UPPSALA
UNIVERSITET

Removal of cyanobacterial toxins (LPS endotoxin and microcystin) in drinking-water using the BioSand household water filter

Hristina Bojcevska
Erik Jergil

Arbetsgruppen för Tropisk Ekologi
Committee of Tropical Ecology
Uppsala University, Sweden

Minor Field Study 91

May 2003 Uppsala

UPPSALA
UNIVERSITET

Removal of cyanobacterial toxins (LPS endotoxin and microcystin) in drinking-water using the BioSand household water filter

Hristina Bojcevska
Erik Jergil

Minor Field Study in Mozambique
September – November 2002
Supervisors: Heléne Annadotter, Gertrud Cronberg Department of Ecology/Limnology Lund University, Sweden and Aidate Mussagy Department of Biological Sciences, Eduardo Mondlane University, Maputo, Mozambique
Evolutionary Biology Centre, Uppsala University, Sweden
Supervisors: Christina Skarpe, Roger Bergström and Bo Tallmark

ABSTRACT

The efficiency of the BioSand household water filter (BSF) in removing the cyanobacterial toxins LPS endotoxin and microcystin was investigated. The study also evaluated if the removal efficiency of the toxins would be higher if the BSF was implemented with a layer of granulated active carbon (GAC) or charcoal. Three BSFs were used in the study: one ordinary, one implemented with a layer of charcoal and one implemented with a layer of GAC. To investigate if the removal efficiency varied with different genera of cyanobacteria three different raw water sources were used. The study was performed in Maputo, Mozambique. A major part of the raw water samples and all of the filtered water samples contained microcystin concentrations below the guideline level (1.0 μg/L) set by the World Health Organization. As a consequence of the low microcystin concentrations in raw water the conclusion is that more studies are necessary to confirm if the BSFs work efficiently in removing microcystin. Analyses during the first 22 days study period indicated that the concentrations of LPS endotoxin generally increased after filtration through the ordinary- and the charcoal BSFs. The BSF containing the GAC layer showed a positive mean removal rate of LPS endotoxin during this period. The most likely explanation might be that the GAC layer increased the removal efficiency. Twenty-eight days after installation the removal rates of all the BSFs had increased. Although, it is not clear if the increased removal rates are a consequence of the increased biodegradation by the biological layer or a result of the retention of cyanobacteria cells within the BSFs and thus their cell-bound toxins. The BSF containing the GAC layer and the ordinary BSF showed similar removal rates twenty-eight days after installation. This might be an indication that the biological layer probably is more important for the removal efficiency of LPS endotoxin when the BSFs have been in use for more than 3 weeks.
No connections could be found between LPS endotoxin and chlorophyll *a* respectively cyanobacterial biomass in either of the raw- or filtered water samples.

Keywords: Mozambique, cyanobacteria, LPS endotoxin, microcystin, ELISA, slow sand filtration, BioSand household water filter, cyanobacterial toxins, raw water and drinking-water.

TABLE OF CONTENTS

INTRODUCTION

Cyanobacteria are frequent components of many freshwater- and marine ecosystems. Where waters are rich in nutrients and exposed to sunlight cyanobacteria may multiply to high densities – a condition referred to as a water bloom (Bartram *et al*., 1999). Eutrofication of water supplies by urban and agricultural sources has increased in the last century resulting in more frequent and intensified cyanobacterial blooms (Keijola *et al.*, 1988). However, cyanobacterial blooms do not exclusively occur in eutrophic waters. The blooms can also appear in mesotrophic and oligotrophic waters (Annadotter and Cronberg, 1994). The developments of cyanobacteria containing toxins are common phenomenon in inland water systems worldwide and in some coastal waters. Toxins from cyanobacteria blooms have also been found in drinking-water reservoirs (Lawton *et al*. 1998). Rich cyanobacteria growth in water creates severe practical problems for water use, particularly for drinking-water treatment. There are important differences in algal and cyanobacterial growth in tropical compared to temperate areas (Bartram *et al*., 1999). In countries with year-round warm weather in combination with high nutrient leaking the growth of cyanobacteria is especially favoured (Mohamed and Carmichael, 2000). Seasonal differences in environmental factors are often not great enough to induce the replacement of cyanobacteria by other phytoplankton species in the tropics. The practical problems associated with high cyanobacterial biomass and the potential health threats from their toxins increase if cyanobacteria are present or dominant during most of the year (Bartram *et al*., 1999).

Cyanobacterial toxins

There are three different types of cyanobacterial toxins: neurotoxins (anatoxin-a, anatoxin-a (s) and saxitoxins), hepatotoxins (microcystin, nodularin and cylindrospermopsin) and lipopolysaccharide endotoxin (LPS endotoxin). Microcystins, nodularins and anatoxins are all water-soluble. Toxins can either be retained within the cyanobacterial cells or released into the water column (Hrudey *et al.,* 1999). The hepatotoxin microcystin is regarded as being the most significant potential source to human injury from cyanobacteria, being both poisonous to the liver and tumour promotor (Bartram *et al*., 1999, Lawton *et al*., 1998). Human health effects associated with ingestion of water containing toxic *Microcystis* sp. and/or other microcystin producing genera are well documented. The effects range from elevated plasma liver enzyme levels suggesting liver damage to gastroenteritis and may even sometimes be lethal (Pouria *et al*., 1998). The World Health Organization has set the guideline level of microcystin concentration in drinking-water to 1.0 _g/L (Falconer *et al.,* 1999).

Toxicity to animals from ingestion of water containing *Nodularia spumigena* is well known with the necrosis, haemorrhage and other effects in the liver being similar to those seen from microcystins. Exposure to cell material of some cyanobacterial taxon can cause illness such as fever which may be evoked by the LPS endotoxin contained in the cell wall of cyanobacteria. Outer exposure to LPS endotoxin might cause skin rashes (Fitzgerald, 2001). According to recent studies cyanobacterial LPS endotoxins may inhibit the key enzymes in the detoxification of microcystins (Rapala *et al*., 2002). No clear evidence of chronic toxicity from cyanobacterial neurotoxins have been reported. The neurotoxins´ mechanisms of blocking crucial biochemical pathways are reversible i.e. depuration of toxins occurs if acute toxicity effects are not lethal (Chorus *et al.,* 2001). There is a risk of cancer stimulation caused by long time exposure of cyanobacterial toxins. The risk of liver cancer is especially high in poor countries (Falconer, 2001).

Slow sand filtration

The toxins from cyanobacteria present a challenge to drinking-water treatment (Mohamed and Carmichael, 2000). One possible way of water treatment to remove cyanobacterial toxins is to use a slow sand filter (Hrudey *et al.,* 1999). Filtering water through sand is not an advanced technology yet it is regarded as an effective method for water purification. The upward facing surfaces of each sand grain can be seen as a microscopic sedimentation bed, helping to remove cloudiness, odour, taste and harmful organisms from the water. Water that needs filtration usually contains different kinds of organic material including living organisms. During the filtration particles in the water collide with individual sand grains. Since most of the collisions take place in the upper layer of slow sand filter the particles and organisms will accumulate in great number here. Eventually a biological layer will develop in the upper portion of the sand. The biological layer mostly consists of algae, protozoa, rotifers and bacteria living in an active food chain which breaks down the organic matter in the water. The lower portion of sand with little biological activity removes the remaining particles (Samaritan's Purse, 1998).

Most literature recommends hard, durable and angular grains free from loam, clay and organic matter for water filtrations. The grains in a slow sand filter should be fairly similar and of accurate size (∅ 0.10 – 0.90 mm). If the sand is too fine it tends to decrease the flow rate and there is a risk of clogging the sand filter. Coarse sand will on the other hand increase the flow rate and allow suspended solids and bacteria to pass through the pores between the sand grains (Hardenbergh, W.A, 1946, Samaritan's Purse, 1998).

Results from laboratory and pilot-scale experiments with sand filters made by Keijola *et al*. (1988) suggested some removal of cyanobacterial toxins from raw water mixed with freeze-dried toxic cyanobacteria. This method is, however, uncertain since various sand filters have different biological layers and their ability to remove cyanobacterial toxins may thus vary (Keijola *et al*., 1988). Experiments conducted by Grützmacher *et al.* (2002) with water, containing microcystins, on full-scale slow sand filters yielded a high elimination rate for both dissolved- and cell-bound microcystins. The study also showed that the elimination potential for microcystins with slow sand filters decreased with falling temperatures due to the lower biodegradation activity in the biological layer. A study made by Lawton *et al* (1998) showed that domestic jug water filters, based on activated carbon adsorption and ion exchange, removed cyanobacterial cells and microcystins from drinking-water. The percentage removal appeared to be closely related to colony morphology of the cyanobacteria. The filamentous forms, e.g. *Anabaena* sp., were removed to a greater extent than the colony-shaped cyanobacteria, e.g. *Microcystis* sp. Activated carbon can also be implemented in slow sand filters. This technique is called sand-GAC sandwiching (Hrudey *et al.,* 1999). Although high concentrations of organic material and toxins might decrease the toxin removal as the competition for binding on the GAC increases (Lawton *et al.,* 1998).

The BioSand household water filter (BSF) in Mozambique

The BSF was designed by Dr. David Manz at the University of Calgary, Canada. It is a slow sand filter designed to be used intermittently on a household level. A big difference compared to large-scale continuous slow sand filters is the maintenance of the filter bed. It is done by hand and the whole filter can be inspected in a few minutes. The BSF has a lid preventing algal growth and protecting the filter from its surroundings e.g. debris and insects. The unique characteristic of the BSF is its ability to sustain the biological layer between uses. While at rest the BSF, if correctly built, is designed to hold five cm of water above the top surface of the sand column. A constant aquatic environment is essential for the organisms in the biological layer. A diffuser level prevents the input water from disturbing the top layer of sand (Figure 1). The diffuser level should not rest directly on the sand since this would deplete the oxygen supply which is important for the growth and efficiency of the biological layer. It can take up to three weeks for the BSF to reach an optimum removal rate of bacteria, viruses and protozoa. The more living organisms in the water that needs filtration the more the biological layer will grow and raise the effectiveness of the filter. Therefore, it is desirable to pour water into the BSF every second day.
When the flow rate of the BSF diminishes to a level that is unsatisfying to the user it is a sign that it needs maintenance not that it is failing to clean the water.

According to a recent study, made in Valle Menier Nicaragua, the BSF yielded a mean removal rate of faecal coliforms of 97 % ranging from a low 86 to a high of 100 %. Within the community where the study was performed some of the water sources contained contamination in the range of 100 fcb (faecal coliform bacteria) colonies per ml water (Samaritan's Purse, 1998).

