agroecology research team have participated in several meetings with Putahi farm management to embed project goals and practical components of the research. This has involved direct kanohi ki te kanohi interaction with James Daniels (Farm Director), members of the governing board and the farm manager.

This review therefore describes:

- The use of lysimeters in soil contamination assessment
- Influence of dairy effluent on soil invertebrates (pasture field)
- Remediation by organic mulch
- Evaluating mitigation strategies in the literature with respect to their practicability and cost effectiveness.
- Recommending those considered both effective and feasible for implementation at the farm

Background

1. Transfer of pathogens from soil to water

The transport mechanisms of microorganisms within soils can be split into physical, geochemical, and biological processes. The physical processes are where potential pathogens are carried in bulk water and move according to the water velocity, and dispersion, which involves the spreading of micro-organisms as they move along the water path. The geochemical processes delay the microbial transfer through the soil matrix and consist of filtration, sorption, and sedimentation mechanisms. Finally, biological processes, such as growth and chemotactic (movement of organisms or cells across a chemical gradient) responses, may influence pathogen transfer through the soil habitat as demonstrated with other introduced micro-organisms (Oliver et al. 2005). There are a variety of available transfer routes such as soil type, hydrological pathways, slope gradient and overland travel distance. From here the potential pathogens may be transported from soil to waterways, but the factors that control the transfer of microbes through soils are not well understood (Hornberger et al. 1992). Figure one shows a conceptual model of microbial transmission through available routes from source to receptor and the die-off of microorganisms. This helps with determining what contaminants will intercept in the route and take remediation action at points of interest.

