FINAL REPORT

Innovative Hydroponic Treatment of Municipal Wastewater Generating Commercially Valuable Plants

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Environmentally Sound Effluent for Local Communities

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1.0 SUMMARY

The objectives of this project were to design an inexpensive and simple system that can treat primary municipal wastewater to discharge standards, produce commercially valuable plants for small communities in arid and semi-arid areas and provide an increased supply of water suitable for safe, yet with low operational costs.

A commercial hydroponic system was adapted for studying the potential recycling of nutrients from primary treated municipal sewage effluent. The system consisted of five gullies, 3 meters long by 100 mm wide. Primary treated effluent was used to irrigate lettuce in one series, and a commercial nutrient solution was used to irrigate the same type of lettuce in another series as a control, both by nutrient film technique (NFT). Nutrient and suspended solids were efficiently removed by the NFT-plant system. Lettuces however, appeared to take up model viruses (fluorescent 0.1 μm microspheres) and possibly spores of the faecal bacterium, *Clostridium perfringens*. Microbial data was used in a β – Poisson dose respond model and indicated that the probability of infection for a single ingestion event of NFT grown lettuce fed primary treated municipal effluent was about 1.7% for viruses. Moreover, plants accumulated heavy metals in leaf tissues at concentrations higher than the maximum recommended levels for Australia and New Zealand Food (As = 6.5, Cd = 3.8, Pb = 20 mg kg^{-1}). Hence, it is recommended to grow ornamental or non-edible crops, such as essential oils, pyrethrum or flowers. The subsequent design of a full-scale production treatment hydroponic farm (PTHF) for small communities was based on modelling phosphorus removal with the hydroponic NFT experimental pilot plant. With an influent total phosphorus (TP) concentration 2-6 mg L^{-1}, the PTHF would be expected to be economical for small communities (< 400 people) and produce effluent with TP < 0.15 mg L^{-1}, SS < 2.5 mg L^{-1} and BOD < 55 mg L^{-1}.

This project focused on modelling phosphorus removal with the hydroponic NFT experimental pilot plant to design a full-scale production treatment hydroponic farm (PTHF) for small communities. It was concluded that the phosphorus removal model developed could be used to design an inexpensive and simple full scale PTHF for small communities (<400 people). Such a farm would treat wastewater to provide an alternative resource of nonpotable water and commercially valuable plants. Non-edible plants are recommended at this stage due to possible microbial and heavy metal contamination.

2. INTRODUCTION

Though Australia is the driest inhabited continent on Earth, demands on its very limited raw water resources are not sustainable, given current trends and an expanding population with high standards of living. Only 5% of Australia's wastewater is currently reused (Curran and Drinnan, 1992; Anderson, 1994). Less than 11% of effluent is reused for restricted municipal purposes in the NT (Burgess, 1991) where the climate is harsh with extended dry periods and water resources are relatively expensive. Less than 10% of Australia's water diverted for urban and industrial use is recycled for direct beneficial reuse (Anderson, 1996a).

If all effluent could be reused then the demand on potable water sources could be reduced by 30% (Anderson, 1996a). Nonetheless, various studies have been carried out to extend the permitted areas of reuse as reviewed by Curran and Drinnan (1992). In Sydney, municipal reuse of wastewater includes irrigation of several local golf courses. Furthermore, six million litres of secondary treated wastewater is used every day to cool molten slag at BHP's Port Kembla steel works. Commercial polymers, Victoria, has used treated effluent as cooling water for over 15 years. Cattle, sheep and goats are grazed on 1500 ha grass infiltration area which is flooded by effluent from the Werribee, Victoria, land based wastewater facilities. Some of the wastewater is also applied to 3,200 ha for land filtration. Results from several studies carried out on reuse of wastewater to irrigate plants like banana, tea, and forest trees were favourable and in Adelaide, wastewater from the Bolivar STP is intended for vegetable irrigation. In

Western Australia, there is one large groundwater recharging scheme in Perth, recharging its groundwater with one megalitres of reclaimed water every day. The use of wetlands for wastewater treatment is practised in many regions in Australia. Sydney Water Corp, (formally Water Board, Sydney, Blue Mountains & Illawarra) had a wetland system receiving secondary treated wastewater at North Katoomba before that plant was closed down, and now operates a wetland at Rouse Hill plant, largely for nutrient reduction..

The following forms of wastewater recycling were reviewed by Anderson (1996a):

2.1 Horticultural and Forestry Reuse

In temperate zones of Australia, reclaimed water is being used to irrigate a variety of crops, including sugarcane and tea-tree as a cash crop. In arid zones, reclaimed water is being used to irrigate tree plantations. Major trials have been undertaken at Albury, Branxton, Dubbo, Wagga Wagga, Mildura and Loxton. In the Wagga Wagga Trails, Australian eucalypts have outperformed other species.

2.2 Industrial Reuse

In the industrial field, reclaimed water is widely used for low grade industrial purposes including treatment works washdown, road marking, vehicle washing, dust suppression, fire fighting etc. At the Eraring power station near Newcastle, up to 400 m^3 day^{-1} of reclaimed water from the local sewage treatment plant receive advanced treatment using microfiltration and reverse osmosis before being fed into the demineralisation plant to provide boiler feed water. The power station was excpected to save approximately A$1 million per year in water charges, while the water authority will save the cost of a 15 km transfer main required for ocean discharge (Williams, 1997).

2.3 Urban and Residential Reuse for Non Potable Purposes

The first major residential recycling system in Australia is at Rouse Hill (9,400 ha) in Sydney. The development will ultimately accommodate 235,000 people. Though the application of reclaimed development will use treated wastewater for non-potable domestic purposes such as garden watering, toilet flushing and fire fighting (Law, 1996; Williams, 1997). A smaller system is under construction at Wagga Wagga.

At the Taronga Zoological Park in Sydney, wastewater from animal exhibits and visitor facilities will be treated before being recycled for site irrigation. This system will save 250 m^3 day^{-1} (A$ 50,000 per year savings) (Williams, 1997).

The rivers of the Murray-Darling Basin have deteriorated because of nutrients from agriculture, urban wastewater and storm water inputs. Similar deterioration has occurred in the Hawkesbury-Nepean Basin. Nutrient removal facilities or land application facilities are being installed in all significant plants. Furthermore, NSW has initiated a statewide "Phosphorus Action" program which aims to minimise phosphorus inputs to both wastewater and stormwater (NSW EPA, 1995). Hence, methods to reuse effluent and reduce eutrophication are clearly needed.

2.2 Hydroponically Reclaimed Water

Virtually every terrestrial plant appears to be capable of growing in some form of hydroponic system (Jewell, 1990; Cooper, 1996).

Worldwide, Environmental Protection Authorities require sewage to be treated to discharged standards (acceptable standards) (see section 2.5.1) before being reused for the desired applications. In New South Wales, the Environmental Protection Authority (NSW EPA) is contemplating very strict levels and soon loadingsfor phosphorus, nitrogen and other pollutants in effluent discharged to water bodies (section 2.6).

Many communities have very limited capacity from surface water bodies. In addition, many surface waters can not be used for any purpose due to contamination by effluent discharged with high levels of pollutants. In some parts of the world farmers use such effluent for plant production for human consumption, resulting in serious health and social problems. The common reasons for the high levels of pollutants in industrial municipal effluents are the difficulties and unafordable cost of operation and maintenance of necessary treatment facilities. A major part of the operating cost is due to energy used to transfer oxygen to the aerobic bacteria in order to oxidise organic matter to carbon dioxide, water and new cells. Moreover, operating and maintaining a conventional sewage treatment plant (STP) requires skilled people.

Even with conventional treatment, it is estimated that a conventional treatment system after treating sewage from 10,000 people, discharges more than a quarter of a ton per day of suspended and biodegradable matter (Jewell, 1994). With future legislation this will not be permitted, therefore treatment costs are expected to increase dramatically. In addition to the suspended and biodegradable matter that is discharged, the disinfection of conventional effluent with chlorine gas is an expensive process that generates significant toxic by-products. Hence it is clear that alternative or more advanced sewage treatment methods have to be adopted for a safer environment and yet allow development. Jewell (1986) used roots of many kinds of plants to remove pollutants from sewage. After eight years of research with plants grown in a modified hydroponic system feed partially treated sewage, he indicated that plants removed more than 80% of nutrients and 90% of faecal coliforms (Jewell, 1994). A further claim was that sewage of 10,000 people could produce biogas from a nutrient film technique hydroponic system worth more than US$ 250,000 per year.

In one of the few cases in which a hydroponic concept has been used to purify sewage, British researchers from Portsmouth Polytechnic (Butler *et al.,* 1989) filled inclined impermeable channels with gravel and planted them with reeds in the first stage and sugar beet in the second. A primary settled sewage was used in the hydroponic system for wastewater treatment, and for crop production, beans, sunflower and cotton. The system achieved 90% BOD removal, 95% ammonia-N removal, and more than 99% faecal coliforms removal (Bulter and Dewedar, 1991; Stott *et al.,* 1997).

Further work in Japan demonstrated a hydroponic system utilising channels in porous blocks of concrete, for the treatment of a small polluted municipal river and for the production of tomatoes (Ohta *et al.,* 1993). Their system removed more than 99% of total organic carbon (TOC). Nevertheless, none of the above authors developed models for their hydroponic systems, which could have be used for general design considerations.

In addition to possible long term efficiency, the hydroponic approach used in this project offers a simple and less expensive treatment of wastewater that small communities can afford to use to clean their wastewater and benefit from commercial crops. This type of hydroponic system is often refer to as a nutrient film technique (NFT) which was developed in England to grow plants without soil in green houses (Cooper, 1996). The proposed treatment allows plants to grow in a thin film of wastewater in impermeable channels to prevent groundwater pollution and soil salientian.

When wastewater effluents are used for crop irrigation, the concentration of trace elements in generally is not high enough to cause any short-term (acute) harmful effects (Pettygrove and Asano, 1985). Since most trace elements tend to accumulate in the soil, the trace-element contents of the receiving soil could be substantially elevated by the long-term use of the wastewater. Subsequently, the ground water quality may deteriorate. Hydroponic applications of wastewater for irrigation will not have such effects as long as plant biomass is utilised appropriately.

2.3 Guidelines

The use of untreated water is not recommended by the National Health and Medical Research Council (NH&MRC) Draft Guidelines (1996). Table 2.1 shows the relative health risks involved where untreated wastewater is used for crop production as identified by WHO (1989).

Table 2.1 Relative health risks (NH&MRC, 1996)

Highest Risk		Lower Risk		Least Risk	
Category	Examples	Category	Examples	Category	Examples
Helminths	Roundworm and flatworm in humans and livestock	Bacteria	cholera typhoid shigellosis	Viruses	viral gastroenteritis infectious hepatitis
		Protozoa	amoebiasis giardiasis		

Levine *et al.* (1997) attempted to identify different risk levels in wastewater reuse guidelines for different countries (Table 2.2). These different risk categories varied between I and IV, where IV is the most stringent. In some countries, such as Australia, III is the most stringent which is for crops eaten raw to those eaten cooked. Categories in Table 2.2 are not clearly identified for each country and hence the relationship between categories of different countries was not identified.

Table 2.2 Agricultural reuse categories* use in different countries (Levine *et al.,* 1997)

<table>
<tr><th>Countries</th><th>No exposure</th><th>Forest/ restricted areas</th><th>Cotton sugar beets, cereals, dry fodder seeds</th><th>Green fodder Orchards</th><th>Deciduous fruits, vegetables cooked or peeled, Pastures</th><th>Sport fields and golf courses</th><th>parks and lawns</th><th>unrestricted crops and vegetables eaten raw</th></tr>
<tr><td>Australia</td><td>I</td><td colspan="3">II</td><td colspan="4">III</td></tr>
<tr><td>France</td><td>I</td><td colspan="4">II</td><td colspan="3">III</td></tr>
<tr><td>Israel</td><td colspan="3">I</td><td>II</td><td colspan="3">III</td><td>IV</td></tr>
<tr><td>Kuwait</td><td colspan="7">I</td><td>II</td></tr>
<tr><td>California</td><td colspan="4">I</td><td colspan="3">II</td><td>III</td></tr>
<tr><td>WHO</td><td>I</td><td colspan="4">II</td><td colspan="3">III</td></tr>
</table>

*Category I is the least stringent and IV is the most stringent.

The discharged effluent which is planned to be considered as an alternative resource of non-potable water used for irrigation and the commercially valuable plants produced should comply with the standards outlined below.

2.3.1 Reclaimed Water

The NSW Recycled Water Coordination Committee guidelines (NSW RWCC, 1993) and the NH&MRC Draft Guidelines (1996) identified reclaimed water quality, treatment and permissible uses for urban, recreation, aquaculture and residential reuse as outlined in the following sub-sections.

2.3.1.1 Microbial Quality

A comparison of reuse guidelines illustrates that generally only thermotolerant (faecal) coliforms are the principal microbial group specified (Table 2.3). Reasons for excluding other microbial groups (Table 2.2) vary, but are largely due to the high cost and low accuracy of such tests. For Example the NH&MRC (1996) listed the following reasons for not recommending virus limits in wastewater reuse:

- evidence of viral removal by filtration and disinfection
- virus monitoring is slow, expensive and imprecise (28 days is needed for virus identification)
- there is no consensus on the health significance of low levels of viruses in reclaimed wastewater, and
- there are insufficient studies available to define appropriate benchmark levels for setting viral standards.

Nevertheless, it is essential to set viral and other parasite standards for wastewater reuse in the human sphere. Hence, research for simple, cost effective methods are required, and a possible utilising microbial risk assessment is discussed in section 4.4.

Table 2.3 Comparison of thermotolerant coliforms guidelines

Water use application	Thermotolerant coliforms per 100 mL (median)		
	(Australia)[1]	California[3]	WHO[4]
Non-human food chain	<10,000	No guidline recommended	No guidline recommended[3]
Low contact, eg. horticulture	<1000	No guidline recommended	No guidline recommended[3]
Medium contact (recreational)	<150	<23	<200 [3]
High contact, e.g. irrigation of salad vegetables	<10	<2.2	<1000 [2]

[1](NH&MRC, 1996)

[2](Levine *et al.,* 1997)

[3] (Asano and Levine, 1996)

[4] The WHO guideline for developing countries also requires <1 helminth egg per 100 mL in agricultural effluent.

2.3.1.2 Physical Quality

Turbidity can protect pathogens from the effects of disinfection, enhance bacterial growth and cause a significant disinfection demand (NSW RWCC, 1993). For irrigation reuse, reclaimed water must satisfy the turbidity levels shown in Table 2.4.

At a pH below 6.5, water may be corrosive to plumbing fixtures. At a pH above 8, chlorine disinfection efficiency is impaired. Hence pH must be within the limits shown in Table 2.4. Californian and WHO guidelines did not include values for pH or colour (ie organics which give a chlorine demand).

Table 2.4 Comparison for physical quality guidelines

Parameters	Australia[1]	California[2]	WHO[2]
Turbidity NTU	5 (95% of samples)	2	NGR*
pH	6.5-8.0	NGR	NGR
Colour TCU	15	NGR	NGR

[1](NH&MRC, 1996; NSW RWCC, 1993)
[2](Levine *et al.*, 1997)
*NGR = No guideline recommended

2.3.1.3 Chemical Quality

Chemical guidelines for wastewater reuse in irrigation are summarised in Table 2.5 for Australia and California .

(a) General

The Australian and New Zealand NH&MRC (1996) requires that the following types of wastes should be excluded from reclaimed water for general reuse:

- radioactive material with a half-life of more than a few days;
- pharmacologically active compounds;
- non-biodegradable toxic organic materials;
- high concentration of metals;
- petrochemical and mining industries output.

Free residual chlorine in the treated effluent for reuse purposes should not exceed 0.5 mg L^{-1} at the point of use. Clearly achieving the absence of pharmacologicall active chemicals is problomatice given the very low levels and absence data (e.g. xenoestrogens [Nogel *et al.*, 1997]).

(b) Metals, Salts and Nutrients

Application of reclaimed water for irrigation in excess of plant requirements may lead to adverse long term environmental effects from accumulation of salt (Table 2.5) or nutrients in soils or groundwater. Excessive application of reclaimed water may also cause runoff of nutrients to local waterways. Guidelines for organic compounds are represented by biochemical oxygen demand (BOD), whereas salinity is represented by total dissolved solids (TDS). Australian standards for nitrogen, phosphorus, BOD and

TDS are complex. The Water and Cathment Branch of NSW EPA sets standards for each individaul wastewater treatment site depending on the effluent recieving body of that site (Marxen, 1997). BOD and TDS standards for secondary effluent were 20 $mg.L^{-1}$ and 500 $mg.L^{-1}$, respectively (NSW RWCC, 1993). Limits, however, will shortly be set on a total loading basis by the NSW EPA.

Table 2.5 Recommended maximum concentrations of metals and salts in irrigation water used continuously on various soil types

Metal	Australia[1] on all soils ($mg\ L^{-1}$)	California/USA on Sandy soil[2] ($mg\ L^{-1}$)
Al	5.0	5
As	0.1	0.1
B	3.0	0.75
Cd	0.01	0.01
Co	0.05	0.05
Cr	0.1	0.1
Cu	0.2	0.2
Fe	1.0	NGR[3]
Hg	NGR	0.01
Li	2.5	2.5
Mg	NGR	NGR
Mn	0.2	0.2
Mo	0.01	0.01
Se	0.02	0.02
Be	0.1	0.1
Ni	0.02	0.2
Pb	0.2	5.0
Zn	0.20	1.0

[1](NSW RWCC, 1993; NSW EPA, 1995; NH&MRC, 1996)

[2] (Chang *et al.,* 1996)

3 NGR = no guilines recommended

2.3.1.4 Permissible Uses for Reclaimed Wastwaters

The treatment and quality requirements for the irrigation uses covered by the Wastewater Reclamation Criteria in California and New South Wales are summarised in Table 2.6.

Table 2.6 Wastewater treatment criteria and quality for irrigation

California[1]		Australia[2]	
Treatment Level	**Type of Reuse**	**Treatment Level**	**Type of Reuse**
Primary	Surface Irrigation for orchard and vineyards Fodder, fibre and seed crops	Secondary plus Pathogen reduction (disinfection by chlorine, ponds or lagoons)	Indirect potable: Pasture and fodder for dairy cattle, crops not in direct contact with reclaimed water, cooked crops, (controlled public access) irrigation parks, sportsgrounds
Oxidation and disinfection	Pasture for milking animals Landscape impoundment Landscape irrigation (golf courses, cemeteries) Surface irrigation of food crops (no contact between water and edible portion of crop)	Secondary+ filtration Pathogen reduction	Urban (non-potable) residential garden watering, irrigation parks, sportsgrounds Agricultural: food production spray irrigation
Oxidation, coagulation, calcification, filtration[3], and disinfection	Spray irrigation of food crops Landscape irrigation (play grounds, parks)	Secondary	Pasture and fodder Horticulture

[1](Pettygrove and Asano, 1985; Chang *et al.,* 1996)

[2](NH&MRC, 1996)

[3]The turbidity of filtered effluent cannot exceed an average of 2 turbidity unit during any 24-hour period

2.3.2 Food Quality

The Australia and New Zealand Food Authority (ANZFA) does not set guidelines for microbial levels in food. The ANZFA however, sets guidelines of selected heavy metals for food Table 2.7. Details about health risk associated with effluent application for food production is discussed in Chapter 6.