Mozambique's opportunity to develop in a positive direction as a country was seriously disturbed in February-March 2000 when severe floods devastated the country. These floods were caused by the heaviest rains seen in Mozambique in over 50 years (SIDA, 2001). Government officials in Maputo reported that tens of thousands of hectares of farmland were washed away after the Incomate, Sabie, Umbeluzi, Movene and Maputo rivers burst their banks in February 2000. The areas around Chokwe, the district capital of the Gaza province, and Xai-Xai suffered most during the floods. The Limpopo river delta expanded to over 30 km across at Xai-Xai in March 2000 and left Chokwe completely under water. Mozambique's capital, Maputo, was cut off from the rest of the world when the roads to South Africa and Swaziland were washed away. The water works were flooded leaving the city without any drinking-water for days. Cases of cholera and diarrhoea were reported in all affected areas (www.afrikagrupperna.se). The shortage of safe drinking-water remains a problem both in cities and villages (SIDA, 2001).

In August 2000 a team of volunteers, working for the help organizations Samaritan's Purse and Medair, introduced the BSF in villages around Chokwe. According to Medair the BSF removed 99-100 % of the diarrhoea bacteria and 85 % of the total bacteria amount in raw water. The BSF's ability to eliminate toxins from cyanobacteria was never tested (Björk, personal comm., 2002).

There are still many cases of illness, mostly among children, in Mozambique that can be related to drinking-water but where a diagnosis has not been established (Ernst, personal comm., 2002). Therefore it was important to investigate the efficiency of the BSF in removing LPS endotoxin and microcystin and also to study if the toxin removal rate varied with different genera of cyanobacteria. Small unicellular algae usually pass through slow sand filters especially at the end of a *Microcystis* bloom when colonies disintegrate. Large, unicellular or filamentous algae can cause clogging of slow sand filters (Hutson *et al.,* 1987).

Granulated active carbon (GAC) has been associated with a high removal rate of cyanobacterial toxins (Hrudey *et al.,* 1999, Keijola *et al*., 1988) and therefore it was predicted that a BSF could remove a higher amount of cyanobacterial toxins if it was complemented with a GAC layer. According to studies at nine water works made by Rapala *et al*. (2002) activated carbon filtration either increased or had no effect on LPS endotoxin concentration in drinking-water in their studies of nine water works. In addition, it was investigated if charcoal could be used in the BSF as an alternative complement instead of the more expensive GAC.

The study took place in Mozambique. The main drinking-water supplier to Maputo, the capital of Mozambique, is the Pequenos Libombos reservoir – a man-made impoundment located 35 km west of Maputo. Studies at the Pequenos Libombos reservoir have shown that the reservoir can develop heavy blooms of potentially toxic cyanobacteria. The last investigations in 2000 showed that there were toxins from cyanobacteria in the water from this reservoir. Many people take untreated drinking-water from the Umbeluzi River where the Pequenos Libombos reservoir has its outlet (Mussagy, personal comm., 2001). The Chokwe irrigations canals (also used as drinking-water) and a lagoon near Chitenguele north of Xai-Xai have both proved to contain toxins from cyanobacteria (Annadotter personal comm., 2002). The locations of the Pequenos Libombos reservoir, the Chokwe canals and the Chitenguele lagoon are shown in Appendix 1.

MATERIALS AND METHODS

Filter construction

Three BioSand filters, designed according to the BioSand filter manual written by Samaritan's purse (1998), were used in the experiment. The difference between the three BSFs was the sand column. The first BSF was designed with a 46 cm high sand column. The second was designed with a five cm thick charcoal layer in the middle of the sand column. The charcoal was placed inside a textile bag to prevent it from floating when placed inside the BSF. A five cm thick GAC layer was implemented in the middle of the sand column of the third BSF (Figure 1). The filters were named BSF1 (ordinary BioSand filter), BSF2 (BioSand filter with a charcoal layer) and BSF3 (BioSand filter with a layer of GAC).

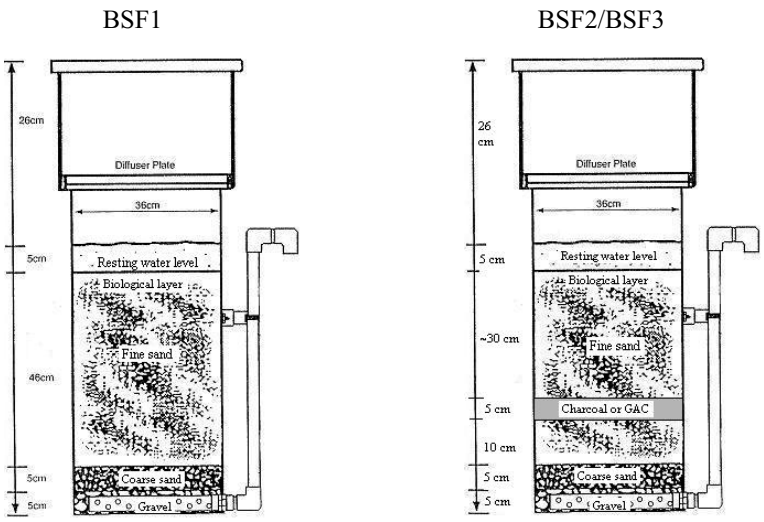

Figure 1. Ordinary BioSand filter (left) and BioSand filter with implemented layer of charcoal or granulated active carbon (right).

Sampling sites

Water for filtration in the BSFs was taken from Maputo's drinking-water reservoir the Pequenos Libombos reservoir. The reservoir is situated in the south of Mozambique and receives its water mainly from the four rivers Umbeluzi, Impolvale, Mabelabele and Calichane. It has a surface area of approximately 41 km^2 and a maximum depth of 33 meters (mean depth 9 m). The Pequenos Libombos reservoir has a high algae diversity with various cyanobacteria e.g. *Microcystis* spp., *Cylindrospermopsis raciborskii*, *Anabaena* spp. and *Raphidiopsis* sp. (Mussagy, 1997).

At one occasion water for filtration in the BSFs was collected from the irrigation canals around Chokwe. This water contained a relatively high density of the small filamentous *Cylindrospermopsis raciborskii*.

Filtration in the BSFs was also carried out with water from a lagoon situated near the city Chitenguele. This lagoon was dominated by large colonies of *Microcystis botrys* and *M. novacekii*.

The differences between the phytoplankton communities of the sampling sites – the Pequenos Libombos reservoir, Chokwe canals and Chitenguele lagoon can be seen in Appendix 2.

Sampling methods

Raw water

Sampling at the Pequenos Libombos reservoir was performed on the 9th, 11th, 14th, 18th, 25th, 28th and 31st September 2002. The water was collected 200-300 meters from the outlet, i.e. near the dam wall from a boat. Raw water was collected vertically with a 0.5 meter long plexi glass tube (∅ 44 mm) and poured into a plastic bucket. The procedure was repeated five times. Samples were taken from the bucket while gently stirring to ensure a homogenous mixture of cyanobacterial toxins and algae. A pipette was used for collection of LPS endotoxin- and microcystin samples. These samples were poured into LPS endotoxin free tubes and microcentrifuge tubes respectively and frozen upon arrival in the laboratory. The quantitative samples were poured into glass bottles and preserved with Lugol's acid solution. Water for chlorophyll *a* analyses was taken with the plexi glass tube and poured directly into dark 2 x 1 L plastic bottles and refrigerated upon arrival in the laboratory. Qualitative phytoplankton samples were collected with a plankton net (15 _m). The samples were poured into dark glass bottles and preserved with Lugol's acid solution. Raw water, for filtration in the BSFs, was collected in three 20 L dark plastic containers. At the shore four 20 L dark plastic containers were filled with water. This water was used in the BSFs between the sampling days for maintenance of the biological layer.

Raw water from the irrigation canals in Chokwe was collected in nine 20 L plastic containers on the 6th of November. Qualitative phytoplankton samples were taken with a plankton net (15 μm) and preserved with Lugol's acid solution. At the laboratory three out of nine containers were mixed in a barrel on the day of collection. From the barrel samples for LPS endotoxin, microcystin, chlorophyll *a* and quantitative phytoplankton were collected using the same methods as described above.

The remaining six containers were used on the 7th and on the 8th mixing three at a time in the barrel each day and collecting the samples as described above.

On the 12th of November raw water from the lagoon near Chitenguele was collected in nine 20 L plastic containers. A qualitative phytoplankton sample was collected with plankton net (15 μm) and preserved with Lugol's acid solution. At the laboratory the same sampling procedures were used as for the Chokwe raw water. The nine containers were used on the 12th, 13th and 14th November mixing three each day and collecting the samples as described above.

Filtered water

Twenty litres of raw water the Pequenos Libombos reservoir were poured into each BSF. Ten litres of water were let to flow through each BSF to minimize the risk of contamination from previously used raw water in the BSFs. This water was disposed. The next eight litres of water from each BSF were collected in plastic buckets. Samples were taken from each bucket while stirring to ensure a homogenous mixture of cyanobacterial toxins and algae. Quantitative phytoplankton samples were transferred from the bucket to glass bottles. LPS endotoxin and microcystin samples were collected with a plastic pipette. Chlorophyll *a* samples were poured into dark 1 L plastic bottles. LPS endotoxin and microcystin samples were immediately frozen. Chlorophyll *a* samples were refrigerated and phytoplankton samples were conserved with Lugol's acid solution. Twenty litres of maintenance water were poured through each BSF approximately every second day (sampling days excluded). Filtration with raw water from the Pequenos Libombos reservoir was performed on the 9th, 11th, 14th, 18th, 25th, 28th and 31st September.

Filtration in the BSFs with raw water from Chokwe was carried out on the day of collection and the two following days (6th, 7th and 8th of November). Sixty litres of Chokwe raw water was mixed in a barrel and distributed equally between the BSFs pouring twenty litres into each filter. Samples from the filtered water were then collected in the same way as for water from Pequenos Libombos. The water was stored in dark plastic containers at app. 25-30 °C during the time from collecting the raw water until filtration in the BSFs.

The raw water from the Chitenguele lagoon was treated, filtrated and sampled in the same way as the Chokwe water. This was performed on the 12th, 13th and 14th of November. The raw water was stored in dark plastic containers at app. 25-30 °C from the time of collection until filtration.

Before every sampling occasion the flow rate of each BSF was measured. This was done to be able to ensure that different flow rates were not a dominating factor for different cyanobacterial toxin reductions. The flow rates were measured when the surface of the water in respective BSF was 10 cm above the head of the sand column using a stop watch and a measuring cup.

Analyses

Phytoplankton

To be able to determine if there was a relationship between cyanobacterial biomass and cyanobacterial toxins, phytoplankton analyses were carried out.

The identification of the phytoplankton was carried out with a light microscope. The identification was done, when possible, to species level for cyanobacteria and to genera level for remaining phytoplankton. The taxonomic classification followed Tikanen & Willén (1992).

The quantitative phytoplankton samples were let to sediment for at least 12 hours in appropriate sedimentation chambers (2, 10 or 25 ml). The biomass of cyanobacteria and the most commonly present phytoplankton was estimated by direct counts and measurements of cells, colonies or filaments using inverted phase contrast microscopes (Nikon TMS and Olympus CK2) at 200x and 400x magnification. The mean volumes of cyanobacteria and phytoplankton were calculated using geometric formulas and the biomass was calculated assuming a density of 1 g/cm^3 (Cronberg, 1982).

Chlorophyll *a*

To investigate if chlorophyll *a* concentrations were associated with concentrations of cyanobacterial toxins, chlorophyll *a* analyses were carried out.

