THE UNIVERSITY OF HULL

Toxic cyanobacteria, cyanotoxins and drinking water production in Ghana; implications to human health

Being a Thesis submitted for the Degree of Doctor of Philosophy in the University of Hull

By

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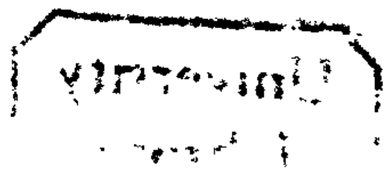

Thesis dedicated to my sons, Kwamena and Kobina de-Veer and to my late parents,

Abu and Naa Abia Addico. They instil in me hard work, happiness and God-fearing.

They will never be forgotten

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Gloria Naa Dzama Addico

Abstract

The management and control of cyanobacteria and their toxins in drinking water reservoirs and water supplies have engaged the attention of many scientists worldwide due to their negative effects on population health. The cyanotoxin, microcystin, the main focus of this research has been responsible for much documented illness in humans and is the most wildly studied cyanotoxin. The World Health Organization has set a guideline limit of 1 μg/l in drinking water.

A number of methodologies have been used in this research. These include the use of an inverted microscope for the identification and quantification of cyanobacteria species after sedimentation in counting chambers. The biomass of picocyanobacteria was determined by epifluorescence microscopy after staining with DAPI. Extraction, purification and concentration of dissolved microcystins were done using the Solid Phase Extraction method. Identification of microcystins was done through comparison with commercial standards and their characteristic UV- spectra, and quantified by extrapolations of HPLC peak areas at 238 nm to a linear calibration curve for microcystin-LR standard. Nutrients (NO_2-N, NO_3-N and PO_3-P) in reservoirs in Ghana were analysed using the American Public Health Association standard methods.

The results which are the first of their kind from Ghana, and for most part in West Africa, highlight that the water treatment processing currently in place is not effective in removing cyanobacteria cells from the final drinking water. Positive correlations were obtained between cyanobacteria biomass and nutrients concentrations in the reservoirs. Fifteen new cyanobacteria species were identified for the first time in Ghana of which *Cyanogranis ferruginea* is reported for the first time in tropical waters. Four known microcystin variants -LR, -RR, -LF and -YR in both dissolved and intracellular samples were identified in four drinking water reservoirs.

The study concludes that, the presence and dominance of potentially toxic small sized cyanobacteria such as *Aphanocapsa nubilum*, *Cyanogranis ferruginea*,

Geitlerinema unigranulatum and other toxic cyanobacteria species like *Cylindrospermopsis raciborskii*, *Planktothrix agardhii* and *Microcystis* spp in the Weija, Kpong, Barekese and the Owabi reservoirs all with basic conventional drinking water treatment facilities, shown to be ineffective in removing cyanobacteria and their toxins, present a potential risk to human health through exposure to cyanotoxins such as microcystins and cylindrospermopsin. Even though the concentrations of dissolved microcystins obtained in the reservoirs and drinking water supplies were lower than the WHO limit, there is potentially a risk to public health and ongoing monitoring would be a good idea.

STRUCTURE OF THESIS

This thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Cyanobacteria species identified in the Weija and Kpong reservoirs, Ghana, and their implications for drinking water quality with respect to microcystin (2006). *African Journal of Marine Science* **28** (2): pp 451- 456.

II. Cyanobacteria diversity and biomass in relation to nutrient regime of four freshwater reservoirs sourced for the production of drinking water in Ghana (submitted to *Journal of Algological Studies*).

III. First report of the hepatotoxins, microcystin-LR, -YR, -RR and-LF in extracellular (dissolved) and intracellular samples from four drinking water reservoirs and water supplies in Ghana (Manuscript).

IV. Demethylated microcystin-RR ([D-Asp^3] MCYST-RR), ([D-Asp^3] MCYST-LR) and four other putative microcystins identified in a culture of *Planktothrix* sp. strain CCAP 1460/13 (Manuscript).

V. Microcystin-RR like toxin in the cyanobacterium *Anabaena flos-Aquae* strain 1403/13B (Manuscript).

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1.0 General Introduction

The presence of toxic cyanobacteria blooms in water bodies used for drinking water or recreational purposes may present serious health risks for the human population (Hoeger et al., 2004; 2005; Orr et al., 2005). Safe drinking water is one of the most critical factors to guarantee long-term population health. In Ghana the increasing pollution of surface water bodies and reservoirs used for the production of drinking water is creating a shift in the phytoplankton composition to mainly cyanobacteria (Addico and Frempong, 2004). Recent studies on phytoplankton in some Ghanaian lagoons, estuaries and reservoirs indicated that cyanobacteria composition of the phytoplankton biomass is either the highest or the second highest (Addico and Frempong, 2004). The most common cyanobacteria identified were *Anabaena*, *Oscillatoria*, *Microcystis*, *Lyngbya*, *Merismopedia* and *Gomphosphaeria*. Pollution originates from either point sources such as municipal wastes or diverse sources from agricultural farmlands with heavy fertilizer use. Other sources are domestic garbage and liquid wastes disposed indiscriminately into the open environment which ends up in the water bodies during the rains. Industrial wastes are also increasingly becoming a problem with waste discharged into open drains and water bodies mostly without any form of treatment. Reservoirs, due to their normally high water retention time invariably undergo excessive eutrophication which leads to algal blooms most of which are cyanobacteria. Eutrophication is one most important factors accounting for the growing number of cyanobacteria blooms of which approximately 50% are known to be toxicogenic in rivers, lakes and reservoirs (National Rivers Authority, 1990). Cyanobacteria are the most important fresh water phytoplanktonic organisms due to their ability to produce strong hepatotoxins such as microcystins, nodularins and neurotoxins (Sivonen and Jones, 1999; Senogles-Derham, 2003; McElhiney and Lawton, 2005; Molica et al., 2002, 2005; Hoeger et al., 2002, 2004, 2005; Dietrich and Hoeger, 2005). Knowledge of cyanobacteria, their toxins and effects on public health is low in Ghana whilst in addition drinking water production procedures do not consider this important health hazard. Water treatment plants do not only have to reduce cyanobacterial cells, odour and colour during the water treatment process but also have to eliminate the toxins produced by the cyanobacteria. Cyanobacteria synthesize a variety of toxins usually classified by their chemical structure, and fall into three

groups namely cyclic peptides (hepatotoxins i.e. microcystin and nodularin), alkaloid neurotoxins (anatoxin-a, anatoxin-a (s) and Saxitoxins) and lipopolysaccharides (LPS) (Sivonen and Jones 1999; Molica et al., 2002, 2005). Drinking water treatment process in Ghana uses coagulation and flocculation with alum followed by sedimentation, filtration and chlorination, whilst others like the Kpong water treatment plant in addition employ pre-chlorination as an additional treatment stage. Most cyanotoxins are cell bound and are released when the cells age, die or are lysed through pre-chlorination or algaeciding with copper sulphate (Sivonen and Jones, 1999; WHO, 1999; Kennewick *et al.*, 1993). Thus the pre-chlorination employed at the Kpong water works could put consumers at the risk of ingesting the resultant cyanobacteria toxins if present. Microcystins, the toxin, commonly found in most of the cyanobacteria genera identified in drinking water bodies in Ghana are known to be active tumour promoters in humans at very low concentrations (Nishiwaka-Matsushima *et al.*, 1992) and in acute doses result in progressive liver damage (Runnegar *et al.*, 1988). Concerns over these public health risks have prompted the World Health Organisation (WHO) to adopt a provisional guideline value of 1.0 μg/l microcystin-LR for drinking water (WHO 1996; 2001). Thus potable water producers in Ghana need to develop methods to either completely remove cyanotoxins or reduce their concentrations (with regard to human health) to acceptable levels.

Cyanobacteria

The name cyanobacteria, blue-green algae and cyanophyceae are all valid and compatible systematic terms. Cyanobacteria are an ancient group of organisms whose habitat range from hot springs to temporarily frozen ponds in Antarctica (Whitton, 1992; Whitton and Potts, 2000)) and occurring both in freshwater and marine environment. According to current taxonomy, 150 genera with about 2,000 species are known with at least 40 of which are known to be toxicogenic (Skulberg et al., 1994). This group of algae comprises both unicellular and multicellular organisms either in filamentous or colonial forms some of which are enclosed in mucilaginous sheaths either individually or in colonies (Wetzel, 1983). They are described as prokaryotes, which mean their cells lack membranous structures including a nuclear membrane, mitochondria and chloroplasts (Wetzel, 1883; Fay and Van Baalen, 1987; Bryant, 1994). They

possess chlorophyll-*a*, and have the ability to undergo oxygenic photosynthesis associated with photo-systems I and II (Castenholz and Waterbury, 1989). With these two accessory pigments mentioned above, they are able to use effectively the region of the light spectrum between the absorption peaks of chlorophyll-*a* and the caroteniods. The majority of cyanobacteria are aerobic photoautotrophs and their life processes only require water, carbon dioxide, inorganic substances and light (Wetzel, 1983; Luuc et al., 1999). Photosynthesis is their principal mode of energy metabolism. Certain species of cyanobacteria mostly filamentous forms such as *Anabaena* and *Nostoc* are capable of fixing atmospheric nitrogen through specialised cells known as heterocyst (Stewart, 1973). Many cyanobacteria species possess gas vesicles that allow them to regulate their position in the water column and give them a distinct competitive advantage over other planktonic species. Cyanobacteria can normally be found in salty, brackish or fresh water bodies, in cold and hot spring and in environments where no other micro-algae can exist, mostly as benthic or part of the plankton. Cyanobacteria are able to withstand high salinity and temperature ranges (Luuc et al., 1999). Reproduction in cyanobacteria is asexual, filamentous forms reproduce by trichome fragmentation or by the formation of special hormogonia (Luuc et al., 1999). Hormogonia are distinct reproductive segments of the trichome, which exhibit active gliding motion upon their liberation and gradually develop into new trichomes. Cell colours vary from blue-green to violet-red. The green of chlorophyll-*a* is usually masked by caroteniods and accessory pigments such as phycocyanin, allophycocyanin and phycoerythrin (phycobiliprotein). All cyanobacteria contain chlorophyll-*a* and phycocyanin. Cyanobacteria have a remarkable ability to store essential nutrients and metabolites within their cytoplasm as reserves under conditions of excess supply of particular nutrients. Prominent cyatoplasmic inclusions are the glycogen granules, lipid globules, cyanophycin granules, polyphosphate bodies and carboxysomes (Fay and Van Baalen, 1987). Many species of cyanobacteria possess gas vesicles, these are cytoplasmic inclusions that enable buoyancy regulation and are gas-filled cylindrical structures. Their function is to give planktonic species ecological important mechanisms enabling them to adjust their vertical position in water column and poise themselves within vertical gradients of physical and chemical factors (Walsby, 1987). Cyanobacteria in order to optimise their position and thus

find a suitable niche for survival and growth use different environmental stimuli such as photic, gravitational, chemical and thermal as clues.

Cyanobacteria are capable of producing a wide range of toxins including hepatotoxins (microcystins and nodularin), alkaloids neurotoxins, endotoxins and cytotoxins (Carmichael, 1997; Codd et. al., 1997; Sivonen and Jones, 1999; Senogles-Derham, 2003; Hoeger et al., 2002, 2004, 2005; Molica et al., 2005; McElhiney and Lawton, 2005; Dietrich and Hoeger, 2005). The reasons for synthesis of toxins are not well understood, but it is believed to be an adaptation that allows cyanobacteria in resource-limiting environment to ameliorate the effects of herbivory and competition with other phototrophs (Arnold, 1971; Demott et al., 1991; Richman and Dodson, 1983), and inhibit the growth of other phototrophs (Stein, 1973; Smayda, 1974; Gross et al., 1991), bacteria (Flores and Wolk, 1986) and fungi (Patterson and Bolis, 1997). Cyanobacteria have both beneficial and detrimental properties when judged from human perspectives. Their extensive growth can create considerable nuisance for management of inland waters such as water supply, recreation and fishing and can also release substances into the water which may be unpleasant leading to taste and odour problems or toxic conditions (Gorham and Carmichael, 1988). Water quality problems caused by dense populations of cyanobacteria are intricate, many and varied (Skulberg, 1996a) and can have great health and socio-economic impacts, as such, the negative impacts of cyanobacteria have gained research attention and public concern. However, in contrast blue-green algae have numerous qualifications for possible positive economic use. They are sources of many valuable products (Richmond, 1990) and carry promising physiological processes, including light-induced hydrogen evolution by biophotolysis (Skulberg, 1994). Cyanobacteria may be used for food and fodder due to the high protein content, vitamins and other essential growth factors and vital pigments of interest produced by certain species. Cyanobacteria are also sources for substances of pharmaceutical interest such as antibiotics (Falch *et. al.*, 1995). These and other uses and applications of cyanobacteria have made their exploitation among the many challenges for biotechnology in the next millennium.

An overview of physical and chemical characteristics of cyanotoxins

Cyanotoxins are a diverse group of natural toxin both from the chemical and the toxicological point of view. Globally, the most frequently occurring cyanotoxin encountered in fresh and brackish waters are the cyclic peptides known as microcystin (Sivonen and Jones 1999; McElhiney and Lawton 2005; Moreno et al., 2005) and are therefore very important regarding treatment of drinking water and pose a major challenge for the production of safe drinking water from surface waters containing cyanobacteria with these toxins. The effects of cyanotoxin on mammals including humans are skin irritation, allergic responses, mucosa blistering, muscular and joint pains, gastroenteritis, pulmonary consolidation, liver and kidney damage among other neurological effects (Oberholster et al., 2004; Hitzfeld et al., 2000). Likely symptoms associated with cyanotoxin poisoning include piloerection, anorexia, cold extremities, diarrhoea, weakness and vomiting (De Figueiredo et al., 2004)

Hepatotoxic cyclic peptides

Microcystin

Microcystins have been characterised from many cyanobacteria species such *as Microcystis* (Botes *et. al.*, 1984), *Anabaena* (Krishnamurthy *et. al.*, 1986), *Planktothrix* (Meriluoto *et. al.*, 1989), *Nostoc* (Sivonen and Jones, 1999; Namikoshi et al., 1990), *Oscillatoria* (Brittain *et. al.*, 2000), *Radiocystis* (Veira *et. al.*, 2003; Lombardo et al., 2006), *Anabaenopsis* and *Cylindrospermopsis* (Senogles-Derham *et. al.*, 2003; Veira *et. al.*, 2003). It has recently also been reported in several other species including *Aphanothece* (Sant Anna et al., 2004; Dasey et al., 2005), *Aphanocapsa* (Domingos et al., 1999), *Arthrospira* (Ballot et al., 2004; 2005), *Synechocystis* (Oudra et al., 2002) and *Scytonema* (Kumar et al., 2000)

The cyclic peptides (microcystins) contain seven amino acids (Sivonen and Jones, 1999), with two terminal amino acids of the linear peptide being condensed (joined) to form a cyclic compound (Sivonen and Jones, 1999) (Fig.1). The reason for the synthesis of microcystins like any other secondary metabolites are not well understood even though it has been suggested that they act as chemical defence mechanisms against grazing by zooplankton and fish (Hitzfeld et al.,

2000; De Figueiredo, et al 2004). Microcystins are known to be synthesised non-ribosomally and by thiotemplate mechanisms (Dittmann et al., 1997; Meissner et al., 1996; Arment and Carmichael, 1999). About 60 structural variants of microcystins have been characterised so far from bloom samples and isolated strains of cyanobacteria (Sivonen and Jones, 1999; Zegura et al., 2004). Microcystins are comparatively large natural products, with molecular weight (MW) of 800 –1000 (Sivonen and Jones, 1999; Kaebernick and Neilan, 2001). The cyclic nature of microcystins means they are highly stable in water across a wide range of pH and temperatures (Fischer and Dietrich, 2000; Dietrich and Hoeger, 2005). The most toxic and commonly occurring microcystin is microcystin-LR, which has a toxicity of 60 μg/kg (Carmichael, 1998; Bell and Codd, 1996).). Microcystins are water soluble and except for a few hydrophobic types, microcystins are unable to penetrate directly the lipid membrane of animals, plants and bacterial cells, (Sivonen and Jones, 1999). Therefore to elicit their toxic effects, uptake into cells occurs through membrane transporters (adenosine triphosphate ATP) for which bile acids (choate and taurocholate) among others are the natural substrates. These organs otherwise carry essential biochemicals or nutrients and this restricts the target organ range in mammals largely to the liver (Runnegar *et.al.*, 1981, 1982; Kaya, 1996; Meier 1996; Codd, 2000; Meier and Stieger, 2002; Takikawa, 2002). Damage to the liver by microcystin includes morphological and functional changes in hepatocytes. In addition to hepatic cells, glomeruli and renal proximal tubule cells, brain, lungs and intestine are also affected by microcystin (Kullak-Ublick et al., 1998; Hagenbuch and Meier, 2003; Dietrich and Hoeger, 2005) as organic anion transporters required for the transport of microcystins are also expressed in some of these organs (Hoeger et al., 2005). The mammalian toxicity of microcystin is mediated through their specific and irreversible inhibition of the catalytic subunit of protein phosphatases 1 and 2A, which results in hyperphosphorylation of cellular proteins (Makintosh *et al.*, 1990; Carmichael, 1994; Mastusushima *et. al.*, 1990; Yoshizawa *et. al.*, 1990; Toivola *et. al.*, 1994; Maatouk et al., 2002; De Figueiredo et al., 2004), leading to disruption of liver cell structures, followed by loss of cytoskeletal integrity and conductance, subsequent cytolysis or apoptosis and intrahepatic bleeding resulting in death (Codd et al., 2005; Dietrich and Hoeger, 2005; Weigand and Pflugmacher, 2005). As a result of this biochemical activity, microcystins have been described as potent liver toxins as well as tumour

promoters, and there are also indications that they may also act as tumour initiators (Fujiki and Suganuma, 1993; Ito *et. al.*, 1997; Mackintosh *et. al.*, 1990; Humpage et al., 2000; Falconer *et. al.*, 1994, 2004). Recent studies have shown that the Adda-side-chain is crucial for the interaction with the protein phosphatase protein molecule, and hence crucial for the toxicity of microcystin (Barford and Keller, 1994; Goldberg *et. al.*, 1995; De Figueiredo et al., 2004). It has also been reported by Repavich *et al.*, (1990) that microcystin induces chromosomal aberrations in human lymphocytes in vitro. Recently Bojana *et al.*, (2003) have demonstrated that microcystin-LR at doses that were not cytotoxic (0.01-1 μg/ml), induced dose and time dependent DNA strand breaks in human hepatoma cell line HepG2. These DNA strands breaks were transient, reaching a maximum level after 4 hrs of exposure and declining with further exposure. Most of the structural variants of microcystins are highly toxic within a comparatively narrow range (intra-peritoneal i.p. mouse toxicities largely in the range 50-300 μgkg^{-1} body weight (BW) (Sivonen and Jones, 1999). Only a few non toxic strains had been identified, (Harada *et. al.*, 1990a, b; Reinhart *et .al.*, 1994). The routes for microcystin poisoning have been identified as absorption, direct ingestion of cell bound and dissolved microcystin through drinking water (Falconer, 1996, 1999) and ingestion of organisms such as shell fish that have accumulated the toxin and contaminated agricultural products (Abe at al., 1996; Codd et al., 1999; McElhiney 2001; Ernst et al., 2001; De Figueiredo et al., 2004). Dermal contact with cyanobacteria and their toxins through recreational activities (swimming, canoeing, etc) and inhalation of the toxins have all been identified as possible routes of intoxication in humans (Picanto et al., 1997; 2004; Chorus et al, 2000; Hoeger et al., 2005). Consumption of algal health food tablets has also been identified as a possible route (Gilroy and Chu, 1998; Gilroy et al., 2000).

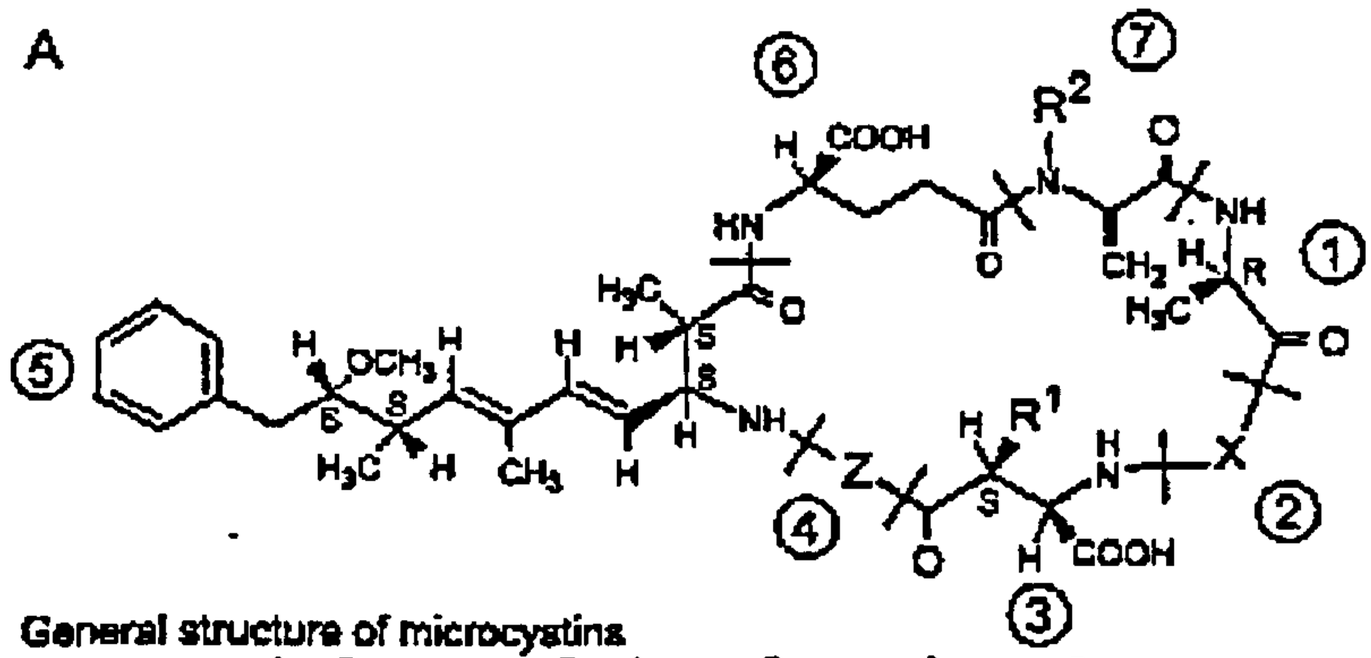

Figure 1 General Structure of Microcystin

In aquatic environments, cyanobacteria toxins remain confined within the cells and are released substantially into the environment on cell lysis, senescence or death (Berg *et.al.*, 1987; Kennewick *et.al.*, 1993; Jones and Orr, 1994; Hitzfeld et al., 2000). This along with their high chemical stability and water solubility has important implications for their environmental persistence and exposure to humans in surface waters. Microcystins, being cyclic peptides are extremely stable in water and resistant to chemical hydrolysis or oxidation at near neutral pH. Microcystins and nodularins are resistant to boiling and in natural waters and in the dark microcystins may persist for months (Sivonen and Jones, 1999). Teixera et al., (1993), reported of gastroenteritis among many people in Itaparica, Brazil, after boiling their water before drinking. It was concluded that boiling actually caused lyses of the cyanobacterial cells resulting in toxin release. Slow hydrolysis had been found to occur at high temperatures and at elevated or low pH (Harada et al., 1996) Rapid chemical hydrolysis is noted to occur only under conditions that are unlikely to be attained outside the laboratory, for example with the use of 6N hydrochloric acid or with trifluoroacetic acid under reflux (Welker and Steinberg, 1999). Microcystins are very stable under sunlight (Tsuji et al., 1994) but can be oxidized by Ozone and other strong oxidizing agents such as chlorine and hydrogen peroxide and under intense ultra violet (UV) light at wavelength around the absorption maxima of the toxins (Tsuji et al., 1995), and the degree of decomposition dependent on the intensity of the UV light used (Nicholson *et. al.*, 1994; Tsuji et al., 1994). A photocatalytic process using a TiO2 catalyst and UV radiation quickly decomposed microcystin-LR, -YR with half lives of less than 5 min (Shephard et al., 1998). However, all these agents produce

chemical residues which may in themselves be toxic to man such as the chlorination by-products, trihalomethanes and haloacetic acids (Rositano *et.al.*, 1995). In full sunlight microcystins undergo slow photochemical breakdown and isomerisation (Tsuji *et.al.*, 1993; Mazur and Plinski, 2001). This can take two to six weeks depending on the presence and concentration of water-soluble cell pigments (phycobiliproteins) in the cyanobacteria cells. The most important degradation pathways for microcystins are enzymatic mineralization by bacteria degradation and degradation photosensitised by humic substances (Welker *et. al.*, 2001).

Neurotoxic Alkaloids

Neurotoxins are less common as compared to the cyclic peptide microcystin. They are predominantly produced by *Anabaena circinalis*, *Cylindrospermopsis raciborskii*, *Aphanizomenon flos-aquae*, *Lyngbya wollei* and *Planktothrix* sp., (Humpage et al., 1983; Hawkins et al., 1985; Lagos et al., 1997; Mahmood and Carmichael, 1986; Sivonen, 1996; Onodera et al., 1997, Falconer, 2004). Mass occurrences of neurotoxic cyanobacteria have been reported in North America, Europe and Australia where they have caused animal and human poisoning. In mouse bioassays death by respiratory arrest occurs rapidly within 2-30 minutes (Sivonen and Jones, 1999). The alkaloids toxins are diverse, both in their chemical structures and in their mammalian toxicities and the more commonly occurring neurotoxins are relatively unstable for extended periods (greater than 24 hours) in aqueous solution at neutral pH (Stevens and Krieger, 1991; Hall *et al.*, 1990). They are in general, a broad group of heterocyclic nitrogenous compounds (i.e. they contain ring structure with at least one carbon-nitrogen bond) usually of low to moderate molecular (less than 1000) weight (Sivonen and Jones, 1999). Alkaloids have varying chemical stabilities, often undergoing spontaneous transformations to by-products which may have higher or lower potencies than the parent toxin.

Anatoxin-a

Anatoxin-a (Fig. 2) had been described from *Anabaena* sp., *Oscillatoria*, *Aphanizomenon* sp., *Cylindrospermopsis* sp. and in small amounts in *Microcystis* sp. (Sivonen, 1996; Park *et. al.*, 1993a and b; Molica et al., 2002, Gugger et al., 2005). Anatoxin-a exerts its neurotoxic effects by mimicking acetylcholine and can cause rapid death by respiratory arrest after ingestion, with clinical symptoms such as muscle fasciculation, decreased movements, abdominal breathing, gasping and convulsion (Fawell *et al.*, 1999; Carmichael, 1994; Hunter, 1995). It has an LD_{50} of 50-250 $\mu g\ kg^{-1}$ bw with a survival time of 4-7 minutes (Carmichael *et. al.*, 1975; Kluge and Szinicz, 2005). No known therapy exists for anatoxin-a, although respiratory support may allow sufficient time for detoxification to occur followed by recovery of respiratory control (Falconer, 1993; Carmichael, 1994) It is a bicyclic secondary amine 2-acetyl-9-azabicyclo (4-2-1) non-2-ene, (Devlin *et.al.*, 1977) and a structural analogue of cocaine. It has also been described by Molloy *et al.*, (1995) as a potent nicotinic agonist and by Carmichael *et. al.*, (1979) as a potent post-synaptic depolarizing neuromuscular blocking agent and an inhibitor of acetylcholinesterase activity (Molica, 2005). Anatoxin-a is a low molecular weight alkaloid (MW 165) (Sivonen and Jones 1999). Anatoxin-a is relatively stable in the dark, but undergoes rapid photochemical degradation in sunlight. It is resistant to degradation on boiling at neutral pH, but breakdown is more enhanced in alkaline conditions (Stevens and Krieger, 1991). The half-life of photochemical breakdown is 1-2 hours (Sivonen and Jones, 1999). Under normal day and light conditions at pH 8 and pH 9, and at low initial concentrations (10 μg per litre) the half-life of anatoxin-a was found to be approximately 14 days (Smith and Sutton, 1993). Anatoxin-a is also known to decompose rapidly in basic solutions but relatively stable under neutral conditions (Matsunaga *et. al.*, 1989).

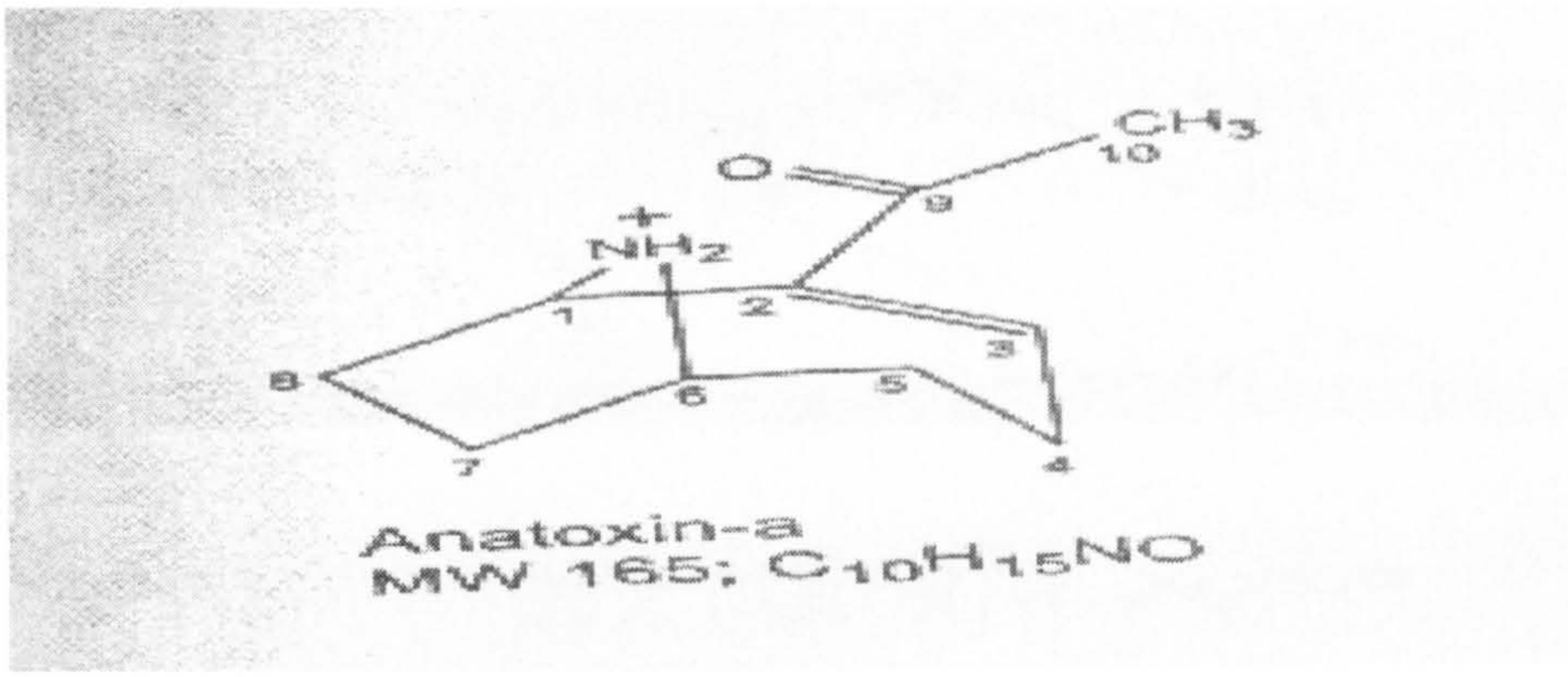

Figure 2 General Structure of Anatoxin-a

Anatoxin-a(s)

Anatoxin-a(s) (Fig. 3), is an organophosphorus anticholinesterase similar to many organophosphate insecticides (Falconer, 1993 Sivonen and Jones, 1999; Molica et al.,, 2002) and the only naturally occurring organophosphate (Matsunaga *et.al*., 1989). The s in anatoxin-a (s) denotes salivation in vertebrates. It has been isolated from *Anabaena flos-aquae* and *Anabaena lemmermanni* (Matsunaga *et. al*., 1989; Henriksen *et. al*., 1997; Onodera *et. al*., 1997). It is a highly and swiftly toxic compound with an LD_{50} of 20 $\mu g\ kg^{-1}$ bw and ten times more lethal than anatoxin-a (Carmichael *et. al*., 1990), with molecular weight of 252. No structural variants of anatoxin-a(s) have been detected (Sivonen and Jones, 1999). Anatoxin-a(s) is highly unstable and becomes inactivated with elevated temperatures above 40°C and also under alkaline conditions (Mahmood *et al*., 1988; Cook *et al*., 1989). Clinical signs of anatoxin-a(s) toxicosis in animals include hypersalivation, mucoid nasal discharge, tremors and fasciculation, ataxia, diarrhoea and recumbency and death by respiratory arrest (Codd *et al*., 1989; Viaggiu et al., 2004). Anatoxin-a(s) appear not to cross blood-brain barrier, therefore it is possible to use a cholinergic blocker such as methyl atropine nitrate or glycopyrolate as a therapy (Beasley *et al*., 1989).

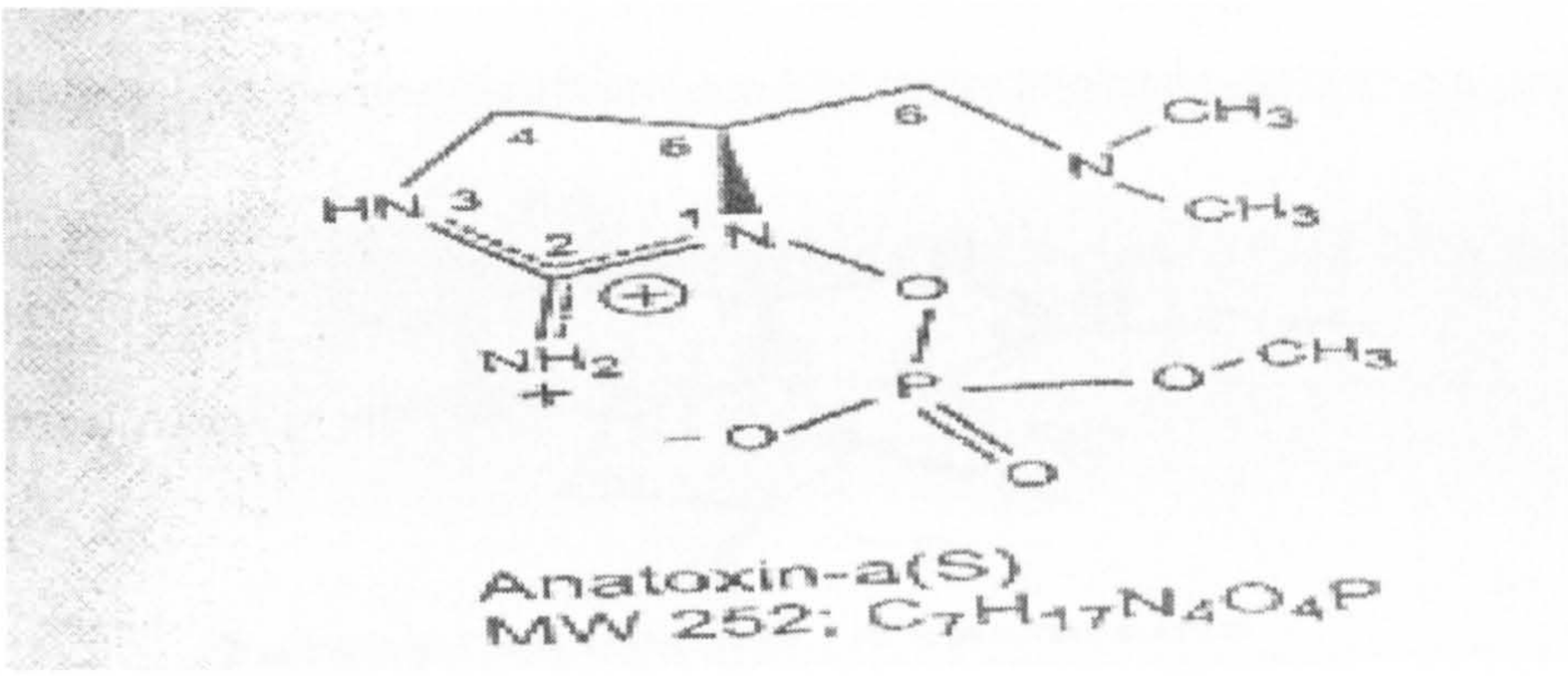

Figure 3 General Structure of Anatoxin-a (s)

Saxitoxin

Saxitoxin, also known as paralytic shellfish poison (PSP) in marine literature, blocks nerve sodium channels and prevent the transmission of nervous impulses with major impact on liver cells, and has also been associated with gastro-intestinal diseases (Sivonen and Jones, 1999; Molica et a., 2002, 2005). Death may occur through respiratory arrest (Carmichael, 1994). For such toxicosis, therapy is best approached by trying to limit further absorption from the gastrointestinal tract by using activated charcoal. In addition, artificial respiration is needed.

They are a group of carbonate alkaloids neurotoxins which are either non-sulphated (Saxitoxin), singly sulphated or double sulphated. Saxitoxins were originally isolated from shellfish, where they are concentrated in dinoflagellates (red tides) and have caused deaths in humans (Anderson, 1994). Saxitoxins have also been found in the cyanobacteria *Aphanizomenon flos-aquae*, *Anabaena circinalis*, *Lyngbya wollei* and *Cylindrospermopsis raciborskii*, (Sivonen and Jones, 1999; Mahmood and Carmichael, 1986; Negri and Jones, 1995; Humpage *et.al*., 1994) and two of these species have been identified from the study sites from Ghana. Saxitoxins undergo slow degradation in the dark at room temperature with the half-life for the breakdown between 1 to 10 weeks, with more than three months required for greater than 90 percent breakdown (Jones and Negri, 1995). Degradation of paralytic shellfish poisons (PSPs) seems to be

more chemically mediated process, and these toxins appear to be able to persist in water bodies for more than three months (Jones and Negri, 1997).

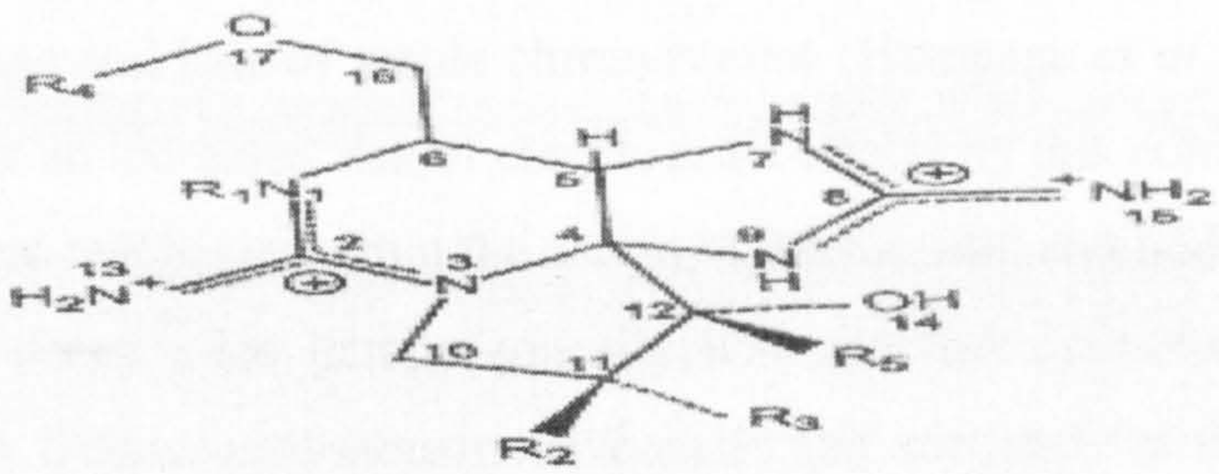

Figure 4 General structure of Saxitoxin

Cylindrospermopsin

Cylindrospermopsin (CYN) a cytotoxic alkaloid with a cyclic guanidine unit produced by several freshwater cyanobacteria genera including *Anabaena* (Schembri *et al*., 2001), *Aphanizomenon* (Banker *et al*., 1997; Shaw *et al*., 1999; Falconer 2004), *Cylindrospermopsis* (Hawkins *et al*., 1997; Griffiths and Saker, 2003; Falconer, 2004), *Raphidiopsis* (Li *et al*., 2001) and *Umezakia* (Harada *et al*., 1994). The toxin and its toxicity came to attention as a result of a severe gastroenteritis outbreak among children who were drinking water from a dam with the cyanobacterium *Cylindrospermopsis raciborskii* (Byth 1980; Hawkins et al., 1985; Falconer, 2004). Toxicological studies on the properties of this compound have shown that it is a cytotoxin with similar toxicity in the mouse bioassay as those of other cyanotoxin including microcystins and nodularins (Kuiper-Goodman *et al*., 1999). The LD 50 of pure cylindrospermopsin to mouse (intra-peritoneal administration) is 2100 μg/kg at 24 hours and 200μg/kg at 5-6 days. The toxin cylindrospermopsin from *Cylindrospermopsis raciborskii* undergoes very slow breakdown at an elevated temperature of 5O°C (Chiswell *et. al*., 1999) in the dark. Under sunlight and in the presence of cell pigments breakdown occurs more rapidly being more than 90 percent complete within 2-3 days (Chiswell *et. al*., 1999). However, pure cylindrospermopsin is relatively stable in sunlight. Studies on the mechanism of action of cylindrospermopsin have shown that in mouse hepatocytes (liver cells) invitro the toxin disrupts

protein synthesis (Tereo *et. al.*, 1994) and in rat hepatocytes (liver cells) invitro the toxin is metabolised by cytochrome P-450 whereupon a metabolite acts to inhibit glutathione synthesis. Studies by Humpage *et al.*, (2000) and Shen *et al.*, (2002) have shown that genotoxic activity of cylindrospermopsin causes DNA strand breakage and loss of whole chromosomes (Humpage *et al.*, 2000), which might indicate an increased risk of carcinogenic effects by this compound. This is a very different mechanism from the action of microcystin and nodularin. Several groups have dosed mice intraperitoneally with purified cylindrospermopsin or extracts from *Cylindrospermopsis raciborskii* and reported on the progressive necrosis in liver and injuries in kidney, lungs, heart and intestines (Hawkins *et al.*, 1985; Harada *et. al.*, 1994; Falconer *et. al.*, 1999, 2004). In particular relevance to human exposure, laboratory studies in mice have been recently conducted using oral dosing of *Cylindrospermopsis raciborskii* extract containing 0.2% cylindrospermopsin. The toxin was found to be bioavailable by this route (Seawright *et. al.*, 1999). The median lethal dose being in the range of 4.4 to 6.9 mg toxin-equivalent kg^{-1} (Seawright *et. al.*, 1999). This oral administration resulted in the same liver pathological changes as those caused by interperineally dosing with the No Observed Adverse Effect Level (NOAEL) for repeated oral dosing of about 0.1 mg kg^{-1} (Moore *et. al.*, 1998). At minimally toxic doses the liver was the primary target organ with some evidence of spleen lymphophagocytosis, while at higher doses effects included thymus atrophy, renal ischaemic acute tubular necrosis, subepicardial and myocardial haemorrhage, adrenal cortex sinusoid congestion and microscopic ulcerations of the oesophageal part of the gastric mucosa with moderate stomach wall oedema (Seawright *et. al.*, 1999). Recently a guideline value for the presence of cylindrospermopsin in human potable water supplies had been given as 1 μg/l (Humpage and Falconer, 2003).

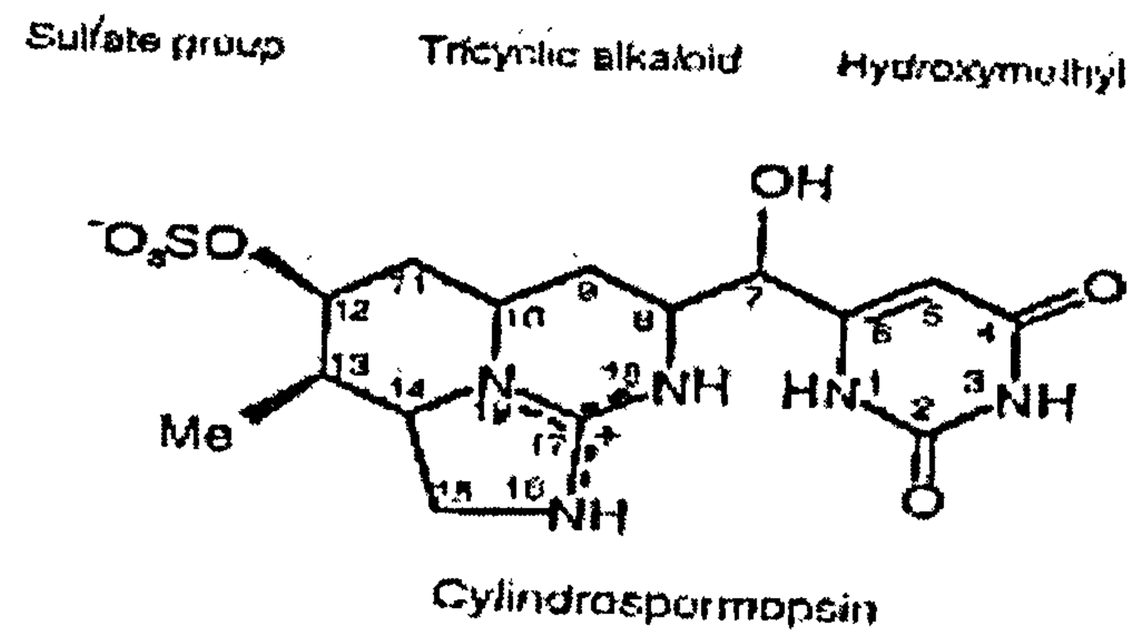

Figure 5 General structure of Cylindrospermopsin

Cyanotoxin and human health

Public health significance of cyanotoxins in humans

Evidence of human poisoning by cyanobacteria and its toxins range from health effects after recreational exposure to poisoning following consumption of contaminated drinking water or other sources of water (Kuiper-Goodman et al., 1999). These evidences had been derived from epidemiological sources, animal poisoning and toxicological studies. Epidemiological evidence attributed to cyanotoxins includes the fatal intoxication by microcystins of over 50 dialysis patients in Brazil and the hospitalization of 140 children supplied with water from a dam containing *Cylindrospermopsis raciborskii* in Australia (Falconer *et. al.*, 1983; 2004). Reports of gastro-enteritis after the appearance of cyanobacteria blooms in drinking water sources had been reported in North and South America, Africa and especially Europe (Kuiper-Goodman *et.al.*, 1999). Animal toxicity test, though not conducted under natural conditions of exposure provide plausible evidence of the role of cyanotoxins in humans and animals poisoning and provide information on their comparative toxicity. Oral toxicity tests are especially important and have been carried out on large animals. Of all the cyanobacterial toxins currently known the cyclic peptides (microcystins) represent the greatest concern to human health because of their potential risk of long-term exposure at

comparatively low concentrations of toxins in drinking water supplies (Kuiper-Goodman *et.al.*, 1999; Falconer 2004). This is because microcystins have been found to be specific liver toxins in mammals and following acute exposure at high doses cause death from liver haemorrhage or from liver failure (Kuiper-Goodman *et.al.*, 1999; De Fegueiredo et al., 2004) and may promote the growth of liver and other tumours following chronic exposures at low doses (Runnegar *et. al.*, 1995; Sueoka *et.al.*, 1997; Carmichael, 1994; Falconer, 1996; 1998, 2004; Nishiwaki-Mastushima *et.al.*, 1992). The alkaloid neurotoxins (anatoxin-a and saxitotoxins) have only shown acute effects in mammals (Kuiper-Goodman *et.al.*, 1999). The toxicity of pure cylindrospermopsin has only recently been studied following the identification of the toxin in 1992 (Ohtani *et. al.*, 1992). However, it is not known whether cylindrospermopsin will elicit histopathological damage following chronic (long-term) exposure at low concentrations as normally found in drinking water (Hawkins *et.al.*, 1997; Falconer *et. al.*, 1999; Seawright *et. al.*, 1999).

Short-Term (acute) effects of cyanotoxins in humans attributed to drinking water

Recorded cases of gastro-intestinal and hepatic illness that can be reliably attributed to cyanobacteria toxins in water supplies have all been conducted either during the breakdown of natural blooms of cyanobacteria or by application of copper sulphate. Both of these situations lead to the release of cyanotoxin from decomposing cells. The earliest cases of gastro-enteritis from cyanobacteria were in the populations of a series of small towns along the Ohio River in 1931 where low rain fall had caused the development of blooms along the banks of the river which was washed into the river (Tisdale, 1931).

In Harare, Zimbabwe, children living in an area of the city supplied from a particular reservoir developed gastro-enteritis as well as influenza-like symptoms each year at the time when a natural bloom of *Microcystis* was decaying in the reservoir (Zilberg, 1968). Other children in the same city but receiving drinking water from different reservoirs were not affected and no infectious agent was identified in the reservoir. Skin rashes, itching and eye sores were also reported in workers coming into contact with cyanobacterial blooms at an aquacultural facility.

A lethal outbreak attributed to cyanobacteria toxins from drinking water occurred in Bahia, Brazil in 1988, when a newly flooded dam (Itaparica) developed blooms and about 88 children were recorded to have died due to this incident (Teixera et.al., 1993) from over 2000 cases of gastro-enteritis over a period of 42 days. Clinical data gathered from blood and faecal specimens from patients subjected to bacteriological, virological and toxicological analysis and also the drinking water conclusively demonstrated that there was no other infectious agent or toxin in the water. All the cases occurred in people drinking boiled water and cases were restricted only to populations supplied with drinking water from the dam. Cyanobacteria genera identified in the untreated raw water at the time of the outbreak were *Anabaena* and *Microcystis* at between 1,104 to 9,755 units/ml. Another disastrous event reported by Jochimsen et.al., (1998) was the poisoning episode involving 117 dialysis patients in a dialysis clinic in Brazil. The patients developed cholestatic liver disease and over 50 deaths occurred due to the exposure of patients to the cyanotoxin, microcystin in the dialysis water used for the treatment. This was the first documented lethality of hepatotoxins (microcystins) to humans occurring via the intravenous route during dialysis in Brazil (Pouria et al., 1998; Azevedo et al., 2002). The syndrome was characterised by painful, extreme hepatomegaly, jaundice, bleeding diathesis manifested by ecchymosis, epistaxis and methorrhagia, elevated transarminases, variable hyperbilirubinemia, disruption of liver plates, liver cell deformity, necrosis, apoptosis and cholestasis (Carmichael *et al.*, 2001). Examination of phytoplankton showed a dominance of the cyanobacteria genera *Microcystis*, *Anabaena*, *Anabaenopsis*, *Oscillatoria* and *Cylindrospermopsis* representing 99% of the total phytoplankton density. Blood sera provided by health officials in Pernambuco, Brazil and sent to the Centre for Disease Control in Atlanta Georgia, USA from affected and control patients and liver tissues from deceased patients showed microcystin content up to 10 ng/ml for sera and 0.1 to 0.5 ng/ml in liver tissues. All exposed patient sera and tissues were positive for microcystin (Jochimsen *et.al.*, 1998; 1996; Pouria *et. al.*, 1998). Intoxication through haemodialysis has also been reported from Portugal (Pereira et al., 2000), USA (Hindman et al., 1975)

In Armidale, Australia, an epidemiological study of the local population after water supply authorities had treated a dense population of *Microcystis aeruginosa* with 1 ppm of copper sulphate after complains of taste and odour in the water

indicated liver damage occurring among the population simultaneous with the termination of the bloom (Botes *et.al.*, 1985). The water treatment process involved pre-chlorination, alum flocculation, sedimentation, rapid sand filtration, post-chlorination and fluoridation. This process was unable to remove or breakdown the toxin in the water. The toxin identified in the water was microcystin-YM (Botes *et.al.*, 1985). Still in Australia, a more severe outbreak occurred in human populations due to cyanobacteria toxins from drinking water. After treatment of the water with copper sulphate, within a week, numerous children developed hepatoenteritis and a total of 140 children and 10 adults were hospitalised for treatment (Byth, 1980). Diagnostic information from the hospitals included detailed examinations with major symptoms as malaise, anorexia, vomiting, headache, painful liver enlargement, and initial constipation followed by bloody diarrhoea and varying levels of dehydration (Byth, 1980). Urine analysis showed electrolyte loss together with glucose, ketones, protein and blood in the urine. Blood analysis also showed elevated serum liver enzymes in some children indicating liver damage. Sixty-nine percent of the patients were reported to require intravenous therapy and in more severe cases, the individuals went into hypovolamic/acidotic shock (Byth, 1980). The affected populations all received water from the same source, the Solomon Dam. Examination of faecal samples and food eliminated a range of infectious organisms and toxins as possible cause of the outbreak. Organisms from the dam were isolated and cultured and administered to mice. After several days the mice developed widespread tissue injury involving the gastrointestinal tract, kidney and liver (Hawkins *et. al.*, 1985). Following subsequent monitoring of the algal blooms in the dam, the cyanobacteria *Cylindrospermopsis raciborskii* was identified as the cause of the bloom with concentrations as high as 300,000 cells per ml (Hawkins and Griffiths, 1993). One disturbing factor about *Cylindrospermopsis raciborskii* is that it does not form scums at the water surface and grow well below the water surface where it is not easily visible and can be abstracted into drinking water intake points. To control this alga the authorities introduced destratification in the reservoir. The presence of this species in the Ghanaian drinking water reservoirs certainly is a cause for concern.

