Bioremediation of Nitrates in groundwater

K. Melvani[1] *and S. Pathmarajah*[2]
[1]Postgraduate Institute of Agriculture
University of Peradeniya
Peradeniya, Sri Lanka

ABSTRACT

The Kalpitiya Peninsula is low-lying with sandy soils and located on the North West coast of Sri Lanka. Of significance is the underlying Gyben-Herzberg lens of fresh water that is extensively pumped for irrigation and potable water supply. Ground water quality throughout the Peninsula was acceptable until concentrations of nitrate, nitrite, chloride and potassium increased beyond WHO drinking water standards due to massive applications of inorganic fertilizer and chemical pesticides.

The National Water Supply and Drainage Board declared unsafe many wells in the Peninsula because of nitrate and nitrite contamination. In 2001, they contracted with Neo Synthesis Research Centre (NSRC) to conduct research on bioremediation of water in a well in Nawakkaduwa that involved planting its micro watershed with plants analogous to the natural forest. The surroundings were cultivated with annual and tree crops using organic regimes.

The vegetation had matured by 2004 and formed canopies above ground and a dense root mat below ground. Eight piezometers were installed in the micro watershed and water from piezometers, model well and two control wells located along the hydraulic head were tested from February 2004 to January 2009 on a monthly basis for potability.

Statistical analysis of data demonstrated that there were significant mean differences (P<0.05) in the concentration of nitrates and electric conductivity between the water in piezometers and control wells indicating that the intended bioremediation was effective in the removal of nitrates and in reducing the electrical conductivity of groundwater. No significant differences were recorded for concentrations of other parameters. Further the hydraulic retention time (HRT) for bioremediation was calculated; in 2007 and 2008 values ranged from 104 days to 197 days while by 2011, HRT had decreased to 51 days. The maturity of the micro watershed ecosystem (in terms of the growth in vegetation and soil development) was attributed to the change in HRT.

Key words: Bioremediation, Kalpitiya, nitrates, electrical conductivity, hydraulic retention time

INTRODUCTION

The land use planning in modern agriculture includes, among many other facets, the intensive use of agrochemicals, monocultures of one or two crop species requiring the

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[1] Corresponding Author, email*:* neosynth@sltnet.lk

2 Board of Study in Agricultural Engineering, Postgraduate Institute of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka

removal of all other vegetation, introduction of new crop varieties and continuous cultivation to support three cropping cycles.

The intensity of modern agriculture in sensitive environments is rife with negative impacts. For instance, in many countries, ground water from shallow aquifers is used to supply potable water and for irrigating crops grown with the use of chemical fertilizers and pesticides. The leaching of these agrochemicals into the water table significantly impacts ground water quality (Fetter, 2008). Excessive applications of nitrogen fertilizers to certain soils contribute to the concentration of nitrates in ground water (Dissanayake, 1988).

The Kalpitiya Peninsula is a good case in point. It is a low-lying, sand peninsula located on the north west coast of Sri Lanka. The climate is characterized by an average annual rainfall between 500-650 mm from the North East monsoon between October and January and annual temperatures that range from a maximum of 30.6^0C to a minimum of 23.9^0C (Cooray, 1995).

Sandy regosols occupy the central portion and a greater part of the elevated beach plain that is adjacent to the Puttalam lagoon. Dune sands are mainly present on the seaward side of the beach plain.

There is a strong edaphic control of vegetation along the coastal strip (Holmes, 1951) that can be divided into zones of low, creeping vegetation, low shrubs and littoral woodland. Of significance to this study is the composition of the littoral woodland that is mainly comprised of species like *Pandanus tectorius, Callophyllum inophyllum, Pongamia pinnata, Barringtonia asiatica, Terminalia catappa* and *Phoenix zeylanica.*

The Kalpitiya aquifer that is located on a coastal spit is bound by sea on both its eastern and western flanks and comprised of coarse grain sands. A thin fresh water, *Gyben-Herzberg* type lens occurs in the sand and is present at a depth of 1-3m over most of the Peninsula. This permits stable human settlement and agricultural production on this landscape even in the very dry environment (Panabokke, 1996). The lens is extensively pumped for both irrigation and potable water supply and recharge is through direct infiltration from both rainfall and return irrigation flows. The highly permeable sands permit an excess of 50% of water applied to the surface to reach the water table (Panabokke, 2007). The circulation of groundwater has important implications on groundwater quality.

Ground water quality throughout the Peninsula had been good until massive amounts of inorganic fertilizer and chemical pesticides began to be used in agriculture that increased ground water concentrations of nitrate, nitrite, chloride and potassium beyond WHO drinking water standards (Lawrence & Kuruppuarachi, 1986). WHO Standards for nitrate concentrations in drinking water are 10 mg N/l (nitrate nitrogen/l) or 50mg No_3/l (nitrate/l)(WHO, 2006)

Studies conducted on groundwater within intensively cultivated areas typically had nitrate nitrogen concentrations in the range of 10-15 mg N/l compared to 0.2 mg N/l within non-cultivated lands. There is thus seen a correlation between ground water quality and land use (Kuruppuarachchi & Fernando, 1999). Further, the build up of nitrate-nitrogen concentrations of ground water within the intensively cultivated areas were estimated (Lawrence & Kuruppuarachi, 1986) following the introduction of intensive agriculture in the Peninsula at 2 mg/l per annum. The study revealed that groundwater in dug wells located within areas that had been cultivated for more than 20 years recorded concentrations in the range of 10-50 mg N/l while non-cultivated or lightly fertilized coconut plantations recorded less than 0.2 mg N/l. The latter concentrations were assumed to be the background concentrations prior to cultivation.

Another study carried out in 1999 (Liyanage *et. al*, 2000) confirmed widespread contamination. This study analysed drinking water from 225 wells in 11 Family Health Worker Areas in the Kalpitiya peninsula and showed that only 56% of the wells contained nitrate concentrations considered to be safe by the WHO (<50mg No_3/l). 31% of these wells contained nitrate concentrations between 50 and 100mg No_3/l while the balance contained very high nitrate concentrations ranging from 101-300 mg No_3/l.