To determine the chlorophyll *a* concentrations in raw- and filtered water one litre of each water was filtrated through GF/C micro glass filters. The GF/C filters were frozen for at least 24 hours and extracted with 10 ml acetone each. After extraction the samples were centrifuged for 10 minutes at 10 000 g. The analysis of the chlorophyll *a* concentrations were done with a Hitachi U-1100 Spectrophotometer and the equation of Ahlgren & Ahlgren (1976) was used to calculate the chlorophyll *a* concentrations.

The final values were then calculated as means between duplicates and expressed as microgram per litre (_g/L μg/L) except for the value from the Chokwe raw water filtrated in BSF3 on the 6th of November were one single value was used.

Microcystin

The occurrence of microcystins in samples were analysed with Enzyme-Linked Immuno Sorbent Assay (ELISA). Samples were frozen and thawed three times and sonicated for 5 minutes to dissolve cell-bound microcystin in the water. After centrifuging for 20 minutes at 10 000 g, samples were treated according to the manual for EnviroGard® Microcystins Plate Kit 75400 and analysed spectrophotometrically with a Microreader Hyperion III (450 nm detection). Concentrations were read from a standard curve. The detection limit was 0.10 μg/L.

Lipopolysaccharide endotoxin

The presence of LPS endotoxin was analysed with the Limulus Amebocyte Lysate - test method, kinetical and turbidimetrical, with an ECX8081V Ultra Microplate Reader (Biotek Instruments, INC). For the calculation of the results a computer program, Endotoxin Data Collection Analyses and Reporting Software (Endoscan V™ version 1.0.8c) was used (Annadotter, personal comm., 2002). The LPS endotoxin concentrations were calculated as mean values between duplicates and were expressed in Endotoxin Units per millilitre (EU/ml) where 10 EU is equal to 1 ng.

RESULTS

Flow rate

The flow rates of the three BSFs varied between 0.07 and 0.18 L/min during the study period. During this time the highest mean flow rate was measured from BSF2 (0.15 L/min). Initially BSF2 and BSF3 had higher flow rates than BSF1. In the last days of the study the flow rates of BSF1 and BSF3 became similar and decreased while the flow rate of BSF2 remained higher than the others.

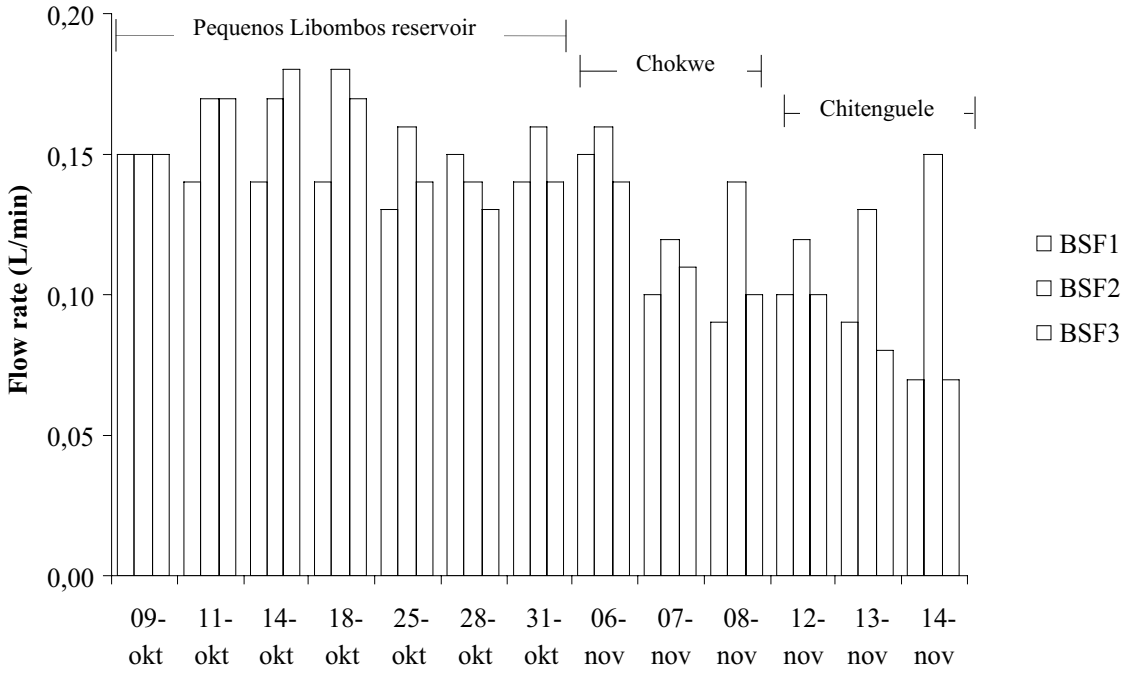

Figure 2. Flow rates of the three BSFs during the 36 days study period (9th of October to 14th of November 2002).

The flow rates remained relatively stable during the period when raw water from the Pequenos Libombos reservoir was filtrated through the BSFs. When the raw water from Chokwe canals was used the flow rates for all BSFs started to decrease. However, when raw water from the Chitenguele lagoon was used, the flow rate of BSF2 showed an increasing pattern while BSF1 and BSF3 continued to decrease (Figure 2).

Phytoplankton

In the raw water from the Pequenos Libombos reservoir 26 genera of phytoplankton were identified during the sampling period. Thirty-five percent of these were cyanobacteria genera containing a total number of 14 species. During the period of sampling the dominating phytoplankton, by biomass per litre, in the raw water from the reservoir was *Mougeotia* sp. and in the filtered waters was *Cryptomonas* sp.

The mean cyanobacterial biomass in the raw water from the reservoir was calculated to 0.38 mg/L with a range between 0.08 and 1.59 mg/L. The mean cyanobacterial biomass in the filtered water was about 55 (BSF2 and BSF3) to 170 (BSF1) times lower than in the raw water. The removal rate of BSF1 varied between 95 - 100 %, of BSF2: 81 - 100 % and of BSF3: 73 - 100 % (Table 1).

Table 1. Cyanobacterial biomass in raw water from the Pequenos Libombos reservoir and percentage reduction of cyanobacterial biomass in the water filtered in the BSFs during the sampling period between the 9th and 31st of October 2002. *) No data for BSF3 on the 9th of October 2002.

Sampling dates	*021009*	*021011*	*021014*	*021018*	*021025*	*021028*	*021031*
Raw water (mg/L)	1.59	0.08	0.40	0.18	0.11	0.17	0.10
BSF1 reduction (%)	100	99	99	95	100	100	100
BSF2 reduction (%)	98	81	100	100	97	100	98
BSF3 reduction (%)	*	89	100	99	73	100	99

On the 6th of November 14 genera of phytoplankton were identified in the qualitative sample from the raw water of the Chokwe canals. Forty-three percent of these were cyanobacteria genera divided in seven species. The dominating phytoplankton in the raw water from the canals was the cyanobacteria *Cylindrospermopsis raciborskii* with a mean biomass of 1.14 mg/L during the sampling period. The most dominating genera of phytoplankton, by biomass per litre in the filtered waters was *Cryptomonas* sp.

In the Chokwe raw water the mean cyanobacterial biomass was calculated to 1.37 mg/L with a range between 1.00 and 1.71 mg/L. In the BSF1 and BSF3 the mean cyanobacterial biomasses were 1400 and 1150 times lower, respectively. In the BSF2 it was only 36 times lower than in the raw water. All the filtered waters showed equal removal rates during the study period except for the water filtered through BSF2 on the second sampling occasion (7th of November) that showed a slightly lower rate (Table 2).

Table 2. Cyanobacterial biomass in raw water from the Chokwe canals and percentage reduction of cyanobacterial biomass in the water filtered through the BSFs on the dates 6th, 7th and 8th of November 2002.

Sampling dates	*021106*	*021107*	*021108*
Raw water (mg/L)	1.40	1.00	1.71
BSF1 reduction (%)	100	100	100
BSF2 reduction (%)	100	89	100
BSF3 reduction (%)	100	100	100

The cyanobacterial biomass in raw water from the Chitenguele lagoon was more than 10 times higher than from the Chokwe canals and almost 50 times higher than from the Pequenos Libombos reservoir.

In the qualitative sample collected from the Chitenguele lagoon on the 12th of November ten genera of phytoplankton were identified of which 40 % were cyanobacteria represented by eight species. During the period of sampling the dominating phytoplankton in the raw water from the lagoon was *Microcystis novacekii and M. botrys* with a mean biomass of 16.39 mg/L. The most dominating genera, by biomass per litre, of phytoplankton in the filtered waters were *Geitlerina unigranulatum and Cryptomonas sp.*

The mean cyanobacterial biomass in the raw water was 17.13 mg/L (sampling period 12th to 14th of November). The filtered waters from all the BSFs contained similar cyanobacterial biomasses varying between 170 to 315 times lower than the raw water concentration. Consequently, the different BSFs also showed similar removal rates of cyanobacterial biomass ranging between 98 and 100 % (Table 3).

Table 3. Cyanobacterial biomass in raw water from the Chitenguele lagoon and percentage reduction of cyanobacterial biomass in the water filtered through the BSFs on the 12th, 13th and 14th of November 2002.

Sampling dates	*021112*	*021113*	*021114*
Raw water (mg/L)	14.53	8.52	28.33
BSF1 reduction (%)	100	98	100
BSF2 reduction (%)	99	99	100
BSF3 reduction (%)	100	99	100

All cyanobacteria species and other dominating phytoplankton genera found in raw water from the Pequenos Libombos reservoir, Chokwe canals and Chitenguele lagoon are listed in Appendix 3. The cyanobacteria species and two of the most common phytoplankton genera in raw water from respective sampling sites are listed in Appendix 4. The biomasses of the cyanobacteria- and the most common phytoplankton genera in raw- and filtered water are shown in Appendix 5.

Chlorophyll a

The chlorophyll *a* concentrations in the filtered waters from the Pequenos Libombos reservoir were in all cases at least 89 % lower and in the best case even 97 % lower than the concentration in the raw water (Figure 3).

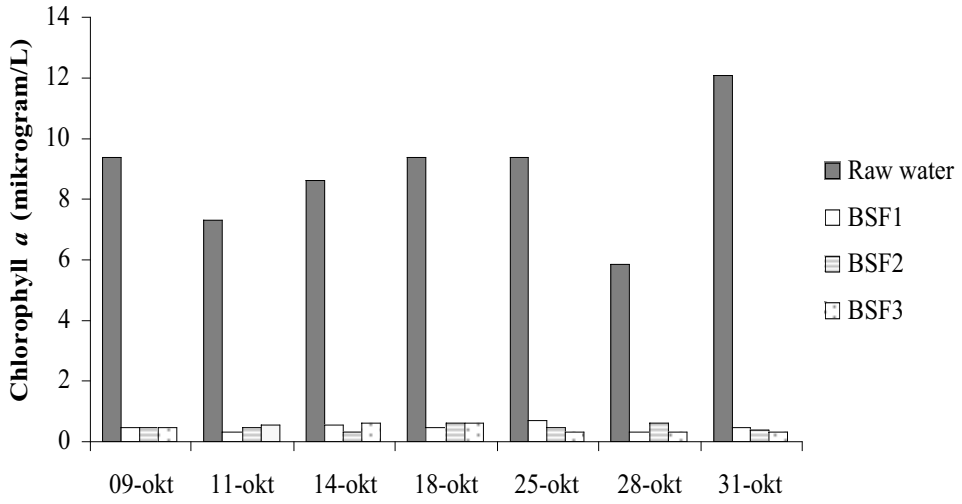

Figure 3. Chlorophyll *a* concentrations in raw water from the Pequenos Libombos reservoir and in the water filtered through the BSFs during the period 9th and the 31st of October 2002.