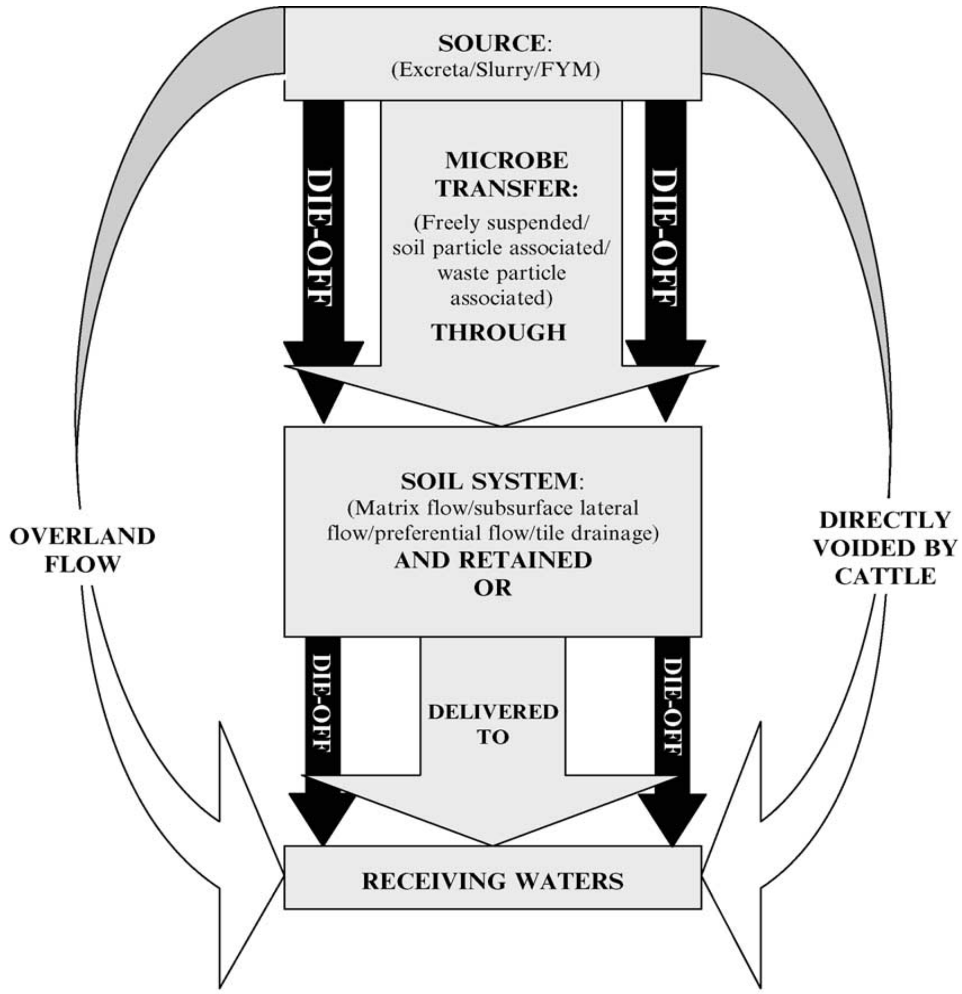


Figure 1 Conceptual model of micro-organism transmission from surface applied fecal waste to surface waters (Oliver et al. 2005).

Environmental factors are key elements to the die-off of the pathogens between the phases shown in figure one above. Drought and high exposure to UV (from the sun) are natural remediation mechanisms of bacteria such as *E.coli* as these provide unsuitable conditions for bacterial growth (Chadwick et al. 2008). However, many gram positive bacteria produce dormant spores, a response mechanism to allow the survival through harsh conditions and prosper once the environment is favorable again. Gram negative bacteria differ as they tend to produce resistant cells without dormancy allowing survival in common unfavorable conditions (Llorens et al. 2010).

Some pathogens can bypass soil and the die off phase through being deposited by livestock directly into the waterways, increasing the degradation of water quality. Solutions for this without capital expenditure are difficult to find. Livestock need good access to water supplies. One alternative is too fence the waterways completely and provide troughs, if this is not feasible then waterways should be fenced and access points provided as a temporary measure until troughs are available. Medicinal plants can easily be grown in the riparian margins and provide additional benefits including the trapping of pathogens transported in run off. A plantation of species with medicinal and remedial qualities would have to be efficient for a farm and require minimal requirements; this is where such things as good soil systems and biowalls can become very useful.

2. Soil Systems and Quality

Soil systems directly affect the rate at which pathogens and other contaminants move via water. The structure is managed by several factors such as microbial activity, climatic cycles, soil type and vegetation. The pore space and size distribution influences the infiltration efficiency of the soil and therefore the rate at which water can run through. Pathogens tend to be carried along the pathway that bulk water follows so the Infiltration, hydraulic conductivity, and water holding capacity are important measures (Lewandowski & Zumwinkle 1999) for understanding how much water will be retained and how much will enter waterways potentially carrying pathogens. *Infiltration* is the rate at which water enters the soil. *Hydraulic conductivity* is a measure of the rate of movement of water through soil. It is affected by the amount of organic matter, soil porosity, soil structure, and amount of water in the soil. *Water holding capacity* (the amount of water that can be held by soil) depends on the texture, organic matter, structure and percent of sand, silt and clay in the soil.

There is variability of soils between landscapes due to climate, slope, terrain etc that affect the soil systems and assessments of these factors need to be taken into consideration. Differences such as soil depth, density and compaction can have a significant impact on of the mechanisms stated previously and any results obtained in remediation trials.

Soil quality is “the capacity of soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health” (Doran and Parkin, 1994). A major contribution of the soil quality literature has been its emphasis on the importance of microorganisms to understanding the soil system. Soil organisms are continually adapting to changes in their environment, and therefore are rapid and sensitive indicators of soil quality changes(Lewandowski & Zumwinkle 1999).

Microorganisms and soil invertebrates are considered incredibly important due to their degradation of organic matter for nutrient cycling. The more microorganisms and invertebrates present, the more nutrients being recycled and the richer the soil becomes. Thus a good indicator of soil quality is a measure of these organisms in the area or interest. There are two common and cost efficient methods to indicate micro and macro invertebrate activity in the soil, the *cotton thread test* and the *bait laminar test*.