Collins (1979) reported on the results of an epidemiological investigation into a high birth defect rate that suggested a link to two drinking water reservoirs.

Subsequent studies showed that the water was mutagenic in laboratory animals at the time of a cyanobacterial bloom.

In a sugar refinery in Scania, Sweden, Annadotter et al., (2001) reported that the drinking water distribution system was erroneously coupled to untreated river water. Coincidentally, a high density of *Planktothrix agardhii* occurred in this river and the water contained approximately 1.0 ug/l microcystin-LR equivalent. In the following days, 121 persons developed numerous symptoms including diarrhoea, headache, vomiting, fever and muscular and abdominal pain. Pathogenic bacteria or viruses could be excluded as the cause for the illness. Another interesting fact was that, 100% of the tea-drinkers of the sugar refinery were sick in the days following of the accident, while none of the coffee drinkers were affected. It was likely that the toxins were released from the cells by boiling the water and in the case of the coffee-drinkers, filtered out by the use of a coffee filter and the coffee grinds (Dietrich and Hoeger, 2005).

Chronic effect of cyanotoxins in Humans

Acute toxicity from cyanotoxins is the most obvious effect, however, long term chronic effects are possible and should be of most concern to drinking water authorities. Experiments with animals have shown chronic liver injury from continuing oral exposure to microcystins particularly the possibility of carcinogenesis and tumour growth promotion e.g. chronic administration of *Mirocystis* extract at low concentrations in the drinking water of mice resulted in increased mortality, particularly in male mice, together with chronic active liver injury. Deaths were largely due to endemic bronchopneumonia, indicating an impairment of disease resistance (Falconer, 1993).

With regards to humans, in China a high incidence of primary liver cancer among villagers drinking untreated surface water were associated with three risk factors, hepatitis-B virus, intake of aflatoxin from food items such as corn and microcystin from drinking water (Yu, 1989, 1995). In an epidemiological studies of liver cancer incidence in China, it was demonstrated that particular groups of villagers showed greatly elevated rates, some above 60 per 100,000 (Yu 1989, 1995). Overall, liver cancer is a major source of cancer deaths in China and in some areas second to stomach cancer. It was observed that mortality rates from

liver cancer was higher in villages drinking water drawn from ponds and ditches as compared to those drawing water from deep wells. Cyanobacteria are highest in South East China where the incidence of hepatocellular carcinoma is highest. The populations were encouraged to eat rice instead of maize, and wells were provided to supply cleaner drinking water. Children were vaccinated against hepatitis B. These measures were effective as all three risk factors are being reduced concurrently in China and liver cancer rates have dropped to non epidemic rates, particularly in villages with new wells for water supply (Yu, 1995). It was proposed that the reason why the drinking of ditch and well posed a higher risk of liver cancer was due to microcystin contamination. Surveys of microcystins in these water supplies have shown relatively low concentrations of up to 0.46 ug/l (Ueno *et al.*, 1996). These are relatively lower compared to concentrations recorded for lakes and rivers during *Microcystis* blooms elsewhere in the world, however, it is possible that people in these villages were vulnerable to microcystin as a result of constant exposure to strong carcinogenic aflatoxin and infection with hepatitis B, there is also the potential presence of other carcinogens, pesticides and other natural toxins in shallow surface waters (Falconer, 2004).

Recent epidemiological study of colorectal cancer and its relationship with microcystin in drinking water in china has also proved very informative. This was carried out as a retrospective case control study of eight townships in Haining City. The colorectal cancer rate in the city was 8.37 per 100,000 averaged over the accumulated population of nearly 5 million people. The relative risk increased sharply with drinking water of river (7.9) and pond water (7.7) compared to well water (Falconer 2004). Tap water showed a relative risk of 1.9. Analysis of microcystin in drinking water samples showed negligible concentrations in well and tap water, with average concentrations of 0.14 and 0.11 ug/l in river and pond water respectively. The maximum concentrations of microcystin measured were 1 μg/l in rivers and 2 μg/l in ponds. The concentration of microcystin in the water supply significantly correlated with colorectal cancer rate (Zhou *et al.*, 2002). These epidemiological data for both human liver cancer and colorectal cancer in china are supported by the experimental demonstrations of tumour promotion by microcystin in the livers and colons of rodents (Falconer, 2004). *Lyngbya* one of the common cyanobacteria species found in Ghana is known to cause skin irritation on contact and contains the well-characterised tumour-

promoting toxin, lyngbyatoxin-A (Hakii *et al.*, 1984) and aplysiatoxin (Fujiki *et al.*, 1985). However, nothing is known about their oral toxicities.

Recreational exposure to cyanotoxin

One of the earliest reports of microcystin poisoning through recreational activity as a result of *Microcystis* scum occurred in England in 1989. It was reported that a group of 20 army recruits were carrying out exercises in Rudyard reservoir in Staffordshire. These involved Eskimo roles in which the canoe capsized and righted again. The recruits were also swimming while carrying packs. Two of the recruits were admitted to the medical centre after 4 to 5 days of illness of malaise, vomiting, sore throats, blistering around the mouth, dry cough and pneumonia confirmed by x-ray examination (Falconer, 2004). They also had abdominal tenderness and elevated temperature. Eight other soldiers who had been canoeing were also examined and found to have developed the same symptoms as previously described. There were no evidence of pathogens, but the reservoir contained mass development of cyanobacteria, dominated by *Microcystis aeruginosa* (Turner *et al*, 1990). Microcystin-LR was identified in the sample of the bloom. While inhalation toxicity of microcystin has not received much research attention evidence available shows that nasal application in rodents has a toxicity close to that of intraperitoneally injection, or some 10 to 50 times toxic than oral toxicity (Fitzgeorge *et al.*, 1994).

A coroner in Wisconsin, USA, recently concluded that a teenager who had been wrestling and diving in a golf course pond with heavy scums of toxic *Anabaena flos-aquae* died through exposure to blue-green algal toxins. The cyanobacterial cells were found in faecal samples and the toxin was measured in scum samples. The teenager and his friend both suffered severe vomiting, diarrhoea and abdominal pain. In the more severely affected individual, this ended in shock and seizer after 48 hrs of exposure (Behm, 2003). The toxin implicated was anatoxin-a. Anatoxin-a is a fast acting neurotoxin, but there are no data on human exposure.

Management of cyanobacteria and cyanotoxins in drinking water reservoirs

Cyanobacteria blooms are an ancient phenomenon, however, their frequency and intensity appear to have increased in the last 50 years (Falconer, 2004). The reasons being largely due to anthropogenic effects (whether through population increase, intensification of agriculture or global warming). Population increase affects water quality in many ways. Increased demand for drinking water results in the construction of new dams to provide reservoir capacity leading to decrease in river flow, resulting in high concentrations of salt and nutrients concentrations in the rivers. Urbanization contributes to nutrient enrichment of lakes and rivers through storm water runoff containing fertilizer from farm lands and gardens, septic tanks overflow and domestic and animal wastes. These serve as a source of nutrient to cyanobacteria resulting in high blooms in urban lakes and rivers, rending them unsuitable for the production of drinking water and recreational use due to potential hazard to consumers and increased water treatment cost. Population growth directly contributes to nutrients to rivers systems through sewage discharge. It has been reported by Falconer (2004) that approximately half of the phosphorus in sewage comes from human waste, with the reminder coming from detergents and industrial products. Siegrist and Boller (1977) estimated that human waste contains 2 to 4 g of phosphorus per person per day. The use of fertilizer on agricultural lands is also a major factor in eutrophication in rivers and lakes. The most widely used form of fertilizer is a mixture of phosphates and phosphoric acids, commonly called superphosphates (Falconer, 2004). In light sandy soils large proportions of this applied soluble phosphate are not retained and will wash out into rivers during heavy rains. Soil erosion, in which clay particles carrying absorbed phosphorus washed into rivers, provide majority of non sewage phosphate. This has resulted in extensive lake and river sediments that carry phosphorus. Such particulate-bound phosphorus can be mobilized to soluble phosphorus under anaerobic conditions in the hypolimnium of lakes and slow moving rivers, becoming available for cyanobacteria growth (Wetzel, 1983; Falconer, 2004). Intensive livestock industries have also been recognised as a potential source of nitrogen and phosphate in animal waste. Global warming is now accepted as a real process and some of the effects of this phenomenon are the increased lake and river temperatures as a result in increased atmospheric and sea temperatures. The distribution of the formally tropical species

Cylindrospermopsis raciborskii into the Northern Hemisphere has been suggested to be the response of global warming (Padisak, 1997; Briand et al., 2004).

The ultimate approach in reducing cyanobacteria blooms in rivers, lakes and reservoirs is to reduce nutrients inputs into these water bodies (WHO, 1999; Zalewski, 2002; Falconer, 2004). However, increasing emphasis is been put on whole-Catchment management for the reduction of nutrient inputs into surface water bodies. Since catchment areas may contain land with wide range of ownership, such as land totally owned by water utility, national parks, leasehold lands, freehold lands used for farming, small urban areas and isolated housing, considerable cooperation is required. Catchment groups with interest in education and coordination role have to be established with representation from landlords, water supply agency, local councils and other concerned groups. However, due to the economic and financial implications in catchment management, direct participation of political and government participation of is needed for a successful change. Changing land-use practices is difficult and education through catchment groups and provision of expect help is needed. Changes in fertilizer type, to lower-solubility slow release forms, has reduced the magnitude of cyanobacteria blooms (Lukatelich and McComb, 1981). Apart from nutrients control and catchment management, the management of reservoir hydrology such as reservoir mediation, destratification, flow management, phosphorus precipitation, sediment capping, dredging, algaecides and biological mediation have all been suggested as possible ways of controlling cyanobacteria blooms (WHO, 1999; Falconer, 2004).

Drinking water treatment with respect to cyanobacteria and cyanotoxins

From the viewpoint of safety of drinking water supplies, the major area of concern is the water-soluble cyanobacterial toxins. Lipid-soluble toxins are bound to cells or particulate fragments and could be removed by coagulation and sedimentation in standard water treatment (Falconer 2004). Drinking water treatment began in response to high levels of waterborne diseases such as dysentery, typhoid and cholera, transmitted through faecal contamination of food and water in urban populations (Falconer, 2004). Of all the routes of cyanotoxin exposure to humans, drinking water is in a worldwide view, the main source (Dietrich and Hoeger, 2005). The published cases of cyanobacterial toxins in raw

water, during drinking water treatment and even in finished water are numerous (Dietrich and Hoeger, 2005). Microcystins have been reported in final drinking water in many countries including Argentine, Australia, Bangladesh, Canada, Czech Republic, China, Finland, France, Germany, Latvia, Poland, Thailand, Turkey, Spain, Switzerland and USA (Westrick, 2003; Hoeger et al., 2004a, 2004b). The most common and older water treatment systems follows the flow diagram illustrated below (from left to right, across the top of the figure and down the right hand side) adopted from Falconer (2004). This involves pre-oxidation with chlorine, flocculation and coagulation of suspended materials. To obtain optimal performance, the pH of the incoming water is adjusted. The most common and frequently used coagulant is aluminium sulphate, but ferric and ferrous salts are also used. This is followed by sedimentation of flocs of metal hydroxide with entrapped organisms and organic debris, which is the main element of water purification (Gray, 1994).

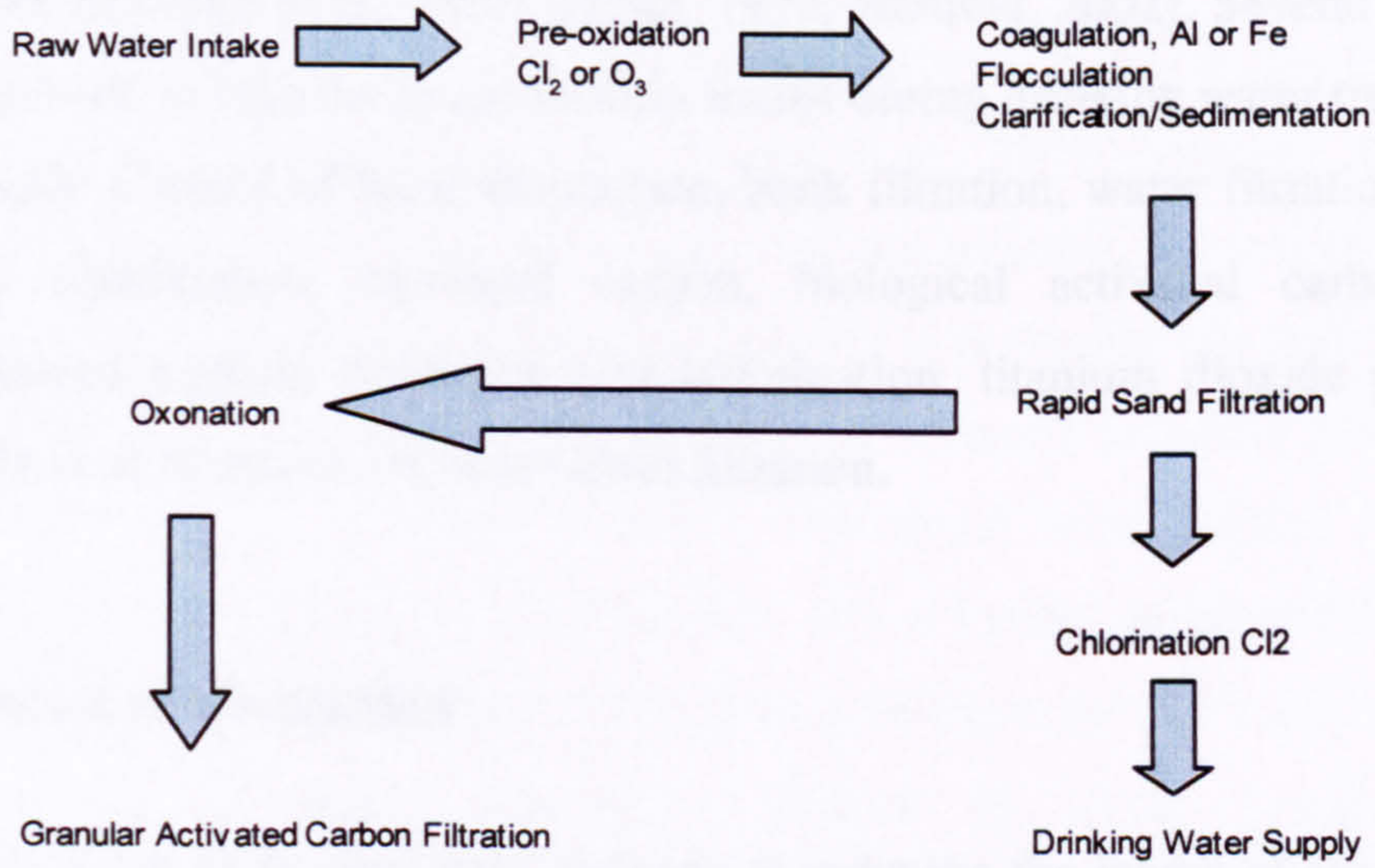

Figure 6 Simplified diagram of a drinking water treatment plant (adopted from Falconer, 2004)

Sedimentation is mostly achieved by three methods, either by gravitation in slow moving tanks after stirring, sludge blanket, in which the water and new flocs are forced upwards through a layer of old flocs which act as a filter or finally dissolved air flotation, in which compressed air is dissolved in water and then released from pressure at the base of the tank holding the flocs (Falconer, 2004). This forms fine bubbles, which trap the flocs and carry them up and out of the tank. Even though this removes most of the sedimented materials, filtration of the water is needed to bring about a bright, clear drinking water and this is done by rapid sand filtration with a mixture of crushed antracite (hard coal) and sand in the filter bed (Gray, 1994). The final stage in drinking water treatment prior to distribution to consumers is chlorination to render the water safe from bacteria and if operated well prevent almost all pathogens from being distributed in the water. The disadvantage of this process is that harmful organic compounds such as pesticides, some industrial wastes pharmaceuticals heavy metals, cyanobacterial toxins, unpleasant taste and odour producing substances in solution will not be removed and will cause adverse human health effects (Byth, 1980; Falconer et al., 1983; WHO, 1999; Hiztfeld, 2002). Several methods have been used to remove cyanobacteria toxins during drinking water treatment. These include Control of bank abstraction, bank filtration, water filtration, coagulation and clarification, activated carbon, biological activated carbon, powdered activated carbon, ozonation and chlorination, titanium dioxide photocatalysis, slow sand filtration and membrane filtration.

Control of Abstraction

One of the most applicable methods of reducing the intake of cyanobacteria cell into drinking water treatment plants is to regulate the depth of the water intake. Cyanobacteria due to their dependency on sunlight for production are normally found in the first few meters of the water column normally referred to as photic zone. Intakes well below the surface will lesson the possibility of drawing cyanobacteria cells into the treatment plant. The location of intakes is also a major factor determining the immediate cyanobacteria concentrations, as intake downwards of the prevailing air movement across the lake surface and especially in sheltered bays will accumulate cyanobacteria scum (Hrudey et al., 1999; Falconer, 2004). Cyanobacteria species like *Cylindrospermopsis raciborskii* and

Planktothrix rubescens will form peak cell concentrations deep in the water, taking advantage of the higher nutrient concentrations in the metalimniom and their ability to photosynthesize at low light intensity (Skulberg et al., 1999; Humbert et al., 2001; Reynolds et al., 2002; Falconer, 2004). When these species are known to be present depth profiles of cyanobacterial concentrations are required to determine the best level for water abstraction (Hrudey et al., 1999). Floating barriers have been known to prevent cyanobacterial scums forming around intakes in small systems with single depth intake (Hrudey et al., 1999), this will reduce the quantity of toxin released in the vicinity of the intake when lysis of a bloom is occurring in an adjacent scum. This physical barrier often only extends to a depth of 0.5 to 1 m and does not affect bulk flow or horizontal water significantly.

Bank Filtration

A widely used method of obtaining good quality water for a drinking water treatment plant is to draw water from shallow wells along the banks of a reservoir or river beneath the river beds (Hrudey et al., 1999; Falconer, 2004). In these cases, the ecology of the river or lake bed and adjacent floodplains is very crucial for success. A study by Chorus et al., (1992) to determine the removal of taste and odour from cyanobacterial blooms by bank filtration provided promising results. Because of the generally positive experience with respect to removal of suspended materials, micro-organisms and a variety of chemical contaminants (Laszlo, 1984; UNDP/WHO, 1992) it may be expected that bank filtration will be a highly promising abstraction method to avoid contamination with cyanobacterial cells as well as dissolved toxins. Laboratory based experiments have demonstrated that both adsorption and degradation of microcystin can occur in lake sediments. Lake water to which both microcystin and *Microcystis* cultures were added showed over 90% removal of free toxins when passed through sediment and soil column over a week (Lahti et al., 1996). Soils with high clay content or organic carbon contents showed the highest adsorption when the hepatotoxin nodularin was passed through five different soils (Miller et al., 2001). The disadvantages of bank filtration have been identified as channelling, whereby water moves directly from the lake or river into wells without any filtrations or

delay, impervious substratum, such as clay and highly saline ground water which is unsuitable for drinking water (Falconer, 2004).

Use of Algicides

Algicides are used in reservoirs to control cyanobacterial growth and to prevent or reduce to some extent the problems of toxins in the associated drinking water supply (Hrudey et al., 1999). Their role in the management scheme may be to provide effective short-term control of growth of cyanobacteria at one point in time, particularly in circumstances where alternative drinking water sources are not available. Algicides treatment has been reported to be more cost effective than toxin removal in drinking water treatment plants, as has been suggested for the control of off-flavour problems (McGuire and Gaston, 1988). Experience with abatement of off-flavours caused by cyanobacteria using algicides has demonstrated that this treatment actually enhances the problem by development of species resistant to the treatment (Izaguirre, 1992). Environmental concerns have been raised because the most commonly used algaecide, copper sulphate, has broad ecological impacts (Hrudey et al., 1999) and should be used only in dedicated water supply reservoirs in special circumstances but not for long-term solution. Algicides, like all management techniques, must be applied correctly in order to be effective. Algicides must be applied at the beginning of the growing season when cell densities are low in order to avoid potential liberation of high concentrations of intracellular toxins that are associated with large blooms (Hrudey et al., 1999). This has an added advantage of enhancing the effectiveness of treatment because cyanobacterial cells can form a major part of the copper demand along with other organic matter in natural water. A major limitation of any agent which disrupts cyanobacterial cells is the release of toxins and taste and odour compounds from the cells. Cyanotoxins are substantially retained in healthy cells in the water and are only released at an advanced stage of bloom senescence or following treatment with chemicals such as algicides (Lahti et al., 1996). The release of cyanotoxins can be very rapid and has been shown to occur within 3-24 hours (Jones and Orr, 1994; Kenefick et al., 1993). If there are no additional facilities for removing dissolved toxins then conventional water treatment with flocculation and filtration will not be able to remove the dissolved toxins. The dangers of treating dense blooms with algicides was demonstrated in

an incident which occurred on tropical Palm Island, Australia, where members of the community became ill with hepatoenteritis following treatment of the water supply reservoir with copper sulphate (Bourke et al., 1983). Jones and Orr (1994) suggested that if algicides are used to control toxic cyanobacteria, the reservoir should be isolated for a period to allow the toxins and odour to degrade. Unfortunately there are no data available on the withholding period but it could be in excess of 14 days. Algicides treatment is not always successful due to inadequate dispersal and contact with target organisms, variable sensitivity of cyanobacteria and reduced toxicity due to complexation of copper (Burch et al., 1998). The form of copper most toxic to aquatic organisms if the free cupric ion (Cu^{2+}) and this can be reduced by complexation with both inorganic ligands under alkaline condition (McKnight et al., 1983). The most commonly used algicides is copper sulphate. Records of use of copper sulphate date from 1890 in Europe (Sawyer, 1962). Copper sulphate has been described as algicide of choice because it is economical, effective, relatively safe and easy to apply. It is considered to be of limited significance to human health at the doses commonly used (WHO, 1996) and has been considered not to cause extensive environmental damage (Elder and Horne, 1978; McKnight et al., 1983). The latter point has been an issue of debate as copper sulphate tends to accumulate in lake sediments (Sanchez and Lee, 1978; Hanson and Stephan, 1984). In a study of 10 dugouts (small reservoirs) in Canada, sediment copper previously accumulated from copper sulphate treatment was released back into the open water under low dissolved oxygen conditions in the hypolimnium in summer (Prepas and Murphy, 1988). Hanson and Stephan (1984) reported that sediment-bound copper could have an adverse impact on the benthic macroinvertebrate community. Fish kills may also occur following copper sulphate treatment, although it is not clear whether this is due to copper toxicity or oxygen depletion (Hanson and Stefan, 1984). The problem of precipitation loss of toxic copper under alkaline conditions has been long over come by the use of Chelated copper algicides. Chelated copper algicides are available as commercial preparations (Hoffman et al., 1982) or by simultaneously dosing copper sulphate and citric acid (Raman, 1988). It is claimed that the use of citric acid as a chelating agent enhances the solubility of copper allowing it to remain in solution longer under alkaline conditions (Raman, 1985; 1988). Raman (1988) recommended the application of copper sulphate:citric acid in the weight ratio 2:1 in high alkalinity waters (>40 mg/l

$CaCO_3$). Other chemicals used as algicides are potassium permanganate and chlorine (Holden, 1970)

Aeration and Air stripping

Aeration and air stripping are methods for contacting air with water in drinking water treatment that is required for various purposes, such as oxidation of iron and manganese from soluble to insoluble forms to prevent reducing conditions which may yield odorous compounds (Hrudey et al., 1999). It is also to remove dissolved gases such as carbon dioxide, hydrogen sulphide and other reduced sulphur compounds and volatile organic compounds (Hamann et al., 1990). However, neither aeration nor air stripping will be effective for removing soluble toxins, nor could they be effective for removal of cyanobacterial cells.

Water filtration, Coagulation and Clarification

Cyanotoxins, especially microcystins, nodularins and a substantial portion of cylindrospermopsins are contained within the cyanobacteria cells, therefore, the first priority in the removal to cyanobacterial toxins is the removal of whole live cells from the drinking water ((Berg *et.al.*, 1987; Kenefick *et.al.*, 1993; Jones and Orr, 1994; Lahti et al., 1996; Hitzfeld et al., 2000; Drikas et al., 2001). Cells may lyse during transportation through pipelines (Dickens and Graham, 1995), particularly, those with a substantial drop in height between the reservoir outlet and treatment work. The extent of cell lysis depends on the degree of pressure reduction and the cyanobacteria genus involved (Falconer, 2004). Dickens and Graham (1995) reported that *Microcystis* are more robust whereas *Anabaena* is easily disrupted, *Planktothrix* and *Cylindrospermopsis* are intermediate. Preoxidation by chlorine dosing of the incoming water will lead to cell lysis, liberating the free toxins into the water (Hitzfeld et al., 2000). In an experimental evaluation of the effects of prechlorination, Lam et al., (1995) found 64% release of intracellular microcystin after dosing with chlorine. An additional disadvantage of prechlorination is the formation of chlorinated organic molecules early in the treatment process called disinfectant by-products. These include a wide range of chloro- and bromoorganic molecules, many of which had been reported as

harmful (Gray, 1994; Rositano et al., 1995). According to Narotsky et al., (1997), trihalomethane a chlorination by-product in drinking water may lead to rectal, intestinal and bladder cancer, similarly haloacetic acids are also known to induce liver cancer in mice (Daniel et al., 1992). Thus prechlorination of raw water with cyanobacteria must be avoided in drinking water treatment as it results in the release of free toxins and chlorinated by-products both with potential adverse effects on human health. The World Health Organization (WHO) has set up a provisional guideline values for both dichloroacetic acids (5μg/l) and 100μg/l for trichloroacetic acid (WHO, 1998)

Ozone as a preoxidant appears to be less active in cell lysis at low dose of 1 mg/l (Mouchet and Bonnelye, 1998). Ozone rapidly destroys microcystins in solution and hence preozonation may reduce soluble microcystins while not liberating cell-bound microcystins. Ozone is also known to assist in coagulation and removal of cells (Falconer, 2004). On the basis of these advantages, preozonation as an initial step in drinking water production from raw water with live toxic cyanobacteria cells have significant benefits. Karner (2001) reported that potassium permanganate pretreatment reduce total microcystins by about 50% which was likely to include cell-bound toxins as well as dissolved microcystin. If preoxidation with chlorine, potassium permanganate and ozone is omitted in drinking water treatment, then majority of live cyanobacterial cells will enter the coagulation and clarification stage. The proportion of cyanobacterial toxin contained in the cells at this step will depend on the species present and growth phase of the cyanobacterial cells (NRA, 1990). In toxic *Anabaena* and *Cylindrospermopsis*, large proportion of the toxins will be free in the water phase, hence cells removal at the flocculation/clarification step in drinking water treatment can not be relied upon for total toxin removal, however, it will have a remarkably beneficial effect (Rositano and Nicholson, 1994; Falconer, 2004). The process of coagulation, clarification and filtration in drinking water treatment are efficient in removing particulate content of raw water, including protozoa, bacteria, cyanobacteria, viruses and general debris (Falconer, 2004). Coagulation promotes the aggregation of small, dispersed particles into larger particles which can be separated by sedimentation, filtration or floatation (Grohman et al., 1985; Hamann et al., 1990). Gray (1994) reported that alum coagulation removes about 90% of faecal indicator bacteria and up to 99% of viruses present in raw water. Hart et al., (1997) reported that application of alum reduced intracellular

microcystins with the percentage removal increasing with increasing alum dosage reaching a plateau of about 90% of the original content, while dissolved microcystin concentration remained unchanged. The alum coagulation dosage needed for effective flocculation is determined by the alkalinity of the water and the cyanobacteria cell concentration (Mouchet and Bonnelye, 1998). It is proportional to the sum of alkalinity and the logarithm of cells number. Alum and ferric salt flocculation do not lyse *Microcystis* cells (Drikas et al., 2002) which are effectively removed intact. Soluble toxins present in raw water are essentially not affected by the flocculation/coagulation process in drinking water production. Measurement of dissolved toxin removal across the alum flocculation/sedimentation process in a small water treatment plant showed only 0-39% microcystins removal present in the natural raw water (Lambert et al., 1996). In general the conventional water treatment system illustrated above will remove intact cyanobacterial cells at moderate concentrations with reasonable effectiveness, but will not remove dissolved toxins (Hoffman, 1976; Keijola et al., 1988; Himberg et al., 1988; 1989; Hitzfeld et al., 2000; Lahti et al., 2001). The process used for the removal of flocculated material containing cyanobacteria cell may affect the outcome of the flocculation/clarification stage of drinking water process. Three alternative methods are in widespread use as mentioned before; sedimentation tanks followed by rapid sand or dual-medium filters are the most common. Dissolved air floatation followed by rapid dual-medium filtration can be highly effective in removal of *Microcystis* and *Anabaena* from raw water and had been applied to both high and moderate capacity treatment plants (Falconer, 2004). Sludge blanket filtration with upward flow has been suggested as the most effective system of water clarification and is capable of use at a range of rates of water flow (Gray, 1994). Water extracted from the sludge of flocculants is added back to the raw water intake. In the case of trapped cyanobacteria cells, the death of the cells in the sludge will release toxin back into solution. This has been recently investigated by Drikas et al., (2002) and has shown an almost quantitative release of microcystin from cells in sludge within 2 days of separation. The soluble toxin concentration in sludge remained high for a further 4 days, when microbial degradation reduced the concentration over a subsequent 10 days (Drikas et al., 2002).

Activated Carbon

To reduce the potential risk to human health from cyanobacterial toxins through drinking water, an additional treatment step has been investigated in which activated carbon is included in the process. This material has also been used extensively to absorb organic pollutants such as industrial chemicals in water treatment (Falconer, 2004). The earliest research into the use of activated carbon for the removal of cyanobacterial toxins from water supplies was done in South Africa as a result of substantial contamination by toxic *Microcystis* in a major water supply reservoir near Pretoria (Hoffmann, 1976). An investigation at a pilot plant with granular activated carbon have shown high performance in toxin removal, with greater than 90% removal of microcystins from an initial concentrations of 30 to 50 μg/l. In this study, 7,000 to 10,000 litres of water were treated via the activated carbon bed before its absorption efficiency dropped to less than 63% (Bernezeau, 1994). Under large scale commercial water treatment plant conditions, a granular activated carbon filtration system may have a bed depth of 1 to 3 m with a contact time of 10 to 15 min and a flow rate of 12 m/h (Falconer, 2004) and this will provide for an extended working life of the filter. High organic matter content of the water reaching a granular activated carbon filter would reduce the working life of a granular activated carbon filter; therefore the effectiveness of the coagulation/clarification system prior to activated carbon filtration will determine the life of the filter. It has also been showed by Lambert and co-workers (1996) using an electron microscope, that extended use of granulated activated carbon leads to the development of biofilm on the surface. In this study, activated carbon that had been in use for about 5 month continuous was compared with a new unused carbon from the same supplier. A reduction in absorption capacity in the used carbon was observed, which could be eliminated by crushing and or exposing fresh adsorption sites. Biodegradation of biofilm does not seem to occur (Falconer and Runnegar, 1983; Falconer, 1989; Lambert et al., 1996). The operational difficulty associated with activated carbon treatment is that the normal filter backwashing practices will displace the carbon into the backwash water at considerable cost to the treatment plant. This can be overcome by gentle backwashing to retain the carbon in the filters. Experimentation has shown that chemically activated wood-based carbons are more effective in microcystin adsorption (Drikas et al., 2002). The performance of the carbon was

rated as (Coal, >wood>peat/coconut), the reason being probably due to the different pore sizes relative to the microcystin molecule (Donati et al., 1994). It is recommended that measurement of toxin adsorption capacity be carried out prior to purchase of large quantities of granular activated carbon.

Powdered activated carbon is recommended for temporary toxin removal for water treatment plants that are subjected to intermittent contamination by cyanobacterial blooms. For example, during only one or two summer months each year (Falconer, 2004). Powdered activated carbon can be added directly to the water prior to coagulation or filtration. This technique is widely used when taste and odour occurs in treated supply, which may be at cyanobacterial cell concentration too low to be of a health hazard. Powdered activated can be incorporated at several points of a standard water treatment system. The effectiveness of toxin absorption is affected by competition between organic material in the water and toxins for absorption sites and also by the presence of ionic compounds and chlorine and its by-products (Falconer, 2004). For effective toxin removal a contact time of 30 min has been recommended (Falconer and Runnegar, 1983; Falconer et al., 1989; Hart et al., 1993) with a dosing capacity of 10 mug/l toxin: >200 mg/PAC/l.

Ozonation

Ozone is an exceptionally powerful oxidizing agent widely used in water treatment in developed countries to remove potentially harmful organic pollutants and for disinfection (Langlais et al., 1991). It has therefore been extensively examined as a technique for the removal of a wide range of cyanotoxins. Ozonation may be used as a single or multiple applications in the water treatment plant as an early (pre-ozonation) or late phase (intermediate ozonation) in the water treatment process. Pre-ozonation with 0.5-1.0 mg/l in water treatment will inactivate bacteria (Lee and Deininger, 2000), virus and protozoa and detoxify harmful compounds such as phenols, polycyclic aromatics and microcystins. Undesirable taste and odor substances are also eliminated (Lee and Deininger, 2000). Furthermore other natural organic matter is modified to products that are more easily absorbed and filtered (Siddiqui et al., 1997). In water, two pathways for oxidation of organic pollutants by ozone have been described (Langlais et al., 1991; Masten and Davis, 1994). These are, direct attack by molecular ozone via

cycloaddition or electrophilic reaction and indirect attack by free radicals (primarily OH) formed by decomposition of ozone. The mechanism involving cycloaddition in water usually results in the formation of aldehydes, carboxylic acids, ketones and or carbon dioxide (Hitzfeld at al, 2000). The electrophilic attack by molecular ozone probably occurs on atoms carrying negative charge such as N, P, O or nucleophilic C. An indirect attach by free radicals generally occurs via one of the following three pathways, hydrogen abstraction, electron transfer or radical addition (Hitzfeld et al., 2000). Studies of the effect of ozone on the removal of microcystins and nodularins under laboratory conditions have shown complete toxin removal within seconds to minutes (Rositano et al., 1998; Shawa and Smith, 2001; Newcombe et al., 2002; Hoeger et al., 2002). Hoeger et al., (2002) showed in their study that *Microcystis* cells lyse at concentrations of 1 mg O_3/l, a concentration routinely used in water treatment plants. Ozonation with 1.5 mg O3/l with a contact time of 9 min provides enough oxidation capacity to ensure the destruction of the protein phosphate inhibiting toxins including microcystins after 60 min of reaction time (Hoeger et al., 2002). Ozone is an unselective oxidizing agent and any organic material present in the water will be attached (Bruchet et al., 1998; Hart and Scott, 1993; Rositano et al., 1998; Shawwa and Smith, 2001), so for effective oxidation of toxins present in the water an ozone demand above that of the water is required for complete oxidation of organic material. A summary of microcystins removal by ozone shows that only partial destruction of microcystin has resulted when organic matter was present in the water (Hitzfeld et al., 2000; Drikas et al., 2002). Drikas et al., (2002) recommended that ozone residual should be maintained after oxidation for over 1 min to ensure all microcystins oxidation. Commonly a post clarification zone dose of 1 mg/l is used, however, this has been found to be insufficient to maintain ozone residual under heavy cyanobacterial bloom due to increased soluble organic load. The major problem associated with ozonation is the danger of cell lysis and release of toxin. A second postozonation step, using ozone concentration high enough to oxidized the remaining organic matter and toxin has been recommended by Hitzfeld et al., (2000). A very important parameter in oxidation efficiency is pH ((Hitzfeld et al., 2000). According Hitzfeld et al., (2000) at pH values >7.5, toxins can still be detected in samples. This was due to the lower oxidation potential of ozone under alkaline conditions (1.24V) compared to acidic conditions (2.07V). Ozonation is also known to result in by-

products that they themselves may have adverse health effects (Hitzfeld et al., 2000). These by-products were found to exhibit phosphatase inhibitory activity. Falconer (2004) recommends that water filtration through granular activated carbon after oxidation is advisable. This eliminates the surplus ozone, absorb hydrophobic compounds and acts as substrate for bacteria, which mineralise most of the organic by-products (ketones, aldehydes, acids) produced by the Ozonation step (Lambert and Graham, 1995).

Chlorination

Chlorine disinfection of water has been practised for over a century and has been credited with saving a significant number of lives worldwide on a daily basis (Freese and Nozaic (2004). Numerous references can be found attesting to the significant beneficial effects of chlorine disinfection of water. For example, figures from the United States show that in 1900, the death rate from typhoid was 36 per 100,000 populations, this dropped to 20 per 100,000 in 1910, 3 per 100,000 in 1935 and by 1960 only 20 people were recorded as having died from typhoid fever throughout the entire USA (Tiernan, 1984; Laubusch, 1964). Today typhoid is virtually unknown in the USA and other developed countries (Freese and Nozaic (2004). This dramatic reduction in the typhoid death rate can be solely attributed entirely to chlorination of potable water supplies. Similar figures exist for other water-borne diseases such as cholera and dysentery ((Freese and Nozaic (2004). Unfortunately, untreated or inadequately treated potable water supplies are still the greatest threat to public health in developing countries where it has been estimated that around half of the population do not have access to drinking water (Freese and Nozaic (2004). Statistics provided by Christman (2004) for developing countries states that in 1990 deaths in children under the age of five due to diarrhoeal diseases were a staggering three million. It has been conservatively estimated that in the region of about 25,000 lives are saved daily due to water chlorination already being practiced, but this figure may go as high as 70,000 ((Freese and Nozaic (2004). Chlorination of water alone has been shown to remove microcystins provided the pH is 8.0 or below (between 5 and 8) and there is a chlorine residual of at least 0.5-1 mg/l for a period of 30 min (Nicholson et al., 1994; Senogles et al., 2000c; Drikas et al., 2002). However, it has been found in several operating treatment plants in which chlorine was used

as a postclarification disinfectant, that microcystins were not effectively removed, probably because the total chlorine demand was not been met under conditions of raised organic load (Keijola et al., 1988; Himberg et al., 1989). Chlorine dissolves in water forming hypochlorous acid which is the most effective disinfectant agent. Above pH 5, this starts to dissociate forming hypochlorite ions, with 100% dissociation above pH of 10 (Lawton and Robertson, 1999), therefore pH is the most important parameter in chlorination. In most injury to human health as a result of microcystins poisoning through drinking water, chlorine had been used as post-clarification disinfectant. The efficiency of chlorination has been found to depend largely on the chloride compounds and the concentrations used (Hitzfeld, 2000). Aqueous chlorine and calcium hypochlorite at ≥ 1 mg/l remove more than 95% of microcystins and nodularin, while sodium hypochlorite at the same dose or chloramines achieve 40-80% removal at most (Rositano and Nicholson, 1994; Nicholson et al., 1993; Nicholson et al., 1994). Chlorine has been shown to destroy cylindrospermopsin under conditions that can be met during water treatment at a pH of about 7.5 ($6 > pH < 8$) with a residual of 0.5 to 0.7 (Senogles et a., 2000c; Falconer, 2004). The pH limits for drinking water treatment is 6.5-8.5 (WTH, 1991). Anatoxin-a or saxitoxins could neither be destroyed with chlorine doses exceeding a 30 min chlorine demand nor changes in Ph (Nicholson et al., 1993). The advantages of chlorine are many. It is relatively easy to handle, capital cost for chlorine installation low, it is cost effective, simple to dose, measure and control and has a relatively good residual effect (Freese and Nozaic, (2004). However, the disadvantages of chlorination have to do with the discovery in the 1970s by the US Public Health Services that chlorination of water with organic compounds could result in the formation of trihalomethanes (USPHS Report, 1970; Rook, 1974; Bella et al., 1974; Symons et al., 1975). It has been reported by Freese and Nozaic (2004) that the advantages of chlorinating water far out ways the perceived ill effects of trihalomethanes on human health. This can be seen in Peru in 1991 where based on epidemiological evidence suggesting a statistically weak link between the consumption of chlorinated water and liver cancer (Murphy and Craun, 1990). The Peruvian officials took the decision not to chlorinate much of their country's potable water supply. The ensuing disastrous result was a cholera epidemic which claimed over 4, 0000 lives (Anderson, 1991). In conclusion Freese and Nozaic, (2004) state that there is still no conclusive evidence to indicate that THMs at the levels at which they occur in potable water,

pose any serious health threats to humans. They also concluded that chlorine disinfection has probably been the most successful mass mediation ever administered and chlorination of water still provides obvious benefits to public health that greatly exceed the dangers posed by THMs.

Slow Sand Filtration and Rapid Filtration

Slow sand filtration is an old established method of water purification which relies on the formation of a layer of micro-organisms forming on the surface of the sand, which acts both as a particulate filter and as an active metabolic degradation system for organic molecules (Ellis, 1985). The water flow through the filter is slow, allowing uptake and metabolism of xenobiotics and natural toxins by bacteria and fungi. In a full scale test filter, flow rates of 0.8 and 0.2 m/day were used (Grutzmacher et al., 2002) with ambient temperature between 15 to 20°C. Experimental verification of the effectiveness of slow sand filtration as a method for microcystins removal from drinking water was carried out by Grutzmacher et al., (2002). In this study, a full scale sand filter system was used, employing a holding reservoir supplying two slow sand filter beds, each with a 0.5 m of water above 0.8 m of sand. The filters had earlier been exposed to water containing microcystins, so that the need to precondition the microflora to metabolised microcystin was reduced. Total microcystins removal was about 85%. The conclusions from these experiments is that slow sand filtration operating at warm ambient temperatures can be expected to remove microcystins effectively, so that the technique has application in warm climates where low operating costs and low maintenance are major considerations for drinking water treatment. The large land area needed for these filters is a disadvantage in locations with high land values (Falconer, 2004). Due to evaporative water loss, they are inappropriate for arid areas with high daytime temperature. Rapid sand filtration, another method for removing the floc after flocculation does not effectively remove cyanobacterial cells (Lepisto et al., 1994; Steffensen and Nicholson, 1994). Conventional water treatment requires regular backwashing of the filters, but if this process is not performed adequately, lysis of cyanobacterial cells on the filters can lead to release of toxins into the water (Hoeger et al., 1999).

Aims and Objectives

1. The ultimate objective of this research is to determine the quality of drinking water in Ghana with respect to the cyanotoxin microcystin.
2. The research also assessed the cyanobacteria species and biomass of four reservoirs used for the production of drinking water for the two biggest cities in Ghana and to determine whether current treatment processes in place are capable of removing cyanobacteria cells from the drinking water.
3. The study also looked at the relationship between nutrients and cyanobacteria diversity in the four reservoirs.
4. The experience and results obtained from this research will be extended to cover all reservoirs used for the production of drinking water.
5. Agencies responsible for drinking water production will be informed of the drinking water quality and advice on ways to improve the drinking water quality to World Health Organization acceptable limits suitable for human health.
6. Finally a nation-wide education and public awareness campaign about toxic cyanobacteria, and associated toxins on human and animal health would be undertaken in Ghana.

2.0 General Materials and Methods

Study area

Four drinking water reservoirs were chosen, these are the Weija and Kpong Reservoirs which supply Accra, the capital city of Ghana with drinking water and the Owabi and Barekese Reservoirs situated in the Ashanti Region of Ghana and supplying drinking water to the Kumasi metropolis.

Kpong Reservoir

The Kpong reservoir, located in the Eastern Region of Ghana on 6^{o} 9′ N (Fig. 1) was constructed in 1982 to provide hydroelectricity to supplement power generated from the Volta River dam. It has a total surface area of 38.0 km^2 and a maximum depth of 15 m with a mean depth of 5 m (Ansa-Asare and Ansong Asante, 1998). It is characterised by small Islands forming the Senchi rapids to the North and the Kpong rapids to the south. The water retention time for the Kpong reservoir is 5 days and has a mean annual flow of 1183 m^3 s^{-1}. According Gyima-Amoako (1989), 20 to 25 % of its surface area is covered with aquatic vegetation. The Kpong reservoir is on the Volta river system, which is also used for the production of hydroelectricity, irrigation, recreation and also well known for its fisheries especially the Tilapias.

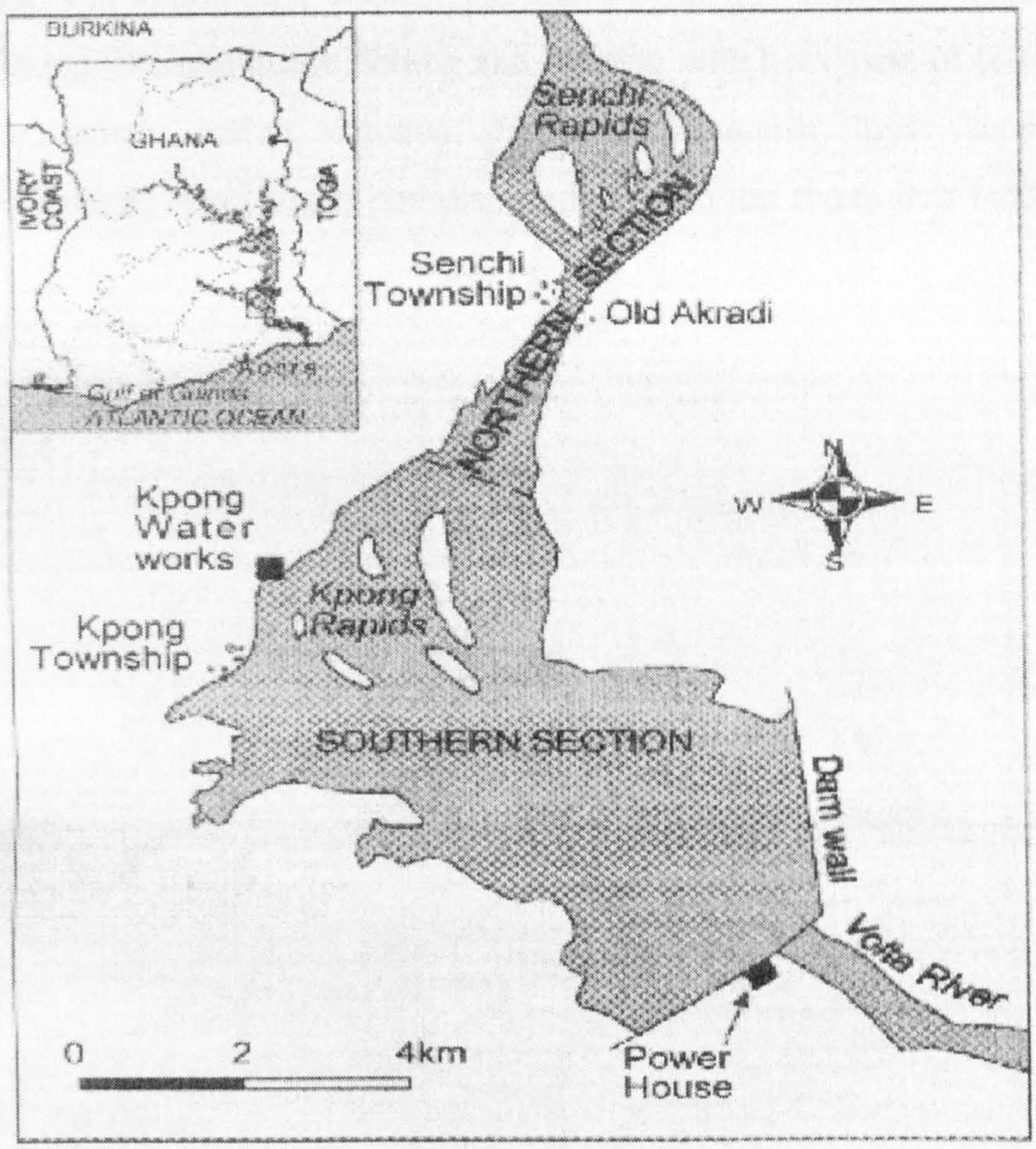

Figure 1 Map of the Kpong reservoir and its catchment area

Weija Reservoir

The Weija reservoir situated in the Greater Accra Region of Ghana lies on latitude 5° 35′ N and 0° 22′ W (Fig. 2). It has a surface area of about 3 km^2 and a depth of 1 to 7 m (Dassah and Abban, 1979) and a mean annual flow of 54.2 $m^3 s^{-1}$ (Ansa-Asare and Ansong Asante, 1998). The Weija reservoir lies in the coastal savannah zone characterised by seasonal rainfall with two peaks in June and October. Rainfall within this study area is generally erratic and low averaging 840 mm a year (Boateng, 1970). The vegetation consists of dense thickets interspersed with small patches of grass. The nature of the soils in the catchment have been reported to be well drained, friable, porous loam savannah ochrosols which are low in nutrients especially phosphorus and nitrogen (Boateng, 1970; Dickson and Benneh, 1970). The monthly mean temperature ranges from 21.7 °C in August to 32°C March (Boateng, 1979). The Weija reservoir is built on the Densu river

system. This river system is under intensive threat from heavy pollution mainly from domestic and agricultural wastes. The main economic activities within the Weija and Kpong catchments are fishing and farming with heavy use of fertilizer. Major crops include maize, cassava, pineapples, banana, sugar cane and vegetables. Domestic wastewaters are disposed off into the rivers that feed both reservoirs.

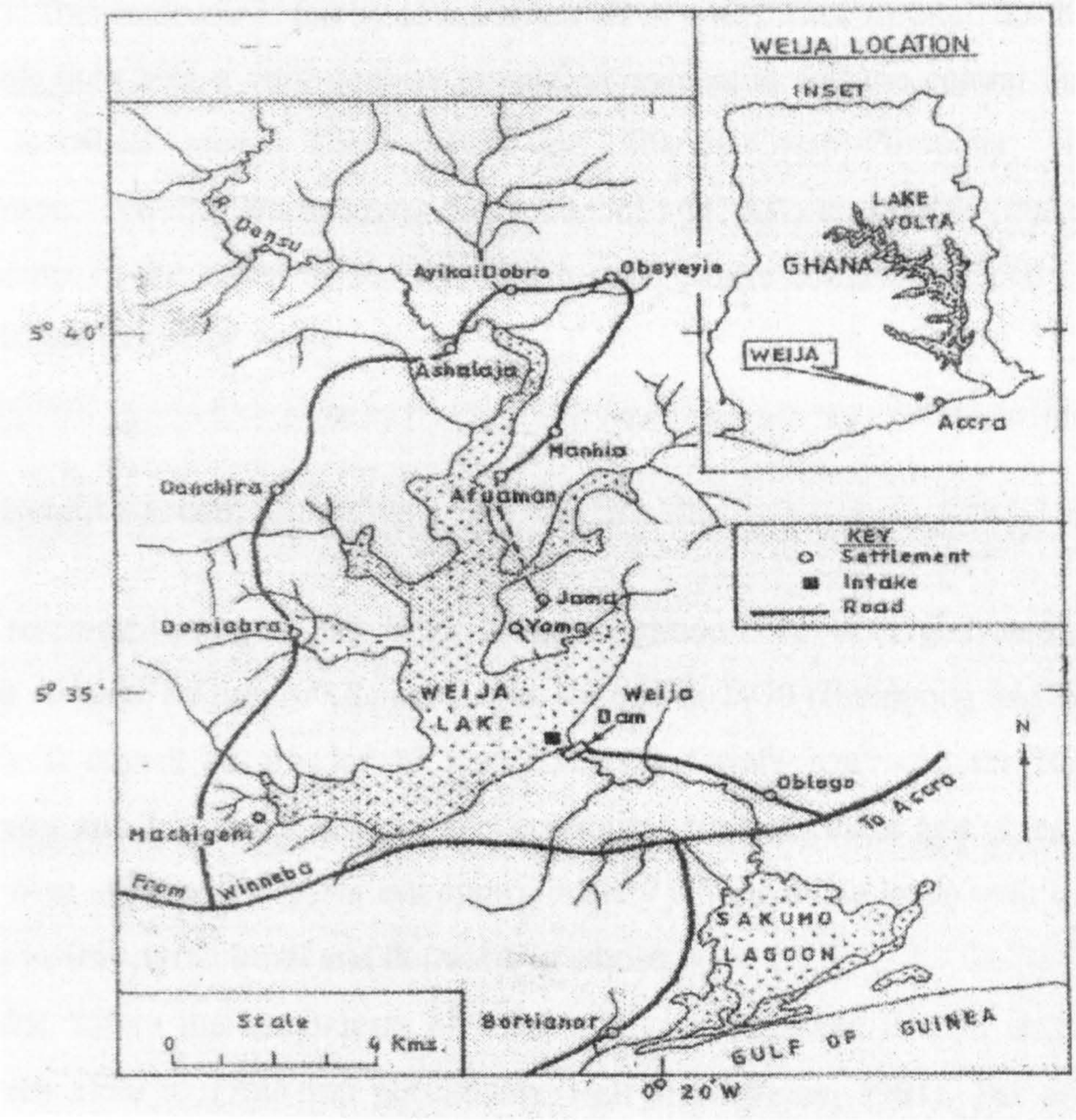

Figure 2 Map of the Weija reservoir in the Greater Accra Region of Ghana

Owabi Reservoir

The Owabi reservoir lies on latitude 6° 52′ N and longitude 1° 43′ W (Fig. 3) and situated in the Ashanti Region of Ghana. It was constructed in 1928 and resulted in the formation of the reservoir with a surface area of about 7 km^2 (Amakye, 2002) and was upgraded in 1954. At present it has capacity to produce 13.6 million litres of water per day from 4.5 million litres per day. It covers an area of 16 km^2. It lies in the closed forest ecological zone of Ghana (Hall and Swaine, 1981). This reservoir is fed by seven rivers all of which flow through the Kumasi Metropolitan area a very densely populated residential and the central business and industrial areas. These rivers are Sukobri-Owabi-Pumpuna, Ntikyei, Anyinasu, Nwabi, Bunkunfuo, Lakyeanpon and Asuokuu. This makes the pollutants in the reservoir mostly of industrial nature consisting mostly heavy metals and industrial waste.

