Project title:	**Monitoring the commercial development of slow sand filtration in HNS.**
Project number:	**HNS 88**
Project leader:	**Dr Tim Pettitt** **HRI Efford** **Lymington** **Hants SO41 0LZ**
Report:	**Final report (October 1998)**
Previous reports:	**None**
Key workers:	**Tim Pettitt - plant pathologist HRI Efford** **Mike Wainwright - assistant plant pathologist HRI Efford**
Location:	**HRI Efford; Commercial nurseries in Suffolk and Surrey**
Project co-ordinator:	**Mr Brian Humphrey** **Ramblers** **Benhall Low Street** **Saxmundham** **Suffolk IP17 1JE**
Date commenced:	**1 August 1997**
Date completed:	**31 October 1998**
Key words:	**Hardy nursery stock, irrigation water, recirculation/ recycling, slow sand filtration, biofiltration, *Phytophthora*, water cleaning, water testing**

CONTENTS

PRACTICAL SECTION FOR GROWERS

Objectives and Background

Although used since the early nineteenth century for purification of drinking water, slow sand filters (SSF) have only very recently been tested for their ability to remove plant pathogens from irrigation water. The results of research at Geisenheim, Boskoop, Wageningen and HRI Efford have shown that SSF successfully removes pathogens such as *Phytophthora* and *Pythium* from contaminated water in experimental-scale rigs. In an ongoing MAFF research project looking at a range of methods for cleaning pathogens from recycled HNS irrigation water (HH1708SHN & HH1733SHN), slow sand filtration was identified as a very promising method in terms of efficacy, flexibility, sustainability and cost.

The basic concept of SSF is simple. Raw water, (collected from nursery beds, greenhouse roofs or any other sources where there is a risk of contamination with plant pathogens), is passed through a column of sand at a slow flow rate (0.1-0.2 mh^{-1}) and run to waste until a biofilm layer has built up over the sand grain surfaces. Once this priming period is complete the filter will effectively remove pathogen spores and organic carbon particles from the raw water by a complex combination of physical and biological processes in which the bacteria of the biofilm are of key importance.

Two pioneering HNS nurseries recently decided to install SSF to clean their recycled water. With the backing of HDC funding it was possible to monitor the progress of these systems for pathogen removal under fully commercial conditions, using sensitive microbiological techniques developed in the MAFF research programme for testing for pathogen presence. The objectives of this project were to:

- Monitor raw and SSF effluent waters for pathogens during the priming period;
- Determine when filters were fully primed;
- Monitor raw and SSF effluent waters through complete filter runs to determine their efficacy and consistency once primed;
- Determine the time for filters to be 're-primed' after cleanups and winter 'shutdowns';
- Monitor practical filter management under commercial conditions and prepare some basic guidelines for other HNS growers considering SSF;
- Identify any areas were further research would benefit the development of biofiltration technology for the UK horticulture industry.

Brief description of SSF

A schematic representation of a transverse cross-section through a SSF is shown in Figure 1. Raw water to be treated enters the filter at (A) and percolates through a sand layer between 0.4 and 1.5 m deep under the force of gravity and the head pressure of the supernatant raw water. The sand is normally supported by a layer of gravel, through which the water drains, either to pass through a porous base into a system of under-drains (the large systems used by the water industry operate this way) or to be drawn up *via* a pump as in Figure 1. The rate of flow of water through the filter is controlled either by an exit valve on an under-drain system, or by the flow rate of the pump in a lifting system such as that illustrated in Figure 1 and used in the SSF studied in this project. The efficacy of SSF against plant pathogen propagules is the result of the activity of a biofilm layer, which builds up on the sand grain surfaces. As raw water is passed through a new SSF its efficacy improves as the biofilm layer builds up until the filter becomes mature. This maturation process is often referred to as priming and may take several weeks, during which time the filtered water is run to waste. Once primed the SSF is a very effective and flexible system for removing potential pathogens from water. SSF is also flexible in terms of size. The volume of output is governed by filter size and 1 m^2 of filter surface area will produce between 1 and 3 m^3 (approximately 220-660 gallons) of clean water per day, depending on the sand grain size. SSF must be run continuously and when considering installation, the size the filter needs to be able to produce at least the maximum daily need of the site in 24 h. The flow rate from a filter is too slow to apply water directly to irrigation systems and some form of water storage is needed. The best configuration for using an SSF would be to have a collection reservoir for raw water, which is fed continuously to the SSF. The SSF in turn feeds treated water to a clean water storage reservoir or tank of sufficient capacity to allow for potential filter down times of 2-3 days and from which water is drawn for the irrigation system.

Summary of Results

By carrying out detailed microbiological assessments of samples of water going into and passing through the SSF based at HNS Nurseries 1 and 2 at regular intervals throughout the season it was possible to show that:

- Before the filters were primed, infective pathogen spores passed straight through the sand column;

- Initial priming of fresh new sand took approximately 20 days (in MAFF-funded experiments this figure is generally between 10 and 20 days, depending on raw water quality);

- Once primed, the SSF were 100% effective in removing *Phytophthora* and *Pythium* spores throughout the season and at water temperatures down to 6°C (N.B. this is the lowest temperature monitored and not necessarily the minimum temperature for filter efficacy - MAFF-funded experimental filters have been effective down to at least 4°C);

- SSF took up to 24 h to re-prime following cleanups and approximately 11-12 days after a winter shut-down of 4 months.

In addition, observations on the general operation of SSF on the two nurseries highlighted that the main problem with their management was the frequency of cleanups made necessary by the clogging of the surface sand layer with silt, detritus, algae and peat fines. This is a problem which could be greatly reduced by the use of some kind of coarse pre-filtration system. Otherwise SSF management was straightforward and successful under commercial conditions.

Action Points for Growers

These are best expressed as a series of guidelines:

- Before a newly installed SSF can be used to remove pathogen propagules from irrigation water it must be primed. To prime a new SSF raw water is passed through the filter and run to waste until an active biofilm layer has built up over the sand grain surfaces. Taking samples of the raw and filtered water and getting assessments of the micro-organisms present in them gives a very good measure of when a filter is primed, and during the filter priming period it is a good idea to have water samples tested frequently (at least weekly).

- A continuous flow of water must be maintained through the sand column and a high degree of oxygenation is desirable. For this reason it is best to avoid ice developing on the head of raw water. Spraying the water into the filter head area is a good way of both introducing oxygen and keeping the water moving and thereby avoiding ice formation.

- Since SSF are run continuously at a slow flow rate, it is inefficient to have their output fed directly into the irrigation system. It is best to install a SSF in line between a collection reservoir/pond for raw water and a clean storage reservoir/tank for treated water. With the the continuous flow of water from the SSF, the clean water reservoir needs to have a capacity capable of holding at least 2-3 and preferably 5-6 times the 24 h production capacity of the SSF. This gives buffering for possible filter down-time for maintenance and also provides some space for excess clean water storage during periods of lower demand. Once the storage reservoir is full the SSF can either be run to waste, run back to the collection reservoir or run in recycle mode by feeding the filtered water back into the supernatant head water.

- The size of the SSF installed is governed by the maximum daily demand for water on the nursery and it is advisable to install a filter capable of delivering 1.5-2 times the estimated maximum daily demand over 24 h of filtering. The 24 h output can be roughly estimated as we know that 1 m^2 of filter surface area will produce between 1 and 3 m^3 (220-660 gallons) of water over 24 h, depending on the sand grain size and the head of water over the filter.

- The optimum depth of the sand is about 1.0 m, which would allow for some cleanup operations. The range of sand quality suitable for horticultural filters is still under investigation but effective pathogen control will be achieved with a uniform fine sand that contains no more than 10% grains < 0.2 mm and no more than 10% > 1.0 mm.

- It is advisable to put a cover over the filter as this will cut down the development of algal blooms in the raw head water and will also prevent leaves from blowing in and clogging the sand.

- Pre-filtration of some kind is desirable for efficient SSF use as this reduces the rate at which clogging of the surface sand layer occurs and thereby increases the time between filter cleanups. With pre-filtration, filter cleanups can be reduced to once or twice per season, whereas without it they can be as frequent as every two weeks.

- Filter cleanups are straightforward, but can be disruptive by: (a) causing the filter to be out of production for 1-2 days; (b) labour input required to scrape the clogged sand out (approximately 30 m^2 of filter surface can be scraped in 1 man-hour) and (c) causing sand loss (the more frequent the cleanups, the more often the sand will need to be replaced). The filter cleanup operation is simple. First the water is drained down to below the sand surface, then the clogged surface layer of sand (approx. 1-3 cm deep) is removed with a shovel. After levelling the scraped surface with a rake, the sand is recharged with clean water from below until the water level is about 5-10cm above the sand. This allows the surface to settle, prevents the raw water inlet from scouring the sand surface and reduces the formation of air pockets in the filter profile. Once the water depth above the sand is between 5-10 cm the raw water inlet can be switched back on and the SSF is run to waste for 24 h to reprime, after which it can be switched back into production.