Table 2.7 Australian food standards for food crops

Metal	Food guidelines* for vegetables (mg kg^{-1})
As	1.0
Cd	1.25
Cu	10.0
Pb	0.5
Zn	150

*(ANZFA, 1995)

2.4 Plant Needs and Effluent Quality

It is essential to understand the relationship of plant-effluent in the design and implementation of nutrient film technique (NFT) for wastewater treatment and plant production. Effluent has most nutrients and micronutrients (Table 3.1) required for plant growth (Metcalf & Eddy, 1991). Since the NFT liquid is stirred, nutrients are immediately available at the root surface. Moreover, the proportion of the nutrient in the effluent in direct contact with the root is large compared to that for plants grown in soil. Plant roots also modify their environment to survive the toxic nature of effluent (Waisel *et al.*, 1996).

The proposed NFT gydroponic systemis expected to produce highly treated effluent even at low levels of nutrient because of the sorption treatment mechanisms in addition to the nutrient, uptake by plant roots. For example, root system of vegetable plants (eg lettuces) at age of 38 days continue to absorb nutrients from wastewater even at concentrations as low as 0.0075 mg.L^{-1} (Barber, 1984).

In summary, hydroponic systems can achieve a high degree of wastewater treatment for almost all pollutants (Butler and Dewedar, 1991; Ohta *et al.,* 1993; Jewell, 1994).

2.4.1 Constituents of Effluent Utilised by Plants

Effluent is mostly water which is important (Barber, 1984) as :-

(1) a medium for diffusion of solutes and mass transport of nutrients to roots,

(2) a temperature-regulating liquid, and

(3) a solvent for biochemical reactions.

Reuse of effluent for agriculture is popular because it is an alternative resource of water and contains essential elements for plant growth. Nevertheless, it contains some elements which alter plant growth (Kourik, 1990).

The nutrients present in effluent most likely to be utilised by plants are nitrogen, phosphorus, potassium, and in smaller quantities, trace elements such as boron and zinc (Larcou and Hadjivassilis, 1989; Kuribayashi, 1992). Since full utilisation of nutrients is the goal of effluent irrigation schemes, nutrients should not be removed from the effluent at the treatment stage except for agronomic systems where nutrients would accumulate to unacceptable levels in soils and waters (Katterman, 1987).

Since nutrient levels may influence the amount of additional nutrients that can safely be applied in effluent, they should be determined prior to the establishment of an irrigation scheme (Laughton *et al.*, 1990; NSW EPA, 1995). Further, the composition of nutrients taken up by the crop should be determined at key stages of crop growth to ensure that nutrient balance is maintained (Esen and Puskas, 1989).

2.4.1.1 Nitrogen

Effluent nitrogen, 15-50 mg L^{-1} (total as N) may be present in a variety of chemical forms depending to some extent on the treatment and stabilisation processes employed: organic nitrogen, ammonia (NH_3), ammonium (NH^+_4), nitrate (NO^-_3), and nitrite (NO^-_2) (Metcalf and Eddy, 1991). About 40% of the nitrogen is usually present as organic nitrogen with the remainder as ammonia or ammonium ions (Kuenen and Robertson, 1994; Law and Walmslwy, 1994). Discharge levels for selected parameters are listed in Table 2.8.

Table 2.8 NSW discharged effluent guidelines for organic matter, nutrients and salinity for irrigation waters

Parameters	Guideline Value* (mg L^{-1})
BOD	<5
TDS	<175
N	<8
P	<0.3

*(NH&MRC, 1996; Williams, 1997)

2.4.1.1.1 Assays for Forms of Nitrogen

Total nitrogen is comprised of organic nitrogen, ammonia, nitrite and nitrate. Organic nitrogen is determined by Kjeldhal method (APHA, 1995). The aqueous sample is first boiled then it is digested. During the digestion, the organic nitrogen is converted to ammonium. Total Kjeldhal nitrogen is determined in the same manner as organic nitrogen except that the ammonia is not driven off before the digestion step. Total Kjeldhal nitrogen is, therefore, the total of organic and ammonia nitrogen. Ammonia nitrogen exists in aqueous as either the ammonium ion or ammonia, depending on pH of the solution (Metcalf and Eddy, 1991). The total nitrogen (as N) concentration in typical sewage effluent ranges from 15 to 50 mg L^{-1} (Lockwood *et al.*, 1994; NSW EPA, 1995). It is recommended that irrigation water should contain nitrate in concentration ranges between 75-151 mg.L^{-1} (Table 2.10).

Any excess nitrogen in irrigation water may be carried through the soil to the water table, where it becomes incorporated into the groundwater (Marecos *et al.,* 1989; Oron *et al.*, 1991; Lobartini *et al.*, 1994). Elevated levels of nutrients in the groundwater may render the groundwater unsuitable for stock and domestic water supplies, or may provide nutrients for unwanted plants and algae when groundwater eventually joins surface waters (Lowe *et al.*, 1986; Murphy, 1986). Significant nitrate concentrations are only encountered in freshly treated sewage effluent if the treatment process employes extended aeration for nitrification (NSW EPA, 1995). From an environmental perspective, nitrate is the most critical form of nitrogen. Its solubility, mobility and stability mean that it is readily leached to groundwaters, it has an active role in the eutrophication process, and, in drinking water, it poses a threat to human and animal health (Oswald, 1989).

Inorganic nitrogen in any soluble form (NH_3, NH_4^+, NO_3^-, NO_2^-) is a nutrient and needs to be largely removed from wastewater to control algae growth in receiving bodies (Payne *et al.,* 1989; Rausch and Heydon, 1991).

2.4.1.1.2 Nitrogen Balance

The nitrogen balance is estimated by comparing the total nitrogen usage of the crop cultivated, with the amount of total nitrogen applied in effluent (Overman and Schanze 1985; Oron and DeMalach 1987a; Readron, 1994). This is only an approximation since there are other processes which use or convert various constituents of the applied nitrogen. These include volatilisation of ammonia, denitrification of both nitrate and nitrite to gaseous nitrogen forms which then return to the atmosphere, and some residue left on the ground which may cycle nitrogen back into the plant soil system (Pettygrove and Asano, 1985).

The fraction of the applied nitrogen lost through denitrification, volatilisation, and soil storage, depends primarily on effluent characteristics and climate (Darji, 1988). Nitrogen losses are generally lower in cold climate and higher in warm climates. Generally, fractions of applied nitrogen lost to the atmosphere range between 15% and 25% for secondary treated municipal effluent (Christoulas and Andereadakis, 1989).

2.4.1.1.3 Nitrogen Forms and Plants

Nitrogen is present in wastewater in different organic forms and in two inorganic forms, ie. ammonium and nitrate (De Wet and Barnard, 1992). The behaviour of nitrogen in effluent is therefore complex and many processes influence uptake by the plant (Jung and Ito, 1994). Ammonium may be volatilise, it can be adsorbed at the cation exchange complex or it can be converted into nitrate. Nitrate and nitrite may be lost through denitrification. Both ammonium and nitrate can be converted into organic nitrogen by microorganisms; which may then be remineralised (Karia, 1985). The net balance of inorganic nitrogen determines availability of nitrogen for plants (Gee *et al.*, 1985; Goronszy, 1994).

2.4.1.2 Phosphorus

Wastewater and diffuse agricultural runoff have been identified as the principal sources of phosphorus responsible for the stimulation of aquatic plants and for contributing to eutrophication in general. Typically, the phosphorus enters wastewater from human body wastes, from food kitchen wastes, and from the condensed inorganic compounds used in detergents (Metcalf and Eddy, 1991). Phosphorus in effluent is usually present in three chemical forms: orthophosphate, polyphosphate and organic phosphate (Ohta *et al.*, 1993). The orthophosphates are available immediately for biophysical reaction in the plant/effluent system (Bayly *et al.,* 1994). The availability of polyphosphates is limited by their hydrolysis which proceeds slowly in most cases (Tebbutt, 1983; Smethurst and Comerford, 1993). Organic phosphates are broken down biologically to polyphosphates and then to orthophosphates (Stoner, 1977; Sund, 1986). Phosphorus is removed from effluents through biological, chemical and physical processes as well as uptake by plants (Sheikh *et al.*, 1986; Van Oorschot and French, 1994).

In soil irrigation, the existing soil phosphorus sorption capacity, and the phosphorus uptake of the vegetation to be cultivated would determine how much phosphorus may be introduced before the site is saturated (White and Dornbush, 1988; Readorn, 1994). The useable lifetime of an irrigation site is evaluated from this information. Physical and chemical soil reactions provide significant phosphorus removal pathways and are not necessarily renewable (Breen, 1990; Goronszy, 1994). Thus, applying effluent with very high phosphorus concentration could shorten the useable lifetime of a site (NSW EPA, 1995). On the other hand, high levels of phosphorus in effluent does not affect the useable life time of a hydroponic system (Rababah and Boyden, 1995).

Spencer (1997) analysed data obtained from different authors for nutrient uptake by plants. His analyses for nutrient uptake by tea tree plants showed that nitrogen would be depleted before phosphorus from wastewater, therefore for the full phosphorus utilisation, nitrogen might need to be added to wastewater. Collected data (Tables 3.2 and 3.3 and Appendix D) from the experimental trials carried out for this project however, showed that nitrogen was not completely depleted from the utilised effluent although higher nitrogen removal rates than phosphorus removal rates were achieved. Results of this project and Spencer's (1997) analyses showed that phosphorus is an important and common problem in wastewater treatment, and hence the focus on phosphorus for the model developed for this project.

2.4.1.3 Potassium

Potassium is an essential nutrient for plant growth and it assists plants to take up nitrogen. In sewage effluent, however, it is usually present at concentrations which may be too low for the optimum uptake of nitrogen (Hutchins *et al.,* 1986; Wilson *et al.,* 1988). Since a sewage effluent reuse scheme depends on the growth of cultivated vegetation for nitrogen utilisation, a potassium supplement may be required to maximise plant growth and so optimise nitrogen uptake. On the other hand, the application of an excess amount of potassium, as could happen in effluent irrigation, can alter the uptake of other elements by plants (Nissen, 1989; Abdelrrahman and Shahalam, 1991).

Lettuce plants contain some 3% of their dry weight as potassium (Table 2.9). Potassium has two major types of nutritional roles in plants. Firstly, potassium is an activator of many enzymes hence, is biochemically important in various metabolic processes including photosynesis respiration and protein synthesis. Secondly K^+, is the most important inorganic atom involved in the control of osmotic potential (Lüttge and Pitman, 1976; Smethurst and Comerford, 1993). So K is essential for plant growth as well as for stomatal and leaf movements (Gutschick, 1993). The recommended concentration in irrigation water ranges between 124 and 248 mg L^{-1} (Table 2.10) (Robb and Pierpoint, 1983; Glendinning, 1990; NSW EPA, 1995).

Table 2.9 Typical composition of plant material (Robb and Pierpoint, 1983)

oven dry weight (%)				oven dry weight (mg kg^{-1})				
N	P	Ca	K	Cd	Cu	Mn	Ni	Zn
3.8	0.57	0.3	3	0.9	14	51	6	104

2.5 Effects of Wastewater Constituents on Plant Growth

Effluent contains valuable resources of salts and elements (Cathcart, 1989). Nevertheless, these elements may exist at unacceptable levels which resulting in phytotoxicity (Pettygrove and Asano, 1985). The more important elements are described in the following sections, together with their implications for plant growth and removal by plants.

2.5.1 Trace Elements

Trace elements are those that occur in the effluent at concentrations less than a few mg L^{-1} with usual concentrations less than 100 µg L^{-1} (Adin and Elimenlech, 1989). Some may be essential for plant growth at very low concentrations (micronutrients) but quickly become toxic as the concentration increases (Chansler, 1991). Others are not essential (Young and Holliman, 1991). The term trace element is used to denote a group otherwise unrelated chemical elements present in the natural environment in low concentrations (Pettygrove and Asano, 1985). In small quantities, many elements (e.g. F, Si, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Mo, Sn, I, Cl, B) are essential for biological growth. At slightly higher concentration, many elements may become toxic to plants and/or animals (Bisio, 1990). There are also elements (e.g. As, Cd, Pb, Hg) that have no known physiological function and are always considered biologically harmful (Pettygrove and Asano, 1985).

Salinity refers to the quantity and type of salts dissolved in the irrigation water (Wareing and Phillips, 1981). It is usually determined by measuring the electrical conductivity of water; the saltier the water, the greater its conductivity. Salinity is the single most important parameter in determining the suitability of water for irrigation, as it relates directly to possible problems caused by the total salt load in the water (Collins *et al.,* 1990). Plants damage from either salinity or specific ions is usually tied closely to an increase in salinity (Azov *et al.,* 1991). Salt sensitive crops show drastic reductions at Electrical Conductivity > 3000 µS cm^{-1} even under otherwise best management (Pettygrove and Asano, 1985). Arsenic in wastewater is an interesting by-product due to its use to improve animals growth rates. Hence depending on the source of wastewater as toxicity may be a problem (Premier, *pers. comm.*)

The ions of most concern in wastewater are sodium, chloride, and boron. The most prevalent phytotoxicity from the use of reclaimed municipal wastewater is from boron (Kirk, 1987). The source of boron is usually household detergents or discharges from industrial plants (Pettygrove and Asano, 1985).

Trace elements are effectively removed from the effluent by removal of suspended solids (Pettygrove and Asano, 1985), whereas salinity is not easily removed, but requires exchange resins and/or reverse osmosis (RO).

2.5.2 Mineral Salts in Effluent

Effluent contains dissolved mineral salt such as sodium, calcium potassium, magnesium, boron, chloride, sulphate, carbonate and bicarbonate (Overman and Schanze, 1985). The term "total dissolved solids" (TDS) is commonly used to express the combined concentration of these salts (Marecos *et al.,* 1989). Most salts are present in effluent as dissolved charged particles, or ions, which can conduct electric current. An estimate of TDS is obtained through measurement of the electrical conductivity of the effluent, usually measured in $\mu S\ cm^{-1}$ (Herman, 1991; APHA, 1995). It is not recommended under normal circumstances to use effluent of more than 2500 $\mu S\ cm^{-1}$ for irrigation (Shukla, 1987; NSW EPA, 1995). The recommended levels of micronutrient and some nutrients in irrigation solutions are listed in Table 2.10. Salt toxicities arte generally reduced at neutral or pH > 6 (Pettygrove and Asano, 1985).

Table 2.10 Recommended nutrient balance* of different elements in nutrient solution for lettuce plants (Finlayson, 1996)

Element	High limit ($mg\ L^{-1}$)	Low limit ($mg\ L^{-1}$)
Aluminium	5	0
Boron	0.412	0.20
Calcium	101	50
Copper	0.04	0.02
Iron	3.1	1.6
Magnesium	24	12
Manganese	0.42	0.2
Nitrate-N	151	75
Phosphorus	28	14
Potassium	248	124
Sodium	150	0
Sulphur	32	16
Zinc	0.19	0.10

*Same or close ratios may be used to other vegetables

2.5.3 Elemental Uptake by Plants

The factors affecting the amounts of metal absorbed by plants are those controlling (Shariatpanahi and Anderson, 1986; Gardiner *et al.*, 1990):

(i) the concentrations and speciation of the metal in the solution;

(ii) the movement of the metal from the solution to the root surface;

(ii) the transport of the metal from the root surface into the root;

(iv) its translocation from the root to the shoot.

In the case of the strongly adsorbed ions, adsorption is more dependent upon the surface area of root available (Nye and Marriot, 1969; Noggle and Fritz, 1983). Mycorrhizae are symbiotic fungi which effectively increase the absorptive area of the root and can assist in the uptake of nutrient ions, such as orthophosphates and micronutrients (Lüttge and Pitman, 1976). Roots also posses a significant cation exchange capacity (CEC), due largely to the presence of carboxyl groups, and this may form part of the mechanism of moving ions through the outer part of the root to the plasmalemma where active absorption occurs (Robb and Pierpoint, 1983).

Absorption of metals by plant roots can be by both passive and active (metabolic) processes (Broom *et al.,* 1994). Passive (non-metabolic) uptake involves diffusion of ions in the solution into the root endodermis. On the other hand, active uptake takes place against a concentration gradient but requires metabolic energy and can therefore be inhibited by toxins (Enoch *et al.,* 1994). The mechanisms appear to differ between metals; for instance Pb uptake is generally considered to be passive while that of Cu, Mo, and Zn, is thought to be either active metabolic uptake, or a combination of both active and passive uptake (Robb and Pierpoint, 1983).

Relative differences in the uptake of metal ions between plant species and cultivars is genetically controlled and can be due to various factors including: surface area of the root, root exudates and the rate of evapotranspiration. The latter mechanism affects the mass flow of the solution in the vicinity of the root and thus the movement of ions to the root absorbing surface (Waisel *et al.,* 1996).

2.6 Specific Ion Phytotoxicity

Growth depression due to excessive concentrations of specific ions is called specific ion toxicity (Pettygrove and Asano, 1985). Further details follow for important trace metals in wastewater.

Arsenic

Arsenic is a constituent of most plants (Farago, 1994), but can be phytotoxic (Peterson *et al.*, 1981). The levels of As in edible plant is generally low, often being close to the detection limit (Pettygrove and Asano, 1985). Natural arsenic levels in plants seldom exceeds 1 mg kg^{-1} (Peterson *et al.*, 1981). Kitagishi and Yamane (1981) showed that the relationships between As content of lettuce and concentration in soil solution was significant, hence As levels in leaves increases with increasing soil solution concentration.

Boron

For plants, boron is an essential micronutrient (Berthet *et al.,* 1989) and H_3BO_3 is the principal form in which it is taken up (IAEA, 1984). At levels found in some effluents, boron can be toxic to crops/plants (EPA NSW, 1995). Plants greatly vary in their tolerance to boron, Table 2.11 shows the levels of boron tolerated in irrigation water by selected plants.

Boron can become toxic at levels only slightly greater than required for good plant growth (Table 2.11) (Leeper, 1978). Symptoms of excess boron include leaf tip and marginal burn, leaf cupping, chlorosis (yellowing leaves), drop branch dieback, and reduced growth. The basic characteristics of B deficiency as shown first in the youngest leaves, then by stem shortining, with severely affected plants liable to have a shrunken appearance, death of growing points and impaired root growth (Berthet *et al.,* 1989).

Table 2.11 Limits of boron in irrigation waters for plants with different levels of boron tolerance (Reid, 1990)

Tolerant 2- 4 mg L^{-1}	**Semi-Tolerant** 1- 2 mg L^{-1}	**Sensitive** 0.3-1 mg L^{-1}
date palm	sunflower	plum
cabbage	tomato	pear
lettuce	corn	apple
broad bean	capsicum	avocado

Cadmium

Cadmium has aroused concern less as a possible reducer of yields than as a possible contaminant in human food (Leeper, 1978). Cadmium is considered to be a toxic element to plants, and the main cause of its toxicity lies in that it disturbs enzyme activities (Adriano, 1986). When CdO or $CdSO_4$ was added to lettuce crop pots at pH 6.5 their yield was reduced. For example, when the Cd content was ≥ 200 mg L^{-1} the crop failed (Amoros *et al.*, 1989) which is more likely, however is tha it may accumulate in healthy plants in concentrations dangerous to the animal or human consumer. The animal body however, has learned to protect itself against cadmium by producing the specific molecule metallothioneion (Leeper, 1978; Broom *et al.,* 1994).