However, the problem of nitrate contamination in Kalpitiya is not limited to ground water; the same study analysed seven types of food items commonly grown in the peninsula (sweet potato - *Ipomaea batatas*, kurathampala- *Amaranthus viridis*, gherkins - *Cucumis sp*, anguna leaves- *Timonius jambosella*, red onions- *Allium cepa*, potatoes - *Solanum tuberosum* and long beans - *Vigna cylindrica*) and revealed that anguna leaves, long beans and kurathampala contained high nitrate concentrations (2.79g/kg, 1.15g/kg and 3.25g/kg respectively) on a wet weight basis. Of these, anguna leaves is one vegetable that is eaten daily by a majority of the local population. This study revealed that on average the daily intake of anguna leaves by a person living in the Kalpitiya area is approximately 100g and this would contribute 300 mg of nitrate to the diet. If that person consumed 3l of water daily from a well that contained >100mg No_3/l, this would contribute another 300 mg of nitrate that was ingested. If only both of these inputs are taken into consideration, the total intake of nitrates would be close to 600 mg per day that is a very high intake. If the contribution from other vegetables consumed were also taken in to account, the daily nitrate intake of these individuals would be even higher (Liyanage *et.al*, 2000). This is almost 3 times higher than the Acceptable Daily Intake (ADI) for nitrate for 3.7 mg/kg body weight/day that is equivalent to 222 mg nitrate per day for a 60 kg adult as established by the former Scientific Committee on Food (SCF) and reconfirmed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2002 (EFSA, 2008).

Hence it is possible that high nitrate levels in the vegetables grown and groundwater in intensively cultivated areas in the Kalpitiya peninsula could attribute to the development of toxic *methaemoglobinaemia* in its populace. The impact of nitrate-contaminated ground water on human health has been assessed from 1945. In a 1950 report, 144 cases of infant *methaemoglobinemia* were reported with 14 deaths in a 30-day period in Minnesota (Johnson *et al*., 1987). *Methaemoglobinaemia* is a condition in the blood that causes infant cyanosis, or *blue-baby syndrome*. This condition is seen more frequently in infants than in adults due to the higher pH of the gastrointestinal tract in new borns favouring bacterial growth; including those (especially *Escherichia coli*), which converts nitrates to nitrites, the latter being responsible for *Methaemoglobinaemia* (Mirvish, 1991). Because of oxygen deprivation, the infant will often take on a blue or purple tinge on the lips and extremities, hence the name, *blue baby syndrome* (Comly, 1987). In addition, high nitrate levels have been associated with congenital abnormalities in infants whose mothers have been exposed to high nitrate levels in food or water during pregnancy. Nitrates and nitrites are also reduced to N-nitroso compounds that are reported to cause gastro intestinal cancers. Single oral doses of 2-4g nitrate or 60-500 mg nitrite have been shown to cause toxic *methaemeglobinemia* in humans; the lethal dose range in human adults is 4-50g for nitrates and 1.6-9.5 g for nitrite (Mirvish, 1991)

In 1993, Doctors from the Faculty of Medicine, University of Ruhuna, carried out a study of the impact of nitrate contamination on human health in Thalawila and Etale areas in the Kalpitiya Peninsula. Infants from 171 families who engaged in intensive cultivation from the two areas were sampled. It was revealed that 41% of the infants consumed water that had very high nitrate levels (>50 mg/l) that were higher than the WHO standards. The balance were found to be consuming water that had nitrate concentrations from 31-49.9 mg/l. There is an obvious correlation between nitrate levels in drinking water consumed by infants in the Etale and Thalawila areas and the *methaemoglobin* levels found in their blood. This was revealed through Haematological estimations that were carried out on 162 infants of the sample. It was found that only 36.4% of the infants were non anaemic where their *haemoglobin* levels were greater than the WHO standards (1968) of 11 mg/dl. It was also found that 29% of the infants had *methaemoglobin* levels within the normal range (<2%) while 71% were above the normal range (Liyanage *et.al*, 1993). Symptoms are usually minimal until *methaemoglobin* concentrations exceed 20% (Wright *et. al,* 1999). Anaemic children will display toxicity at lower *methaemoglobin* concentrations (Greer, 2005).

The National Water Supply and Drainage Board (NWSDB) initiated 12 community based drinking water supply schemes in the Puttalam District with the assistance of the Asian Development Bank in 2001. They were compelled to abandon many schemes since the water was contaminated with nitrates and nitrites. In 2002, they contracted with Neo Synthesis Research Centre to conduct research on the bioremediation of nitrates in well water in the Kalpitiya peninsula.

RATIONALE

Dietz and Schnoor (2001) state that "one of the most promising frontiers of environmental research is bioremediation; the use of natural means to breakdown or degrade hazardous substances into less toxic compounds and thus repair environmental damage. Underneath the umbrella of bioremediation there are two main categories: microbial remediation and phytoremediation.

Microbial remediation refers to the use of fungi or bacteria to break down pollutants and is a proven method for environmental remediation. For microbial remediation to be successful there must be a nutrient source and conditions that are chemically hospitable for organisms to thrive.

Phytoremediation is defined as the use of green plants and their associated microorganisms, soil amendments and agronomic techniques to remove content or render harmless environmental contaminants. According to Schnoor (1997) phytoremediation is best applied at sites with shallow contamination of organic, nutrient or metal pollutants that can use any of the following applications: Phytotransformation or Phytodegradation, Rhizosphere Bioremediation, Phytostabilization, Phytoextraction, or Rhizofiltration. Of these Phytotransformation or Phytodegradation and Rhizosphere Bioremediation are pertinent here.