The raw water from the Chokwe canals indicated an increasing trend in chlorophyll *a* concentrations during the sampling period. The concentrations in the filtered waters were between 95 and 100 % lower than the raw water (Figure 4).

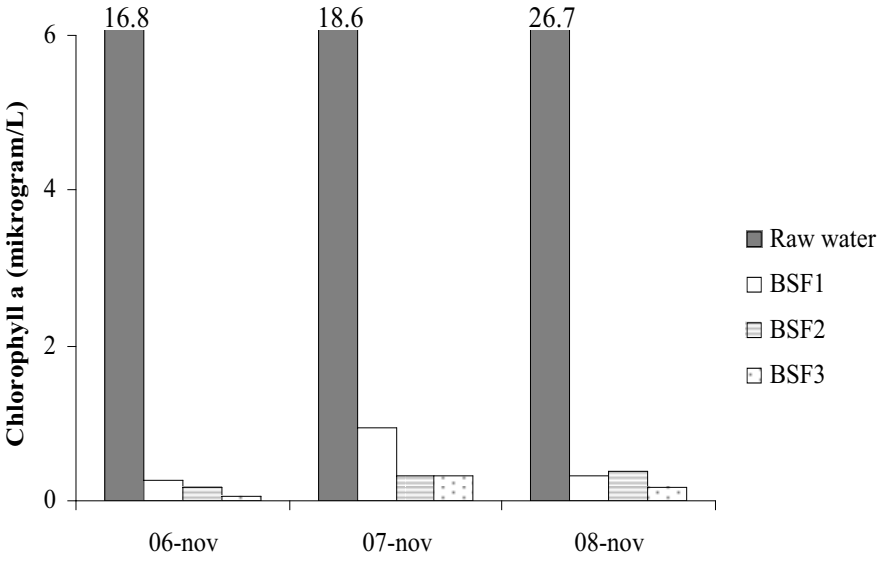

Figure 4. Chlorophyll *a* concentrations in raw water from the Chokwe canals and in the water filtered through the BSFs on the dates 6th, 7th and 8th of November 2002.

The chlorophyll *a* concentrations in the raw water from the Chitenguele lagoon showed a decreasing trend. The filtered waters from the BSFs all had lower concentrations than the raw water (Figure 5).

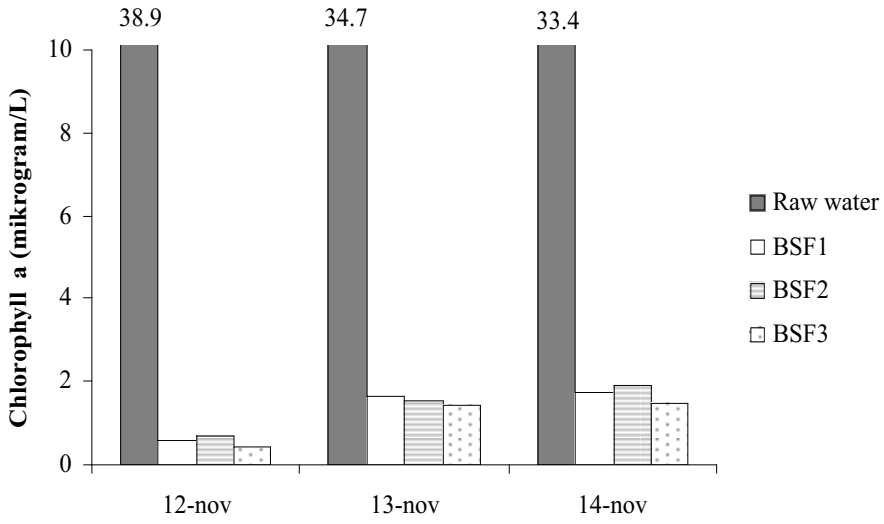

Figure 5. Chlorophyll *a* concentrations in raw water from the Chitenguele lagoon and in the water filtered through the BSFs on the dates 12th, 13th and 14th of November 2002.

The highest chlorophyll *a* concentrations in both raw- and filtered water were found when the Chitenguele lagoon raw water was used as test water. The lowest chlorophyll *a* concentrations in raw water, and also the lowest removal rates of chlorophyll *a*, were measured when the Pequenos Libombos raw water was used.

Microcystin

The microcystin concentrations in the Pequenos Libombos raw water ranged between < 0.10 and 0.28 μg/L. In the filtered water the microcystin concentrations ranged between < 0.10 and 0.22 μg/L. The highest concentrations, both in raw- and filtered water, were detected on the 18th of October when the water was collected from the shore. On this date the three BSFs reduced the microcystin concentrations with 22; 46 and 61 % respectively. Water filtered in BSF1 and in BSF2 on the 25th of October showed an increased microcystin concentration compared to the raw water (BSF1 +16 % and BSF2 +2 %). The rest of the samples, both raw- and filtered water, indicated concentrations less then 0.10 μg/L which is under detection level (Table 4).

Table 4. Microcystin concentrations in raw water from the Pequenos Libombos reservoir and in the water filtered through the BSFs during the period 9th and the 31st of October 2002. Detectable values (> 0.10 μg/L) are in bold numbers.

Sampling dates	*021009*	*021011*	*021014*	*021018*	*021025*	*021028*	*021031*
Raw water (μg/L)	0.05	0.09	0.05	**0.28**	**0.10**	0.07	0.05
BSF1 (μg/L)	0.00	0.05	0.03	**0.22**	**0.12**	0.06	0.05
BSF2 (μg/L)	0.04	0.04	0.05	**0.15**	**0.11**	0.04	0.05
BSF3 (μg/L)	0.03	0.04	0.00	**0.11**	0.05	0.04	0.05

The microcystin concentration in the samples from the Chokwe raw-and filtered water were all below detection level (< 0.10 μg/L) (Table 5).

Table 5. Microcystin concentrations in raw water from the Chokwe canals and in the water filtered through the BSFs on the 6th, 7th and 8th of November 2002. All values are below detection level.

Sampling dates	*021106*	*021107*	*021108*
Raw water (μg/L)	0.08	0.05	0.04
BSF1 (μg/L)	0.05	0.05	0.03
BSF2 (μg/L)	0.04	0.04	0.05
BSF3 (μg/L)	0.04	0.05	0.04

The raw water from the Chitenguele lagoon contained higher amounts of microcystin compared to raw water from the Pequenos Libombos reservoir and the Chokwe canals. The mean removal rates, on the dates 12th, 13th and 14th, for all the BSFs were > 99; > 98 and > 83 % (the removal rates are based on a reduction to 0.10 μg/L for all the BSFs). The microcystin concentrations in raw water decreased during the three-day sampling period but were in two cases approximately six times higher than the guideline level of 1.0 μg/L set by the World Health Organization (Table 6).

Table 6. Microcystin concentrations in raw water from the Chitenguele lagoon and in the water filtered through the BSFs on the 12th, 13th and 14th of November 2002. Detectable values (> 0.10 μg/L) are in bold numbers.

Sampling dates	*021112*	*021113*	*021114*
Raw water (μg/L)	**6,83**	**5,72**	**0,57**
BSF1 (μg/L)	0,05	0,08	0,05
BSF2 (μg/L)	0,05	0,08	0,06
BSF3 (μg/L)	0,04	0,04	0,04

Lipopolysaccharide endotoxin

Raw water from the Pequenos Libombos reservoir showed a continuous decrease of LPS endotoxin concentration during the sampling period. The highest value 38.9 EU/ml was measured on the 9th and the lowest 2.8 EU/ml on the 31st of October. The filtrated waters pointed out a decreasing pattern of LPS endotoxin during the study period. The highest concentrations of LPS endotoxins in filtered water were measured in the water filtrated through BSF2. This water showed a higher LPS endotoxin concentration in comparison to the raw water in six of seven sampling occasion. Even water filtrated in BSF1 showed higher concentrations compared to the raw water on several occasions (Figure 6).

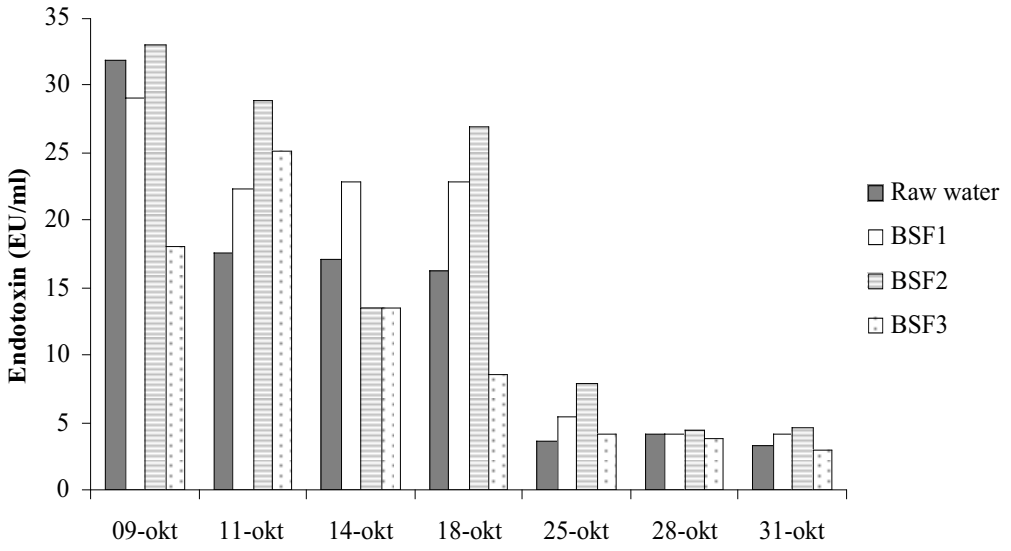

Figure 6. Lipopolysaccharide endotoxin concentrations in raw water from the Pequenos Libombos reservoir and in the water filtered through the BSFs during the period 9th and the 31st of October 2002.

The raw water from the Chokwe canals showed a peak in LPS endotoxin concentration, compared to the other days, on the second sampling day. The filtered waters from BSF1 and BSF3 contained similar and stable LPS endotoxin concentrations all lower than the raw water. The filtered water from BSF2 showed stable but higher concentrations compared to the waters from the other BSFs. In one case (6th of November) BSF2 pointed out a slightly higher LPS endotoxin concentration than the raw water (Figure 7).