Barekese Reservoir

This reservoir lies on latitude 6° 52′ N and longitude 1° 42′ W (Fig. 3) and located in the Ashanti Region of Ghana. It was formed in 1970 (Frempong and Nijjhar, 1973). It covers an area of 16 km^2. Like the Owabi reservoir, the Barekese reservoir also lies in the closed forest ecological zone of Ghana and is fed by the Ofin river system. The basin lies approximately within arable lands with land use being mainly agricultural and domestic in nature.

Rainfall within the catchments of the Owabi and Barekese is high and ranges between 1500 to 1750 mm per annum (Hall and Swaine, 1981). The soils are therefore deeply weathered and erosion very high and soil nutrients mainly derived from decomposition of leaf fall that accumulates on the forest floor. Both reservoirs also lie in the area with high intensity of poultry farms and wastes from these activities with high nutrient loading find their way directly into the reservoirs. The chemistry of these two reservoirs is therefore expected to be heavily influenced by these land use activities.

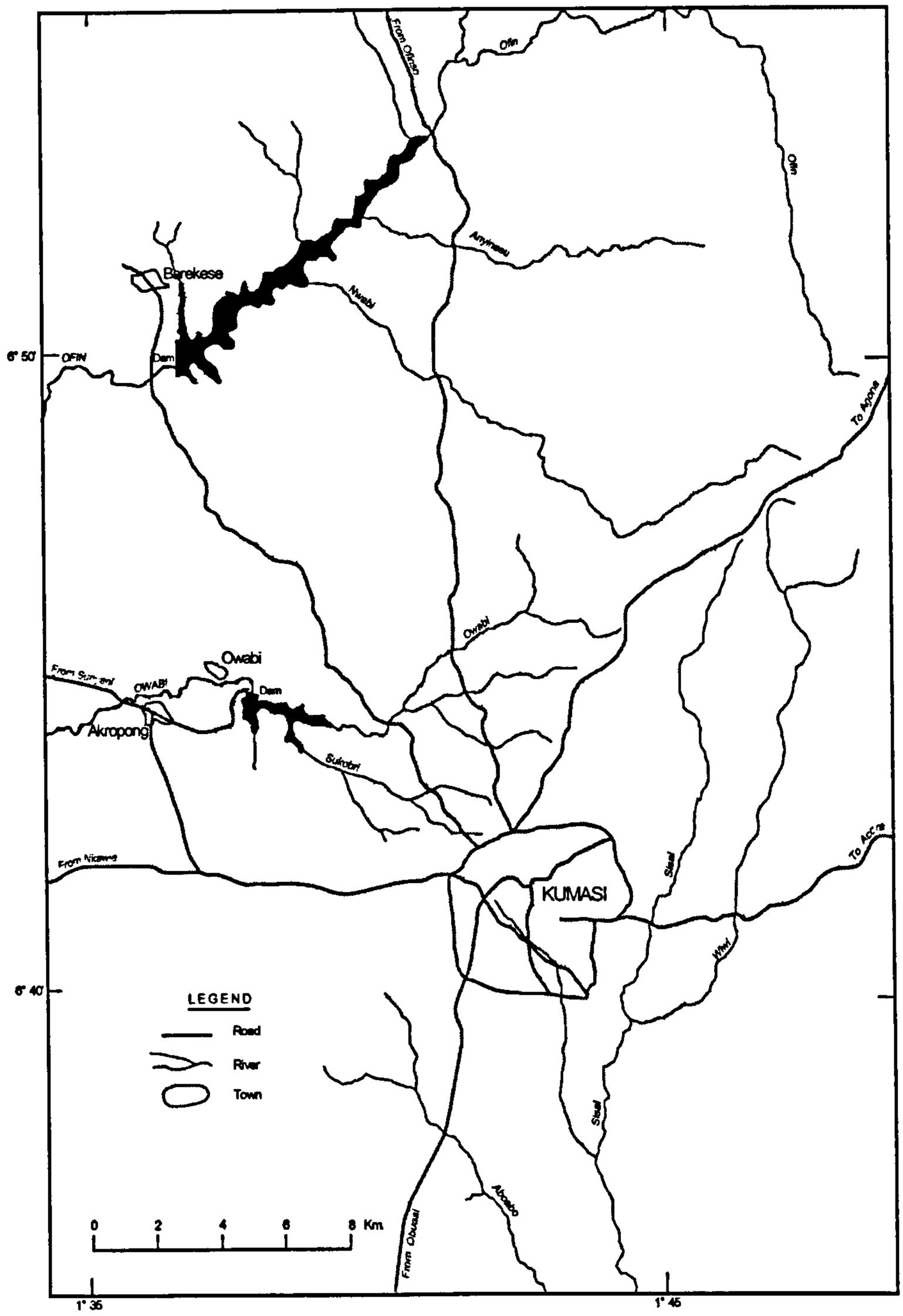

Figure 3 Map of Owabi and Barekese reservoirs in the Ashanti Region Of Ghana

Sampling, sample preservation and identification of cyanobacteria

Samples for cyanobacteria species identification were collected from all the four study sites during a six months field work in Ghana from January to June 2005. In all a total number of 72 samples were collected from the intake and final chlorinated water points of each reservoir. Samples were collected with either a phytoplankton net with a mesh size of about 20 to 25 μm or by simply filling a bucket directly from the reservoir. Net samples were preserved to a final concentration of 2% formalin, whilst water samples for quantitative analysis were preserved in Lugol's solution (Guillard and Sieracki, 2005). Work on identification of cyanobacteria species was carried out at the Institute of Botany of the Czech Academy of Sciences in Trebon, with the assistance of Prof. Jiri Komarek of the University of South Bohemia and the Institute of Botany of the Czech Academy of Sciences, Trebon Czech Republic. Identification was done using a model: Olympus light microscope BX 51 manufactured in Japan with objectives 10, 20, 40, 60 and 100. Pictures were taken using a model: Olympus camera C-5050 with a zoom and mounted on the microscope.

Cyanobacteria biomass determination

Cyanobacteria biomass determination was done by direct counting of cell using an inverted microscope as described by Lund et al., (1958 and Lawton et al., (1999). Sedimentation of cells was carried out in counting chambers with a settling time of 4 hours for every 1 cm of water column of the sample (Wetzel and Likens, 1990). This method had been described as the most effective way of handling water samples with a mixture of green algae, diatoms and cyanobacteria (Falconer, 2004) and gives an indication of the potential concentration of toxins in the water. All colonies and filaments were counted as individuals, and the average number of cells determined for 20 individuals and cell concentrations was calculated as described by Addico et al., (2006). The method used will provide data on both the number and genus or species of cyanobacteria present, and the analysis of samples collected over a period of six months will show trends in species composition as well as numbers of cells of each species or genus present at a particular time. Cyanobacteria cell numbers can be used as an information base for health warning or treatment modifications and are of value

for recreational water use as well as drinking water (WHO, 1998). The disadvantage of this method is the time consuming task of cell counting, especially in a mixed population of organisms (Falconer, 2004).

Picocyanobacteria

Autotrophic picoplankton (APP) are small sized cyanobacteria in the range of 1-4 μm (Crongerg and Annadotter, 2006). Picoplankton have been recognized as an important component within the pelagic communities of both freshwater (Hawley and Whitton, 1991; Petersen, 1991; Voros et al., 1991) and marine environments (Takahashi et al., 1985). Sub-samples for autotrophic picoplankton (APP) counting were preserved in formalin to a final concentration of 1.2 to 2%. A Volume of 1 to 2 ml depending on the concentration of the sample was filtered through a 0.2 μm Nuclepore filter (2 cm diameter) prestained with Irgalan black. Dapi (4'6-diamidino-2-phenylindole dihydrochloride) was used to stain the cells for fluorescence of DNA as described by Porter and Feig (1980); Stockner et al., (2000). The APP cells were counted in red fluorescence of phycocyanin and partly chlorophyll -a using Olympus BX60 microscope in the epifluorescence modification under green excitation (510-560 nm) and checked under UV excitation (330-385 nm) for the DNA distribution in the cells. Eukaryotic picoplankton differed from cyanobacteria picoplankton with the chromatophore always occupying on a part of the cell. The images of the cells counted were strong and clearly defined. About 300-400 APP cells were counted per each sample and the counts were converted to APP cells per ml. All data on abundance were expressed in numbers of cells, including the cells inside colonies.

Culture of cyanobacteria strains

In the laboratory preparation for the cultures of cyanobacteria as an experimental material for toxicity analysis was started with the purchase of seven cultures from Culture Collection of Algae and Protozoa, Windermere Laboratory, Scotland. All cultures were grown in a growth chamber in 2 litre flasks. Species were purchased from the culture laboratory of CCAP, Windermere Laboratory, Scotland. The species were *Anabaena flos-aquae* (CCAP 1403/13B), *Microcystis aeruginosa*

(CCAP 1450/3), *Oscillatoria limnetica* (CCAP 1459/18), *Oscillatoria agardhii* (CCAP 1459/23), *Planktothrix* sp. (CCAP 1460/13), *Lyngbya* spp (CCAP 1446/10) and *Merismopedia punctata* (CCAP 1448/2). All these species with the exception of *Merismopedia punctata* are known to produce toxins and have cosmopolitan distribution all over the world. *Merismopedia punctata* a non-toxic cyanobacteria was set up as a control. *Anabaena flos-aquae*, *Microcystis aeruginosa*, *Oscillatoria limnetica* and *Oscillatoria agardhii* were cultured in Jaworski medium (JM) (Thompson *et. al.*, 1988) whilst *Lyngbya*, *Planktothrix* and *Merismopedia punctata* were cultured in BG11. Both media were prepared in the laboratory.

Culture conditions were temperature 25°C, continuous incident irradiance of 20 μmol photon/m^2/s, with constant aeration. Humidity was kept below 60% as described by Senogles-Derham *et.al.*, (2003).

Harvesting of cultured cyanobacteria cells

Cultured cells were harvested in two ways. This depends on the volume and density of the culture.

Filtration

A glass fibre filter of 70 mm diameter was placed in a desiccator under vacuum, removed at regular intervals and weighed until a constant weight was obtained. The filter is then placed in a Petri dish and the weight of the filter recorded. Using the pre-weighed filter a known volume (250-500 ml) of the culture was filtered and the cells collected on the filter. The filter was then returned to the labelled Petri dish and placed in a freezer at -20 °C over night and then lyophilized/dried using the freeze-drier for 24 hours. After the cells were dried, the filter with the dried cells were placed in a desiccator and reweighed. The difference between the initial weight of the filter and the final weight of the filter and the cells gave the dried weight of cells harvested. The filtrate was stored in glass bottles at -20 °C and later used for the determination of dissolved toxins.

Centrifugation

Large volumes of cultures (1 litre) were harvested through centrifugation at 5000 rpm for 15 min using a model: BR401 Denley refrigerated centrifuge, the clear supernatant was stored for determination of dissolved toxins. The cell pellets obtained after centrifugation were transferred into pre-weighed glass tubes and frozen over night and later dried/lyophilized in a freeze-drier for 24 hours. The final weight of a tube and dried cells was determined and stored at -20 °C until needed for cell-bound toxin.

Extraction of intracellular microcystins

Extraction of cell-bound intracellular toxins from culture and field samples were also done by two different methods (Sonication and rotary evaporation method) this was to compare the efficiency of the two extraction methods.

Sonication

A known weight of dried cyanobacteria cells (10-20 mg), exact weight was extracted in a 2 ml Eppendorf tube with 1 ml of 50% aqueous methanol (v/v) using the ultrasonic probe for 30 sec.

The resultant extract in Eppendorf tube was centrifuged using a model: BR401 Denley refrigerated centrifuge for 15 min at 5000 rpm and supernatant transferred into a glass vial. To clean the supernatant prior to HPLC analysis, the supernatant was passed through a 4 mm nalgene nylon syringe filter with pore size 0.45 μm.

Rotary Evaporation

a. Dried cells on filters were extracted in 20 ml of 75% aqueous methanol for 1 hour as described by Harada et al, (1999). The 75% methanol had been recommended by Faster et al., (1998) and used by many authors (Spoof et al., 2003, 2004; Hoeger et al., 2005). The extract was decanted into a rotary evaporation flask and dried in vacuo at 45 °C. This process was repeated three times each time decanting the extract into the same rotary flask and drying. After

all three extracts had been dried, 200 μl of pure methanol was added to the dried extract, mixed and resuspended extract decanted into a 500 μl glass vials. This process was repeated with a second 200 μl of methanol, placing both aliquots in the same vial. The final extract was filtered through a 4 mm nalgene nylon syringe filter with pore size 0.45 μm or a 0.45 μm pore sized mini start non-pyrogenic 16555 Millipore syringe filter. This was considered as a Cell Free Extract (CFE).

b. A second method of extraction of intracellular microcystins from filtered samples was used. This involved the use of an ultrasonic probe for 30 seconds, shaking for 30 minutes and then followed by bath sonication for 30 minutes as described by Faster *et al.*, (1998). The extract was centrifuged as described above. This process was done using a 15-25 ml micro-centrifuge tubes. Filter with lyophilised field samples was cut into pieces and extracted in 10 to 20 ml of 75% methanol depending on the number of filters to be extracted. The shaking, sonication and centrifuging were repeated three times and the supernatants pooled together and dried using a rotary evaporator at 45°C. The dry residue in the rotary evaporator was redissolved in 400 μl of pure methanol, cleaned of any debris and stored at – 20°C until analyses.

Extraction and purification of dissolved (extracellular) microcystins by Solid Phase Extraction (SPE)

Water Samples of approximately 1 litre collected from the drinking water reservoirs and different treatment stages in Ghana were passed through filter papers using the process of vacuum filtration. Additional filtrations through membrane filters with pore size 0.45-1.2 μm were done when necessary. The filtrates were collected and stored at – 20°C until extraction. For toxin extraction, concentration and clean up the filtered water samples were passed through reversed-phase octadecyl (C-18) silanised silica gel (ODS) in 3 ml tube cartridges as described by Meriluoto (1997). The cartridges were activated prior to use with 5 ml pure methanol and subsequently washed with 5 ml of distilled or deionised water as described by Falconer (2004). After conditioning the cartridge, water samples were passed through the column and the permeate discarded. The column

was then washed with 5 ml of 20% of aqueous methanol (v/v), this eluate was also discarded and the column dried for 30 min by applying vacuum. The toxin was finally eluated from the column with 15 ml pure methanol and eluate (methanolic eluate) collected. Further concentration was achieved by drying using rotary evaporation flask. The residue from the rotary evaporation flask was redissolved in 500 μl of 100% methanol on ultrasonic bath for 5 min and transferred into vials for HPLC analysis.

Analysis of microcystins by HPLC

Toxins analysis of extracts of samples from the four drinking water reservoirs were analysed using a reverse-phase high performance liquid chromatography (Agilent 1100 series) with a photodiode array detector (DAD) G1315B, autosampler ALS G1313A with a quaternary pump G 1311A. The stationary phase was Phenomenex Luna 5 μ C18 150 ×4.60 μm. Column temperature was kept at 30°C. The mobile phase A was 0.1% trifluoroacetic acid in water and B; acetronitrile with 0.1% trifluoroacetic acid (Lawton et al., 1996) with a linear gradient of 30 % of B at 0 min to 70% of B at 30 min, with a flow rate of 1 ml/min. Volume of extract injected was 20 μl. Data was gathered between 200-300 nm and chromatograms evaluated at 238 nm. Microcystin peaks were identified by comparison of retention times with those of standards used (MC-LR, MC-LF, MC-LW, MC-RR) purchased from Alexis Biochemicals and their characteristic UV-spectra as per Lawton et al., (1994). All other unknown microcystin peaks at 238 um were identified as MC-LR variants with comparism with MC-LR spectrum (Fig. 4) and toxic fractions quantification by extrapolating HPLC peak areas at 238 nm to a linear calibration curve for microcystin-LR standard (n =5, r^2 =0.999). An example of calibration curve is shown as figure 5.

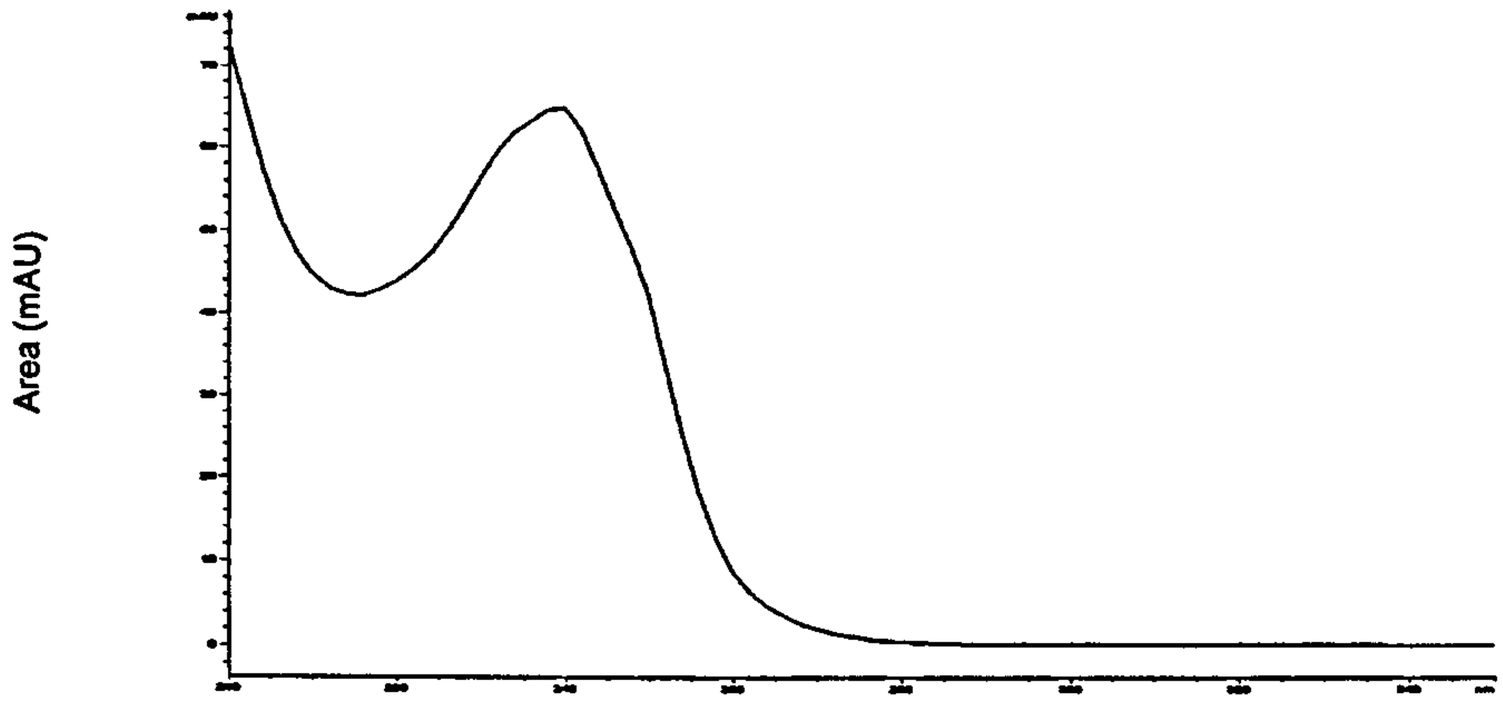

Figure 4 HPLC-DAD spectrum of microcystin-LR spectrum at 238 nm

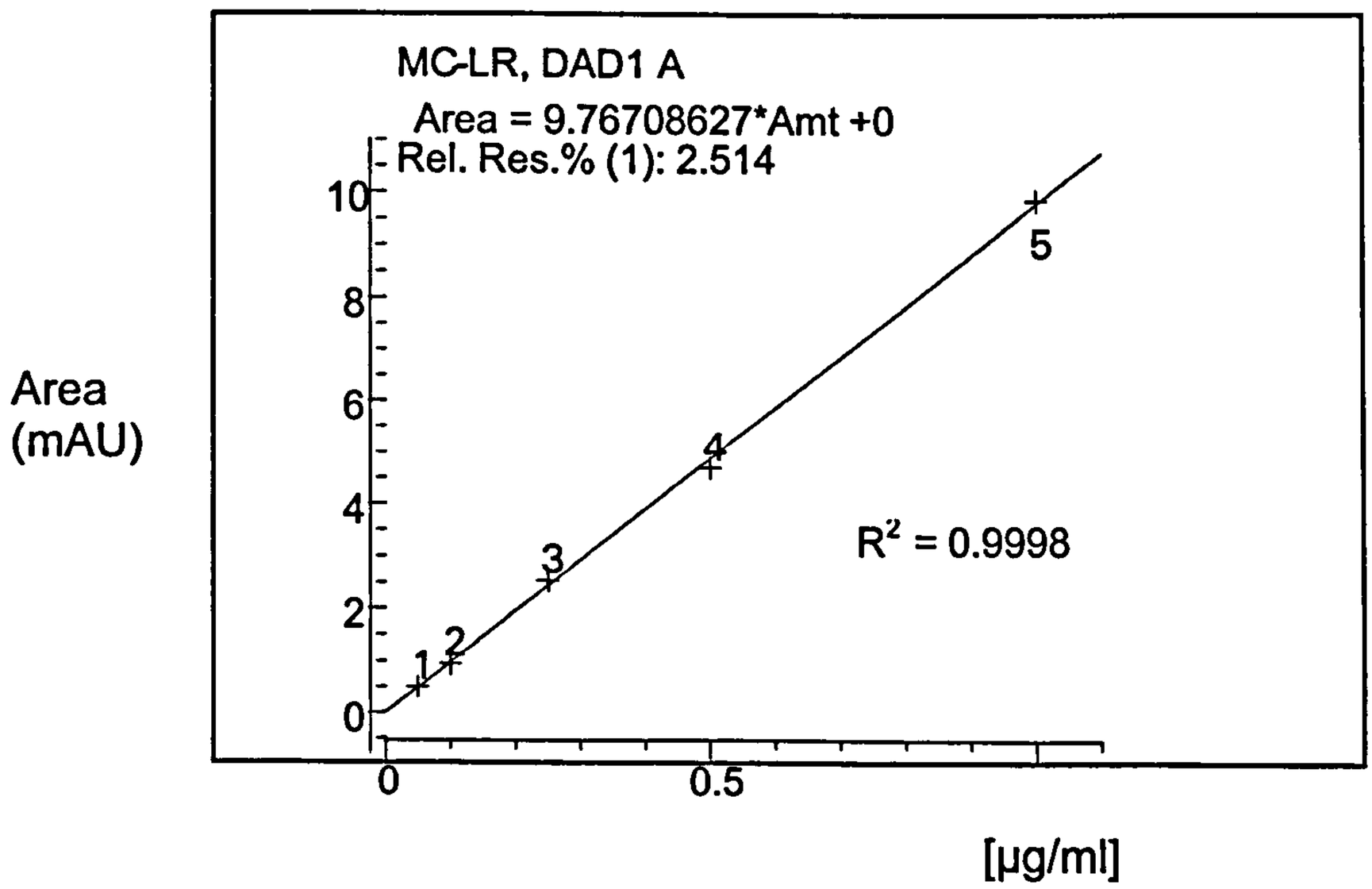

Figure 5 Standard microcystin-LR calibration curve

Nutrients analysis

Water samples for nitrite and nitrate analysis were collected into cleaned 1 litre polyethylene bottles and filtered to remove any cells present in the water. Samples were preserved by deep freezing at – 20°C and 4°C for nitrite and nitrate respectively to prevent bacteria conversion of nitrite to nitrate or ammonia.

Nitrite

Nitrite-Nitrogen was determined by the diazotization method (APHA, 1992) using a spectrophotometer (Ultraspec 11, Model 80-2091-73). The principle behind this method is that nitrite reacts in strongly acid medium with sulphanilamide. The resulting diazo compound is coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride (NED) to form an intensely red coloured Azo compound. The absorbance of the dye is proportional to the concentration (mg/l) of nitrite present. This method is applicable in the range of 0.01 to 1.0 mg/l. The spectrophotometer was calibrated prior to analysis at 5 different concentrations 0.2, 0.4, 0.5, 0.8 and 1 mg/l. The standard curve obtained is shown below. Absorbance was measured at 543 nm.

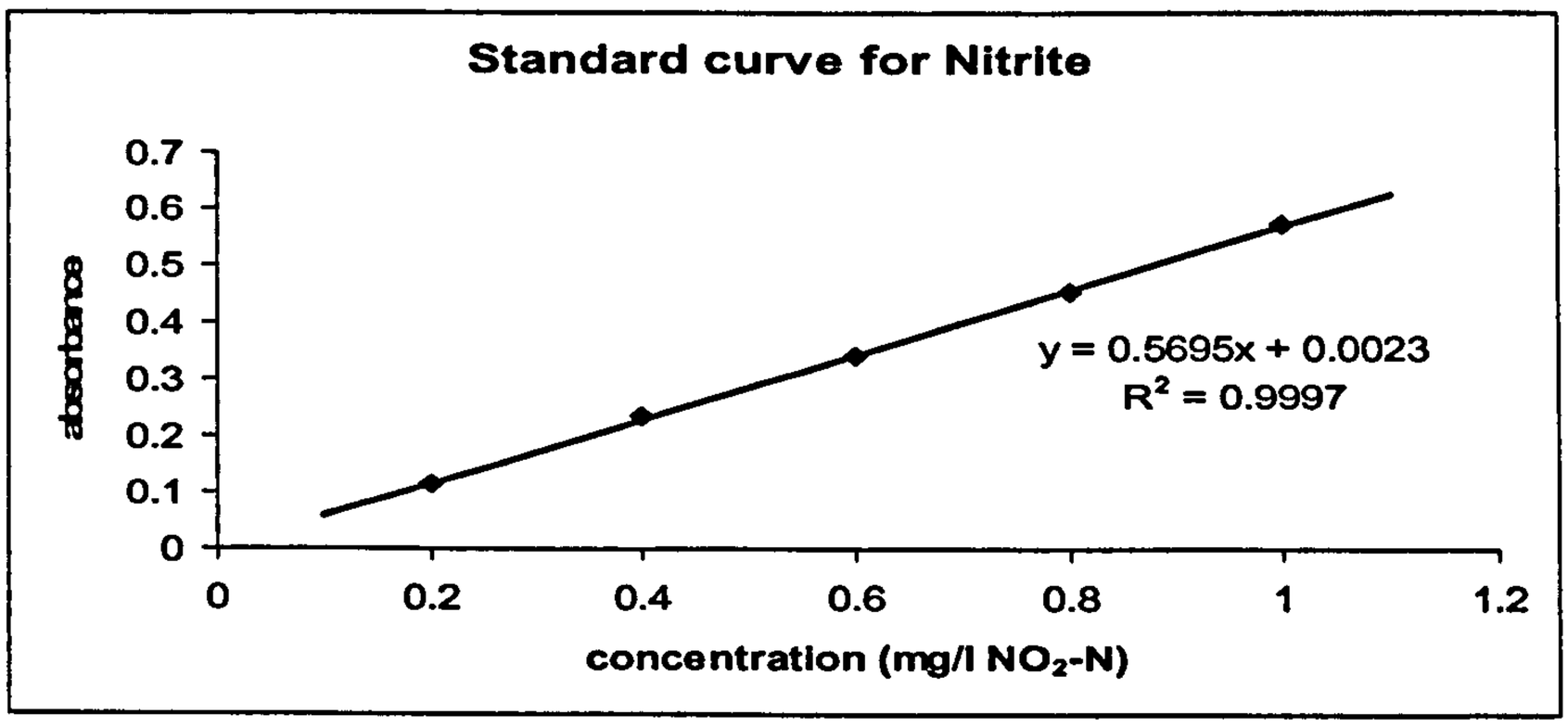

Figure 6 Standard curve for Nitrite (NO_2-N)

Nitrate

Nitrate-Nitrogen was determined by the hydrazine reduction method (APHA, 1992). The principle of this method involves the reduction of nitrate to nitrite with hydrazine sulphate. The nitrite ion originally present plus the reduced nitrate ion is determined by diazotization with sulphanilamide coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly coloured Azo dye which is measured spectrophotometrically. Calibration of the spectrophotometer was done at 5 different concentrations as in the case of nitrite described above. The range of application of this method is 0.01 to 10 mg/l.

Phosphate

Phosphate was determined by the stannous chloride method (APHA, 1992). The minimum detectable concentration is about 0.003 mg/l PO_4-P. Samples for phosphate determination were collected into acid-washed 1 litre polyethylene bottles and preserved at 4°C. Molybdophosphoric acid is formed and reduced by stannous chloride to intensely coloured molybdenum blue. The absorbance of the molybdenum blue at a wavelength of 690 nm is proportional to the concentrations of the phosphate in the samples. Standard phosphorus solutions of known concentrations ranging from 0.01 to 1.0 mg/l were used to calibrate the spectrophotometer prior to analysis of samples. The standard curve obtained is shown below.

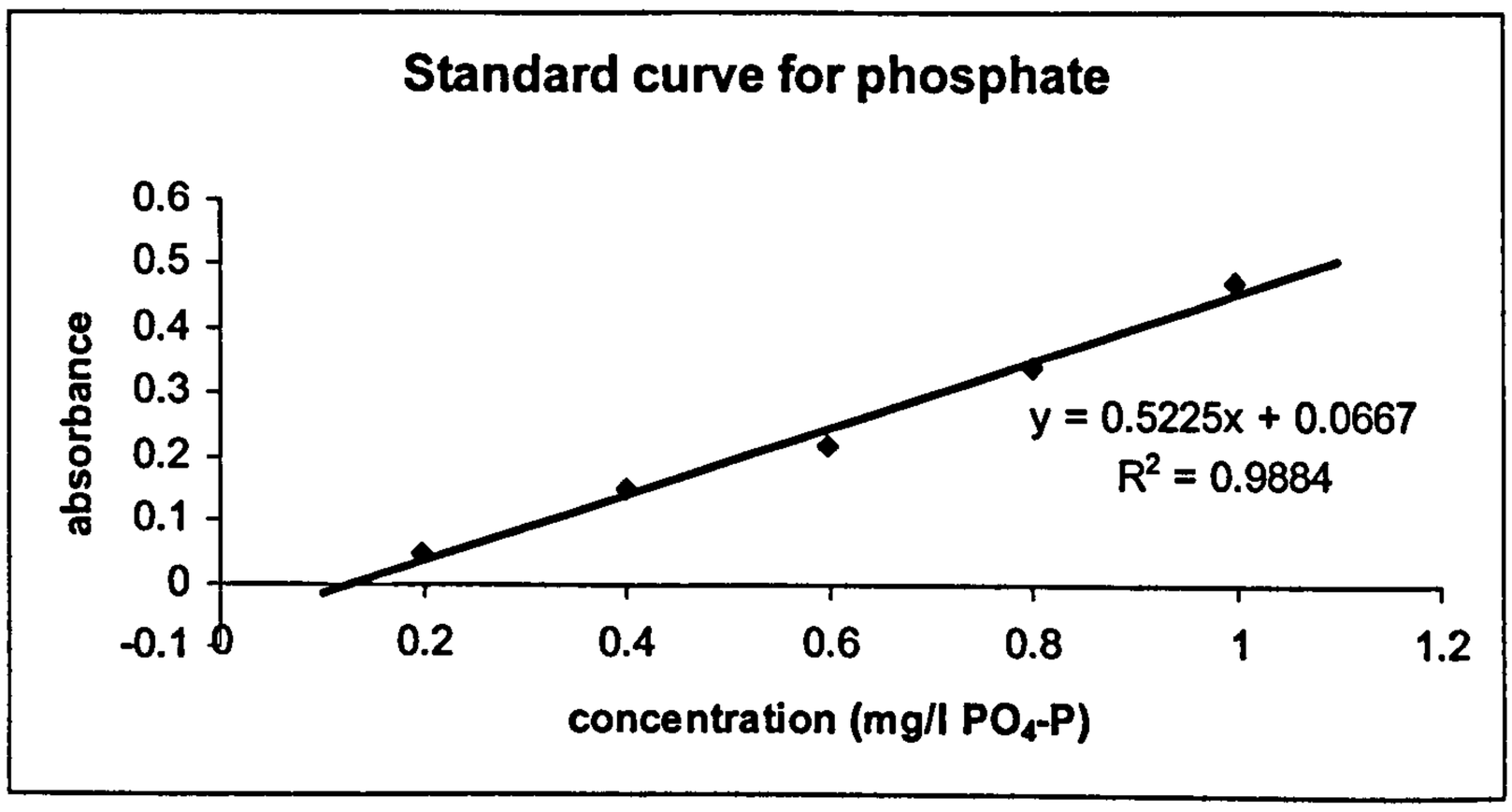

Figure 7 Standard curve for Nitrite (PO_4-P)

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Cyanobacteria species identified in the Weija and Kpong reservoirs, Ghana, and their implications for drinking water quality with respect to microcystin

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The Kpong and Weija reservoirs supply drinking water to Accra, Ghana. This study was conducted to identify the cyanobacteria present in these reservoirs and to ascertain whether current treatment processes remove whole cyanobacteria cells from the drinking water produced. Cyanotoxins are mostly cell bound and could easily be removed during water treatment. However, certain water treatment practices, such as pre-chlorination and the use of algicides, lead to lyses of cyanobacteria cells and the release of toxins into the water. The study shows that the water treatment process in the two reservoirs is not effective in the removal of all cyanobacterial cells. Out of the six cyanobacteria species identified in the reservoirs, four produce toxins: *Anabaena flos-aquae*, *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa* and *Planktothrix agardhii*. These four species constituted about 70–90% of the total algal biomass at all the water treatment stages, including the final product supplied to consumers. Preliminary toxicological analysis of intracellular toxin of samples from the raw water intakes of the two reservoirs indicated the presence of microcystin, with the highest concentration (3.21µg l^{-1}) found in the Weija Reservoir. This study provides the first report of microcystins in drinking water supplies in Ghana.

Keywords: cyanobacteria, drinking water, HPLC, microcystins, toxins

Introduction

In Ghana, the increasing pollution of surface water reservoirs used for the production of drinking water is causing a shift in the phytoplankton composition to mainly cyanobacteria (blue-green algae). Pollution may originate from point sources such as municipal wastes, or from more diffuse sources such as agricultural farmlands with heavy fertiliser use. Other sources are the indiscriminate disposal of domestic garbage and liquid wastes, which are flushed into water bodies when it rains. Recent studies on phytoplankton in some Ghanaian lagoons and estuaries indicated that cyanobacteria comprise either the highest or the second highest component of the phytoplankton biomass (Addico and Frempong 2004). The most common cyanobacteria identified were *Anabaena*, *Oscillatoria*, *Microcystis*, *Planktolyngbya*, *Merismopedia* and *Woronichinia* (Addico and Frempong 2004). Eutrophication is the most important factor accounting for the growing incidence of cyanobacteria blooms, of which approximately 50% are known to be toxic, in rivers, lakes and reservoirs worldwide (National Rivers Authority 1990 cited in Cornish *et al.* 2000).

Cyanobacteria are the freshwater phytoplanktonic organisms of most concern, because of their ability to produce strong hepatotoxins such as microcystins, nodularins and neurotoxins (Sivonen and Jones 1999). Knowledge of cyanobacteria, their toxins and effects on public health is rudimentary in Ghana, and drinking water treatment practices do not specifically address this health risk. Treatment of drinking water is very basic and involves flocculation with alum followed by sedimentation, filtration and chlorination. The Kpong treatment plant uses an additional chlorination step to pre-treat the water. Most cyanotoxins are cell bound and are released when the cells die or are lysed through pre-chlorination or treatment with the algicide, copper sulphate (Kenefick *et al.* 1993 cited in Hrudey *et al.* 1999).

Microcystin, the toxin commonly found in most of the cyanobacteria species identified in drinking water bodies in Ghana, is known to be an active tumour promoter in humans at very low concentrations (Nishiwaka-Matsushima *et al.* 1992) and acute doses result in progressive liver damage (Runnegar *et al.* 1988). Concerns over these public health risks have prompted the World Health Organisation (WHO) to adopt a provisional guideline value of 1.0µg l^{-1} microcystin-LR for drinking water (WHO 1984 cited in Falconer *et al.* 1999). Potable water producers in Ghana

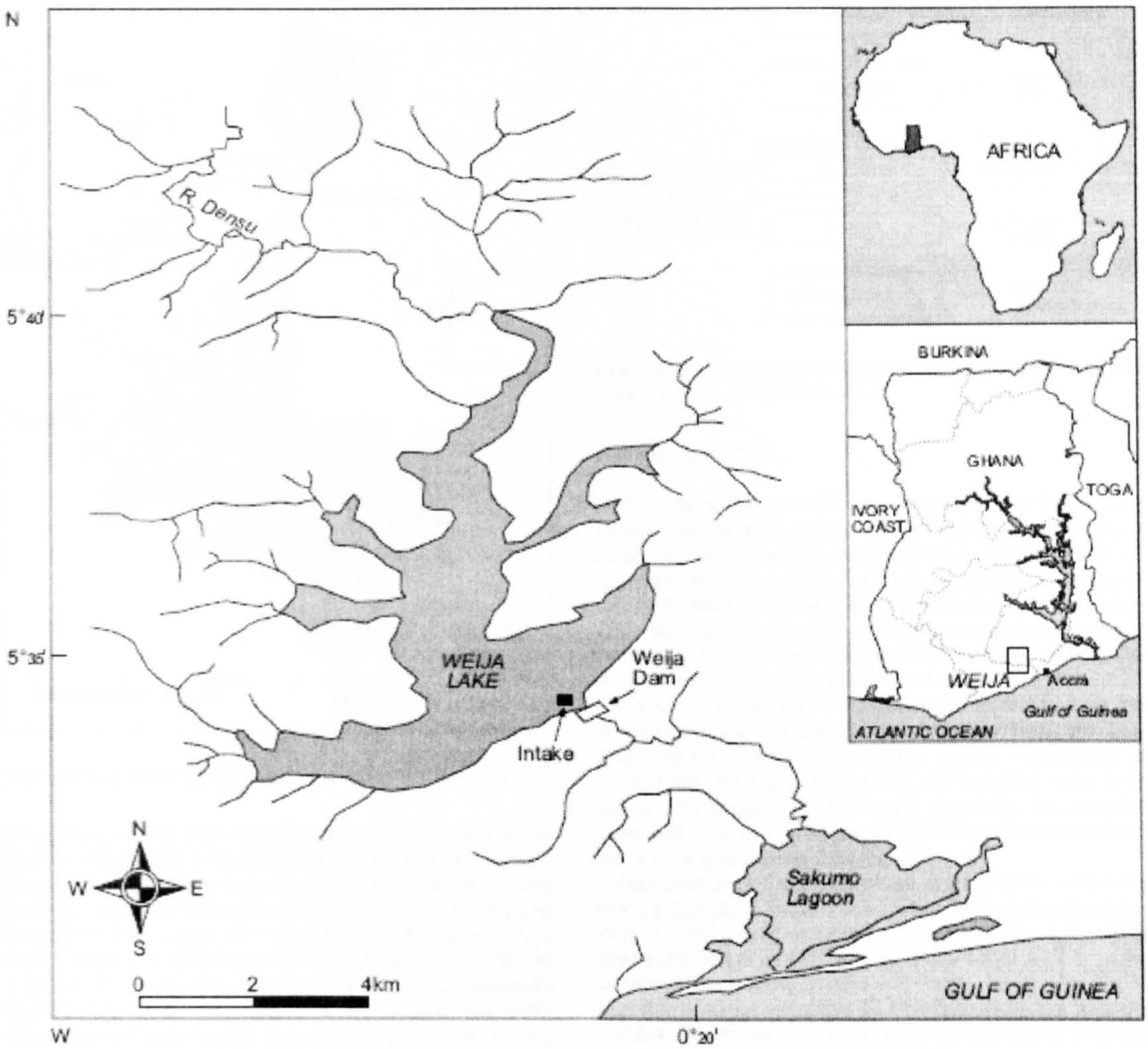

Figure 1: Map of the Weija Reservoir and its catchment area

need to develop methods to either completely remove cyanotoxins or reduce their concentrations to acceptable levels. This study was initiated to identify the cyanobacteria found in water reservoirs in Ghana that supply its capital, Accra, with drinking water, and to assess whether the currently used processes can remove whole cyanobacteria cells from the drinking water.

Material and Methods

Study area

The Weija Reservoir (5°35'N, 0°22'W, Figure 1), which supplies the southern parts of Accra with drinking water, has a surface area of about 300ha (Dassah and Abban 1979) and a mean annual flow of 54.2m^3 s^{-1} (Ansa-Asare and Ansong-Asante 1998). This reservoir lies in the coastal savannah zone and is characterised by seasonal rainfall, which peaks in June and September. The reservoir is built on the Densu River system, which is under intensive pollution threat from mainly domestic and agricultural wastes. The main economic activities within the Weija catchments are fishing, and farming with heavy use of fertilisers. Major crops include maize, cassava, pineapples, banana, sugar cane and vegetables. Domestic waste waters are mainly disposed into the rivers that feed the reservoir.

The Kpong Reservoir is located in the east of Ghana (6°9'N, Figure 2). This reservoir, which supplies the east-

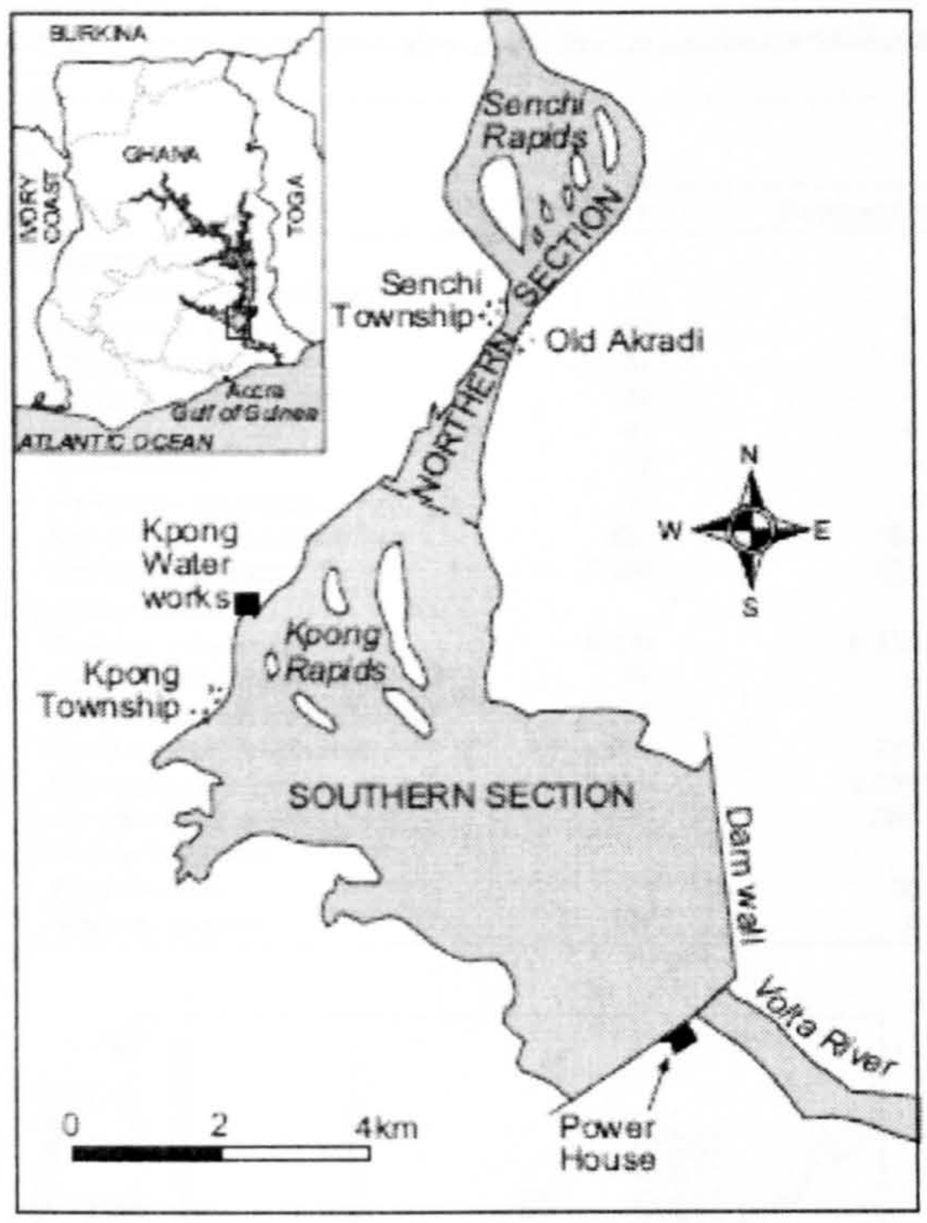

Figure 2: Map of the Kpong Reservoir and its catchment area

ern parts of Accra with drinking water, has a total surface area of 38km² and a mean depth of 5m (Ansa-Asare and Ansong-Asante 1998). It is characterised by small islands forming the Senchi Rapids to the north and the Kpong Rapids to the south. The water retention time of the Kpong Reservoir is 5 days and has a mean annual flow of 1 183m³ s⁻¹. Up to 25% of its surface area is covered with aquatic vegetation. The Kpong Reservoir is on the Volta River system (see Figure 1), which is also used for the production of hydroelectricity, irrigation and recreation and is well known for its fisheries, especially the tilapias *Oreochromis niloticus* and *Tilapia zilli*. Both the Kpong and the Weija reservoirs have a maximum depth of 15m.

Drinking water treatment

Water treatment in the Weija and Kpong waterworks follows the conventional treatment for drinking water, with no consideration of cyanotoxins. This involves abstraction of water from the intake into the treatment train, followed by aeration, flocculation and coagulation using alum, sedimentation, rapid sand filtration and finally pH adjustment and chlorination with hydrated lime or chlorine gas. The Kpong Reservoir has an additional pre-chlorination step (Figure 3). Also at the Kpong Reservoir, alum is used occasionally when the raw water is turbid.

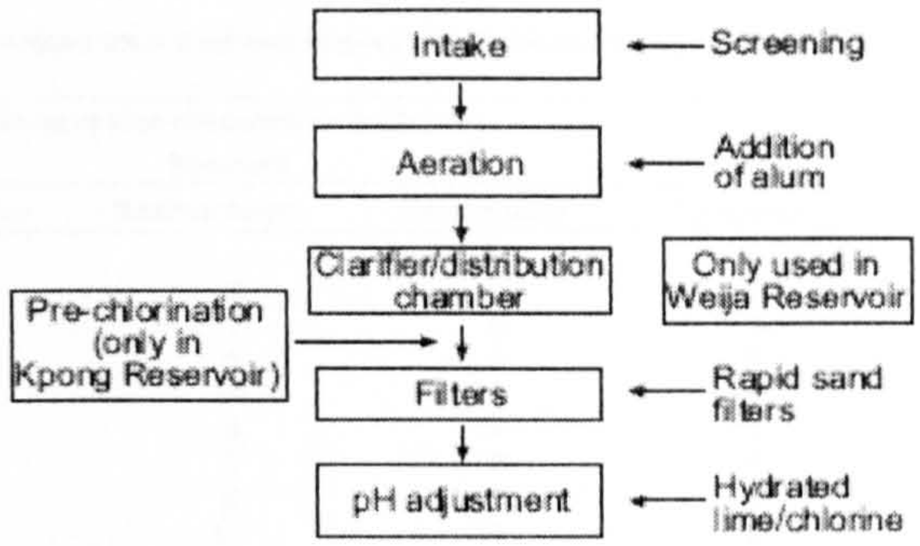

Figure 3: Stages of drinking water treatment train in the Weija and Kpong water reservoirs in Ghana

Sample collection

Water samples were collected monthly in 100ml plastic bottles from the two reservoirs from December 2002 to April 2003. At both reservoirs, samples were collected from all five main treatment stages within the treatment train from a depth of about 5–7m. At the Weija Reservoir, these stages were the intake, the alum application chamber (flocculation/coagulation), the sedimentation tank/clarifier, the filtered water, and chlorination or pH adjustment (Figure 3). For the Kpong Reservoir, samples were collected from the intake/aeration, the distribution tank/clarifiers, the pre-chlorination tanks, the filtered water and the chlorination or pH adjustment stage (Figure 3). All samples were preserved with Lugol's solution. In the laboratory, the samples were well shaken and aliquots of 10–25ml were transferred into counting chambers for analysis.

Identification and enumeration of cyanobacteria was done using a Carl Zeiss inverted microscope as described by Lund *et al.* (1958). Sedimentation was carried out in counting chambers with a settling time of 4h for every 1cm of water column of the sample (Wetzel and Likens 1990). All colonies and filaments were counted as individuals, and the average number of cells was determined for 20 individuals and cell concentration was calculated. In order not to contaminate the samples, counting chambers were cleaned with detergent after each sample and the cover slides were changed.

Toxicological analysis

Preliminary toxicological analyses of intracellular toxins of cells filtered from 1-litre water samples from the two reservoirs using high-performance liquid chromatography (HPLC) were undertaken at the University of Hull, UK, and at the Ecotoxicity Laboratory of the Masaryk University of the Czech Republic. Extraction of toxins from filters and HPLC analysis were done as described by Harada *et al.* (1999). Extraction was carried out with 75% methanol as recommended by Fastner *et al.* (1998). Microcystins were identified based on their retention time compared with external standards and their characteristic UV spectra

Table 1: Mean concentration of the phytoplankton species identified at the various water treatment stages in the Weija and Kpong reservoirs

	Phytoplankton concentration (cells ml^{-1})				
	Treatment				
Species	Intake	Alum application	Sedimentation	Filtered water	Chlorination
Chlorophyceae					
Ankistrodesmus fusiformis	6	1	5	1	1
Actinastrum hantzshii	25	3	1	0	0
Chlorella vulgaris	33	9	6	0	0
Coelastrum reticulatum	32	0	4	0	0
Pediastrum duplex	49	9	5	28	10
Tetrastrum sp.	1	0	3	0	0
Tetraedron caudatum	11	0	0	7	0
Scenedesmus armatus	304	52	13	15	2
Ulothrix tenuissima	1354	386	221	648	33
Cyanophyceae					
Anabaena flos-aquae	9 136	4 238	1 117	662	56
Anabaenopsis tanganyikae	77	0	0	0	0
Cylindrospermopsis raciborskii	221	0	0	0	0
Merismopedia tenuissima	631	232	419	192	43
Microcystis aeruginosa	11 159	1 921	320	657	583
Planktothrix agardhii	825	398	43	11	3
Bacillariophyceae					
Synedra acus	21	20	14	14	1
Navicula simplex	330	0	1	0	0

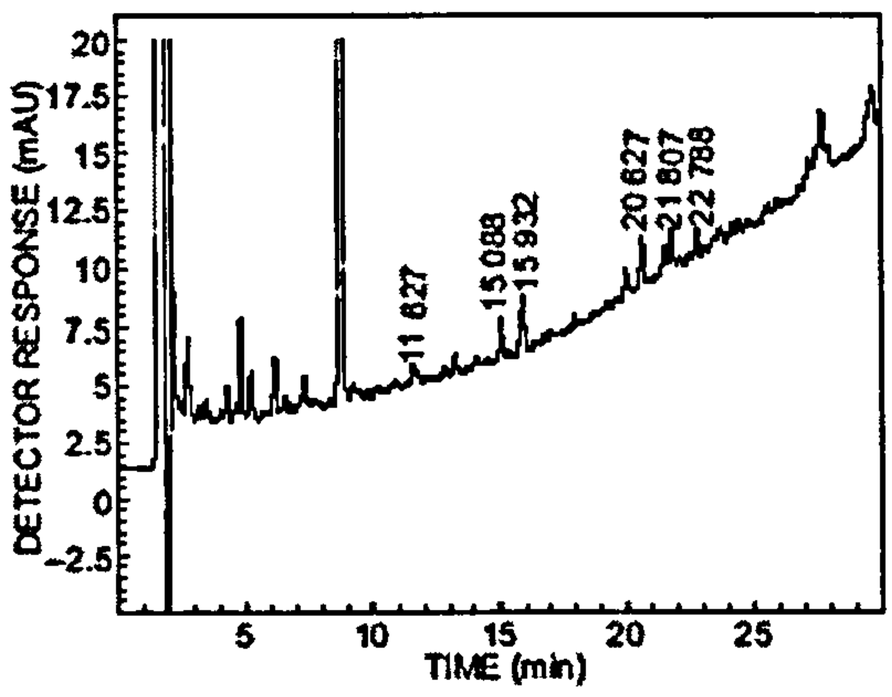

Figure 4: HPLC-UV elution profile at 238nm of the intake sample from the Weija drinking water reservoir showing six microcystin variants

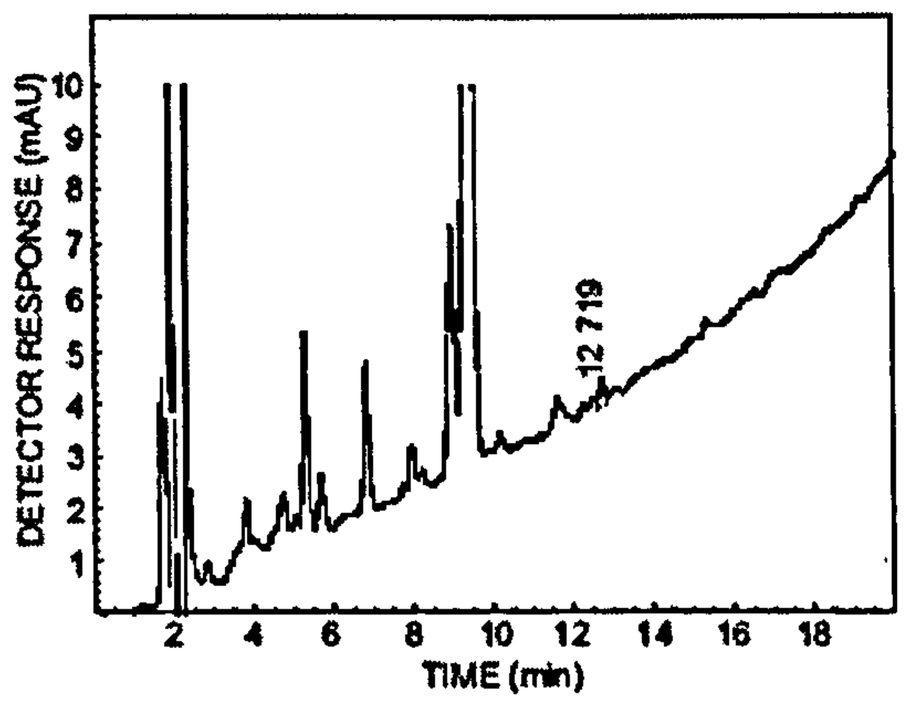

Figure 5: HPLC-UV elution profile at 238nm of the intake sample from the Kpong drinking water reservoir showing the single microcystin variant

(Lawton *et al.* 1994). All other unknown microcystin peaks with absorption maxima measured at 238nm were quantified using microcystin-LR.