Phytotransformation or Phytodegradation

The uptake of organic and nutrient contaminants from soil and groundwater and the subsequent transformation by plants is referred to as Phytotransformation or Phytodegradation. Contaminants often migrate from their sources via groundwater in the form of a plume. Hydraulic control (or hydraulic plume control) is the use of vegetation (as a cap or buffer strip) to influence the movement of ground water and soil water, through the uptake and consumption of large volumes of water. (Pivetz, 2011). Several characteristics of plants, such as local adaptation, metabolism, uptake, and tolerance, are important factors in designing plant-based treatment systems. Direct uptake of chemical into the plant through roots depends on uptake efficiency, transpiration rate and concentration of chemical in soil water (Burken and Schnoor, 1996). The process of nutrient uptake by plants refers to the transfer of the nutrient ions across the soil root interfaces into the plant cells. The energy for the process is provided by the metabolic activity of the plant and in its absence no absorption of nutrients take place. Nutrient absorption involves the phenomenon of ion exchange. The root surface, like soil, carries a negative charge and exhibits cat ion-exchange properties. The most efficient absorption of plant nutrients takes place on the younger tissues of the roots, capable of growth and elongation. The absorption mechanisms of the crop plants use three mechanisms in the soil-water-plant systems viz: (i) contact exchange and root interception, (ii) mass flow or convection, and (iii) diffusion via the apoplastic route. In the case of contact exchange and root interception, the exchangeable nutrient ions from the clay-humus colloids migrate directly to the root surface when plant roots come into contact with the soil solids. Mass flow or convection relates to nutrient mobility with the movement of soil water towards the root surface where absorption through the roots takes place along with water. Nutrient uptake through this mechanism is directly related to the amount of water transpired by plants. (http://www.krishiworld.com/html/soil_ferti1.html) Plant-based remediation systems are biological, solar-driven, pump and treat systems with an extensive, self-extending uptake network (the root system) that enhance the under-ground ecosystem for subsequent productive use (Cobbet, 2000).

To be remediated, ground water must be within the depth of influence of the roots. Hence tree roots that grow deeper than smaller plants reach pollution embedded deeper in the ground. Once inside the plant, chemicals can be stored in the roots, stems or leaves, changed into less harmful chemicals within the plant or into gases that are released into the air as the plant transpires. The time required for phytoremediation depends on the type and number of plants being used, harmful chemicals present, size and depth of polluted area and the prevailing type of soil and ground conditions.

Potential applications include phytotransformation of petrochemical sites/storage areas, ammunition waste dumps, fuel spills, chlorinated solvents pollution, landfill leachates and agrochemicals (pesticides and fertilizers) in soil and groundwater.

Deep-rooted poplars have been used to treat groundwater contaminated with mineral fertilizers that contain large amounts of nitrates and pesticides. Poplars planted as shelterbelts along watercourses prevent migration of contaminants from agricultural sites to rivers and other surface waters via groundwater (Pilipovic, 2006). Research conducted by Licht and Schnoor (1993) showed that concentration of nitrates in groundwater decreased from 150mg/l to 3 mg/l measuring in front and behind rows of poplar trees. They also found that plants take up Nitrate and convert it into nitrogen containing compounds or into nitrogen gas. Deep-rooting techniques can increase the effective depth of this application.

The Tree Remediation Process undertaken by Applied Natural Sciences Inc., states that "with proper coaxing, trees can effectively root into aquifers at least 6.1metres deep. Once established (1-2 years) these root systems can begin pumping water at a rate of 3.78 to 7.56 million litres per two hundred trees per acre per year, creating a significant pumping regime for a given area. Therefore, by planting trees in close proximity over a contaminated aquifer, or as a border around a site, a barrier to the movement of contaminated ground water can be effected". In Oconee, Illinois, U.S.A. in 1988 an experiment in tree remediation was conducted in soil and ground water that was contaminated with nitrates, ammonium and pesticides. The depth of the ground water table was 1.82 – 3.048 metres. The objective was the prevention of off-site migration of the contaminant plume and soil clean up. After collection and assessment of baseline data, a phytoremediation program was established using selected trees and grasses. This resulted in soil pesticide levels falling in three years following implementation, while the growth of trees at a rate of between 1.82 and 2.44 metres per year resulted in a steady decline in nitrogen and pesticide levels in ground water (Argonne National Laboratory, 1999)

Rhizosphere Bioremediation

Understanding contaminant uptake mechanisms includes the study of root physiology and morphology, uptake kinetics, translocation in roots, stems, and leaves, bioaccumulation in specific organs, as well as the role of mycorrhizae and the rhizosphere (Hinchman *et.al.,* 2000). Plants release exudates like sugars, amino acids, organic acids, fatty acids, sterols, growth factors, nucleotides, flavanones, enzymes, and other compounds (Shimp *et al.,* 1993) to the soil environment stimulating the degradation of organic chemicals by inducing enzyme systems of existing bacterial populations; stimulating growth of new species that are able to degrade wastes, and/or increasing soluble substrate concentrations for all microorganisms. Plants and plant roots can also affect the water content, water and nutrient transport, aeration, structure, temperature, pH, or other parameters in the soil thereby creating favorable environments for soil microorganisms, regardless of the production of exudates.

Rhizosphere bioremediation is also known as phytostimulation or plant-assisted bioremediation. Significantly higher populations of total heterotrophs and beneficial bacteria increased in the root zone of hybrid poplar trees compared to an unplanted reference site. Denitrifiers, *Pseudomonas* sp., BTEX (benzene, toluene, xylenes) degraders and atrazine degraders were found in rhizosphere soil around hybrid poplar trees in a field plot when compared with a non-rhizosphere soil (Jordahl *et al.,* 1997).

OBJECTIVES

The overall objective of this study was to test the hypothesis that under the groundwater hydraulic conditions that exist in Kalpitiya, bioremediation can render nitrate contaminated water potable.

The specific objectives of the study were to:
a) Statistically experiment the effectiveness of bioremediation
b) Establish the hydraulic retention time in the process of bioremediation of nitrate-contaminated water.

MATERIALS AND METHODS

Description of the work done

1. Establishment of the demonstration model

The drinking water well that is located in Nawakkaduwa village in the Kalpitiya Peninsula had been constructed in 2000 through an ADB funded 3rd Rural Water Supply and sanitation Project carried out by the NWSDB. Nitrate concentration in well water was 58.5mg N/l. Funds for well construction were donated by the NWSDB to the newly formed community group whose membership comprised 600 families in the Nawakkaduwa area. The water was to be distributed to their homes after bioremediation. The land was approximately 4046.86m^2 in extent and located about 200 m from the seashore. The landscape consisted of sandy soils and had no vegetation. The well was constructed in the front corner of the land adjacent to a farm garden that was cultivated with onions, chillies and long beans using agrochemicals in production.