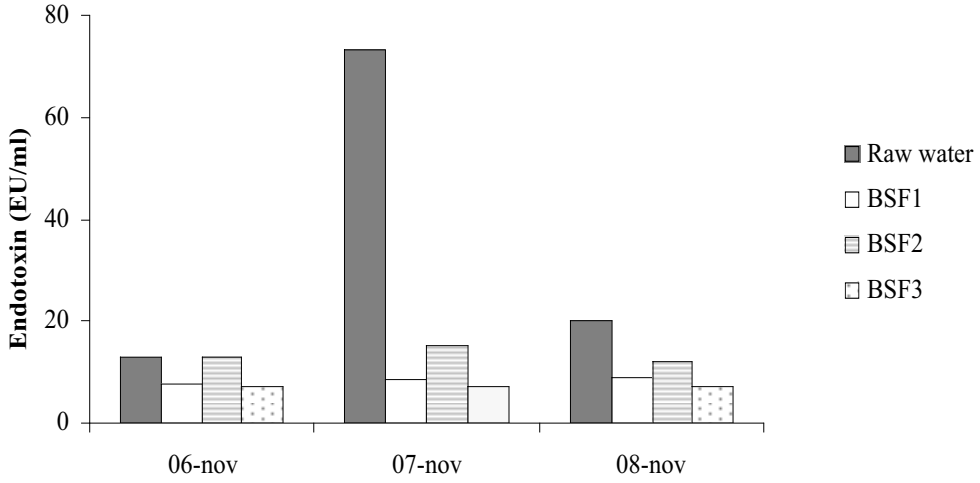

Figure 7. Lipopolysaccharide endotoxin concentrations in raw water from the Chokwe canals and in the water filtered through the BSFs on the dates 6th, 7th and 8th of November 2002.

In the raw water from the Chitenguele lagoon the LPS endotoxin concentrations were similar on the first and third sampling day. However, on the second sampling day the concentration was almost the half compared to the other days. Water filtered through BSF1 and BSF3 showed similar LPS endotoxin concentrations that were in all cases lower than the raw water concentrations. The water from the BSF2 filter always contained higher LPS endotoxin concentrations compared to water from the other filters. In one case the BSF2 water showed a higher concentration than the raw water (Figure 8).

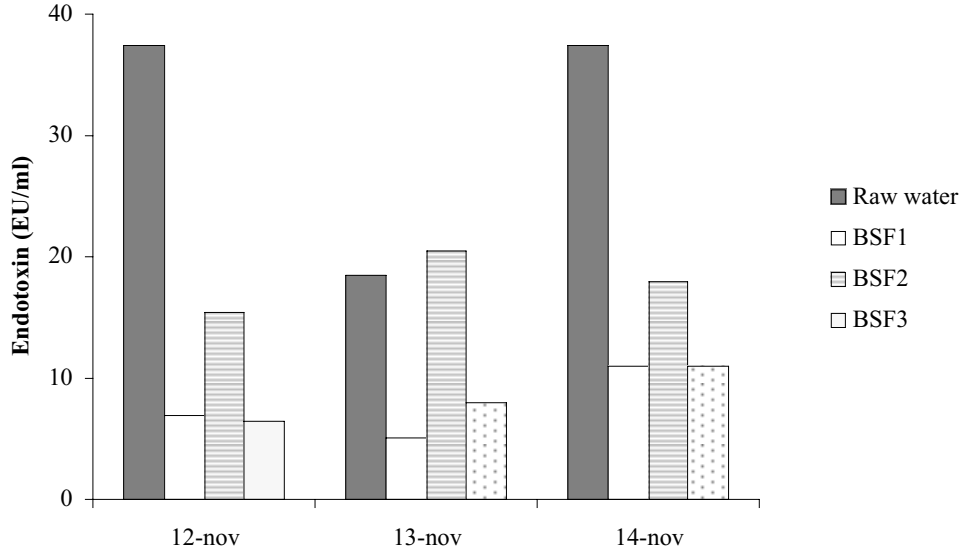

Figure 8. Lipopolysaccharide endotoxin concentrations in raw water from the Chitenguele lagoon and in the water filtered through the BSFs on the dates 12th, 13th and 14th of November 2002.

The highest LPS endotoxin concentrations in filtered water compared to the raw water were found in BSF2 throughout the study. The mean removal rate of LPS endotoxin by BSF2 was -5 % indicating that this BSF generally contaminated the water rather than cleaning it.

The efficiency of BFS1 in removing LPS endotoxin was almost as low as of BSF2 in the first twenty-two days (9th to 31st of October) when raw water from the Pequenos Libombos reservoir was used. BSF3 generally showed a higher removal rate than the other BSFs.

Throughout the study the mean removal rate of BSF1 was 18 % while BSF3 had the best mean removal rate at 37 %. Twenty-eight days after installation (6th of November) the removal rates for all the BSFs had increased compared to the first twenty-two day period. During the last nine day period (6th to 14th of November) the removal rates of BSF1 and BSF3 became similar to each other (Figure 9).

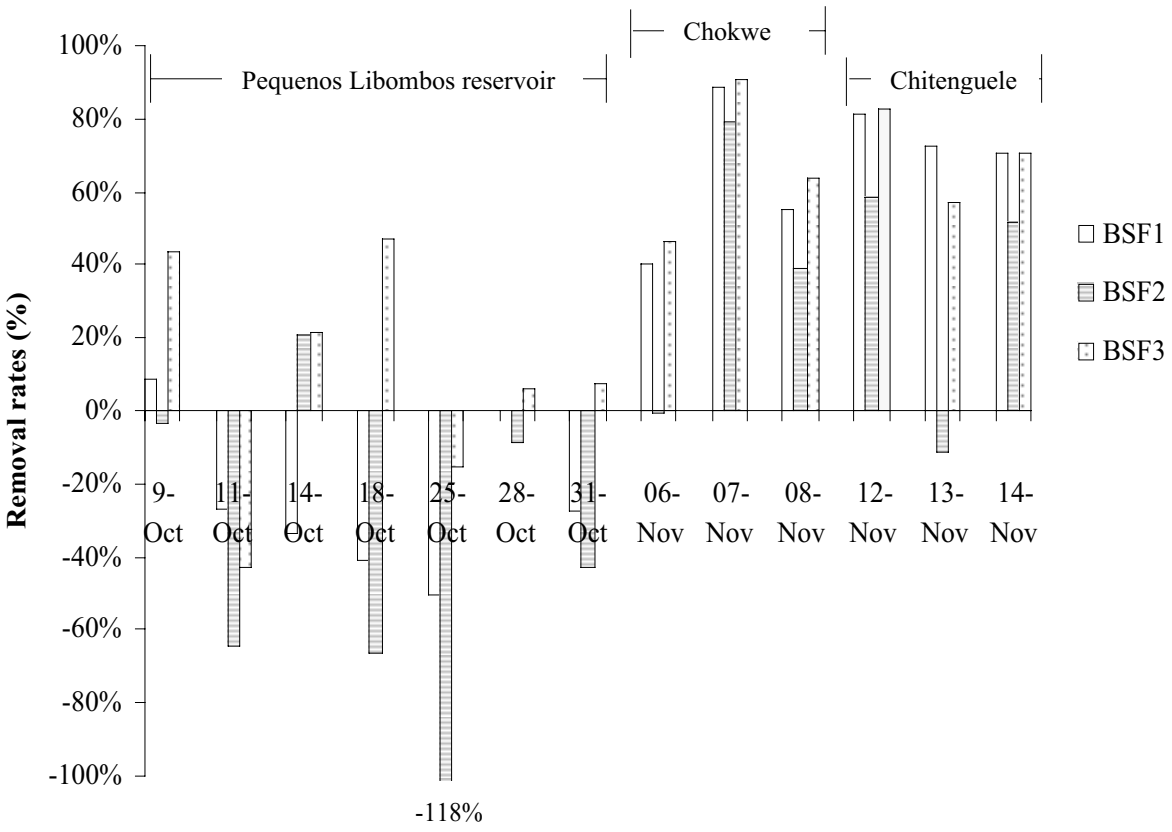

Figure 9. Removal rates of lipopolysaccharide endotoxin by the three BSFs using raw water from Pequenos Libombos reservoir, Chokwe canals and Chitenguele lagoon during the 36 day study period (9th October to 14th of November 2002). Negative removal rates indicate an increase of LPS endotoxin concentrations compared to the raw water in question.

DISCUSSION

The flow rates of all the BSF were similar in the beginning of the study although the flow rates decreased over time. This might have been a direct result of clogging of the sand pores by organic material (e.g. phytoplankton), silt etc. that were present in the raw water as described by Samaritan's purse (1998). The decreased flow rate, resulting in a slow drinking-water production not satisfying for the user, indicates that it is time to maintain the BSF (i.e. clean the sand). The maintenance requires a change or cleansing of the upper portion of sand and thus removal of the biological layer. This will decrease the BSF´s biodegradation ability for some time until the biological layer has developed again. This is one of the disadvantages of the BSF that can not be ignored. One possible way of overcoming this is to complement the BSF with a layer of granulated active carbon. In the starting period when there were no fully developed biological layers in the filters BFS3, containing the GAC layer, generally showed the highest removal rates of LPS endotoxin (Figure 9).

No connections between LPS endotoxin and chlorophyll *a* respectively cyanobacterial biomass could be found in either the raw- or filtered water samples.

On the 18th of October the raw water was collected at the shore of the Pequenos Libombos reservoir instead of the at ordinary collection place near the dam wall. Scum was observed along the shore line and the wind was blowing inshore on this day. Wind blowing towards the shore may cause an accumulation of cyanobacteria at the shore (Falconer *et al.,* 1999). This might be the reason why the microcystin concentrations were higher on the 18th of October compared to the other days. The higher microcystin concentrations in filtered water in comparison to raw water, measured on the 25th of October, may have been caused by contamination from the maintenance water possibly containing microcystin collected at the shore on the 18th of October (Table 4).

Raw water from the Chokwe canals contained microcystin values below the detection level 0.10 μg/L. The low concentrations can be explained by a low amount of microcystin producing cyanobacteria in the raw water.
Results from the microcystin analyses of the filtered water from the Chokwe canals can not be evaluated because all the concentrations were under detection level (Table 5).

The high microcystin concentrations in the Chitenguele raw water are probably caused by the dominating cyanobacteria *Microcystis botrys* and *M. novacekii* which are major microcystin producers.

The decrease of the microcystin concentrations in the raw water, during the three-day sampling period (12th to 14th of November), can be a result of microcystin degradation during storage in the plastic containers. Despite the high concentrations in raw water all BSFs showed a high removal rate of microcystin. However, the lower removal rate (> 83 %) detected on the 14th of November is a direct result of the lower microcystin concentration in the raw water (Table 6).

All of the filtered waters, and a major part of the raw waters, contained microcystins concentrations below the guideline level (1.0 μg/L) set by the World Health Organization. Therefore more studies with raw water containing higher microcystin concentrations are necessary to confirm that the BSFs work efficiently in removing microcystin from raw water. It is difficult, if not impossible, to say if there was any difference between the efficiencies of the three BSFs in removing microcystin since almost all the values of microcystin in filtered water were below the detection level. It is also hard to conclude if the efficiencies of the BSFs in removing microcystin varied with the different raw water sources i.e. with the different genera of cyanobacteria.