Roots take up cadmium rapidly from culture solution and some plants accumulate large amounts in their tops (Farago, 1994). Plants grown hydroponically in Cd readily accumulated 0.01 mg L^{-1} Cd within 3 days with at least 70 % of the cadmium in their roots. Furthermore, about 40 mg kg^{-1} of Cd was found in the tops of many dried plants without any visible damage (Leeper, 1978).

Chromium

It has not been possible so far to establish that Cr is an essential element required by plants. The addition of Cr to soil solution deficient in the element however, has been shown to increase plant yields (Farago, 1994). In an experiment, crops were unaffected at 1000 mg kg^{-1} and damaged at 2000 mg kg^{-1} (Blumenthal *et al.,* 1989).

Copper

Copper is an essential element for plants. Copper plays a significant role in several physiological processes photosynesis, respiration, carbohydrate distribution, nitrogen reduction, protein metabolism and cell wall metabolism. Many plant metalloenzymes contain copper (Lepp, 1981). The Cu content of normal plants, even with high soil applications, rarely exceeds 30 mg kg^{-1} (Farago, 1994). The Cu taken up by roots does not increase with addition to the soil solution at pH< 4.9 (Broom *et al.,* 1994). Copper concentrations in irrigation water higher than 1920 mg L^{-1} were damaging to various plants at pH = 6 (Leeper, 1978).

Lead

Lead is considered to be a non-essential metal to plants and a small proportion of lead is bioavailable to plants in soil solution (Alloway, 1995). Lead ions strongly precipitated in soil, yet many plants take up as much as 30 mg kg^{-1} in their roots (Peterson, 1978). Most plants retain this Pb almost entirely in the roots (Robb and Pierpoint, 1983).

Zinc

Zinc is of major importance in many fields of agriculture and is applied as a fertiliser more widely than magnesium (Leeper, 1978). It is likely that mankind would be healthier if we had more of it in our food (Farago, 1994). There are few reports of damage to plants by excessive Zn in soils (Berthet *et al.*, 1989). Plants continue to absorb Zn in high amounts at pH over 7 (for example, the accumulator Swiss chard may accumulate 630 mg kg^{-1}) (Leeper, 1978). Plants took Zn up to 300 mg kg^{-1} of their dry matter without being damaged. Nonetheless, a thousand mg L^{-1} Zn in an animals diet may be harmful, although zinc will kill the plant before it accumulate in amounts dangerous to an animal that eats the plant (Leeper, 1978).

Recorded concentrations of trace elements and some heavy metals in selected plants grown in soil are listed in Tables 2.9 and 2.12.

Table 2.12 Common values (mg kg^{-1}) of a number of heavy metals in dry matter of selected crops grown in soil

Type	Cd	Cr	Cu	Ni	Pb	Zn
Corn Leafs	10-15	1.5-2.5	12-16	2-4	4-9	100-250
Corn grain	0.1-0.3	0.5	5-7	1.5-3	0.2-0.8	20-35
Soybean leaf	1-5	0.5-1	17	8-14	4	80-160
Soybean grain	1-2	0.5-0.7	15-20	10-15	2	50-110

(Robb and Pierpoint, 1983)

2.7 Effluent Characteristics Considerations

Other effluent characteristics that may directly affect plant growth and not mentioned in the pervious sections are briefly explained in the following sub-sections.

2.7.1 Dissolved Oxygen

Nutrient cannot be taken up by most plants without oxygen at the root zone (Cooper, 1996). Experimental trials conducted for this study showed that the concentration of dissolved oxygen (DO) in the utilised primary treated effluent played the most essential role in plant growth.

2.7.2 pH

Effluent within the pH range 6.5 to 8.5 is acceptable for irrigation (Pettygrove and Asano, 1985). If the effluent is very acid, or very alkaline, it may need to be neutralised before application. Since solution pH affects the availability of nutrients to plants, periodic monitoring is necessary to ensure that nutrient uptake by plants is optimised by maintaining appropriate solution pH levels (NSW EPA, 1995). Plants growing in nutrient culture may increase or decrease the pH of the solution used for irrigation (Barber, 1984; Waisel *et al.*, 1996).

2.7.3 Organic Matter

Organic matter in effluent is presented in the dissolved form as well as in the form of suspended and colloidal solids. They can be measured as biochemical oxygen demand (BOD), chemical oxygen demand (COD), or total organic carbon (TOC). Dissolved oxygen (DO) in wastewater is utilised by microorganisms during the stabilisation process of organic matters. The dissolved oxygen in wastewater is not sufficient for the microorganisms to biochemically oxidise all organic matter. Therefore, continued addition of DO is needed to meet the microbial demand for DO. Hence, aeration is needed in the aerobic stage of wastewater treatment (Metcalf & Eddy, 1991).

The most widely used parameter used to measure organic pollution in wastewater and surface water is the 5-day biochemical oxygen demand (BOD_5). This determination involves the measurement of the dissolved oxygen used by microorganisms in the biochemical oxidation of organic matter over five days at 25 °C (Schreiber and Neumaier, 1987; Metcalf & Eddy, 1991).

Continuous application of wastewater to soil causes waterlogging, accumulation of undecomposed solids and progressive changes in soil structure. Furthermore, soil systems overloaded with applied organic material may develop anaerobic conditions causing odour and insect problems (Adin, 1987). Hence, the NFT system has the advantage of being sustainable when compared to the deterioration of land due to the continuous soil application of organic matter, particularly when natural rainfall makes it unnecessary.

2.7.4 Suspended Solids

By definition, suspended solids are all matter that remain as residue upon evaporation at 73 °C with minimum diameter of 1 micron (Metcalf and Eddy, 1991). Some 75% of suspended solids in a medium strength wastewater are organic in nature. Suspended solids can lead to the development of sludge deposits, anaerobic conditions, and odour problems when discharged to the surroundings. In a hydroponicv system, suspended solids may clog the pipes of a production-treatment hydroponic farm (PTHF) and cause anaerobic conditions at the bottom of the system channels.

2.8 Scope of Project

In the nutrient film technique (NFT) plants benefit from the various nutrients and permanent supply of water supplied by the effluent and offer treatment to that wastewater by biological, chemical and physical mechanisms outlined later in this project. Thus, application of NFT for wastewater treatment was found to fulfil the objectives sought in this project, which were to design an inexpensive and simple system that can treat primary municipal wastewater to discharge standards and produce commercially valuable plants for small communities in water shortage areas. This research aimed to provide an increased supply of water for irrigation essentially free from pathogens that might cause public health problems, with low operational costs, requiring minimal amount of energy, and minimising waste input to the environment.

The proposed NFT farm is an inexpensive and simple alternative technology that may replace conventional wastewater treatment plants for small communities. The NFT approach eliminates the large waste input to the environment by conventional sewage treatment.

Unlike reclaimed water soil irrigation schemes, the adapted nutrient film technique for this project was:

- An intensive production system, producing large numbers of plants in a limited area. This is of special value in many treatment plants built near cities, where land is expensive and limited;
- A sustainable system that will operate continuously because there is no fear of soil salinity problems, or water pollution;
- A pleasant irrigation scheme with no odour problems, because of the continuos passive sewage aeration throughout the system; and
- A clogg-free system.

Phosphorus was used as the initial modelling parameter to design the nutrient film technique farm, mainly because (i) phosphorus is an important and common problem in wastewater treatment, and (ii) it is possible to quantify all forms of phosphorus in the system which is an important issue for this modelling study.

Therefore, the project focused on experiments to develop the phosphorus removal model (P-Model) and concluded with a hypothetic hydroponic treatment plant farm design. In addition, human health concern from heavy metals and pathogen are discussed.

3. LIST OF PEPOLE INVOLVED

A/Prof. Nicholas Ashbolt	Centre for Water and Waste Technology Project Leader Wastewater Treatment

	Environmental Microbiology Health Risk Assessment
Dr Brace Boyden	Sinclair Knight Merz PTY LTD Industrial Partner Wastewater Treatment
Dr Rengel Zdenco	University of Western Australia Model Development
Dr Anthony Haigh	University of Western Sydney Plant physiologist
Accent Hydroponic PTY LTD	Sydney System Supplier
Mr Abdellah Rababah	Centre for Water and Waste Technology University of NSW Research Assistant
Ms Anna Carew	Microbiologist Centre for Water and Waste Technology University of NSW

4. PROJECT DESCRIPTION

4.1 Background

The experimental treatment plant was designed to collect;

1. Phosphorus and nitrogen removal rate data from primary treated municipal effluent by the nutrient film technique (NFT) type of treatment.
2. Growth rate of plants grown in NFT utilising primary treated municipal effluent.
3. Phosphorus removal data for the model to aid the design of a Production-Treatment Hydroponic Farm (PTHF) suitable for small communities:
   - phosphorus content of the plants and on the hydroponic system (e.g. roots and NFT channels, Mu and Ms respectively;
   - Phosphorus concentrations in the utilised effluent (Pi and Pe) at different plant age (t); and
   - root weight (W_R) and plant weight (W) at the same plant age (t).
4. Heavy metal concentration in effluent and in plants grown in NFT compared with licensed discharge limits and food standards for preliminary health risk assessment.
5. Pathogen translocation into plants for preliminary health risk assessment.

The experimental pilot plant set up and methodologies were designed to be capable of investigating recycling, not only the mass flow of wastewater but also the nutrients found in that water. The results from the NFT pilot plant and were used to deveolp design criteria for a full-scale production treatment hydroponic farm (PTHF) suitable for a small community (200 - 400 people). The potential viability of a hydroponic treatment system was also ascertained in this investigation. Lastely, environmental conditions of the experimental sites were considered because of their affects on plants physiology. Hence, three different locations were utilised in the NFT studies.

4.2 Experimental Setup of the NFT Pilot Plant

Criteria for the selection of the plant type to be tested with the NFT wastewater treatment system included:

1. plants grown commonly with NFT hydroponics system to minimise systems modification;
2. concentration on a species requiring large nitrogen and phosphorous inputs;
3. plants able to tolerate the wastewater physical and chemical characteristics;
4. plants able to grow using growing lights and have a short growth cycle (e.g. 8-10 weeks); and
5. plants with a commercial value.

Lettuce (*Lactuca sativa L.*) with an annual output value of £31 million was the third most important crop under protected cultivation in the United Kingdom. An area of approximately 1200 hectares of glass and plastic-film structures are used for cultivation of lettuce in the UK during the period from September to early June (Rose and Edwards, 1981). Comparable statistical data was difficult to obtain for lettuces in Australia, but lettuce is also commonly grown hydroponically. After some consultation, the project was initiated with a local variety, Mignonette Green Lettuce (Accent Hydroponics PTY LTD, Sydney). Moreover lettuce is a common crop eaten raw. The physiology of the plant also make it one of the worst cases, ie pathogens and heavy metals taken up by the root are directly translocated to the edible part.

Two series of commercial hydroponic nutrient film technique (NFT) systems (Cat-2530 Accent Hydroponics PTY LTD, Sydney) were modified to study the potential of recycling nutrients from primary treated municipal sewage effluent to produce lettuces for this study.

4.2.1 Wastewater Plot

The system consisted of the following components and is illustrated in Figure 4.1.

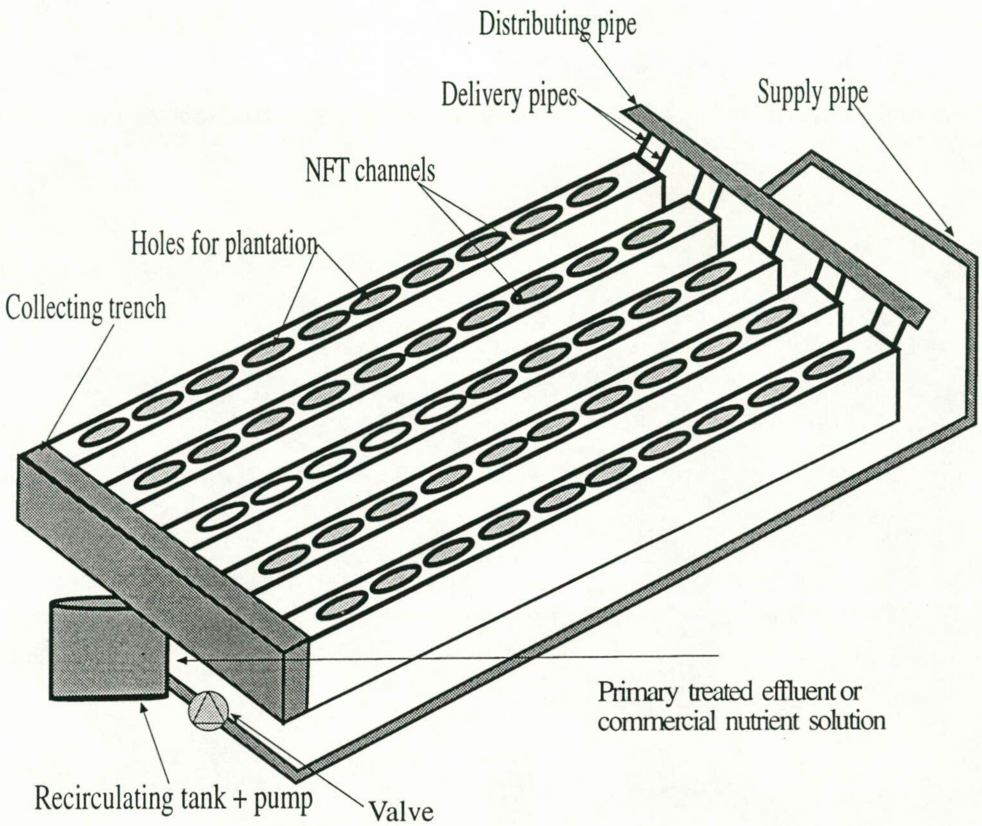

Figure 4.1 Commercial hydroponic system (Accent Hydroponics PTY LTD, Sydney) after modifications to suit the experimental design. Two of these systems were used for the experiment: one as a control and one for the effluent

Feed Recirculating Tank: A cylindrical 1 m^3 plastic tank was chosen to be the circulating tank of the primary effluent collected from an municipal wastewater plant (Bondi Sewage Treatment Plant, Sydney).

Supply Pipe Line: A light 10 mm in diameter PVC pipe (Accent Hydroponics PTY LTD, Sydney) was selected to transmit the primary effluent from the circulating tank to the plants. This type of pipe has been successfully used by commercial growers throughout Sydney for over 14 years. The pipe performed well throughout the study, and was found to be easily handled and shaped with minimum head loss and deposits around its inner surface.

Delivery Tubes: Two small bore 5 mm in diameter polythene tubes per channel delivered the effluent from the supply pipe to the inlet (ie. the upper) ends of the NFT channels. These delivery tubes were prepared (Figure 4.2) by tapering the end of the tubes that is to be inserted in the supply pipe. This was easily done by using a pencil sharpener. A hole was then drilled in the supply pipe which has a smaller diameter than the external diameter of the delivery tube. When the tapered end of the delivery tube was pushed into the under-sized hole, a very tight fit was ensured. Before inserting the delivery tube, an oblique cut was made across the tapered end. This ensures that when the delivery tube was inserted into the supply, the end of the tube cannot be sealed off by resting on the internal face of the supply pipe.

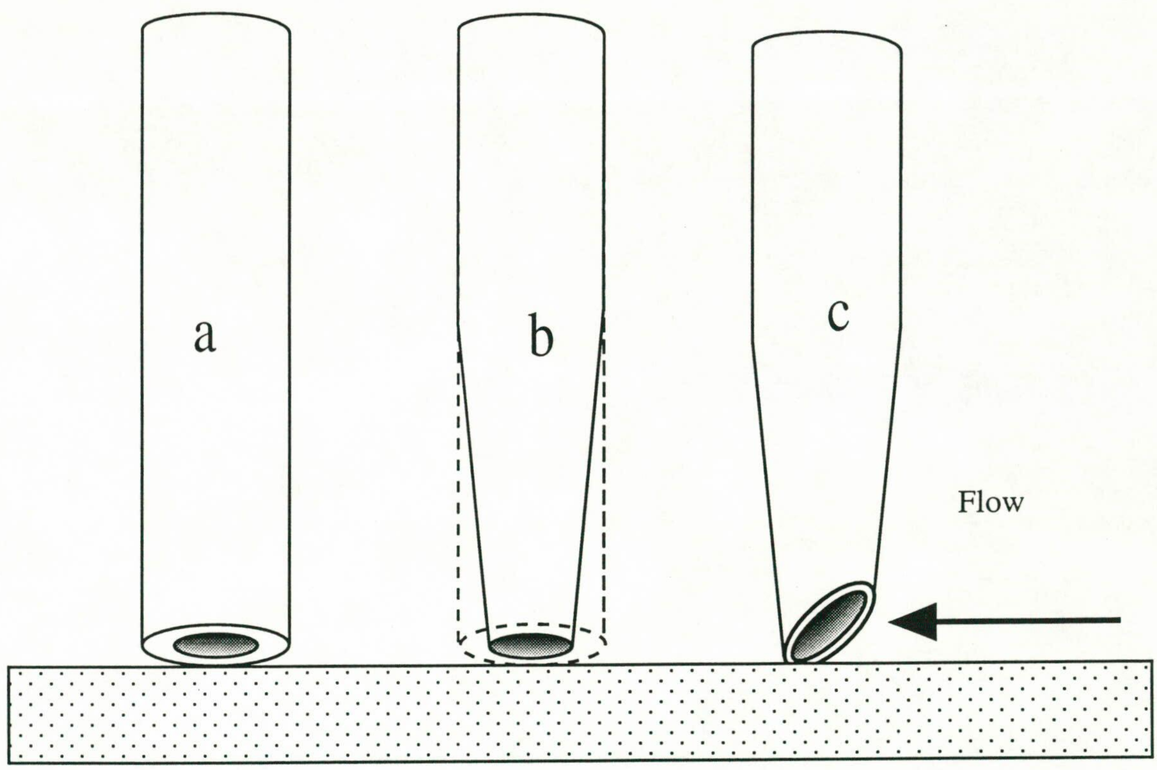

Figure 4.2 Tapered delivery tubes: (a) before modifications, (b) identify removed part to decrease diameter from the end going into supply pipe, and (c) modified tube; note slope cut at the end. From Cooper (1996).

Pump: A Little Giant submersible pump (Model NK1, Trans World Traders Pty Ltd, Sydney) was used to pump from the circulating tank to the head of the NFT channels where the plants were grown bare-rooted. The pump material consisted of: glass-filled nylon housing; nylon volute, plate, impeller and elbow; stainless steel motor shaft and fasteners; nitrile square cut seal (between volute and plate); Viton shaft seal and polyethylene screen. The cord jacket was a PVC material. Other plastic parts were nylon. This recirculation pump delivered up to 15 litres per minute at 5-meter head loss. A 10mm plastic valve was installed after the pump as a by-pass to control the flow rate. In order to maintain the basic NFT principle, thin (2-10 mm) flow of effluent through the 100 mm wide NFT channels, different flow rates and channel slopes were experimented. The suitable flow rate of effluent in the supply pipe was 8 $L.min^{-1}$. This flow rate also ensured sufficient supply of nutrient (phosphorus) to plants in the five NFT channels.