The landscape of the demonstration model was restored primarily using the silvicultural technique of Analog forestry (Falls Brook Centre, 1997). Analog forestry seeks to establish a tree dominant ecosystem that is analogous or similar in architectural structure and ecological function to the original climax or sub-climax vegetation community or natural forest. The forest community so formed will also be analogous in terms of whole forest products and services, such as the production of clean water, environmental stability or biodiversity conservation.

The closest natural forest that is 12,140.58m^2 in extent is located in the village of Daluwa and provided the model on which restoration strategies were based on since it was the best example of a mature ecosystem in the area. The mapping of the vegetation of the Daluwa forest patch was undertaken in 2002 using the notation developed by Kuchler and Zonneveld (1988) and later modified by Senanayake in 1989. The forest architectural structure, floristic species composition in terms of height, growth and frequency were recorded. Further the ecological functions of the forest, soil and visible soil macro fauna were also detailed. The data gathered in Daluwa provided the basis for restoring the micro watershed of the well located in the adjacent village of Nawakkaduwa. The landscape design involved three main aspects:
The micro watershed of the well is the immediate surrounding area and referred to as the buffer zone. In this zone, several deep rooted, mostly native trees with both long and short growth cycles, were densely planted to form a ‘root mat’ below the surface facilitating the uptake of contaminants. Among the species used were Kumbuk (*Terminalia arjuna*), Mee (*Madhuca longifolia*), Palu (*Manilkara hexandra*), Thimbiri

(*Diospyros malabarica*), Halmilla (*Berrya cordifolia*), Wetakeiiya (*Pandanus kaiida*), Karanda (*Pongamia pinnata*), Surya (*Thespepsea populnea*) and Murunga (*Moringa oleifera*).

The demonstration area was fenced using several species that could withstand the salt laden sea breeze and, at the same time, serve as windbreaks. Trees used for the live fence included Mudilla (*Barringtonia speciosa*), Kasa (*Casuarina equisetifolia*), Watha Bhanga (*Pisonia alba*) and Kotta (*Ceiba pentandra*).

Beyond the buffer zone, a production area of both perennial and annual crops was established using organic cultivation regimes. There was an emphasis on fruit plants including Papaya, Banana, Mango, Guava, Pomegranate, Sapodilla, Anona, Lime, Lemon, Mandarin, Orange, Avocado, Jak, Grape, Passion, and a new variety, Acerola. In addition, plants with medicinal value like Nelli (*Phyllanthus embelicus*), Bulu (*Terminalia bellerica*) and shrubs like Katukarosana (*Picrorhiza kurrooa*), Tippili (*Piper longum*) and Aloe (*Aloe vera*) were included. All vegetable crops cultivatable in the Dry Zone of Sri Lanka were grown here.

2. Investigating water quality

a) Selection of sampling locations to test changes in contaminant concentrations in water in the following wells (see *Fig. 1* for greater understanding):

- The drinking water well that is located in Nawakkaduwa village herein after referred to as 'model well'
- 8 piezometers that were installed around the 'model well' in the 'root mat' created by the vegetation that had been planted. These piezometres were named P1, P2, P3, P4, P5, P6, P7 and P8.
- 2 control wells were set up on the upper and lower sides of the model well along the hydraulic grade line:
    - The Well that is located in a farm garden adjacent to the demonstration model (hereafter referred to as the Upper well) and is subject to continuous use of chemical fertilizers and biocides
    - The sea well that is located on the lower side of the model well on hydraulic grade line and adjacent to the shoreline in Nawakkaduwa

b) Sampling of water carried out from February 2004 to January, 2009

- Baseline tests were conducted on the water from the model well in January 2004 to determine:

Physical properties: colour, odour, taste, turbidity, total Solids at 103-105^0, Oil and Grease

Chemical properties: pH at 25°C, Electrical Conductivity at 25°C, Total Alkalinity (as $CaCO_3$), Total Hardness ($CaCO_3$), Chloride (Cl), Total Residual Chlorine (Cl_2), Free Ammonia (NH_3), Albuminoid Ammonia (NH_3), Nitrate (N), Nitrite (N), Fluoride (F) at 25°C, Total Phosphate (PO_4), Total Iron (Fe), Sulphate (SO_4), Phenolic Compounds (phenolic OH), Calcium (Ca), Magnesium (Mg), Copper (Cu), Manganese (Mn), Zinc (Zn), Aluminium (Al), Arsenic (As), Cadmium (Cd), Cyanide (Cn), Lead (Pb), Mercury (Hg), Selenium (Se), Chromium (Cr).

Micro Biology: Total Coliform count and *E.coli*

- Monthly tests were carried out from February 2004 up to January 2009 on water from:

Model well, 8 piezometers, Upper and Sea wells to establish potability of water. Tests done were:
Physical properties: colour, odour, taste and turbidity
Chemical properties: pH at 25°C, Electrical Conductivity at 25°C, Total Alkalinity ($CaCO_3$), Total Hardness ($CaCO_3$), Chloride (Cl), Free Ammonia (NH_3), Albuminoid Ammonia (NH_3), Nitrate (N), Nitrite (N), Total Iron (Fe).

All water tests were carried out at the Laboratory of the NWSDB in Getambe.

3. Measurements taken

- All measurements of chemical, physical and biological parameters were taken manually
- Relative elevation at ground level was taken from an arbitrary point of reference using a Dumbi Level.
- Distance between the Upper well and Piezometer P5 (hereafter referred to as the Lower well) were measured manually.
- Diagonal length of the vegetation established around the model well that signifies the length of the basin for measuring hydraulic retention time was measured as the distance between the fence (on the side of the farm) and the Lower well.

Theory
Flow from upper well to the sea through the experimental plot and the lower well area was assumed to be one-dimensional. According to Darcy's Law (Darcy, 1856), the one-dimensional flow of water through a porous media can be expressed as:

$$v = -K\, i$$

Where:
v= velocity of flow(m/day)
K= hydraulic conductivity (m/day)
$i = dh/dl$ or gradient of hydraulic head (unit less)

The hydraulic conductivity for Mampuri in the Kalpitiya Peninsula was obtained from literature (Dassanayake *et. al.*, 2010) and hydraulic gradient was calculated using field measurements of hydraulic heads in piezometers and all wells.