Results and Discussion

Table 1 shows the cyanobacteria species and the average cell concentrations in the two reservoirs. Six cyanobacteria species were identified, of which *Anabaena flos-aquae* and *Microcystis aeruginosa* dominated with average cell densities of 9 138 cells ml^{-1} and 11 159 cells ml^{-1} respectively. Cyanobacterial cell counts were highest in January and February, the peak of the dry season when water levels in the reservoirs were very low. Bartram *et al.* (1999) report that when cyanobacteria density exceeds 2 000 cells ml^{-1}, an alert level is reached. The total cell counts of *M. aeruginosa* and *A. flos-aquae* far exceeded this value.

The hepatotoxic cyclic peptide, microcystin, the toxin commonly found in most of the species identified in the two reservoirs (Table 1), is the most studied cyanotoxin and the one of most concern with regard to intoxication of humans through drinking water. Preliminary HPLC results of intracellular toxins of intake water samples taken from the two reservoirs showed the presence of six different microcystin

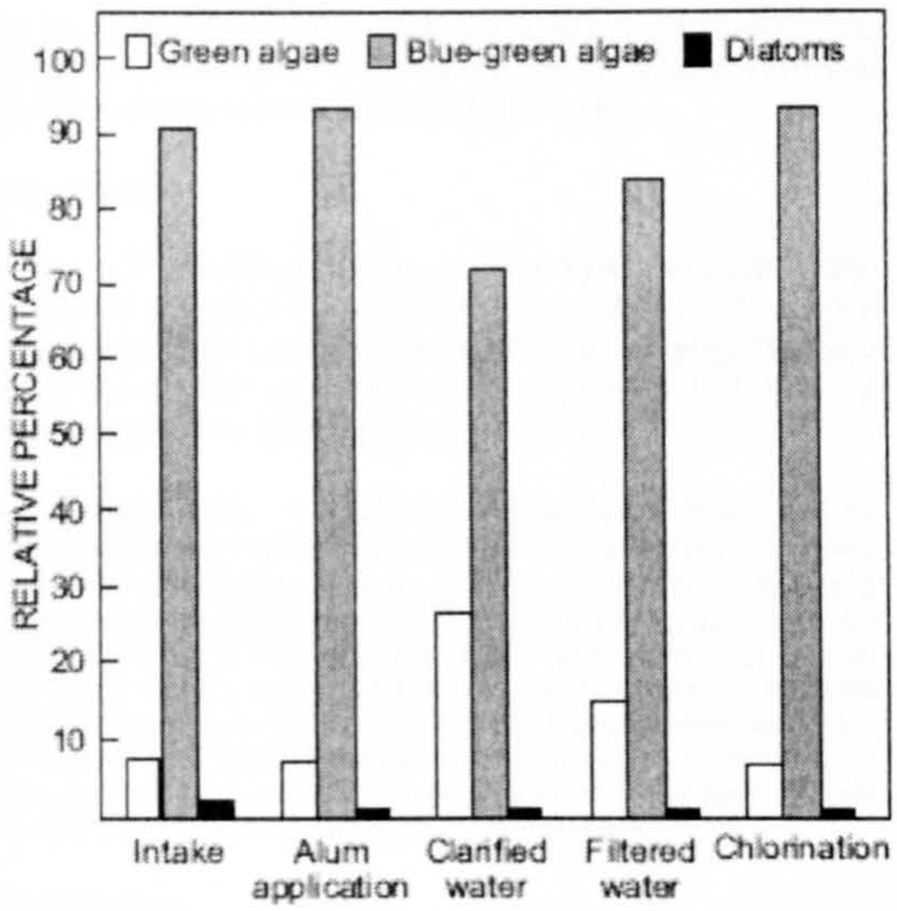

Figure 6: Phytoplankton composition of drinking water at various treatment stages in the Weija and Kpong reservoirs

variants in the Weija Reservoir and one variant (microcystin-RR) from the Kpong Reservoir (Figures 4, 5). Quantification of the total microcystins identified from the intake to the Weija Reservoir showed a production of 3.21µg l^{-1} of microcystin. The fraction of microcystin-RR identified in the intake sample of the Kpong Reservoir was much lower at 0.03µg l^{-1}.

Actual cyanobacterial cell counts in each treatment stage of water production were lower in the Kpong Reservoir than in the Weija Reservoir (actual counts not shown), but the general trend was the same for both reservoirs (Figure 6). Bláha and Maršálek (2003) measured intracellular microcystin concentrations of between 0.001µg l^{-1} and 15.7µg l^{-1} in reservoirs in the Czech Republic. The values obtained here fall within this range.

Incidents of human health problems associated with exposure to cyanobacteria toxins through drinking water have been well documented. For example, Byth (1980) reported a poisoning episode involving 140 children and 10 adults in Australia, where copper sulphate was used to control algae after consumers had complained about the taste and odour of the water. In China, a high incidence of primary liver cancer within certain towns has been attributed in part to cyanotoxin-contaminated drinking water (Yu 1995 cited in Kuiper-Goodman *et al.* 1999). Surveys of microcystins in these above-mentioned water supplies had relatively low concentrations of up to 0.46µg l^{-1} (Ueno *et al.* 1996). In addition, the death of over 60 dialysis patients in Caruaru, Brazil, following intake of contaminated drinking water has also been documented (Jochimsen *et al.* 1998 cited in Kuiper-Goodman *et al.* 1999). In Harare, Zimbabwe, in southern Africa, children living in an area of the city supplied with drinking water from a particular water reservoir developed gastroenteritis each year at the time when a natural bloom of *Microcystis* spp. was decaying (Zilberg, 1966). In Ghana, there are no substantiated records of human poisoning associated with cyanobacterial toxins. However, diseases such as gastroenteritis, diarrhoea, vomiting, and skin irritations, which are symptoms of intoxication by cyanotoxins, and, more seriously, kidney cancer, are reported from hospitals on a daily basis.

Water treatment techniques and their ability to remove cyanotoxins from drinking water have been the focus of a number of studies (e.g. Rositano and Nicholson 1994, Lawton and Robertson 1999, Hitzfeld *et al.* 2000). All have provided conclusive evidence that conventional methods of treating drinking water containing cyanobacteria, as practised in Ghana (Figure 3), are not effective in the removal of cyanobacteria cells, and/or cyanotoxins. Moreover, even the sophisticated and expensive methods such as ozonation and treatment with activated carbon have their limitations. Table 1 shows that the Ghanaian water treatment system is inefficient at removing cyanobacteria cells from the drinking water, as indicated by the presence of cyanobacteria in all the treatment stages, comprising over 70–90% of the total phytoplankton biomass. In addition, substantial cell lysis can occur, resulting in the release of dissolved cyanotoxins into the drinking water system. Conditions promoting cell disruption include pumping regimes, senescence or sudden death of blooms, flocculation with alum, pre-chlorination and filtration (Hitzfeld *et al.* 2000), all of which form part of the Ghanaian drinking water treatment system.

Coagulation is known to be an effective method for eliminating intact cyanobacteria cells, but it is not effective for the removal of dissolved toxin (Rositano and Nicholson 1994). It also has the disadvantage of causing cell lysis. The filtration system, especially in the Weija Reservoir, was found to be ineffective because of the large numbers of cyanobacterial cells present in the water; the filters had also deteriorated.

Chlorination can be effective at removing cyanobacteria from drinking water, but its efficiency depends on the chlorine compound used and concentration (Hitzfeld *et al.* 2000). Rositano and Nicholson (1994) found that aqueous chlorine and calcium hypochlorite at a concentration of about 1mg l^{-1} removed >95% of microcystin compared with sodium hypochlorite at the same dose, which achieved only a 40–80% reduction. The formation of toxic chlorination by-products, such as trihalomethane and haloacetic acids, must also be considered (Rositano *et al.* 1995 cited in Hitzfeld *et al.* 2000). According to Narotsky *et al.* (1997), trihalomethane in drinking water may lead to rectal, intestinal and bladder cancer. Haloacetic acids are also known to induce liver cancer in mice. In addition, for chlorination to be completely effective in the destruction of microcystins, a chlorine residue of at least 0.5mg l^{-1} should be present 30min after application (Hitzfeld *et al.* 2000). Because no chemical analysis was carried out on the drinking water, it is not possible to ascertain if the above conditions were met. However, the presence of microcystins in the intake samples of the two reservoirs, especially Weija, requires more rigorous monitoring of the drinking water for both intracellular and dissolved toxins.

Outreach programmes on cyanotoxins and their effect on human health are needed to educate the Ghanaian population

on good health practices. The responsible authorities should institute measures to minimise the risk of human exposure to microcystins from drinking water supplies.

Conclusion

This study provides the first report of cyanobacterial microcystins in drinking water reservoirs in Ghana. As current water treatment practices are inefficient at removing cyanobacterial cells, cyanotoxin contamination of drinking water constitutes a high public health risk.

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II

Cyanobacteria diversity and biomass in relation to nutrient regime of four freshwater reservoirs sourced for the production of drinking water in Ghana

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Abstract

The cyanobacteria diversity, biomass and nutrients (nitrite, nitrate and phosphate) of the Weija, Kpong, Owabi and Barekese reservoirs in Accra and Kumasi Metropolitan Areas of Ghana, were monitored from January to May 2005. The results show that the reservoirs were dominated by nanocyanobacteria not recorded in earlier studies. The Weija reservoir was the most diversified in terms of cyanobacteria species, which was dominated by the nanocyanobacteria *Aphanocapsa nubilum* accompanied by *Merismopedia tenuissima*, *Planktolyngbya minor* and *Pseudanabaena recta*. The Kpong reservoir was dominated by *Geitlerinema unigranulatum*, whilst the Owabi and Barekese reservoirs both situated in the closed-forest region of Ghana with high rainfall and human activity were dominated by the nanocyanobacteria *Cyanogranis ferruginea*, an entirely new cyanobacterium in Ghana and in fact the whole of the tropical Africa. A high and positive correlation was obtained between the total cyanobacteria cell counts/ml and nitrite in the Weija reservoir ($r = 0.9997$) and also a low but positive correlation was observed for phosphate ($r = 0.5791$), whilst a negative correlation was obtained between total cyanobacteria cell counts/ml/month and nitrate. In the Owabi reservoir, positive correlations were obtained between total cyanobacteria cell counts/ml/month for all the three nutrients ($r = 0.8479$, 0.9286 and 0.8424) respectively. In the Kpong and Barekese reservoirs, low correlations were obtained between total cyanobacteria cell counts/ml/month and the three nutrients. In the Kpong reservoir a high positive correlation was obtained between total monthly rainfall and total cyanobacteria cell counts/ml ($r = 0.8285$). Intracellular microcystins have been identified in all four reservoirs.

Key words: Cyanobacteria, biomass, diversity, nutrients, reservoirs, microcystins

Introduction

The occurrence of cyanobacteria blooms in eutrophic lakes, reservoirs and recreational waters has become a worldwide problem. Cyanobacteria blooms often create unsightly surface scums, decreased water column transparency, unpalatable drinking water and noxious odour (Bernadette and Jean, 2003). Cyanobacteria are ancient, cosmopolitan inhabitants of fresh, brackish and marine waters (Hitzfeld et al. 2000; Whitton and Potts, 2000). Cyanobacteria are said to fulfil key roles in the biogeochemical cycling of matter and in the structure, maintenance and biodiversity of microbial and higher organism community (Codd et al. 2005). Contrary to this benign view of the cyanobacteria according to Codd et al (2005) from the human standpoint is the increasing realization that cyanobacteria produce potent toxins, which have been implicated in livestock, wildlife as well as human poisoning throughout the world (Codd et al. 1999a; Chorus, 2001). These toxins, defined by their chemical structure fall into three main groups; cyclic peptides, alkaloids and lipopolysaccharides (Sivonen and Jones, 1999; Falconer, 2004; McElhiney and Lawton, 2005; Molica et al. 2005; Dietrich and Hoeger, 2005). The reasons for synthesis of toxins by cyanobacteria are not well understood, but it is believed to be an adaptation that allows cyanobacteria in resource-limiting environment to ameliorate the effects of herbivory and competition with other phototrophs (Arnold, 1971; Richman and Dodson, 1983; Demott et al. 1991; Legrand et al. 2003), bacteria (Chrost, 1975; Flores and Wolk, 1986) and fungi (Patterson and Bolis, 1997). The toxins produced by cyanobacteria are resistant to boiling and can also pass through conventional water treatment. Due to these reason cyanobacteria has been described as the most important freshwater phytoplanktonic organisms from the view point of human health hazard (Sivonen and Jones, 1999). Water quality problems caused by dense populations of cyanobacteria are intricate, many and varied (Skulberg, 1996a; Falconer, 2004) and can have great health and socio-economic impacts, as such, the negative impacts of cyanobacteria have gained research attention and public concern. Human health effects caused by cyanobacteria and associated toxins include among others: gastroenteritis, nausea, vomiting, fevers, flu-like symptoms, sore throat, ear and eye irritations, abdominal pains including painful hepatomegaly, visual disturbances, kidney and liver damage (Oberholster et al. 2004; Hitzfeld et al. 2000; Codd et al. 2005) and in some cases fatal as reported in Brazil where over 50 dialysis patients died due

to exposure to microcystin via haemodialysis (Azevedo et al. 2002; Carmichael *et al.* 2001). Again in Brazil, phytoplankton blooms consisting of 20% *Microcystis* was reported to be the cause of mass mortality of the fish *Parapimelodus nigribarbis* in the Northern parts of the Patos lagoon in Brazil (Joao et al. 1998). As a result, the presence of toxic cyanobacterial blooms in water used for the production of drinking water, fisheries and recreational purposes may present a serious health risks for both the human population and wildlife resources (Hitzfeld et al. 2000; Hoeger et al. 2004; Falconer, 2004). Addico et al. (2006) reported the dominance of cyanobacteria over green algae and diatoms in two of the four reservoirs studied in Ghana. Blooms of cyanobacteria in Ghana have been associated with influxes of soluble phosphate from irrigation, storm water, industrial discharges, fertilizer application and sewage effluents and natural phosphate sources from catchments of water bodies (Biney 1990, Frempong and Addico, 2004). The dominance of cyanobacteria in freshwater bodies is a common phenomenon due to increasing eutrophication and warming of surface waters (Hoeger et al. 2004; Bouvy et al. 2006). According to Sommer (1989), the annual variation of predominant species can be predicted, even though the taxa that dominate the communities will depend upon complex factors such as retention time, nutrient load and grazing pressure. Cronberg and Annadotter (2006) attributed eight different theories on the dominance of cyanobacteria. These are total nitrogen/ total phosphorus ratio, low light hypothesis, buoyancy hypothesis, elevated temperature hypothesis, zooplankton grazing hypothesis, trace elements hypothesis, storage strategy hypothesis and inorganic nitrogen hypothesis. Codd et al. (2005) recommended a hazard characterization of cyanobacterial cells (biomass) to contribute to the monitoring and control of drinking water reservoirs, which is necessary for the risk management of recreational waters that support cyanobacteria growth. In this study we monitored the changes in cyanobacteria cells (biomass), diversity and relationship to nutrients concentrations in four freshwater reservoirs sourced for the production of drinking water in Ghana, which are also important in fishery resource and recreational activities.

Study Area

The study areas of Weija and Kpong reservoirs have been described in Addico et al. (2006). The Barekese and Owabi reservoirs, both situated in the Ashanti Region of Ghana are described below.

Owabi Reservoir

The Owabi reservoir lies on latitude 6° 52′ N and longitude 1° 43′ W (Fig. 1) and situated in the Ashanti Region of Ghana. It was constructed in 1928 and resulted in the formation of the reservoir with a surface area of about 7 km^2 (Amakye, 2002). The reservoir was upgraded in 1954. At present it has the capacity to produce 13.6 million litres of water per day from 4.5 million litres in 1928 per day. It covers an area of 16 km^2. It lies in the closed forest ecological zone of Ghana (Hall and Swaine, 1981). The Owabi is reservoir is fed by seven rivers all of which flow through the Kumasi Metropolitan area, a very densely populated residential and the central business and industrial areas. These rivers are Sukobri-Owabi-Pumpuna, Ntikyei, Anyinasu, Nwabi, Bunkunfuo, Lakyeanpon and Asuokuu.

Barekese Reservoir

The Barekese reservoir lies on latitude 6° 52′ N and longitude 1° 42′ W (Fig. 1) and located in the Ashanti Region of Ghana. It was formed in 1970 (Frempong and Nijjhar, 1973). It covers an area of 16 km^2. Like the Owabi reservoir, the Barekese reservoir also lies in the closed forest ecological zone of Ghana and is its major tributary is the Ofin river system. The basin lies approximately within arable lands with land use being mainly agricultural and domestic in nature.

Rainfall within the catchments of the Owabi and Barekese reservoirs is high and ranges between 1500 to 1750 mm per annum (Hall and Swaine, 1981). The soils are therefore deeply weathered with very high erosion and soil nutrients mainly derived from decomposition of leaf litter that fall accumulates on the forest floor. Both reservoirs lie in areas with high intensity of human activities and poultry farms with large industrial estates. Wastes waters from these activities with high nutrient loading find their way directly into these reservoirs. The chemistry of the

two reservoirs is therefore expected to be heavily influenced by these land use activities

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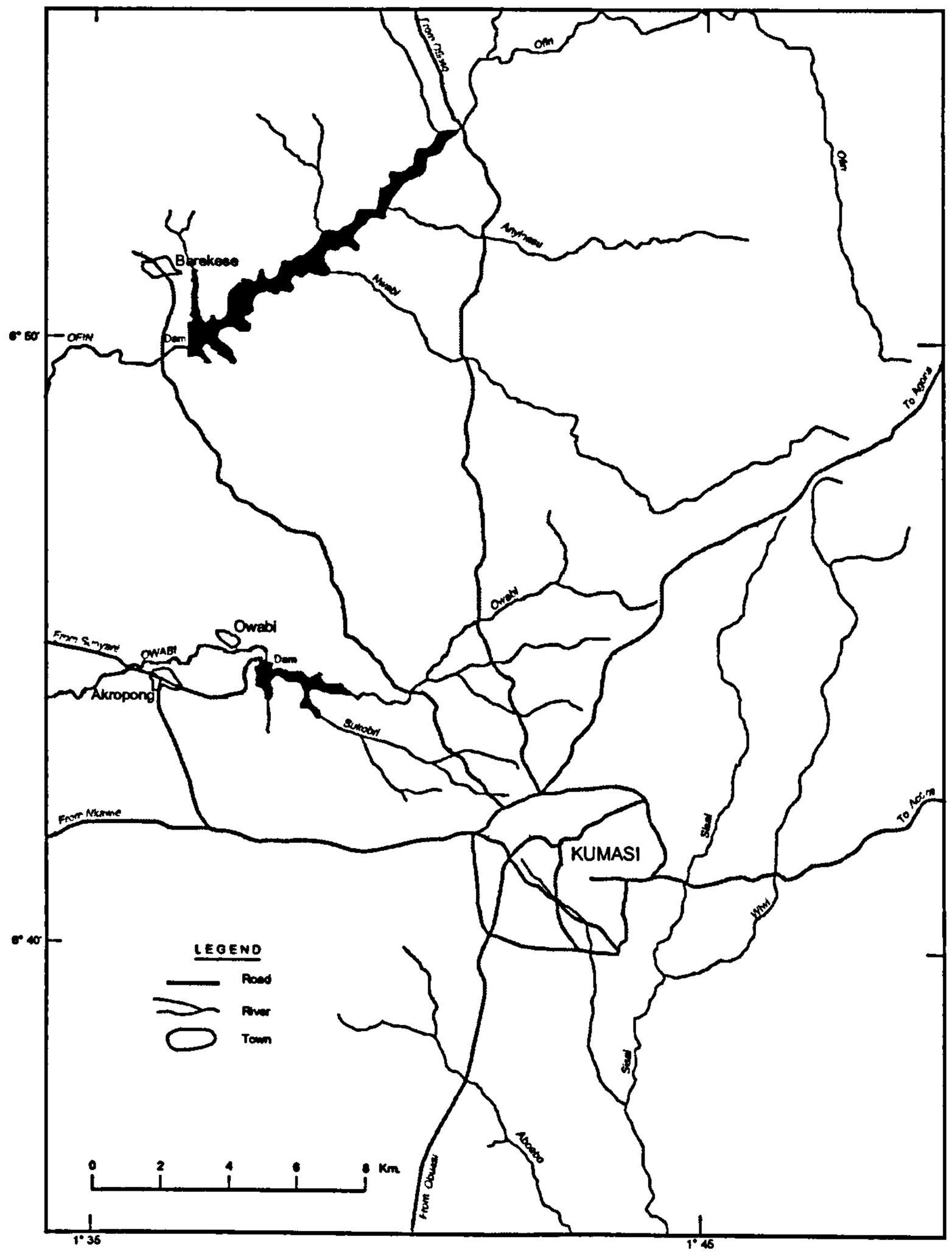


Figure 1 Map of Owabi and Barekese reservoirs in the Ashanti Region of Ghana and its catchment area

Material and Methods

Identification of cyanobacteria

Samples for cyanobacteria species identification and biomass determination were collected from all the four study sites during a six months field work in Ghana from January to June 2005. Samples were collected with plankton net or by simply filling a bucket directly from the reservoir. Net samples were preserved to a final concentration of 2% formalin, whilst water samples for quantitative analysis were preserved in Lugol's solution (Guillard and Sieracki, 2005). Work on identification of cyanobacteria species was carried out at the Institute of Botany of the Czech Academy of Sciences, Trebon, Czech Republic. Identification of species was done using a model: Olympus light microscope BX 51 manufactured in Japan with objectives 10, 20, 40, 60 and 100. Pictures were taken using a model: Olympus camera C-5050 with a zoom and mounted on the microscope.

Cyanobacteria biomass determination

Cyanobacteria biomass determination was done by direct counting of cells using an inverted microscope as described by Lund et al., (1958) and Lawton et al., (1999). Sedimentation of cells were carried out directly in counting chambers with a settling time of 4 hours for every 1 cm of water column of the sample (Wetzel and Likens, 1990). This method had been described as the most effective way of handling water samples with a mixture of green algae, diatoms and cyanobacteria (Falconer, 2004). All colonies and filaments were counted as individuals, and the average number of cells determined for 20 individuals and cell concentrations was calculated as described by Addico et al., (2006).

Picocyanobacteria

Sub-samples for counting picocyanobacteria were preserved in formalin to a final concentration 2%. A Volume of 1 to 2 ml depending on the concentration of the sample was filtered through a 0.2 μm Nuclepore filter prestained with Irgalan black. Dapi (4-diamidino-2-phenylindole dihydrochloride) was used to stain the cells for fluorescence of DNA as described by Porter and Feig (1980) and Stockner et al., (2000). The cells were counted in red fluorescence of phycocyanin and partly chlorophyll -a using Olympus BX60 microscope in the epifluorescence modification under green excitation (510-560 nm) and checked under UV excitation (330-385 nm) for the DNA distribution in the cells. The images of the cells counted were strong and clearly defined. About 300-400 cells were counted per each sample and the counts were converted to picocyanobacteria cells per ml. All data on abundance were expressed in numbers of cells, including the cells inside colonies.

Nutrient analysis

Water samples for nitrite and nitrate analysis were collected into cleaned 1 litre polyethylene bottles and filtered to remove any cells debris present in the water. Samples were preserved by deep freezing at – 20°C for nitrite and 4°C for nitrate to prevent bacteria conversion of nitrite to nitrate or ammonia.

Nitrite

Nitrite-Nitrogen was determined by the diazotization method (APHA, 1992) using a spectrophotometer (Ultraspec 11, Model 80-2091-73). The principle behind this method is that nitrite reacts in strongly acid medium with sulphanilamide to form a diazo compound which is coupled with N-(1-naphthyl)-ethylenediamine dihydrochloride to form an intensely red coloured Azo compound. The absorbance of the dye is proportional to the concentration (mg/l) of nitrite present. The spectrophotometer was calibrated prior to analysis (n =6), (r^2 =0.9997). Absorbance was measured at 507 and 356 nm respectively.

Nitrate

Nitrate-Nitrogen was determined by the hydrazine reduction method (APHA, 1992). The principle of this method involves the reduction of nitrate to nitrite with hydrazine sulphate. The nitrite ion originally present plus the reduced nitrate ion is determined by diazotization with sulphanilamide coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly coloured Azo dye which is measured spectrophotometrically. Calibration of the spectrophotometer was done at 6 different concentrations (n =6), (r^2 =0.9994).

Phosphate

Phosphate was determined by the stannous chloride method (APHA, 1992). Samples for phosphate determination were collected into acid-washed 1 litre polyethylene bottles and preserved at 4°C. Molybdophosphoric acid is formed and reduced by stannous chloride to intensely coloured molybdenum blue. The absorbance of the molybdenum blue at a wavelength of 690 nm is proportional to the concentrations of the phosphate in the samples. Standard phosphate solutions of known concentrations ranging from 0.1 to 1.0 mg/l were used to calibrate the spectrophotometer prior to analysis of samples (n =6), (r^2 =0.9884).

Rainfall

All rainfall data were obtained through the Ghana Meteorological Service recorded at 09 GMT (9 am in Ghana) for the respective catchments of the four reservoirs and data analyses were done using Microsoft Excel.

Results

Weija reservoir

Cyanobacteria in the Weija reservoir showed a high species diversity. A total of twenty six species were identified belonging to sixteen genera (Appendix 4). These are *Anabaena*, *Anabaenopsis*, *Aphanocapsa*, *Chroococcus*, *Coelomoron*, *Cyanogranis*, *Cylindrospermopsis*, *Geitlerinema*, *Lyngbya*, *Microcystis*, *Merismopedia*, *Planktolyngbya*, *Planktothrix*, *Pseudanabaena*, *Radiocystis* and *Romeria* (Fig 2). Two species of *Anabaena* were identified, straight *Anabaena austro-africana* and coiled *Anabaena nygaardii* both new species found in Ghana as against *Anabaena flos-aquae* known to exist in this reservoir (Addico et al., 2006). Two species of *Anabaenopsis* were also identified, *Anabaenopsis tanganyikae* and *Anabaenopsis ambigua*, both identified for the first time in this reservoir. *Aphanocapsa holsatica* and *Aphanocapsa nubilum* have been identified recently in the Weija reservoir but the dominance of *Aphanocapsa nubilum* comprising 21.4% of the total biomass in this study has not been observed before probably due to improvement of counting technique. The genus *Chroococcus* is known to be very common in Ghanaian freshwater bodies but the species C. *cronbergae* is a new identification and this may be due to access to improved technical assistance during identification. *Coelomoron tropicalis* and *Cyanogranis ferruginea* are entirely new species identified in the Weija reservoir and in Ghana. *Cylindrospermopsis raciborskii* is a very common cyanobacterium species in Ghana as in many African countries. However, the second species of *Cylindrospermopsis* described from large East African lakes, *Cylindrospermopsis cuspis* is a new one, which we believe had not been reported from many parts of tropical Africa. *Geitlerinema unigranulatum* is also a new species to be identified in this reservoir even though it occurred in negligible amount. Three species of *Microcystis* were identified, *Microcystis aeruginosa*, *Microcystis wesenbergii* and *Microcystis viridis* (Fig. 2). Out of these three *Microcystis* species, *Microcystis aeruginosa* is a common known species in the reservoir. Two species of *Planktothrix* were identified, *Planktothrix agardhii* and *Planktothrix lacustris* var. *solitaria*, of which the latter is a new species in Ghana and so is *Romeria elegans*. Generally, there were no marked differences in species diversity of cyanobacteria throughout the study. *Aphanocapsa nubilum* was the dominant species occurring

together with *Merismopedia tenuissima*, *Planktolyngbya minor* and *Pseudanabaena recta* (Fig 2). *Aphanocapsa nubilum* dominated the cyanobacteria population throughout the sampling period with a highest cell count/ml of 26,883 cells/ml in April after the highest rainfall recorded in March to 19,510 cells/ml in May (Fig. 3). *Cylindrospermopsis raciborskii* though low in biomass as compared to *Aphanocapsa nubilum* was consistently present throughout with the highest biomass of 5,050 cells/ml also obtained in April (Fig. 2). *Planktothrix agardhii*, a well know toxin producing cyanobacterium was also present throughout the sampling period as well as *Planktothrix lacustris* var. *solitaria*. A positive and significant correlations were obtained between nitrite, phosphate and total monthly cyanobacteria cell counts/ml (r = 0.9997, 0.5791) respectively but negative for nitrate (r = - 0.4269). Similarly positive correlations were also obtained between total monthly rainfall and nitrite and phosphate (r =0.1227, 0.7151) respectively for nitrite and phosphate but negative with nitrate (r = - 0.5441). Out of the twenty six species of cyanobacteria identified in the Weija reservoir during the study, twenty (76.9 %) of them are non nitrogen fixing cyanobacteria. The Weija reservoir had been described as eutrophic, figure 4 shows that for most part of the study period nitrate and phosphate concentrations were high. Figure 5 is a summary of mean nutrient composition in the four reservoirs with the Weija reservoir having an average nitrate and phosphate concentrations of about 0.05 and 0.04 mg/l respectively. Out of the sixteen cyanobacteria genera identified in the Weija reservoir, 11 genera making over 69% are confirmed toxin producers, producing the hepatotoxin, microcystin and neurotoxins. These are *Anabaena*, *Anabaenopsis*, *Aphanocapsa*, *Cyanogranis*, *Cylindrospermopsis*, *Geitlerinema*, *Lyngbya*, *Microcystis*, *Planktothrix*, *Pseudanabaena* and *Radiocystis*. The high cyanobacteria diversity in the Weija reservoir is a manifestation of the multiple microcystin peaks associated with this reservoir.

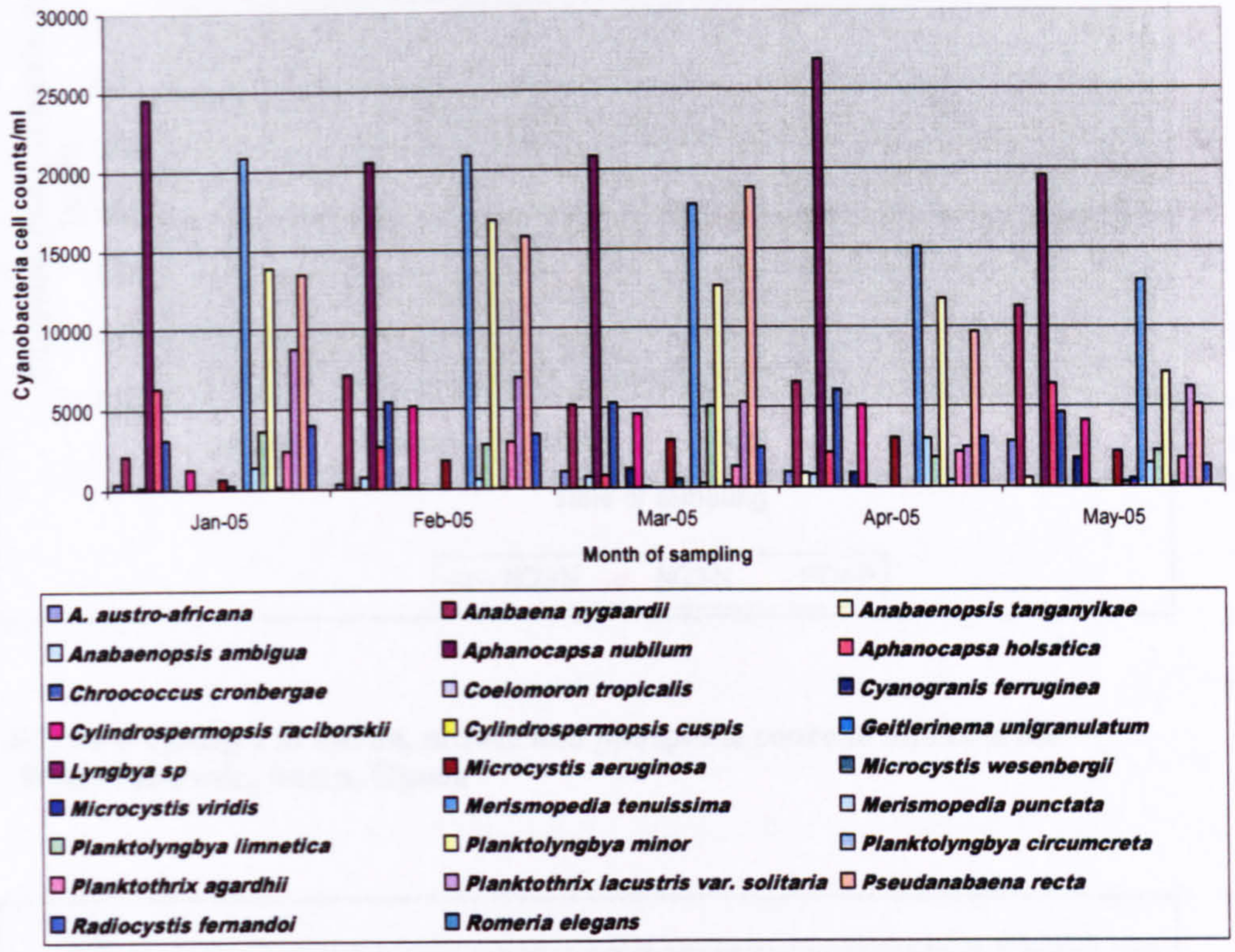


Figure 2 Changes in cyanobacteria composition and biomass in the Weija reservoir, Accra, Ghana

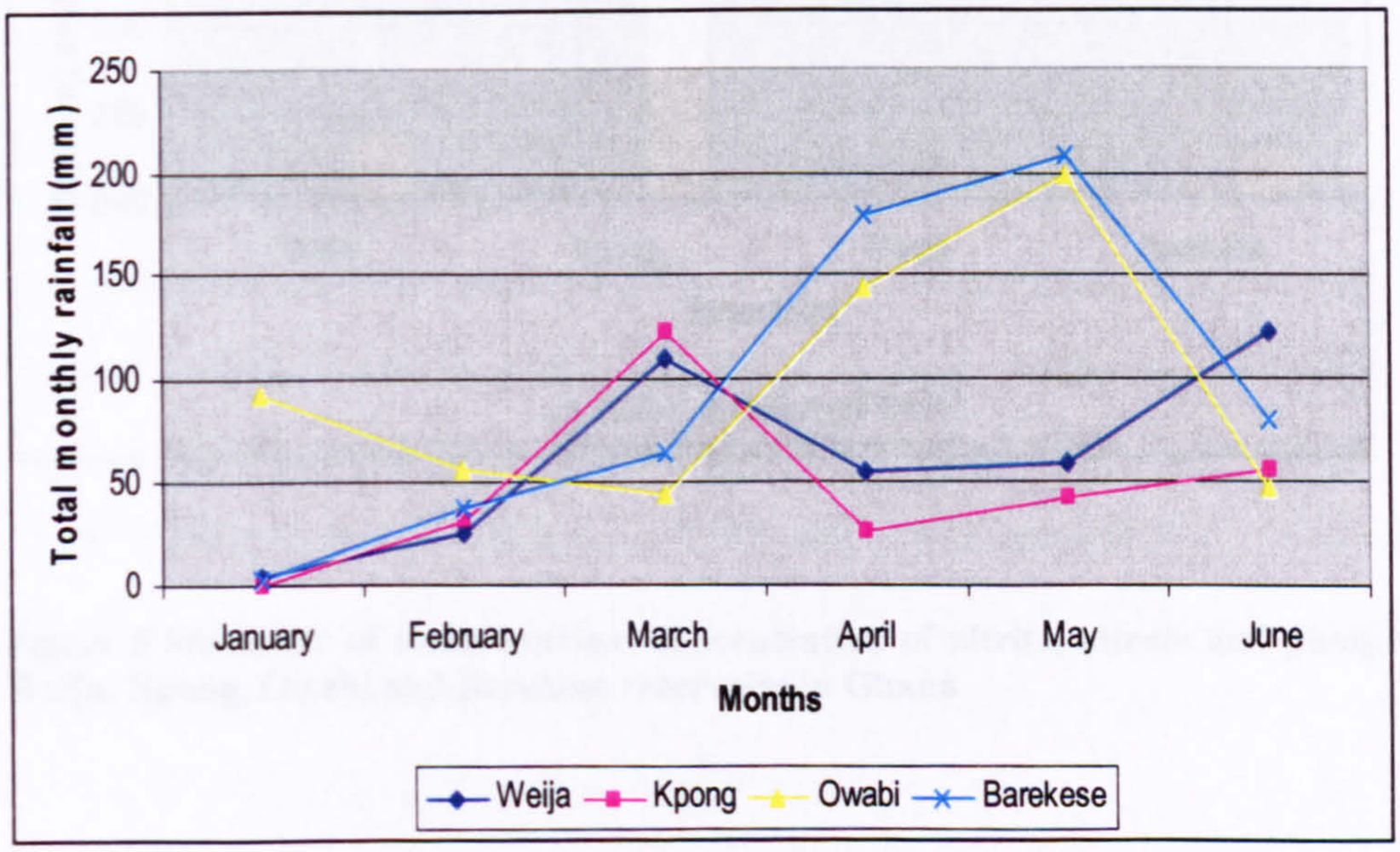


Figure 3 Changes in total monthly rainfall (mm) measured at 9 am GMT

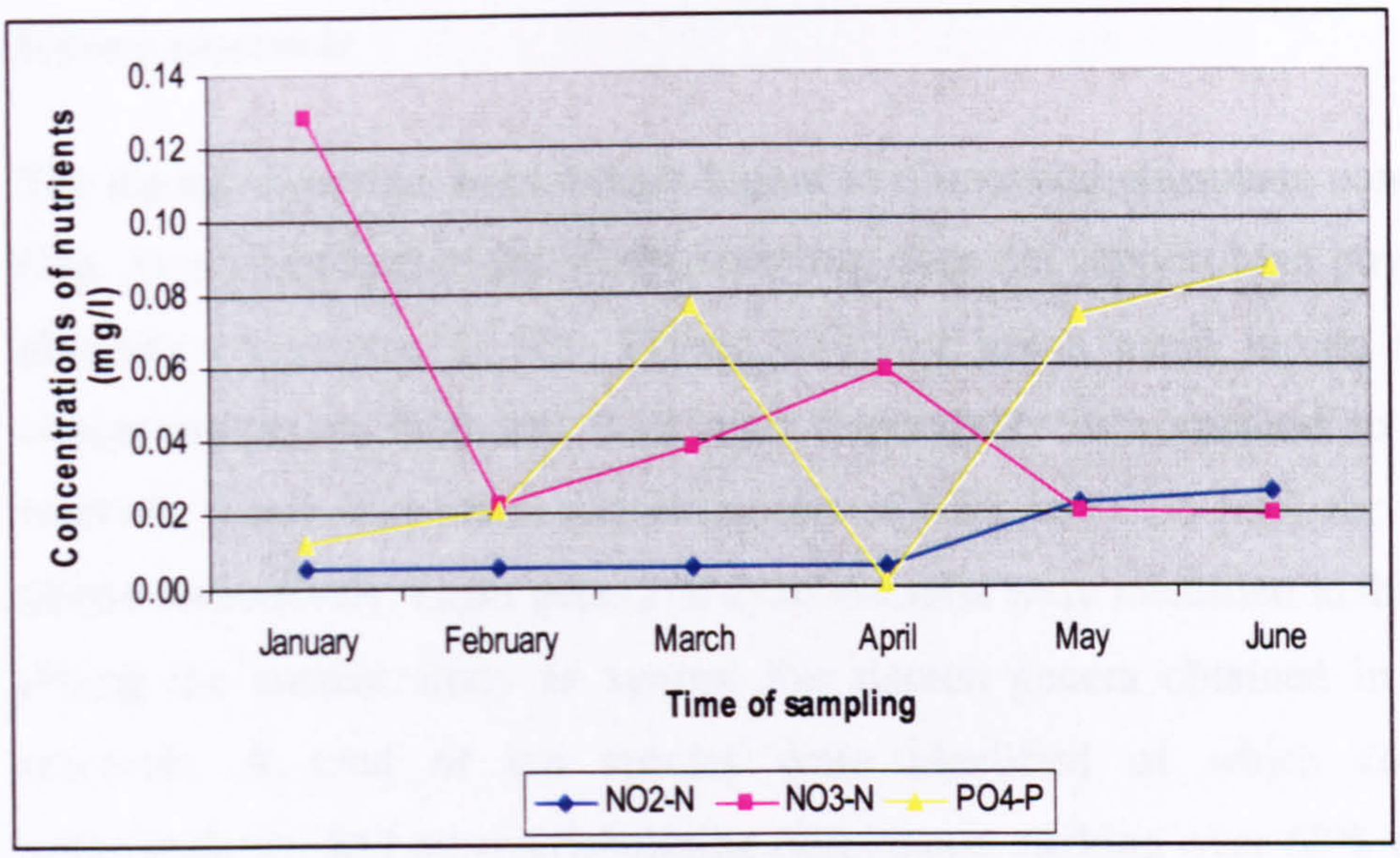


Figure 4 Changes in nitrite, nitrate and phosphate concentrations in the Weija reservoir, Accra, Ghana

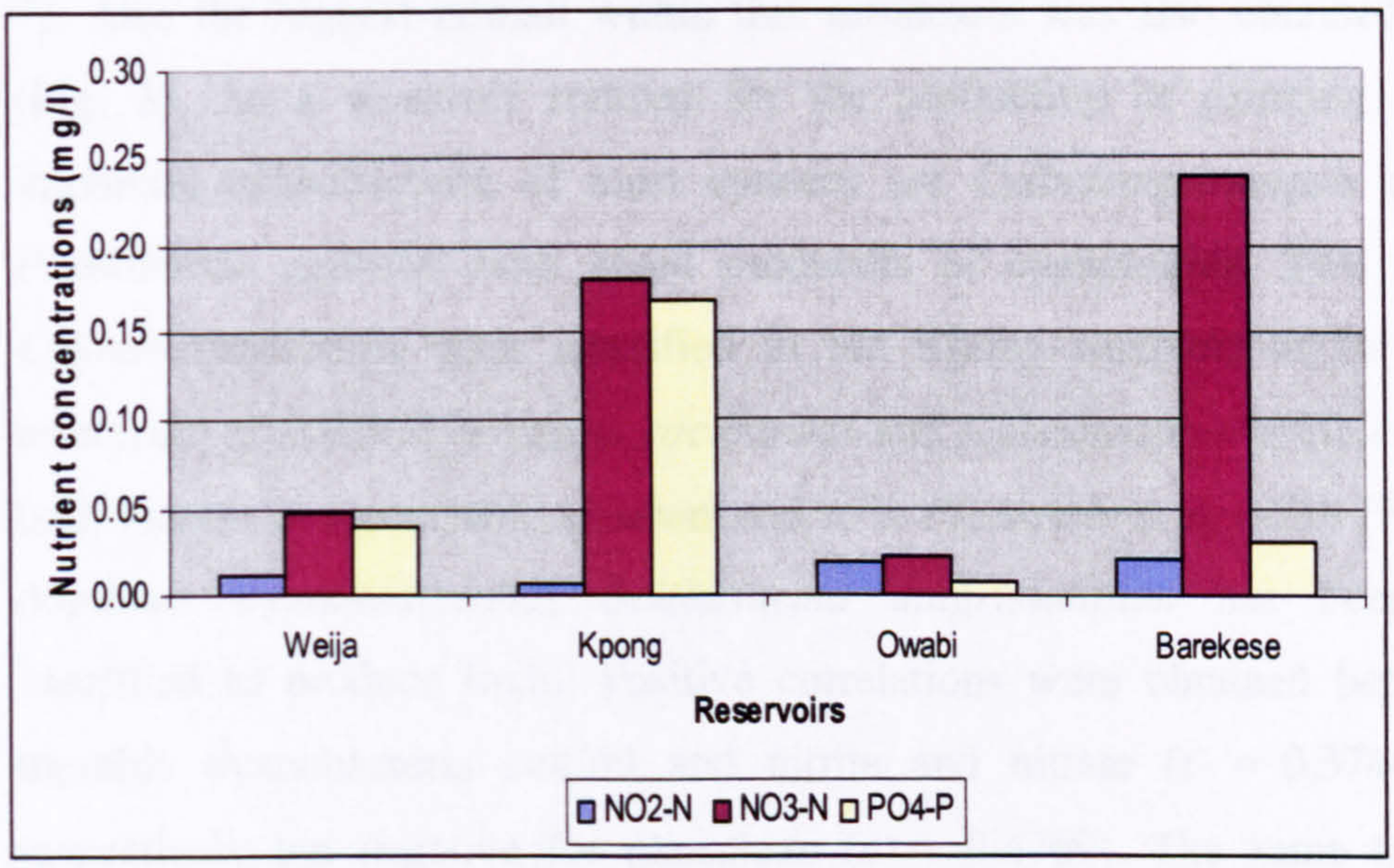


Figure 5 Summary of mean nutrient concentration of nitrite, nitrate and phosphate in the Weija, Kpong, Owabi and Barekese reservoirs in Ghana

Kpong reservoir

The Kpong reservoir, even though higher in nitrate and phosphate concentrations (Fig. 5) as compared to the Weija reservoir, does not support high phytoplankton diversity (Appendix 4). The Kpong reservoir has a mean nitrate and nitrate concentrations of 0.18 and 0.17 mg/l respectively as compared to the Weija reservoir which had mean concentrations of 0.05 and 0.04 mg/l for nitrate and nitrate respectively. Eight genera of cyanobacteria were identified in this reservoir during the current study as against the sixteen genera obtained in the Weija reservoir. A total of ten species were identified of which *Geitlerinema unigranulatum* had an overwhelming dominance, making over 68% of the total biomass (Fig. 6) with a maximum cell concentration of over 49, 000 cell/ml in March when the highest nitrate concentration of 0.29 mg/l was obtained (Fig. 7), which also coincided with the lowest phosphate concentration of 0.001 mg/l (Fig. 7). Also the highest rainfall within this catchment was also obtained in March (Fig. 3). As a reservoir sourced for the production of drinking water, the important cyanobacteria of most concern are *Cylindrospermopsis* as well as *Planktothrix agardhii* both noted producers of cyanotoxins. Two species of *Cylindrospermopsis* were identified in the Kpong reservoir as in the Weija reservoir. *Cylindrospermopsis raciborskii* and *Cylindrospermopsis cuspis* were both present in appreciable numbers and so is *Planktothrix agardhii* (Fig. 7). The dominant cyanobacterium, *Geitlerinema unigranulatum* has been recently identified to produce toxin. Positive correlations were obtained between total monthly cyanobacteria cell/ml and nitrite and nitrate ($r = 0.3744, 0.6286$) respectively but negative for phosphate ($r = -0.4769$). The same trends were obtained between total monthly rainfall and nitrite and nitrate ($r = 0.0603, 0.3786$) respectively but negative with phosphate ($r = -0.5703$). However, a high positive correlation was obtained between total monthly rainfall and total monthly cyanobacteria cell counts/ml ($r = 0.8286$).

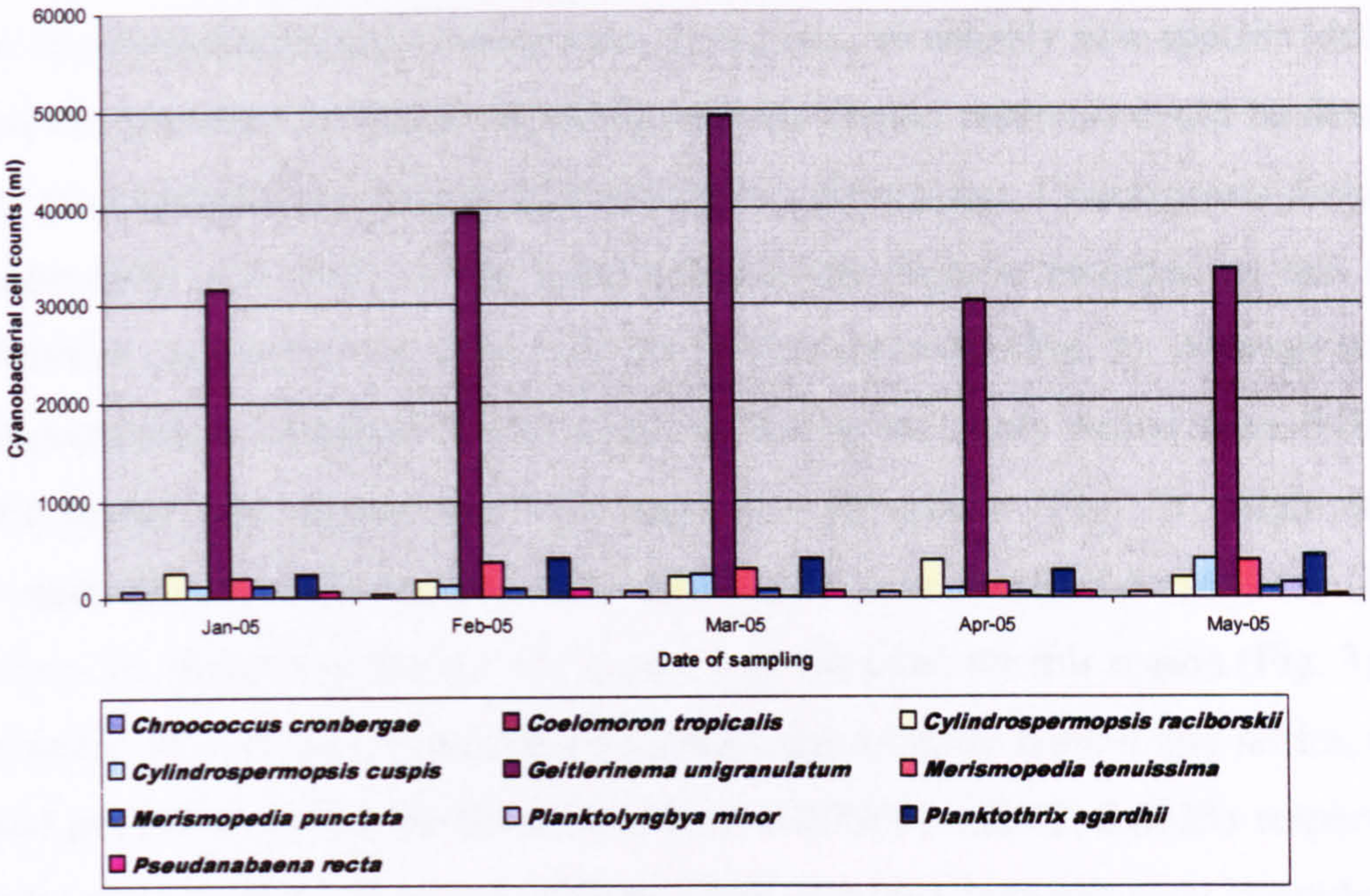


Figure 6 Changes in cyanobacteria composition and biomass in the Kpong reservoir, Accra, Ghana.