NFT Channels: Two series consisting of five plastic rectangular NFT channels measuring 3 meters in length, 100 mm in width, 700 mm in height were used. Primary treated effluent was used to irrigate lettuce in one series (effluent plot), and a commercial nutrients solution (Culture-S; Cat-28015, Accent Hydroponics PTY LTD, Sydney) was used to irrigate the same type of lettuce in another series as a control (control plot).

Each NFT channel was a hollow rectangular plastic tube with 10 holes of 75 mm in diameter drilled in the top side of the channel. The effluent flowed as a very shallow stream ($\approx$ 4 mm) on the inner surface of the bottom channel by gravity. The bottom inner surface of the NFT channel was modified by installing a grooved polyethylene surface (Accent Hydroponics PTY LTD, Sydney) for even flow distribution across the bottom of the NFT channel and for better effluent aeration. Each channel discharged directly into a trench which is a bigger channel into which the lower end of each NFT channel was inserted (Figure 4.1). The trench collected the effluent from the five NFT channels then discharged it into the circulating tank.

The plants were grown bare-rooted in the thin film of the feed wastewater. Hence the name, nutrient film technique (Cooper, 1996).

Growing Pots: White coloured plastic re-useable growing pots (Cat-39505; Accent Hydroponics PTY LTD, Sydney) (75 mm x 46mm dia) with a perforated base were used to hold the plants in the NFT channels. The perforated base ensured capillary action to draw water in, and a way out for the growing roots.

Growing Medium: Perlite and Vermiculite were used as support media for seedlings in the growing pots. A ratio of two parts perlite to one part vermiculite (v/v) was utilised. The vermiculite keep the medium moist while the perlite helped in aeration. The growing cups were filled with medium up to 5 mm in depth. The medium aided in protecting the roots from light.

Growing Lights: Lighting systems were used to control light dosage to both plots described in section 4.2.1. Six; 400 W halide lights (Accent Hydroponics PTY LTD, Sydney) with a light intensity of 450 $\mu W\ m^{-2}$ and a wavelength around 500 nm were installed overhead of the hydroponics plots. A 400 Watt light suits an area of 1 m^2, which accommodated 10 plants for this project. The lights were kept 300 mm over the plants. Initially, the lights were operating for 18 hours until the plants reached 4 weeks of age, then the lights were timed for up to 16 hours per day. This was partly to save energy and reduce the possibility of plants being affected by the light heat as they grew closer to the light, where it was difficult to move the lights in two experimental sites (the glasshouse and Bondi sewage treatment plant).

Wastewater: Primary settled municipal wastewater was utilised in this study. Bondi and Liverpool sewage treatment plants (STP) primary effluent was almost free from bulk objects to avoid clogging of the effluent distribution system within the pilot plant. Moreover, the selected effluents were primary settled without chemical treatment of nutrients that are essential for plants growth. For the first experimental site which was an old covered building, effluent was collected weekly from Liverpool STP. Bondi STP effluent was utilised in the two other experimental sites, a glasshouse and Bondi STP as described in section 4.3. Table 4.1 shows phosphorus and nitrogen levels in Bondi and Liverpool wastewater treatment plants effluents along with other parameters for Bondi STP. Every batch of wastewater was analysed in the laboratory before being used for growing plants.

4.2.2 Control Plot

The control plot was in principle the same hydroponic system used as the test. It consisted of a 60 L recirculation tank for nutrient solution storage, a small submersible pump (10 litres at 1.4 m head) and the same NFT channels. The pump was the same as for the standard and quality required for the use in hydroponics, ie. able to tolerate nutrient solution salt levels without causing contamination of nutrients or rapid deterioration of the pump itself. A plastic by-pass valve was used to control the quantity of water being pumped to the plants.

Commercial nutrients "Culture-S" were purchased from Accent Hydroponics (Sydney). The nutrients starter is packed in two separate A&B bags to keep concentrated phosphate and calcium salts separated so as to prevent precipitation. Five hundred grams of mixture A were dissolved in two litres of water (solution A), and 500 grams of mixture B were dissolved in two litres of water, solution B. Four mL of solution A and 4 mL of solution B were added to every litre of irrigation water. The plants were irrigated at 8 $L.min^{-1}$. Table 4.1 shows the concentrations of macronutrients and micronutrients in the final nutrient solution that was added to the circulating tank of the control plot as compared to Bondi and Liverpool STPs effluent.

Table 4.1 Average concentrations of nutrients and other elements in Bondi and Liverpool STPs (n=3) effluent compared to Accent Hydroponics final nutrient solution and standard deviations

Parameter	Bondi STP Effluent Concentration ± standard deviation (mg L^{-1})	Liverpool STP Effluent Concentration ± standard deviation (mg L^{-1})	*Nutrient Solution Concentration (mg L^{-1})
Nitrogen (Total as N)	56 ± 6	64 ± 14	208**
TP (as P)	4.4 ± 0.8	6 ± 3	62
K	30.7±3.15	NM	332
Ca	41.4 ± 28	NM	168
Mg	15.4 ± 3.9	NM	49
S	NM**	NM	65
Fe	1.97 ± 1.14	NM	5.6
Mn	0.03 ± 0.01	NM	2.2
B	2.7±0.96	NM	0.3
Cu	1.29±0.79	NM	0.06
Zn	0.18± 0.12	NM	0.06
Conductivity (μs.cm^{-1})	1325 ± 215	NM	2850 ± 320
pH	6.2 ± 0.8	6.8 ± 0.7	6.9 ± 1.1

* (Accent Hydroponics PTY LTD, 1994), **Nitrate as (N), NM = Not measured.

4.3 Experimental Methodology

The hydroponic pilot plant was set up in three locations (Figure 4.3):

a) Large Covered Building

b) Glasshouse

c) Underground Bondi Sewage Treatment Plant

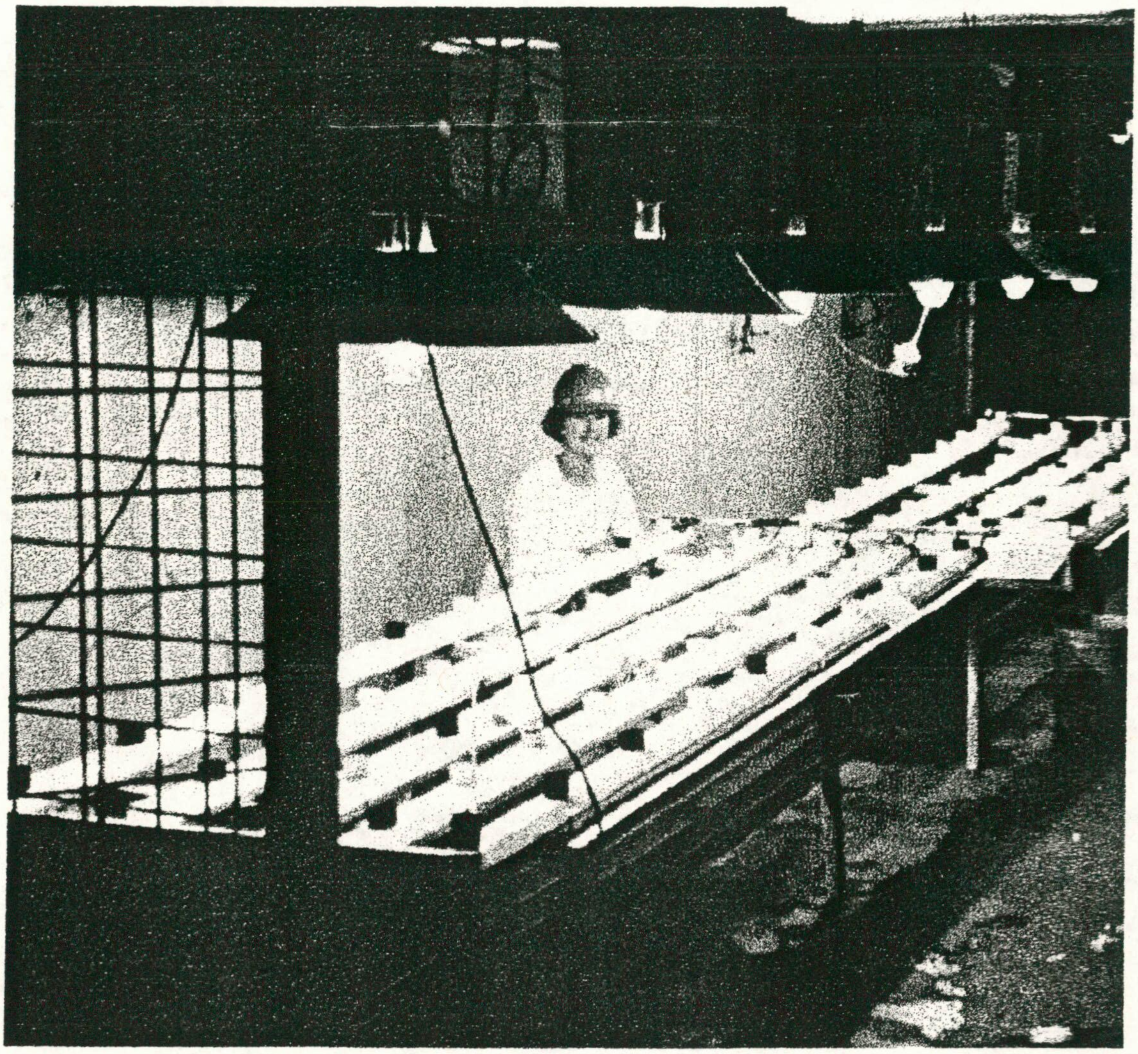

Figure 4.3c The NFT experimental set up at Bondi STP

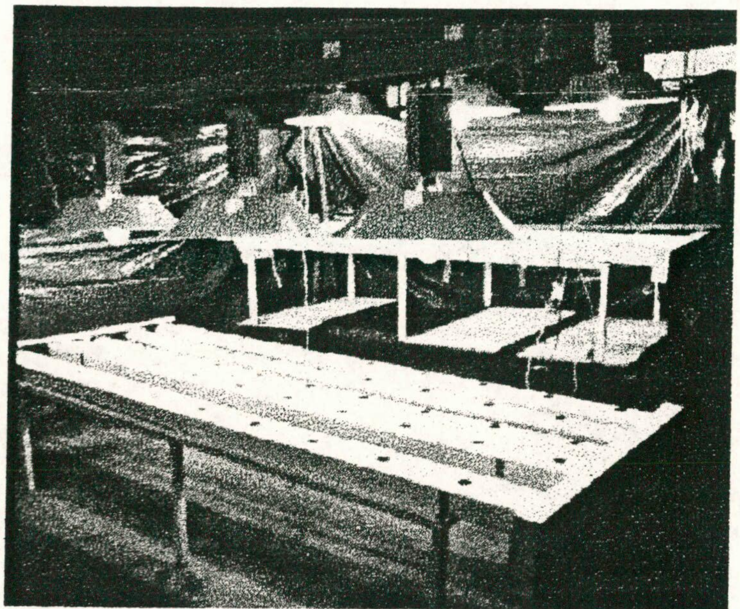

Figure 4.3a The NFT experimental set up in the large covered building

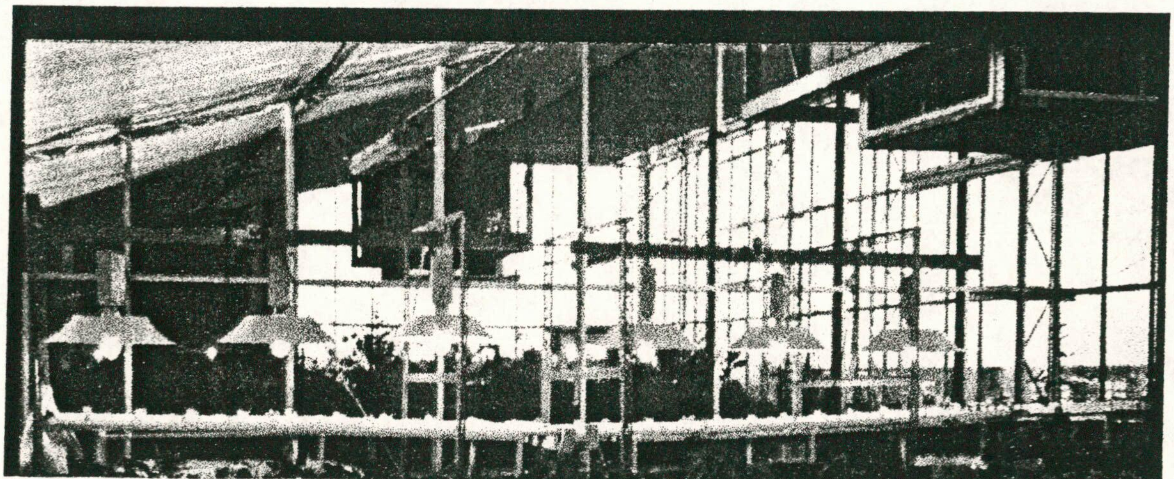

Figure 4 3b The NFT experimental set up in the glasshouse

4.3.1 Large Covered Building

The system was initially set up in a large covered tram shed, where the primary light source was artificial growing lights (400 W m^{-2} light per m^2). Plants were grown initially with light for 18 hours per day for the first 5 weeks, then they were grown with 16 hours per day of light.

4.3.1.1 Experimental Setup

Wastewater Plot

Unless otherwise stated, primary treated effluent was collected weekly from Liverpool STP in Western Sydney. The effluent was pumped from the primary settling tank into a mobile fibreglass tank, and transported to the experimental pilot plant. This tank was fixed onto a trailer, along with a petrol driven pump. The mobile system was specially designed and manufactured for the project. From the mobile fibreglass tank, four hundred litters of effluent were transferred into a 1 m^3 plastic tank (the recirculating tank) from which the effluent was pumped to the lead of the NFT channels for gravity feed via the lettuce plants (section 4.2.1) in a closed-loop hydroponic-NFT configuration.

Control Plot

Parallel with the wastewater plot, a commercial hydroponics system was operated which utilises a commercial nutrients solution dissolved in tap water. This solution was pumped from a 60-litre tank to the plants in a similar hydroponic system to those of the wastewater plot at 8 L min^{-1}. The utilised solution flowed back to the 60-litre plastic tank by gravity.

4.3.1.2 Purpose of Experiment

This was the first experiment undertaken with the aims of:

- becoming familiar with the NFT as an irrigation and treatment system,
- determining whether lettuce plants could be grown with effluent using a NFT system and measure their growth rate, and
- to collect primary data about nutrients, suspended solids (SS), chemical oxygen demand (COD) and biochemical oxygen demand (BOD) removal by NFT treatment.

4.3.1.3 Sampling and Analyses

Three samples from the effluent and control plot were taken from each batch of effluent before and after being used to irrigate the plants. These samples were tested in the laboratory to examine their quality in terms of COD, BOD, pH, TKN according to standard methods (APHA, 1995). Nitrate and TP concentrations were determined according to Hach methods (Hach, 1990).

At each plant sampling, a random number generator was used to select lettuce plants from the test and control plots. Each whole lettuce plant sampled was dried in the oven at 60 °C for 72 hours and weighed to determine plant mass.

4.3.1.4 Results and Discussion

Two major objectives were achieved from this first experimental trial; assessment of plant production capability and wastewater treatment efficiency of the proposed NFT-plant system. Both objectives are discussed separately below:

4.3.1.4.1 Plant Growth

Mass of the dried plants (leaves and roots) are plotted vs plant age in Figure 4.4.

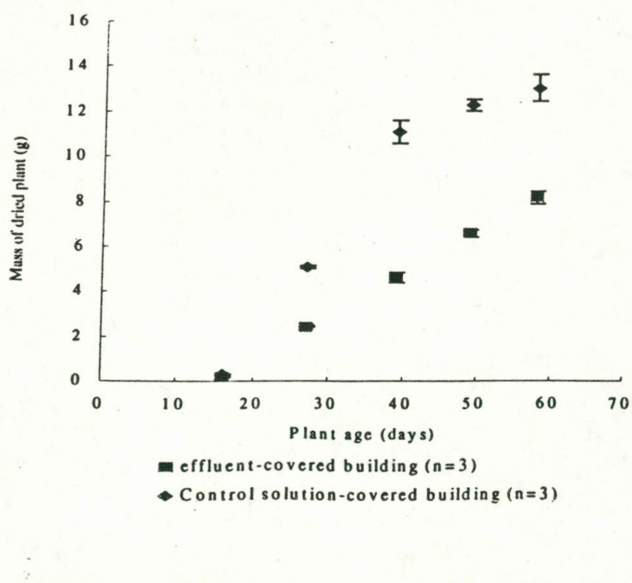

Figure 4.4 Average growth rate of lettuce plants irrigated by primary effluent from Liverpool Sewage Treatment Plant and for control plants irrigated with commercial nutrient solution in a covered building (error bar = 1 SD).

The plants irrigated with commercial nutrient solution (the control plot plants) were generally larger than those irrigated with municipal primary treated effluent (plants of the effluent plot). The plants in both plots, however, generally followed a three-phase growth cycle similar to bacterial growth, viz a lag phase, a phase of exponential growth and the transition into a stationary phase at maturity (Figure 4.4). The slope of the control plants growth curve is steeper than the slope of the effluent plants growth curve, showing that the control plants grew at a faster rate than the effluent plants until they reached 40 days of age. The lettuces utilising the effluent apparently did not achieve mature growth by 58 days, possibly due to inadequate potassium supply or toxicity. The potassium concentration was about 30 mg L^{-1} compared to 332 mg L^{-1} in the nutrient solution (Table 4.1).

Figure 4.4 also shows that crops irrigated with primary effluent (without disinfection) appeared as healthy as the control plants utilising commercial nutrient, but yielded about 40% less mass.

4.3.1.4.2 Treatment of Effluent

About 400 litres of Liverpool STP primary effluent was recirculated in the NFT pilot plant and replaced at about weekly intervals. Each fresh batch of effluent was analysed for selected parameters listed in Table 4.2. The same batches were also analysed for the listed parameters after one week of being recycled in the wastewater plot. Table 4.2 shows the average concentrations and standard deviations of the fresh and recycled effluent over the growth period (58 days).

Table 4.2 Average wastewater characteristics (± standard deviations) before and after being recirculated within the NFT-lettuce system for 7 days (n=24 averaged over 58 d growth cycle)

Parameter	Initial Concentration ($mg\ L^{-1}$)	Final Concentration ($mg\ L^{-1}$)	% REMOVAL
TKN (N)	64	13	80
TOTAL P (P)	6 ± 3	1.2 ± 0.2	77
NITRATE-N	18 ± 4	36 ± 6	Not Applicable
BOD	195 ± 13	25 ± 2	87
COD	366 ± 16	53 ± 5	86
SS	150 ± 10	2.4 ± 1.7	99

About 80% of TKN (total Kjeldhal nitrogen, total of organic and ammonia nitrogen) and 77% of total phosphorous was removed from the effluent over the 7-day time period of circulation in the NFT. In Table 4.2, TKN and Nitrate were presented separately to demonstrate the oxidation process of ammonia into nitrate by the nitrifying bacteria. The overall total nitrogen (as N) removal efficiency was therefore about 40%. Most of the nitrogen applied is utilised by plants during days 26-36, and little net removal occurred during periods when vegetation is in the early growth stage.