It was assumed that the piezometers on the lower fence side of the experimental plot namely; P4, P5, P6 and P7 represent remediated water while the Upper well represents contaminated water. The comparison of contaminant concentration levels was monitored between the upper well and piezometers P4, P5, P6 and P7.

4. Statistical Analysis

All together, eleven points (wells and piezometers) were sampled and 57 sets of data were recorded between February 2004 and January 2009. Paired T-Tests were done using “MINITAB 11” software to test whether there was a significant mean difference between the contaminant concentrations in the water in the upper well and that of the Piezometers (P4, P5, P6 and P7).

RESULTS

1. **Establishment of the demonstration model**

Vegetation Cap

The vegetation cap in Nawakkaduwa was planted from 2001 around the model well on sandy, barren land. By 2008, canopy closure of approximately 60% had been achieved. The growth of the canopy cover over the area around the well was concomitant with the growth of the root mat as evidenced when soil was dug up around each piezometer. The landscape design of the model well micro watershed used pioneer, sub climax and climax species identified in the natural forest of the area. Hence, even though species like Timbiri (*Diospyros malabarica*) and Palu (*Manilkara hexandra*) grew slowly, pioneer species like *Thespepsea populnea* and *Moringa oleifera* grew rapidly to deposit leaf litter, establish a canopy cover and with it the skeleton of the root mat.

Organic agriculture

While farmers in the Kalpitiya Peninsula only cultivate seasonal crops like Chillies, Onions, Tobacco, and Melons, several varieties of vegetables were grown successfully in the experimental plot including Snake gourd, Bitter Gourd, Ridge gourd, *Amaranthus* sp., Maize, Spinach, Ladies Fingers, Cucumber, Pumpkin, Long Bean, Tomato, Spring Onions, Winged Bean, and several varieties of Chillies using purely organic cultivation methods. Green manures like *Gliricidia maculata*, *Pavetta indica* and *Vitex negundo* were propagated, and formed the basis of the compost made. Other materials like rock phosphate were added. The sandy soil was periodically treated (upto 2004) with approximately 67,000 kgs of a special mix containing carbon rich inputs like Coconut peat and paddy straw as well as lagoon soils (clay) and gravel (NSRC, 2004)

2. Water quality monitoring

Data was gathered from:

- baseline tests conducted on water from the model well in January 2004
- microbiology of the water
- tests carried out 57 times from February 2004 up to January 2009 on a monthly basis on water from 8 piezometers, model well, Upper and Sea wells

3. Measurements of hydraulic retention time

a) Direction of flow

At the beginning of the experiment (i.e. in April 2001) water levels in the wells and the piezometers were measured from an arbitrary datum to establish the direction of groundwater flow. Accordingly:

Water level in Upper well (ha) = 3.77m
Water level in Lower well (hb) = 2.47m
Difference in level, *dh* = (ha-hb) = 1.30m

There is a substantial difference in head between the water in the Upper well (3.77m) and that of the Lower well (2.47m) of 1.30m. This means that the direction of flow is from the Upper well to the Lower well.

b) Hydraulic gradient: $i = dh/dl$

Water was assumed to move through the 'basin' between the Upper and Lower wells
Linear distance, *dl*, as measured between the Upper well and the Lower well
(see *Figure 1)* is 34.9 m

Then for data recorded in April, 2011 the hydraulic gradient, i

$$i = dh/dl = 1.30\text{m}/34.9\text{ m} = 0.04$$

c) Calculation of Velocity of Flow, v

Assumption: Water is flowing with uniform velocity

If hydraulic conductivity, K for regosols of the Mampuri series is 9.6 m/day (Dassanayake *et.al*, 2010)
Then flow rate of water through the basin, $v = -K\,i$
After substitution with data recorded in April, 2011,

$$v = 9.6\text{ x m/d x } 0.04 = 0.384\text{m/day}$$

Table 1: Calculating Hydraulic Head

Measurement (m)		**Upper Well**	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**Lower Well**	**Sea Well**
Elevation at ground level From datum		3.87	3.88	3.94	3.88	3.96	3.89	3.91	4.06	3.88	3.57	3.74
Height of piezometer to ground level	-	3.81	4.23	2.25	2.50	2.71	1.97	2.26	2.36	2.57	3.82	2.64
Ground Water Level	+	3.71	2.72	0.74	1.00 2	1.09	0.55	0.71	0.68	1.07	2.58	1.12
Height of water in well	=	3.77	2.37	2.43	2.38	2.34	2.47	2.36	2.38	2.38	2.33	2.22

a) Hydraulic retention time (HRT)

In most treatment basins a "plug flow" is maintained where essentially a plug of water enters and exits the basin area (Jenkins, 2003). The time that this 'plug' of water stays in the system is referred to as hydraulic retention time. In ideal conditions, water moving through the system travels from the upper well at the same velocity through the distance (*d*) reaching the exit (the lower well) at the same nominal (theoretical) time (*t*).

Hence the HRT in ideal plug flow conditions:

$$t = d/v \text{ where,}$$

t = HRT (in days)
v= velocity of flow (m/d)
d= length of the root mat/basin (m)
Substituting with data recorded in April of 2011:

If v= 0.384 m/day and d= 19.71m

$$\text{Then, } t = 19.71\text{m}/0.384 \text{ m/day} = 51\text{days}$$

However in order to comprehend hydraulic retention time in the context of an extended period of time, the eight-month period between August 2007 and April 2008 was assessed. The assumption is that across the short distance of 19.71m between the Fence and the Lower well (Piezometer P5) within the root basin, the gradient remains constant throughout the year irrespective of rainfall. However since data was taken at different times in the day, the water levels in the wells assessed were subject to pumping and therefore reflected differing values. The data recorded is seen in Table 2.