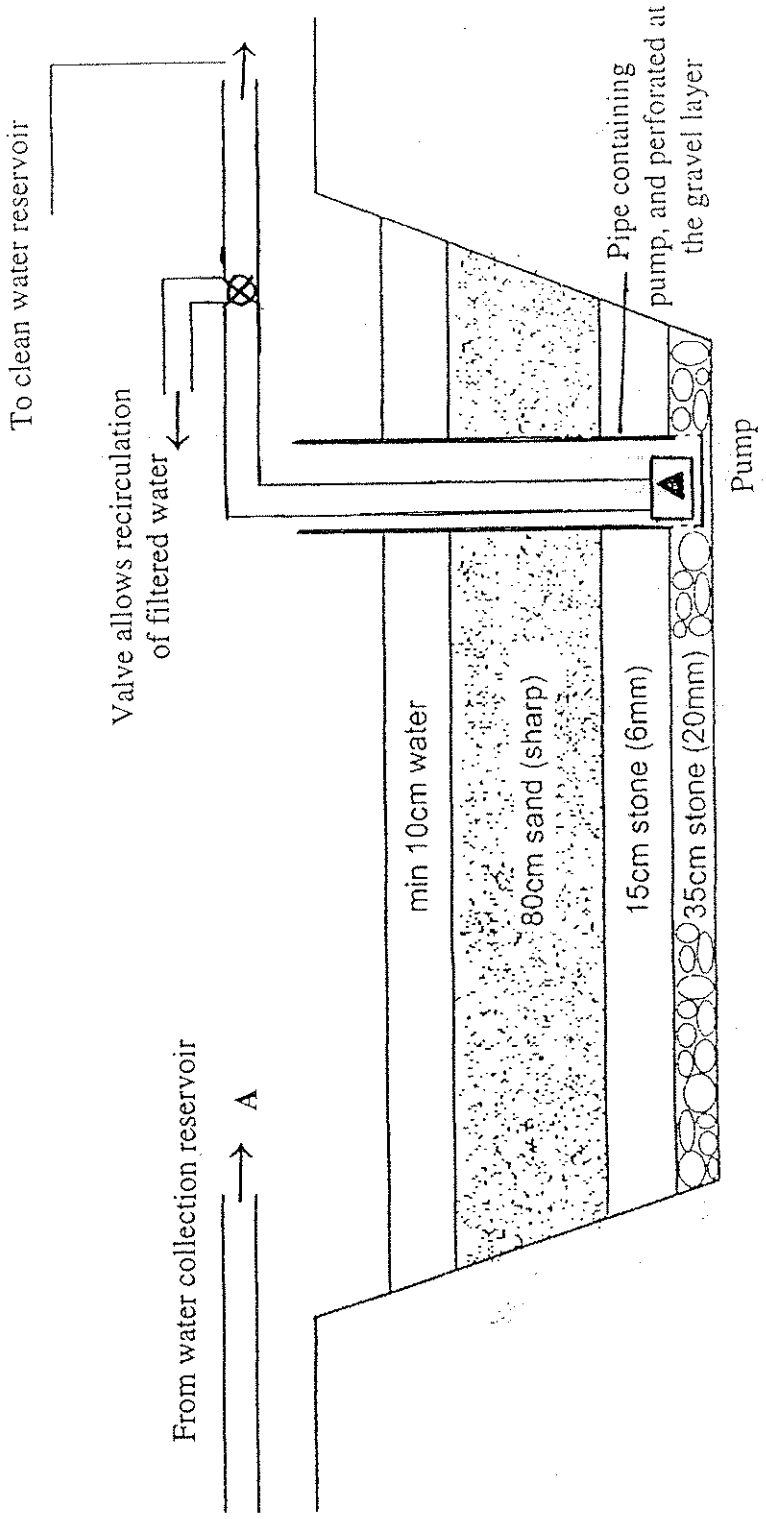
- After a large number of cleanups the sand depth will be reduced to a level where SSF efficacy will start to be reduced, (MAFF-funded experiments have shown that 40 cm of sand is the minimum depth for consistently effective SSF operation). Once the depth of sand in the SSF is reduced to 50 cm, the filter will need to be re-sanded. The re-sanding operation is a process similar to 'double digging'. A trench approximately two spits wide is dug to the bottom of the filter sand layer at one end of the filter and the sand from this trench is stored on top of the sand at the other end of the filter. Fresh sand is placed in the trench to the required top-up depth. The trench in the old sand is then extended a further two spits along the filter and the sand taken from this excavation is placed on top of the new sand in the first trench. This process continues right across the filter surface until a layer of fresh sand is in place with the old sand on top of it. The filter is then recharged from below and re-primed as described above. In the present project this process was carried out once and the re-priming in this case was very rapid, taking only a few days.

- Once the SSF is installed and fully primed, water samples for microbiological assessment can be collected less frequently (at least monthly). However, when using a system like SSF to clean water it is still important to monitor the treated and untreated water for presence/absence of pathogens and to make sure that the filter is still fully primed (this is true for any system for water treatment, or for any system using water likely to be contaminated with pathogen spores).

Anticipated Practical and Financial Benefits

The use of SSF allowed the two nurseries studied in this project to use surplus water collected from their sites for irrigation. In one case this reduced their dependency on mains water and in the other it provided a reliable source of water during the summer months of high demand and low water availability. In both cases this resulted in significant cost savings which both nurseries consider justified the expense of installation.

Figure 1 **Schematic representation of a transverse cross-section through a slow sand filter.**



SCIENCE SECTION

Introduction

The danger of contamination of irrigation water derived from surface waters with infective pathogen spores has long been recognised (Bewley & Buddin, 1921). Often, exposed nursery irrigation ponds can be contaminated with spores/propagules of a wide range of plant pathogens (Shokes & McCarter, 1979). In recent years rising water costs, possible restrictions in water availability during dry seasons and the increasing prospects of more restrictive legislation are encouraging many UK growers to consider recycling their excess irrigation water (Pettitt, Finlay, Scott & Davies, 1998). The main risk from this practice is the possibility of spreading disease from small numbers of infected plants to entire nurseries (Braune, 1987; Neher & Duniway, 1992; VanLuik, 1992; Kemp, Behrens & Wohanka, 1992; Pettitt *et al.*, 1998). In both of these situations it is sound growing practice to treat water from such sources, to eliminate pathogen propagules, before using it to irrigate potentially susceptible crops.

Comparative trials funded by MAFF at HRI Efford identified slow sand filtration (SSF) as a very promising technique for use in the HNS sector because of its flexibility in terms of size and design and its relatively low cost. Although SSF has been successfully used for cleaning drinking water since at least 1804 (Sinclaire, 1808), the technique has only very recently been tested for its ability to remove plant pathogens from irrigation water (Wohanka, 1988 & 1992). The results of research at Geisenheim, Boskoop, Wageningen and HRI Efford have shown that the system is largely successful at removing pathogens such as *Phytophthora* and *Pythium* spp. from irrigation water in experimental-scale filtration rigs. Recent results from MAFF-funded work at HRI Efford indicate that a significant part of SSF action is biological in origin and that under extreme conditions (e.g. autoclaving) this can break down (Pettitt, 1996). Improvements in techniques for pathogen detection in irrigation water samples have also resulted from this work (Wakeham, Pettitt & White, 1997; Pettitt *et al.*, 1998) and these allow reasonably accurate quantitative as well as qualitative assessments of SSF performance.

During 1996 a large-scale producer of HNS installed a large SSF (capable of dealing with at least 200 m^3 of water per day) on one of their nurseries. A small number of water samples were taken from this filter during its pilot running period and these gave some interesting and useful results, particularly those relating to influent water quality and also the priming periods required for the filter to recover its efficacy after being shut down. This work was not funded, and consequently the amount of time and facilities available for it were very restricted. However, the results of operating this filter were sufficiently encouraging for the company to consider installing a SSF on a second site. This situation provided an excellent opportunity to monitor the progress of a new and an established filter over the 1997 and 1998 seasons in order to collect information that would be of great value to any HNS growers considering installing SSF. Such a study also complemented (as well as benefited from) the programme of strategic research on SSF funded by MAFF at HRI Efford (HH 1733 SHN). The objectives of this project were to examine the performance of SSF in commercial situations and to provide some guidance to growers about the future application of this technique to HNS production systems.

Materials and Methods

Sample collection

The bulk of the monitoring work carried out in this project consisted of collection and microbiological analysis of water samples. Water samples were collected in sterile bottles (either autoclaved 1 litre Nalgene polypropylene bottles or freshly-emptied spring water bottles rinsed with boiling water). The minimum sample size collected was 1 litre and all samples were processed and plated within 24 h of collection. Whenever SSF effluent samples were collected an influent water sample was also collected as a standard. For convenience in sampling, these two samples were collected at the same time without taking the filter retention time into account. This was justified by observations in MAFF-funded work indicating that the retention time would range between <1 and 3 hours, over which time the quality of a large supernatant water volume would not drastically alter.

Analysis of water samples

On arrival in the laboratory, water samples were measured for pH and EC and divided into three portions: 10 ml for direct and dilution plating, 750 ml for plating, following concentration by membrane filtration, and the remainder (usually 240 ml or more) for bait assay.

For direct plating, 0.1 aliquots were pipetted onto 9 cm plates of potato dextrose agar (PDA) and King's B medium (King, Ward & Raney, 1954) and spread over the agar surface using a glass spreader. Identical aliquots of 1 in 10 and 1 in 100 dilutions in sterile distilled water (SDW) were similarly plated onto PDA and King's B. These plates were incubated at 20°C for 36 h, after which, the numbers of bacterial colony forming units (cfu) plus fungi (if present) were counted on PDA and the fluorescent pseudomonad cfu were counted on King's B under a UV lamp.

Samples for membrane filtration were passed under vacuum through 47 mm diameter, 3.0 µm cellulose nitrate membrane filters housed in autoclaved Nalgene reusable membrane filtration funnels. Membrane filters were cut into approximately 1 cm squares and placed in sterile glass universal bottles containing 5 ml of a re-suspension medium (0.1% w/v aqueous agar) and shaken for 5 minutes at 500 rpm on a flask shaker (Stuart). Aliquots (0.5 ml) of the resulting suspensions were plated out on PDA, *Fusarium*-'selective' agar (Pettitt, Parry & Polley, 1993) and on Phycomycete-selective agar (modified BNPRA - Pettitt & Pegg, 1991). All plates were incubated at 20°C for 48 h and counts (cfu l^{-1}) were made of total fungus, *Fusarium* spp., *Trichoderma* spp., total Phycomycete, *Pythium* spp., and *Phytophthora* spp.

Bait tests were carried out with surface sterilised *Rhododendron* leaf disks using the method described by Pettitt *et al.* (1998). The water samples were retained in their original sample bottles, to which 10 leaf disks were added. After 24 h incubation at 20°C, leaf disks were

collected in sterile sieves, blotted dry, on autoclaved tissue paper, and plated onto BNPRA. After a further 36 h incubation, the percentage of baits infected was determined.