The lowest removal rates of LPS endotoxin concentrations by all the BSFs were found in the beginning of the study when Pequenos Libombos was used as raw water. This may be explained by an undeveloped biological layer and thus low biodegradation activity in the sand. In several cases the concentrations of LPS endotoxin even increased with the filtration through the BSFs (Figure 6). A possible reason for this might be that the sand contained LPS endotoxin from an unidentified source. LPS endotoxin is an integral component of all gram-negative bacteria (Sivonen & Jones, 1999) and the test method used for analyses (Limulus Amebocyte Lysate) can only tell the amount of LPS endotoxin not where it originates from. The LPS endotoxin removal rate increased for all the BSFs from the 6th of November i.e. 28 days after installation (Figure 9). According to the BioSand filter manual by Samaritan's purse (1998) this is approximately the time for the biological layer to develop to a satisfying level for drinking-water treatment.

A negative correlation between removal rates of LPS endotoxin and the flow rates of the BSFs could be seen when put in a diagram where a higher flow rate was connected to a lower removal rate of LPS endotoxin. This correlation was however not strong with low or very low R^2-values (Appendix 6). Therefore, it is uncertain to identify the flow rate as a dominating factor for the varying toxin reductions between the BSFs.

Of the three BioSand filters BSF2 showed the lowest removal rates of LPS endotoxin in all cases but one throughout the study (Figure 9). This might be a result of contamination from different sources e.g. the charcoal, the sack textile, used to keep the charcoal in place, and LPS endotoxin in the sand as described above. It is however unlikely that the sand is the major factor for the higher LPS endotoxin amounts in BSF2 since the same sand was used for all BSFs. The highest flow rates were generally measured from BSF2 (Figure 2) which might have reduced the removal rate of LPS endotoxin. The higher flow rate can be a result of the charcoal layer that creates less resistance for the water flowing through the filter and therefore allowing more LPS endotoxins to reach the drinking-water. Complementing the BioSand filter with a charcoal layer did not indicate a higher removal rate of LPS endotoxins when compared to the other BSFs.

The removal rates of LPS endotoxins by BSF1 were generally negative during the first 22 day period (9th to 31st of October). This period is approximately the development time for the biological layer in the BSF as mentioned above. Throughout the rest of the period BSF1 showed only positive removal rates which indicates that the biological layer was important for the removal of LPS endotoxins (Figure 9).

In the first twenty-two day period of the study BSF3 generally had higher removal rates of LPS endotoxin compared to the other BSFs (Figure 9). One possible explanation might be that the adsorption of LPS endotoxin on the GAC layer was, to some extent, compensating for the undeveloped biological layer and its low biological activity. According to Samaritan's purse (1998) the biological layer should reach an optimum biodegradation capability three weeks after installation. On the other hand, the adsorption on the GAC decreases due to increased competition between organic matter, cyanobacterial toxins etc (Lawton *et al*., 1998). The similar removal rates of LPS endotoxin that can be seen between BSF1 and BSF3 in the later period of the study might be an indication that the biological layer probably is more important for the removal efficiency than the GAC (Figure 9).

The filtrations with raw water from the three different sources were carried out on different dates and therefore the biological layers in the BSFs had had different time to develop. It is therefore difficult to conclude if the efficiencies of the BSFs in removing LPS endotoxins varied with raw water i.e. with different genera of cyanobacteria. To ensure that the biological layer in the BSFs would have been the same when the different raw water sources were used the filtrations should have been carried out on the same occasion. This was however not performable for various practical reasons.

Assuming that the amount of cell-bound LPS endotoxins is directly related to the amount cyanobacterial biomass and assuming that the higher cyanobacterial biomass there is in raw water the more cyanobacteria are retained within the BSF as a consequence of clogging. This could have resulted in higher removal rates of LPS endotoxins in waters with high cyanobacterial biomass. This can be seen when the Chokwe- and Chitenguele raw waters, that contained higher biomasses of cyanobacteria than raw water from the Pequenos Libombos reservoir, were filtered through the BSFs (Figure 9). The dominance of the extremely large colonies of *Microcystis* spp. in the Chitenguele raw water might have contributed to the increased removal rates of LPS endotoxin. However, the increased removal rates of LPS endotoxin seen in the later period of the study (6^{th} to14^{th} of November) are more likely a result of the increased activity in the biological layer.

According to the BioSand filter by Samaritan's purse (1998) it takes at least three weeks for the biological layer to develop to a satisfying level for drinking-water treatment. However the BSFs were constantly leaking (approximately 1 to 2 litres per day) and thus became dry on some occassions which may have disturbed the growth of the biological layer. The leakage might have been caused by too much sand in the cement mixture increasing the porosity of the cement folder.

The high LPS endotoxin peak on the 7^{th} of November in the Chokwe raw water could be incorrect because the same raw water was used on the 6^{th} and 8^{th} of November collected at the same place and time (Figure 7). It is however difficult to say whether it is a true value or a measuring error. Assuming the value is wrong and excluding the values for LPS endotoxin concentrations obtained on the 7^{th} of November would decrease the mean removal rates of LPS endotoxins by all BSFs.

It is difficult to say if the concentrations of LPS endotoxins in the filtered waters from the BSFs are safe, because of the lack of guideline concentrations for LPS endotoxins in drinking-water.

The material, e.g. buckets, pipettes etc, used to collect raw- respectively filtered water could have contained LPS endotoxin. This might have affected the empirical values however assuming that all material contaminated the water equally this would have resulted in systematical errors i.e. all filtered waters received equal amounts of LPS endotoxin from the material.

It would have been desirable to be able to produce and analyse more replicates in order to confirm and support the results by statistical analyses. This was however not possible because of the high costs associated with the analytical methods, Enzyme-Linked Immuno Sorbent Assay (ELISA) and Limulus Amebocyte Lysate (LAL) - test method, making more replicates economically unrealistic. Implementing the BioSand household water filter with a GAC layer could be a good way of increasing the removal efficiency of LPS endotoxin especially during the time when the biological layer is undeveloped. However, implementing a GAC layer would increase the price of the BioSand filter. A BioSand filter costs approximately 4 US$ (Muchanga, personal. comm., 2002) and the amount of GAC used in one BSF would cost approximately 1.4 US$ on the Swedish market exclusive transportation expenses (Hägermark, personal comm., 2003). The Biosand filter is with current prices hard to sell to people living in one of the poorest countries in the world.

ACKNOWLEDGEMENTS

We would like to thank our supervisors Heléne Annadotter, Gertrud Cronberg at the department of Limnology, Lund University and Aidate Mussagy at the department of Biological Sciences, Eduardo Mondlane University in Maputo, Mozambique. Special thanks to Luis Mateulane Junior who helped us out with the practical details and transports. Thanks to Victorino Muchanga and Dr. Pieter Ernst for providing us with the BioSand household water filters and Göran Hägermark that provided us with granulated active carbon. A special thanks to Linda Lantz for English proof reading.

We would also like to thank SIDA and the Committee of Tropical Ecology at Uppsala University for giving us the opportunity to perform this study.

REFERENCES

Personal communication:
Annadotter, H. Ph D, University of Lund, Department of Ecology/Limnology. Personal communication, 2002-01-17 and 2002-01-30.

Björk, N. Member of Medair and Samaritan's purse, Sweden. Personal communication, 2002-02-11

Ernst, P. Child survivor program manager. World Relief. Chokwe. Mozambique. Personal communication. 2002-09-26.

Hägermark, G. Ascania AB. Personal communication. 2003-03-12.

Muchanga, V. Mic Ind & BD program. World relief. Chokwe. Mozambique. Personal communication. 2002-09-26.

Mussagy, A. Lic, University of Lund, Department of Ecology/Limnology; Eduardo Mondlane University, Maputo, Mozambique. Personal communication, 2001-12-14, 2002-01-17 and 2002-01-30.

Internet references:
Wallenlind, A. 2002. Regnkatastof i södra. Afrikagrupperna.
URL: www.afrikagrupperna.se/fakta/mocambique/mocfeb.htm

Printed references:

Ahlgren, I & Ahlgren, G. 1976. Metodos de analisis quimicos del aqua. Version revisada por Ahlgren, I & Figueroa, R. 1992. Limnologiska institutionen, Uppsala Universitet. Sverige.1992

Annadotter, H and Cronberg, G.1994. Toxinproduktion vid blomning av blågröna alger (cyanobakterier). Vatten 50:299-303. Lund.

Bartram, J. Carmichael, W, W. Chorus, I. Jones, G. Skullberg, O, M. 1999. Introduction. In: Chorus, I and Bartram, J. Toxic cyanobacteria in water. St Edmundsbury Press, Bury St Edmunds, Suffolk: 1-13.

Chorus, I. Bumke-Vogt, C. Lindenschmidt, K-E and Jaime, E. 2001. Cyanotoxin occurence in freshwaters. In.: Chorus, I. Cyanotoxins-occurrence, causes and consquences. Springer Verlag Berlin Heidelberg New York.: 5-45.

Cronberg, G. 1982. Phytoplankton changes in Lake Trummen induced by restoration. – Folia limnol. scand. 18: 1-119.

Falconer, I. R. Bartram, J. Chorus, I. Kuiper-Goodman, T. Utkilen, H. Burch, M & Codd, G.A. 1999. Safe levels and safe practices. In: Chorus, I and Bartram, J. Toxic cyanobacteria in water. St Edmundsbury Press, Bury St Edmunds, Suffolk: 155-178.

Falconer, I.R. 2001. Toxic cyanobacterial bloom problems in Australian waters: risks and impacts on human health. Phycologia 40 (3): 228-233.

Fitzgerald, J. 2001. Cyanobacterial toxicity and human exposure. In: Chorus, I. Cyanotoxins-occurrence, causes and consequences. Springer Verlag Berlin Heidelberg New York: 178-238.

Grützmacher, G. Böttcher, G. Chorus, I & Bartel, H. 2002. Removal of microcystins by slow sand filtration. Toxicol 17: 386-394.

Hardenbergh, W.A. 1946. Water supply and purification. International textbook company. Great Britain: 370-371.

Hrudey, S. Burch, M. Drikas, M & Gregory, R. 1999. Remedial Measures. In: Chorus, I and Bartram, J. Toxic cyanobacteria in water. St Edmundsbury Press, Bury St Edmunds, Suffolk: 275-312.

Hutson, R.A. Leadbeater, B.S.C. Sedgwick, R.W. 1987. Algal interference with water treatment processes. Progress in physiological research. Vol 3: 265-299.

Keijola, A.M. Himberg, K. Esala, A.L. Sivonen, K. & Hiisvirta, L. 1988. Removal of cyanobacterial toxins in water treatment processes: laboratory and pilot-scale experiments. Toxicity Assessment: An international journal 3: 643-656.