Table 4.2 also shows that 86% COD, 87 % BOD, and 99% suspended solids (SS) were removed. These removals could well be attributed to the attached microorganisms on the root system forming a biological treatment system (attached-growth process), as in a trickling filter treatment process. Removal by surface sorption is discussed in Chapter 6.

Nutrient solution samples were taken from the control plot simultaneously with the effluent plot samples to be analysed for total nitrogen (as N) and total phosphorus (as P). Table 4.3 shows that plants grown in the control hydroponic system removed most of the available nutrients in the weekly batch of nutrient solution.

Table 4.3 Average weekly characteristics of nutrients in the control hydroponic system and standard deviations (n=24 averaged over 58 d growth cycle of NFT lettuce)

Parameter	**Concentration (mg L^{-1})***	**Concentration After ± standard deviation (mg L^{-1})**	**% Removal**
Total N (N)	208	30 ± 11	86
Total P (P)	62	8 ± 8	88

*(Creevey,1996)

4.3.2 Glasshouse

The hydroponic NFT system was moved to a glasshouse belonging to the School of Biological Sciences (UNSW, Sydney) with a controlled temperature of 22-26°C, where the primary light source was natural light during the months of May to June. The glasshouse provided a controlled environment to grow plants in the adapted hydroponic system at any time of the year under natural light. The growing lights however, were installed and used to give uniform lighting through the experiment for 16 hours a day.

4.3.2.1 Purpose of Experiment

As effluent flows by gravity through long (>30 m) commercial NFT channels, its nutrient concentration is expected to decrease. Therefore this experimental trial was conducted in a glasshouse environment and the possible effect of plant's position within the NFT channel with regard to growth and nutrient removal rates.

The main objectives for the glasshouse experimental trial were therefore:

- to obtain growth and nutrient removal data in a temperature controlled environment largely under natural light;
- determine heavy metal plant accumulation rates along the length of each NFT channel;
- to compare heavy metal concentration in the edible parts of lettuce with the Australian food standards for health risk assessment; and
- to compare heavy metal concentrations in primary treated municipal effluent with the maximum recommended concentrations for irrigation water.

4.3.2.2 Description of Experiment

The primary settled municipal effluent was regularly collected from Bondi STP (Eastern Sydney) and transported to the system in the glasshouse using the tanker described in 4.3.1.1.

Two series of five NFT channels with capacity of 45 plants each (Figure 4.5) were used so that the effluent was pumped from the one recirculating tank through two supply pipes, each delivering effluent at 8 L min^{-1} to each series. The effluent was recirculated as described in 4.3.1.1.

In addition to the two-series effluent plot, a 90-plant capacity hydroponic system (the nursery plot) was operated using a nutrient solution. Plants of this system were transferred to the effluent plot to substitute the sampled plants of the same age. Hence the same number and aged plants were maintained throughout the experimental trial.

The effluent plot experimental set up is represented by Figure 4.5. One series of the effluent plot was named as the left series and referred to by the letter L. The other series was named the right series and referred to by the letter R.

During the course of the experiment, plants were harvested on five occasions. Eighteen plants were removed and replaced at each sampling period. There are 45 plants in each plot placed in five channels forming nine rows (Figure 4.5). The growing period was about 58 days and samples were taken about every ten days (Table 4.4). Theoretically, consecutive plants in each NFT channel were exposed to lowering concentrations of phosphorus along the length of each channel. Duplicate plants were harvested from each row at each sampling time. The position of plants at different harvest periods is given in Table 4.4.

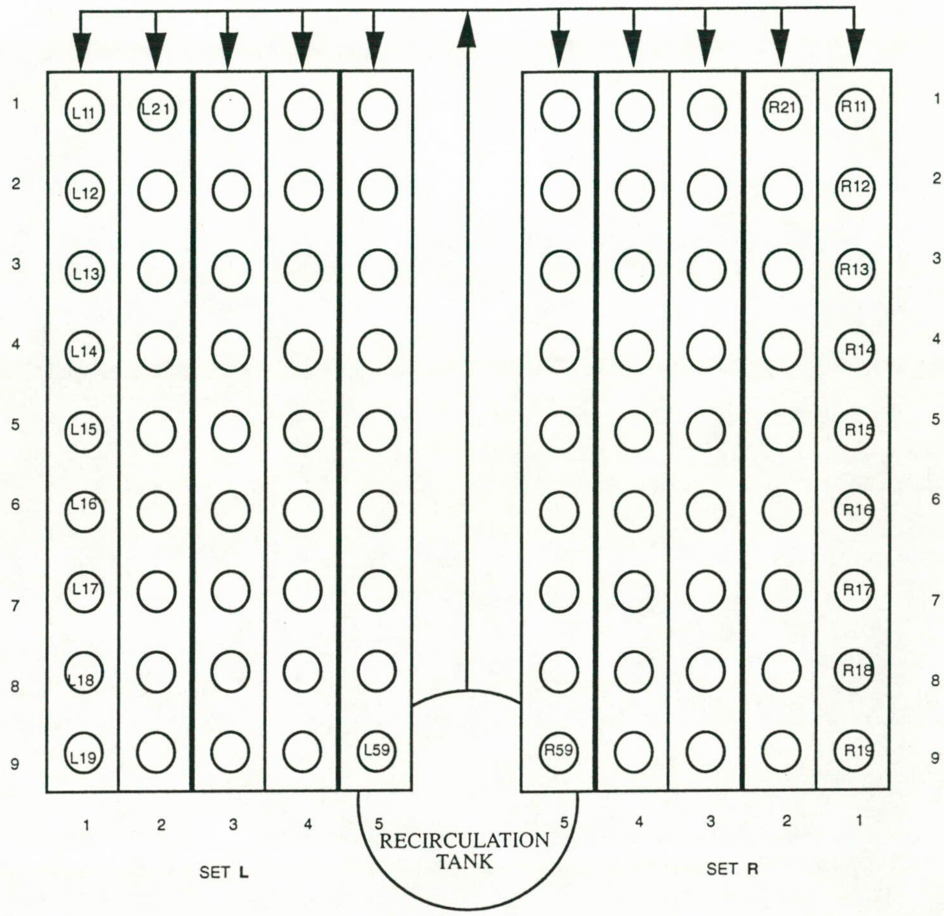

Figure 4.5 the experimental effluent plot showing plant numbering system

Table 4.4 Position of lettuce plants harvested and replaced in the NFT experimental pilot plant *

Sampling group	**Lettuces removed and replaced with one of the same age from nursery**
First (day 16)	L11&R11, L22&R22, L33&R33, L44&R44, L55&R55, L16&R16, L27&R27, L38&R38 and L49&R49.
Second (day 27)	L21&R21, L32&R32, L43&R43, L54&R54, L15&R15, L26&R26, L37&R37, L48&R48 and L59&R59.
Third (day 39)	L31&R31, L42&R42, L53&R53, L14&R14, L25&r25, L36&R36, L47&R47, L58&R58 and L19&R19.
Fourth (day 49)	L41&R41, L52&R52, L13&R13, L24&R24, L35&R35, L46&R46, L57&R57, L18&R18 and L29&R29.
Fifth (day 58)	L51&R51, L12&R12, L23&R23, L34&R34, L45&R45, L56&R56, L17&R17, L28&R28 and L39&R39

* See Figure 4.5 for plant numbering system

4.3.2.3 Laboratory Analyses

Trace elements and phosphorus[1] were determined by Inductively Coupled Plasma-AES (ICP-AES) as detailed below (Anderson, 1996b):

1. Immediately after harvest, tissue samples were washed with P-free detergent (extrain 300, BDH, Sydney) and rinsed with milli-Q water to remove dust particle residues.

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11 Phosphorus in plant samples was determined by ICP-AES only for Bondi STP trials otherwise by Hach
(1992).

The risk involved in washing plant tissues, however, is that some nutrients (for example potassium) may be leached out of the tissue (Noggle and Fritz, 1983).

Roots were further washed with 0.06M $CaCl_2$ for fifteen minutes, then rinsed with milli-Q water. This technique excluded any adsorption contribution to the absorbed phosphorus, but rinsings were collected for analyses.

2. To express the element composition of plant tissues in terms of dry rather than fresh weight, the tissues were dried in a forced draft oven at a temperature of 70°C.
3. The dried tissue was subsequently ground to powder using a ceramic pestle and mortar.
4. After weighing the total mass of powdered tissue, a known weight (50-100 mg) of plant powder was added to a digestion vessel, mixed with 2 ml of 70% nitric acid (HNO_3) and digested at 150°C for three hours.
5. After cooling to room temperature, the digest was diluted to form a 2% HNO_3 final solution (prepared sample).
6. Two-mL of the prepared sample was analysed via a fully calibrated ICP-AES according to standard methods for Metals by the Inductively Coupled Plasma Method (APHA, 1995).

Effluent samples were digested with nitric acid according to the 3030 E. Nitric Acid Digestion Method for Metals according to (APHA, 1995). The digested sample then analysed via a fully calibrated ICP-AES according to standard method for wastewater (APHA, 1995).

4.3.2.4 Results and Discussion

Growth rate of the NFT lettuces, wastewater treatment efficiency and heavy metals concentrations in the NFT letytuces are discussed below.

4.3.2.4.1 Plant Growth

Though no control lettuce plants were grown in a nutrient solution in the glasshouse, the growth rate of the plants in effluent at this location with natural light exceeded the growth rate of the plants in effluent at the covered building with artificial light (Figure 4.6). The favourable air temperature (22-26 °C) in the glasshouse compared to 5-26 °C in the large covered building may be the main reason for different growth rates.

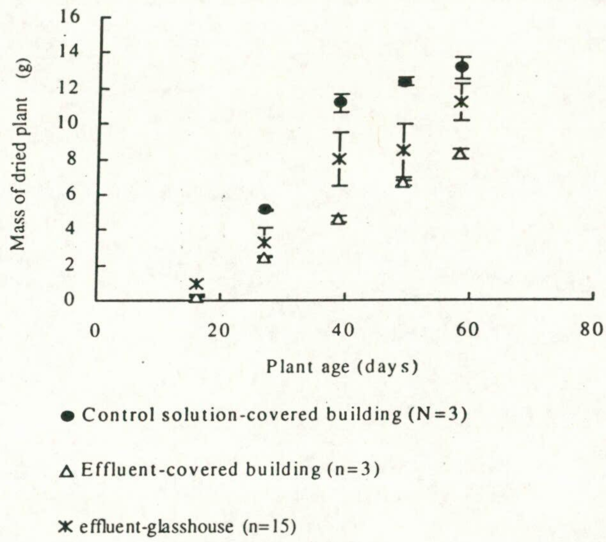

Figure 4.6 Average weight vs age of lettuce plants irrigated by effluent or nutrient solution in the glasshouse and in the covered building

4.3.2.4.2 Nutrient Removal

The daily average nitrogen and phosphorus concentrations of Bondi STP effluent before being recirculated within the NFT channels and after one week of recirculation were measured and averaged on a daily bases. The average percentage daily removal of nitrogen and phosphorus are presented in Figure 4.7 for different plant growth stages.

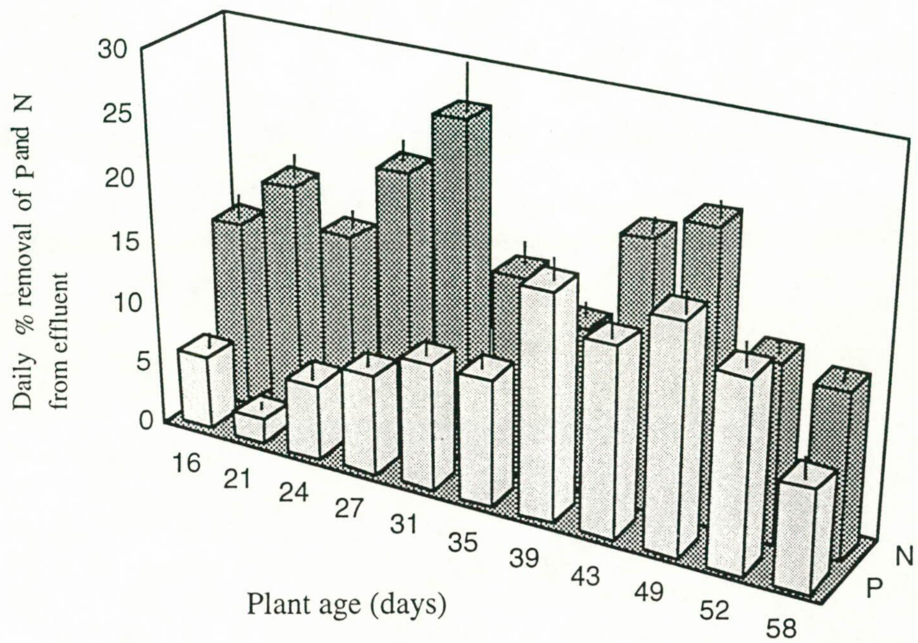

Figure 4.7 Apparent daily percentage removal of nutrients from effluent recirculated in the NFT experimental pilot plant (n=3, error bar = 1SD)

The average daily removal percentage of total nitrogen (as N) was 10 ± 5.4 % (mean ± SD) from the utilised effluent in the hydroponic system. The average daily removal percentage of total phosphorus (as P) was 18 ± 4.5% (mean ± SD). Based on the Student's T-test there was no significant difference ($p>0.05$) between the means of these nutrients for the average daily percentage removal. Nonetheless, average mean comparisons are invalid as there were significant differences versus days (Figure 4.7) with more N removal compared to P for the first 31 days. Highest phosphorus removal was achieved when plants were 39-52 days of age. Nitrogen removal data however, did not show a distinct trend although removal rate appeared to decrease after age of 49 days.

4.3.2.4.3 Metal Accumulations in Lettuce

Theoretically, roots of the plants in the NFT channels are exposed to different levels of macronutrients and trace elements depending on their location within the NFT channels. Hence, the experimental trial in the glasshouse was conducted to investigate element concentrations in each plant grown in the system. In addition to effluent quality indications along the channels, the obtained concentrations will aid in the final design of the hydroponic treatment farm, ie. distributing plants of different ages within the proposed full-scale treatment farm based on the plant content of the measured elements.

Trace elements concentrations were also used to observe metals accumulation in the plant tissues for health risk assessment. Results showed that plants grown in the holes near the inlet (upper) end of the channels did not accumulate more heavy metals than those grown at the outlet end of the channels. An example of the obtained results is presented in Table 4.5. Hence, length of channels were insufficient to show a concentration gradient effect.

Table 4.5 Concentrations and standard deviations of Zn in plants grown in different locations of the NFT experimental pilot plant (mg per gram of dry leaves)

Plant location	**Concentration ± standard deviation (mg g^{-1})**
L 21	2.4 ± 1.2
L 32	1.8 ± 0.6
L 43	1.6 ± 0.9
L 54	3.6 ± 1.5
L 15	1.1 ± 0.9
L 26	2.0 ± 0.3
L 37	not determind
L 48	3.1 ± 1.7
L 59	3.2 ± 0.9

* See Figure 4.5 for plant numbering system

The primary objective of using lettuce in this hydroponic wastewater treatment system was not to remove or concentrate metals from the wastewater but to produce a crop which is both commercially valuable and primarily capable of removing the nutrients nitrogen and phosphorus for acceptable effluent discharge. Nonetheless, excessive heavy metals content would obviously preclude the use of these crops as fodder for animals or humans.

Figures 3.8 through 3.10 summarise the results obtained from the ICP analyses conducted on the lettuce plants at various stages in their growth cycle. The numbers represented composite averages from sacrificing fifteen plants per sampling period.

In Figure 4.8, concentrations of four macro elements are represented. The average concentrations in mature plants and average standard deviations were about: K, $1.2\text{x}10^5 \pm 1.6\text{x}10^4$ mg kg^{-1}; Na, $3.4\text{x}10^4 \pm 7.0\text{x}10^3$ mg kg^{-1}; Ca, $2.3\text{x}10^4 \pm 2.8\text{x}10^3$ mg kg^{-1}; and Mg, $1.2\text{x}10^4 \pm 687$ mg kg^{-1}. Lettuce plants at 39 days of age seems to show greater difference for Na and Ca than at other ages. The significant difference between the means of K and Na concentrations in plants of different ages was tested according to the null hypothesis tests (Student's T-test, Miller and Miller, 1993). The means of the two macro elements were significantly different ($p < 0.05$). However, following the same approach the means of Mg and Na were not significantly different ($p > 0.05$).

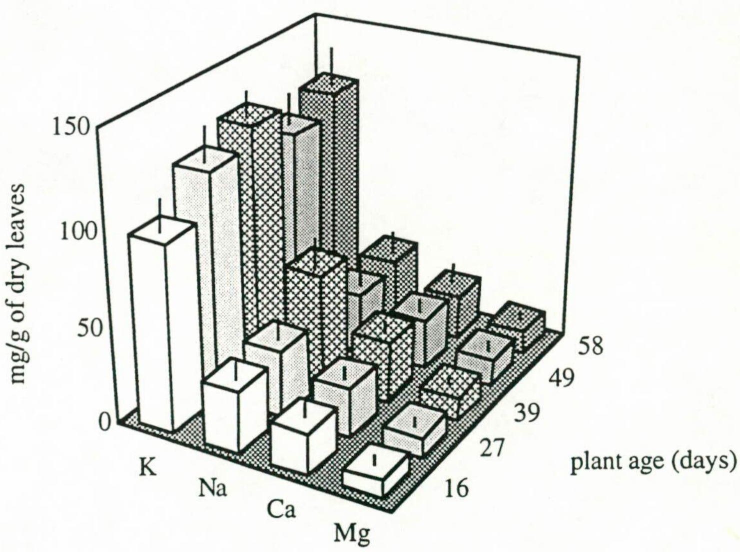

Figure 4.8 Concentration of different macro elements at different growth stages in the leaves of lettuce plants grown in a NFT utilising primary effluent (n=15, error bar = 1 SD)

Concentrations of another group of different trace elements are presented in Figure 4.9. B, Fe, Mn and Zn, were all below 3,000 mg kg^{-1}. The Mn and Zn concentrations followed similar pattern, but at 58 days concentrations were 1,454 ± 219 and 2,042 ± 378 mg kg^{-1}, respectively. At 58 days the concentrations of Fe and B were 647 ± 154 mg kg^{-1} and 85 ± 78 mg kg^{-1}, respectively. Neither of the differences between means of Mn and Zn, nor Fe and B were significant ($p > 0.05$).