Programme of sampling

Regular samples of filtered and 'raw' water were taken over the period of investigation at approximately one-month intervals. In addition to this 'routine' monitoring, more frequent sampling was carried out during the initial priming period of the newly constructed SSF, during the re-priming of the older filter after the '96/'97 winter shut-down period and during re-priming of both SSFs following routine filter cleaning operations. The precise timing of sampling varied according to the comvenience of the nurseries concerned, but the aim was to collect samples of 'raw' and filtered water approximately 1 h, 3-5 h, 24 h, 2-4 days and 7-10days after start/re-start of the SSF.

Sand samples

Before the start of filtration a sample of 1 kg of sand was collected from each of the two SSF studied. Sieve analysis was carried out on these samples in order to determine their grain size distributions. In research carried out in the water industry two parameters of sand grain size are used to judge whether a sand is suitable for SSF use and these were determined for the sands in the present study using sieve analysis. The effective size (ES) of a sand is the sieve mesh diameter through which 10% by weight of the sand will pass. Reasonably uniform sand is required for SSF and a good measure of this is obtained by using the second parameter, the uniformity coefficient (UC). The UC is calculated by dividing the sieve mesh diameter through which 60% by weight of the sand passes by the ES of the sand. For drinking water production using SSF, recommendations for ES vary between 0.15 and 0.40 mm (Ellis, 1986) and the UC should always be below 3.0 and preferably be less than 2.0 (VanDijk & Oomen, 1978).

Samples of the top layer of primed sand were also collected when filter cleaning/scraping operations were under way. These samples consisted of approximately 50 g of wet sand from the upper 'clogged' layer and were taken for immediate microbiological assessment. The microbiological assessment was a simple extraction-dilution plating procedure. Sand samples (1 g wet weight) were suspended in 20 ml of a sterile 0.01% (w/v) agar suspension medium and shaken vigorously for 5 minutes. Alliquots of the resultant suspension were taken through a dilution series in sterile distilled water and cfu counts were carried out on spreader plates on PDA, BNPRA and King's B at the most appropriate dilutions. Counts were made of Phycomycetes, total filamentous fungi, *Trichoderma* spp., total bacteria and fluorescent pseudomonads. Pre-weighed sub-samples of the sands were dried and reweighed to determine their moisture content and the number of propagules of each group of organisms was expressed as cfu per gram dry weight of sand.

Results

Routine water sampling

The results of the regular sampling of filtered and raw water from the two SSF at Nurseries 1 and 2 during the seasons of 1997-98 are presented in Tables 1 and 2. In addition, the small number of observations made in 1996, at the start of SSF operation at Nursery 1, are included in Table 1. From these data it can be clearly seen that once primed, both SSF were 100% effective against Phycomycete propagules. The predominant groups of Phycomycetes detected in samples were *Saprolegnia* spp. and *Pythium* spp.. Pathogenic *Phytophthora* species (*P. cactorum, P. cryptogea* and *P.cinnamomi*) were also occasionally detected in 'raw' water samples, although these propagules were normally present in comparatively small numbers (the largest concentration was 52 cfu* l^{-1} of *P. cryptogea*, detected in raw water from Nursery 2 on 19/11/97).

Other groups of fungi assessed in detail were *Fusarium* spp. and *Trichoderma* spp. (Tables 1 and 2). The main *Fusarium* species isolated were *F. oxysporum* and *F. avenaceum*. Once fully primed, both the SSF successfully removed all *Fusarium* propagules. The only observed incidence of these passing through a SSF were at Nursery 1 on 10.6.98, when the filter was not functioning properly due to blockage, and a small number (3 cfu l^{-1}) of *F. oxysprum* propagules (probably microconidia) were detected in the SSF effluent.

Trichoderma spp. were commonly detected in raw water samples. These fungi can cause blockage problems in irrigation lines, particularly in drip irrigation nozzles. An interesting difference was observed in the activity of the two SSF in removing *Trichoderma* propagules. This appeared to be related the numbers of propagules in the raw water entering the SSF. With comparatively small numbers of propagules (for details of propagule numbers see Appendix), the filter at Nursery 1 gave a variable performance, with 100% removal on only 6 sample dates and a removal rate of just 44% on one sample date. However, in the presence of consistently greater numbers of cfu, the filter at Nursery 2 was consistently 100% effective at removing *Trichoderma* propagules. One reason for the apparently variable performance of the SSF at Nursery 1 may be the disruption to filters caused by cleaning operations. The numbers of *Trichoderma* propagules detected in SSF effluent were strongly influenced by filter scraping operations (Table 3) and appeared to be a good indicator of filter recovery or 'repriming' following a clean-up operation. *Trichoderma* spp. appear to be one of the colonisers of SSF and are regularly isolated from primed SSF sand at virtually all depths in the sand profile down to at least 35 cm, but are especially common in the top 5 cm and in the so-called Schmutzdecke layer. Cleaning the top layer of sand appears to briefly disrupt the stability of the filter causing large numbers of propagules to pass from the upper layers straight through the entire profile. This also appears to be the case with bacterial populations, where the numbers of cfu l^{-1} in the SSF effluent after cleaning are far greater than in the raw water entering the filter.

*cfu = colony forming units

Total counts of bacteria growing on PDA were very variable and the conditions under which samples were taken and the time taken for them to be delivered to the laboratory for analysis mean that these results need to be treated with caution. During transportation to the laboratory the populations of bacteria in water samples could greatly multiply and change, profoundly affecting the determinations of SSF efficacy. However, the fully primed SSF on both nurseries assessed did remove large numbers of bacteria from the raw water and were especially effective at removing fluorescent pseudomonads (Tables 1 and 2).

Of all the groups of organisms assessed in this study, yeasts appeared the least affected by SSF. Although, generally removed in large numbers by the fully-primed SSF, yeast populations in the effluent water fluctuated greatly (Tables 1 and 2) especially in the SSF at Nursery 1 (Table 1), indicating that SSF efficacy was not consistent. However, similar caution to that expressed for bacterial populations is required for the interpretation of data presented here for yeasts, and it is possible that occasionally, in the absence of competitors (removed by SSF!), large populations of yeasts could develop in effluent water samples during transit from the sample site to the laboratory.

The temperature was not routinely monitored during this study, but in the case of winter samples collected at Nursery 2 on 9/2/98, the temperature of the water entering the filter was 6°C and this low temperature did not have any adverse effect on overall SSF efficacy (Table 2).

Visual assessments of water quality were found to be fairly meaningless and are not reported in detail here. Raw water samples were invariably turbid, whilst SSF effluent samples were clear (see Appendix Plate I), although, if a SSF effluent sample was found to be turbid this would be a good indication of problems with the SSF activity and would warrant immediate, more detailed investigation.

Filter 'priming', cleaning and restarting

Results of samples collected from the newly installed SSF at Nursery 2 over the first 24h of operation are presented in Table 4a. Results of samples collected over the rest of the priming period for this filter are presented at the top of Table 2. This SSF took approximately 20 days to become fully primed. The results in Table 4a indicate that at the start of filtration virtually all Phycomycete propagules, and the majority of the propagules of the other organisms studied, were able to pass straight through the un-primed filter. After 22 h the filter was beginning to remove propagules (Table 4a), and by 6 days this was greatly increased with the infection of baits in effluent water down to zero and the percentage removal of fluorescent pseudomonads up to 59% (Table 2). This steady improvement in filter efficacy continued, until by 20 days removal of Phycomycetes was 100%, of filamentous fungi was 99.5%, of *Trichoderma* spp., *Fusarium* spp. and fluorescent pseudomonads was 100% and of total bacteria on PDA was 77% (Table 2). Only efficacy against yeasts remained low at 10%, although in the previous sample collected less

than 24h before, yeast removal was at 78%, again illustrating the great variability of efficacy against these fungi mentioned above.

Once fully primed, the time taken for the two SSF studied to become re-primed after shutting down was comparatively short. In the case of short-term shut-downs for cleaning, a re-priming period of 24 hours was sufficient to restore SSF activity against fungal pathogens, and efficacy against Phycomycetes in particular, was restored after less than 8.5 h (Table 3). After longer periods of shut-down the re-priming period was longer, at between 10 and 16 days (Table 1). This was still a shorter time than the initial priming period and there was some evidence of reduced filter activity over the first hours of filter operation (Table 4b).

Filter cleaning becomes necessary when the flow of water through the SSF is reduced by clogging of the sand surface with fine particles of silt, detritus and algae. The speed with which this clogging happens is dependent on the quality of the raw water being filtered, and the times between filter cleanups can be greatly increased by the use of coarse pre-filtration techniques. The two filters monitored in this study were installed without any form of pre-filtration and consequently became clogged at fairly frequent intervals of between two and four weeks. Clogging was especially a risk after heavy rainfall, when the raw water contained large amounts of suspended silt and peat fines, and the intervals between cleanups could be greatly increased by switching the filter out of production and into recirculation mode (Figure 1) during such weather. Filter cleanups were relatively straightforward but were disruptive, with the filter being out of production for about 1.5 days. After the raw water was drained down to below the sand surface, the top 1 - 3 cm of clogged sand was removed by shovel (see Appendix Plates E & F) and the cleaned surface was smoothed down with a rake. This was a relatively simple process and took three people about 1 h on a SSF with a surface area of 110 m^2. Once scraping was completed, the filter was recharged with clean water from below to give a water depth of about 5 10 cm above the sand. This allowed the filter surface to settle, prevented scouring of the filter surface by the jets of water from the raw water inlet and prevented the formation of air pockets in the filter profile (see Appendix Plates G & H). Once the water level reached 5 -10 cm above the sand surface, raw water was applied to the filter and the filter was run to waste util re-primed (about 24 h, see above).