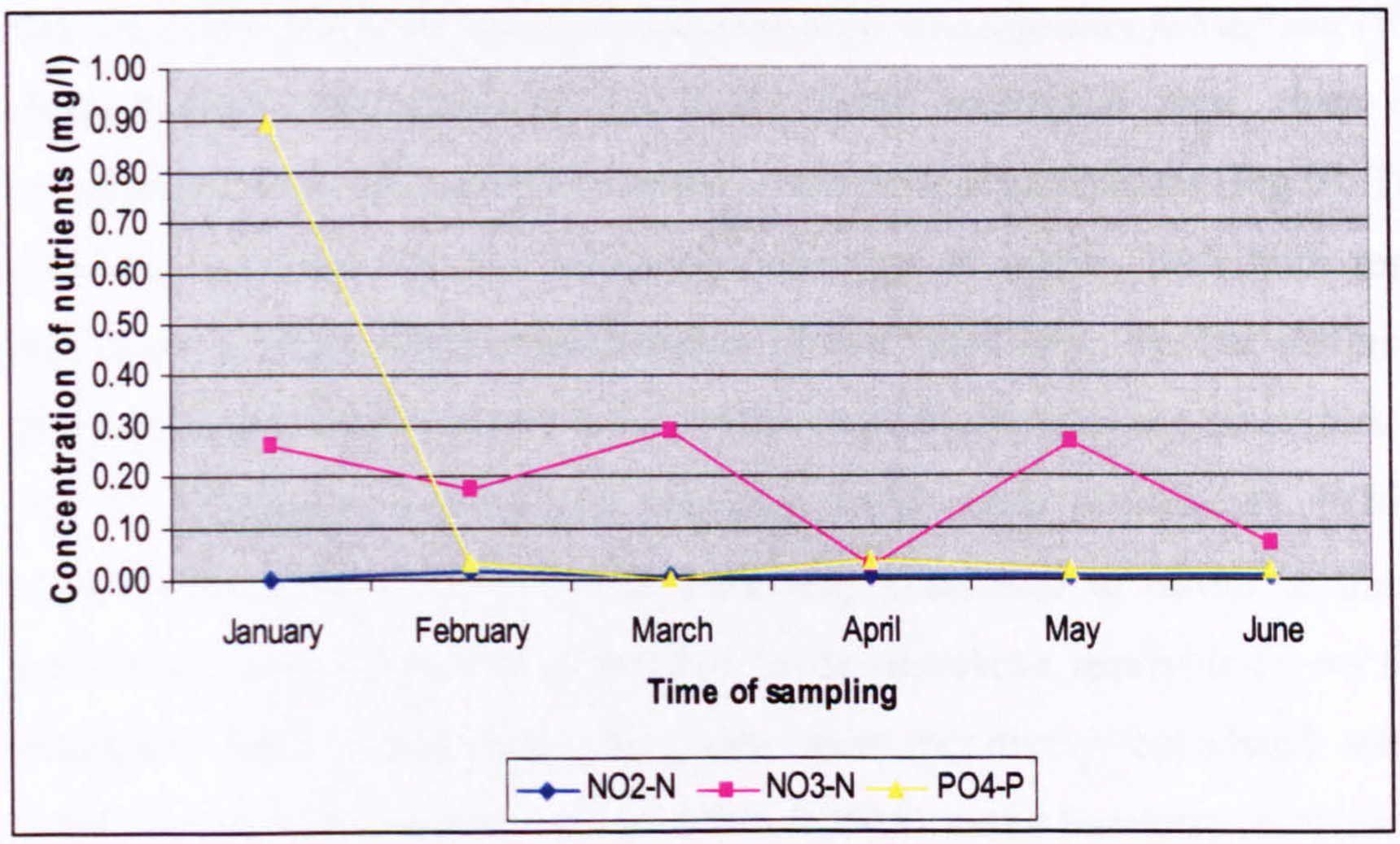


Figure 7 Changes in nitrite, nitrate and phosphate concentrations in the Kpong reservoir in Accra, Ghana

The Barekese and Owabi reservoirs both situated in the Ashanti region, ecologically described as the closed-forest zone of Ghana were both dominated by the cyanobacterium *Cyanogranis ferruginea*, an entirely new species identified for the first time in Ghana. From figure 8 the Owabi reservoir could be described as monospecific population of *Cyanogranis ferruginea*. *Cyanogranis ferruginea* comprised over 96% of the total biomass the highest recorded in this study. Nutrient concentrations were very low in this reservoir (Fig. 5). Average nutrient concentrations obtained in this reservoir during the study period were 0.02 mg/l for nitrite and nitrate and 0.01 mg/l for phosphate (Fig. 5). High nutrient concentrations of all the three nutrients studied were obtained during May (Fig. 9) when the highest rainfall of 197.6 mm was recorded for this region (Fig. 3). This resulted in a positive correlation between total monthly rainfall and nitrite, nitrate and phosphate in the Owabi reservoir (r =0.7909, 0.6542, 0.6625) respectively. Also very high positive correlations were obtained between total cyanobacteria cell counts/ml and nutrients (r =0.8479, 0.9286, 0.8424) for nitrite, nitrate and phosphate respectively. In addition, positive correlation was obtained between total monthly rainfall and total monthly cyanobacteria cell counts/ml (r =0.4706). The Barekese reservoir was also dominated by *Cyanogranis ferruginea* (Fig. 10). Even though both reservoirs lie in the same ecological zone, their nutrient concentrations varied greatly, in terms of nitrate and phosphates (Fig.9). The only similarity between the two reservoirs was that of nitrite, with both reservoirs having an average nitrite concentration of 0.02 mg/l (Fig. 5). The differences in phosphate and nitrate concentration in the Owabi and Barekese reservoirs may be due to differences in land use practices within their catchments. While total cyanobacteria cell counts/ml was positively correlated to nitrite in the Owabi reservoir it was negatively correlated in the Barekese reservoir (r =- 0.3008). However, both nitrate and phosphate were positively correlated with total cyanobacteria cell counts/ml (r =0.5380, 0.2943) in the Barekese reservoir as in the Owabi reservoir respectively. This may have accounted for the dominance of *Cyanogranis ferruginea* in both reservoirs. Looking at figures 9 and 11, it is obvious that in both reservoirs nitrate concentration was higher than the other two nutrients. The highest nitrate concentration recorded in the Barekese reservoir (over 1.2 mg/l) in February (Fig. 11) coincided with the beginning of the raining season, which also coincided with the highest biomass of *Cyanogranis ferruginea*. The Barekese reservoir was also more diversified than the Owabi

reservoir, but the Owabi reservoir had higher biomass of the dominant cyanobacterium *Cyanogranis ferruginea*. The toxicity of *Cyanogranis ferruginea* has not been well established, but microcystins have been recently identified in both the Owabi and Barekese reservoirs. Microscopic pictures of most of the cyanobacteria species discussed in the results section can be seen in Plate 1, 2 and 3. All photos were taken with a magnification of 400X and 600X.

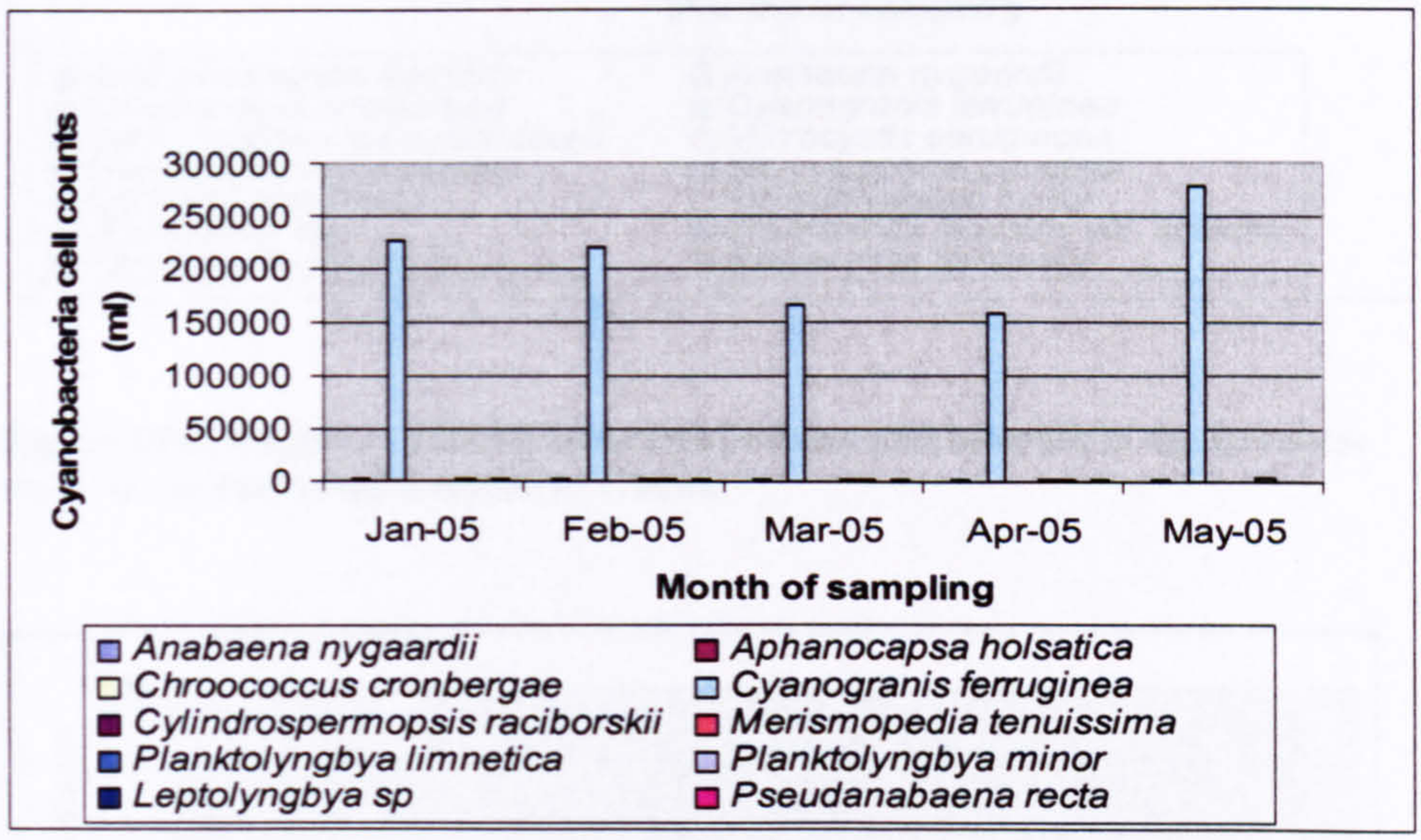


Figure 8 Changes in cyanobacteria composition and biomass in the Owabi reservoir in the Ashanti region of Ghana.

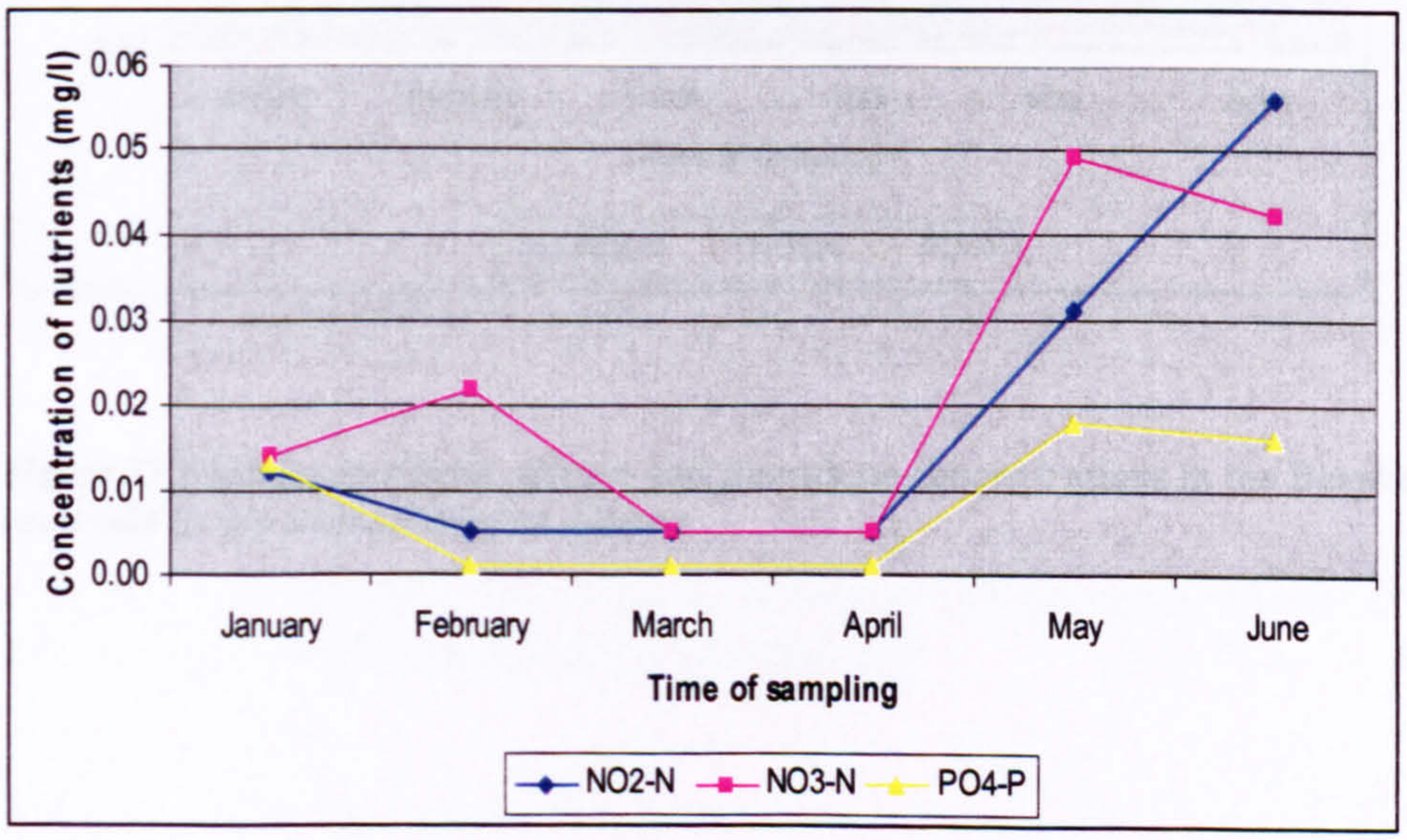


Figure 9 Changes in nitrite, nitrate and phosphate concentrations in the Owabi reservoir in the Ashanti Region, Ghana

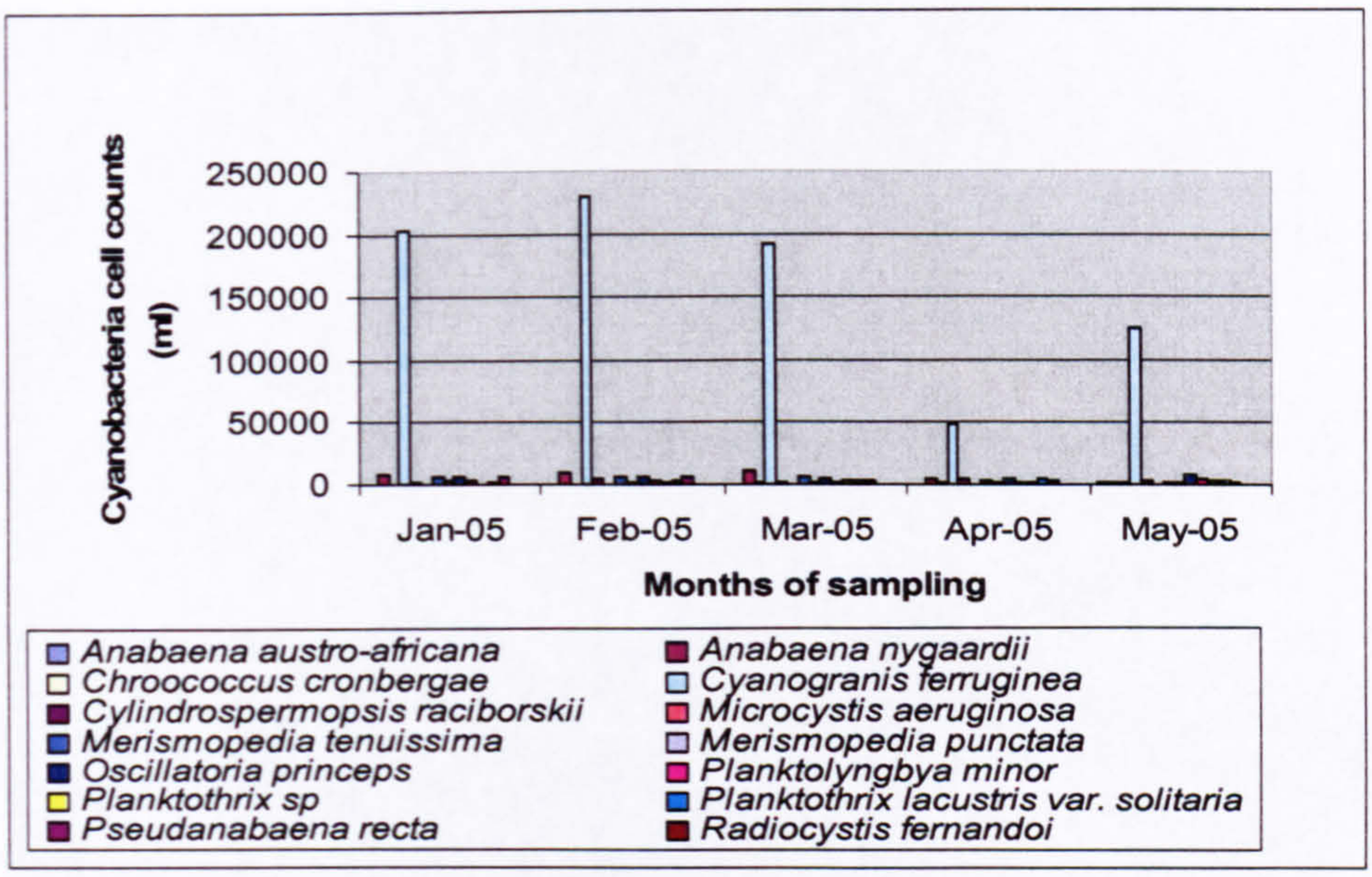


Figure 10 Changes in cyanobacteria composition and biomass in the Barekese reservoir in the Ashanti region of Ghana

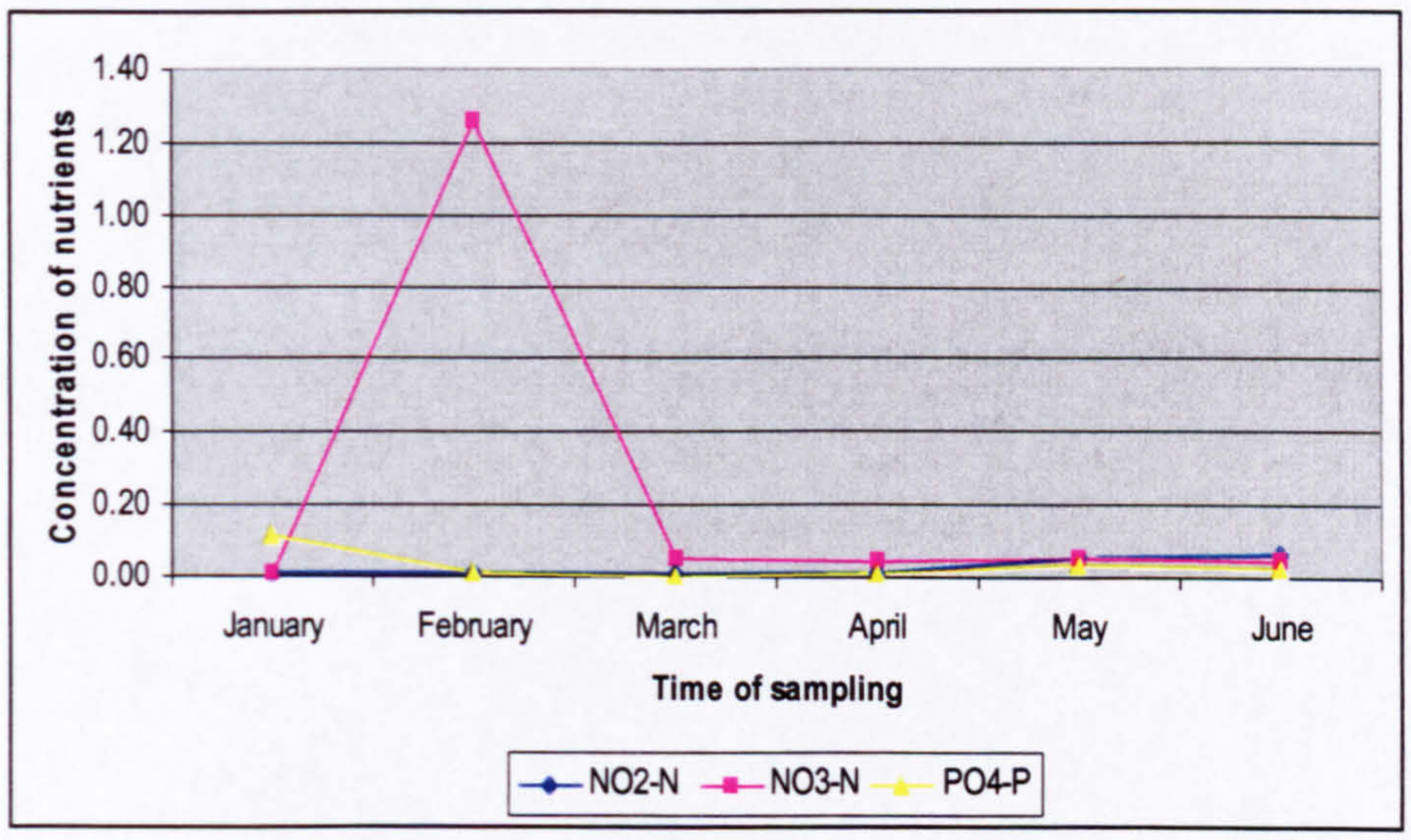


Figure 11 Changes in nitrite, nitrate and phosphate concentrations in the Barekese reservoir in the Ashanti Region, Ghana

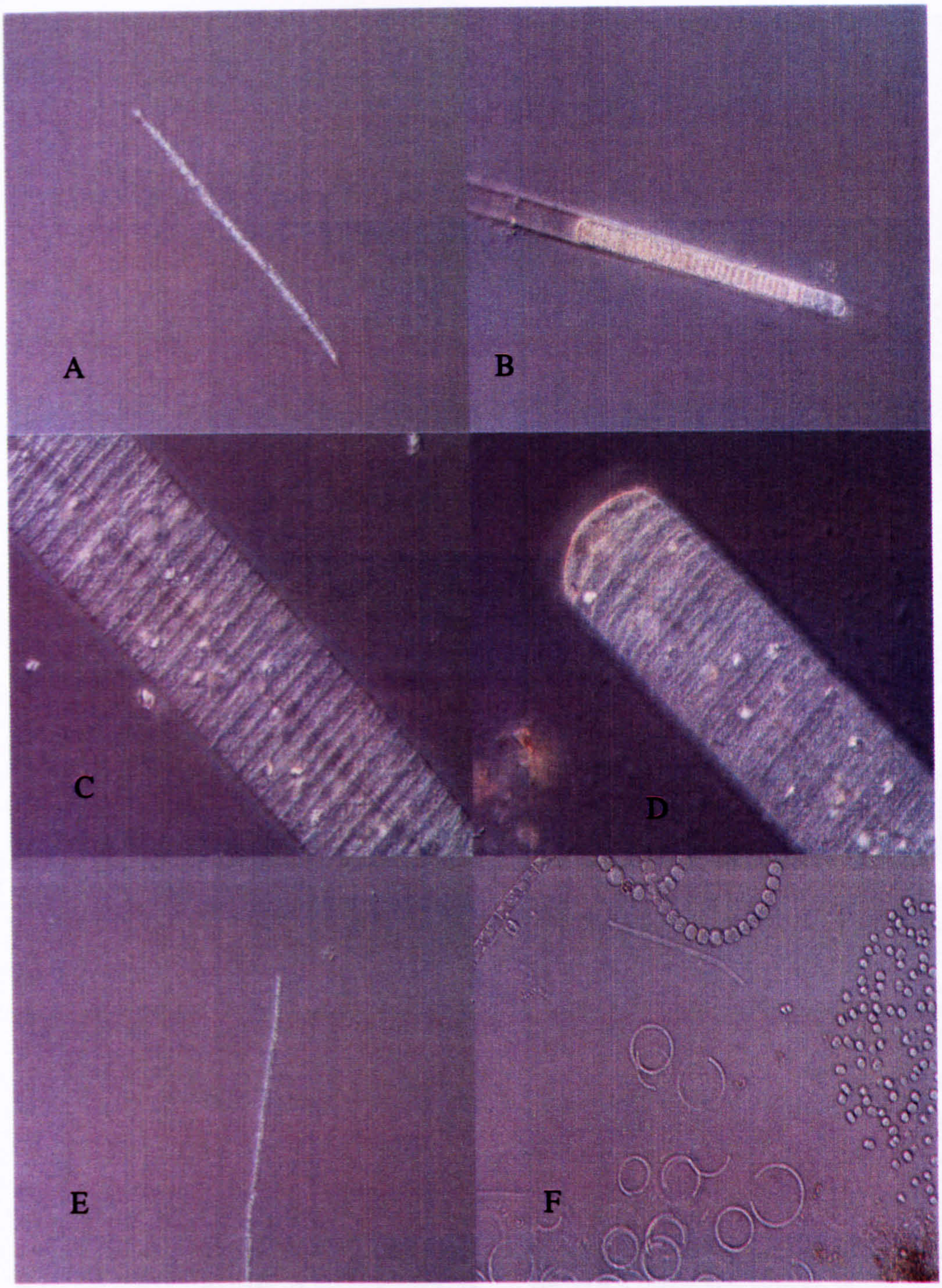
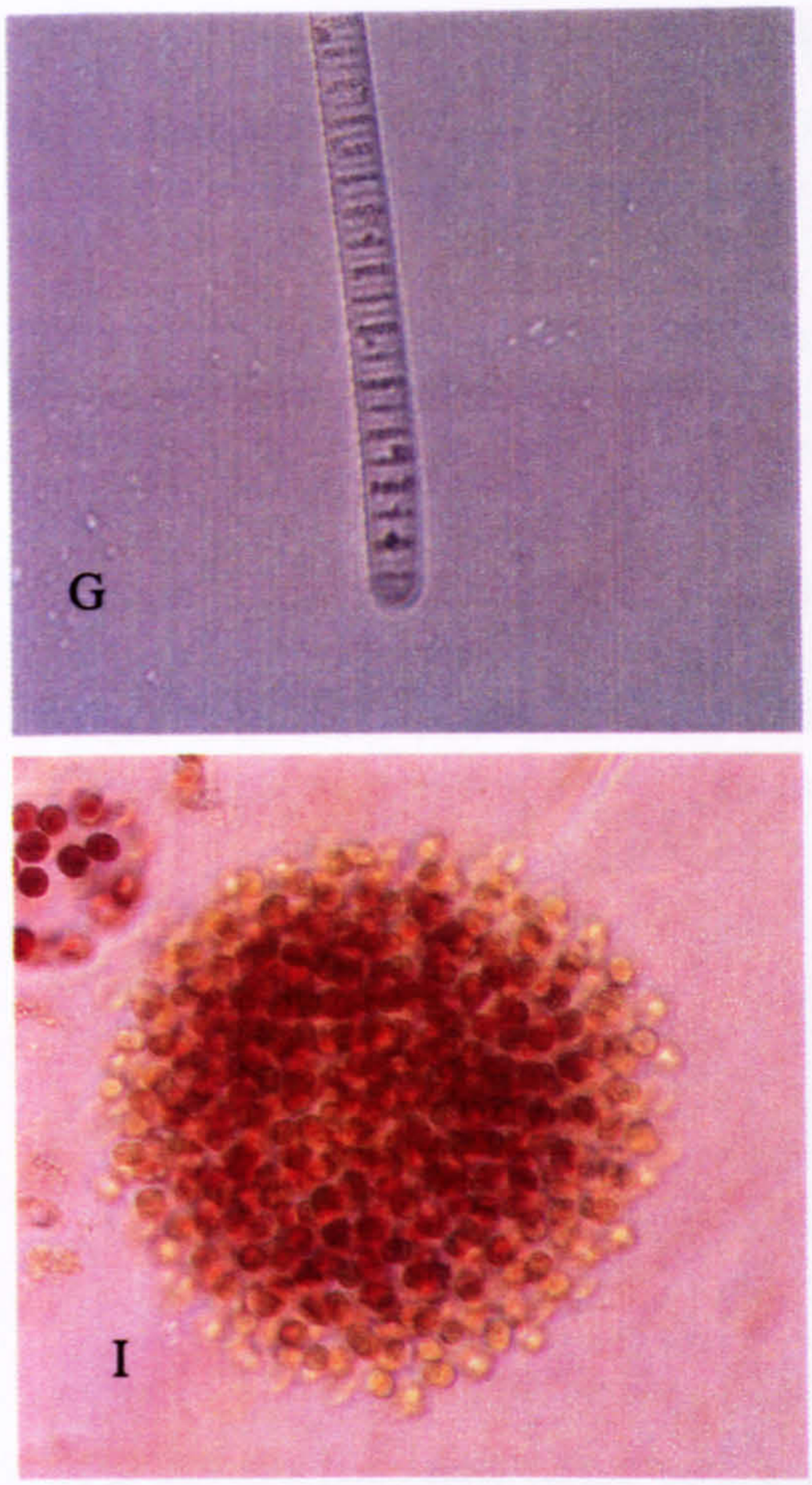
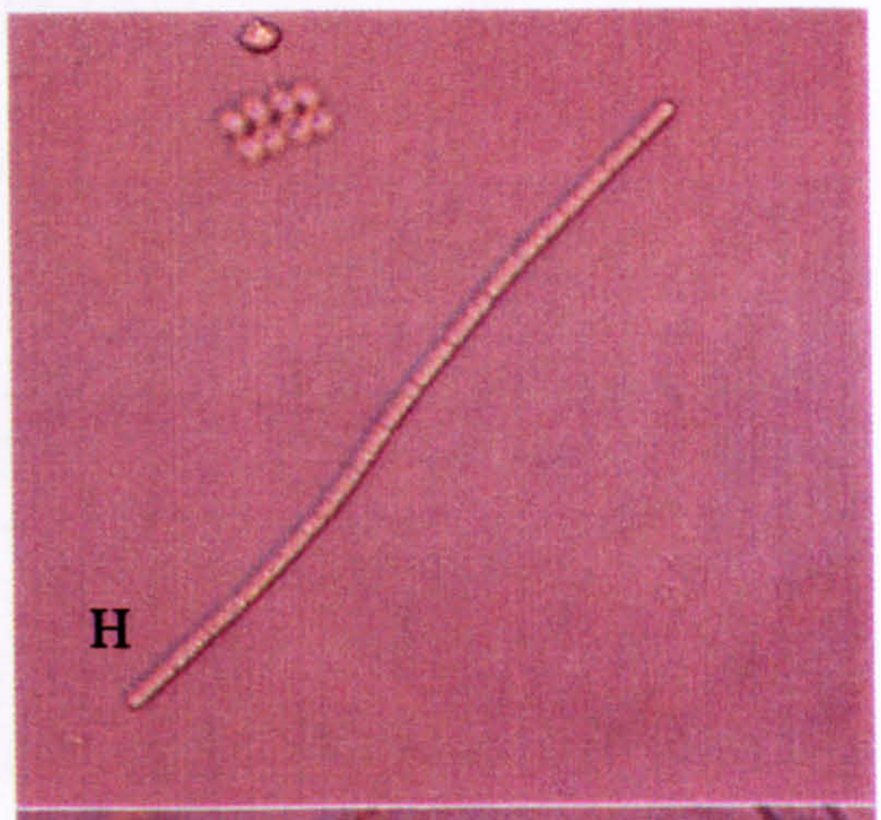
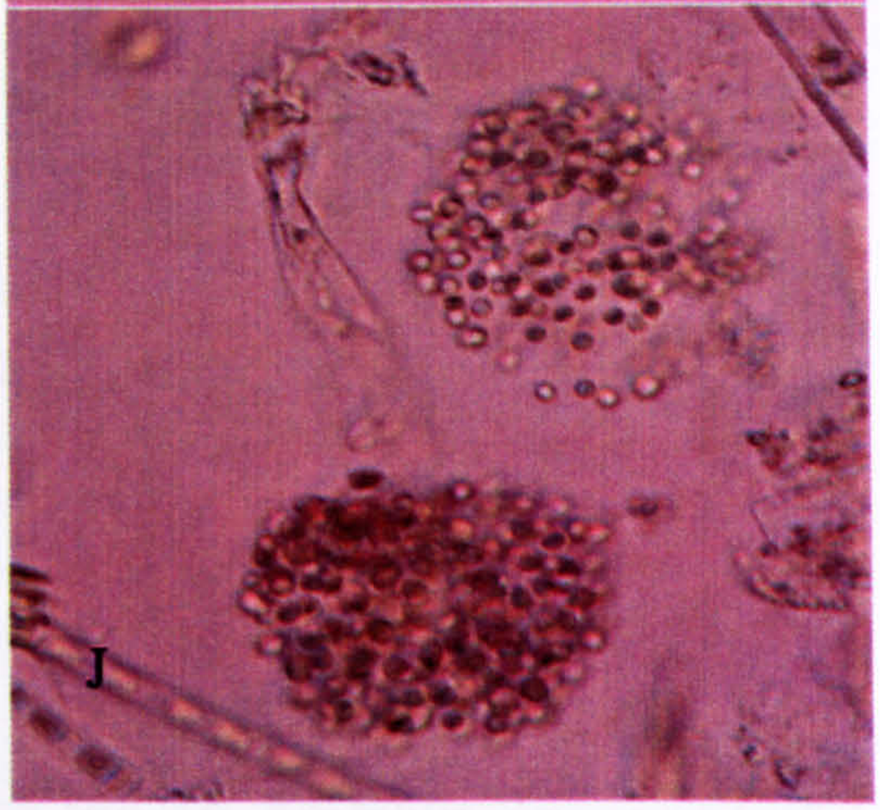
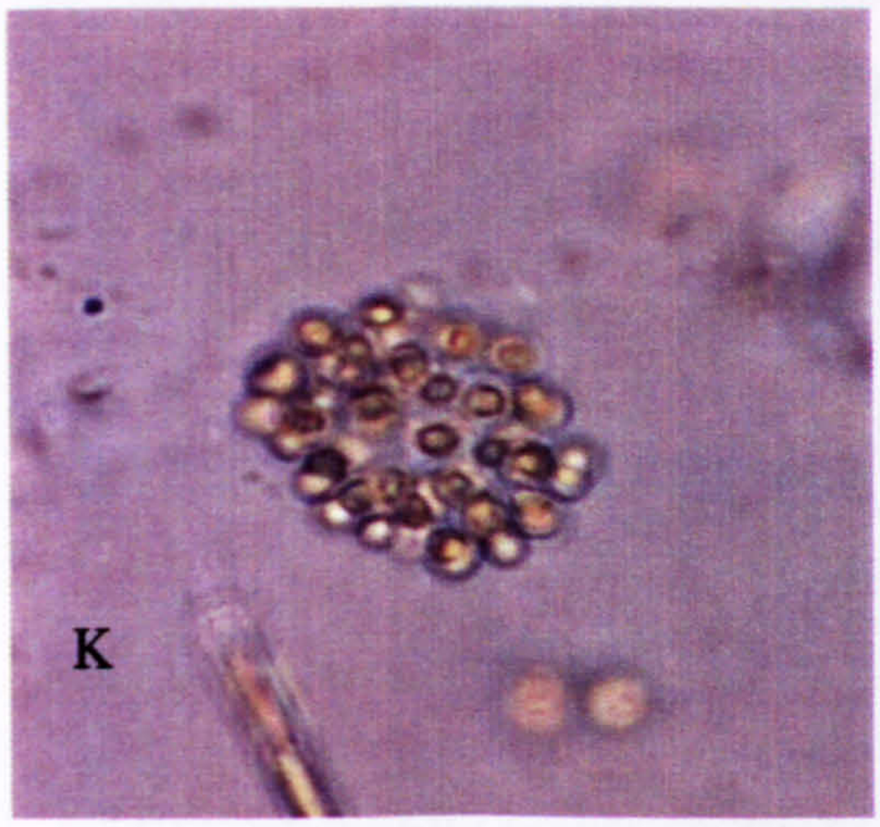


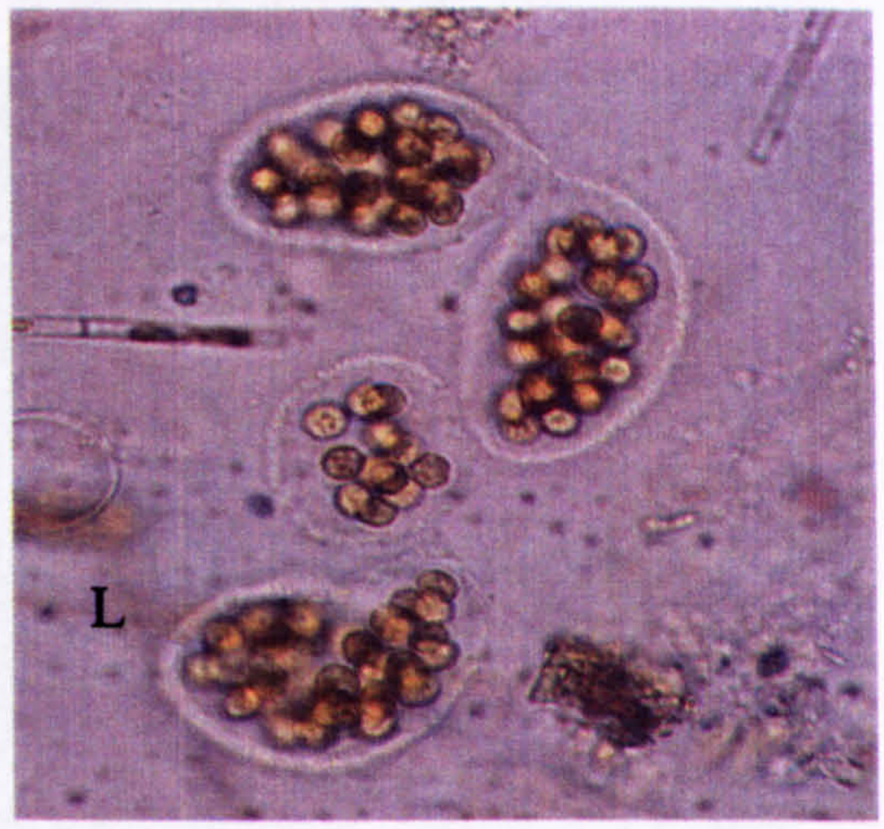
Plate 1: A. *Cylindrospermopsis raciborskii*, B. *Lyngbya* sp., C and D. *Oscillatoria princep* E. *Planktothrix* cf *suspensa*, F. Mixture of cyanobacteria (*Planktolyngbya circumcreta*, *Anabaena* sp and *Microcystis* sp)











.Plate 2: G. *Oscillatoria miniata*, H. *Planktolyngbya limnetica* I. *Radiocystis Fernandoi* J. *Aphanothece nubilum*, K. *Coelomoron* sp, L. *Microcystis wesenbergii*

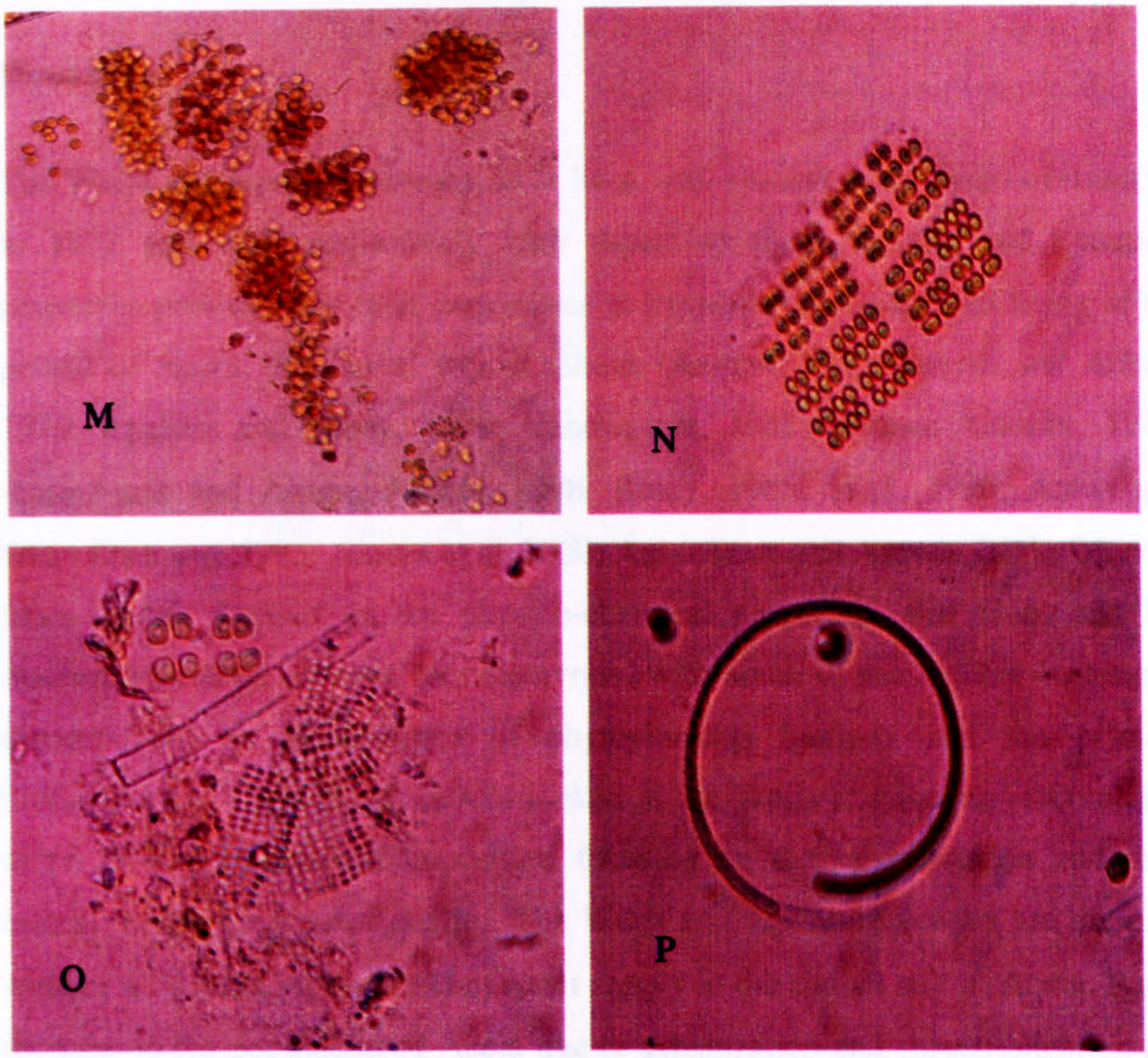


Plate 3: M. *Microcystis* sp, N. *Merismopedia punctata*, O. *Merismopedia tenuissima*, P. *Planktolyngbya circumcreta* Q. *Pseudanabaena recta* and R. *Romeria elegans*

Discussion

The Weija and Kpong reservoirs have been well studied since their construction in 1977 and 1981 respectively with regard to their physical and chemical properties probably due their importance in the socio-economic well being of the people of Accra, the capital city of Ghana (Amuzu, 1970; Dassah and Abban, 1979; Kpakata and Biney, 1979; Vanderpuye, 1982; Gyimah-Amoako, 1989; Ansa-Asare and Ansong-Asante, 1998; Biney, 1985, 1987, 1990; Antwi and Ofori-Dannson, 1993; Ameka et al. 2000; Ansa-Asare and Asante, 2005). Most of these studies focused on the physico-chemical properties, fish fauna and the macrophytes flora of these two reservoirs, with some of the authors looking at chlorophyll-*a* as an estimate of phytoplankton biomass and the primary productivity. Addico et al. (2006) looked at the Weija and Kpong reservoirs in terms of phytoplankton composition focusing on the cyanobacteria and their effects on drinking water quality. The Weija and Kpong reservoirs are the only sources of potable piped drinking water supply in the capital city of Accra. Rapid increase in the population of Accra due to urbanization and industrialization has led to an increased demand for water for domestic, agricultural and commercial uses. This situation has led to an increased pressure on the already over exploited resources of the Weija reservoir. The south-western parts of Accra which depends on the Weija reservoir for its drinking water supply are constantly in short of drinking water as a result of algal pollution which results in delays in water treatment as the filters are constantly been clogged. The cost of drinking water production in the Weija reservoir has significantly increased as compared to that of the Kpong reservoir. Addico et al. (2006) showed that cyanobacteria composed from 70 to 90% of the total phytoplankton biomass in both reservoirs. This current study is focussed on the diversity and biomass of the cyanobacteria in the four reservoirs sourced for the production of drinking water. The Weija and Kpong reservoirs are typical tropical waters with mean temperature range of 29.3-31.0°C and 28.9-30.8°C respectively (Ansa-Asare and Ansong-Asante, 1998; Ameka et al. 2000) with a near neutral to neutral pH ranging from 7.0 to 7.6. The Weija reservoir has been reported to be saturated with oxygen with mean oxygen saturation ranging from 85 to 90% (Ansa-Asare and Ansong-Asante, 1998), which was attributed to high photosynthesis by phytoplankton. The Weija reservoir is mostly turbid with mean transparency of 59.1 cm (Ameka et al. 2000),

attributed to high suspended matter including phytoplankton (Ameka et al., 2000) and silt contained in discharges of domestic effluents and run-off from agricultural lands within the catchment. On the contrary, the Kpong reservoir is clear with secchi disc reading of 180 cm (Biney, 1985). The Weija reservoir had been described as eutrophic (Ansa-Asare and Asante, 2005). Most of the cyanobacteria identified in this reservoir are known to thrive in eutrophic waters (Komarek, 2003; Cronberg and Komarek, 2004; Sant' Anna, et al. 2004; Komarek, 2005; Bouvy et al. 2000, 2006). *Aphanocapsa* cf. *nubilum* Komarek et Kling 1991, the dominant species in the Weija reservoir occurs among the plankton mostly in lakes, ponds and artificial reservoirs, with a cosmopolitan distribution (Sant' Anna et al. 2004). There are no literature regarding to the toxicity of this particular species but another species of *Aphanocapsa*, *Aphanocapsa incerta* described in Brazil a sub tropical region has been described as potentially toxic (Sant' Anna, et al. 2004). *Aphanocapsa nubilum* with cells diameter of 1.2-2 μm can be described as nanocyanobacteria. Small-coiled, colonial nanoplanktons are typically not scum forming. They have been described as small unicellular simple cyanobacteria of cell size 0.2-2 μm (Blaha and Marsalek, 1999; Luuc et al. 1999) and by Cronberg and Annadotter (2006) as ranging from 1-4 μm. As a reservoir sourced for the production of drinking water the presence of potentially toxic nanocyanobacteria in such high biomass is health risk. Blaha and Marsalek (1999) cautioned that the finding that such small sized cyanobacteria produces microcystin is an important situation in drinking water treatment, since they can leak through filter systems more easily than scum-forming genera like *Anabaena*, *Microcystis* and *Aphanizomenon*. Their results of invitro toxicity testing based on the picocyanobacteria *Cyanobium rubescens* and *Synechococcus nidulans* showed that low molecular weight picocyanobacterial product can cause several adverse effects of mammal cell models. They also found in vitro cytotoxic, hepatotoxic and immunotoxic activities. Blaha and Marsalek, (1999) observed slight immunotoxic effects targeted especially on T-lymphocytes, proving production of antimitogenic compounds in picocyanobacteria strains. As mentioned earlier Addico et. al. 2006 obtained six different microcystin peaks at the intake of this reservoir, with a total toxin concentration of 3.21 μg/l and microcystin-RR in the Kpong reservoir. The dominant cyanobacterium in the Kpong reservoir, *Geitlerinema unigranulatum* (R.N. SINGH) comb. nova with thin trichomes of about 0.8-2 μm in diameter has

been recently identified as a toxin producer (Azevedo pers com), and also commonly found in eutrophic freshwater reservoirs in tropical regions (Komarek and Azevedo, 2000). Other cyanobacteria found in the Kpong reservoir with toxic implications are *Planktothrix agardhii* (Gom.) Anagnostidis et Komarek 1988 producing both hepatotoxins and neurotoxin (Krishnamurty *et al.*, 1986; Meriluoto et. al., 1989; Sivonen and Jones, 1999; Chorus, 2001; Laub et al., 2002; Falconer, 2004) and *Cylindrospermopsis raciborskii* (Wolosz) Seenaya et Subba Raju 1972, which produces the toxin cylindrospermopsin (Sivonen and Jones 1999, Falconer, 2004). This cyanobacterium was present in all the four reservoirs studied and was present through out the sampling period. In Australia this cyanobacterium was first recognised as a result of substantial human poisoning episode through a bloom in a drinking water supply reservoir (Byth, 1980; Bouke et al. 1983; Hawkins et al. 1985). *Cylindrospermopsis raciborskii* is a pervasive and a cosmopolitan species which is commonly found in Ghana as well as other African countries (Ganf, 1974; Komarek and Kling, 1991; Cogels et al. 2001; Cronberg and Komarek, 2004; Addico et al. 2006; Dufour et al. 2006; Bouvy et al. 2000, 2006). *Cylindrospermopsis raciborskii* is very toxic with a wide range of toxins (Humpage et al. 1994; Sivonen and Jones, 1999; Falconer, 2004; Cronberg and Komarek, 2004) and can cause significant changes in the phytoplankton components (Bouvy et al. 2001; Leonard and Paerl, 2005). In addition, the ability of *Cylindrospermopsis raciborskii* to grow well below the water surface close to water intake points makes it a high risk cyanobacterium to public health through drinking water (Fabbro, 1999; Falconer, 2004). Falconer (2004) reported three phylogeographical groups of *Cylindrospermopsis raciborskii*, with strains isolated from the US and Australia producing only cylindrospermopsin, those from Brazil producing either saxitoxins and/or cylindrospermopsin and the final group from Europe producing an unknown neurotoxin. One of the reasons attributed for the proliferation of *Cylindrospermopsis raciborskii* is its ability to compete well for nutrients. Even though the Weija reservoir had much lower nutrient concentrations than the Kpong reservoir, the Weija reservoir had higher cyanobacteria counts in general and of *Cylindrospermopsis raciborskii* than the Kpong reservoir. *Cylindrospermopsis raciborskii* takes advantage of its ability to assimilate ammonium and phosphate at low nutrient concentration (Presing et al. 1996; Istvanovics et al. 2002; Briand 2002) and also ability to fix atmospheric nitrogen using its terminal heterocyst (Padisak, 1997). In addition it can migrate

to deeper and more nutrient-enrich layers through buoyancy changes (Branco and Senna, 1994). These same reasons can be attributed to the high cyanobacteria diversity of the Weija reservoir as compared to the Kpong reservoir. Both the Kpong and the Weija reservoirs had low ratios of nitrogen to phosphorus (1.32 and 1.10) respectively, implying nitrogen limitation in these reservoirs. Also Meybeck et al. (1989) reported that in waters with n/p ratio lower than 7, nitrogen will be limiting. Falconer (2004) also reported in general, the higher the ratio towards nitrogen excess, the more likely diatoms or green algae will dominate, while the lower the ratio the more likely that cyanobacteria will dominate. Bouvy et al. (2006) reported that in Lake Guiers, in spite low nutrient concentrations during their study, cyanobacteria community exhibited high abundances and biovolumes especially *Cylindrospermopsis raciborskii*, similar to our results and also to those observed in other tropical ecosystems (Havens et al. 1998; Bouvy et al. 1999; Lung'ayia et al. 2000). Flushing and dilution of the reservoir waters by the Volta Lake which discharges into the Kpong reservoir as well as the resident time of the water in the Kpong reservoir may also be the cause of low cyanobacteria diversity in this reservoir. The mean annual water flow through the Kpong reservoir is 1,183 m^3 s^{-1} as compared to the 54.2 m^3 s^- for the Weija reservoir (Ansa-Asare and Ansong Asante, 1998; Antwi and Ofori-Danson, 1993). In addition, the high macrophytes cover of the Kpong reservoir which may be in competition for nutrients and light input with phytoplankton could also be responsible for the low diversity and biomass of cyanobacteria in the Kpong reservoir. It has been reported that about 20-25% of the reservoir surface is covered by aquatic macrophytes (Gyima-Amaoko (1989; Antwi and Ofori-Danson, 1993). This estimate may be higher as at now. Falconer (2004) reported of *Cylindrospermopsis raciborskii* occurring in mixed population with *Aphanizomenon*, *Aphanocapsa*, *Limnothrix* and *Planktolyngbya* in the Fitzroy River in Australia similar to the situation in Ghana. *Cylindrospermopsis raciborskii* grows to bloom proportions only in water with temperature above 25°C (Padisak, 1997), most water bodies in Ghana have mean water temperatures higher than this and this makes our reservoirs ecologically favourable for this cyanobacterium. The second species of *Cylindrospermopsis*, *Cylindrospermopsis cuspis* identified in these reservoirs is hardly mentioned in literature probably because it is not a commonly occurring species. The positive correlation obtained between nitrite and phosphate and that of rainfall and also with total monthly

cyanobacteria cell counts/ml, may imply that whilst nitrite and phosphate were largely controlled by land based sources via runoff from the catchment during rainfall, nitrate on the other hand may be from other sources apart from land, probably through nutrient cycling from the sediment and decomposition of dead cyanobacteria. The catchment of Weija reservoir is a densely populated area with high human activities both domestic and commercial horticulture and crop farming. Ansa-Asare and Ansong-Asante (1989) observed similar trends, with nitrite concentration higher during the rainfall season in the Weija reservoir. The highest biomass of *Aphanocapsa nubilum* obtained in April may be nutrient induced as this time coincides with the main ploughing season in the Weija catchment when most fertilizer is applied to the land. Biney (1983) reported that ammonia in the presence of high oxygen is readily oxidized through nitrite to nitrate. The Weija reservoir as mentioned earlier is very saturated with oxygen. Again Biney (1990) reported that, while phosphorus may be introduced as a result of domestic and industrial activities, high levels of ammonia-nitrogen in these waters may be as a result of nitrogen fixation by cyanobacteria. 69 % of the cyanobacteria genera in the Weija reservoir are potentially toxic as compared to 25 % for the Kpong reservoir. These are *Anabaena* (Krishnamurthy et al. 1986; Watanabe et al. 1989; Sivonen et al. 1999), *Anabaenopsis* (Sivonen et al., 1999), *Aphanocapsa* (Domingos et al. 1999; Oudra et al. 2002; Sant' Anna, et al. 2004; Pavlova et al. 2006), *Cyanogranis* (Komarek pers com), *Cylindrospermopsis* (Sivonen et.al., 1999; Senogles-Derham et al. 2003; Veira et al. 2003; Falconer, 2004), *Geitlerinema* (Azevedo pers com), *Lyngbya* (Sivonen et al. 1999), *Microcystis* (Botes et al. 1984; Sivonen et al. 1999; Sant Anna et al 2004), *Planktothrix* (Meriluoto et al. 1989; Sivonen et.al. 1999; Luukkainen et al. 1993), *Pseudanabaena* (Oudra et al. 2002) and *Radiocystis* (Veira et at. 2003; Lombardo et al. 2006). This situation presents a high risk to human health through exposer to microcystins and cylindrospermopsin through drinking water. Drinking water treatment facilities in Ghana are very basic (Addico et al. 2006) and incapable of completely removing cells from the water. Eutrophication has been reported as the most important factor accounting for the growing incidence of cyanobacteria blooms of which approximately 50% are known to be toxic in rivers, lakes and reservoirs (National Rivers Authority, 1990). Nitrate and phosphate concentrations were very high in the Kpong reservoir. Mean nutrient concentrations obtained for the reservoir in this study was 0.17, 0.18 and 0.01

mg/l for phosphate, nitrate and nitrite respectively. Previous studies on this reservoir obtained similar results for phosphate and nitrite of 0.15 and 0.01 mg/l respectively (Antwi and Ofori-Danson, 1993). It is obvious that over the past decade nitrate concentration has increased drastically in the Kpong reservoir. The construction of the Kpong reservoir opened opportunities for large scale irrigation project in the Kpong catchment, with attendant increased use of nitrate fertilizer and this may have resulted in the current high nitrate concentration in the reservoir. UNESCO (1982) reported that the increasing use of artificial fertilizers on farmlands, especially in developing countries has resulted in increasing concentrations of nitrates in aquatic systems.