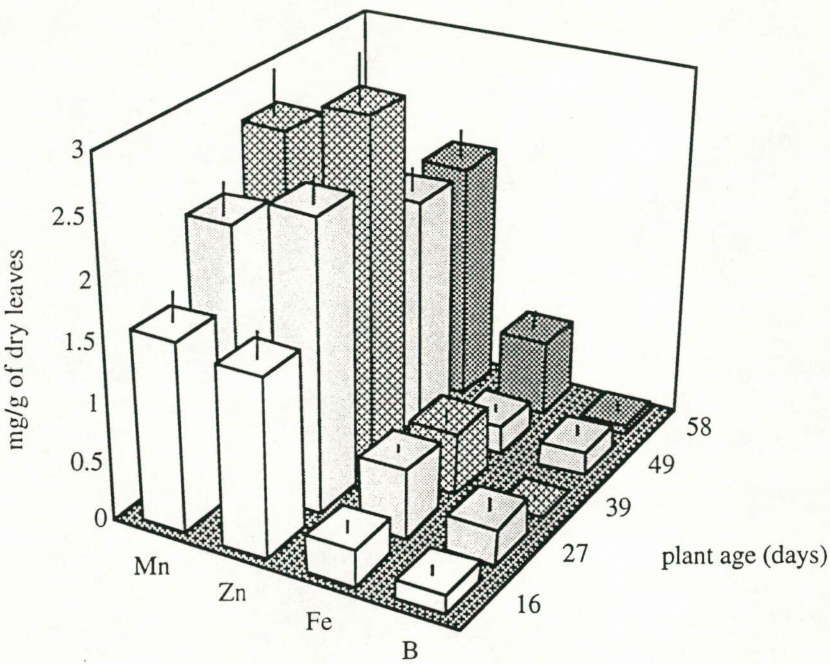

Figure 4.9 Concentration of different trace elements at different growth stages in the leaves of lettuce plants grown in a NFT utilising primary effluent (n=15, error bar = 1SD).

Figure 4.10 shows concentration of the heavy metals Ni, Pb, Cu, Cr and Cd. Concentration and standard deviation of Ni in mature lettuce plants was 46.6 ± 18.8 mg kg^{-1}, lead 20 ± 8.2 mg kg^{-1}, copper 12 ± 2.5 mg kg^{-1}, chromium was 3.1 ±0.4 mg kg^{-1} and cadmium 4.8 ± 0.96 mg kg^{-1}. The average concentration of Cd was lower than the average concentration of either Ni, Pb or Cu (ie the average concentration of Cd was signicantly different compared to either Ni, Pb or Cu, $p \leq 0.05$). Similarly, the average concentration of Cr compared to the average concentration of either Ni, Pb or Cu differ significantly (for $p \leq 0.05$) (average Cr concentration < the average concentration of either Ni, Pb or Cu). However, when compared to the average concentration of either Cu or Pb, the average concentration of Ni in plant tissues did not differ significantly ($p > 0.05$).

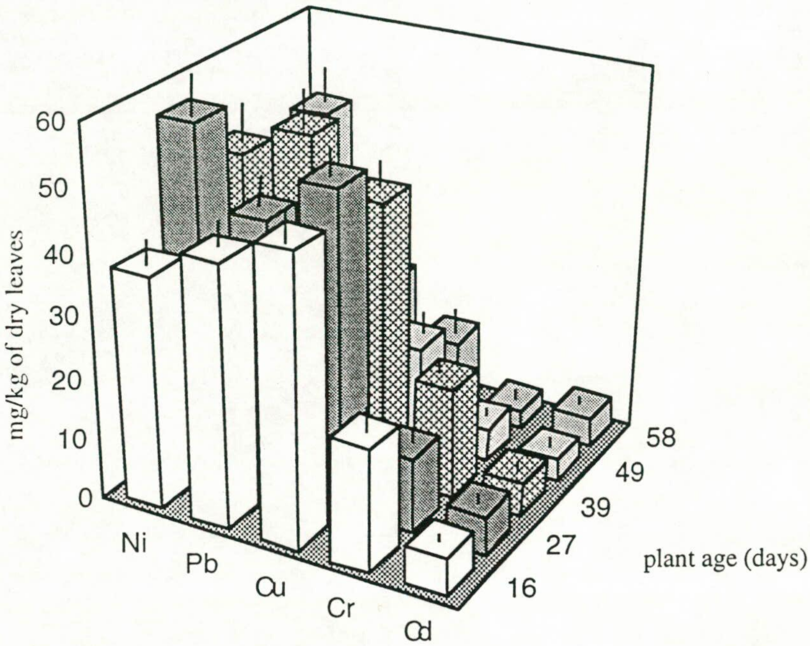

Figure 4.10 Concentration of different heavy metals at different growth stages in the leaves of lettuce plants grown in a NFT system with primary effluent (n=15, error bar = 1 SD).

The concentrations of the five metals (Ni, Pb, Cu, Cr, Cd) in the plants generally decreased after 39 days of age. Consultations with Dr Barrow (CSIRO, WA, 1997) concluded that the decrease in concentration with plant age was due to the fact that these concentrations were diluted by the faster rate of mass increase than the rate of absorption. Furthermore, young plants have greater capacity to take up nutrient per unit of root growth than older plants.

Average levels of trace elements found in the edible part of plants grown in the NFT systems in the glasshouse and Bondi STP (4.3.3) are listed in Table 4.6. In column 3, typical values of these elements in market plants are also listed. Four values for heavy metals were found in the reports of Australia New Zealand Food Authority (1995), as guidelines for the acceptable levels of metals in vegetable plants. Looking at Table 4.6, the NFT-grown lettuce in effluent concentrated K, Mg, Zn, Pb, Mn, Fe, Cr, and Na in the lettuce leaves.

The plants grown in the NFT experimental set up contained As, Cd, Cu, Pb and Zn in excess to what may be considered an acceptable level even though some (eg Zn) were within limits set for irrigation. This is one of the main reasons to recommend the use of non-edible plants such as flowers or pyrethrum as value added crops for the proposed system.

Table 4.6 Average concentrations of nutrients and heavy metals in the leaves of lettuce grown on wastewater NFT compared with typical values in foliage plants along with food standards for vegetables

Metal	Measured concentration in NFT fully grown lettuce leaves± standard deviation (mg kg^{-1})(n=15)	Typical values* in leaves of foliage plants (mg kg^{-1})	Food standards** for vegetables (mg kg^{-1})
As[1]	6.5 ± 1.2	0.1-5 [2]	1.0
B	85 ± 78	15-100	NA
Ca	23 338 ± 2 870	10 000-50 000	NA
Cd	3.8 ± 0.96	1-5	1.25
Co	2.8 ± 0.4	0.2-29	NA
Cr	3 ± 0.07	0.5-1	NA
Cu	12 ± 1.7	5-15	10.0
Fe	647 ± 154	50-300	NA
K	120 049 ± 16 837	15 000-50 000	NA
Mg	12 819 ± 687	2500-10 000	NA
Mn	1 454 ± 219	25-250	NA
Na	33 983 ± 7 032	200-2000	NA
Ni	47 ± 18.8	8-14	NA
Pb	20 ± 15	4-6	0.5
Zn	2 042 ± 378	15-75	150

* From Robb and Pierpoint (1983)

** Douch (1997)

[1] From Bondi STP results only (n=3) (see section 4.3.3)

[2] From Pettygrove and Asano (1985). NA = not available

Arsenic

Arsenic concentrations in Bondi STP effluent exceeded the recommended levels for irrigation waters (Table 4.7). The As content of lettuce plants grown in the experimental NFT pilot plant was also well over the recommended value.

Boron

Bondi STP effluent is suitable for growing lettuce, because the plant can tolerate boron level which is higher than the concentration found in effluent (Table 4.7). Bondi STP effluent however, would not be suitable for many plants such as tomatos or even apples (Leeper, 1978).

Cadmium

Lettuce plants grown in the NFT experimental pilot plant accumulated Cd in their leaf tissues at concentrations typical for leaves of foliage plants (Table 4.6).

Copper

Lettuce plants grown in the adapted hydroponic system accumulated Cu at concentration higher than the recommended value (Table 4.6).

Lead

Australia and New Zealand Food Authority (ANZFA, 1995) does not allow more than 0.5 mg kg^{-1} of Pb in food (Table 4.7). About 20 mg kg^{-1} of Pb was accumulated in the leaves of the lettuce plants grown in the experimental NFT pilot plant with Bondi effluent which contain 0.2 mg L^{-1} of Pb. Though, the maximum recommended concentration of Pb in irrigation water is 0.2 mg L^{-1}, which is equal to the concentration in the utilised effluent (Table 4.7), the grown lettuce in the NFT system do not comply with the food standard.

Nickel

About 47 mg kg^{-1} of Ni was accumulated in the leaves of lettuce grown in the NFT channels irrigated with Bondi STP effluent. Typical plant content of Ni is between 8 and

14 mg kg^{-1}. Nickel concentration in Bondi STP effluent was about 2.0 mg L^{-1} which is higher than the recommended maximum concentration for irrigation waters (Table 4.8).

Zinc

Lettuce plants accumulated about 2,042 mg kg^{-1} of Zn which is over an order of magnitude higher than the maximum recommended concentration for crops (150 mg kg^{-1}). The Zn concentration in Bondi STP effluent averaged 0.18 mg L^{-1}, being less than the maximum recommended concentration for irrigation water 0.2 mg L^{-1} (Table 4.8).

4.3.3 Bondi STP

The proposed full-scale PTHF will be located downstream of a primary settling tank, which will receive the variable flow rate of the targeted small community's wastewater. The use of this variable flow data in designing the NFT system is discussed in Chapter 6. Hence, Bondi STP experiments were undertaken to enable various dilutions of primary effluent to be readily delivered to the experimental NFT set up.

4.3.3.1 Purpose of Experiment

The Bondi STP trials were conducted to aid the development of a phosphorus removal model which predicts the size of the proposed PTHF.

The data required for this model development were: (i) root and total plant weights, and (ii) phosphorus removal rates from effluent of different dilutions by plants of different ages.

4.3.3.2 Material and Methods

The following samples were collected and analysed as indicated:

Effluent Samples

Fresh effluent before being recirculated in the NFT system and effluent samples after 24 hours of recycling. These samples were analysed for nitrogen and phosphorus.

System surfaces were washed five times during the course of each experimental trial. Root surfaces were washed as in 4.3.2.3. The rinse material was analysed for total phosphorus separately and referred to as phosphorus removed from effluent by surface sorption.

The phosphorus concentration in effluent was measured according to Hach method for total phosphorus using the Acid Persulfate Digestion Method (Hach, 1990).

Nitrate concentration in effluent was measured according to Hach method for nitrate using the High Range Method (Hach, 1990). Total Kjeldhal nitrogen was measured for effluent and control nutrient solution samples only. The sample was first digested using the 4500-N_{org} B. Macro-Kjeldhal Method (APHA, 1991). Then the sample left to cool to room temperature and distilled. The distilled sample was titrated according to

Standard Method (APHA, 1995). Nitrate and TKN were considered to account for total nitrogen (as N) in samples assuming nitrite occured in very small concentrations (Pettygrove and Asano 1985; Metcalf and Eddy, 1991).

Plant Samples

Phosphorus and trace elements concentrations in plant tissues were measured as in 4.3.2.3.

4.3.3.3 Description of Experiment

The experimental hydroponic pilot plant was moved from the glasshouse to the underground tunnel of Bondi STP (Sydney). The effluent of Bondi STP is primary treated (78% solid removal) and allowed to settle in four separate sedimentation tanks. The location of the pilot plant was chosen so that the effluent from the four sedimentation tanks was well mixed before being pumped into the NFT system. The underground air temperature was almost constant at 22 ^{o}C and the only source of light for the plants was the growing lights, one per m^2, (Metal Halide, supplied by Accent Hydroponics Pty Ltd. Australia), which operated initially for 18 hours then, for 16 hours a day once the plants were at 16-day of age. Two experimental trials were carried out at Bondi:

I. In the first trial, one hydroponic system was used with commercial nutrient solution (Culture-S, Accent Hydroponics, Sydney) as a control and one with primary effluent. When the effluent was continuously pumped from the discharged effluent in the main channels of Bondi STP into the NFT channels, the plants did not grow due to the low level of dissolved oxygen in the effluent, about 0.9 mg L^{-1}. Therefore, the effluent was pumped into a 1 m^3 plastic tank and allowed to be recirculated within the NFT pilot plant to maintain a dissolved oxygen level ≈ 5 mg L^{-1}. The effluent was replaced every 24 hours to keep plants growing in relatively fresh effluent. The largest reduction in total phosphorus at any day was 21%.

II. In the second trial, the two hydroponic series were used to grow plants in two levels of diluted effluent. One series was used with effluent mixed with 50% tap water

([P]≈2.24 mg L^{-1}). The other series was used to grow lettuce with effluent mixed with 75 % tap water ([P]≈1.07 mg L^{-1}). Both mixes were maintained in 1 m^3 tanks and replaced daily. Hence in combination with trial I, three dilutions of P were examined at Bondi STP, P of 4.5, 2.2, and 1.1 mg L^{-1}.

Microorganisms were analysed according to the following methods:

1. Bacteriological Methods: A twenty four hour sampling run was undertaken to determine variability in indicator numbers in the source effluent over time and to identify the most appropriate time of the day for sample collection. Duplicate assays were carried out on each sample for *E. coli, C. perfringens* and F-RNA phage enumeration from solutions. Estimates of thermotolerant coliforms and faecal streptococci were carried out according to Standard Methods (APHA, 1995) to provide comparative bacterial indicators with which to assess variation and reliability of E. *coli* occurrence.

Separate hydroponic tanks were utilised for wastewater as nutrient reduction culture of lettuces for examine pathogen uptake.

E. *coli* Enumeration: The microbiological quality of the utilised nutrient solution and effluent was monitored prior to plant installation and on day eight. Three samples were taken from effluent and nutrient solution every other day throughout the growth cycle. Samples were analysed for *E. coli*, according to Standard Methods (APHA, 1995), except for the use of Membrane Lauryl Sulphate Agar (MLSA, Oxoid, UK) in place of m-FC agar (Mossel *et al.*, 1995). Enumeration entailed; decimal dilution in quarter strength Ringer's solution (Oxoid, UK), membrane filtration through 0.45 μm nitrocellulose filters (Millipore, Australia) and water bath incubation of membranes on MLSA for 24±2 hours at 44.5 °C. Confirmation of 10% of typical colonies was undertaken according to Standard Methods (APHA, 1995) and for plants International Standards Organisation (1991) methods were used.

Clostridium Perfringens Spore Enumeration: Wong *et al.* (1994) method was used to examine nutrient solution and effluent for *C. perfringens* spores. Samples were incubated for ten minutes at 75 °C to kill vegetative cells and then diluted in quarter strength Ringer's solution (Oxoid, UK). Following membrane filtration through 0.45μm nitrocellulose filters, membranes where incubated on Perfringens Agar OPSP (Oxoid, UK) supplemented with selective agents R076 and SR077 (Oxoid, UK) and 0.29 g L^{-1} methylumbelliferyl phosphate (Sigma Aldrich, St. Louis). Incubation at 37 °C for 24±2 hours was under anaerobic conditions using anaerobic jars (Oxoid, UK and Becton Dickinson, USA) and BBL Anaerobe Gaspaks (Becton Dickinson, USA). Typical colonies were black and exhibited fluorescence under UV illumination using a 100 Watt mercury arc lamp with 265 nm band pass filter (Rofin, UK). Confirmation of *C. perfringens* colonies was by inoculation into lactose gelatin and nitrate motility media (Amyl Media, Dandenong-Australia).

C. perfringens Spore in plant tissue: The International Standards Organisation (1991) and Wong *et al.* (1994) methods were adapted to enumerate *C. perfringens* spores from lettuce leaf tissues. The leaf samples were stomached (Colewell, UK) in 50 mL of 1.0% peptone water and the resulting slurry was incubated at 75 °C for ten minutes before dilution, membrane filtration and enumerated as previously described.

2. F-RNA Phage Methods

Solution Samples Enumeration: F-RNA phage titre in control (nutrient) solution and effluent samples were assessed every second day according to the F-specific RNA bacteriophages method (International Standard Organisation, 1991). The method utilises the double agar layer technique with *Salmonella typhimurium* WG-49 as the host.

Leaf Enumeration: The diluent was based on a diluent employed for dilution of one of the F-specific phages which plaques on S. typhimurium WG-49 (MS2 phage) during barrier testing (Lytle and Routson, 1995). The diluent was supplemented with 0.5% of a surfactant polyoxyethylenesorbitan monooleate (Sigma Aldrich, St. Louis-Sydney) to counteract the hydrophobic nature of F-RNA phages. Following masceration the homogenate was plated out according to the double agar layer technique as recommended for the detection and enumeration of F-specific RNA bacteriophages by the International Standard Organisation (1991).

Assessment of the efficacy of F-RNA phage recovery by the above method was undertaken in the form of seeding trials. Lettuce samples were prepared for bacteriological seeding trials seeded with 300 pfu of MS2 phage stock and assayed for F-RNA phage as detailed above.

3. Fluosphere Methods, Lettuce Leaf Tissue Enumeration: Five millilitre aliquot's of lettuce homogenate were filtered through hydrophilic polycarbonate 47 mm diameter 0.22µm filters (Nuclepore, Cambridge USA). The polycarbonate filters had been pre-treated with 0.15% ethanol diluted with distilled water. Pre-treating prevented the filters from autofluorescing. Forty fields of view (fov) were counted for each plant assayed.

4.3.3.4 Results and Discussion

The two trials allow the design of the hydroponic treatment plant in terms of plant age and their location within the treatment plant. During the first growth stage plants require less nutrients than during the exponential growth stage. Moreover, during the initial stage plant growth might be inhibited by the toxins in the wastewater.

4.3.3.4.1 Phosphorus and Nitrogen Removal

The average daily phosphorus and nitrogen concentrations in effluent were measured before and after being used to irrigate plants for the three effluent mixes (see II in section 4.3.3.3). Phosphorus and nitrogen removal percentages are summarised in Figures 4.11-4.12. Phosphorus results were used to distribute the plants within the NFT farm according to the availability of nutrient in the flowing effluent and nutrient demand of plants in that location.

Nutrient removal results (Figures 4.11-4.12) and growth data (Figure 4.13) indicate that plants removed most nutrients when they were 30-49 days of age, except for plants grown in the 25% effluent mix, where plants grew slowly and the %TP removal at 58 days of age was the highest. Plants grown in the 25% effluent removed the least amount of phosphorus presumably because, these lettuce plants were least developed. About 75% of the removed phosphorus was by plant uptake in the 25% effluent dilution. In contrast, most of the removed phosphorus in the 50% and 100% effluent treatments was due to surface sorption, 67% and 72% of the total removed phosphorus, respectively. Hence, plants absorbed into their tissues 33% and 28% of the total removed phosphorus in the 50% and 100% plots, respectively. Highest recorded daily phosphorus removal efficiency however, was achieved by plants grown in the 50% dilution. The more concentrated toxins in the 100% effluent presumably suppressed plant growth potential.

The two-way analysis of variance (ANOVA) showed that there was a statistically significant difference ($p<0.001$) among the means of the %TP removed for the three levels of effluent. Note that %TP removals were $\log_{10}$ transformed to normalise the data. The effect of different levels of effluent dilution on %TP removal depended on plant age, ie there was a statistically significant interaction ($p<0.001$) between level of dilution and plant age. Age stages where the %P removal for each effluent level also differed significantly ($p<0.001$) (Tukey Test). Tukey's test was also used to compare the means of %TP removed for each level of effluent dilution for each growth stage. Overall there was a statistically significant difference ($p<0.001$) amongst the means for %TP removal, but that differed for each age group (Figure 4.11).