With regular cleanup/scrapings over two seasons, the depth of the sand in the SSF at Nursery 1 was reduced from a depth of approximately 100 to 40 cm which was found in the MAFF work at HRI Efford to be the minimum depth for 'safe' and consistent SSF operation against plant pathogens. Fresh sand was applied to this filter on 23/1/98 using the trenching technique described by Visscher, Paramasivam, Raman & Heijnen (1987). This technique is rather like 'double digging'. A trench approximately two spits wide is dug to the bottom of the filter sand layer at one end of the filter and the sand from this trench is stored on top of the sand at the other end of the filter. Fresh sand is placed in the trench to the required top-up depth. The trench in the old sand is then extended a further two spits along the filter and the sand taken from this excavation is placed on top of the new sand in the first trench. This process continues right

across the filter surface until a layer of fresh sand is in place with the old sand on top of it. The filter is then recharged from below and re-primed as described above. The re-priming in this case was very rapid as evidenced by the water samples collected on 26/1/98 (Table 1).

Sand samples

Both Nurseries 1 and 2 used sands with the simple classification of grain size between 0.2 and 2 mm diameter. The results of sieve analysis of the two sands (Figure 1) provided similar data for estimated size (ES) and uniformity coefficient (UC). These were ES = 0.26 mm and UC = 2.33 for the SSF sand used at Nursery 1 and ES = 0.20 mm and UC = 2.25 for that used at Nursery 2.

The results of microbiological assessments carried out on sand samples collected during the cleaning operations at Nursery 1 on 23/4/97, 26/6/97 and 30/7/97 and from Nursery 2 on 4/8/97 are presented in Table 5. The results of these preliminary investigations were inconclusive. The majority of Phycomycetes isolated were unidentified members of the Saprolegniacae. *Trichoderma* spp. were isolated in relatively similar numbers from all four samples and are very frequently present in sand samples taken from throughout mature sand filter profiles. The total numbers of bacteria were slightly less than anticipated at between 2.25×10^6 and 3.71×10^7 cfu g^{-1}, although the proportions of total bacteria to fluorescent pseudomonads were similar to those detected in raw water samples. A small number of isolates of fungi and bacteria collected from these samples were kept for further study in the MAFF-funded project on the biology of SSF activity.

Table 1: **Results of 'routine' microbiological assessments of efficacy of the SSF based at Nursery 1 during 1996, 1997 and 1998**

Date	t (Days)	% Removal by SSF							
		Phycomycetes		Filamentous Fungi (*cfu)	*Trichoderma* spp. (*cfu)	*Fusarium* spp. (*cfu)	Bacteria on PDA (*cfu)	Fluorescent Pseudomonads (*cfu)	Yeasts (*cfu)
		Baits	*cfu						
6.6.96	0	-	-	-	-	-	-	-	-
19.6.96	13	80	0[a]	-	-	-	99.6	-	-
25.6.96	19	100	100	-	-	-	99	-	-
10.7.96	34	100	100	-	-	-	99.8	-	-
9.3.97	0	27	27	16	54	100	26	40	28
19.3.97	10	98	98	98	94	100	97	90	0[a]
25.3.97	16	100	100	92	100[b]	100[b]	71	97	0[a]
2.4.97	24	100	100	99.8	100	100	92	99	0[a]
24.4.97	46	100	100	92	100[b]	100[b]	74	99.2	80
21.6.97	104	100	100	85	100	100	60	83	74
26.6.97	109	100	100	98	100	100	77	88	22
30.7.97	143	100	100	99.6	44	100	61	0	12
3.9.97	178	100	100	95	100[b]	100	90	98	60
23.10.97	228	100	100	74	100	100	54	95	57
26.1.98	323	100	100	69	100	100	50	61	73
4.6.98	452	100	100	97	100	100	94	98	85
10.6.98	458	100	100	52	85	95	42	35	61

[a] Filtered sample contained more *cfu than raw water sample

[b] No *cfu in raw water for these samples

- For raw data see Appendix Table 1 and 2

*cfu = colony forming units

Table 2: **Results of 'routine' microbiological assessments of the efficacy of the SSF based at Nursery 2 during 1997 and 1998**

Date	Time from filter start-up (Days)	% Removal by SSF: Phycomycetes Baits	Phycomycetes *cfu*	Filamentous Fungi (*cfu)	*Trichoderma* spp. (*cfu)	*Fusarium* spp. (*cfu)	Bacteria on PDA (*cfu)	Fluorescent Pseudomonads (*cfu)	Yeasts (*cfu)
16.7.97	0	0[a]	15	81	46	100	15	13	17
17.7.97	1	0[a]	68	88	34	100	0[a]	0[a]	17
22.7.97	6	100	63	73	62	100	59	82	0
28.7.97	12	90	89	38	27	100	3	92	20
4.8.97	19	100	91	64	100	73	50	87	78
5.8.97	20	100	100	99.5	100	100	77	100	10
14.8.97	29	100	96	58	100	100	27	92	10
1.9.97	47	100	100	79.5	100	100	49	99	49
30.9.97	76	100	100	61	100	100	82	100	64
19.11.97	126	100	100	98	100[b]	100	86	81	56
14.1.98	182	100	100	71	100	100[b]	52	98	87
9.2.98	208	100	100	80	100	100	92	100	93
23.3.98	250	100	100	93	100	100	47	96	90
11.5.98	299	100	100	81	100	100	63	99.3	85
22.6.98	341	100	100	85	100	100	64	99	76
6.7.98	355	100	100	81	100[b]	100	85	99.9	72
21.9.98	432	100	100	10[c]	100	100	69	95	59
17.10.98	458	100	100	92	100	100	78	65	20

[a] Filtered sample contained more *cfu than raw water sample

[b] No *cfu in raw water for these samples

[c] Filtered sample was contaminated with large numbers of *Penicillium* spp. Spores

- For raw data see Appendix Tables 3 and 4

*cfu = colony forming units

Table 3: **Recovery of SSF activity after clean up operations (scraping) on Nurseries 1 and 2**

Date of cleaning operation	Nursery	Time after Restart (h)	Phycomycetes		% Removal by SSF		
			Baits	*cfu	Filamentous fungi (*cfu)	*Trichoderma* spp. (*cfu)	Bacteria on PDA (*cfu)
23.4.97	1	0.5	100	100	0[a]	0[a]	0[a]
		1	100	100	0[a]	23.7	0[a]
		2	100	100	60.4	59.2	0[a]
		5	100	100	77.8	75.0	0.2
		24	100	100	95.0	100	95.5
26.6.97	1	0.33	80	89.2	0[a]	0[a]	0[a]
		1	90	95.1	63.1	0[a]	0[a]
		8.5	100	100	84.0	67.1	77.4
		24	100	100	95.7	100	42.7
		36	100	100	94.4	100	91.9
30.7.97	1	0.5	100	100	70.6	7.7	0[a]
		1	0	97.8	40.4	38.5	58.7
		24	100	100	61.5	100	93.8
		72	100	100	73.4	100	50.0
4.8.97	2	1	100	92.5	68.3	100	0[a]
		17.5	100	100	99.5	100	0[a]
		41.5	100	100	97.1	100	17.8

[a] More *cfu in SSF effluent than in the raw water
- For raw date see Appendix tables 5 and 6

*cfu = colony forming units

Table 4a: **Results of microbiological assessment of new SSF start-up at Nursery 2 Start Date 16.7.97**

Time (h)	% Removal Phycomycetes Baits	Phycomycetes *cfu	Filamentous Fungi (*cfu)	*Trichoderma* spp. (*cfu)	Bacteria on PDA (*cfu)
1	0	93	38	100	0[b]
2	0	80	84	0[b]	0[b]
5[a]	0[b]	15	81	41	15
22[a]	0[b]	68	88	34	0[b]

Table 4b: **Results of microbiological assessment of re-start of SSF at Nursery 1 following a winter shut-down of approx. 4 months. Restart date 9.3.97**

Time (h)	% Removal Phycomycetes Baits	Phycomycetes *cfu	Filamentous Fungi (*cfu)	*Trichoderma* spp. (*cfu)	Bacteria on PDA (*cfu)
2	10	32	27	71	0[b]
4[c]	5	27	16	54	26
24	25	47	81	64[b]	53
240[c]	72[b]	98	98	94	97
384[c]	100[b]	100	92	100[d]	71

[a] Data also presented in Table x
[b] More *cfu detected in SSF effluent than in raw water sample
*cfu = colony forming units
[c] Data also presented in Table y
[d] No *cfu in the raw water sample
- For raw data see Appendix Table 7

Figure 2 Grain size distributions for sands in SSF at nurseries 1 and 2

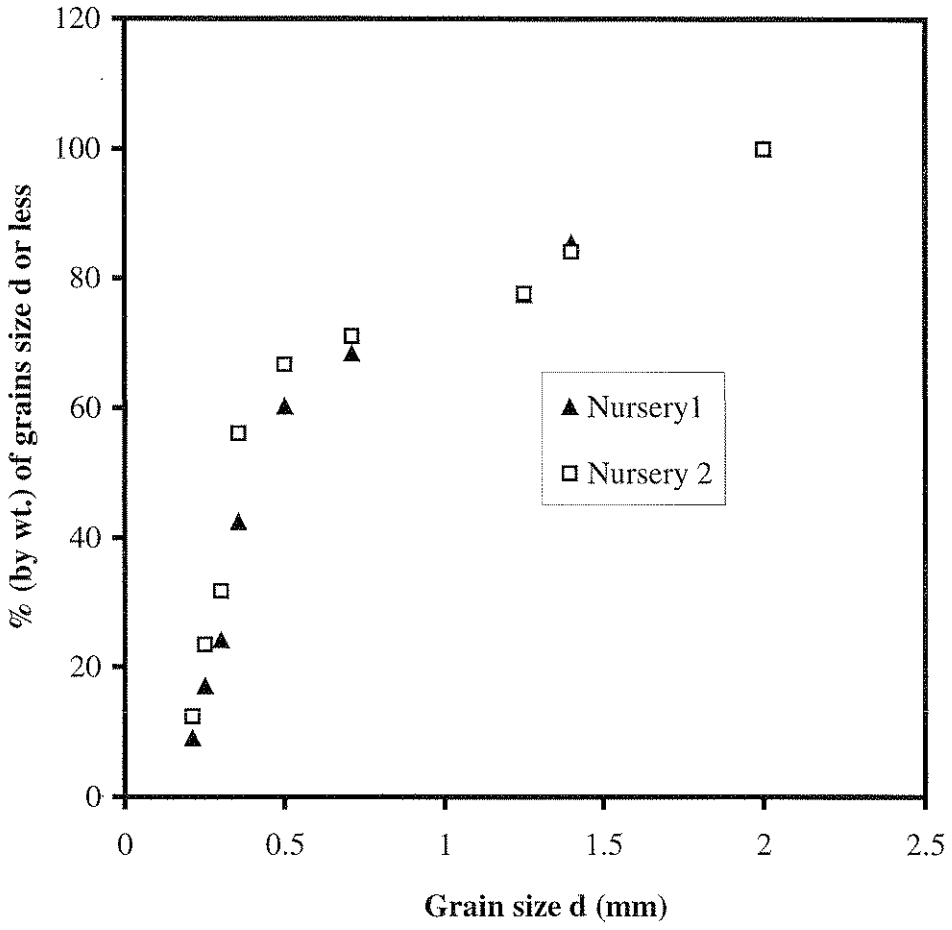


Table 5 **Preliminary microbiological assessment of colonisation of filter sand samples collected during clean-up operations**