3. Remediation using Organic Mulch Biowalls

Biowall systems can potentially be used in the subsurface for the reduction of contaminants using a porous medium that intercepts the contaminants and prevents their transport to areas which it may be difficult to locate and mitigate. To enhance the efficiency of a permeable biowall, organic mulch can be used for biofiltration and bioremediation processes for some hydrophobic organic pollutants. Lignin is known to have a high affinity and sorption capacity for nonionic organic compounds which is considered a good property for a biowall as well as sand for a support medium (Seo et al. 2007).

In a permeable biowall system, the contaminated groundwater is diverted through the wall/mulch where filtration, sorption and biodegradation will occur. These barriers can treat groundwater in the long term with minimal maintenance costs, compared to pump and treat methods which require high-energy costs (Miller et al. 2001; Seo et al. 2007).

Livestock may deposit bacteria such as *E.coli* on pastures, so plants such as Manuka, *Leptospermum scoparium*, can become very important because the antimicrobial activity of its oils which contain triketones. These triketones show activity against Gram-positive bacteria including antibiotic-resistant strains and therefore would be a good host for a biowall system. More recently, it has emerged that there are significant geographical variations affecting the composition of these oils (Maddocks-Jennings et al. 2005). Manuka oils from different sites differed widely in composition and could be separated into four groups by the presence and levels of distinctive components (Porter & Wilkins 1999).

The many variations of manuka around New Zealand and Australia all have different chemotypes, (a distinct chemical makeup with differences in secondary metabolites), that influence the antimicrobial activity. The manuka in the East Cape of New Zealand all showed similar high triketone contents, approx 20% triketones (Christoph et al. 1999), this is the highest amount of triketone content found among any variation. Manuka from Marlborough Sounds has also been shown to have high concentrations of triketones although not as high as individuals in the East Cape. With little seasonal variation manuka could be harvested all year round for the antibacterial oils (Douglas et al. 2004) making this a very efficient tool for remediation projects.

Kanuka, like manuka, is a native oil producing species found in New Zealand, and these oils have medicinal properties which were traditionally used for rongoa (Maori medicinal remedies) (Brooker & Cooper 1961). Kanuka is similar to manuka in appearances as well as some of the compounds present in the oils like leptospermone, a beta-triketone that holds some antimicrobial and also antifungal activity(Perry et al. 1997).

Methodology

Laboratory Experiment

A preliminary laboratory experiment was done to test the microbial remediation using manuka to ensure there was a good foundation to base the field work on. Manuka collected from the Marlborough Sounds had been dried for several weeks and mulched finely (leaves and branches) using a ceramic blades mixer (Büchi M-400).

A volume of 1:4 of water was added and the remaining non-reduced organic matter was ground with a mortar and pestle for an additional 15 min. The ground paste was pressed to obtain liquid filtered through a mousseline and a Whatman #1 filter paper, and further sterilised by passing through Millipore syringe filters (0.22 µm). Effluent containing *Escherichia coli* strain 0157 (non-toxigenic strain) was added and water was poured over the trials to mimic a rain effect.

The plant extract was diluted in deionised water to give 12 concentrations such that when 50 µl was diluted to 200 µl in one well of a 96 well microtitre plate the final extract concentrations would be as follows: 25 %, 12.5 %, 6.25 %, 3.13 %, 1.56 %, 0.78 %, 0.39 %, 0.20 %, 0.10 %, 0.05 %, 0.02 %, and 0.01 %. The antibacterial activity of manuka extract was tested using absorbance (Patton et al. 2006) on a microplate reader at 595nm for 36hrs at 30°C.