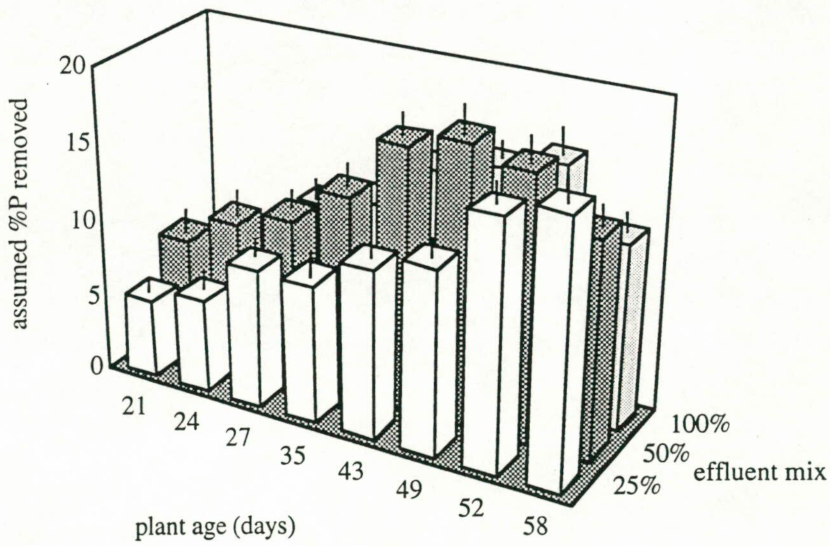

Figure 4.11 Percent daily phosphorus removal from diluted and undiluted effluents by the NFT experimental pilot plant (Bondi STP) at various ages of lettuce plants. Average TP concentrations = 25% effluent (1.1 mg L^{-1}), 50% (2.2 mg L^{-1}), 100% (4.48 mg L^{-1}) (n=3, error bar = 1 SD)

The nitrogen percentage removal data for the three effluent levels (25%, 50% and 100%) through the growth cycle was analysed using the same (ANOVA) statistical test used for %TP. Again there was a statistically significant interaction ($p<0.001$) between level of dilution and plant age. The statistical difference amongst the three means of %TN removed for the three levels of effluent dilutions is significant ($p<0.001$) after allowing for effects in age and level of dilution.

Though the 100% effluent mix contained more nutrients, the highest recorded nitrogen removal efficiency was achieved by plants grown in the 50% effluent mix. In general, the %TN removed from three effluent dilutions increased until plants reached 49 days of age. After 52 days of age the %TN removal rate decreased. At 52 days, %TN removed did not differ significantly ($p > 0.05$) for 25% and 50% effluent dilutions, but the 100% effluent mix resulted in significantly higher nitrogen removal than measured for either the 25% or 50% mixes ($p < 0.05$). The %TN removal was however, higher ($p < 0.05$) for the 50% and 100% effluent dilutions than for the 25% effluent dilution between 35 and 49 days of age.

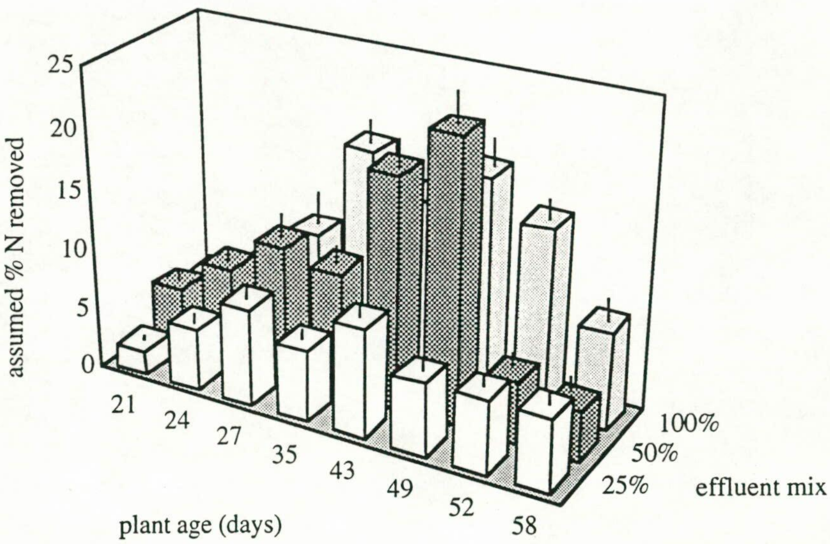

Figure 4.12 Percent daily totalnitrogen removal from diluted and undiluted effluents by the NFT experimental pilot plant (Bondi STP) at various ages of lettuce plants. Average TN concentrations = 25% effluent (15 mg L^{-1}), 50% (27 mg L^{-1}), 100% (4.8 mg L^{-1}) (n=3, error bar = 1 SD)

During the first growth stage, plants of the 100% mix did not show good growth nor nutrient uptake rates compared to those grown in the 50% effluent dilution. After 24 days of age, nutrient uptake and growth rates of some plants improved, indicating plant adaptation to the effluent environment. Hence, plants that can not tolerate the concentrated effluent environment and with small nutrients demand are better allocated where concentrated effluent is not encountered in the treatment plant, ie. at the end of long NFT channels.

Clearly, more plants would be used in the full system to further reduce nutrients levels, but this data were collected to estimate growth rates versus uptake rates at different plant growth stages for three phosphorus concentrations.

4.3.3.4.2 Growth Rate

Growth rates from the Bondi STP experiments are summarised in Figure 4.13. The plants grown with the control nutrient solution generally showed the highest growth rates. Of the effluent mixes, the plants grown in the 50% effluent showed highest growth rates, followed by the plants in the 100% mixture, then plants in the 25% mixture. This can be related to the two following facts; (i) although the undiluted effluent was rich in phosphorus, it was also rich in fatty acids and toxic heavy metals, which suppressed plants growth, and (ii) the initial dissolved oxygen (DO) concentration in the 50% effluent was higher than the intial DO in the 100% effluent (DO ≈ 0.9 mg L^{-1}), gradually this level increased to about 5 mg L^{-1} after 24 hours of being circulating within the NFT system.

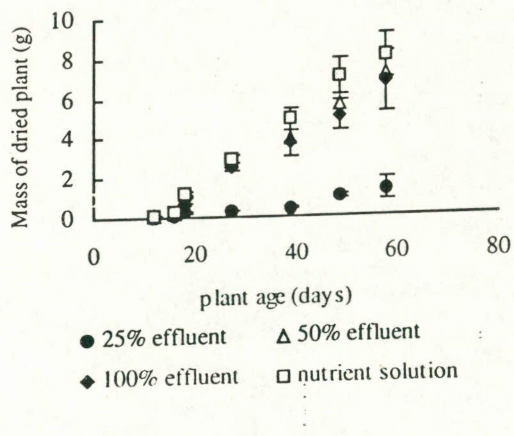

Figure 4.13 Average growth rate of lettuce plants grown in the NFT, using effluent diluted to different levels or nutrient solution control (n=3, error bar = 1 SD).

4.3.3.4.3 Heavy Metals

Concentration of Metals in the Utilised Effluent

Concentrations of common elements in Bondi STP effluent before and after treatment in the NFT system are summarised in Table 4.7. More than 50% of B, 90% of Ni, 50% of Pb, 20% of Cu and 100% of Cr were removed from Bondi STP effluent by the NFT system.

Samples from the utilised effluent were analysed for different elements before and after being used to irrigate the plants. The maximum recommended concentrations for heavy metals in irrigation water for soil applications and that found in the utilised effluent are listed in Table 4.7. Concentrations of arsenic, cadmium, lead and copper exceeded the maximum concentration and need to be reduced to acceptable levels before reusing Bondi effluent for growing edible plants.

Table 4.7 Recommended maximum concentrations of metals in irrigation water used continuously on all soil types and in Bondi effluent before and after one week's recirculation in the NFT pilot plant[1]

Metal	Initial Concentration and Standard Deviation ($mg\ L^{-1}$)	Final Concentration and Standard Deviation ($mg\ L^{-1}$)	Recommended Maximum Concentration in Irrigation Waters* ($mg\ L^{-1}$)
Al	NM**	NM	5.0
As	0.435 ± 0.439	0.403 ± 0.278	0.1
B	2.7 ± 0.96	1.28 ± 1.14	3.00
Cd	0.061 ± 0.067	0.045 ± 0.06	0.01
Co	<0.002	<0.002	0.05
Cr	0.026 ± 0.018	<0.002	0.1
Cu	1.29 ± 0.79	1.022 ± 0.464	0.2
Ni	2.02 ± 1.38	0.4 ± 0.28	0.02
Pb	0.214 ± 0.102	0.12 ± 0.15	0.2
Zn	0.18 ± 0.12	0.1608 ± 0.063	0.20

[1] Form the glasshouse trials. * (NSW EPA, 1995). **not measured

4.4 Microbial Risk Assessment of Produced Crops

Prior to this research, crops irrigated by primary treated municipal effluent in a hydroponic system has not been tested in the laboratory for pathogen uptake. In this study samples of plants grown in the hydroponic system were analysed for viral and bacterial uptake, as well as uptake by virus sized fluorescent microspheres.

The aim of this microbial risk assessment was to aid evaluation of the effluent hydroponic system products for human consumption or animal fodder.

4.4.1 Conventional Risk Assessment Based on Infective Dose

As shown in Table 4.8, infective doses of bacteria are generally considerably greater than for viruses. *Shigella* and some toxigenic strains of *E. coli*, such as O157:H7 however, may cause infection with doses as low as 10 cells. Attack rate is the rate of illness (disease) given infection (growth of the pathogen) (Ashbolt *et al.,* 1996). All of these bacteria may be transmitted to sewage via the faeces of infected individuals (Fattal *et al.,* 1986).

Table 4.8 Infective dose for selected bacterial pathogens

Bacteria	Infective dose	Attack rate	Disease dose
Salmonella spp.	10^5-10^8	50%	10^5
Shigella spp.	10^1-10^2	50%	10^1
Escherichia coli	10^6-10^{10}	25-75%	10^6
Vibrio cholerae	10^3-10^{11}	25-75%	10^3
Streptococcus faecalis	>10^{10}	25-75%	10^{10}

From Ashbolt *et al.* (1996)

Table 4.9 shows dose responses for some key viruses. Microbiologists however, are still unable to culture or enumerate most viruses, the major agents along with protozoa which causes gastroenteritis from faecally contaminated seafoods and waters (Ashbolt *et al.*, 1996).

Table 4.9 Infective dose for a 50% attack rate for viruses in humans

Viruses	50% Infective dose	Mode of administration
poliovirus 1 (Sabin)[a]	72 ($TCID_{50}$)	oral
poliovirus 3 (Fox)[a]	4 ($TCID_{50}$)	stomach
Echovirus 12	35 (pfu)	oral
Coxsachievirus A21	28 ($TCID_{50}$)[b]	nasal
Adenovirus 4	1 ($TCID_{50}$)	nasal

[a] study conducted in healthy infants. ($TCID_{50}$) tissue culture infective dose for 50% .
[b] infective dose was found to approximate the illness dose.
From Ashbolt *et al.* (1996)

Concentrations of thermotolerant coliforms represented by *E. coli* in the utilised effluent was higher (2.9×10^5 cfu) than Australian guidelines for reclaimed water (*E. coli* ≤25 cfu 100 mL^{-1}). Viruses were also not removed from the effluent before being utilised in the NFT channels. Moreover, since analytical results showed the possibility of viral particle transport into lettuce leaves, it is therefore possible that the consumer will be infected, some of whom may develop disease. Lettuce plants grown in the NFT by primary treated municipal effluent, therefore, are not recommended to be consumed by humans. On the other hand, as human viruses do not infect other domestic animals, they could be safely fed to animals.

4.4.2 Quantitative Risk Assessment Using Dose Respond Models

Quantitative Microbial Risk Assessment Using Dose Respond Models

Ideally the events leading from exposure to infection and then disease are probabilities that can be simulated by various dose response models (Teunis *et al.,* 1996). This preliminary health risk study only considered infection by ingestion of lettuce as evidence for health risk.

Assuming at least one pathogen survived and reached a target site within the host, then the probability of infection (P_{INF}) from that one microorganism may be calculated by the β – Poisson model for many viruses and bacteria (Haas, 1983; Fazil and Haas, 1996):

$$P_{INF} = 1 - \left[1 + \frac{D}{N_{50}}\left(2^{1/\alpha} - 1\right)\right]^{-\alpha} \qquad (1)$$

α describes the slope of the dose response curve (eg $\alpha = 0.27$ for rotavirus and 0.313 for *Salmonella*)

D = dose

N_{50} = Number of organisms for 50% infection (eg $N_{50} = 5.6$ for rotavirus and 23600 for *Salmonella*)

Equation 1 was used here to calculate the probability of infection for rotavirus and *Salmonella* spp, and the exponential model (equation 2) for parasitic protozoa (Rose *et al.,* 1991; Hass and Rose, 1994).

$$P_{INF} = 1 - e^{-(D/K)} \qquad (2)$$

K = constant (K = 238.6 and 56.23 for *Cryptosporidium* and *Giardia* respectively [Teunis *et al.,* 1996]).

Estimated number of enteric viruses in one gram of lettuce leaves was calculated based on the assumption that the relationship between translocation of rotavirus and the translocation of fluorosphere model viruses was linear (Rababah, 1998). Hence, a conservative dose for rotavirus was calculated by multiplying the average estimated lettuce leaf content of (fluorosphere g^{-1}) by the average daily intake of lettuce (11.5 g day^{-1}) that an individual consumes (Records, 1997). The result then was multiplied by the ratio of the assumed number of rotaviruses (1500 rotavirus L^{-1}) to number of fluorosphere (1 x 10^{12} fluorosphere L^{-1}) in primary effluent. The provided average lettuce intake value was a broad estimate, based on a national survey conducted by CSIRO in 1993. Uptake of *E. coli* and *C. perfringens* spores versus control was inconclusive, possibly due to cross-contamination, and no F-RNA phage were detected (<8-18 pfu g^{-1}). Hence, acceptable doses for *Cryptosporidium*, *Giardia* and *Salmonella* were estimated by the dose response models (equations 1 and 2) and using an "acceptable" risk level of $P_{INF} = 10^{-4}$ (Tables 4.10 and 4.11). As is often the case in microbial risk assessment, the estimated numbers of the *Cryptosporidium*, *Giardia,* and possibly rotaviruses in the ingested material are probably too low to be enumerated by existing microbial methods.

Table 4.10 Types of dose response models

Pathogen	Model PINF = probability of infection
Giardia	exponential: $P_{INF} = 1-e^{-(D/K)}$ K= 50.23
Cryptosporidium	exponential: K= 238.6
Rotavirus	β - Poisson: $P_{INF} = 1 - \left[1 + \frac{D}{N_{50}}\left(2^{1/\alpha} - 1\right)\right]^{-\alpha}$ $N_{50} = 5.6$, $\alpha = 0.27$
Salmonella	β - Poisson: $N_{50} = 23600$, $\alpha = 0.313$
Shigella	β - Poisson: $N_{50} = 1120$, $\alpha = 0.209$

Table from Ashbolt *et al.* (1997). D= pathogen dose

Table 4.11 Probability of infection (P_{INF}) for rotavirus, and numbers of *Cryptosporidium, Giardia* and *Salmonella* to give 10^{-4} risk if 11.5 g lettuce consumed

Pathogen group	Number L^{-1} in primary effluent[a]	Dose or "acceptable" No. in 11.5 g lettuce	P_{INF} [d]
Rotavirus	1500	0.047[b]	0.017
Cryptosporidium (oocysts)	107	0.02	10^{-4}[c]
Giardia (cysts)	39,000	0.005	10^{-4}[c]
Salmonella spp.	80,000	0.0009	10^{-4}[c]

[a] from Ashbolt *et al.* (1997).

[b]estimated based on fluorosphere model virus uptake into lettuce leaves (Rababah, 1998) and that rotaviruses represented 10% of assumed total virus load.

[c]acceptable risk level assumed. [d]using equations 1 and 2.

The very conservative dose response model predictions indicated that the probability for an individual to be infected due to the consumption of 11.5 g of lettuce grown in the

NFT system was about 1.7% for rotavirus-like pathogens. Clearly subsequent storage prior to ingestion may significantly reduce this pathogen risks. Nonetheless, the produced lettuce plants are not recommended for human consumption, due to pathogens, metals and possibly the uptake of other contaminants, such as various xenoestrogens (Nogel *et al.,* 1997).

Stochastic models fitting the dose response data indicate that the probability of infection after digestion of a single infectious unit or particle may not be negligible. This implies that there is no dose threshold for microbial safety as implied in Tables 4.10-4.11. Hence, modern safety regulations and legislature should be based upon the definition of tolerance levels, based in turn upon publicly acknowledged risk limits (Haas, 1983; Ashbolt *et al.,* 1996).

It must be kept in mind that some individuals will consume more than the average intake value. Hence, the infection probability for such individuals will be higher. Moreover, the estimated probability of infection was based on one path that the organism took to reach the food sample. This path is from the effluent in the NFT channels to the roots and then translocated into the leaf tissues (root path). The root path, however, is not the only possible pathway that the pathogen can take to reach the food samples. Accidentally, pathogen can reach the lettuce leaf due to cross contamination during handling of plants. The surface of lettuce plants can also be contaminated by accidental splashing of effluent due to occasional system faults.

Design of PTHF Using P-Model

Experimental phosphorus removal rates and other data (e.g. root weight, effluent phosphorus concentration and growth rate) were used to develop a phosphorus removal model (P-Model) to scale-up the NFT-experimental pilot plant system (Figure 4.1) to a full-scale PTHF (Figure 4.14) suitable for small communities. Phosphorus concentration was selected as the limiting design parameter to monitor the effect of the variable effluent constituents on plant production and wastewater treatment by the PTHF.

The full scale PTHF was designed so that the utilised effluent continues to loose its constituents while flowing through the (> 30 m) NFT channels. Subsequently, the lowest end of the channels delivers effluent meeting discharge standards. The removal of pollutants from wastewater is accomplished by absorption, surface sorption, filtration and biological treatment. The simulation equations used in the P-Model also included the sorbed phosphorus, which was shown to be the major route for phosphorus removal as described above. The vast majority of surface sorption occurred within the root mat in the channels (87-95%) which may be similar for a range of NFT grown plants. Sorption to plant roots did decrease however, with the older plants indicating, that saturation of P binding sites was approached by week 8 for lettuces.

The chosen model involved developing empirical relationships which related plant phosphorus content over time and growth rates. The statistical equations developed were based on Williams (1946) mechanistic model, and used to predict phosphorus removal rates from the treated effluent. The predicted phosphorus removal data were mathematically translated into design parameters for the hydroponic treatment farm. Design parameters included number of plants of different age groups needed to achieve the required phosphorus removal rates, dimensions of land required to achieve the required removal rates, and full-scale NFT treatment farm configuration. Clearly the final configuration of a proposed hydroponic farm will depend on locality of the site, ie shape and slope of the available land, along with characteristics and flow rate of wastewater.