Sample Date	Nursery	*cfu/g dry weight of sand				
		Phycomycetes	Filamentous Fungi	*Tichoderma* spp.	Total bacteria	Fluorescent Pseudomonads
23.4.97	1	50	2.15×10^4	490	2.25×10^6	1.80×10^4
26.6.97	1	95	1.60×10^4	400	-	-
30.7.97	1	80	$1.1.0 \times 10^4$	400	3.71×10^7	3.40×10^4
4.8.97	2	40	3.80×10^4	300	1.37×10^7	2.20×10^6

Discussion and Conclusions

The installation and operation of both the SSF units monitored in this project has been considered a commercial success by the two nurseries concerned. Both of the SSF were successfully primed, have maintained 100% efficacy against Phycomycete propagules, and have been running continuously since early 1997, only needing regular routine shut-downs for cleanups and one re-sanding at Nursery 1. The frequency of cleanups was undoubtedly far greater than might be necessary in this initial phase of SSF use due to the lack of pre-filtration. However, a substantial risk was involved in setting these two filters up relative to the unknown factors involved in scaling the process up from small experimental prototypes, and at the time of construction the additional cost of pre-filtration was felt to be unjustified. However, there is the possibility that the poorer quality of the raw water resulted in faster priming times, although this has not been confirmed experimentally.

Using the combination of baiting and plate tests for fungal and bacterial cfu to assess raw and filtered water, the priming of the SSF was readily monitored. The known efficacy of these filters against Phycomycetes under experimental conditions (Friedel, Wohanka & Molitor, 1991; Behrens, Kemp & Wohanka, 1992; Wohanka, 1992, VanKuik, 1994; Runia, 1995; Pettitt, 1996) was confirmed and shown to be consistent through the season on a commercial scale in this study. In addition, the two SSF studied were consistently effective against cfu of *Trichoderma* spp. and *Fusarium* spp.. In work carried out at Geisenheim in Germany, the efficacy of glasshouse-based SSF against *Fusarium oxysporum* removal was consistently between 99.9 and 100% (Wohanka, 1992), although when spores did get through the filter they were applied at artificially high concentrations. The presence of *Trichoderma* spp. propagules in SSF sand virtually throughout the filter profile has not been reported before and neither have detailed records of removal of propagules of this genus by SSF. Although *Trichoderma* spp. are not plant pathogens and may even have a certain amount of biocontrol ability, they can cause problems in irrigation systems by blocking irrigation nozzles and clogging pump filters, and their removal by SSF is desirable. The presence of easily identifiable colonies (to genus at least!) of *Trichoderma* spp. in plates from SSF filtrate samples also provides a useful indicator of potential filter failure in laboratory tests.

Consistent SSF efficacy was observed throughout the winter of 1997-98 on both nurseries and temperature records taken at Nursery 2 confirmed that good activity was obtained at water temperatures as low as 6°C. This result is in agreement with the findings of MAFF-funded work with experimental-scale SSF rigs where the water temperature has been taken down to 4°C without any deleterious effects on efficacy. These results contradict anecdotal evidence from Holland linking SSF failures against *Phytophthora cinnamomi* with operation at temperatures lower than 15°C. A threshold temperature for SSF efficacy as high as 15°C, however, is unlikely as evidenced by the year-round operation of large outdoor SSF for drinking water production by companies such as Thames Water (over 70% of London's water supply is treated by SSF). The

main concern in these filters is the formation of ice which can starve the aerobic bacteria, vital to SSF activity, of oxygen.

In conclusion, the positive results from this study have allowed the formulation of a series of guidelines on filter construction/management for HNS growers considering SSF as a method for removing pathogens from their irrigation water. These are set out in the beginning of this report under the Practical Section For Growers. However, it must be stressed that SSF are a form of biofilter and that biofiltration technology is still in the early stages of its development for the treatment of irrigation water. SSF can still be further improved, and more work is required to investigate the suitability of a much wider range of filter media and operating conditions. Particular areas worth further investigation include:

- The impact of prefiltration techniques
- The use of fabric covers to extend the period between cleanups and reduce the time taken and sand loss during cleaning.
- The development of an *in situ* filter cleaning apparatus similar to that designed by Burman & Lewin (1961).
- The efficacy of different filter media: coarser materials in deeper filters may allow faster flow rates and be less prone to clogging.
- The use of filter amendments such as zeolite clays which can remove fertilizers and also change the balance of surface algal populations, creating a similar physical effect to a fabric cover on the filter (McNair, Sims, Sorensen & Hulbert, 1987), and granular activated carbon (GAC), which can remove pesticide contaminants from the water being treated (Bauer, Colbourne, Foster, Goodman & Rachwal, 1996).

Work on this project will continue under HNS 88a, looking into the question of suitability of the wide range of locally available sands for SSF and developing a pilot test rig which will allow growers to assess the suitability of the technique on test sites in a cost-effective manner.

References

Bauer, M.J., Colbourne, J.S., Foster, D.M., Goodman, N.V. & Rachwal, A.J. 1996. GAC Enhanced slow sand filtration (GAC Sandwich ™).

Behrens, V., Kemp, J. & Wohanka, W. 1992. Wasser wiederverwenden trotz Pilz-Gefahr. *Deutsche Baumschule* **7**, 310-312.

Bewley, W.F. & Buddin, W. 1921. On the fungus flora of glasshouse water supplies in relation to plant disease. *Annals of Applied Biology* **8**, 10-19.

Braune, L. 1987. Ausbreitung von *Phytophthora cinnamomi* an *Erica gracilis*: Wiederverwendung van Drainwasser begünstigt den Pilz. *Zierpflanzenbau* **9**, 350-352.

Burman, N.P. & Lewin, J. 1961. Microbiological and operational investigation of relative effects of skimming and *in situ* sand washing on two experimental slow sand filters. *Journal of the Institute of Water Engineers* **15**, 355-367.

Ellis, K.V. 1986. Slow Sand Filtration. *CRC Critical Reviews in Environmental Control* **15**, 315-354.

Friedel, S., Wohanka, W. & Molitor, H.D. 1991. *Erica*: *Phytophthora* in Fliessrinnen bekämpfen. *Gärtnerbörse und Gartenwelt* **91**, 69-72.

Kemp, J., Behrens, V. & Wohanka, W. 1992. Lansamsandfilter verhinderten Ausbreitung von Phytophthora: Kultur von Chamaecyparis im geschlossenen Bewässerungssystem. *Gartenbau Magazin* **5**, 58-60.

King, E.O., Ward, M.K. & Raney, D.E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. *Journal of Laboratory and Clinical Medicine* **44**, 301-307.

McNair, D.R., Sims, R.C., Sorensen, D.L. & Hulbert, M. 1987. Schmutzdecke characterization of clinoptilolite-amended slow sand filtration. *Journal of American Water Works Association* **79**, 74-81.

Neher, D. & Duniway, J.M. 1992. Dispersal of *Phytophthora parasitica* in tomatofields by furrow irrigation. Plant Disease **76**, 582-586.

Pettitt, T.R. 1996. Clean water, the slow sand way. *Grower*, 26 September, pp. 18-20.

Pettitt, T.R., Parry, D.W. & Polley, R.W. 1993. Improved estimation of the incidence of *Microdochium nivale* in winter wheat stems in England and Wales during 1992, by use of benomyl agar. *Mycological Research* **97**, 1172-1174.

Pettitt, T.R. & Pegg, G.F. 1991. The quantitative estimation of *Phytophthora cactorum* in infected strawberry tissue. *Mycological Research* **95**, 233-238.

Pettitt, T.R., Finlay, A.R., Scott, M.A. & Davies, E.M. 1998. Development of a system simulating commercial production conditions for assessing the potential spread of *Phytophthora cryptogea* root rot of hardy nursery stock in recirculating irrigation water. *Annals of Applied Biology* **132**, 61-75.

Runia, W.T. 1995. A review of the possibilities for disinfection of recirculation water for soilless culture. *Acta Horticulturae* **382**, 221-229.

Shokes, F.M. & McCarter, S.M. 1979. Occurrence, dissemination, and survival of plant pathogens in surface irrigation ponds in South Georgia. *Phytopathology* **69**, 510-516.

Sinclaire, J. 1808. *The code of health and longevity.* Edinburgh: Constable & Co.

VanDijk, J.C. & Oomen, J.H.C.M. 1978. *Slow sand filtration for community water supply in developing countries: A design and construction manual.* The Hague, The Netherlands: WHO International Reference Centre for Community Water Supply.

VanKuik, A.J. 1992. Spread of *Phytophthora cinnamomi* Rands in a recycling system. *Mededelingen van der Faculteit Landbouwwetenschappen Universiteit Gent* **57/2a**, 139-143.

VanKuik, A.J. 1994. Eliminating *Phytophthora cinnamomi* in a recirculated irrigation system by slow sand filtration. *Mededelingen van der Faculteit Landbouwwetenschappen Universiteit Gent* **59/3a**, 1407-1415.