Field Experiment

I. Soil Quality Tests

The *bait laminar test* is a good indicator of soil invertebrates and soil microorganisms. The method behind it is simple, a mixture usually consisting of cellulose, bran flakes and active coal is fixed into the holes on a bait laminar stick (Andre et al. 2009). The sticks are made of PVC which is sturdy enough not to break when putting into soil. Soil invertebrates and microorganisms will then (if present) degrade the mixture in the holes over a period of time (dependant on how many are present) and the bait in the holes is counted as a percentage as an estimate of their biological activity. In our current experiment, 48 bait laminar sticks were placed around the lysimeters evenly throughout the three sites (16 per site) to give a comparison of activity between our chosen locations.

The laminar sticks were left under normal conditions and controls were checked after one week but as there was no activity they were left until after the rain period for removal (approximately 4 weeks). Each hole was assessed and recorded as a percentage of bait that was gone.

The *cotton thread test* is an indicator of microorganisms. Microorganisms are necessary for the decomposition of plant residue into humus and into nutrients that plants can use for growth. Minerals and ions are immobilized by the microbial community then released when organisms die (Lewandowski & Zumwinkle 1999). The method involves cotton threads being placed in the soil contained in the lysimeter then checking for tensile strength over a certain period of time. A tensile meter is used to determine the threads strength and in some cases it is weighed before and after treatment. A decrease in strength means microorganism activity and the bigger the decrease the more activity, and the better quality of soil. Although this is a very easy and efficient soil indicator this could not be carried out as the lysimeters were too small in diameter and depth to be done successfully. It is a method that will be looked into for further projects related to the current experiment.

II. *Lysimeter uses in Soil Assessment*

Lysimeters are cylindrical structures with two compartments and holes between the two that allow the movement of substances between the first open compartment to the bottom collection compartment. Inserting filtration paper between the compartments allows the collection of samples which reflect what would normally be infiltrated into surrounding waterways. Four lysimeters were placed at three different sites along the waterway (in this case up and down stream). Lysimeters were deployed at Te Putahi on 4/02/2013, and the soil layer 1-2 cm and left to 'equilibrate' through one rain period (to flush through minerals and elements released from the disturbed soil). The lysimeters were checked again on the 13/02/2013 and the collection cup was cleaned thoroughly with bottled drinking water.

Four treatments were set up at each site and consisted of:

1. Soil only
2. Soil with cow effluent
3. Manuka mulch added to soil with cow effluent
4. Kanuka mulch added to soil with cow effluent

Freshly deposited cow effluent was collected at the site and 10mls was mixed with spring water to 50:50 ratios. The diluted effluent was then poured into the lysimeter to form an even 'cake.'

The lysimeters and bait laminar sticks were left under normal conditions for the area until a considerable amount of rain had fallen. The collection cups of the lysimeters were then emptied and sent to Nelson for microbial as well as general toxicity analysis.

III. *Organic Mulch*

Manuka (*Leptospermum scoparium)* and Kanuka *(Kunzea ericoides)* are indigenous to New Zealand. They both contain oils which have antiseptic and anti microbial attributes of which is very unique and useful.

Of most interest is β-triketones present in manuka oil, these triketones (especially leptospermone) are believed to significantly contribute to the antimicrobial and antifungal actions (Perry et al. 1997).

The Manuka used for this experiment was from Marlborough Sounds whilst Kanuka was from the Banks Peninsula (Te Putahi Farm). The branches were mulched by entering an electric garden shredder several times and rough foliage was removed from the end product. This was then applied to an area with the effluent of interest as well as a rain effect to cause hydrology of the effluent through the mulch and into the soil.