Table 2: Hydraulic Retention Time for wells from August 2007 to April 2008

Period	**Aug-07**		**Sep-07**		**Oct-07**		**Nov-07**		**Dec-07**		**Feb-08**		**Mar-08**		**Apr-08**	
Well	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
	ha	hb	ha	hb	ha	hb	ha	hb	ha	hb	ha	hb	ha	hb	ha	hb
Level of water in well (m)	2.13	1.56	2.27	1.57	2.18	1.75	2.17	1.74	3.05	2.33	1.28	2.07	2.44	2.25	1.76	2.18
dh (ha-hb) (m)	0.57		0.70		0.43		0.43		0.72		-0.79		0.19		-0.42	
***dl*= Linear distance between Upper and Lower Well(Piezometer P5) = 34.9 m**																
***i*=dh/dl**	0.02		0.02		0.01		0.01		0.02		-0.02		0.01		-0.01	
If v = k *i*, (m/day)	0.19		0.19		0.10		0.10		0.19		-0.19		0.10		-0.10	
d = Linear distance of root mat in basin (between Fence and Piezometer P5) = 19.71 m																
If t=d/v= (days)	104		104		197		197		104		104		197		197	

2. Statistical Analysis

Though data was recorded 57 times from February 2004 to January 2009 on all contaminants in the water from the model well, Upper well, sea well and 8 piezometers, only the concentrations of nitrates (as nitrogen), nitrites (as nitrogen), Chloride, Iron, Alkalinity and Electrical Conductivity were statistically evaluated.

The mean of the most contaminated well, referred to as the Upper well was compared with the mean of the aggregate data recorded for piezometers P4, P5, P6 and P7 where water is deemed to be the least contaminated using the Paired 'T' Test. The results of the analysis are indicated in Table 3.

Table 3: Statistics from analysis of data

1	**Nitrate (N)**	**N**	**Mean**	**St. Dev.**	**SE Mean**	**T-Value**	**P-Value**
	μ_1	57	26.6	15.0	2.0	6.86*	.0000
	μ_2	57	12.28	4.94	0.65		
2	**Nitrite (N)**						
	μ_1	57	.0504	.0609	.0081	-2.77	1.0
	μ_2	57	.132	.215	.028		
3	**Chloride**						
	μ_1	57	252.9	97.8	13	.52	.30
	μ_2	57	243.8	87.0	12		
4	**Iron**						
	μ_1	57	.0332	.0312	.0041	-4.45	1.0
	μ_2	57	.144	.186	.025		
5	**Alkalinity**						
	μ_1	57	290	101	13	-2.17	.98
	μ_2	57	324.8	68.6	9.1		
6	**Electrical Conductivity at 25^{0}C**						
	μ_1	57	1422	290	38	2.79*	.0031
	μ_2	57	1274	275	36		

These results: are *significant**

- Nitrate (N); T-Value is 6.86 that is greater than the table value 1.96 (56 degrees of freedom)
- Electrical Conductivity at 25^{0}C; T-Value is 2.79 that is greater than table value 1.96

While the following results are *not significant*

- Nitrite (N); T-Value is -2.77 that is less than the table value 1.96
- Chloride; (Cl); T-Value is 0.52 that is less than the table value 1.96
- Iron (Fe); T-Value is -4.45 that is less than the table value 1.96
- Alkalinity (OH); T-Value is -2.17 that is less than the table value 1.96

Therefore, it can be concluded that:

1. Nitrate (N); Water in Upper well has a higher concentration than in treated plot
2. Electrical Conductivity at 25^{0}C is higher in the upper well than in treated plot
3. Nitrite (N); Water in Upper well has a lower concentration than in treated plot
4. Chloride (Cl); Water in Upper well has a lower concentration than in treated plot
5. Iron (Fe); Water in Upper well has a lower concentration than in treated plot
6. Alkalinity (OH); Water in Upper well has a lower alkalinity than in treated plot

The change in the concentration of contaminants, specifically nitrates and electrical conductivity with time are graphically represented in *Figures 2 and 3* respectively**.**

DISCUSSION

The establishment of vegetation around the drinking water well increased canopy closure and leaf litter on the sandy soil thereby increasing soil organic matter therein. Mineralization of leaf litter resulted in the formation of humus. This increased plant nutrition that enhanced plant growth and the rapid development of the root mat around the model well since planting had been carried out in a dense manner.

Soil organisms occur wherever organic matter occurs (Ingham, 2000). They concentrate around roots, in litter, on humus, on the surface of soil aggregates and in spaces between aggregates. For this reason, they are most prevalent in forested areas and cropping systems that leave a lot of biomass on the surface. There are three sources of organic matter *viz*: Mucilage, a polysaccharide that is produced at the root tip; low molecular mass substances like sugars, organic and amino acids that are excreted by root hairs and intact cells and cellular material that is released be senescence of root epidermis and cortex and root hairs.

Plants create a favorable microenvironment around their root-zone that facilitates contaminant degradation. Plant-associated, endophytic and rhizospheric bacteria carry out degradation of toxic organic compounds. Endophytic bacteria are usually non-pathogenic bacteria occurring naturally in the internal tissues of plants which may promote plant growth, be beneficial to the plant host by producing a wide range of natural products while contributing to the biodegradation of soil pollutants as well (Bacon and White, 2000; Sessitsch *et al*., 2002). The major endophytic bacterial species isolated from plants, include *Acetobacter, Arthrobacter, Bacillus, Burkholderia, Enterobacter, Herbaspirillum* and *Pseudomonas, (*Lodewyckx *et al*., 2002). The unique status of the rhizosphere as a treatment zone has great potential for remediation (Anderson *et al.,* 1993; Cunningham *et al.,* 1996; Davis *et al*., 1998; 2003). The enhanced rate of biodegradation in the rhizosphere may be due to co metabolism and/or larger microbial populations that stimulate root exudates, root turnover, improved soil moisture, oxygen, and nutrient conditions.

Most of the effects on the soil occur within 1-2mm of the root surface but can extend further if the roots have mycorrhizas. Some of the effects include absorption of water and nutrients creating gradients of water potentials and nutrient concentrations close to the root surface. Further the ratio of cations and anions taken up by the root influences rhizosphere pH. For instance, most plants assimilate nitrogen from soil water for growth in the form of nitrates and ammonium compounds (Taylor *et. al,* 1997). The form of nitrogen taken up by plants has a strong influence on soil pH. Plants dominantly taking up nitrate (using the enzyme nitrate reductase) will release bicarbonate and/or hydroxyl ions to maintain an internal charge balance, thereby raising soil pH. Plants mainly taking up ammonium will release protons to maintain their charge balance, and this will lead to soil acidification (Wild, 1993, Killham, 1994).