The Owabi and Barekese reservoirs both situated in the Ashanti region, ecologically described as the closed-forest zone of Ghana were both dominated by the cyanobacterium *Cyanogranis ferruginea* an entirely new species identified for the first time in Ghana. *Cyanogranis ferruginea* up to now has only been identified in European temperate waters. It is the first case of development of this species in a tropical country. The Owabi reservoir can be described as a monospecific of *Cyanogranis ferruginea*, the dominant cyanobacterium comprising over 96% of the total biomass. Even though both the Owabi and Barekese reservoirs lie in the same ecological zone, the nutrient concentrations varied greatly, in terms of nitrate. Both reservoirs had the same mean nitrite concentration of 0.02 mg/l. The differences in the mean nitrate concentration in the Owabi (0.02 mg/l) and Barekese (0.24 mg/l) reservoirs may be attributed to differences in land use practices within their immediate catchments. The Barekese reservoir is located in a heavy agricultural (crop farming) of the Ashanti region and could be receiving nutrients inputs from these activities while the Owabi reservoir is in the part of the Kumasi metropolis which is mainly built up with residential facilities and well protected by a heavily forested Ramsar site, which could have silted off some of the nitrates in runoff before reaching the reservoir. There appear to be very little information on the ecology or toxicity of *Cyanogranis ferruginea* (Wawrik) Hindak (1992), the dominant cyanobacterium in both the Owabi and Barekese reservoirs. However, according to Komarek pers com this cyanobacterium is potentially toxic. Pelechata et al., (2006) reported the dominance of *Cyanogranis ferruginea* in the Oczko Lake with an n/p ratio of 3 close to the n/p ration of 4 obtained for the Owabi reservoir where it composed of over 96% of the cyanobacteria biomass. Also Szelag-Wasielewska (2004),

observed *Cyanogranis ferruginea* and *Aphanocapsa nubilum* dominating the cyanobacterial population in the polluted eutrophic Warta River in Western Poland exposed to human activities such as agriculture and urban and industrial activities, a similar situation to that of the Owabi and Barekese reservoirs. Intracellular and dissolved toxins have been recently identified in both the Owabi and Barekese reservoirs (Addico et al. unpublished data). Even though the Barekese reservoir has some known toxin producing cyanobacteria, the overwhelming dominance of *Cyanogranis ferruginea* (over 96%) points to the fact that *Cyanogranis ferruginea* may be producing microcystins.

Conclusions

This paper discusses fifteen new cyanobacteria species identified for the first time in drinking water reservoirs in Ghana of which *Cyanogranis ferruginea* is reported for the first time in tropical waters. These are *Anabaena austro-africana*, *Anabaena nygaardii*, *Aphanocapsa nubilum*, *Aphanocapsa holsatica*, *Chroococcus cronbergae*, *Coelomoron tropicalis*, *Cyanogranis ferruginea*, *Cylindrospermopsis cuspis*, *Geitlerinema unigranulatum*, *Microcystis wesenbergii*, *Microcystis viridis*, *Planktothrix lacustris var. solitaria*, *Romeria elegans*, *Leptolyngbya* sp. and *Oscillatoria princes* a hormogonia solitary filament. In conclusion, the presence and dominance of small sized nanocyanobacteria in the Weija, Barekese and the Owabi reservoirs all with basic conventional water treatment facilities known to be ineffective in removing neither cyanobacteria nor their toxins present a high risk to human health through exposure to cyanotoxins such as microcystins and cylindrospermopsin. The situation with the Owabi reservoir with low nutrient levels as compared to the Barekese reservoir both situated in the same region emphasises the importance of protecting water catchments or watershed.

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III

First report of the hepatotoxins microcystin-LR, YR, RR and LF and other microcystin variants in extracellular (dissolved) and intracellular samples from four drinking water reservoirs and water supplies in Ghana

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Abstract

This chapter reports the identification of microcystin in four drinking water reservoirs in Ghana. These were the Weija reservoir in Accra, Kpong headpond (reservoir) at Kpong, Accra, and Barekese and Owabi reservoirs in the Ashanti region of Ghana. 130 water samples were analysed for intracellular microcystin whilst 57 water samples were analysed for dissolved microcystin using the High Performance Liquid Chromatography (HPLC) method. Results showed that out of the 130 samples analysed for intracellular microcystin 81 samples representing 62.3% were negative for microcystin whilst 49 samples (37.7%) were positive. The Owabi reservoir, dominated by the nanocyanobacteria *Cyanogranis ferruginea*, identified for the first time in any tropical water body was the worst reservoir, with 54.2% of the samples analysed containing microcystin. The commonly known microcystin variants identified in this reservoir were MC-LR, YR, RR and LF. The Barekese reservoir also dominated by *Cyanogranis ferruginea* had the highest total microcystin concentration per litre of 15.50 μg/l. With regard to water treatment the stage of raw water flocculation with alum yielded the highest concentration of intracellular microcystin. Five samples from the final drinking water stage were positive for intracellular microcystin while 2 samples were positive for dissolved microcystin. Values of 0.81μg/l and 0.57 μg/l dissolved microcystins were obtained in the final drinking water stage of the Weija and Kpong drinking water treatment plants respectively. These values are lower than the 1 μg/l of microcystin-LR recommended for drinking water by the World Health Organization (WHO, 1996) but higher than Levels of dissolved microcystin obtained in Zimbabwe, Brazil, Australia, China and Sweden where human poisoning have occurred. This puts the health of the drinking public at

risk, as microcystin is known to initiate and promote tumours in humans at exposure to chronic low concentration.

Key words: Intracellular microcystin, dissolved microcystin, cyanobacteria, reservoir, drinking water

Introduction

Concerns regarding the presence of microcystins in drinking water and their attendant negative health effects has raised research interest worldwide. Microcystins are secondary metabolites and are therefore produced only by certain strains of cyanobacteria (McElhainey and Lawton, 2005). Microcystin, the most studied cyanotoxin and the focus of this paper has been characterised from several cyanobacteria species most of which has been identified in Ghana recently. These are *Anabaena* (Krishnamurthy et al. 1986; Watanabe et al. 1989; Sivonen et al. 1999), *Anabaenopsis* (Sivonen and Jones 1999), *Aphanocapsa* (Domingos et al. 1999; Sant' Anna, et al. 2004), *Cylindrospermopsis* (Sivonen et.al. 1999; Senogles-Derham et al. 2003; Veira et al. 2003; Falconer, 2004), *Lyngbya* (Sivonen et al. 1999), *Microcystis* (Botes et al. 1984; Sivonen et.al. 1999; Sant Anna et al 2004), *Planktothrix* (Meriluoto et al. 1989; Sivonen et al. 1999; Luukkainen et al. 1993), *Pseudanabaena* (Oudra et al. 2002) and *Radiocystis* (Veira et at. 2003; Lombardo et al. 2006). Over 70 microcystin variants has been characterised to date (McElhainey and lawton, 2005) of which microcystin-LR is the most commonly encounted variant (Sivonen and Jones 1999) and with a toxicity of 60 μg/kg (Bell and Codd, 1996). According to Kuiper-Goodman et al. (1999), human beings are most likely to be exposed to microcystin through contaminated drinking water or recreational activities such as swimming. The cyclic nature of microcystin means they are highly stable in water across a wide range of pH and temperatures (Fischer and Dietrich, 2000; Dietrich and Hoeger, 2005). The toxicity of microcystin is mediated through their specific and irreversible inhibition of serine/threonine protein phospahates 1 and 2A which results in hyperphosphorylation of cellular proteins (Makintosh et al. 1990; Carmichael, 1994; Maatouk et al. 2002; De Figueiredo et al. 2004) leading to disruption of liver cell structures, followed by loss of cytoskeletal integrity and conductance, subsequent cytolysis or apoptosis and intrahepatic bleeding

resulting in cell death (Codd et al. 2005; Dietrich and Hoeger, 2005; Weigand and Pflugmacher, 2005). As a result of this biochemical activity, microcystins have been described as potent liver toxins as well as tumour promoters. There are also indications that exposure to low concentrations of microcystin in drinking water can cause chronic effects in mammals due to their potent tumour promoting activity (Ito et al. 1997; Humpage et al. 2000; Falconer et al. 1994, 2004). It has also been reported by Repavich et al. (1990) that microcystin induces chromosomal aberrations in human lymphocytes in vitro. There are indications that orally administered microcystin is readily taken up via the bile acid transport system and transported to the liver where it can be reintroduced into the small intestine by entero-hepatic recirculation (Falconer et al. 1992). Recently Bojana et al. (2003) have demonstrated that microcystin-LR at doses that were not cytotoxic (0.01-1 μg/ml), induced dose and time dependent DNA strand breaks in human hepatoma cell line HepG2. It has also been reported that microcystins could induce sterility (Irvine et al., 2003). The numerous incidences of animal poisoning and fatalities caused by microcystin have lead the World Health Organization (WHO) to set a guideline limit of 1 ug/l of microcystin-LR in drinking water (WHO, 1996). There are only few documented cases of report of microcystin poisoning in Africa, even though numerous cases of human poisoning by cyanotoxins have been reported worldwide (Byth, 1980; Bourke et al. 1983; Hawkins et al. 1985, 1997; Teixera et al. 1993; Yu et al. 1995; Ueno et al. 1996; Zhou et al. 2000; Carmichael, 2001). In Africa, the only documented cases of human poisoning through exposure to microcystin occurred in Harare, Zimbabwe. Incidence of gastroenteritis has been reported in Harare, Zimbabwe linked to an algal bloom in Lake Chievero (Zilberg, 1966; Marshall, 1991). Marshall (1991) discovered, by a retrospective study, a close relationship between the number of gastrointestinal cases and the number of colonies of the cyanobacteria *Microcystis aeruginosa* and *Anabaena flos-aquae*. Again in Harare, Zimbabwe, cases of gastrointestinal infections and liver cancer have risen by over 300% and 400%, respectively between 1991 and 2001. Documented cases of gastroenteritis rose from less than 100-300/1000 people, whereas liver cancer rose from 30-130/1000 people (National health Information and Surveillance unit, 1990-2001; Harare City Department of Health, 1990-2001). Although the extent to which this situation is linked to algal toxins unclear, Johansson and Olsson (1998) reported microcystin concentrations in Lake Chievero to be around 13.9 μg/l. Microcystin

was also detected in the city's tap water. In Ghana, there is no documented evidence of human or animal poisoning by cyanotoxin. However, the high number of potentially toxic cyanobacteria encountered in our water bodies makes these issues very relevant. This study reports the first ever identification of microcystin in four reservoirs used for the production of drinking water in Ghana. These are Weija and Kpong reservoirs which supply drinking water to Accra and Owabi and Barekese reservoirs, supplying drinking water to the Kumasi Metropolitan Area. All four study sites have been described in previous papers in this thesis.

Materials and Methods

Water samples for microcystin analysis were collected with 1 litre clean plastic bottles from the four reservoirs mentioned above from each treatment stage namely: intake, flocculation, sedimentation tanks or clarifies, filtered water and finally chlorinated water. In the case of the Kpong reservoir samples were from four stages since allum is not applied during water treatment.

Extraction of cell bound (intracellular) microcystins

Water samples of 1 litre volume were filtered through pre-weighted GFC filter paper pore size 0.45 μm. The cells collected on the filters were frozen overnight and freeze-dried. Freeze-dried cells on filters were stored at - 20°C until extracted for HPLC. Extraction of cell-bound intracellular toxins from freeze-dried cells was done as described by Harada et al., (1999). Cells were extracted in 20 ml of 75% aqueous methanol for 1 hour and repeated three times as described by Harada et al., (1999). The 75% methanol had been recommended by Faster et al., (1998) and used by many authors (Spoof et al., 2003, 2004; Hoeger et al., 2005). The Final extract was dissolved in 400 μl methanol after drying with rotary evaporator and cleaned prior to HPLC analysis with 0.45 μm pore sized mini start non-pyrogenic 16555 Millipore syringe filter.

Extraction of dissolved (extracellular) microcystins

Extracellular dissolved microcystin was done using the solid phase extraction method. Filtered water samples of about 1 litre collected from the drinking water reservoirs as described above were passed through reversed-phase octadecyl (C-18) silanised silica gel (ODS) in 3 ml tube cartridges concentration and clean up as described by Meriluoto, (1997; Harada et al., (1999). The cartridges were activated prior to use with 5 ml pure methanol and washed with 5 ml of distilled water as described by Harada et al., 1999 and Falconer (2004). Final elution of toxins from cartridge was achieved by using 15 ml pure methanol. Further concentration of methanolic extract was achieved by drying using rotary evaporation. The residue after drying was redissolved in 400 μl of pure methanol on ultrasonic bath for 5 minutes and transferred into vials for HPLC analysis.

Identification and quantification of microcystins

Microcystins were identified and quantified using high performance liquid chromatography (HPLC). HPLC technique has been the most widely used method for both research and routine analysis of natural samples (McElhiney and Lawton, 2005). Extracts of samples from Ghana were analysed using a reverse-phase high performance liquid chromatography system, Agilent 1100 series with a photodiode array detector (DAD) G1315B, autosampler ALS G1313A with a quaternary pump G 1311A. The stationary phase was Phenomenex Luna 5 μ C18 150 ×4.60 μm. Column temperature was kept at 30°C. The mobile phase was A, 0.05% trifluoroacetic acid in water and B; acetronitrile with 0.05% trifluoroacetic acid, which according to McElhiney and Lawton (2005) acts as an ion-pairing agent. A linear gradient of 30 % of B at 0 min to 70% of B at 30 min, with a flow rate of 1 ml/min was used. Volume of extract injected was 20 μl. Data was gathered between 200-300 nm and chromatograms evaluated at 238 nm. Microcystin peaks were identified by comparison of retention times with those of standards (MC-LR, MC-LF, MC-LW and MC-RR) purchased from Alexis Biochemical and their characteristic UV-spectra as per Lawton et al., (1994). All other unknown microcystin peaks at 238 nm were identified as MC-LR equivalence in comparism with MC-LR spectrum (McElhiney and Lawton 2005)

and toxic fractions were quantified by extrapolating HPLC peak areas at 238 nm to a linear calibration curve for microcystin-LR standard (n =5, r^2 =0.999).

Results

Intracellular microcystin

A total of 130 samples were analysed for cell-bound intracellular microcystin in the four reservoirs (Table 1). The minimum microcystin concentration was zero, whilst the maximum total microcystin concentration was 15.50 μg/l obtained in the Barekese reservoir (Appendix 5a). Out of the 130 samples analysed, 81 samples constituting 62.3% were negative for microcystin, and 49 samples representing 37.7 % were positive (Table 1). Out of the microcystin positive samples 24.6% have total microcystin concentration less than 1 μg/l whilst 13.1% have total microcystin concentration above 1 μg/l. Common known microcystin variants identified in these reservoirs were microcystin-LR, LF, RR and YR (Figs. 1a to g).

Toxin Concentration (µg/l)

	Frequency	Percent	Cumulative Percent
negative	81	62.3	62.3
Positive < 1 (µg/l)	32	24.6	86.9
Positive >1 (µg/l)	17	13.1	100.0
Total	130	100.0	

Table 1 Frequency table showing microcystin containing samples

Considering the individual reservoirs using statistical cross-tabulation (Table 2) it can be seen that in Barekese reservoir a total number of 27 samples were analysed for cell-bound microcystin. Out of these 27 samples, 23 (85.2%) were negative for microcystin, with 4 (14.6%) being positive for intracellular microcystin. 28 samples were analysed in the Kpong reservoir for intracellular microcystin of which 21 (75%) were negative with 7 (25%) being positive. The Owabi reservoir was the worst reservoir. Out of the 24 samples analysed, 45.8% were negative with 54.2% positive. This reservoir was dominated by *Cyanogranis ferruginea* which constituted over 96% of the cyanobacteria biomass. *Cyanogranis*

ferruginea until now has only been identified in temperate waters with no information on its toxicity. The Weija reservoir with a high diversity of cyanobacteria had 51% microcystin negative samples with 49% microcystin positive samples out of the 44 samples were analysed in this reservoir. However this reservoir had the highest percentage of microcystin positive samples with concentration higher than 1 μg/l (Table 2) and also the number of microcystin variants (Fig. 2).

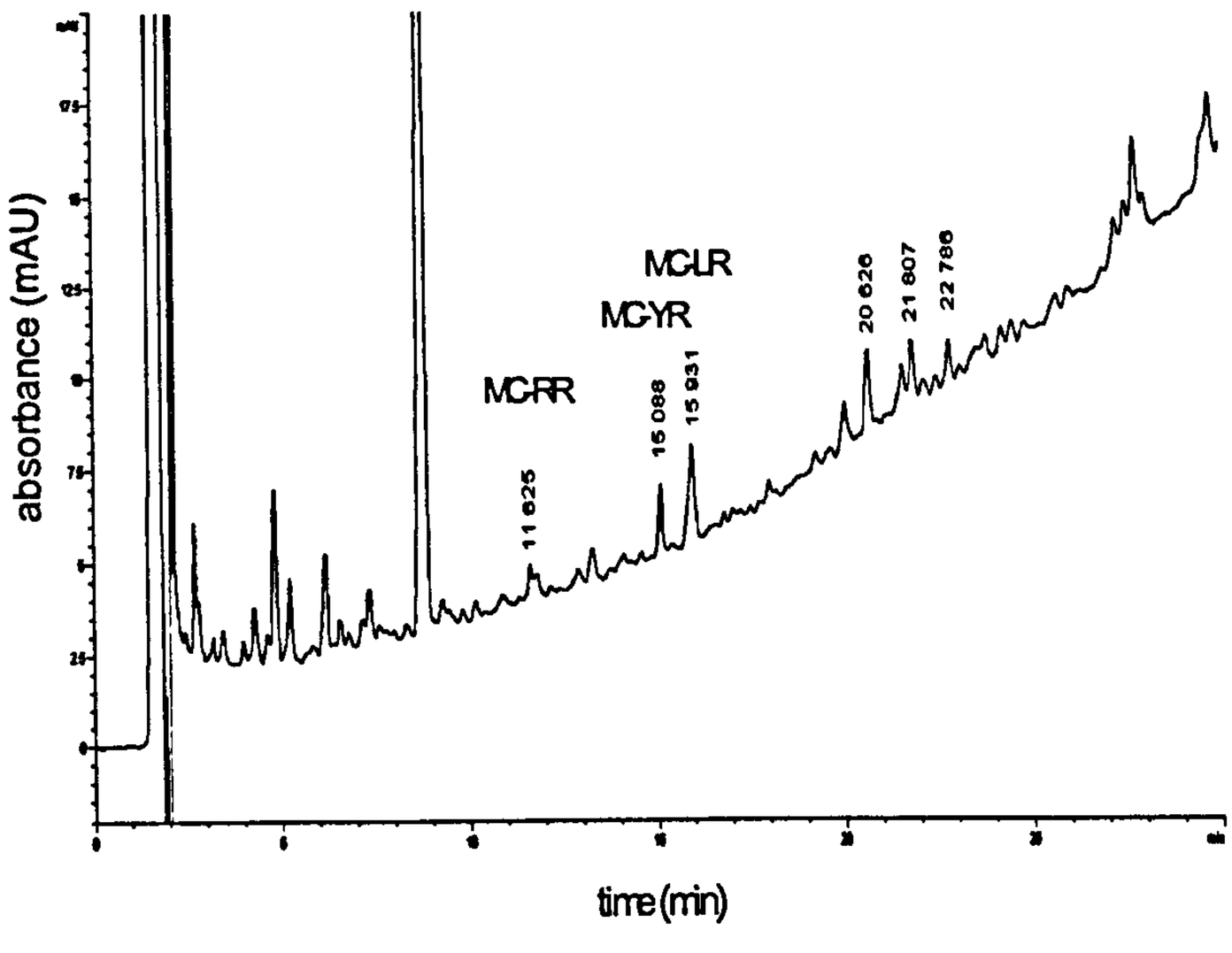


Figure 1a HPLC-DAD chromatogram at 238 nm of the intake sample from the Weija drinking water treatment plant (Ghana) containing six microcystin (MC) variants (MC-RR, -YR, -LR) and three unidentified structural variants

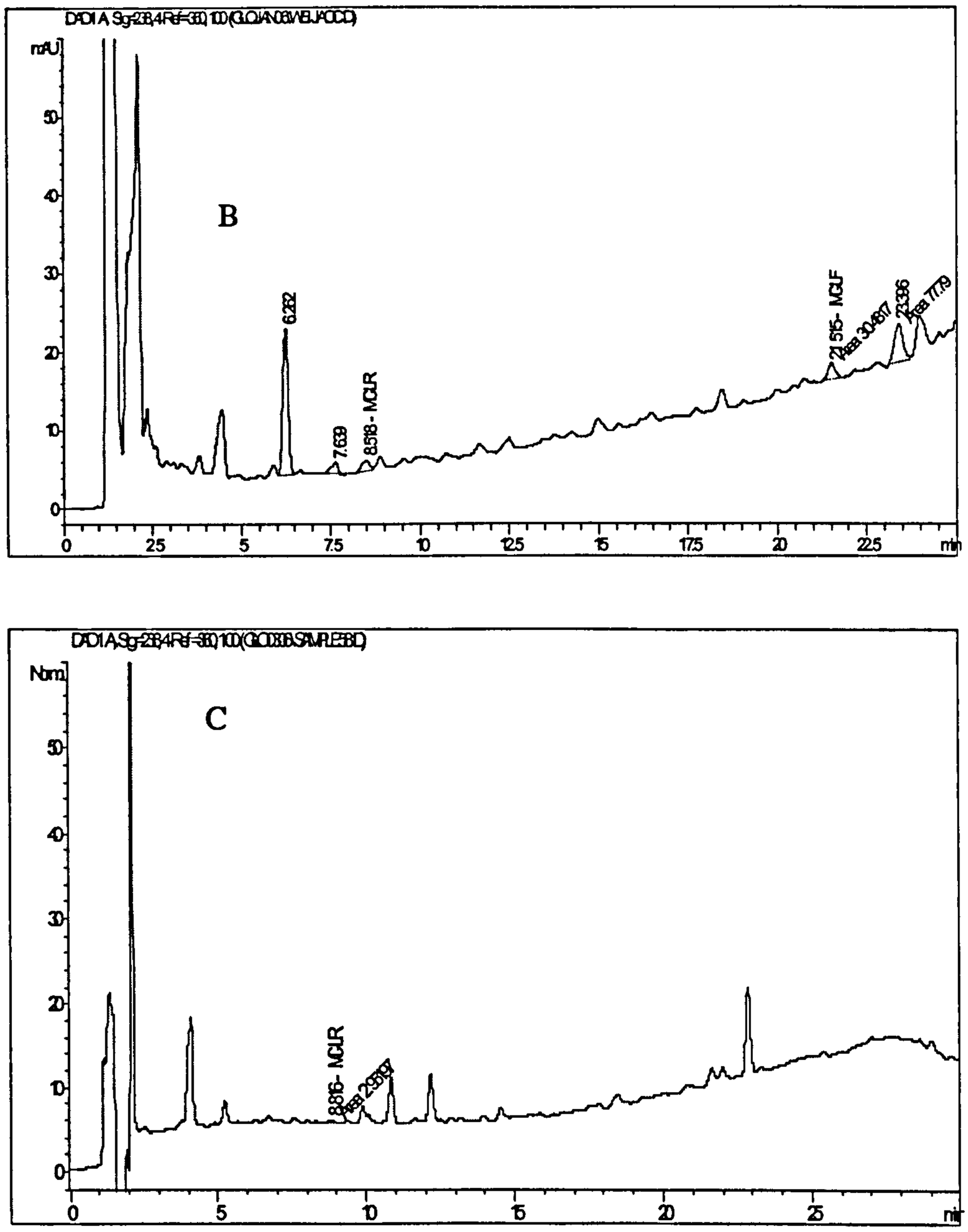


Figure 1 (b and c) HPLC-DAD chromatograms at 238 nm of the sample from the Weija reservoir showing identification of two microcystin variants MC-LR, MC-LF and (c) only MC-LR

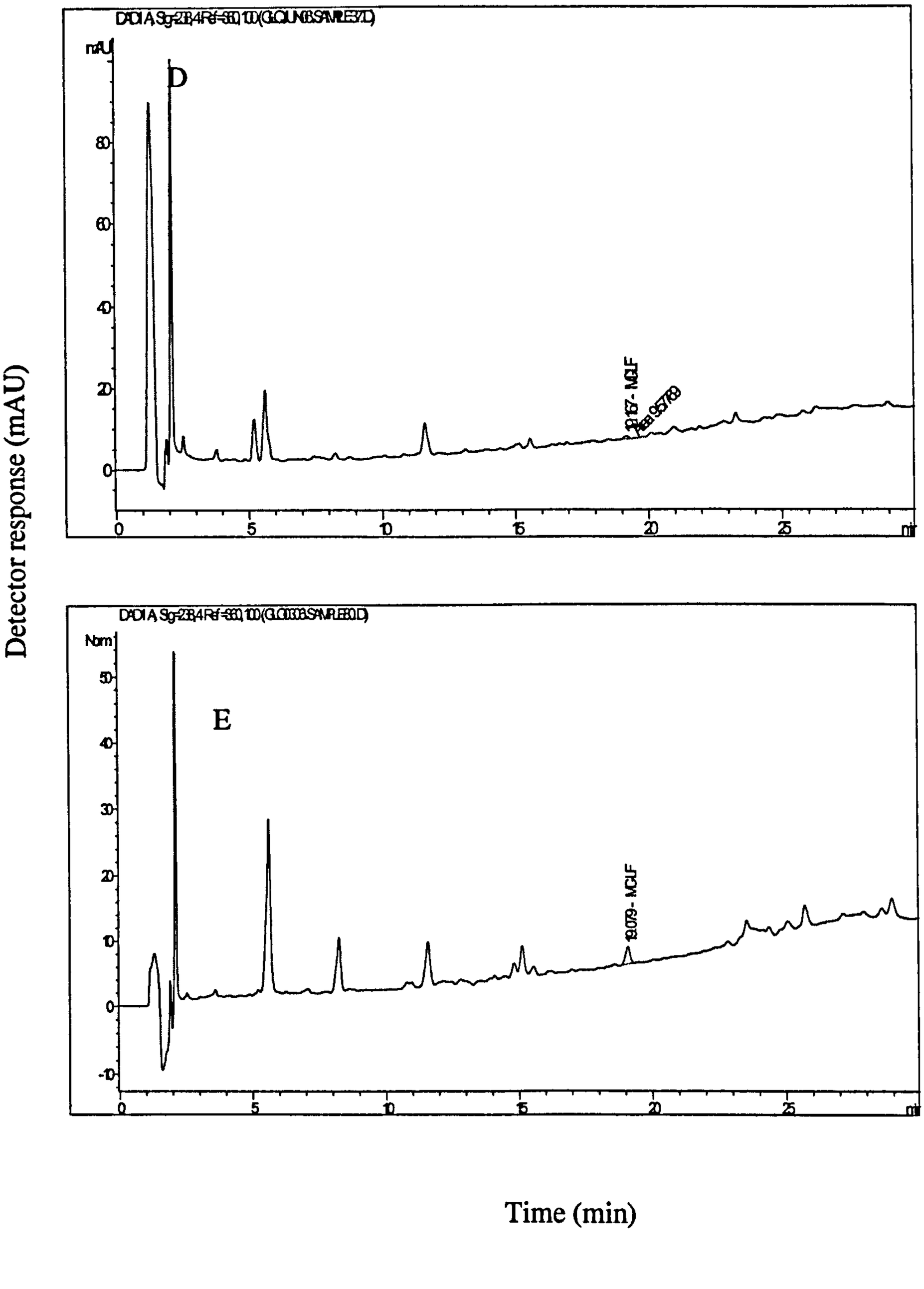
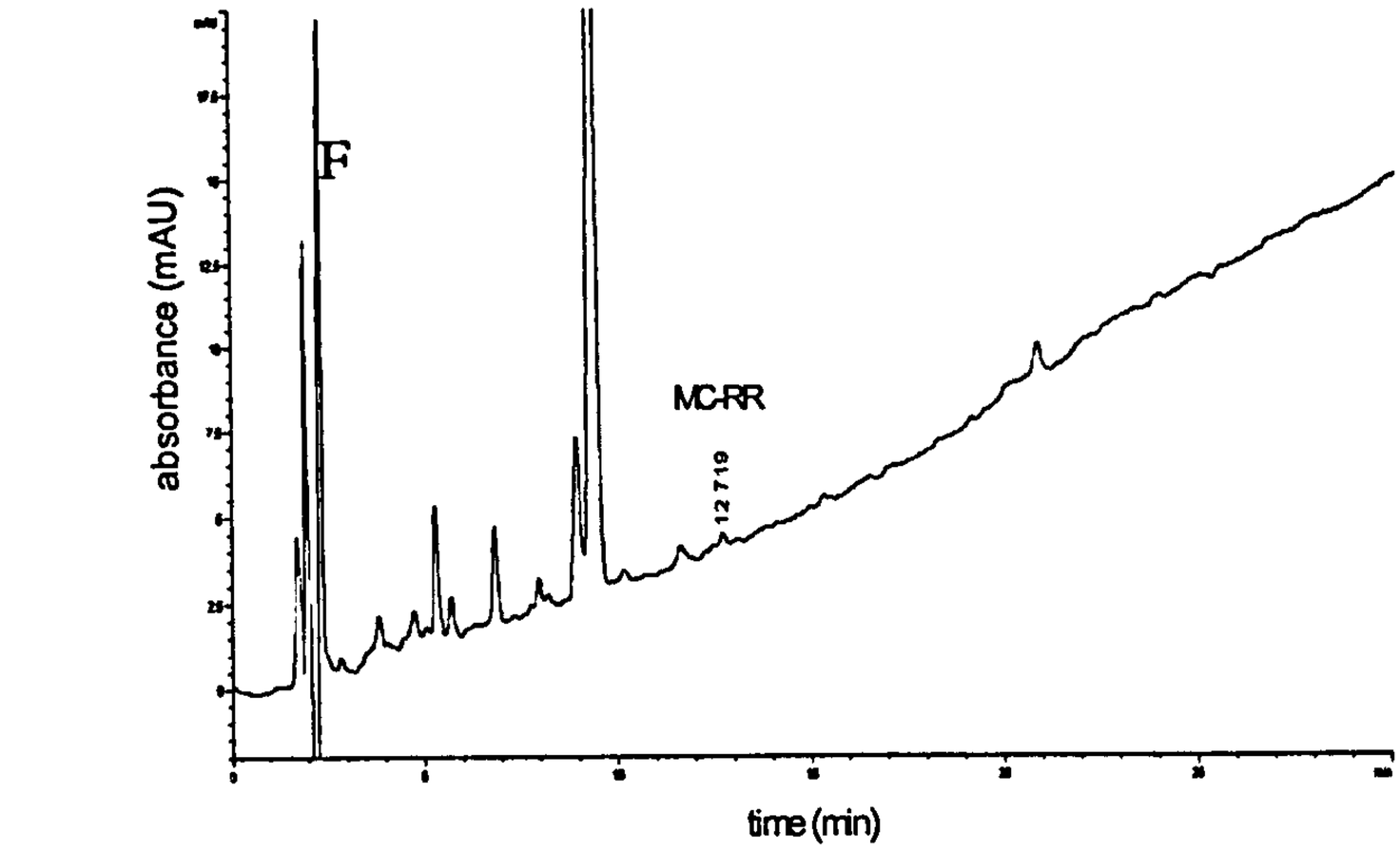


Figure 1 (d and e) HPLC-DAD chromatograms of samples from Owabi and Barekese reservoirs showing identification of a single microcystin MC-LF.



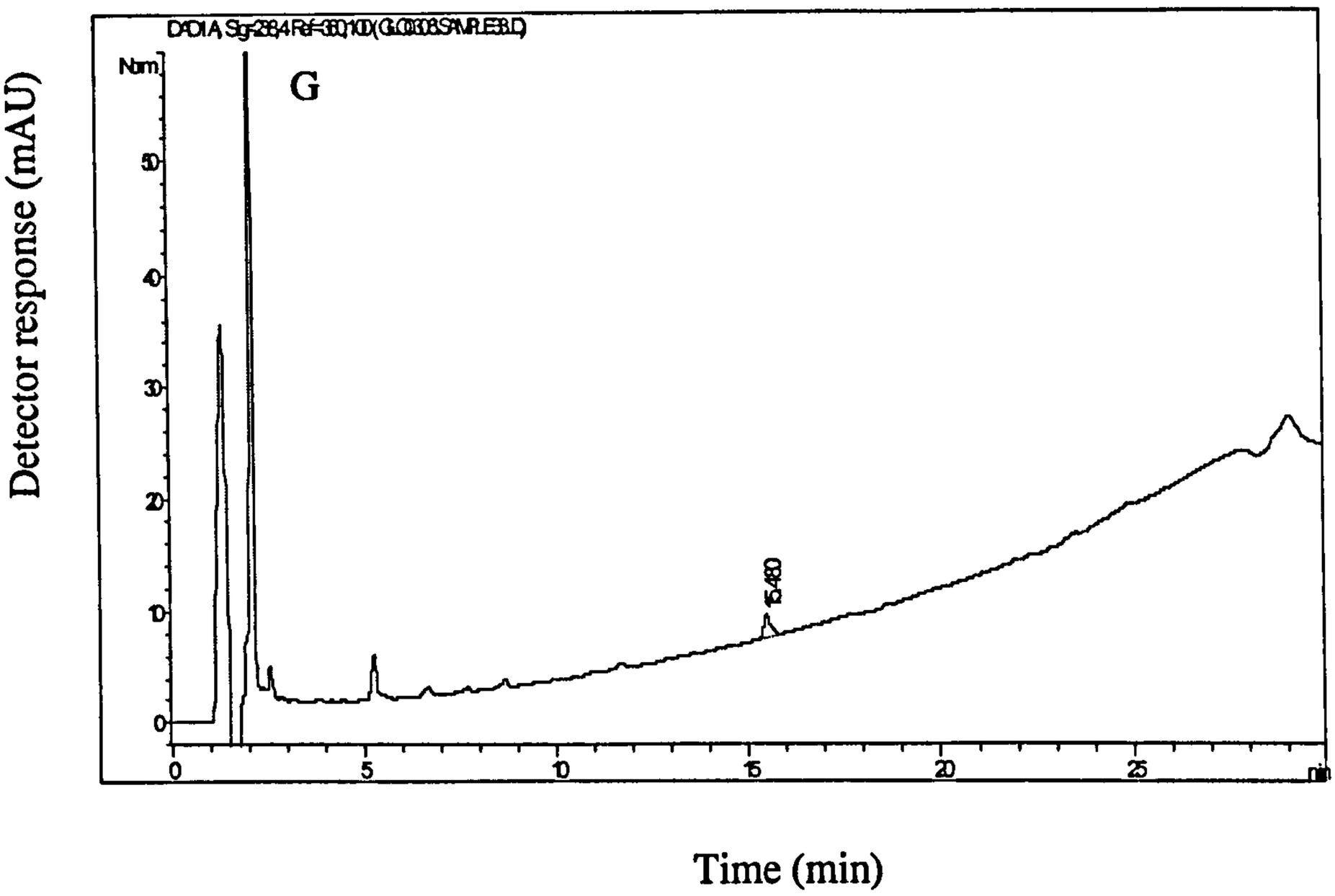
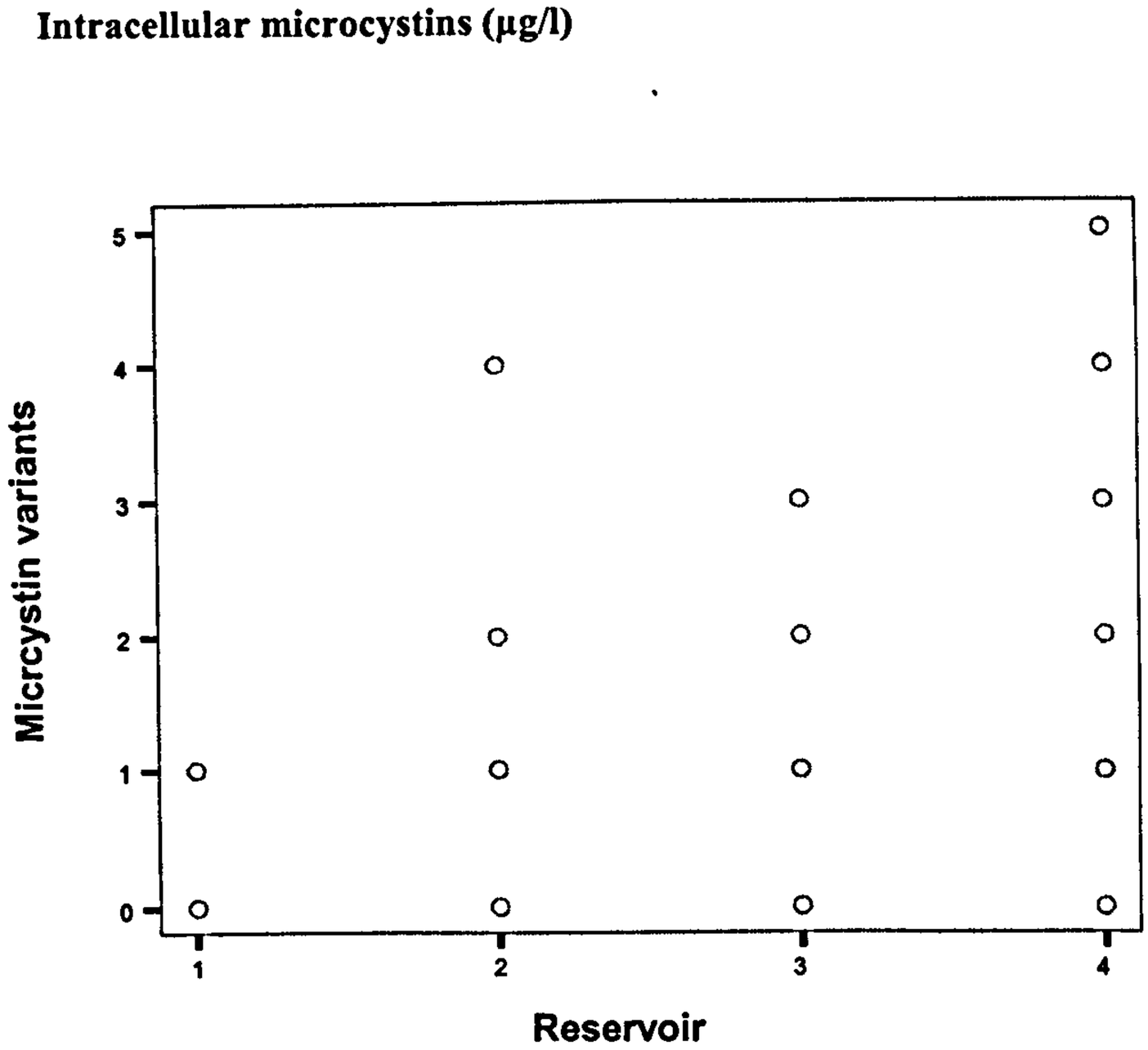


Figure 1 (1f and g) HPLC-DAD chromatogram at 238 nm of the intake samples from the Kpong drinking water treatment plant, Ghana, containing microcystin-RR and unidentified microcystin.

			Toxin Concentration (ug/l)			
			negative	Positive >1 (µg/l)	Positive >1 (µg/l)	Total
Reservoir	Barekese	Count	23	2	2	27
		% within Reservoir	85.2%	7.4%	7.4%	100.0%
		% within Toxin Concentration (µg/l)	28.4%	6.3%	11.8%	20.8%
		% of Total	17.7%	1.5%	1.5%	20.8%
	Owabi	Count	11	9	4	24
		% within Reservoir	45.8%	37.5%	16.7%	100.0%
		% within Toxin Concentration (µg/l)	13.6%	28.1%	23.5%	18.5%
		% of Total	8.5%	6.9%	3.1%	18.5%
	Kpong	Count	21	6	1	28
		% within Reservoir	75.0%	21.4%	3.6%	100.0%
		% within Toxin Concentration (µg/l)	25.9%	18.8%	5.9%	21.5%
		% of Total	16.2%	4.6%	.8%	21.5%
	Weija	Count	26	15	10	51
		% within Reservoir	51.0%	29.4%	19.6%	100.0%
		% within Toxin Concentration (µg/l)	32.1%	46.9%	58.8%	39.2%
		% of Total	20.0%	11.5%	7.7%	39.2%
Total		Count	81	32	17	130
		% within Reservoir	62.3%	24.6%	13.1%	100.0%
		% within Toxin Concentration (µg/l)	100.0%	100.0%	100.0%	100.0%
		% of Total	62.3%	24.6%	13.1%	100.0%

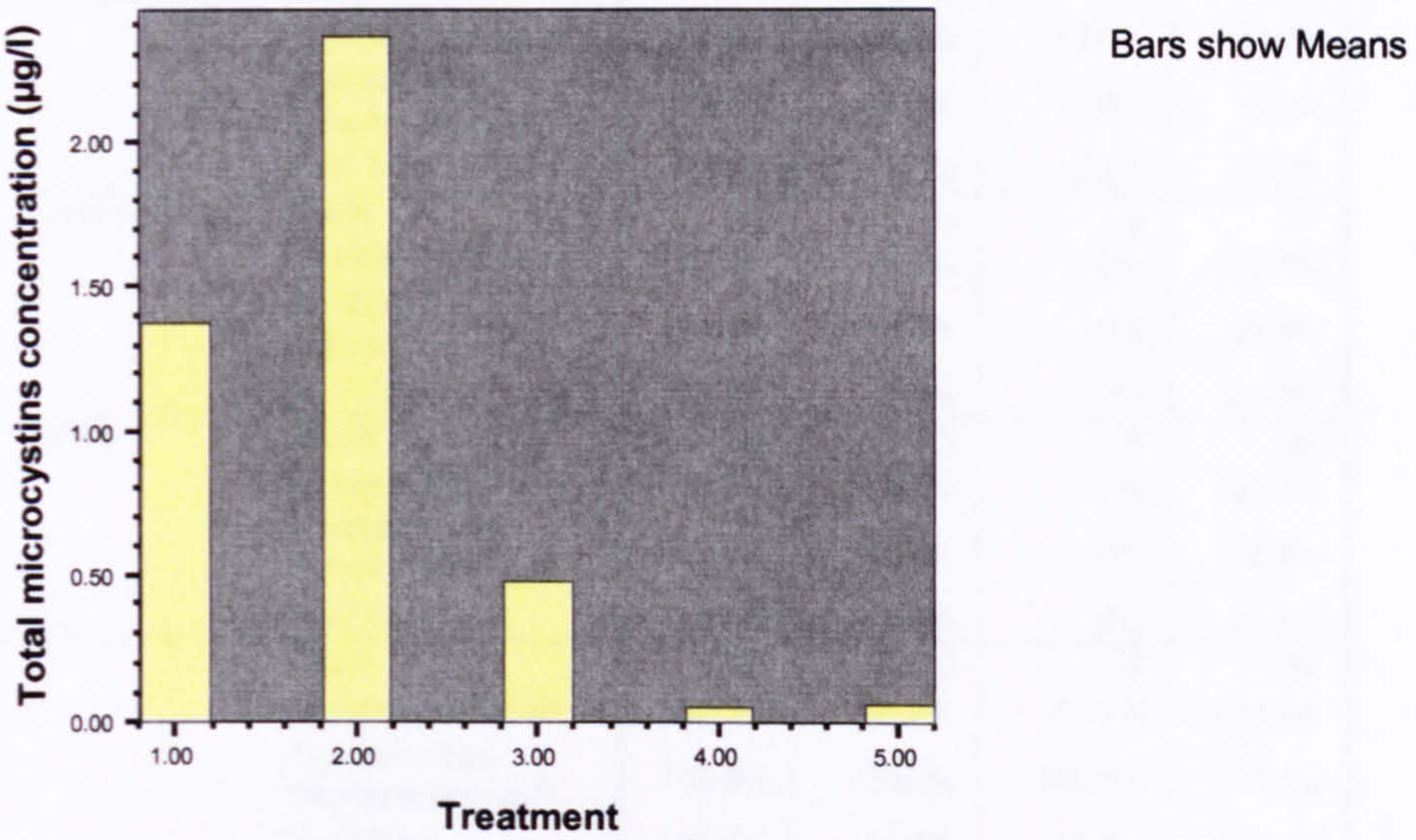
Table 2 Cross-tabulation between the four reservoirs studied and total Intracellular microcystins (µg/l)



Legend: 1 Barekese, 2 Kpong, 3 Owabi, 4 Weija

Figure 2 A scatter plots of intracellular microcystins variants and reservoirs

Figure 3 is a bar chart of total intracellular microcystin concentration as against stages of water treatment. From the Figure 3, it can be seen that the stage of the drinking water treatment where total intracellular microcystin concentrations were highest was flocculation with alum, followed by samples from the intake. The stage of filtration and the final chlorinated water had about the same intracellular microcystin concentration.



Legend: 1. Intake; 2. Alum flocculation; 3. sedimentation of floc; 4. Filtration; 5. Chlorination

Figure 3 A bar chart showing mean intracellular microcystin concentration (µg/l) obtained at each treatment stage of the drinking water production from the intake to the final drinking water stage

A cross-tabulation was also done between the various stages of treatment and microcystin positive samples (Table 3). Once again the result shows that the stage of flocculation with alum had the highest microcystin positive samples than the other four stages of treatment with 61.9% of the samples being microcystin positive. This was followed closely by the intake with 60.7% of the samples positive for microcystin, strongly supporting the information in figure 3 above. The final drinking water stage had over 17% of the samples been microcystin positive.

			Toxin Concentration (µg/l)			
			negative	positive <1 (g/l)	Positive >1 (µg/l)	Total
Treatment	Intake	Count	11	10	7	28
		% within Treatment	39.3%	35.7%	25.0%	100.0%
		% within Toxin Concentration (g/l)	13.6%	31.3%	41.2%	21.5%
		% of Total	8.5%	7.7%	5.4%	21.5%
	Flocculation	Count	8	6	7	21
		% within Treatment	38.1%	28.6%	33.3%	100.0%
		% within Toxin Concentration (g/l)	9.9%	18.8%	41.2%	16.2%
		% of Total	6.2%	4.6%	5.4%	16.2%
	Sedimentation	Count	16	6	3	25
		% within Treatment	64.0%	24.0%	12.0%	100.0%
		% within Toxin Concentration (g/l)	19.8%	18.8%	17.6%	19.2%
		% of Total	12.3%	4.6%	2.3%	19.2%
	Filtered	Count	22	5	0	27
		% within Treatment	81.5%	18.5%	.0%	100.0%
		% within Toxin Concentration (g/l)	27.2%	15.6%	.0%	20.8%
		% of Total	16.9%	3.8%	.0%	20.8%
	Final	Count	24	5	0	29
		% within Treatment	82.8%	17.2%	.0%	100.0%
		% within Toxin Concentration (g/l)	29.6%	15.6%	.0%	22.3%
		% of Total	18.5%	3.8%	.0%	22.3%
Total		Count	81	32	17	130
		% within Treatment	62.3%	24.6%	13.1%	100.0%
		% within Toxin Concentration (g/l)	100.0%	100.0%	100.0%	100.0%
		% of Total	62.3%	24.6%	13.1%	100.0%

Table 3 Cross-tabulation between stages of drinking water treatment and total intracellular microcystins concentration (µg/l) in samples from the four reservoirs

Dissolved microcystin

A total number of 57 samples were analysed for dissolved microcystin. This low number was due to problems with consumables and financial constraints during field work in Ghana. In addition, some of the extracts leaked out of bottles during flight from Ghana to the United Kingdom. No dissolved microcystin was recorded in the treatment train of the Barekese reservoir (Table 4, Appendix 6d). The highest percentage of samples with dissolved microcystin was obtained in the Owabi reservoir (Table 4), which also had the highest number of with intracellular microcystin. 58.3 % of the samples analysed in this reservoir contained dissolved microcystin (Table 4). In this reservoir microcystin-LR was consistently identified. 6 samples from the Kpong reservoir were positive constituting 35% of the samples analysed from the reservoir. The Weija reservoir

had 6 out of the 17 samples been microcystin positive constituting about 36% of the samples analysed in the reservoir. Only in this reservoir was dissolved microcystin higher than 1 μg/l was recorded (Table 4).

			toxin conconcentration (µg/l)			
			Negative	Positive < 1 (µg/l)	Positive > 1 (µg/l)	Total
Reservoir	Barekese	Count	11	0	0	11
		% within Reservoir	100.0%	.0%	.0%	100.0%
		% within toxin concentration (µg/l)	28.9%	.0%	.0%	19.3%
		% of Total	19.3%	.0%	.0%	19.3%
	Owabi	Count	5	7	0	12
		% within Reservoir	41.7%	58.3%	.0%	100.0%
		% within toxin concentration (µg/l)	13.2%	38.9%	.0%	21.1%
		% of Total	8.8%	12.3%	.0%	21.1%
	Kpong	Count	11	6	0	17
		% within Reservoir	64.7%	35.3%	.0%	100.0%
		% within toxin concentration (µg/l)	28.9%	33.3%	.0%	29.8%
		% of Total	19.3%	10.5%	.0%	29.8%
	Weija	Count	11	5	1	17
		% within Reservoir	64.7%	29.4%	5.9%	100.0%
		% within toxin concentration (µg/l)	28.9%	27.8%	100.0%	29.8%
		% of Total	19.3%	8.8%	1.8%	29.8%
Total		Count	38	18	1	57
		% within Reservoir	66.7%	31.6%	1.8%	100.0%
		% within toxin concentration (µg/l)	100.0%	100.0%	100.0%	100.0%
		% of Total	66.7%	31.6%	1.8%	100.0%

Table 4 Cross-tabulation between the four reservoirs studied and total dissolved microcystins concentration (µg/l)

Considering treatment and dissolved microcystin concentration (Table 5), 3 samples from the intake and 4 samples from the flocculation stages contained dissolved microcystin respectively. This constituted 27.3% and 44.4% respectively in the intake and flocculation stages. However, 7 samples from the sedimentation stage contained dissolved microcystin which was the treatment stage with the highest microcystin positive samples with over 58% positive samples, whilst 3 samples from the filtered water stage contained dissolved microcystin. 2 samples from the drinking water stage were positive for dissolved microcystin, both below the 1 μg/l recommended for drinking water set by the World Health Organization (Table 5).