Lawton, L.A. Cornish, B.J.P.A. & Macdonald, A.W.R. 1998. Removal of cyanobacterial toxins (microcystins) and cyanobacterial cells from drinking-water using domestic water filters. Water Research 32: 633-638.

Mohamed, Z.A. & Carmichael, W.W. 2000. Seasonal variation in microcystin levels of river Nile water at Sohag City, Egypt. Annuales de Limnologie. 36 (4): 227-234.

Mussagy, A. 1997. Effects of nutrient addition on the fresh water plankton community in the Pequenos Libombos Reservoir, Mozambique: Enclosure experiments. M.Sc. Degree project. Limnology. Department of Ecology. University of Lund.

Pouria, S. Andrade, A. Barbosa, J. Cavalcanti, R.KL. Barreto, V.T.S. Ward, C.J. Preiser, W. Poon, G. K. Nelid, G.H. Codd, G.A. 1998. Fatal microcystin intoxikation in haemodialysis unit in Carauru, Brazil. – THE LANCET: 352: July 4: 21-26.

Rapala, J. Lahti, K. Rasanen, L.A. Esala, A-L. Niemela, S.I. Sivonen, K. 2002. Endotoxins associated with cyanobacteria and their removal during drinking-water treatment. Water Research. 36: 2627-2635.

Samaritan´s purse. Biosand Household water filter. Third edition. 1998.

SIDA, avdelningen för Afrika. 2001. Underlag till landstrategi för Mocambique - Landanalys Mocambique.

Sivonen, K & Jones, G. 1999. Cyanobacterial toxins. In: Chorus, I and Bartram, J. Toxic cyanobacteria in water. St Edmundsbury Press, Bury St Edmunds, Suffolk: 41-111.

Tikkanen, T & Willén, T. 1992. Växtplanktonflora. Naturvårdsverket.

Appendix 1

Figure 1. Mozambiques location in Africa.

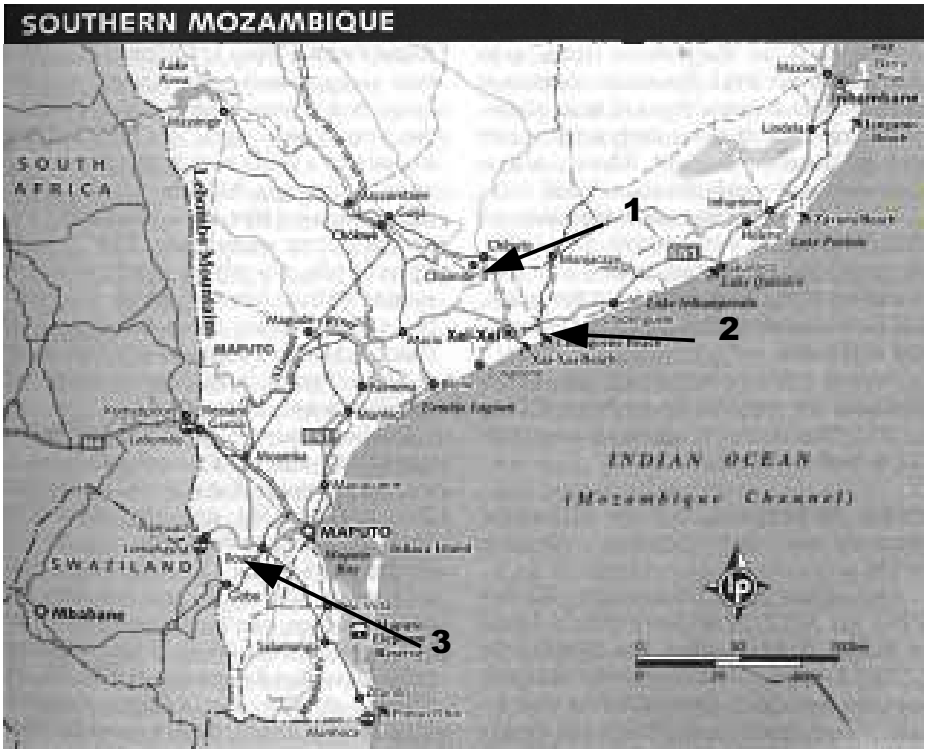

Figure 2. The approximate locations of the three sampling sites. Arrow one (1) points out the Chokwe canals, arrow two (2) the Chitenguele lagoon and arrow three (3) the Pequenos Libombos resevoir.

Appendix 2

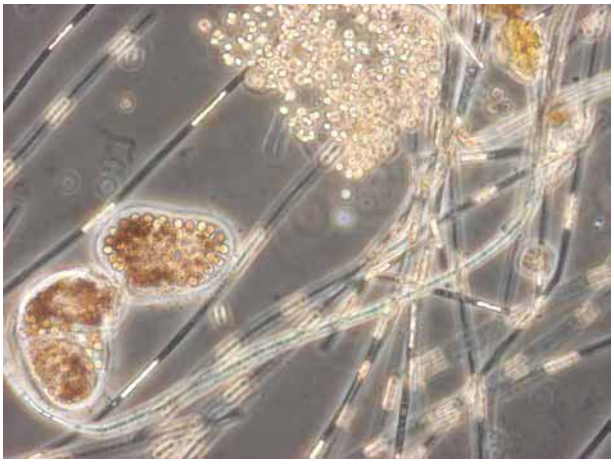

Figure 1. Structure of the phytoplankton community in the raw water of the Pequenos Libombos reservoir (magnification 200x).

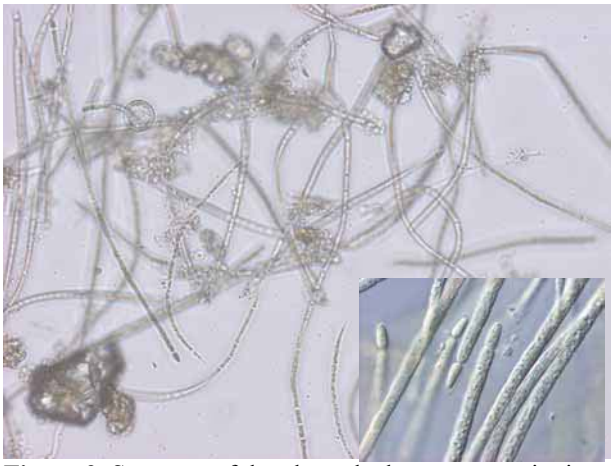

Figure 2. Structure of the phytoplankton community in the raw water of the Chokwe canals (magnification 200x). Inserted photo shows the most common species *Cylindrospermopsis raciborskii* (magnification 1000x)

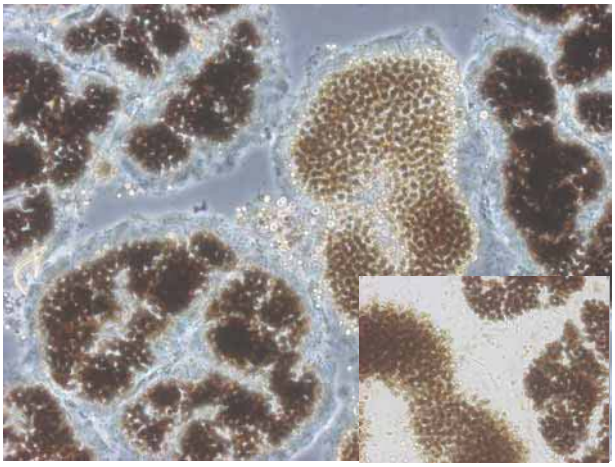

Figure 3. Structure of the phytoplankton community in the raw water of the Chitenguele lagoon (magnification 200x). Inserted photo shows the most common species *Microcystis botrys* and *M. novacekii* (magnification 400x).

Photos by Gertrud Cronberg

Appendix 3

Table 1. Genus and species list of phytoplankton found in raw water from the Pequenos Libombos reservoir, Chokwe canals and Chitenguele lagoon.

CYANOPHYTES
Anabaena spp.
Cyanodictyon imperfectum CRONBERG & WEIB
Cylindrospermopsis raciborskii SEENAYA et SUBBA RAJU
Chroococcus cf *dispersus* (KEISSLER) LEMMERMANN
Chroococcus cf *minutus* (KÜTZIG) NÄGELI
Geitlerina unigranulatum (SINGH) KOMÀREK & AZEVEDO
Microcystis aeruginosa (KÜTZING) KÜTZING
Microcystic botrys TEILING
Microcystis flos-aquae (WITTROCK) KIRCHER
Microcystis novacekii (KOMÀREK) COMPÈRE
Microcystis wesenbergii (KOMÀREK) KOMÀREK in KONDRATEVA
Oscillatoria sp.
Pseudoanabaena mucicola (NAUM. & HUB.-PEST) SCHWABE
Planktolyngbya limnetica (LEMM.) KOM., LENG. & CRONBERG
Planktothrix agardhii (GOM.) ANANG & KOM
Raphidiopsis mediterranea SKUJA
Snowella septentrionalis KOMÀREK et HINDÀK
OTHER DOMINANT PHYTOPLANKTON
Cryptomonas sp.
Koliella sp.
Mougeotia sp.

Appendix 4a

Tabel 1. Genus and species list of phytoplankton in raw water from the Pequenos Libombos resorvoir (depth: 0-0.5 m).

Genera / Date	021009	021011	021014	021018	021025	021028	021031
CYANOPHYTES							
Anabaena spp.	x	x	x	x	x	x	x
Cyanodictyon imperfectum	–	–	–	–	x	–	–
Cylindrospermopsis raciborskii	x	x	x	x	x	x	x
Chroococcus cf *dispersus*	–	x	x	–		–	x
Chroococcus cf *minutus*	x	x	x	x	x	x	x
Microcystis aeruginosa	x	x	x	x	x	x	x
Microcystis flos-aquae	x	x	–	–	–	–	–
Microcystis novacekii	x	x	–	–	–	–	–
Microcystis wesenbergii	x	x	x	x	x	x	x
Oscillatoria sp.	x	x	x	x	x	x	x
Pseudoanabaena mucicola	x	x	x	x	x	x	x
Planktothrix agardhii	x	x	x	x	x	x	x
Raphidiopsis mediteranea	x	x	x	x	x	x	–
Snowella septentrionalis	x	x	–	–	–	x	x
OTHER PHYTOPLANKTON							
Cryptomonas sp.	x	x	x	x	x	x	x
Mougeotia sp.	x	x	x	x	x	x	x

x = recorded
– = not recorded

Appendix 4b

Tabel 2. Genus and species list of phytoplankton in raw water from the Chokwe canals (surface water).

Genera / Date	021106
CYANOPHYTES	
Anabaena spp.	x
Cylindrospermopsis raciborskii	x
Chroococcus minutus	x
Microcystis aeruginosa	x
Microcystis wesenbergii	x
Planktothrix agardhii	x
Raphidiopsis mediterranea	x
OTHER DOMINATING PHYTOPLANKTON	
Cryptomonas sp.	x
Koliella sp.	x

Tabel 3. Genus and species list of phytoplankton in raw water from the Chitenguele lagoon (surface water).