Visscher, J.T., Paramasivam, R., Raman, A, Heijnen, H.A. 1987. *Slow sand filtration for community water supply. Planning, design, construction and maintenance. Technical paper No. 24.* The Hague, The Netherlands: International Reference Centre for Community Water Supply and Sanitation.

Wakeham, A.J., Pettitt, T.R. & White, J.G. 1997. A novel method for detection of viable zoospores of *Pythium* in irrigation water. *Annals of Applied Biology* **131**, 427-435.

Wohanka, W. 1988. *TASPO-magazin*, July/August, pp. 7-8.

Wohanka, W. 1992. Slow sand filtration and UV radiation; low cost techniques for disinfection of recirculating nutrient solution or surface water. *Proceedings of the 8th International Congress on Soilless Culture, 1992.* pp. 497-511.

APPENDIX

APPENDIX

Table 1: **Raw data for text Table 1**

Date	t (Days)	Phycomycetes % Bait Infected		Phycomycetes *cfu l^{-1}		Filamentous fungi (*cfu l^{-1})		*Trichoderma* spp. (*cfu l^{-1})	
		Raw	Filtered	Raw	Filtered	Raw	Filtered	Raw	Filtered
6.6.96	0	-	-	-	-	-	-	-	-
19.6.96	13	83	17	0	6	-	-	-	-
25.6.96	19	100	0	38	0	-	-	-	-
10.7.96	34	100	0	36	0	-	-	-	-
9.3.97	0	100	95	62	45	134	113	28	13
19.3.97	10	90	25	222	4	$1.34x10^4$	233	33	2
25.3.97	16	100	0	551	0	600	47	0	0
2.4.97	24	100	0	364	0	$8.64x10^3$	13	10	0
24.4.97	46	40	0	22	0	$3.26x10^3$	269	0	0
21.6.97	104	100	0	225	0	$1.14x10^3$	172	12	0
26.6.97	109	100	0	358	0	$3.63x10^3$	74	124	0
30.7.97	143	100	0	80	0	$1.50x10^4$	53	72	40
3.9.97	178	60	0	156	0	442	22	0	0
23.10.97	228	40	0	12	0	771	200	35	0
26.1.98	323	10	0	7	0	107	33	5	0
4.6.98	452	40	0	11	0	$4.87x10^3$	167	40	0
10.6.98	458	80	0	71	0	$2.94x10^3$	$1.42x10^3$	47	7

*cfu = colony forming units

APPENDIX

Table 2: Raw data for text Table 1

Date	t (Days)	*Fusarium* spp. (*cfu l^{-1})		Bacteria on PDA (*cfu l^{-1})		Fluorescent Pseudomonads (*cfu l^{-1})		Yeasts (*cfu l^{-1})	
		Raw	Filtered	Raw	Filtered	Raw	Filtered	Raw	Filtered
6.6.96	0	-	-			-	-	-	-
19.6.96	13	-	-	$5.80x10^5$	$2.40x10^3$	-	-	-	-
25.6.96	19	-	-	$6.40x10^5$	$8.30x10^3$	-	-	-	-
10.7.96	34	-	-	$7.80x10^6$	$1.51x10^4$	-	-	-	-
9.3.97	0	15	0	$6.11x10^3$	$4.50x10^3$	541	324	$1.38x10^3$	$1.00x10^3$
19.3.97	10	3	0	$9.12x10^4$	$2.84x10^3$	$1.67x10^3$	170	$8.50x10^3$	$8.11x10^4$
25.3.97	16	0	0	$4.05x10^4$	$1.18x10^4$	$3.78x10^4$	$1.13x10^3$	507	608
2.4.97	24	45	0	$1.32x10^5$	$1.00x10^4$	$1.03x10^4$	103	$1.09x10^4$	$1.42x10^4$
24.4.97	46	0	0	$2.55x10^6$	$6.70x10^5$	$6.61x10^4$	529	924	184
21.6.97	104	135	0	$2.43x10^5$	$9.80x10^4$	$3.15x10^3$	523	$1.31x10^3$	335
26.6.97	109	27	0	$2.65x10^6$	$6.09x10^5$	$5.99x10^3$	719	$1.25x10^3$	978
30.7.97	143	6	0	$3.05x10^7$	$1.19x10^7$	$1.47x10^5$	$1.90x10^5$	411	360
3.9.97	178	17	0	$3.31x10^7$	$3.29x10^6$	$1.25x10^6$	$2.51x10^4$	578	230
23.10.97	228	22	0	$4.49x10^7$	$2.07x10^7$	$1.69x10^6$	$8.46x10^4$	$1.11x10^3$	477
26.1.98	323	6	0	$3.20x10^6$	$1.60x10^6$	$2.80x10^6$	$1.10x10^6$	711	190
4.6.98	452	33	0	$5.50x10^6$	$3.20x10^5$	$1.10x10^6$	$2.00x10^6$	$1.17x10^3$	174
10.6.98	458	60	3	$1.09x10^7$	$6.38x10^6$	$8.76x10^6$	$5.47x10^6$	391	152

*cfu = colony forming units

APPENDIX

Table 3: **Raw data for text Table 2**

Date	t (Days)	Phycomycetes % Baits Infected		Phycomycetes *cfu l^{-1}		Filamentous fungi (*cfu l^{-1})		*Trichoderma* spp. (*cfu l^{-1})	
		Raw	Filtered	Raw	Filtered	Raw	Filtered	Raw	Filtered
16.7.97	0	10	20	102	87	$3.24x10^3$	608	80	47
17.7.97	1	10	20	135	43	$3.28x10^3$	393	80	53
22.7.97	6	10	0	110	41	$3.82x10^3$	$1.03x10^3$	140	53
28.7.97	12	100	10	96	11	213	133	100	73
4.8.97	19	100	0	150	13	$6.36x10^3$	$2.31x10^3$	$1.98x10^3$	0
5.8.97	20	70	0	173	0	$7.30x10^3$	33	$2.03x10^3$	0
14.8.97	29	100	0	$1.45x10^3$	60	$1.61x10^3$	647	107	0
1.9.97	47	10	0	7	0	$1.62x10^3$	333	96	0
30.9.97	76	70	0	20	0	393	153	93	0
19.11.97	126	100	0	187	0	$1.13x10^4$	233	0	0
14.1.98	182	70	0	47	0	$7.30x10^3$	$2.13x10^3$	25	0
9.2.98	208	40	0	36	0	$4.05x10^3$	824	47	0
23.3.98	250	100	0	28	0	709	53	7	0
11.5.98	299	100	0	173	0	350	67	53	0
22.6.98	341	80	0	76	0	$6.08x10^3$	912	33	0
6.7.98	355	80	0	7	0	$1.12x10^3$	216	0	0
21.9.98	432	100	0	382	0	$8.11x10^3$	$7.30x10^3$	73	0
17.10.98	458	40	0	33	0	$1.50x10^4$	$1.13x10^3$	40	0

*cfu = colony forming units

APPENDIX

Table 4: **Raw data for text Table 2**

Date	t (Days)	*Fusarium* spp. (*cfu l^{-1})		Bacteria on PDA (*cfu l^{-1})		Fluorescent Pseudomonads (*cfu l^{-1})		Yeasts (*cfu l^{-1})	
		Raw	Filtered	Raw	Filtered	Raw	Filtered	Raw	Filtered
16.7.97	0	20	0	$1.31x10^7$	$1.12x10^7$	$1.50x10^3$	$1.30x10^3$	608	507
17.7.97	1	20	0	$1.40x10^7$	$1.80x10^7$	$1.33x10^3$	$2.60x10^3$	$1.22x10^3$	$1.01x10^3$
22.7.97	6	36	0	$4.5x10^7$	$1.83x10^7$	$6.10x10^6$	$1.10x10^3$	127	289
28.7.97	12	7	0	$1.95x10^7$	$1.89x10^7$	$1.20x10^5$	$1.00x10^4$	$4.05x10^3$	$3.24x10^3$
4.8.97	19	48	13	$1.83x10^8$	$9.12x10^7$	$2.60x10^6$	$3.33x10^5$	424	93
5.8.97	20	13	0	$9.88x10^7$	$2.31x10^7$	$2.00x10^5$	0	207	187
14.8.97	29	0	0	$2.66x10^4$	$1.95x10^4$	127	10	$4.05x10^3$	$3.66x10^3$
1.9.97	47	20	0	$1.04x10^8$	$5.32x10^7$	$1.04x10^8$	$7.50x10^4$	912	465
30.9.97	76	33	0	$8.36x10^6$	$1.44x10^6$	$5.40x10^5$	0	$1.23x10^3$	447
19.11.97	126	10	0	$8.61x10^7$	$1.25x10^7$	$1.25x10^7$	$2.43x10^6$	$2.01x10^3$	879
14.1.98	182	0	0	$3.10x10^6$	$1.50x10^6$	$2.00x10^6$	$4.50x10^4$	$1.36x10^3$	178
9.2.98	208	180	0	$1.43x10^7$	$1.16x10^6$	$2.73x10^6$	0	$1.74x10^4$	$01.22x10^3$
23.3.98	250	33	0	$3.00x10^7$	$1.60x10^7$	$1.50x10^7$	$6.10x10^5$	$7.73x10^3$	747
11.5.98	299	20	0	$1.55x10^7$	$5.78x10^6$	$1.47x10^7$	$1.00x10^5$	$2.79x10^3$	427
22.6.98	341	58	0	$1.99x10^7$	$7.04x10^6$	$1.59x10^7$	$2.00x10^5$	684	167
6.7.98	355	36	0	$1.82x10^8$	$2.74x10^7$	$8.21x10^7$	$5.00x10^3$	$3.24x10^3$	187
21.9.98	432	33	0	$2.13x10^7$	$6.46x10^6$	$6.99x10^6$	$3.04x10^5$	$1.26x10^3$	518
17.10.98	458	6	0	$5.02x10^7$	$1.13x10^7$	$2.15x10^6$	$7.62x10^5$	300	240