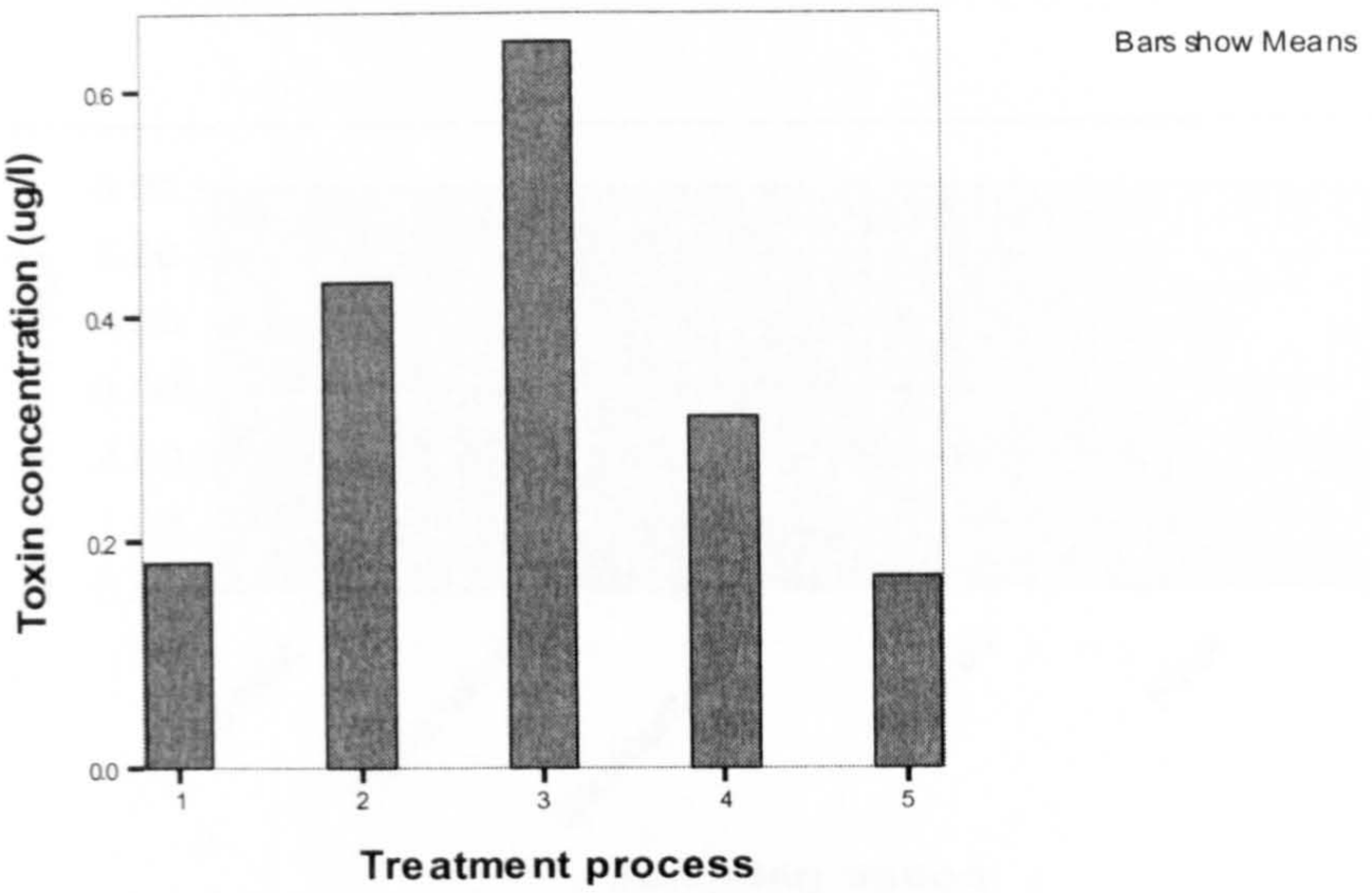
With regards to total dissolved microcystin concentration, the Weija reservoir had the highest concentration of 1.82 μg/l at the sedimentation stage (Appendix 6a)

which follows immediately after flocculation with alum. However, with regards to water treatment process the sedimentation stage had the highest concentration of dissolved microcystin (Fig. 4).

There are not enough data to enable discussion on changes in dissolved microcystin concentration during treatment in individual reservoirs. However, Figure 5 from the Owabi reservoir illustrates a typical reduction in intracellular microcystin concentration as treatment progresses to the final drinking stage. In some cases as mostly encountered in the Weija reservoir, illustrated in Figure 6, intracellular microcystin sharply increases at the flocculation stage and then reduces as the treatment progresses to the final drinking water stage. In the case of the Barekese reservoir intracellular microcystin concentration reduces sharply after flocculation to below the detection limit of 0.125 μg/l (Fig.7)

			toxin concentration (ug/l)			
			Negative	Positive < 1 (µg/l)	Positive > 1 (µg/l)	Total
Treatment	Intake	Count	8	3	0	11
		% within Treatment	72.7%	27.3%	.0%	100.0%
		% within toxin concentration (µg/l)	21.1%	16.7%	.0%	19.3%
		% of Total	14.0%	5.3%	.0%	19.3%
	Flocculation	Count	5	4	0	9
		% within Treatment	55.6%	44.4%	.0%	100.0%
		% within toxin concentration (µg/l)	13.2%	22.2%	.0%	15.8%
		% of Total	8.8%	7.0%	.0%	15.8%
	Sedimentation	Count	5	6	1	12
		% within Treatment	41.7%	50.0%	8.3%	100.0%
		% within toxin concentration (µg/l)	13.2%	33.3%	100.0%	21.1%
		% of Total	8.8%	10.5%	1.8%	21.1%
	Filtered	Count	10	3	0	13
		% within Treatment	76.9%	23.1%	.0%	100.0%
		% within toxin concentration (µg/l)	26.3%	16.7%	.0%	22.8%
		% of Total	17.5%	5.3%	.0%	22.8%
	Final	Count	10	2	0	12
		% within Treatment	83.3%	16.7%	.0%	100.0%
		% within toxin concentration (µg/l)	26.3%	11.1%	.0%	21.1%
		% of Total	17.5%	3.5%	.0%	21.1%
Total		Count	38	18	1	57
		% within Treatment	66.7%	31.6%	1.8%	100.0%
		% within toxin concentration (µg/l)	100.0%	100.0%	100.0%	100.0%
		% of Total	66.7%	31.6%	1.8%	100.0%

Table 5 Cross-tabulation between stages of drinking water treatment and total dissolved microcystins concentration (µg/l) in samples from the four reservoirs



Legend: 1: Intake, 2: Flocculation, 3: Sedimentation, 4: Filtered and 5: Final drinking water

Figure 4 A bar chart of mean total dissolved microcystins concentration (µg/l) obtained at each treatment stage of the drinking water production from the intake to the final drinking water stage

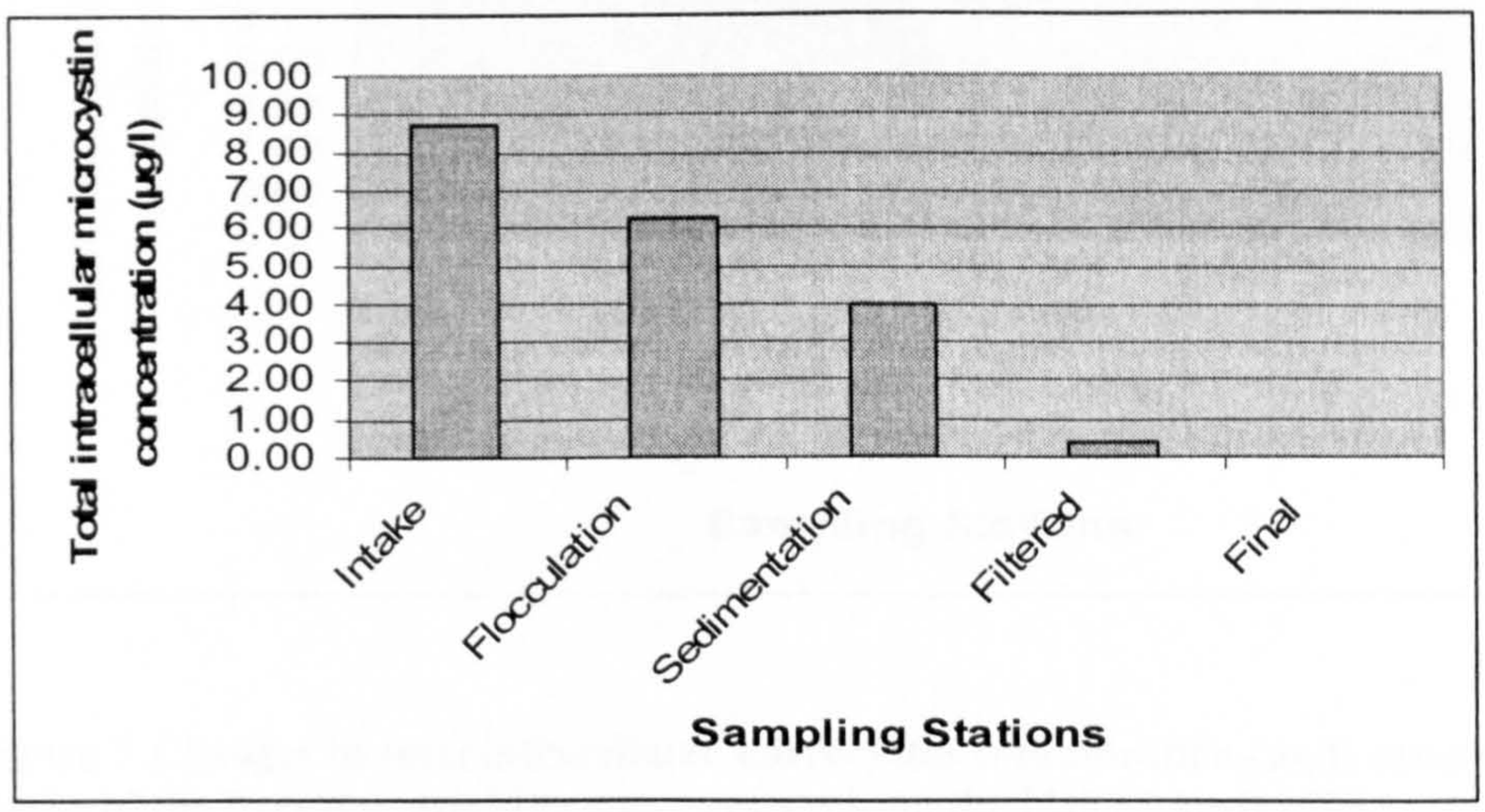


Figure 5 Changes in total intracellular microcystin concentration (µg/l) sampled from the intake of the Owabi drinking water reservoir on the 10th of February 2005.

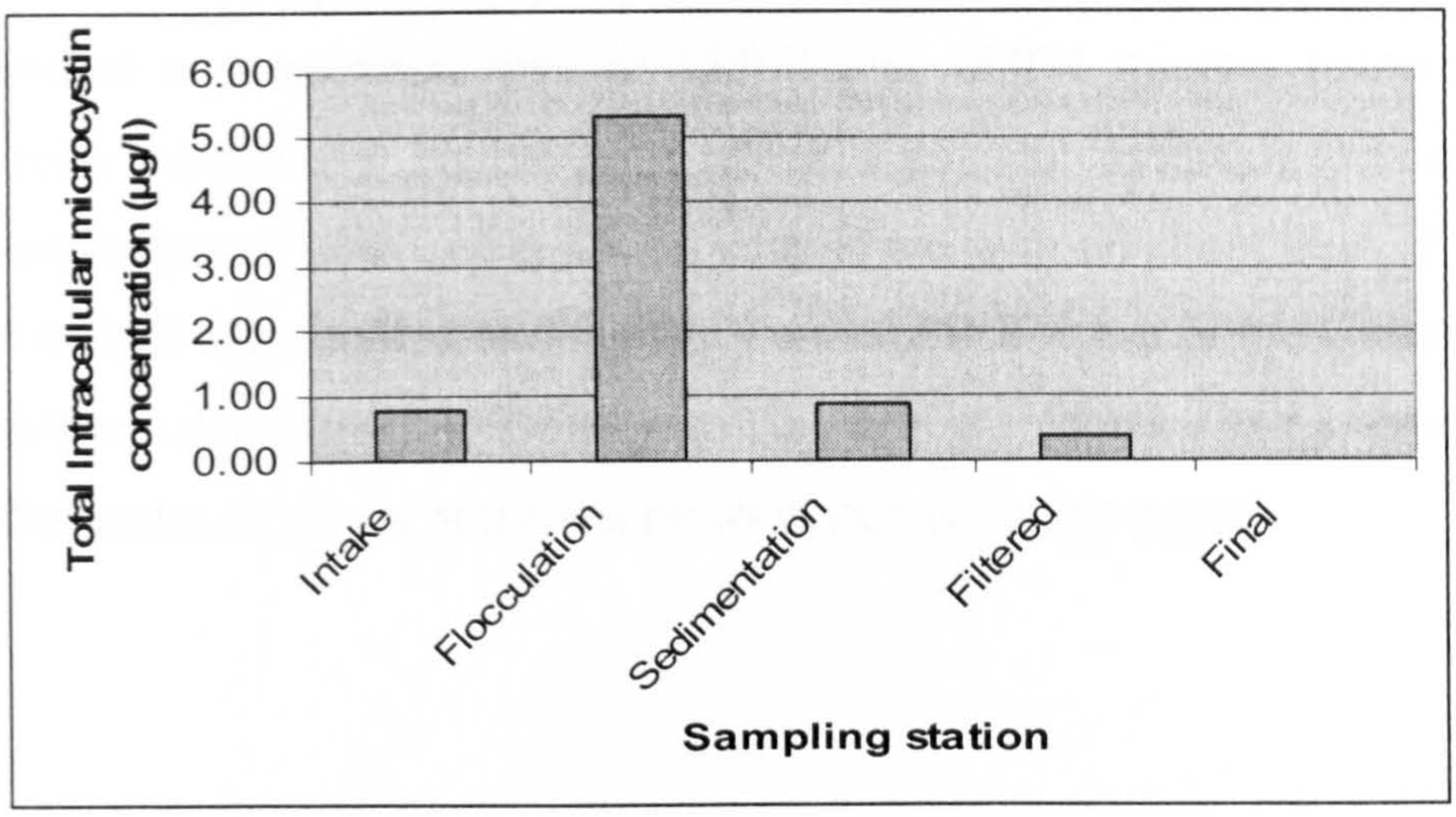


Figure 6 Changes in total intracellular microcystin concentration (µg/l) sampled from the intake of the Weija drinking water reservoir on the 24th of March 2005

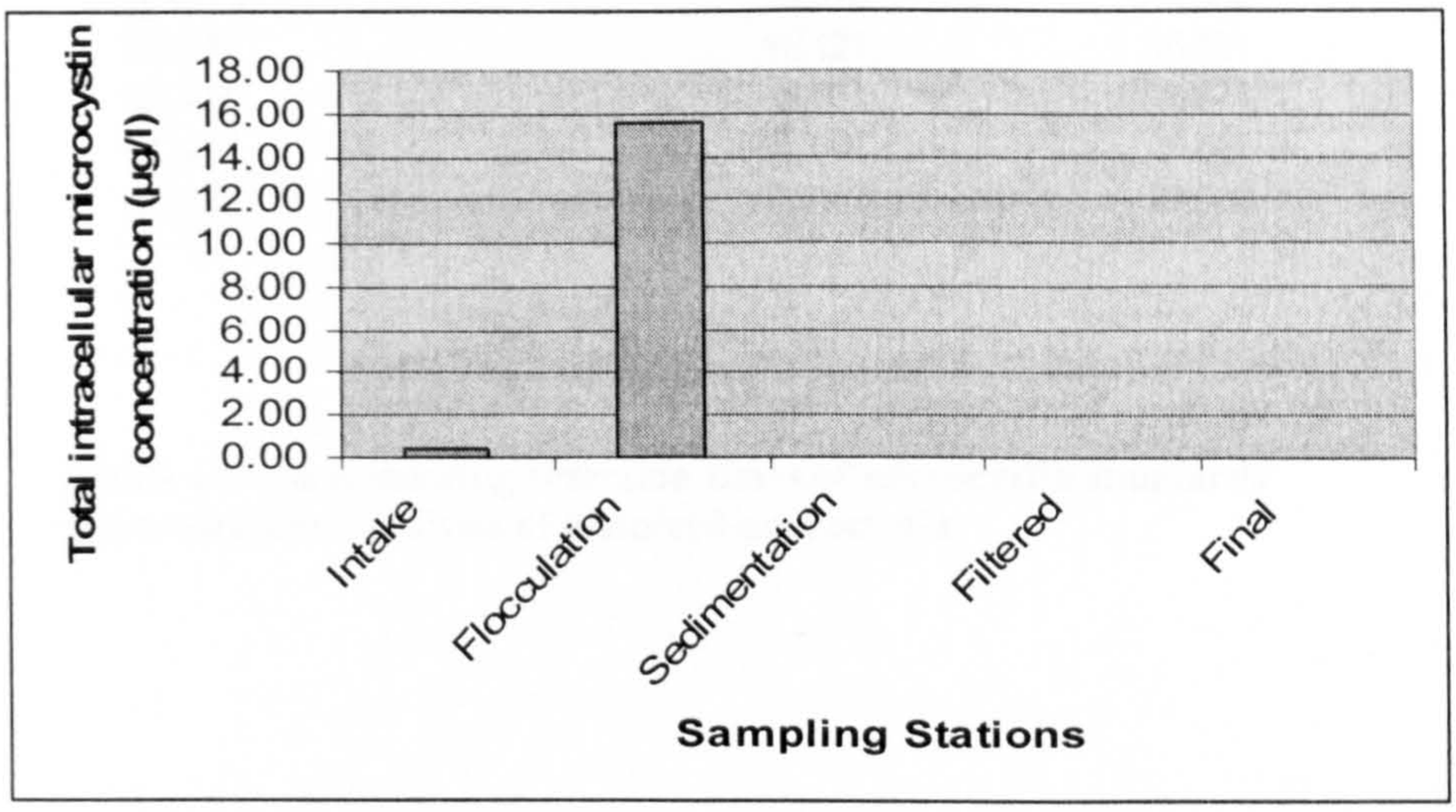


Figure 7 Changes in total intracellular microcystin concentration (µg/l) sampled from the intake of the Barekese drinking water reservoir on the 12th of April 2005.

Microcystin-LR was the common microcystin variant identified in the dissolved microcystin samples. Table 6 is a cocktail of microcystin standards used for dissolved microcystin analysis. From this it can be seen that MC-LR was identified at a retention time of 15.971 and 15.974 minutes before and after analysis respectively. Figures 8a, b, c and d contain chromatogrammes showing identification of microcystin-LR by comparism with retention time of sample to that of MC-LR standard and their UV spectra at 238 nm in the Owabi reservoir which was dominated by *Cyanogranis ferruginea*. Plates 1 and 2 shows pictures of the treatment plants of the reservoirs discussed in this paper.

COCTAIL OF MICROCYSTIN STANDARDS

	Retention times	
structural variant of microcystin	ctxm050.d (analysed prior the set of unknown samples)	ctxm051.d (analysed after the set of unknown samples)
MC-RR	11.663	11.653
NOD	13.579	14.658
MC-YR	15.121	15.121
MC-LR	15.971	15.974
MC-LW	26.646	26.662
MC-LF	27.047	27.066

Table 6 Results showing retention times of microcystin standards prior and after analysis of dissolved microcystin

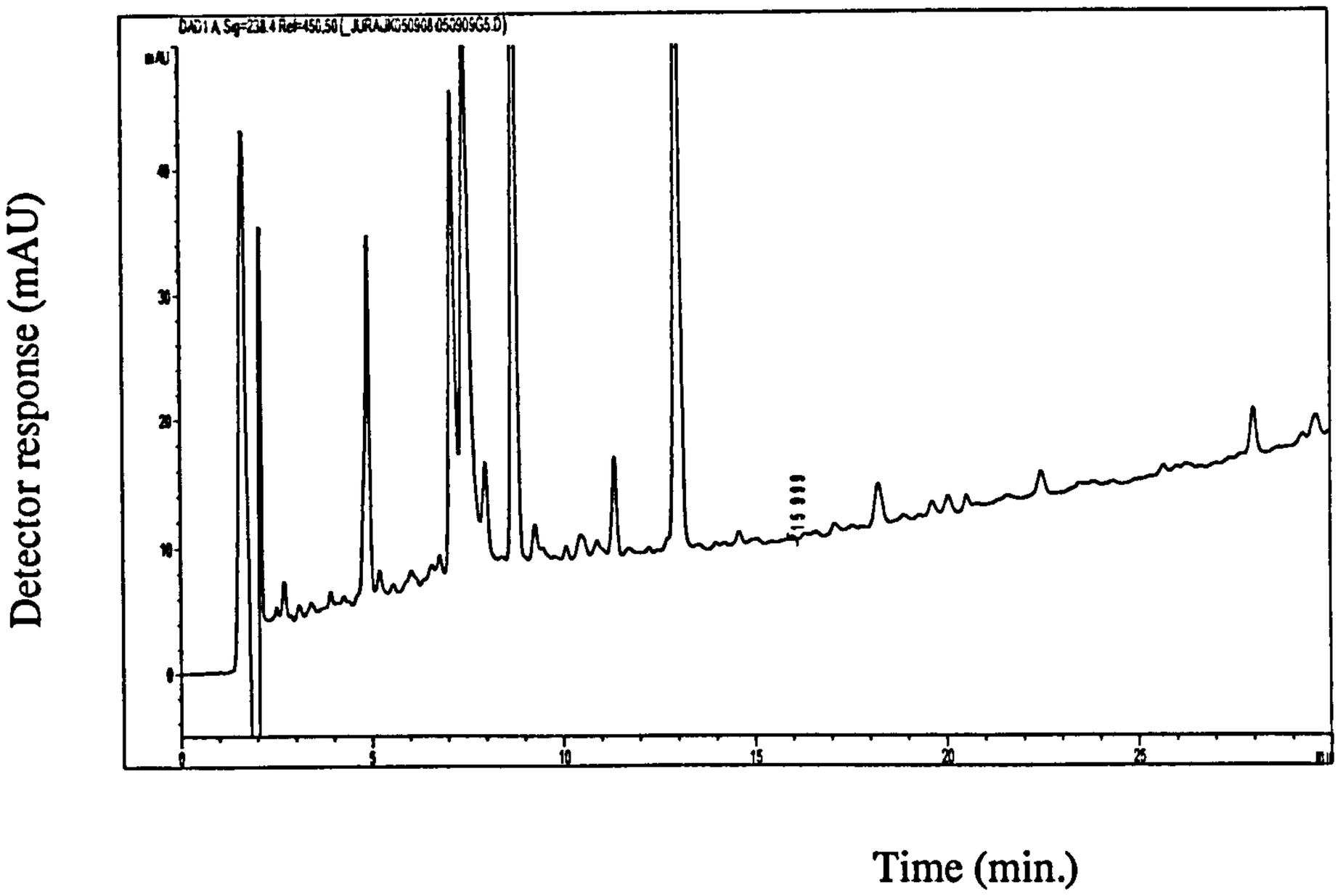


Figure 8a HPLC-DAD chromatograms at 238 nm of the intake sample from the Owabi reservoir (170305) showing identification of MC-LR at a concentration of 0.06 µg/l.

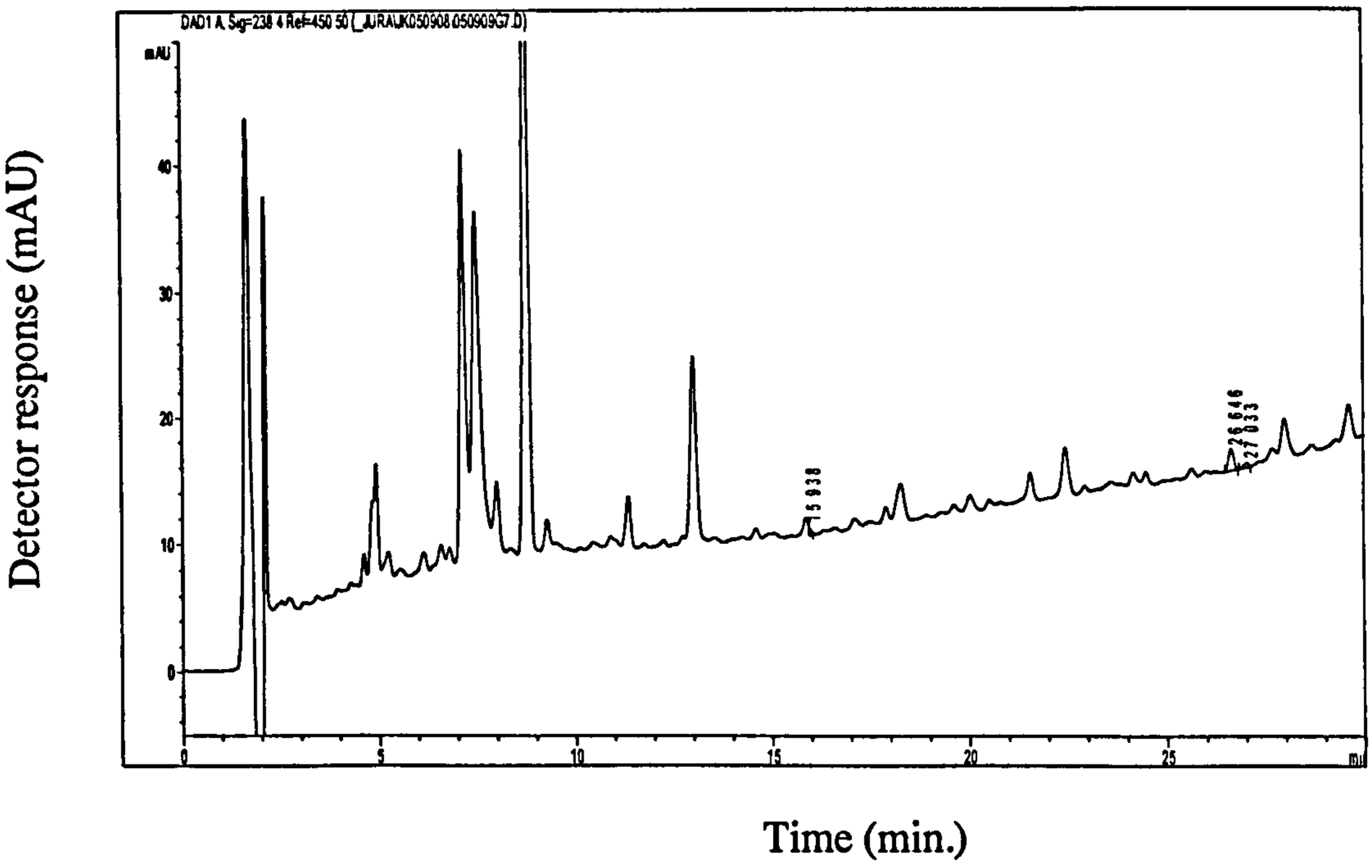


Figure 8b HPLC-DAD chromatograms at 238 nm of the flocculation sample from the Owabi reservoir (170305) showing identification of MC-LR at a concentration of 0.289 µg/l.

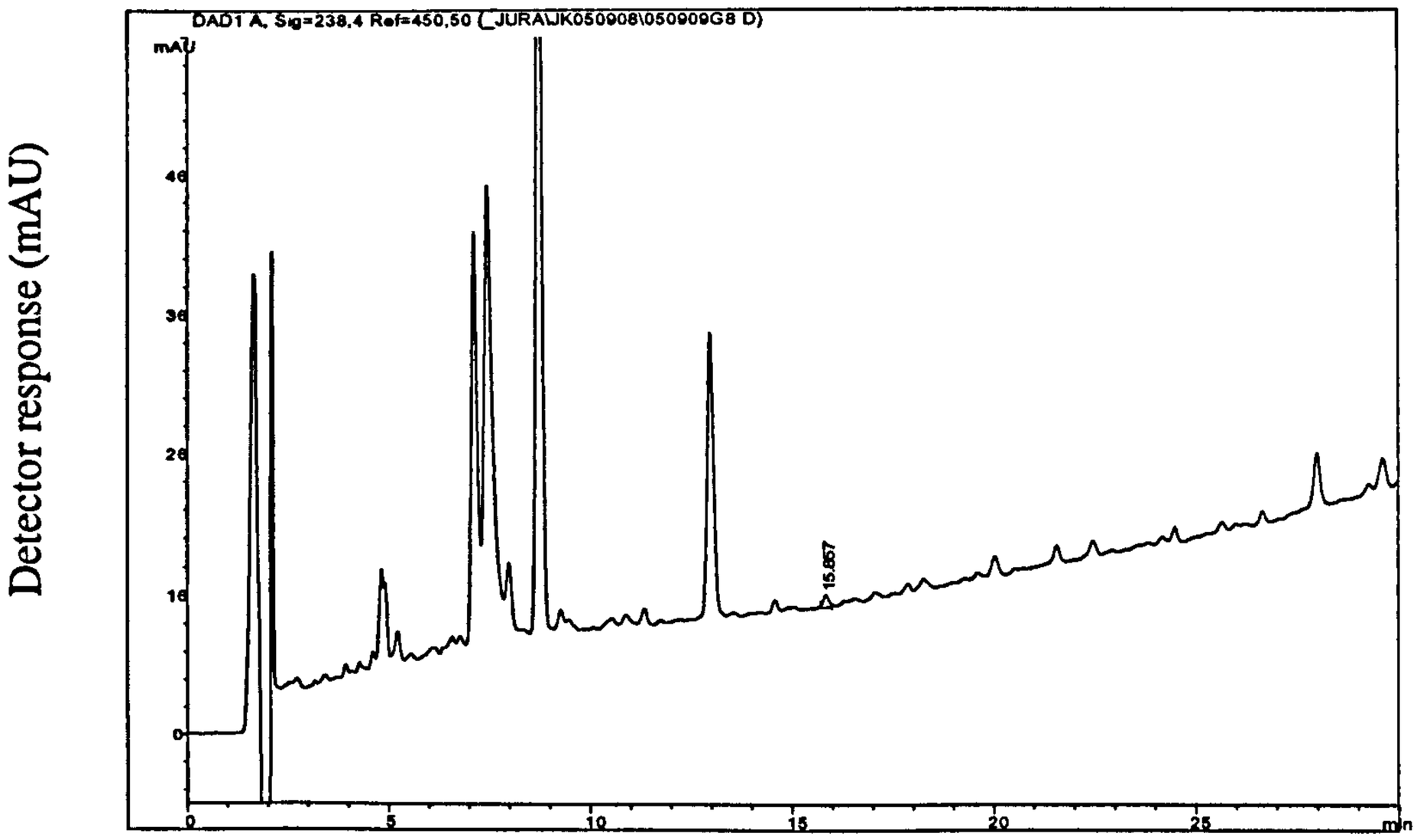


Figure 8c HPLC-DAD chromatograms at 238 nm of the sedimentation sample from the Owabi reservoir (170305) showing identification of MC-LR at a concentration of 0.09 µg/l.

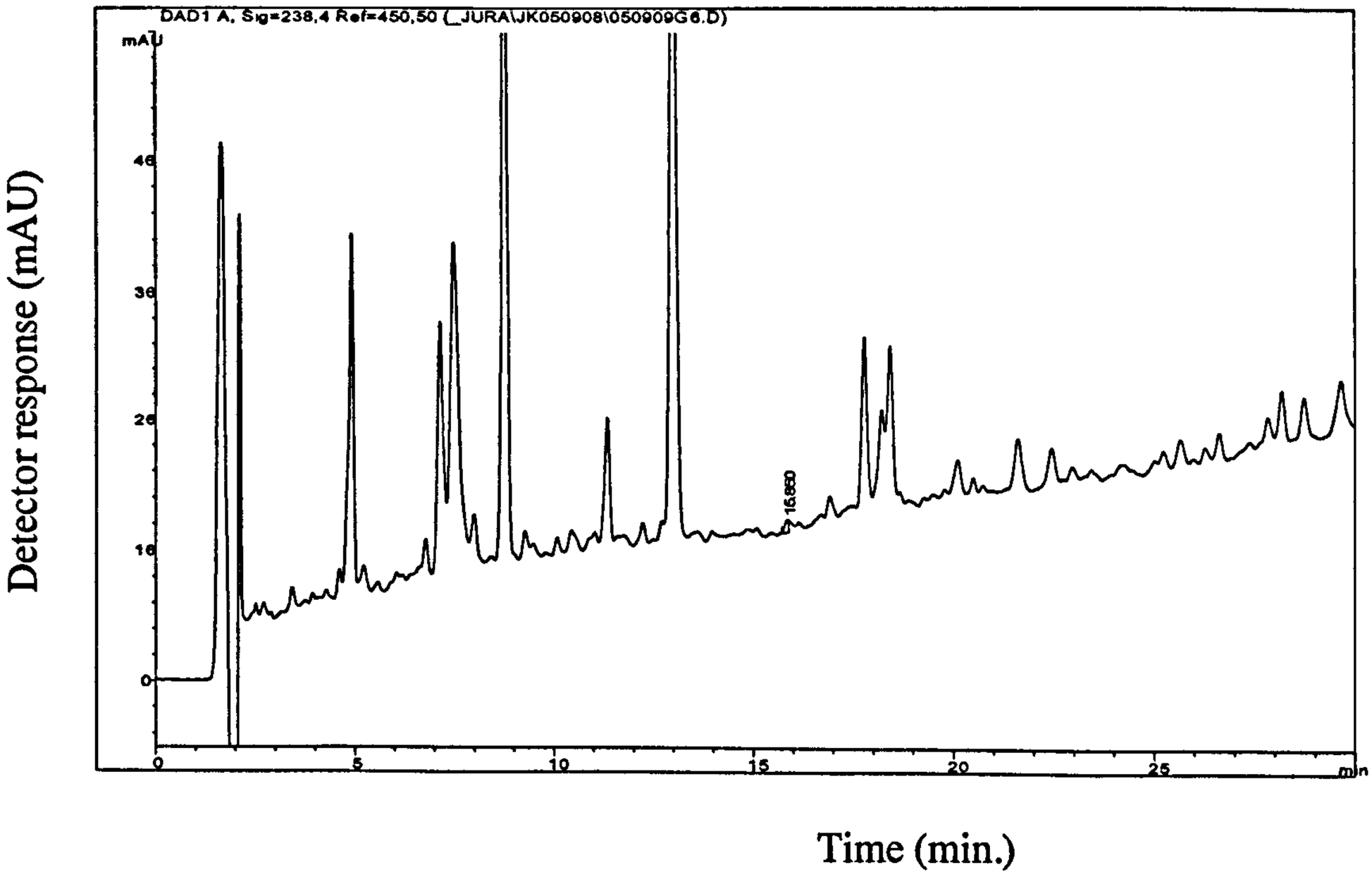


Figure 8d HPLC-DAD chromatograms at 238 nm of the filtered sample from the Owabi reservoir (170305) showing identification of MC-LR at a concentration of 0.156 µg/l

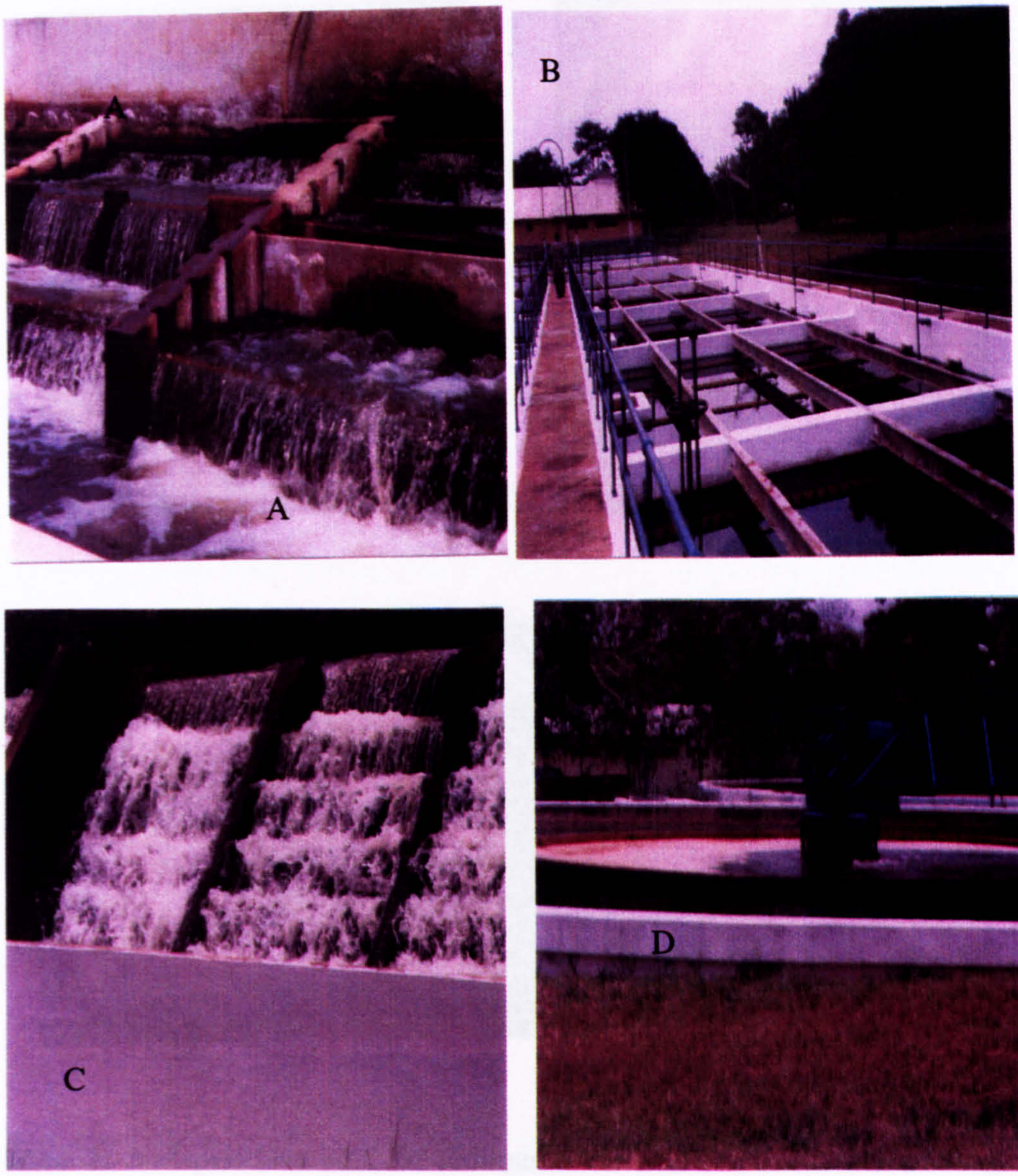


Plate 1: Aeration of raw water as it flows over cascades in (A) Owabi drinking water treatment plant and (B) Barekese drinking water treatment plant, (C) flocculation/coagulation and algaeciding chamber at the Owabi drinking water treatment plant and D: flocculation (inner chamber arrowed) and outer chamber used as sedimentation tanks at the Barekese drinking water treatment plant at the Ashanti region of Ghana.

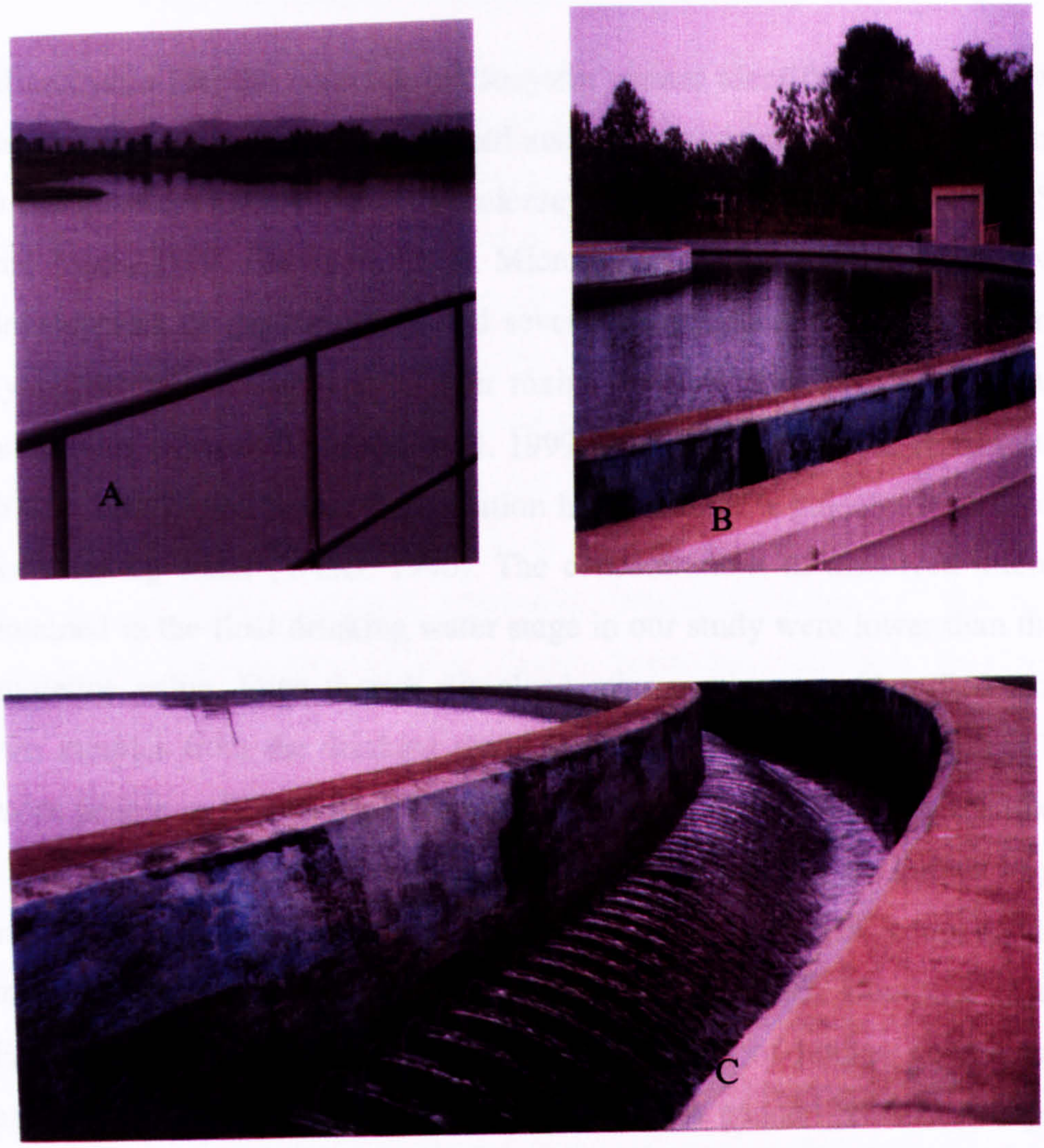


Plate 2: A: Open reservoir, B: sedimentation and C: clear water flowing out of sedimentation tank distribution tank where it is chlorinated before supplied to consumers in the Kpong drinking water treatment plant

Discussion

Microcystin-LR, the common microcystin variant identified in the raw water of the drinking water reservoirs studied and the final drinking water stage in Ghana is the most commonly occurring microcystin variant (Codd et al. 1997; Sivonen and Jones, 1999; Falconer, 2004). Microcystin-LR has been associated with the development of gastroenteritis and severe liver damage of individuals ingesting cyanobacterial blooms and/or their toxins through contaminated drinking water worldwide (Kuiper-Goodman et al. 1999; Azevedo et al. 2002; Annadotter et al. 2001). The World health Organization has instituted a guideline value of 1 μg/l for drinking water (WHO, 1996). The concentrations of dissolved microcystins obtained in the final drinking water stage in our study were lower than the WHO guideline value. Even though dissolved microcystins was identified from only two samples from the drinking water stage, the risk to public health is high, as more people in Ghana drink from open water bodies without any treatment than those that have access to treated piped water. Microcystin are known to promote and initiate tumours at low concentrations over a long term exposure and this is of most concern for public health purposes (Ito et al. 1997; Humpage et al. 2000; Falconer et al. 1994, 2004). A number of reports of human poisoning due to exposure to microcystin-LR and microcystins in general have been reported in several published articles worldwide. As far back as 1965, microcystin was linked to annual outbreak of gastroenteritis in Harare Zimbabwe (Zilberg, 1966). Again in Harare, Zimbabwe, cases of gastrointestinal infections and liver cancer have been reported to have risen by over 300% and 400%, respectively between 1991 and 2001 (National Health Information and Surveillance Unit, 1990-2001; Harare City Department of Health, 1990-2001). Although the extent to which this situation is linked to algal toxins is unclear, Johansson and Olsson (1998) reported microcystins concentrations in Lake Chievero to be around 13.9 μg/l. Microcystin was also detected in the city's tap water. Ndebele and Magadza (2006) also obtained a microcystin concentration (MC-LR) between 18.02 and 22.48 μg/l in cultured *Microcystis aeruginosa* cells from the same lake in Harare, Zimbabwe. In Ghana, our current study had obtained intracellular microcystin concentration as high as 15.50 μg/l in the treatment plant of the Barekese reservoir and dissolved microcystin had been detected in the final drinking water in the Weija treatment plant with significant concentration of 0.81 μg/l and also

0.57 μg/l in the Kpong water treatment plant. Hoeger et al. (2005) cautioned that due to tumour promoting activities of microcystin, chronic exposure of populations to concentration below 0.1 μg/l should be avoided. In Finland a non-fatal outbreak of gastroenteritis among the population was attributed to the mass development of microcystin producing *Planktothrix agardhii* in the raw water supply. A microcystin concentration of 0.1-0.5 μg/l was detected in the drinking water (Lepisto et al. 1993). *Planktothrix agardhii* is a common cyanobacterium which had been identified in all the four reservoirs studied and microcystin concentrations recorded are higher than these values. Carmichael et al. (2001) had reported that microcystin-YR, -LR and –AR were responsible for the death of 76 dialysis patients at a dialysis clinic in Caruaru, Brazil. The symptom reported before the deaths included dizziness, weakness, vomiting, nausea, upper abdominal discomfort, malaise, fever, headaches, vertigo, massive tender hepatomegaly and Jaundice. Microcystin was found in the reservoir water that was supplied to the clinic, water in the delivery tanker truck and the dialysis unit holding tank. Clinical investigations lead to the discovery of microcystin in the sera and liver samples from patients both post-mortem and those alive (Jochimsen et al. 1998) Microcystin-LR was the common microcystin variant identified in the four reservoirs in Ghana along with other variants such as –LF, –LW and YR. Most of the cases mentioned above are due to acute exposure to microcystin. However, as mentioned earlier of more concern is the chronic exposure to microcystin through drinking water. In Southern China, in the cities of Haimen and Qidong, a high incidence of primary liver cancer was associated with the use of water from ditches and pond as drinking water (Yu, 1989; Harada et al. 1996; Ueno et al. 1996). Ueno et al., (1996) investigated microcystin in 989 water samples from pond, ditch, river and shallow well water. He observed that 32% of river water, 17% of pond water and 4% of the shallow well water contained microcystin. Quantification of microcystins in these water supplies showed relatively low concentrations up to 0.46 μg/l, again lower than those obtained from Ghana. It was estimated that humans living in these areas with high primary liver cancer consumed 0.19 pg microcystin per day during four summer months for over 40 to 50 years of their life span. Other factors attributed to the high primary liver cancer in addition to microcystin are potent liver carcinogen aflatoxin B_1 and hepatitis B virus (Ueno et al. 1996). Again, in China Zhou et al. (2000) found a positive correlation between the incidence of colorectal cancer in

China and the use of drinking water from rivers and ponds containing microcystin. Among the four reservoirs studied, the Owabi reservoir was the worst reservoir in terms of both intracellular and dissolved microcystin with 54.2% and 58.3% microcystin positive respectively. This situation is very grievous and higher than most statistics obtained in most places as per the studies in China (Ueno et al. 1996). At the time of sampling the Owabi treatment plant it was observed that algaeciding with copper sulphate, was simultaneously being done during water treatment and this may account for the high level of dissolved microcystin positive samples in this reservoir. Algaeciding with copper sulphate results in cell death and lysis with consequent release of cell-bound microcystin and taste and odour compounds from the cells to surrounding waters where it dissolved in the water (Lahti et al., 1996; Hrudey et al. 1999; Hitzfeld et al., 2000). This may be the reason for the high dissolved microcystin positive samples obtained in this reservoir putting consumers at risk of exposure to microcystin. The dangers of using algaecides in drinking water reservoirs was demonstrated in an incident which occurred on tropical Palm Island, Australia, where members of the community became ill with hepato-enteritis following treatment of the water supply reservoir with copper sulphate after complaints of bad taste and odour from the population (Byth, 1980; Bourke et al. 1983). 140 children and 10 adults were hospitalised for treatment. Jones and Orr (1994) suggested that if algaecides are used to control toxic cyanobacteria, the reservoir should be isolated for a period to allow the toxins and odour to degrade. Unfortunately there are no data available on the withholding period but it could be in excess of 14 days (Falconer, 2004). Our results also indicated that, the stage with alum flocculation during the drinking water treatment yielded the highest total intracellular concentrations and microcystin positive samples followed by the intake of the reservoirs. There is no literature to support this phenomenon but from our experience this may be due to cell weight or numbers harvested at this stage of treatment. Alum is a flocculants that causes the cells to aggregate together (Hrudey et al. 1999; Hitzfeld et al. 2000; Falconer 2004). Our results also showed that total dissolved microcystin increased at the sedimentation stage after flocculation with alum. There is evidence to suggest that alum can lead to lysis of cyanobacterial cells leading to release of cyanotoxins including microcystin (James and Fawell, 1991; Hrudey et al. 1999; Falconer, 2004; Hoeger et al. 2005) and this may result in high dissolved microcystin in the sedimentation tanks. In Ghana there are no cyanobacteria

monitoring neither are there any guidelines for microcystin in drinking water. Conventional drinking water treatment plants as exist in Ghana have been shown to be ineffective in removing dissolved microcystin in water as well as cyanobacterial cells (Lepisto et al. 1994; Grutzmacher et al. 2002; Hoeger et al. 2005). Safe drinking water has been recognised as one of the most critical factors to guaranteed long-term population health. This calls on the Government of Ghana to institute long term guideline values for drinking water so as to protect public health through exposure to microcystin and cyanobacterial cells. Cyanobacterial cells when ingested orally may be digested in the digestive tract and microcystin released into the small intestine and transported through the hepatic portal vein into the liver where it is actively concentrated (Falconer, 1996, 1999). In the Ghanaian situation both cyanobacteria cells and dissolved microcystin are present in the final drinking water. Cyanotoxins, especially microcystins, are contained within healthy cyanobacteria cells, therefore, the first priority in the removal to cyanobacterial toxins is the removal of whole live cells from the drinking water ((Berg *et.al.*, 1987; Kenefick *et.al.*, 1993; Jones and Orr, 1994; Lahti et al., 1996; Hitzfeld et al., 2000; Drikas et al., 2001). There is overwhelming evidence in this chapter to substantiate the fact that the risk to public health and safety is high.

Conclusions

The title of my thesis is toxic cyanobacteria and drinking water production in Ghana and its implications to human health. Enough evidence has been given in this chapter to prove that the risk to public health through exposure to microcystin is high and relevant. Level of dissolved microcystins obtained in the final drinking water stage were higher than those obtained in Zimbabwe, Brazil, Australia, China and Sweden where human poisoning have occurred. There is an urgent need to control the growth and proliferation of cyanobacteria in drinking water reservoirs as well as the levels of microcystin through proper watershed management and monitoring of drinking water quality. Removal of dissolved microcystin can be very expensive and this may not be possible considering the current economic situation of Ghana, however ensuring that intact cyanobacteria cells are efficiently removed during water treatment provide a simple, cheap and convenient way ensuring safe drinking water.

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IV

Demethylated microcystin-RR ([D-Asp^3] MCYST-RR), ([D-Asp^3] MCYST-LR) and four other putative microcystins identified in a culture of *Planktothrix* sp. strain CCAP 1460/13 (Paper IV).

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Abstract

Planktothrix is one of the most common known toxin producing cyanobacterium world-wide and has been reported to produce different types of cyanotoxins especially demethylated microcystins, neurotoxins, and saxitotoxins. We purchased and cultured *Planktothrix* sp strain CCAP 1460/13 in a laboratory using BG11 medium with constant aeration. Temperature was kept at 25°C with continuous illumination from a cool white florescent tube with incident irradiance of 20 μmol photon/m^2/s. *Planktothrix* cells were harvested by centrifugation, lyophilised and stored at -20°C. Toxin extraction was achieved using 20 ml of 75% methanol. HPLC-DAD analysis of the extract showed eight microcystin related peaks of which two peaks were not accepted as microcystin due to the low similarity with microcystin spectra. Quantification of the two dominant toxin fractions demonstrated the presence of high toxin concentrations of 4,036 and 242 μg/g/DW from a cell biomass of 0.042g. This concentration is by far as our knowlegde of the literature is among the highest by any cultured *Planktothrix* strain. Further verification of the toxins in the *Planktothrix* extract using LC-MS identified the two dominant toxins as demethylated microcystin-RR ([D-Asp^3] MCYST-RR) and demethylated microcystin-LR ([D-Asp^3] MCYST-LR) respectively. *Planktothrix* is a highly toxic species known to produce a high amount of microcystin per unit cell. The high concentration of toxin produced by this *Planktothrix* is a confirmation to this fact.