IV. Effluent application rate

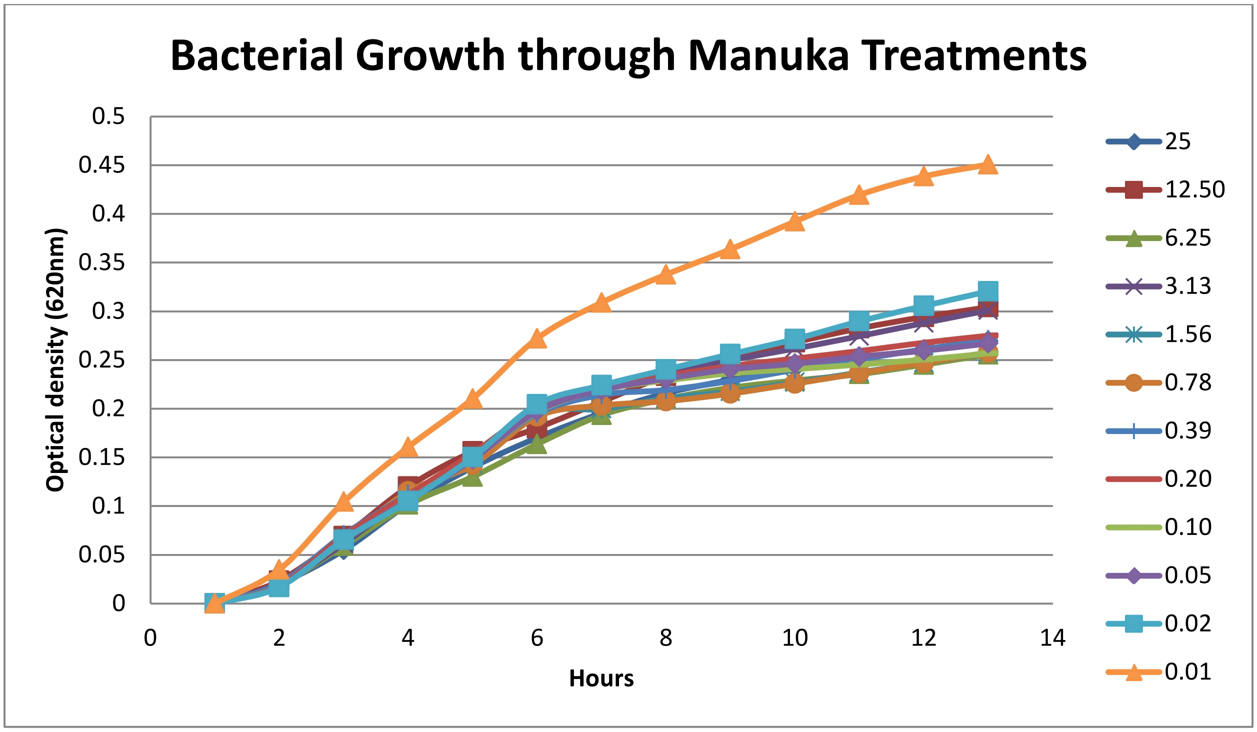
It has been suggested that the higher dry matter content of faeces compared with slurries perhaps allows for a more conducive faecal matrix for microbial survival (Oliver et al. 2006), protects the microbes from degradation and keeps them as a long term source of microbial pollution in suitable environmental conditions (Kay et al. 2007). Controlling manure application rate may help to reduce the risk of some pathogens moving with runoff (Gessel et al. 2004).

The experimental design only allowed a bulk lot to be added to the lysimeters in a dilute concentration. The dilution factor, 50:50 10mls of effluent to 10mls of water may not have been a low enough concentration to see any incriminating results.

Results

The environmental conditions were not ideal as there was a drought throughout New Zealand at the time. This most likely affected the *E.coli* numbers and the soil was also extremely dry preventing normal biological activity from occurring at the surface which is where the laminar sticks were placed. The bait in the laminar sticks could also have been affected by drying out and shrinking, but overall the activity across the sites was very poor.

Due to laboratory errors we could not obtain absolute *E.coli* values for any of the sites for the field experiment, therefore the information we had was inconclusive. The following results are from the preliminary laboratory experiment microbial analysis.



Graph 1: From microbial analysis this graph was produced showing normalized data of the bacterial (*E.coli*) growth through optical density (620 nm) over 13 hours.

Graph 1 only shows the first 13 hours over the total 36 hours because the growth between 0-13hours was most significant as after this period the data reached a plateau. The data has been normalised for better comparisons (starting points at zero). A statistical comparison of *E.coli* growth at 12 hours (maximal growth for un-normalised data) for all replicates between absorbance versus spectrophotometer was correlated using one-way ANOVA.