*cfu = colony forming units

APPENDIX

Table 5: Raw data for text Table 3

Date of cleaning operation	Nursery	Time after Cleaning (h)	Phycomycetes % Baits Infected		Phycomycetes *cfu l^{-1}	
			Raw	Filtered	Raw	Filtered
23.4.97	1	0.5	100	0	47	0
		1	80	0	90	0
		2	80	0	73	0
		5	100	0	153	0
		24	90	0	183	0
26.6.97	1	0.33	100	20	185	20
		1	100	10	185	9
		8.5	100	0	109	0
		24	100	0	145	0
		36	100	0	88	0
30.7.97	1	0.5	100	0	463	0
		1	100	100	463	10
		24	100	0	271	0
		72	100	0	149	0
4.8.97	2	1	70	0	173	13
		17.5	70	0	210	0
		41.5	70	0	125	0

*cfu = colony forming units

APPENDIX

Table 6: Raw data for text Table 3

Date of cleaning operation	Nursery	Time after Cleaning (h)	Filamentous fungi (*cfu l^{-1}) Raw	Filamentous fungi (*cfu l^{-1}) Filtered	*Trichoderma* Spp. (*cfu l^{-1}) Raw	*Trichoderma* Spp. (*cfu l^{-1}) Filtered	Bacteria on PDA (*cfu l^{-1}) Raw	Bacteria on PDA (*cfu l^{-1}) Filtered
23.4.97	1	0.5	680	$2.85x10^3$	507	813	$5.88x10^5$	$1.61x10^6$
		1	680	793	507	387	$5.83x10^5$	$8.13x10^5$
		2	680	269	507	207	$5.83x10^5$	$7.53x10^5$
		5	710	158	507	127	$5.83x10^5$	$5.82x10^5$
		24	$1.01x10^3$	51	442	0	$4.89x10^5$	$2.19x10^4$
26.6.97	1	0.33	$1.95x10^3$	$1.97x10^3$	48	122	$6.00x10^6$	$1.25x10^7$
		1	$1.95x10^3$	719	48	204	$6.00x10^6$	$8.98x10^6$
		8.5	$2.13x10^3$	340	76	25	$6.36x10^6$	$1.44x10^6$
		24	$1.07x10^3$	46	61	0	$8.36x10^6$	$4.79x10^6$
		36	$1.30x10^3$	73	68	0	$1.43x10^7$	$1.16x10^6$
30.7.97	1	0.5	743	233	65	60	$2.88x10^7$	$2.93x10^7$
		1	793	473	65	40	$2.88x10^7$	$1.19x10^7$
		24	698	269	71	0	$2.64x10^7$	$1.63x10^6$
		72	929	247	58	0	$1.90x10^5$	$9.50x10^4$
4.8.97	2	1	$7.30x10^3$	$2.31x10^3$	$2.03x10^3$	0	$2.31x10^7$	$9.12x10^7$
		17.5	$8.11x10^3$	41	41	0	$3.00x10^7$	$9.88x10^7$
		41.5	$8.26x10^3$	240	240	0	$2.69x10^7$	$2.21x10^7$

*cfu = colony forming units

APPENDIX

Table 7: **Raw data for Tables 4a and 4b**

Results of microbiological assessments of new SSF start-up at Nursery 2. Start Date 16.7.97

	Phycomycetes Baits *cfu l^{-1}				Filamentous Fungi (*cfu l^{-1})		*Trichoderma* spp. (*cfu l^{-1})		Bacteria on PDA (*cfu l^{-1})	
Time (h)	Raw	Filtered	Raw	Filtered	Raw	Filtered	Raw	Filtered	Raw	Filtered
1	10	10	102	7	$3.2x10^3$	$2.03x10^3$	80	0	$1.31x10^7$	$3.67x10^7$
2	10	10	102	20	$3.2x10^3$	527	80	100	$1.31x10^7$	$1.47x10^7$
5	10	20	102	87	$3.2x10^3$	608	80	47	$1.31x10^7$	$1.12x10^7$
22	10	20	102	33	$3.2x10^3$	405	80	53	$1.31x10^7$	$1.80x10^7$

Results of microbiological assessments of re-start of SSF at Nursery 1 following a winter shut-down period of approx. 4 months. Restart date 9.3.97

	Phycomycetes Baits *cfu l^{-1}				Filamentous Fungi (*cfu l^{-1})		*Trichoderma* spp. (*cfu l^{-1})		Bacteria on PDA (*cfu l^{-1})	
Time (h)	Raw	Filtered	Raw	Filtered	Raw	Filtered	Raw	Filtered	Raw	Filtered
2	100	80	62	42	134	98	28	8	$6.11x10^3$	$6.34x10^3$
4	100	95	62	45	134	113	28	13	$6.11x10^3$	$4.50x10^3$
24	80	60	58	31	512	95	45	16	$5.98x10^5$	$2.79x10^5$
240	90	25	222	4	$1.34x10^4$	233	33	2	$9.12x10^4$	$2.84x10^3$
384	100	0	551	0	600	47	0	0	$4.05x10^4$	$1.18x10^4$

*cfu = colony forming units

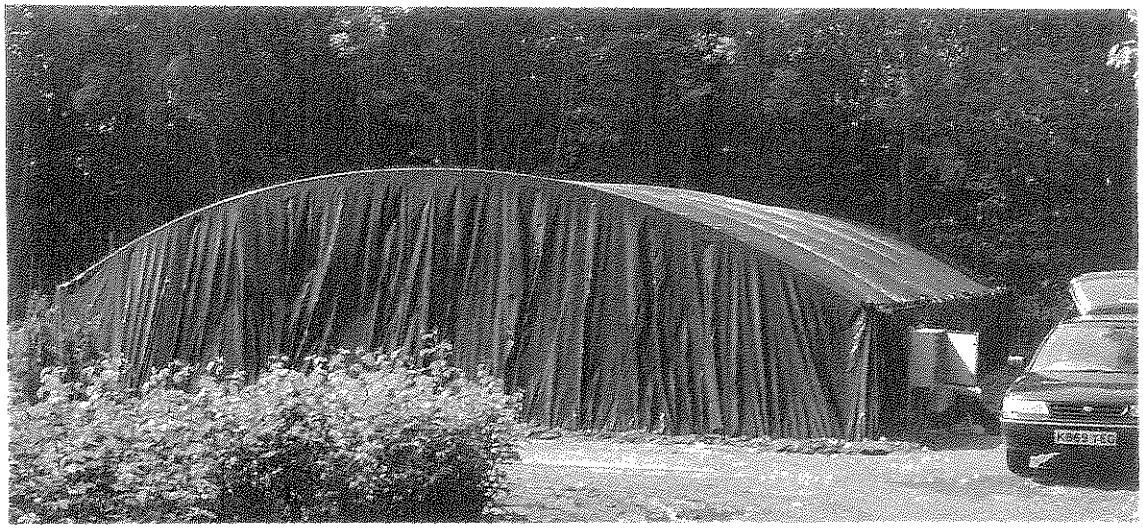


Plate A View of exterior of slow sand filter cover

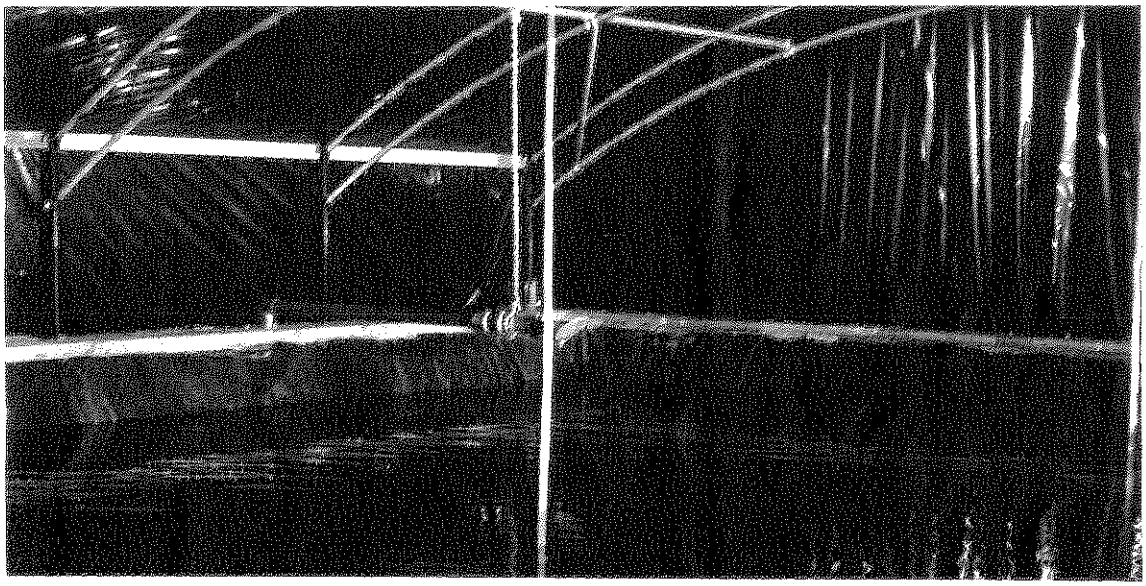


Plate B View of interior of slow sand filter cover, showing the water head level when filter is in operation

Plate C View of water collection 'sump', where surface water from the entire nursery is collected

Plate D

Water collection channel at one of the sampled nursery sites. Water drains from profiled beds into these channels which take water to a collection sump