The sites of phosphorus removal from wastewater were: (i) plant tissues receiving the phosphorus that was taken up by the roots (P_u), and (ii) roots and other NFT surfaces adsorbing phosphorus from the effluent (P_s). For the mass balance of phosphorus associated with plants grown bare-rooted by NFT with primary treated affluent, a number of assumptions were made, most importantly: (a) soluble and particulate phosphorus is uniformly distributed in the primary effluent, (b) all roots on a plant of a given age absorb phosphorus at the same rate, and (c) fresh effluent was used to grow plants in the NFT channels; ie a once through system.

The mass balance statement around the phosphorus removal models (plant tissues, surfaces of roots and other NFT components) can be written as following:
Rate of P accumulation in the plant tissues and on system surfaces = phosphorus entering the control surface – phosphorus leaving the control surface ± net production or depletion.

Using the above assumptions and for flow Q, the mass balance equation can be written as:

$$\frac{dP}{dt} = Q \bullet P_i \quad \text{(notations are listed at the end of the paper)} \qquad (3)$$

Rababah *et al.* (1997) used equation 3 to calculate phosphorus removal rates (for one lettuce plant) due to plant uptake, U (mass of phosphorus absorbed into plant tissues) or due to surface sorption, S (mass of adsorbed phosphorus onto the outer surfaces of plant roots and other system components; ie supply and delivery pipes, recirculating tank and NFT channels). Phosphorus removal rate at plant age (t) due to either plant uptake or surface sorption can be calculated by equation 4:

$$U\ (\text{ or } S) = \frac{W_R}{W} \int I dt \qquad (4)$$

The total phosphorus removal rates (J) from the wastewater by the PTHF of N number of lettuce plants is the summation of phosphorus removal rates due to plant uptake and surface sorption:

$$J = N\ (U + S) \qquad (5)$$

The proposed PTHF is licensed to discharge phosphorus below (P_e). Thus, total phosphorus removal rate (J) of a NFT farm that is suitable for a small community of average wastewater flow rate (Q) and phosphorus concentration (P_i) can also be given by:

$$J = (P_i - P_e) * Q \tag{6}$$

The number of plants (N) required to treat wastewater of a small community by NFT-plant system can be calculated by:

$$N = \frac{Q(P_i - P_e)}{(U + S)} \tag{7}$$

In order to validate the developed model, the predicted phosphorus removal rates were plotted against observed removal rates obtained from different experimental trials carried out under similar conditions to those used to obtain data for the model development at Bondi. Despite the predicted phosphorus removal rates showing an excellent agreement ($R^2 = 0.94$) with measured rates, the model should only be used to design a hydroponic treatment system for small communities with effluent containing phosphorus concentrations within the range used in the three experimental trials, *viz* 0.76-5.5 mg L^{-1}. The PTHF is not recommended for large community because of the very large number of plants that will be required (about 25,000 plants for 200 people) and large area of land which may not be cost effective for NFT treatment. Final polishing of the effluent may require a humus tank, so group A plants are exposed to sufficient nutrients for growth, and disturbed particulates are trapped.

Hence, the model structure described in this paper for P is based on different simulation equations, each of which is suitable for a specific level of phosphorus in effluent and for lettuce plants of different ages.

Distribution of Plants in the NFT Channels According to Age Group

The NFT channels of the proposed PTHF were divided into three sections. Plants of age 44-58 days (group C) were able to tolerate the toxic effluent and provided most of the biological and physical treatment of the effluent because of the relatively large mass of roots. Group C plants therefore, should be located in the upper stream section nearest the inlet of the settled effluent to the channel, followed by plants of the 30-44 days of age (group B) in the intermediate section of the NFT channels (one third in length). Group B plants absorbed most nutrients compared to other age groups, additionally this group grew well in the wastewater environment after aeration and primary settling treatment. Plants of 16-30 days old and less (group A) are more sensitive to the wastewater environment therefore, they are best located in the lower stream section, closest to the receiving water. Hence, in the case of lettuce, every 16 days a new group A is added and the others advances to their new group locations (A→B, B→C, C→harvest).

Layout of the PTHF

The different components of the proposed NFT wastewater treatment and plant production farm are shown in Figure 4.14. A novel idea was to cover the channels with a moving conveyer belt for plant manoeuvring as they progress in age.

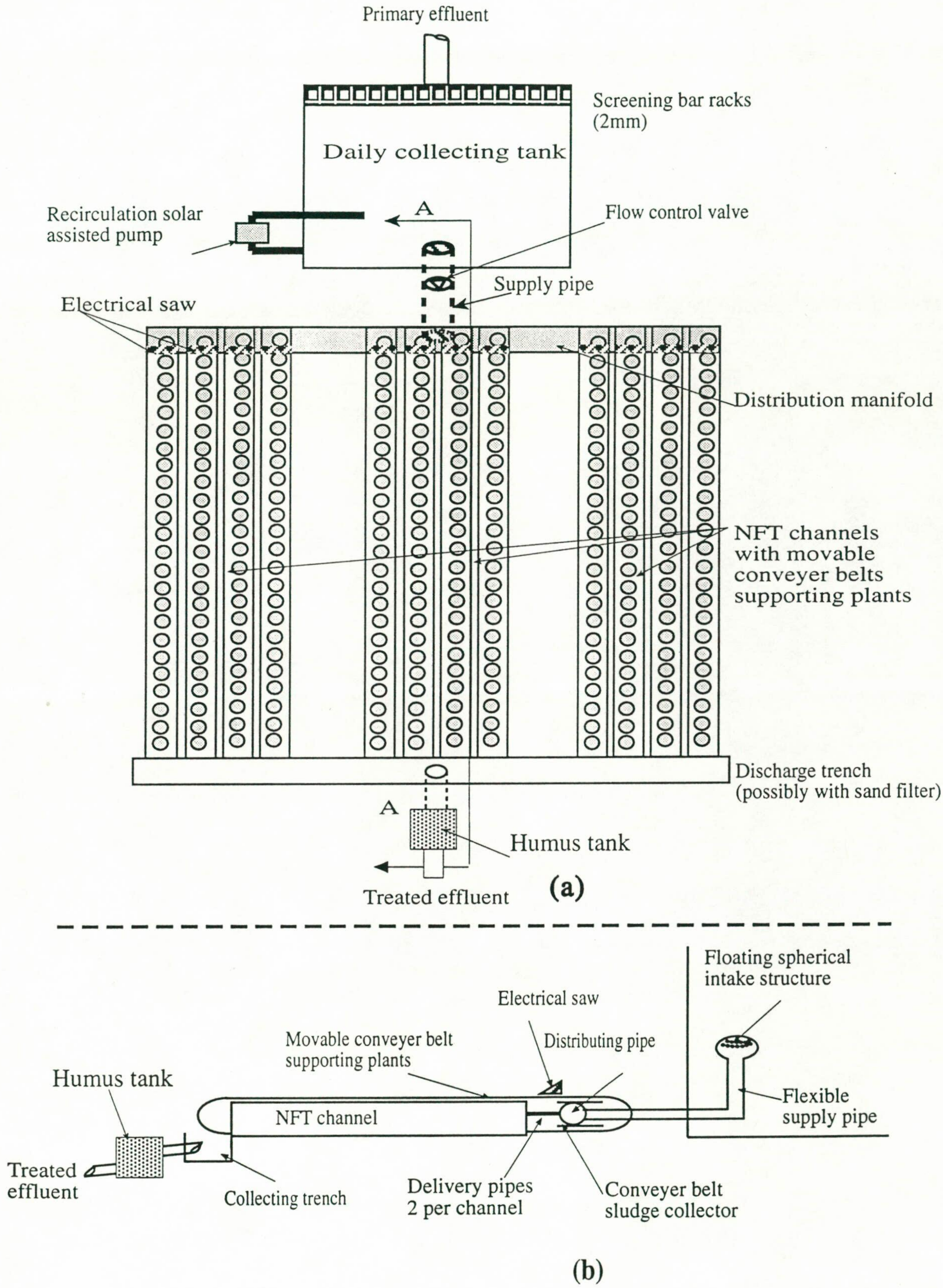

Figure 4.14 Lay out of the proposed NFT farm, (a) top view (b) side view for section A-A showing intake structure and conveyer belt position with respect to NFT channels

Table 4 shows design criteria for a full-scale PTHF for small plants production and wastewater treatment. These criteria were based on observations from the pilot-scale experimental runs.

Table 4.12 Design criteria of a hypothetical PTHF for a small community of 200 people

Design parameter	Definition	Dimension
A_b	area to be allocated for biomass handling	20 m^2
A_s	area of land to be allocated for the buffer tank	84 m^2
B_b	length of land between the upper end of the NFT channels and the buffer tank	10 m
B_s	length of the buffer tank	12 m
D_l	NFT channels width for small plants (e.g. herbs)	100 mm
D_s	NFT channels width for large plants (e.g. tomatoes)	150 mm
h	depth of flow in the NFT channels	2-10 mm
H_l	depth of large NFT channels	150 mm
H_s	depth of small NFT channels	100 mm
k	longitudinal plant spacing	200 mm
Lb	bilateral spacing between channel	100 mm
Y	work and handling space between 10 channels	500 mm
Z_b	width of the allocated space for plant handling between NFT channels and the buffer tank	2 m
Z_s	width of the buffer tank	7 m
Δ	slope of NFT channels	0.1

Plant Handling

The selected type of plant affects the handling technique to be adopted. For short life cycle and small plants such as herbs and peper seedlings of 16 days of age, they can be transferred into plastic growing cups, and then placed into pre-prepared slits (holes) in a moving belt system on top of the NFT channel with the roots of the plants just touching the bottom of the channels. With more than 67% of the primary effluent solids accumulated on root surfaces it is recommended to immediately collect the unwashed roots as they are cut off at the end of the collector belt. Consequently, the sludge does not need any dewatering process as it is all collected on the root surface of the plant. The root/sludge material could be composted or lime treated (pH > 12) for disinfection. The disinfected biosolids may then be sold to landscape contractors or used for gardening. Similar biosolids sale is a profitable practice in California (WEF and ASCE, 1992).

4.4 SUMMARY

The results showed that :

a. Plants grown in the NFT system and irrigated by Bondi STP effluent removed about 80% of TKN (as N), 77% of total phosphorus (as P), 87 % BOD and about 99% suspended solids. Nutrient removal rates will be higher as more plants will be used in the full-scale PTHF.

b. Lettuce plants grew well in a modified hydroponic system (best in the glasshouse) and would be further improved by suitable distribution of plants of different ages along the NFT channels in the proposed full-scale NFT treatment plant which is discussed in Chapter 6. Growth data obtained from the three different locations (covered building, glass house and Bondi STP) are discussed further in Chapter 8.

c. Heavy metals (As, Cd, Cu, Pb) in NFT plants may cause health problems if consumed by humans or animals. Alternative plants (ie. flowers) of minimum health risk and therefore recommended for a NFT system utilising a mixed industrial and/or domestic effluent. Edible plants, however, may be recommended if As, Cd, Cu and Pb are reduced and the effluent is disinfected.

d. Produced lettuces are not recommended for human consumtion. This conclusion was made based on two ways of health risk assessment: firstly, the fact that the analysed leaf samples for virus-like particles were positive and secondly, dose respond model predictions indicated 1.7% probability of infection from effluent pathogen uptake. Animals fed on the crops of the hydroponic system are exposed to a large dose because of their high daily intake of crops. Hence, higher probability of infection for animals from effluent pathogen uptake is expected.

e. The evidence that lettuce plants grown hydroponically in primary municipal effluent absorbed pathogens was not clear. Uptake of *Escherichia coli* and F-RNA phage was not demonstrated. Nevertheless, uptake of spores of *Clostridium perfringens* may occur and uptake of model virus particle was demonstrated.

5. DIFFICULTIES

The following diffuclties were encountered throughout the project:

1. This project involved relatively sensitive plants irrigated with somewhat phytotoxic effluent. On some occasions, the plants could not adapt to the environment of the effluent especially, during their early growth stages. Thus, on several occasions plants died, causing a significant delay in getting the required data and demanded extensive efforts to maintain the pilot plant. This produced unexpected results, which affected the model development plan. Nonetheless, these practical issues were resolved.
2. Despite the extra effort just mentioned, and the modifications that have been taken (see next point, 6) to make it practical to pursue with the experimental trials, poor growth and uptake inhibition continued to dominate the plants in the 100% trial as indicated above. Hence modelling took into account plant age and effluent concentrations for designing a full-scale PTHF.
3. The plants were also growing under confined air conditions at Bondi STP. Concentrations of noxious gaseous emissions from the sedimentation tanks in the tunnels reached high levels. This was especially true, when the air blowers were not in good working order. Consequently, at times air quality was not suitable for plants and affected their growth. This resulted in the cancelling of two growth cycles and starting new runs.
4. Modelling nutrient removal for hydroponic crops is not common in the literature, with most research concentrated on plants grown in soil. On the other hand, model equations for phosphorus uptake in NFT are simple compared to soil, but modifications and additional parameters needed to be included to suite plants grown in NFT with effluent.

Furthermore, research findings have revealed a need for the folowing further investigations which were not clear at the time of the original proposal:

1. Integrate nutrient models to allow modelling of a full scale wastewater treatment system

Hence, the research team applied for extra fund to carry out the above investigations. The requested fund however was not granted, which made it difficult to finalise the study.

6. MODIFICATIONS

The following modifications undertaken to deal with difficulties for better out-comes:

1. The irrigation technique was changed from continuos flow to recycling type of experiment so that the environment of the effluent would suite the plants (largely increasing DO). The level of oxygen increased from about 0.9 to about 5 mg L^{-1}.

2. It was planned to consult only with Dr Zdenco Rengel in University of WA in Perth for the modelling study. This was extended to further consultations with Dr Philip Smethurst, soil scientist, CSIRO Tasmania, Efforts and consultations are in progress to finalise the shape of the model. Model development will take longer than expected due to the sensitive nature of the experimental work.

3. Concentrations of heavy metals in the utilised primary effluent and crops were measured (see Table 3 in progress report 1) for health risk assessment. Investigations for heavy metals removal and effluent sterilisation technologies for a NFT-plant system were also carried out with limiting budget.

7. EVALUATION OF THE ACTUAL PROJECT AS COMPARED WITH PROPOSAL OUTLINED IN THE SUBMITTED APPLICATION

The actual project is an identical application of the proposal outlined in the submitted application. A quantitative microbial risk assessment was added to the project (section 4.4).

8. EVALUATION OF COMPLIANCE WITH THE TIMETABLE SET OUT IN THE SUBMITTED APPLICATION

The proposed objectives to date set out in the timetable were achieved.

9. EVALUATION OF PROJECT

The main product of this project is a concept design for a novel hypothetical PTHF, which provides simple wastewater treatment technology, commercial valuable plants and alternative resource of non-potable water for Australia's inland small communities. The farm produces minimal solid wastes and requires little energy input.

Hydroponic wastewater irrigation is practiced commercially in Melbourne for vegetable growers. Melbourne Water sells treated water to hydroponic farmers to grow Tomatoes and other crops which are sold at market price. Farmers and Melbourne Water find this practice profitable. Nonetheless, pathogen risk assessment is recommended to evaluate such a practice.

The proposed PTHF is ready to be introduced to field application with commercial potential for non-edible crops such as cut flowers and essential oils. The proposed PTHF could also be used for crop production using disinfected effluent.

10. ASSESSMENT OF PROJECT VALUE

The investigation team has successfully met the objectives originally outlined from the research projects. Simple and inexpensive technology for wastewater treatment and commercially valuable plant production was achived for small communities not only in Australia, but also in arid and semi-arid regions around the world.

11 RECOMMENDATIONS FOR FUTURE IMPLICATIONS

It is not recommended to consume lettuce leaves of plants grown in the adapted NFT by primary treated municipal effluent, because of high concentrations of heavy metals in plant leaves. Moreover uptake of pathogenic bacteria may occur (Lamb *et al.,* 1996, Toh *et al.,* 1998) and uptake of model virus particles was demonstrated.

It is recommended to investigate mathematical models for a wide range of plants, further concerning nutrient uptake principles from hydroponic solutions, so that these factors can be taken into account for a comprehensive nutrient removal model.

12. RELEVANT VISUAL DOCUMENTAION

Please see attached photos and slides.

13. PROMOTIONAL MATERIAL

Journal Papers

Boyden, B., Rababah, A. (1996) “Recycling Nutrients From Municipal Wastewater”. *Desalination* 106 (1-3):241-246.

Rababah, A., Ashbolt, N. (1998) "Innovative Production Treatment Hydroponic Farm". Submitted to *Water Research.*

Conference Papers:

1. Rababah, A., Rengel, Z., Ashbolt, N., Boyden, B., (1997) “Modelling Phosphorus Removal in a Nutrient Film Hydroponic System Utilising Municipal Wastewater”. Conference Proceedings, Beneficial Reuse of Water and Biosolids, Marbella, Malaga, Spain, Water Environment Federation pp: 241-246.

2. Boyden, B., Rababah, A., Ashbolt N. (1996) “Nutrient and Heavy Metal Uptake from a Thin Film of Municipal Wastewater by Commercial Viable Plants”. Proceedings of New Zealand Water & Wastes Association Annual Conference, Nelson, New Zealand pp: 43-47.

3. Boyden, B., Rababah, A., Ashbolt N., (1995) “Recycling Nutrients from Municipal Wastewater: Application of Hydroponics to Save Water, Energy and the Environment”. IDA World Congress on Desalination and Water Sciences, Abu Dhabi, UAE, Volume IV pp: 447-458.

4. Rababah, A., Boyden, B. (1995) “Removal of Nutrients from Municipal Wastewater to Produce Vegetable Crops Hydroponically” AWWA 16th Federal Convention Sydney. Australia Water and Wastewater Association pp: 1031-1036.

5. Boyden, B, Rababah, A. (1994) “Recycling Nutrients From Municipal Wastewater” International Specialist Conference on Desalination and Water Reuse, Murdoch University, Perth WA. Australia water and Wastewater Association pp: 126-135.

14. MEDIA COVERAGE OF THE PROJECT

- The "Practical Hydroponics and Greenhouses" magazine, made an interview with Dr Brace Boyden and Abdellah Rababah and published a detailed article, in issue 32, 1995, about the project (copy is included).

- The Sydney morning herald made an interview with Dr Brace Boyden and published an article about the project (1995).

- The ABC radio made an interview with Dr Brace Boyden about the project. The interview was broadcasted a few days later (1995).

NOTATIONS

I = net influx of ions (mg g^{-1} s^{-1})

J = mass of phosphorus (P) removed from the wastewater by NFT-plant system, when plants are at a certain (number of days of) age (mg)

N = required number of plants in the proposed hydroponic farm

P_e = recommended phosphorus concentration in farm effluent (mg L^{-1})

P_i = phosphorus concentration in wastewater influent (mg L^{-1})

Q = hydraulic flow rate of wastewater (L day^{-1})

S = mass of phosphorus (P) removed due to surface sorption (mg)

U = mass of phosphorus (P) removed due to plants uptake (mg)

W = total weight of the plant (g)

W_R = plant root weight (g)

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