Genera / Date	021112
CYANOPHYTES	
Chroococcus cf *dispersus*	x
Geitlerina unigranulatum	x
Microcystis aeruginosa	x
Microcystis botrys	x
Microcystis flos-aquae	x
Microcystis novacekii	x
Microcystis wesenbergii	x
Pseudoanabaena mucicola	x
OTHER PHYTOPLANKTON	
Cryptomonas sp.	x
Koliella sp.	x

x = recorded

Appendix 5a

Tabel 1. Phytoplankton biomass mg/L (fresh weight) in raw water from the Pequenos Libombos reservoir (depth: 0-0,5 m).

Genera / Date	021009	021011	021014	021018	021025	021028	021031
CYANOPHYTES							
Anabaena spp.	0,006	0,001	–	0,002	0,006	0,007	0,015
Chroococcus spp.	0,009	0,005	0,007	0,035	0,002	0,050	–
Cylindrospermopsis raciborskii	0,230	0,037	0,081	0,083	0,075	0,110	0,080
Microcystis spp.	0,054	0,031	0,297	0,042	0,016	0,004	0,006
Oscillatoria sp.	–	0,002	–	0,009	–	0,001	–
Planktolyngbya limnetica	–	–	0,003	–	–	0,000	–
Pseudoanabaena mucicola	0,544	–	–	–	–	0,003	–
Raphidiopsis mediterranea	–	0,003	0,010	0,009	0,009	–	–
Snowella septentrionalis	–	0,263	–	–	–	–	–
Total	0,843	0,341	0,398	0,179	0,108	0,175	0,101
OTHER DOMINATING PHYTOPLANKTON							
Cryptomonas sp.	0,003	0,039	0,009	0,009	0,012	0,001	0,036
Mougeotia sp.	0,750	0,383	1,230	1,390	1,140	2,910	2,055

– = not recorded or < 0,0005 mg/L

Appendix 5b

Tabel 2. Phytoplankton biomass mg/L (fresh weight) in water from the Pequenos Libombos reservoir filtered through BSF1

Genera / Date	021009	021011	021014	021018	021025	021028	021031
CYANOPHYTES							
Cylindrospermopsis raciborskii	–	0,001	–	–	–	0,001	0,001
Microcystis spp.	0,001	–	0,004	0,008	–	–	–
Total	0,001	0,001	0,004	0,008	0,000	0,001	0,001
OTHER DOMINATING PHYTOPLANKTON							
Cryptomonas sp.	0,008	0,006	0,003	0,007	0,002	0,005	0,011

Tabel 3. Phytoplankton biomass mg/L (fresh weight) in water from the Pequenos Libombos reservoir filtered through BSF2

Genera / Date	021009	021011	021014	021018	021025	021028	021031
CYANOPHYTES							
Cylindrospermopsis raciborskii	–	0,001	–	–	–	–	–
Microcystis spp.	0,026	0,001	–	0,001	0,003	0,001	0,002
Total	0,026	0,001	0,000	0,001	0,003	0,001	0,002
OTHER DOMINATING PHYTOPLANKTON							
Cryptomonas sp.	0,020	0,004	0,005	0,002	0,002	0,010	0,001

Tabel 4. Phytoplankton biomass mg/L (fresh weight) in water from the Pequenos Libombos reservoir filtered through BSF3

Genera / Date	021009	021011	021014	021018	021025	021028	021031
CYANOPHYTES							
Cylindrospermopsis raciborskii	*	–	0,001	–	0,001	–	–
Microcystis spp.	*	0,009	–	–	0,029	–	0,001
Pseudoanabaena mucicola	*	–	–	0,001	–	–	–
Total	*	0,009	0,001	0,001	0,030	0,000	0,001
OTHER DOMINATING PHYTOPLANKTON							
Cryptomonas sp.	*	0,009	0,002	0,008	0,009	0,019	0,015

– = not recorded or < 0,0005 mg/L
* = no data

Appendix 5c

Table 5. Phytoplankton biomass mg/L (fresh weight) in raw water from the Chokwe canals (surface water).

Genera / Date	021106	021107	021108
CYANOPHYTES			
Anabaena spp.	0,072	0,235	0,028
Cylindrospermopsis raciborskii	1,190	0,680	1,560
Planktothrix agardhii	–	0,018	–
Raphidiopsis mediterranea	0,140	0,072	0,120
Total	1,402	1,005	1,708
OTHER DOMINATING PHYTOPLANKTON			
Cryptomonas sp.	0,038	0,001	0,041
Koliella sp.	0,015	0,580	0,130

– = not recorded or < 0,0005 mg/L

Appendix 5d

Table 6. Phytoplankton biomass mg/L (fresh weight) in water from the Chokwe canals filtered through BFS1.

Genera / Date	021106	021107	021108
CYANOPHYTES			
Cylindrospermopsis raciborskii	–	–	0,002
Total	0,000	0,000	0,002
OTHER DOMINATING PHYTOPLANKTON			
Cryptomonas sp.	0,004	0,043	0,025

Table 7. Phytoplankton biomass mg/L (fresh weight) in water from the Chokwe canals filtered through BSF2.

Genera / Date	021106	021107	021108
CYANOPHYTES			
Cylindrospermopsis raciborskii	–	–	0,004
Pseudoanabaena mucicola	–	0,003	–
Total	0,000	0,003	0,004
OTHER DOMINATING PHYTOPLANKTON			
Cryptomonas sp.	0,027	0,037	0,170

Table 8. Phytoplankton biomass mg/L (fresh weight) in water from the Chokwe canals filtered through BSF3.

Genera / Date	021106	021107	021108
CYANOPHYTES			
Cylindrospermopsis raciborskii	–	0,001	0,001
Microcystis spp.	–	0,001	–
Pseudoanabaena mucicola	–	0,001	–
Total	0,000	0,003	0,001
OTHER DOMINATING PHYTOPLANKTON			
Cryptomonas sp.	–	0,007	0,005

– = not recorded or < 0,0005 mg/L

Appendix 5e

Table 9. Phytoplankton biomass mg/L (fresh weight) in raw water from the Chitenguele lagoon (surface water).

Genera / Date	021112	021113	021114
CYANOPHYTES			
Cylindrospermopsis raciborskii	0,001	0,069	–
Geitlerina unigranulatum	0,490	0,965	0,454
Microcystis spp.	14,040	7,250	27,880
Pseudoanabaena mucicola	–	0,233	–
Total	14,531	8,517	28,334
OTHER DOMINATING PHYTOPLANKTON			
Cryptomonas sp.	0,085	0,039	0,010
Koliella sp.	0,031	0,034	0,019

– = not recorded or < 0,0005 mg/L

Appendix 5f

Table 10. Phytoplankton biomass mg/L (fresh weight) in water from the Chitenguele lagoon filtered through BSF1.

Genera / Date	021112	021113	021114
CYANOPHYTES			
Cylindrospermopsis raciborskii	0,003	–	–
Geitlerina unigranulatum	0,018	0,168	0,093
Microcystis spp.	0,007	0,006	0,002
Total	0,029	0,174	0,096
OTHER DOMINATING PHYTOPLANKTON			
Cryptomonas sp.	0,007	0,010	0,006

Table 11. Phytoplankton biomass mg/L (fresh weight) in water from the Chitenguele lagoon filtered through BSF2.

Genera / Date	021112	021113	021114
CYANOPHYTES			
Cylindrospermopsis raciborskii	0,001	0,002	–
Geitlerina unigranulatum	0,090	0,068	0,004
Microcystis spp.	0,006	0,003	0,002
Total	0,097	0,073	0,006
OTHER DOMINATING PHYTOPLANKTON			
Cryptomonas sp.	0,001	0,056	0,004

Table 12. Phytoplankton biomass mg/L (fresh weight) in water from the Chitenguele lagoon filtered through BSF3.

Genera / Date	021112	021113	021114
CYANOPHYTES			
Cylindrospermopsis raciborskii	0,001	0,000	0,000
Geitlerina unigranulatum	0,029	0,058	0,066
Microcystis spp.	0,004	0,002	0,003
Total	0,033	0,060	0,069
OTHER DOMINATING PHYTOPLANKTON			
Cryptomonas sp.	0,010	0,006	0,028

– = not recorded or < 0,0005 mg/L

Appendix 6

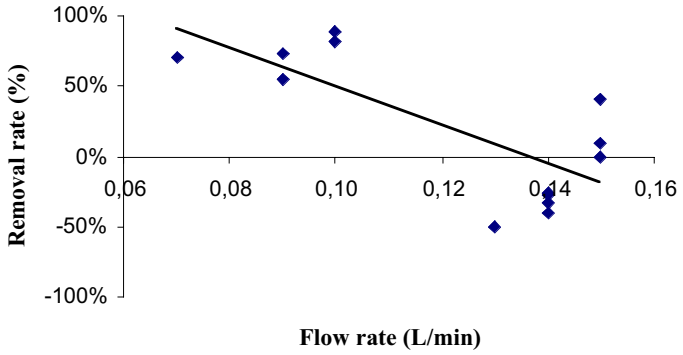

Figure 1 The relationship between removal rate of LPS endotoxin and flow rate in BSF1. $R^2 = 0.55$

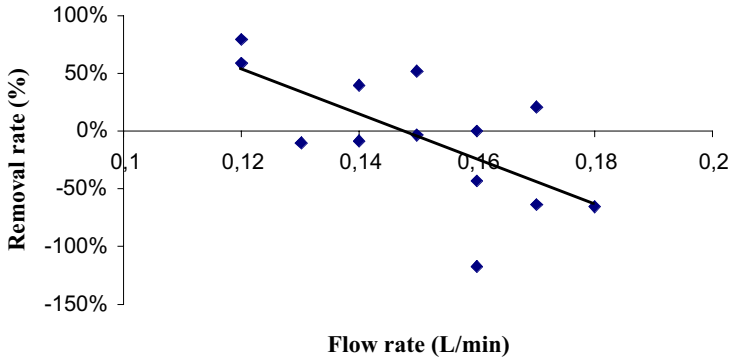

Figure 2. The relationship between removal rate of LPS endotoxin and flow rate in BSF2. $R^2 = 0.45$.

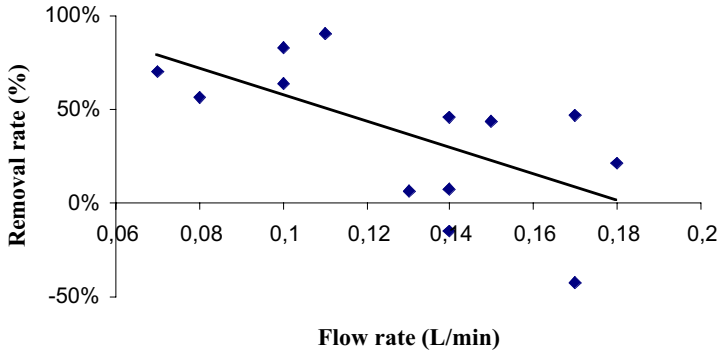

Figure 3. The relationship between removal rate of LPS endotoxin and flow rate in BSF3. $R^2 = 0.40$.