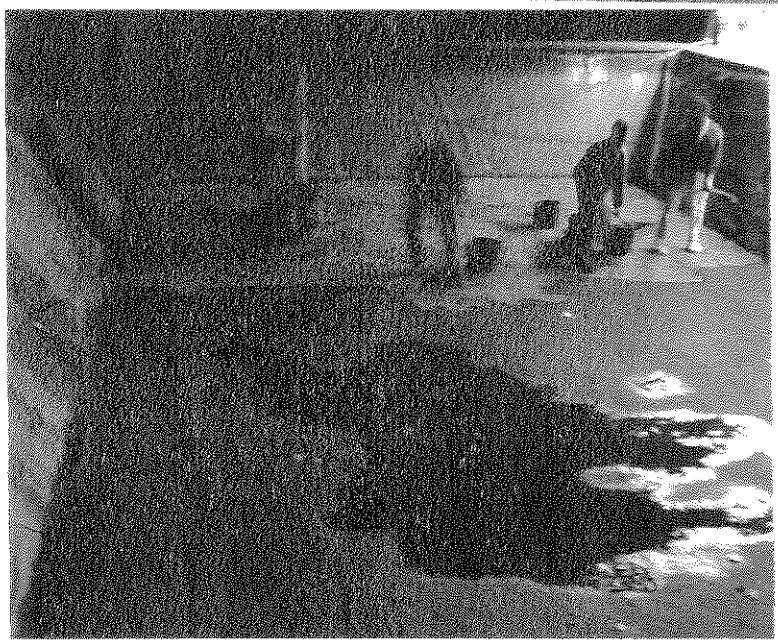


Plate E

Sand filter cleaning operation (scraping)

Plate F

View of clogged surface of a sand filter, illustrating the depth of scrape (0.5 – 1 cm) needed to successfully clean a filter

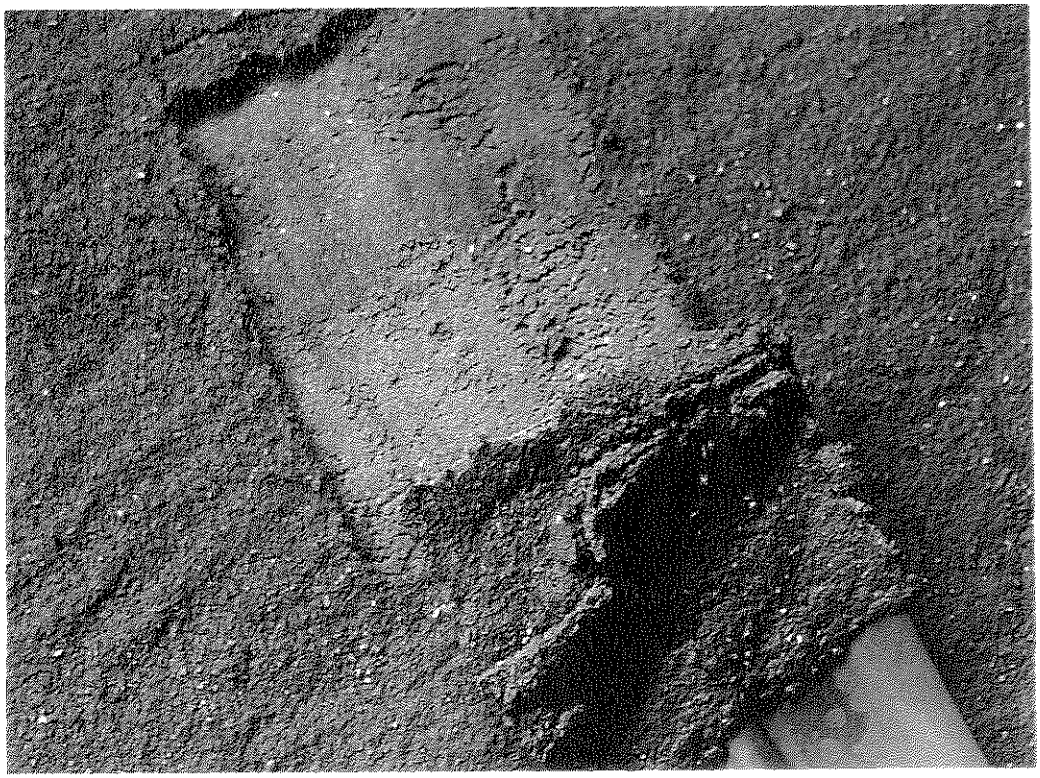


Plate G

Recharging the filter with clean water from below allows the sand to settle, avoids air pockets forming and prevents the sand surfaces from being scoured by the action of water from the 'raw' inlet.

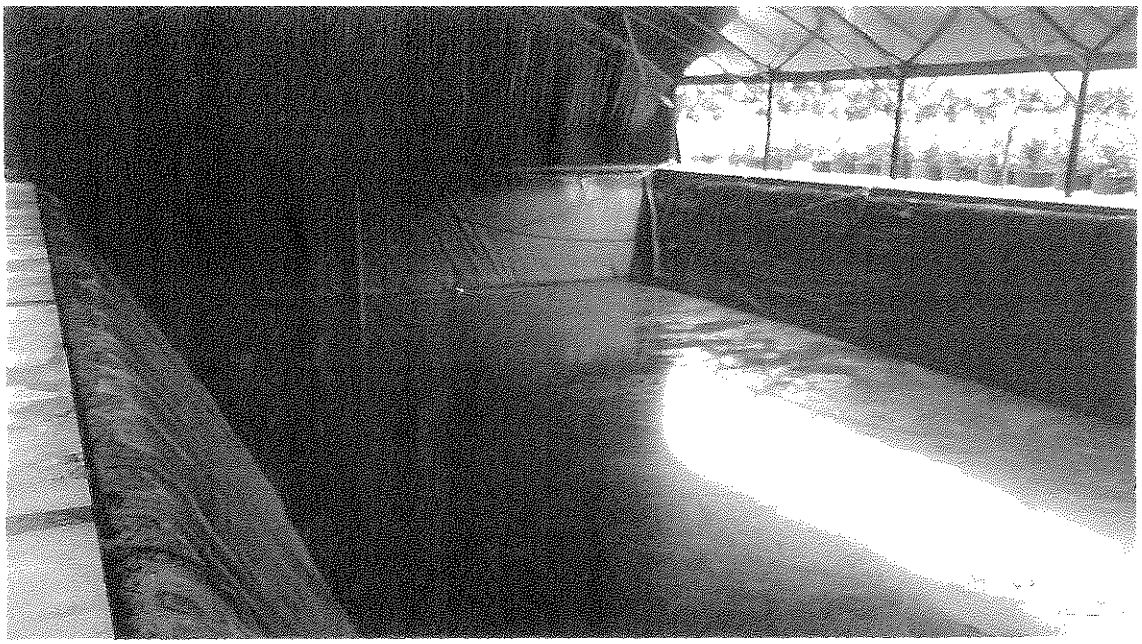


Plate H

Once water depth reaches approx. 5-10 cm, the 'raw' inlet is switched on. Applying water to the filter like this allows good aeration which is important for effective slow sand filtration.

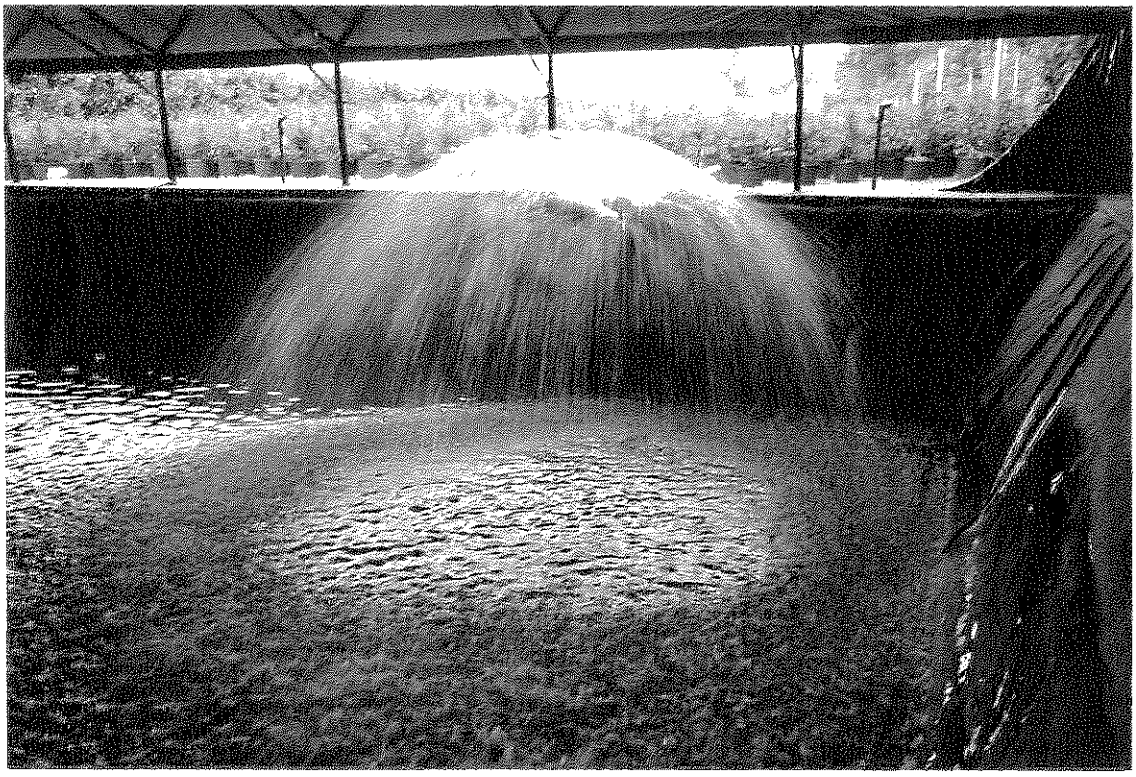
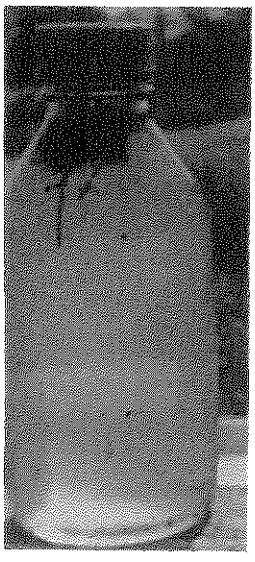


Plate I

Photographs of typical samples of slow sand filtered and 'raw' water.



'Raw' water

Filtered water