The decreasing trend in nitrate levels can also be attributed to the process of denitrification that could be taking place in the ground water/root interface. Denitrification plays an important role in the bioremediation of nitrates and nitrites that have leached into the soil water rendering it unsuitable for human consumption. It refers to the process that occurs during the completion of the nitrogen cycle, and refers to the

reduction of nitrates and nitrites to nitrogen or oxides of nitrogen through microbial activity (Wild, 1993). This is referred to as dissimilatory denitrification and is different to the dissimilatory reduction of nitrate to ammonium. It is also different from assimilatory nitrate reduction where the roots of plants assimilate nitrates for protein synthesis releasing nitrogen or ammonia through respiration into the atmosphere.
The process of dissimilatory denitrification is a stepwise reduction:

Nitrate (NO^-_3) → Nitrite (NO^-_2) → Nitric oxide NO → Nitrous Oxide (N_2O) → Nitrogen (N_2)

The reduction of nitrate to nitrogen gas requires a series of enzymes. The enzyme, Nitrate reductase reduces nitrate to nitrite while nitrite is reduced by Nitrite reductase to gases Nitric oxide, Nitrous Oxide and Nitrogen that diffuse into the atmosphere. Results of experiments on Nitrate Reductase Activity and nitrate accumulation conducted by Pilipovic and others in 2006 demonstrated that the main process of nitrate assimilation is located in leaves, while roots serve as the place for nitrate deposition. Many bacterial genera possess enzymes for each step in the reduction of nitrate to nitrogen gas. Many bacterial genera with the capability to denitrify are found in soils. Facultative anaerobes like *Pseudomonas areuginosa, Pseudomonas fluorescens, Alcaligenes denitrificans, Bacillus licheniformis* have been isolated who feed on soil organic matter. Bacterial species like *Paracoccus denitrifcans, Alcaligenes eutrophus, Thiobacillus denitrificans* and *Rhodipseudomonas sphaeroide*s derive their energy from Hydrogen, Sulphur and from the sun (Kilham, 1994). Denitrification requires the following conditions:

a) presence of nitrate,
b) metabolizable carbon compounds
c) complete absence of oxygen at the site of reduction
d) rate of denitrification increases with temperature and is highest in a soil pH of between 6 and 8 (Wild, 1993).

All conditions required for dissimilative denitrification are met with in the experimental plot in Nawakkaduwa where:

a) Water in the wells is contaminated with high levels of nitrate.
b) In addition to leaf litter, soil organic matter, plant roots and organic manures like coconut peat and straw provide the metabolizable carbon compounds.
c) The below-ground conditions are water saturated and therefore depleted of oxygen. Further, denitrification can occur at micro sites like soil aggregates in the rhizosphere that are water saturated, oxygen restricted and exhibit anaerobic conditions.
d) The Kalpitya Peninsula is subject to high temperatures, low rainfall and relative humidity. The pH of water is between 6 and 7.

Hence conditions are favourable for dissimilative denitrification to occur.

Electrical conductivity (EC) estimates the amount of total dissolved salts (TDS), or the total amount of dissolved ions in the water. The decreasing trend in electrical conductivity could be attributed to the presence of polysaccharides in humus that had built up in the soil. Polysaccharides (repeating units of sugar-type molecules connected in longer chains) promote better soil structure through their ability to bind inorganic soil particles into stable aggregates. Other soil properties affected by polysaccharides include cation exchange capacity (attributed to COOH groups of uronic acids), anion retention (occurrence of NH_2 groups), and biological activity (energy source for microorganisms) (Elliot and Lynch, 1984).

Hydraulic residence time is affected by the shape and bathymetry of the basin and by the vegetation type and distribution. The uneven variation of these parameters produces two-dimensional flow characteristics and the hydraulic residence time of a particular block of water depends on the path taken by water as it flows through the system. A longer HRT allows for more of the treatment processes to be completed (Jenkins, 2003). The HRT for this particular model seems to vary. In the period August 2007 to April 2008, HRT fluctuates from 104 days to 197 days. The variation in HRT is dependent on the variation of the hydraulic gradient *i* during this period. It is pertinent to note that data on water levels in wells was taken in an arbitrary manner and not following a specific period like before or after pumping. It is clear however that the variation in HRT seems affected by the pumping that takes place in many of the wells surrounding the model well. However calculations based on data recorded in April 2011 record a higher hydraulic gradient that contributes to a shorter HRT of 51 days. This could be attributed to the maturing of the vegetation both above and belowground of the model well that had now become more effective in terms of phytoremediation.

In conclusion, calculation of the HRT needs to address the 'time' taken for establishment of the vegetation cap or buffer strip to establish and mature since the vegetation is tree dominant. In this model, planting was initiated in December 2001while testing began in February 2004. Therefore in real terms, the time taken for effective phytoremediation to occur would be 3 years and 51 days.

CONCLUSIONS AND RECOMMENDATIONS

The most appropriate means of controlling contamination of groundwater is to safeguard the source. However this is difficult in places like Kalpitiya where drinking water wells are located in or proximal to areas subject to intensive cultivation. In these circumstances, Phytoremediation offers a low cost solution to providing potable water. The technology also serves to increase soil organic matter and thereby sequester carbon in the soil. The vegetation established serves as habitat for biodiversity.

However, Phytoremediation requires more effort than simply planting vegetation and, with minimal maintenance, assuming that the contaminant will disappear. Phytoremediation requires an understanding of the processes that need to occur, the plants selected, and what needs to be done to ensure plant growth. Phytoremediation requires a commitment of resources and time, but has the potential to provide a lower-cost, environmentally acceptable alternative to conventional remedial technologies at appropriate sites.

Therefore, the roles played by tree roots, soil organic matter and bacteria in the phytoremediation of water are subjects worthy of further research and discussion. In this experiment, the collection of data over an extended period of time enabled the possibility of statistical testing for enhanced verification. The results indicated that the establishment of trees in a micro watershed could (after a three-year period) reduce the nitrate concentration in ground water and lower electrical conductivity as well. However, there is the need to monitor the movement of water and the contaminants in the various wells and piezometers when the process of mass extraction takes place. It is then that the efficacy of this technology can really be ascertained.