Samples are put into a spectrometer at 620nm and this gives us the optimal density of the E.coli growth which is a simple but qualitative method of analysis. Table 1 shows a statistical analysis between optical density (spectrophotometer 620nm) and absorbance (595nm). The probability value for 95% confidence is 0.00 which means that it is highly significant the difference between the two is not due to chance and we can assume with confidence that absorbance is related to optical density.

Table 1: statistical analysis of the spectrophotometer (620nm) assay response correlation/variation with the absorbance (595nm).

One-way ANOVA: 12th hour absorbance versus 12th hour spectrophotometer					
Source	DF	SS	MS	F	P
12th hour spectrophotometer	72	2.143212	0.029767	1105.14	0.000

Discussion

When determining the antimicrobial activity of a plant on bacteria such as *E.coli,* decisions have to be made on the chemotype, the amount of mulch/effluent applied as well as when the plant oils are most active. The choice of manuka was easiest for the preliminary experiment as manuka grows frequently around the Nelson district which is where the laboratory is located. It also seems to contain the same antimicrobial activities throughout the year allowing the harvesting of the plant for such treatments to occur.

Well and Disc diffusion are methods which have been commonly used methods for calculating bacterial growth but in this preliminary experiment we needed more information and less limitations. Spectrophotometric assay increases the automation and efficiency of the evaluation of the antimicrobial properties on *E.coli* growth and can generate larger quantities of data faster.

The results shown in graph 1 show the 12 different dilution factors, high (25) to low (0.01) inhibitory effects on *E.coli* growth. As to be expected the higher the plant extract concentration the higher the inhibitory effect and vice versa with low concentrations. The effect of manuka is seen down to the second lowest concentration (0.02%) as the *E.coli* seems to outgrow the inhibitory effect at 0.01%. It would seem that Manuka has an antimicrobial activity present in its oils that slow down the growth rate of *E.coli*.

The remediation of waterways with plants starts with the destruction of pathogens or organic compounds in soils, groundwater and industrial wastewater. Surface runoff after rain events is one of the main transportation systems for microorganisms to reach waterways and since we cannot completely prevent surface runoff the only logical solution is to block potential water degrading pollutants.

There are complications and limitations to the biowall remedies. Pathogens from livestock wastes are harbored in the feces and can survive for longer periods of time (Gessel et al. 2004), allowing higher concentrations of pathogens to be produced which means that there will be an increased chance of getting to waterways by overcoming the inhibitory effects of biowalls. A solution to these problems has not been well researched, and is a matter of trial and error in the sites of interest that will increase higher chances of remediation. A complication with our field trial was the lysimeter size. The size was too small and some procedures like the cotton thread test couldn't go forward and caused an overall disadvantage to the experiment.

Aside from the limitations which are yet to be fully explored in the field, the results from this experiment have shown that manuka has an inhibitory effect on *E.coli* growth rate. Organic mulches are similar to leaf litter in a forest, and wood process industries are plentiful, inexpensive and readily available, the use of mulch for the removal of organic pollutants in soil and groundwater (Seo et al. 2007) is gaining much attention as a simple, effective and economical means of treatment (Starr & Cherry 1994). If further trials are successful, the inexpensive and easy to put in place systems will hopefully make this initiative more welcomed by famers and the wider communities allowing the waterways to be used for many productive ways and reducing the harmful effects of intensive agriculture on the land.

References:

Andre A, Antunes SC, Goncalves F, Pereira R 2009. Bait-lamina assay as a tool to assess the effects of metal contamination in the feeding activity of soil invertebrates within a uranium mine area. Environmental Pollution 157(8/9): 2368-2377.

Brooker SG, Cooper RC 1961. New Zealand medicinal plants. Economic Botany 15(1): 1-10.