At the same time given that the main offender in ground water contamination in the Kalpitiya Peninsula is the excessive application of agrochemicals, specifically nitrogen based fertilizers it would necessitate authorities to focus on nutrient management that adopts an ecosystem based approach to optimize organic and mineral reservoirs with longer mean residence times that can be accessed through microbial and plant mediated processes. Organic agriculture is one such approach that seeks to manage plants, soil organic matter and soil organisms to maintain internal cycling capacity.

The sensitive nature of the hydrological environment in Kalpitiya requires the promotion of organic agriculture combined with phytoremediation technologies. This could result in greater resilience in the face of the climate change and the knowledge required to better manage sensitive environments.

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FIGURES

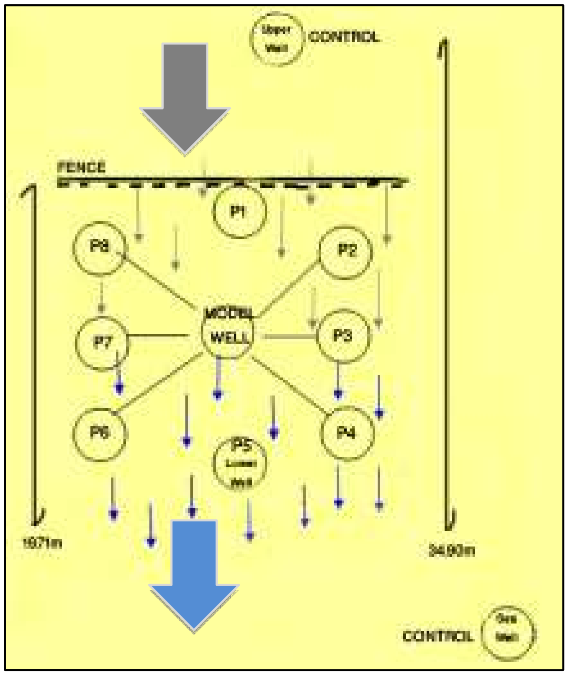

Figure 1: Schematic diagram showing location of wells, piezometers, direction of flow and distances measured

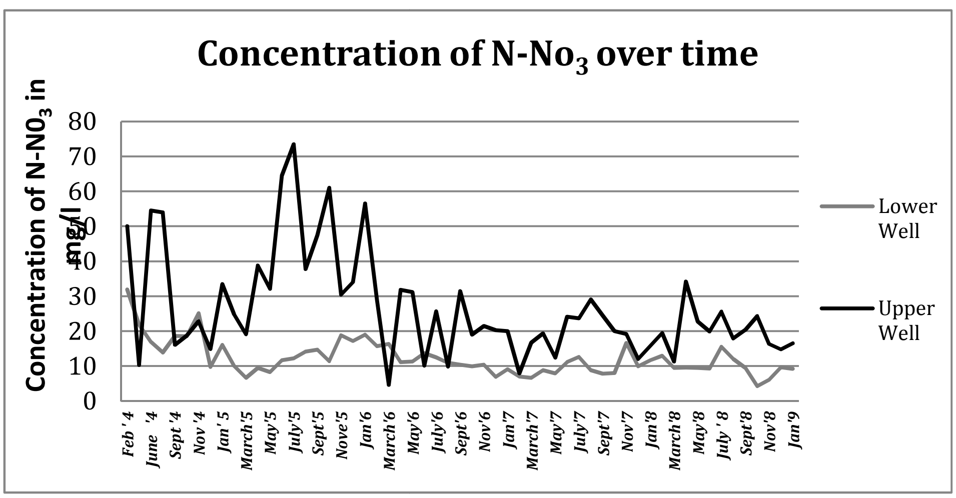

Figure 2: Concentration of Nitrate Nitrogen over time

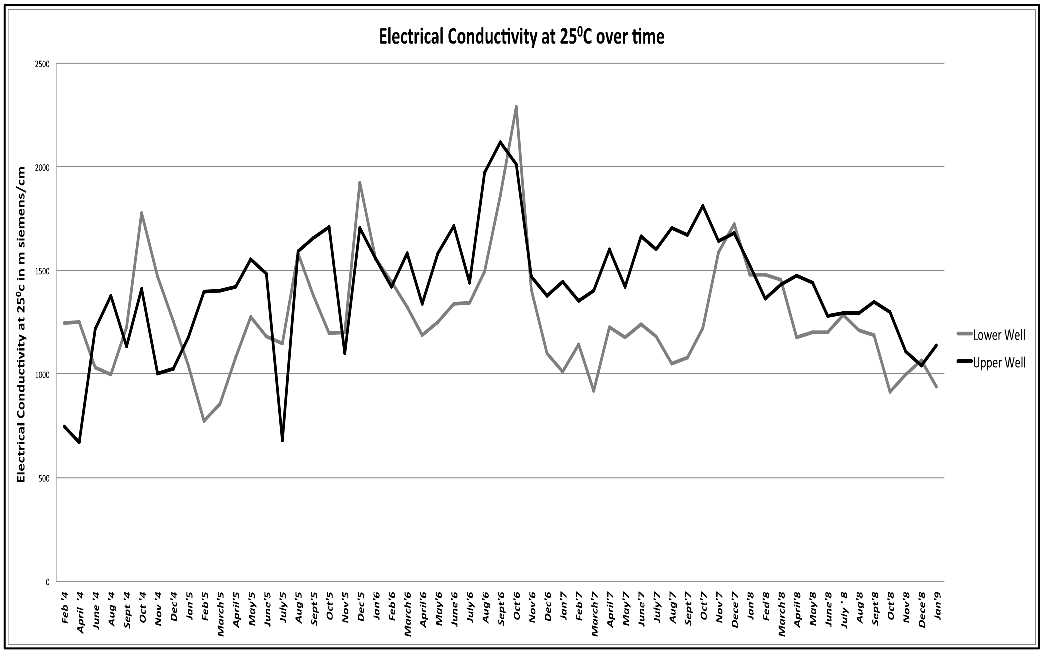

Figure 3: Electrical Conductivity at 25^0C over time