Chadwick D, Fish R, Oliver DM, Heathwaite L, Hodgson C, Winter M 2008. Management of livestock and their manure to reduce the risk of microbial transfers to water – the case for an interdisciplinary approach. Trends in Food Science & Technology 19(5): 240-247.

Christoph F, Kubeczka KH, Stahl-Biskup E 1999. The composition of commercial manuka oils from New Zealand. Journal of Essential Oil Research 11(6): 705-710.

Douglas MH, Klink JWv, Smallfield BM, Perry NB, Anderson RE, Johnstone P, Weavers RT 2004. Essential oils from New Zealand manuka: triketone and other chemotypes of Leptospermum scoparium. Phytochemistry 65(9): 1255-1264.

Gessel PD, Hansen NC, Goyal SM, Johnston LJ, Webb J 2004. Persistence of zoonotic pathogens in surface soil treated with different rates of liquid pig manure. Applied Soil Ecology 25(3): 237-243.

Hornberger GM, Mills AL, Herman JS 1992. Bacterial transport in porousmedia—evaluation of a model using laboratory observations. Water Resources Research 28(3): 915-938.

Kay D, Edwards AC, Ferrier RC, Francis C, Kay C, Rushby L 2007. Catchment microbial dynamics: the emergence of a research agenda. Progress in Physical Geography 31: 59-76.

Kreuger J, Nilsson E 2001. Catchment scale risk-mitigation experiences - key issues for reducing pesticide transport to surface waters BCPC Symposium Proceedings: Pesticide behaviour in soil and water 78: 319-324.

Lewandowski A, Zumwinkle M 1999. Assessing the soil system: A review of soil quailty literature. Minnesota Department of Agriculture Energy and Sustainable Agriculture Program.

Llorens JMN, Tormo A, Mart´ınez-Garc´ıa E 2010. Stationary phase in gram-negative bacteria. Federation of European Microbiological Societies 34: 476-495.

Maddocks-Jennings W, Wilkinson JM, Shillington D, Cavanagh H 2005. A fresh look at manuka and kanuka essential oils from New Zealand. International Journal of Aromatherapy 15(3): 141-146.

Miller KD, Johnson PC, Bruce CL 2001. Full-scale in-situ biobarrier demonstration for containment and treatment of MTBE. Remediation 12: 25-36.

Oliver DM, Clegg CD, Haygarth PM, Heathwaite AL 2005. Assessing the Potential for Pathogen Transfer from Grassland Soils to Surface Waters. Advances in Agronomy, Academic Press. Pp. 125-180.

Oliver DM, Haygarth PM, Clegg CD, Heathwaite AL 2006. Differential E. coli die-off patterns associated with agricultural matrices. Environmental Science and Technology 40: 5710-5716.

Patton T, Barrett J, Brennan J, Moran N 2006. Use of a spectrophotometric bioassay for determination of microbial sensitivity to manuka honey. Journal of Microbiological Methods 64(1): 84-95.

Perry NB, Brennan NJ, Van Klink JW, Harris W, Douglas MH, McGimpsey JA, Smallfield BM, Anderson RE 1997. Essential oils from New Zealand manuka and kanuka: Chemotaxonomy of Leptospermum. Phytochemistry 44(8): 1485-1494.

Porter NG, Wilkins AL 1999. Chemical, physical and antimicrobial properties of essential oils of Leptospermum scoparium and Kunzea ericoides. Phytochemistry 50(3): 407-415.

Rasmussen MA, Cray WC, Casey TA, Whipp SC 1993. Rumen contents as a reservoir of enterohemorrhagic Escherichia coli. FEMS Microbiol. Lett. 114: 79-84.

Seo Y, Jang A, Bishop PL 2007. Organic mulch biowall for PAH contaminated groundwater remediation. European Journal of Soil Biology 43(5–6): 304-309.

Starr RC, Cherry JA 1994. In situ remediation of contaminated groundwater: the funnel and gate system. Groundwater 32: 465-476.