Key words: Cyanobacterium; *Planktothrix*; Microcystin; Toxin; Culture; Cyanobacteria

Introduction

Microcystins are hepatotoxins, chemically classified as cyclic peptides (Sivonen and Jones 1999) of which over 60 microcystin variants have been classified from several species of cyanobacteria including *Microcystis* (Botes et al., 1984), *Anabaena* (Krishnamurthy *et al*., 1986,), *Planktothrix* (Meriluoto et al., 1989; Luukkainen et al., 1993), Napalosiphon (Prinsep et al., 1992), *Nostoc* (Sivonen et al., 1990; Namikoshi et al., 1990) and *Oscillatoria* (Brittain et al., 2000). It has been reported recently, that microcystins can be associated also with cyanobacteria from the genera *Aphanothece* (Sant Anna *et al*, 2004, Dasey et al., 2005), *Aphanocapsa* (Domingos et al, 1999), *Arthrospira* (Ballot et al., 2004; Ballot et al., 2005), *Pseudanabaena* (Oudra et al., 2002), *Radiocystis* (Veira 2003; Lombardo et al., 2006), *Synechocystis* (Oudra et al., 2002) and *Scytonema* (Kumar et al., 2000). Microcystins are comparatively large natural products, with molecular weights (MW) of 800 –1000 (Sivonen and Jones, 1999). The toxicity of microcystins is predominantly mediated through the inhibition of the catalytic subunit of protein phosphatases 1 and 2A, leading to acute liver failure due to disruption of hepatocyte cytoskeletal components (Mastusushima et al., 1990; Yoshizawa et al., 1990; Toivola et al. 1994). Protein phosphatases serve an important regulatory role to maintain homeostasis in the cell (Cohen, 1989). Further intracellular targets of microcystins have been suggested, such as ATP-synthase (Mikhailov et al., 2003) and aldehyde dehydrogenase (Chen et al., 1989). Microcystin also act as tumour promoters after long-term exposure, which had been substantiated by several authors (Runnegar *et al*., 1995; Sueoka et al., 1997; Carmichael, 1994; Falconer, 1989, 1996; Nishiwaki-Matsushima et al., 1992). Microcystins have also been the cause of fatalities in wild and domestic animals (Briand et al., 2003) and can affect a wide range of aquatic organisms (Wiegand and Pflugmacher 2005; Zurawell et al., 2005) and also the cause of misery and illness in humans (Kuiper-Goodman et al., 1999; Falconer 2004). Microcystin were found to be responsible for the death of over 50 dialysis patients in a dialysis clinic in Brazil (Jochimsen et al., 1998; Carmichael et al., 2001).

Planktothrix (formally known as *Oscillatoria*) is a highly toxic cyanobacterium in water bodies known to produce several types of microcystins especially the demethylated microcystins (Krishnamurty *et al.*, 1986; Meriluoto *et al.*, 1989; Sivonen et al., 1995), neurotoxic alkaloids, anatoxin-a and homo-anatoxin-a (Skulberg et al., 1992; Aas et al., 1996; Lilleheil et al., 1997; Fuey et al., 2003; Viaggiu et al., 2004; Araoz et al., 2005) and the paralytic shellfish poisons, saxitotoxins (Pomati *et. al.*, 2000). *Planktothrix* is known to produce the highest levels of microcystins on a dry weight basis of any other cyanobacterium (Fastner et al., 1999b; Chorus, 2001; Falconer et al., 1999, 2004). Emanuela et al., (2004) reported that the death of mice treated with pure extract of *Planktothrix rubescens* occurred within 110-120 minutes. Symptoms shown by dying mice were trembling, convulsion, dyspnea and hemi paralysis (Emanuela et al., 2004), all characteristic signs of poisoning by the neurotoxin alkaloid toxin anatoxin-a. Ecologically *Planktothrix* prefers low light intensities for growth (Skulberg et al., 1999) and produce maximum mass occurrences deep in the water column (Reynolds *et al.*, 2002; Humbert *et al.*, 2001) which may be over looked by surface monitoring of water and therefore be of particular risk in drinking water production (Falconer, 2004).

As part of a PhD research programme to study the cyanobacteria and toxins in drinking water reservoirs in Ghana, we purchased and cultured *Planktothrix* strain CCAP 1460/13 in the laboratory for use as experimental material. Results of high pressure liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS) analysis of the extract of *Planktothrix* strain CCAP 1460/13 are presented in this paper.

Materials and Methods

Culture

Planktothrix strain CCAP 1460/13 was purchased from Culture Collection of Algae and Protozoa (CCAP), Scotland, United Kingdom. *Planktothrix* was cultured in 2 l flask in a growth chamber for 28 days using BG11 medium prepared in the laboratory from recipe supplied by CCAP.

Temperature was kept at 25°C with continuous illumination from a cool white florescent tube with incident irradiance of 20 μmol photon/m^2/s. The culture was continuously aerated with atmospheric oxygen by shaking the flask. *Planktothrix*

cells were harvested on the 28th days by centrifugation using Denley BR401 refrigerated centrifuge at 5000 rpm for 15 min. The harvested cells were frozen, freeze dried and stored at -20°C until chemical analysis.

Extraction

Planktothrix cells on a glass fibre filter of approximately 0.042 g were extracted in 20 ml of 75% aqueous methanol which had been recommended by Faster et al., (1998) as the most appropriate solvent for the recovery of all forms of microcystin, for 1 hr. This process was repeated three times as described by Harada et al., (1999). All three extracts were pooled together and dried using rotary evaporator at 45 °C and dry residue mixed with 0.5 ml pure methanol. The extract was cleaned prior to HPLC analysis using 4 mm Nalgene nylon syringe filter with pore size 0.45um.

High Performance Liquid Chromatography (HPLC) analysis

Toxin analysis of *Planktothrix* extract was achieved by using a reverse-phase high performance liquid chromatography system (Agilent 1100 series) equipped with a photodiode array detector (DAD). The HPLC column was a Supelcosil ABZ + plus with a stationary phase of 150 ×4.6 mm ×5 μm. Column temperature was kept at 30°C. The mobile phase used was constituted of: A: 0.1% trifluoroacetic acid in water and B: acetronitrile with 0.1% trifluoroacetic acid (Lawton et al., 1994), with a linear gradient from 20% of B at 0 min to 59% of B at 30 min, and a flow rate of 1 ml/min. The volume of extract injected was 10 μl. Resulting data were gathered between 200-300 nm and chromatograms were evaluated and shown on screen at 238 nm. Microcystin peaks were identified by comparison of retention times with those of standards used: MC-LR, -LF, -LW, -RR, -YR (Alexis Biochemicals) and nodularin (DHI Water and Environment), and their characteristic UV-spectra (Lawton et al., 1994). The toxic fractions were quantified by extrapolating the HPLC peak areas at 238 nm to a linear calibration curve for microcystin-LR standard (n =5, r^2 =0.999)

Liquid Chromatography/Mass Spectrometry (LC-MS)

Verification of the toxins identified from *Planktothrix* sp strain CCAP 1460/13 was undertaken via HPLC with photodiode array (PDA) and mass spectrometry (MS) detection. A Waters Alliance 2690 solvent delivery system equipped with a 996 PDA and ZQ 2000 MS detector in series was used for all analysis (Waters, Elstree, UK). Separation was achieved using a Symmetry C18 column (2.1 mm i.d. x 150 mm long; 5 μm particle size) which was kept at 40℃. Mobile phase used was water (A) and acetronitrile (B), both containing 0.05% TFA. Test samples (25μl) were separated using a gradient increasing from 5% to 60% B over 25 minutes at a flow rate of 0.3 ml/min followed by ramp up to 100% B and re-equilibration over the next 10 minutes. The eluent was monitored from 200-300 nm with a resolution 1.2 nm and peptides were quantified by external standards using 238 nm. Mass spectrometry analyses were performed using positive ion detection and electro spray sample ionization mode, scanning from the m/z 100 to 1200 with a scan time of 2 s and inter-scan delay of 0.1 s. Ion source parameters were: sprayer voltage, 3.07 kV; cone voltage, 70 V; desolvation temperature, 300℃; and source temperature at 100℃. Instrument control, data acquisition and processing were achieved using Masslynx v3.5 software. Sample volume of 25μl was injected.

Results and Discussion

Planktothrix (Fig. 1) is a well known bloom forming, microcystin producing cyanobacterium (Willen and Mattesson 1997; Luukkainen et al., 1993; Sivonen et al., 1995; Sivonen and Johns 1999; Chorus, 2001; Falconer, 2004). It is also known to produce mainly demethylated microcystin (Luukkainen et al., 1993; Willen and Mattesson 1997; Sivonen et al., 1995; Sivonen and Jones, 1999; Laub et al., 2002). In our HPLC-DAD analysis of *Planktothrix* sp. CCAP 1460/13 we detected eight peaks possessing UV-spectra with maximal absorbances between 238-240 nm (Fig. 2), which are typical for microcystins and nodularins (Meriluoto 1997; Sivonen and Jones 1999; Falconer 2004). However, none of these eight peaks corresponded with the retention times of microcystin and nodularin standards used in our analysis. Four peaks at 8.378, 12.144, 16.529 and 17.689 min (Fig. 3) displayed high similarity (Over 99.8%) to UV-spectrum of

microcystin-LR (Fig. 4). The UV spectra of two other peaks (at 13.005 and 13.542 min) had a resemblance to characteristic microcystin spectra. The peaks at 11.813 and 15.373 min have their absorption maxima at 238-240 nm, but similarity to microcystin-LR spectra was low and therefore these were rejected as microcystin.

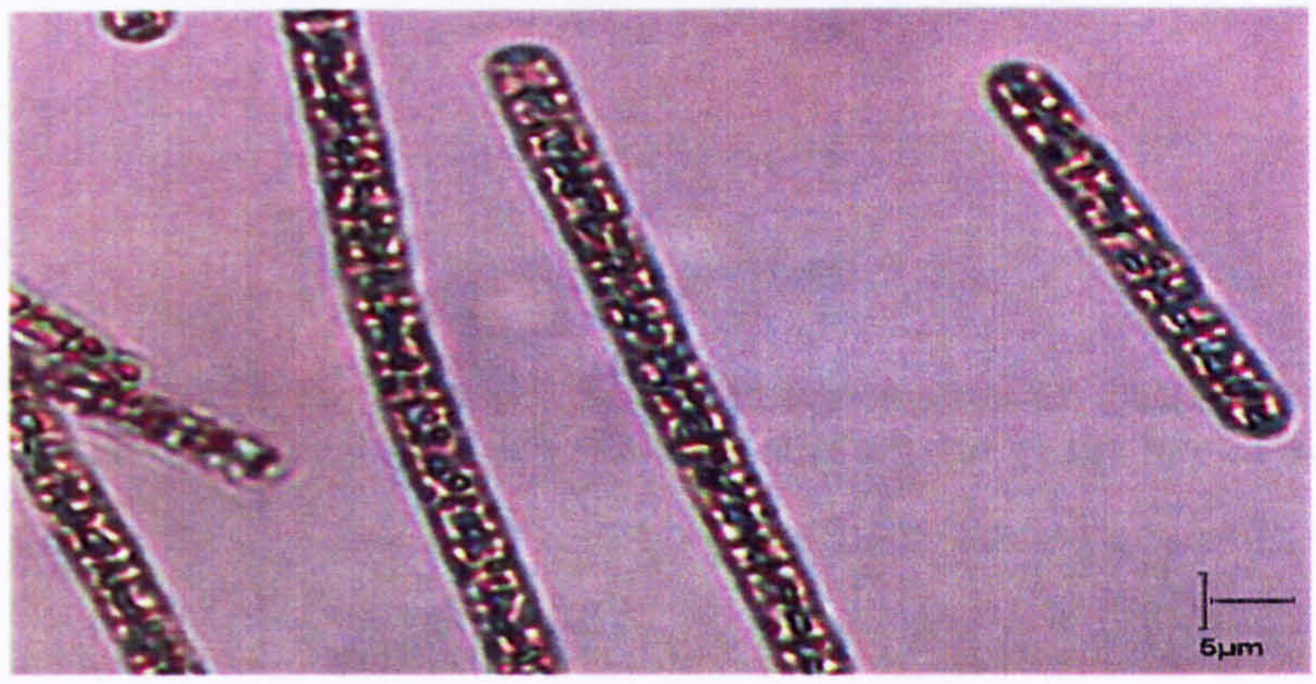

Figure 1 *Planktothrix* sp. Courtesy of Dr John Day, CCAP, Scotland, United Kingdom

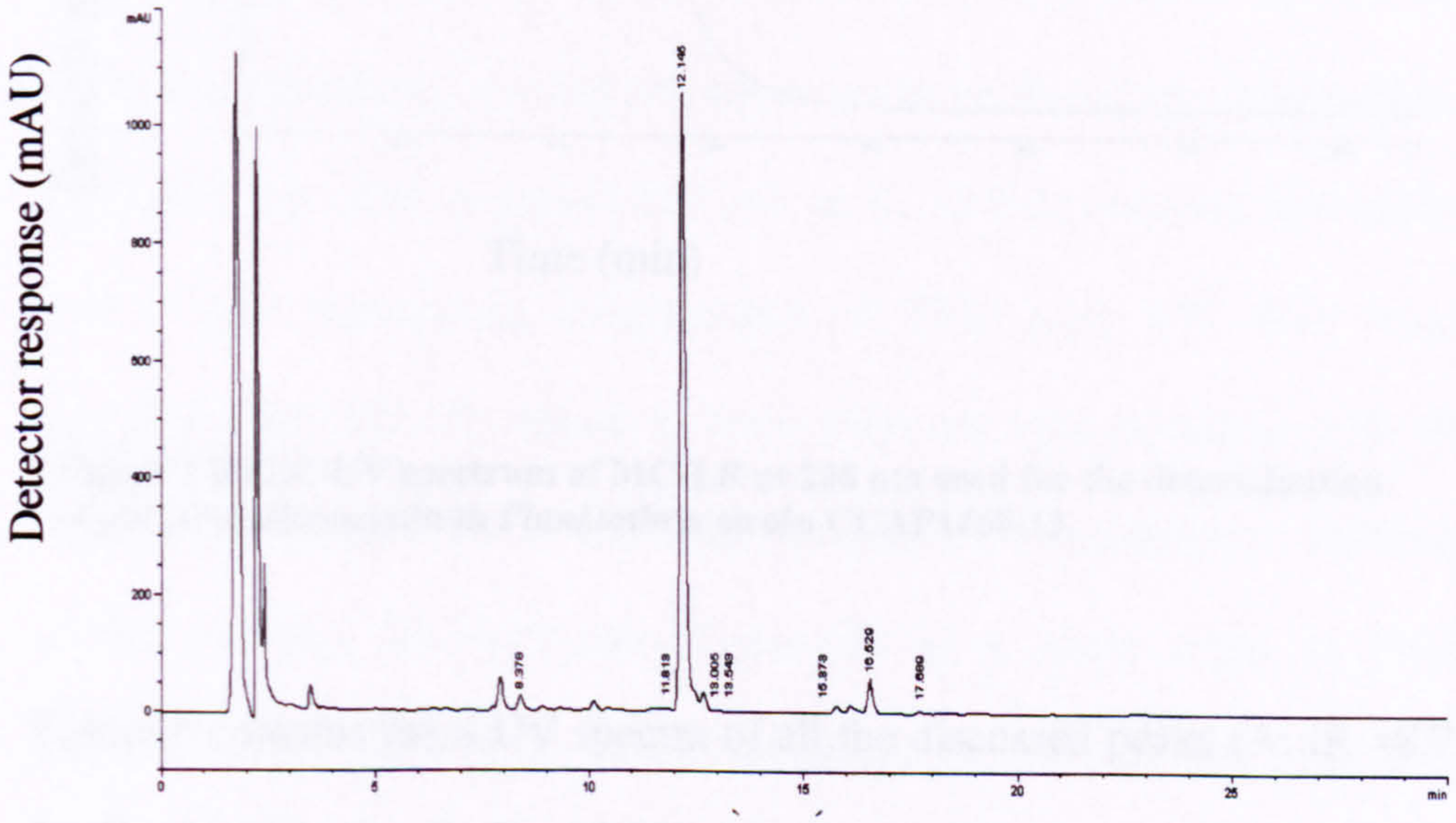

Figure 2 HPLC-DAD chromatogram of *Planktothrix* sp. CCAP 1460/13 extract

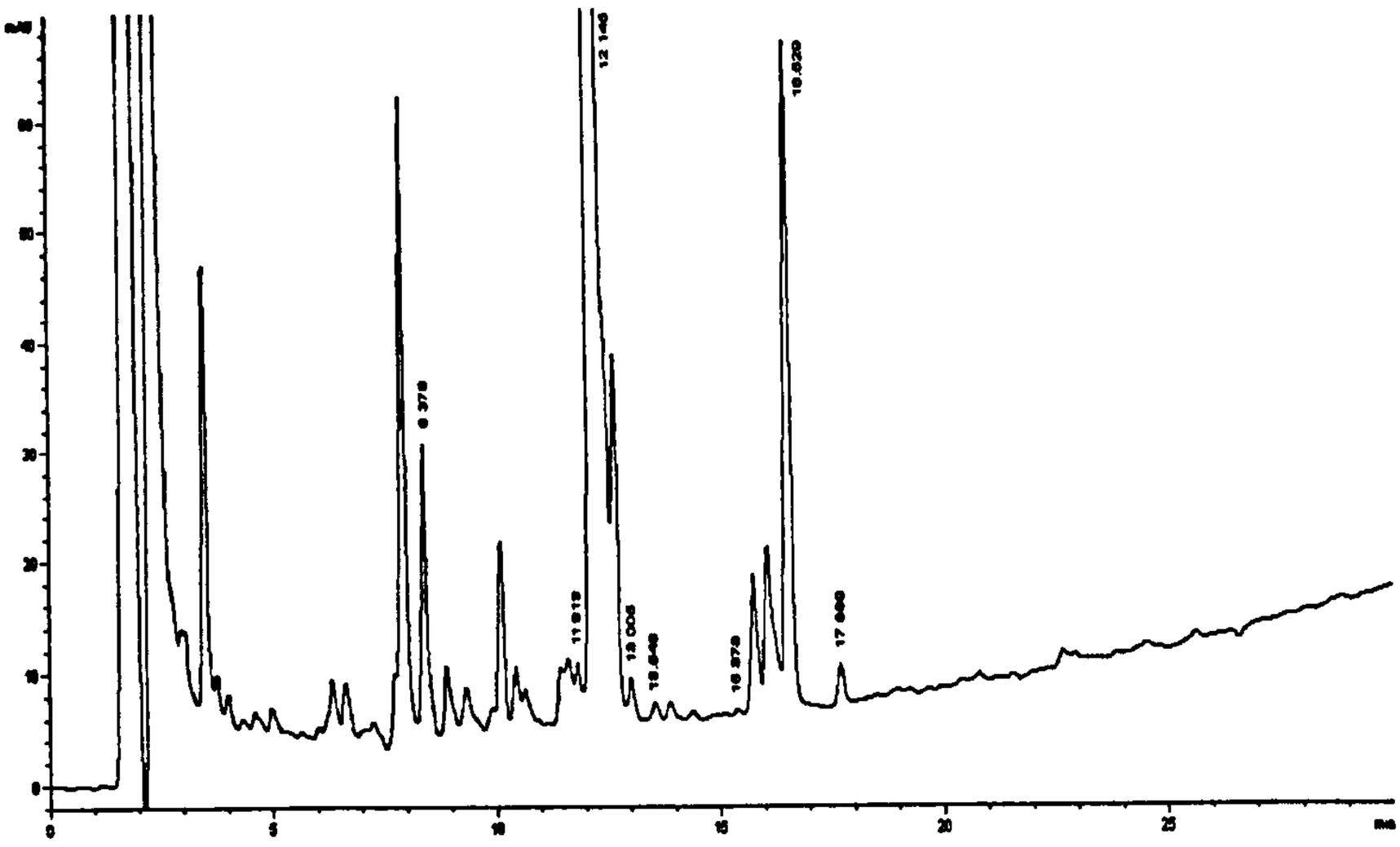

Figure 3 Detail HPLC-DAD chromatogram of *Planktothrix* sp. CCAP 1460/13 extract showing clearly all eight microcystin peaks two of which were rejected as microcystin

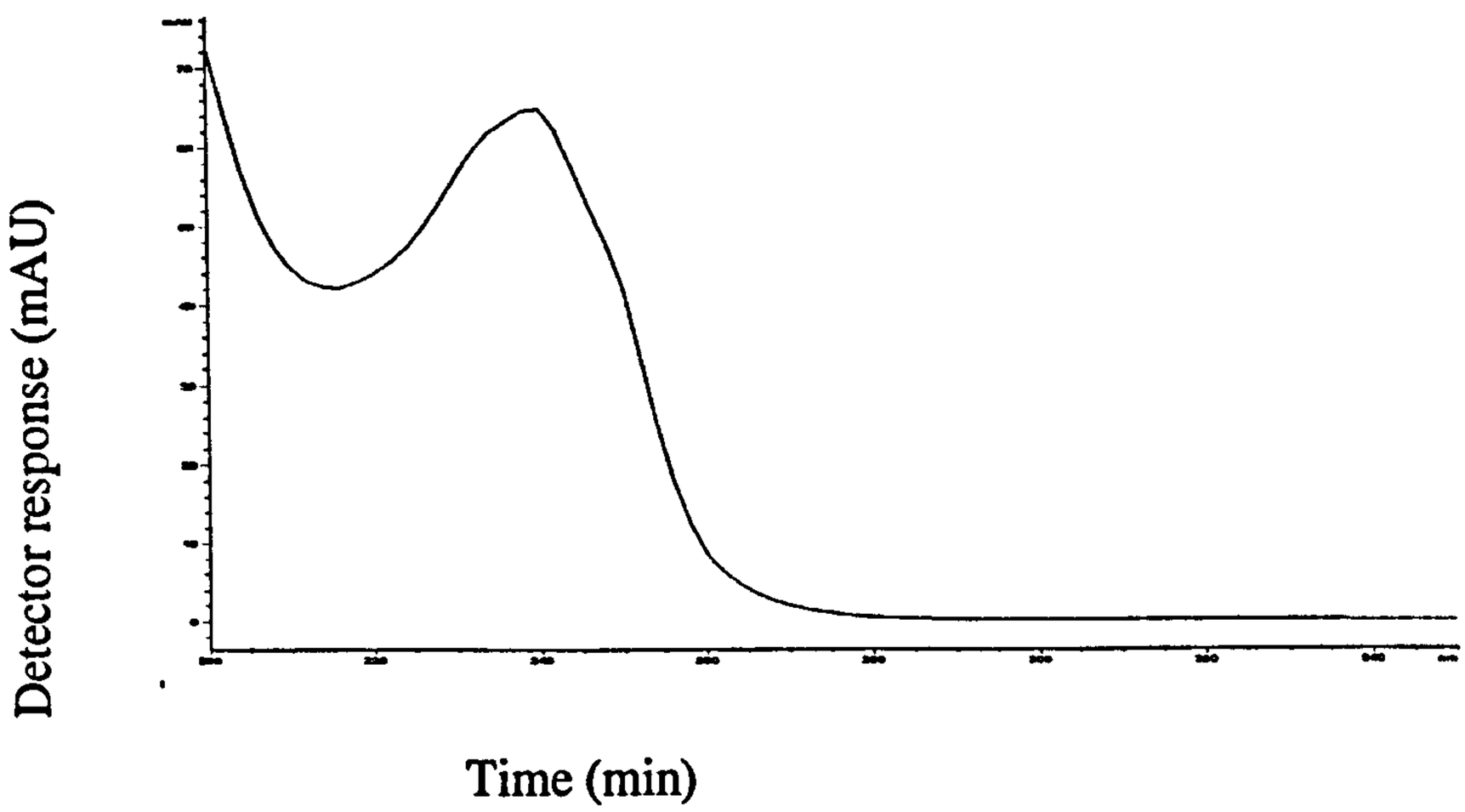

Figure 7 HPLC-UV spectrum of MC-LR at 238 nm used for the determination of putative microcystin in *Planktothrix* strain CCAP1460/13

Figure 5 contains the 8 UV spectra of all the discussed peaks (A: tR =8.738 min, B: tR =11.813 min, C: tR=12.144 min, D: tR=13.005 min E: tR =15.373 min, F: tR = 16.529 min, G: tR = 17.689 min and H: tR = 13.542 min). Although retention times of discussed peaks were different from the retention times of

microcystin and nodularin standards the 6 peaks at 8.378, 12.144, 13.005, 13.542, 16.529 and 17.689 min were evaluated as putative microcystins due to spectral information (Lawton et al., 1994; Harada et al., 1999; Falconer, 2004). To detect six putative microcystins as obtained in our HPLC analysis of *Planktothrix* is not unusual. Luukkainen et al., (1993) obtained eight putative peaks in their study of thirteen *Oscillatoria agardhii* strains now known as *Planktothrix agardhii* (Anagnostidis and Komarek 1988). Welker et al., (2004) obtained eleven putative microcystin peaks in their study with *Planktothrix* strain Max06. Briand et al., 2005 also obtained four microcystin variant in their study on *Planktothrix rubescens* in their survey of Lac du Bourget, France. The HPLC-DAD quantification of all toxin fractions were based on calibration curve previously obtained for microcystin-LR. The estimated concentrations of individual putative microcystin peaks expressed as microcystin-LR concentration equivalents are given in the Table 1. The total microcystin content of the extract was 4523 μg/g DW, to which peaks at 12.144 min and 16.528 min contributed 89.2% and 5.4%, respectively. This high concentration of microcystin produced by *Planktothrix* in our culture is among the highest microcystin produced by *Planktothrix* of any cultured strain cited in literature. Laub et al., (2002) obtained four microcystins with a total microcystin concentration of 3310 μg/g DW from *Planktothrix agardhii* strain PH-123 which is lower than our concentrations with the major toxin contributing 64% of the total microcystin concentration. Fastener et al., (1999) obtained [D-Asp^3] microcystin-RR as a major toxin in *Planktothrix agardhii* strain HUB 076 which amounted to 69% of the total microcystin concentration. In our study the major toxin [D-Asp^3]microcystin-RR contributed 89.2%. Maximum concentration of microcystin measured in German lakes dominated by *Planktothrix* was over 5000 ug/g DW with mean of approximately

2000 ug/g (Fastner et al., 2001). However, these high concentrations were from field samples not cultured strains. Initial results from our HPLC resulted in six unknown microcystin peaks. Determination of these unknown toxin peaks using LC-MS of the extract (Fig. 6A and B) confirmed the toxin fraction our major peak (corresponding to the peak at 12.144 min in HPLC/DAD) as [D-Asp^3]microcystin-RR a toxin with molecular weight 1025 and [D-Asp^3]microcystin-LR (corresponding to the peak at 16.528 min in HPLC/DAD), a toxin with molecular weight of 982. These two toxins, [D-Asp^3] microcystin-RR and [D-Asp^3] microcystin-LR are very common toxins produced by *Planktothrix* in both cultured strains and bloom samples but at lower concentrations (Meriluoto et al., 1989; Carmichael 1989; Krishnamurthy et al., 1989; Luukkainen et al., 1993; Sivonen and Jones 1999; Laub et al., 2002; Welker et al., 2004; Briand et al., 2005).

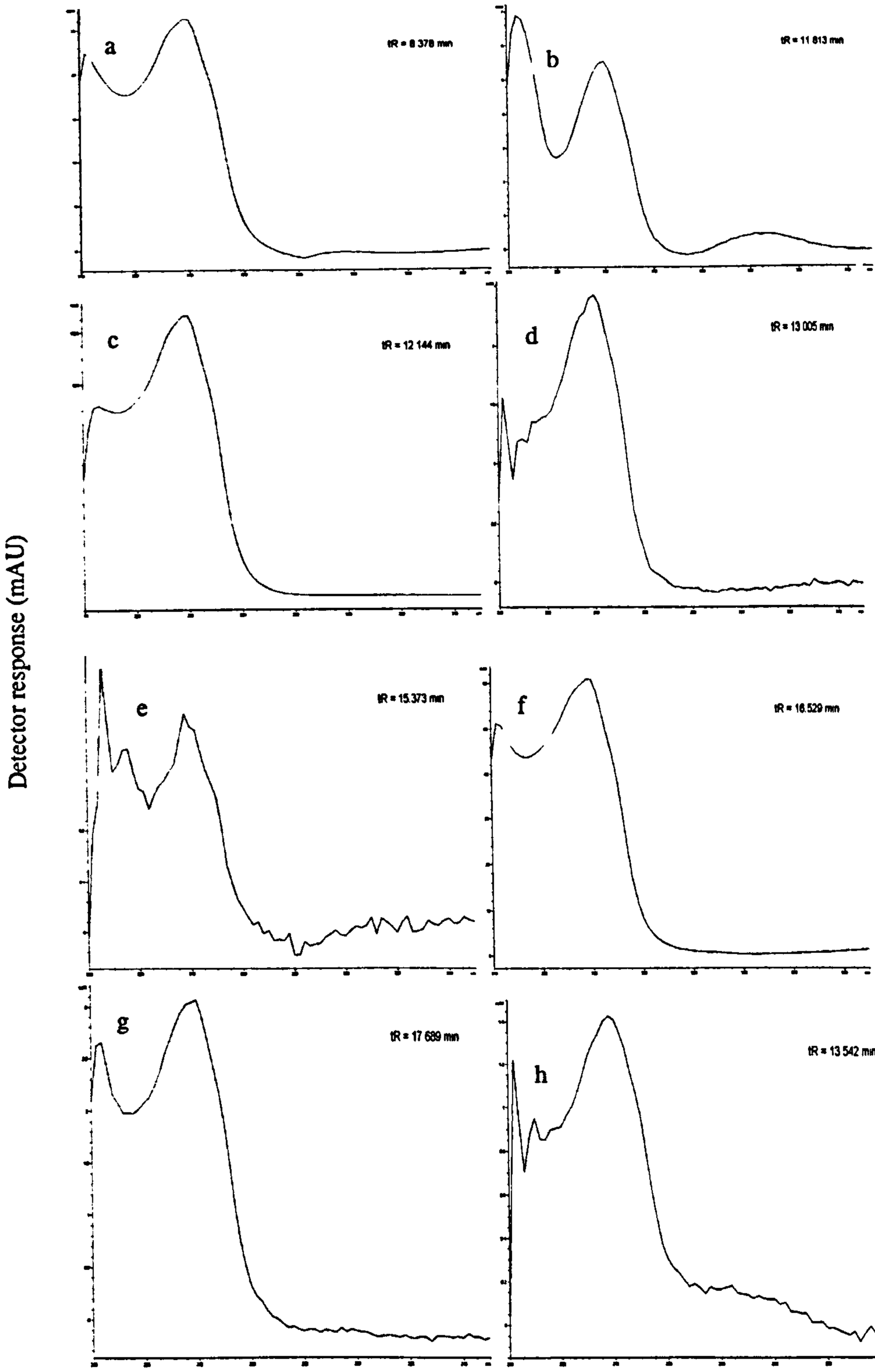

Figure 5 UV- HPLC spectra of all 8 discussed microcystin peaks obtained *Planktothrix* sp. strain CCAP1460/13

Retention time (min)	Concentration in biomass (µg/g DW)	Comment
8.378	113	
12.144	4036	[D-Asp^3]microcystin-RR[a]
13.005	20	
13.542	10	
16.528	242	[D-Asp^3]microcystin-LR[a]
17.689	16	
Total MCYST:	4523	

[a] According to LC/MS analysis

Table 1 Retention times and concentration in biomass of putative microcystins in *Planktothrix* strain CCAP1460/13 extract

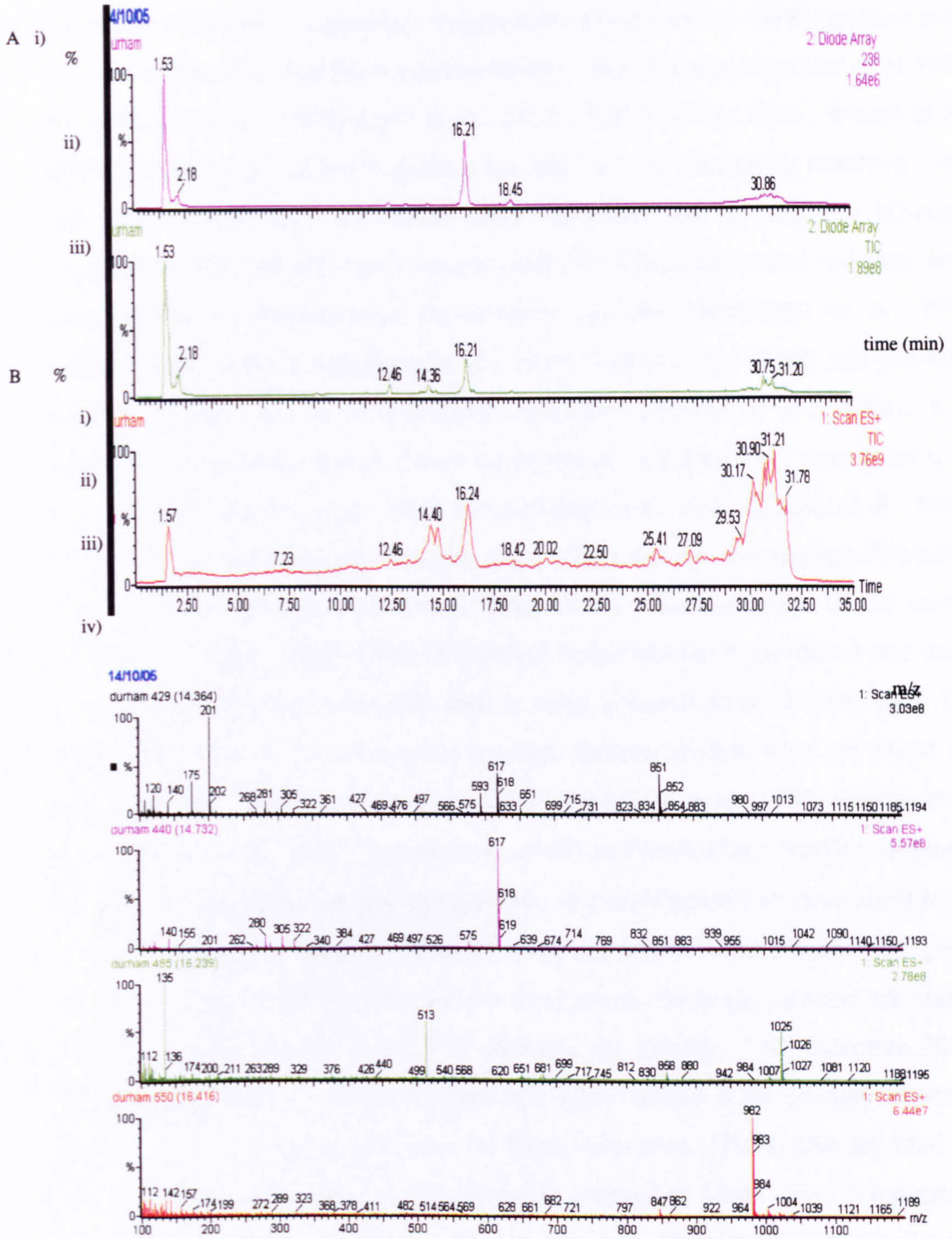

Figure 6a and b. HPLC-LC-MS analytical profile of toxic fraction of *Planktothrix* sp. CCAP 1460/13. Showing 6A; i. HPLC signal from PDA detector at 238 nm. ii. Signal from MS detector at 238 nm and iii. Signal from MS detector, scan ES+TIC. 7B. Mass spectrum of individual peaks in the chromatogram of *Planktothrix* strain CCAP 1460/13, i, Peak at 14.364 min, ii. Peak at 14.732 min, iii. Peak at 16.239 min-putative [D-Asp^3]microcystin-RR and iv. Peak at 18.416 min-[D-Asp^3]microcystin-LR

Luukkainen et al., (1993) were the first to show that *Oscillatoria agardhii* now known as *Planktothrix agardhii* (Anagnostidis and Komarek 1988) produce more than one toxin. This had been collaborated by many authors (Sivonen et al 1995; Sivonen and Jones, 1999, Laub et al., 2002; Welker et al., 2004; Briand et al., 2005). In our study six toxin peaks were obtained by comparing retention times and UV spectra, two of which were identified by LC-MS as [D-Asp^3] microcystin-RR and [D-Asp^3] microcystin-LR. These structural variants have been previously described in *Planktothrix agardhii* (Kivaranta et al., 1991; Fastner et al., 1999; Luukkainen et al., 1993; Welker et al., 2004) and [D-Asp^3] microcystin-RR also in *Planktothrix rubescens* (Briand et al., 2005). It is commonly understood that all *Oscillatoria* strains produce only one major toxin at a time (Luukkainen et al., 1993; Sivonen and Jones 1999; Welker et al., 2003). In contrast to *Anabaena* spp (Sivonen et al., 1992) and *Microcystis* spp (Watanabe et al., 1988) usually produce two or more toxins simultaneously. In our studies *Planktothrix* strain CCAP 1460/13 cultured in the laboratory produced one major toxin, [D-Asp^3] microcystin-RR with a toxin concentration of 4036 ug/g DW which was 89% of the total toxin fraction. Several studies have all found [D-Asp^3] microcystin-RR as the major toxin (Luukkainen et al., 1993; Fastner et al., 1999; Welker et al., 2003; Briand et al., 2005) in *Planktothrix*. Studies on growth requirements have shown that the majority of cyanobacteria produce most toxins under favourable growth condition (Sivonen and Johns 1999). *Planktothrix* prefer low light intensities for growth and for most strains the toxin contents are highest at temperatures between 18 to 25 °C (Robarts and Zohary, 1987; Falconer, 2004). Watanabe and Oishi (1985) concluded that light intensity is the primary important factor for the production of toxins. De Figueiredo et al., (2004) also reported that high temperatures enhance microcystin-RR production while lower temperatures favour the production of microcystin-LR. In our culture studies of *Planktothrix* the temperature was kept at 25 °C and exposed to a continuous illumination from a cool white florescent tube with incident irradiance of 20 μmol photon/m^2/s and these favourable conditions could have been responsible for the high toxin production in this cyanobacterium. [D-Asp^3] microcystin-RR, the major toxin found in our study is the demethylated form of microcystin-RR and the findings of De Figueiredo et al., (2004) could be of significance in our study. Wiedner et al., (2003), concluded that photosynthetically active radiation has a positive effect on microcystin production and content up to a point where maximum growth is

reached. Van Liere and Mur (1980) reported that the growth of *Planktothrix* was inhibited when exposed to extended periods of light intensities above 180 $\mu E/m^2/s$. Briand et al., 2005 suggested that environmental parameters might have an indirect effect on microcystin production, due to their direct impact on cellular growth rates. *Planktothrix* species is one of the common known toxin producing cyanobacterium in lakes, lagoons and drinking water reservoirs in Ghana (Addico and Frempong 2004; Addico et al., 2006). The growth requirements of this cyanobacterium viz a viz temperature, light conditions and nutrients as shown by both laboratory and field studies in literature cited above makes it an important cyanobacterium as far as intoxication by microcystin of both domestic and wild animals including fisheries as well a human beings in Ghana.

Conclusions

In conclusion, eight different microcystin peaks at 238 nm typical for microcystin were isolated from *Planktothrix* spp strain CCAP 1460/13 using HPLC-DAD. Six of these peaks were confirmed as microcystins while two peaks were rejected due to their low similarity with UV spectral data of microcystin-LR. The major toxin in this *Planktothrix* was identified as [$DAsp^3$] microcystin-RR using LC-MS. This compound is a toxin produced by most *Planktothrix* strains, however, the high toxin concentration synthesised by this *Planktothrix* is probably the highest observed in cultured strains. Also the high percentage of 89.2 of the dominant toxin was also significant in this study. *Planktothrix* is well known to synthesise high microcystin concentrations on dry weight basis than any other cyanobacterium and this study is a confirmation to that fact.

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Microcystin-RR-like toxin in the cyanobacterium *Anabaena flos-aquae* strain CCAP 1403/13B

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Abstract

Species of *Anabaena* are common cyanobacteria found in Ghanaian drinking water reservoirs. However, there have been no studies on their toxicity, the toxins they produced and their public health effects in Ghana even though *Anabaena* is known to be highly toxic. We cultured *Anabaena flos- aquae* strain CCAP 1403/13B in the laboratory as part of our research into cyanobacteria and drinking water quality in Ghana. Cultures were kept in a growth chamber with continuous illumination at 20 μmol photon/m^2/s and constant aeration at a temperature of 25°C. HPLC analysis of extract from the culture of *Anabaena flos-aquae* strain CCAP 1403/13B produced a toxin with a retention time similar to microcystin-RR external standard. The concentration of microcystin-RR quantified from *Anabaena flos-aquae* was 10.6 μg/g DW. The biomass of lyophilized cells extracted was 52 mg. *Anabaena flos-aquae* is mainly known to produce neurotoxins, notably anatoxin-a and anatoxin-a(s).

Key Words: culture, *Anabaena flos-aquae*; cyanobacteria, microcystin-RR; toxins

Introduction

Microcystins (cyclic peptide hepatotoxins) have been described and detected in several cyanobacteria genera including *Anabaena* (Krishnamurthy *et. al.*, 1986), *Microcystis* (Botes *et al.*, 1984; Kusumi et al., 1987; Watanabe et a., 1988), *Oscillatoria* (Meriluoto et al., 1989; Brittain *et al.*, 2000) and *Planktothrix* (Meriluoto *et. al.*, 1989). Globally, the most frequently occurring cyanotoxin encountered in fresh and brackish waters are the cyclic peptides known as microcystin (Sivonen and Jones 1999; McElhiney and Lawton 2005; Moreno et al., 2005). Microcystins are hepatotoxic and have been described as potent liver toxins (Fujiki and Suganuma, 1993; Falconer et. al., 1994; Ito et. al., 1997; Mackintosh et. al., 1990), and in chronic doses known to promote and initiate the growth of tumours. It has also been found to inhibit protein phosphatase activity (Yohizawa et al., 1990; Matshushima et al., 1990; MacKintosh et al., 1990). Microcystins are known to be synthesised non-ribosomally and by thiotemplate mechanisms (Dittmann et al., 1997; Meissner et al., 1996; Arment and Carmichael, 1999). In Ghana, there have been no studies on cyanobacteria, cyanotoxins and possible adverse public health effects on humans. This is despite the established knowledge that cyanobacteria have been implicated in several poisoning episodes of humans and animals world wide through drinking water e.g. In Bahia, Brazil, *Anabaena* and *Microcystis* were responsible for a lethal outbreak attributed to cyanobacterial toxins from drinking water which resulted in the death of 88 children from over 2000 cases of gastro-enteritis over a period of 42 days (Teixera *et al.*, 1993). Another human mortality occurred in Brazil, at a haemodialysis clinic where patients were treated with drinking water contaminated with cyanotoxins (Jochimsen *et al.*, (1998)). Over 50 people died in this incident. Examination of the phytoplankton in the reservoir showed the dominance of the cyanobacteria *Microcystis*, *Anabaena* and *Anabaenopsis*. Recent studies on cyanobacteria samples from the Kenyan alkaline lakes Bogoria and Nakuru have shown the presence of the cyanotoxins, microcystins and anatoxin-a (Ballot *et al.*, 2000 and Krienitz *et al.*, 2003). Lake Bogoria was mainly dominated by *Anabaena fusiformis* while Lake Nakuru was dominated by *Anabaena fusiformis* and *Anabaenopsis* sp. In Ghana *Anabaena flos-aquae* and *Anabaena spirodes* are the dominant species of this genera found in most brackish and estuarine water bodies (Addico and Frempong, 2004). *Anabaena* species are

mainly known to produce neurotoxins mainly anatoxin-a and anatoxin-a(s) and other hepatotoxins (Al-Layl et al., 1988; Hara et al., 1991; Sivonen and Jones, 1999). Sivonen et al., (1990) reported a statistical association between neurotoxicity and *Anabaena lemmermanni*, *Anabaena flos-aquae* and *Gomphosphaeria naegeliana*. The same study also reported that *Anabaena* was the most common bloom-forming genus in toxic samples and was suspected to be the causative organism responsible in all cases of cattle poisoning during the study period. Infact *Anabaena flos-aquae* had been described as one of the most toxic strains of cyanobacteria (ARNAT). Neurotoxins are a broad group of heterocyclic nitrogenous compounds which cause death by respiratory arrest in mouse bioassay (Sivonen and Jones, 1999). As part of a study to determine cyanotoxins in cyanobacteria identified in drinking water reservoirs we cultured *Anabaena flos-aquae* strain CCAP 1403/13B and other cyanobacteria species in the laboratory to analyse the cyanotoxin microcystin. Microcystin is the most commonly reported cyanotoxin (Sivonen and Jones 1999) of which the World Health Organization had set up a permissible limit of 1.0 μg/l in drinking water (WHO 1998).

Materials and methods

Culture

The cultures were purchased from the Culture Collection of Algae and Protozoa (CCAP), Windermere Laboratory, United Kingdom. *Anabaena flos-aquae* strain CCAP 1403/13B was cultured in Jaworski medium (JM) (Thompson *et. al.*, 1988). The medium was prepared in the laboratory using recipes provided by CCAP. The cultures were cultivated in 2 litre conical flasks in a growth chamber with continuous irradiance from a cool white florescence tube with a photon flux density of 20 μmol photons/m^2/s and constant aeration. Temperature was kept at 25°C and humidity below 60% (Senogles-Derham *et al.*, 2003). *Anabaena flos-aquae* cells were harvested at the late exponential phase by centrifugation and cell material lyophilized by freeze drying and stored at - 20 °C.

Toxin extraction

Lyophilized cells of *Anabaena flos-aquae* (approximately 52 mg) were extracted using a sonicator in 1 ml 50% aqueous methanol in Eppendorf tubes using the ultrasonic probe for 30 sec. The resultant extract was centrifuged for 15 min at maximum allowed speed (RPM) and supernatant transferred into a vial. To clean the supernatant of particles prior to HPLC analysis, the supernatant was passed through a syringe-filter of 0.45 μm pore size.

High Performance Liquid Chromatography (HPLC) analysis

Toxin analysis of *Planktothrix* extract was achieved by using a reverse-phase high performance liquid chromatography system (Agilent 1100 series) equipped with a photodiode array detector (DAD). The HPLC column was a Supelcosil ABZ + plus with a stationary phase of 150 $\times$4.6 mm $\times$5 μm. Column temperature was kept at 30°C. The mobile phase used was constituted of: A: 0.1% trifluoroacetic acid in water and B: acetronitrile with 0.1% trifluoroacetic acid (Lawton et al., 1994), with a linear gradient from 20% of B at 0 min to 59% of B at 30 min, and a flow rate of 1 ml/min. The volume of extract injected was 10 μl. Resulting data were gathered between 200-300 nm and chromatograms were evaluated and shown on screen at 238 nm. Microcystin peaks were identified by comparison of retention times with those of standards used: MC-LR, -LF, -LW, -RR, -YR (Alexis Biochemicals) and nodularin (DHI Water and Environment), and their characteristic UV-spectra (Lawton et al., 1994). The toxic fractions were quantified by extrapolating the HPLC peak areas at 238 nm to a linear calibration curve for microcystin-LR standard ($n = 5, r^2 = 0.999$)

Results and Discussion

Anabaena flos-aquae is a widely studied strain of cyanobacteria (Mahmood and Carmichael, 1986, 1987; Krishnamurthy et al., 1986; Al-Layi et al., 1988; Matshunaga et al., 1989; Harada et al., 1991; Sivonen et. al., 1990; 1992; Namikoshi et al., 1992). Almost all these authors identified neurotoxins from *Anabaena flos aquae* as a major toxin with the exception of few. Harada et al., (1991), reported the production of a very potent neurotoxin, anatoxin-a from *Anabaeana flos-aquae* strain NRC 525-17 and three other toxic compounds with hepatotoxicity, one of which was identified as 3-desmethylmicrocystin LR. Sivonen et al., (1992), identified microcystin-LR, [D-Asp^3] microcystin-LR and microcystin-RR similar toxin identified in our current study of *Anabaena flos-aquae* strain CCAP 1403/13B. This cyanobacterium is known to be very toxic and had been implicated in numerous animal and human poisoning episodes (Ballot *et al.*, 2000; Krienitz *et al.*, 2003; Jochimsen *et al.*, 1998; Teixera *et al.*, 1993). Drinking water treatment processes in Ghana are very basic comprising mainly of alum flocculation, sedimentation, rapid sand filtration and chlorination. Such treatment trains have been described as conventional and inadequate for the removal of cyanotoxins (Keijola *et al.*, 1999; Lawton and Robertson, 1999). In order to determine the toxins associated with cyanobacteria in Ghanaian drinking water bodies *Anabaena flos-aquae* strain CCAP 1403/13B was cultured, harvested and lyophilized. HPLC analysis of the lyophilized cells of *Anabaena flos-aquae* strain CCAP 1460/13 extract showed a peak at the retention time of 12.642 min (Fig. 1) similar with the retention time of 12.607 min obtained from the external microcystin-RR standard used in the analysis (Fig. 2). Quantification of this fraction showed the production of 10.6 μg/g DW. The high similarity (over 99%) of retention time in our analysis to that of the standard suggests that the toxin produced by *Anabaena flos-aquae* strain CCAP 1460/13 is microcystin-RR. *Anabaena flos-aquae* are mainly known to produce microcystin-LR and neurotoxins, mainly anatoxin-a and anatoxin-a(s) and not microcystin-RR. Recent studies by Ballot *et al.*, (2005) have identified microcystin-RR from Lake Sonachi and Simbi, Kenya dominated by *Anabaena fusiformis* (over 94%) and *Anabaena fusiformis* and *Anabaena abijate* respectively.

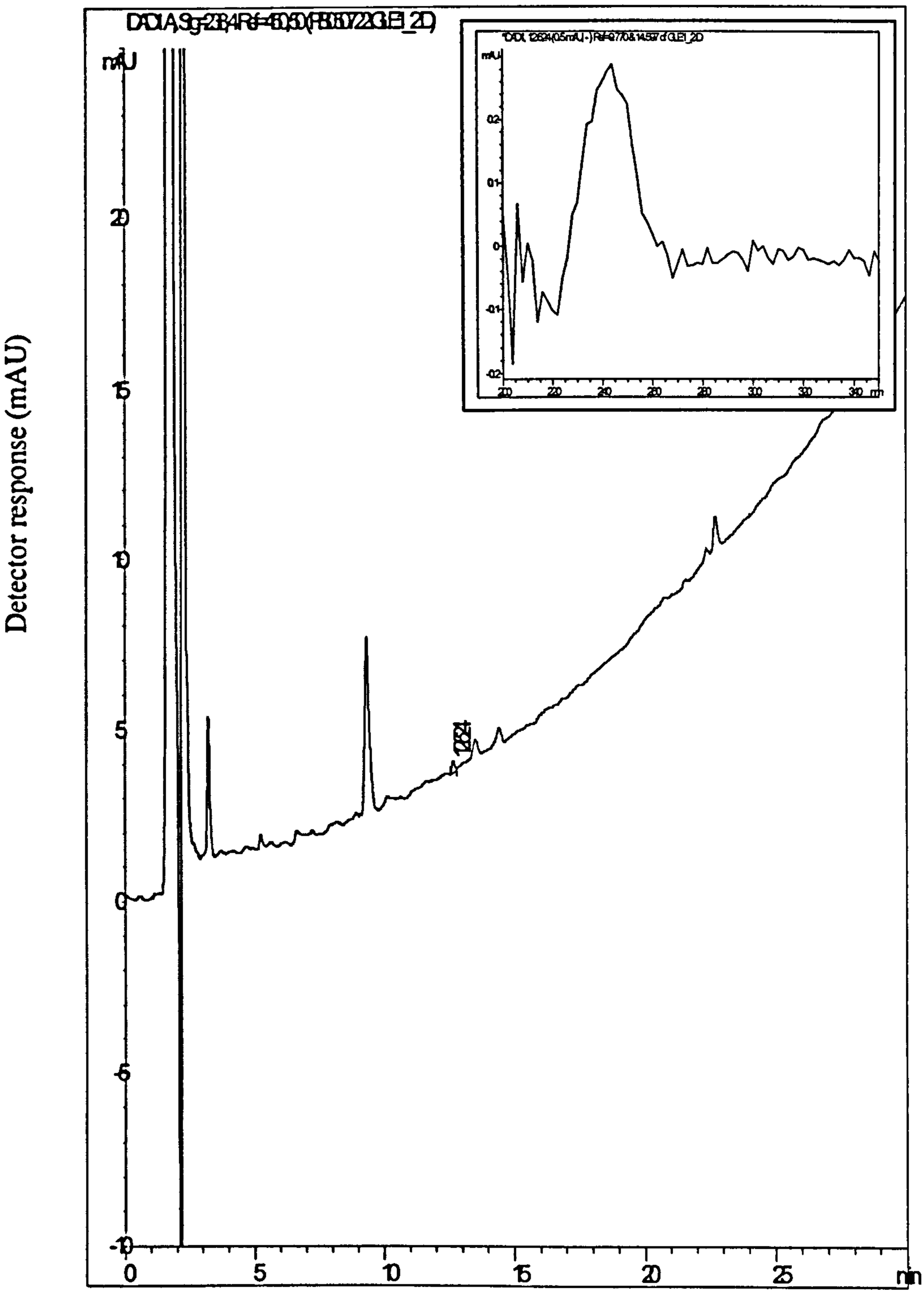

Figure 1 Chromatogram (HPLC) of the extract of *Anabaena flos-aquae* strain CCAP 1403/13B (biomass: 0.052 g) - Ref: 12.642 min, with maximum absorbance of 238-240 nm. Insert: Spectrum of microcystin-RR

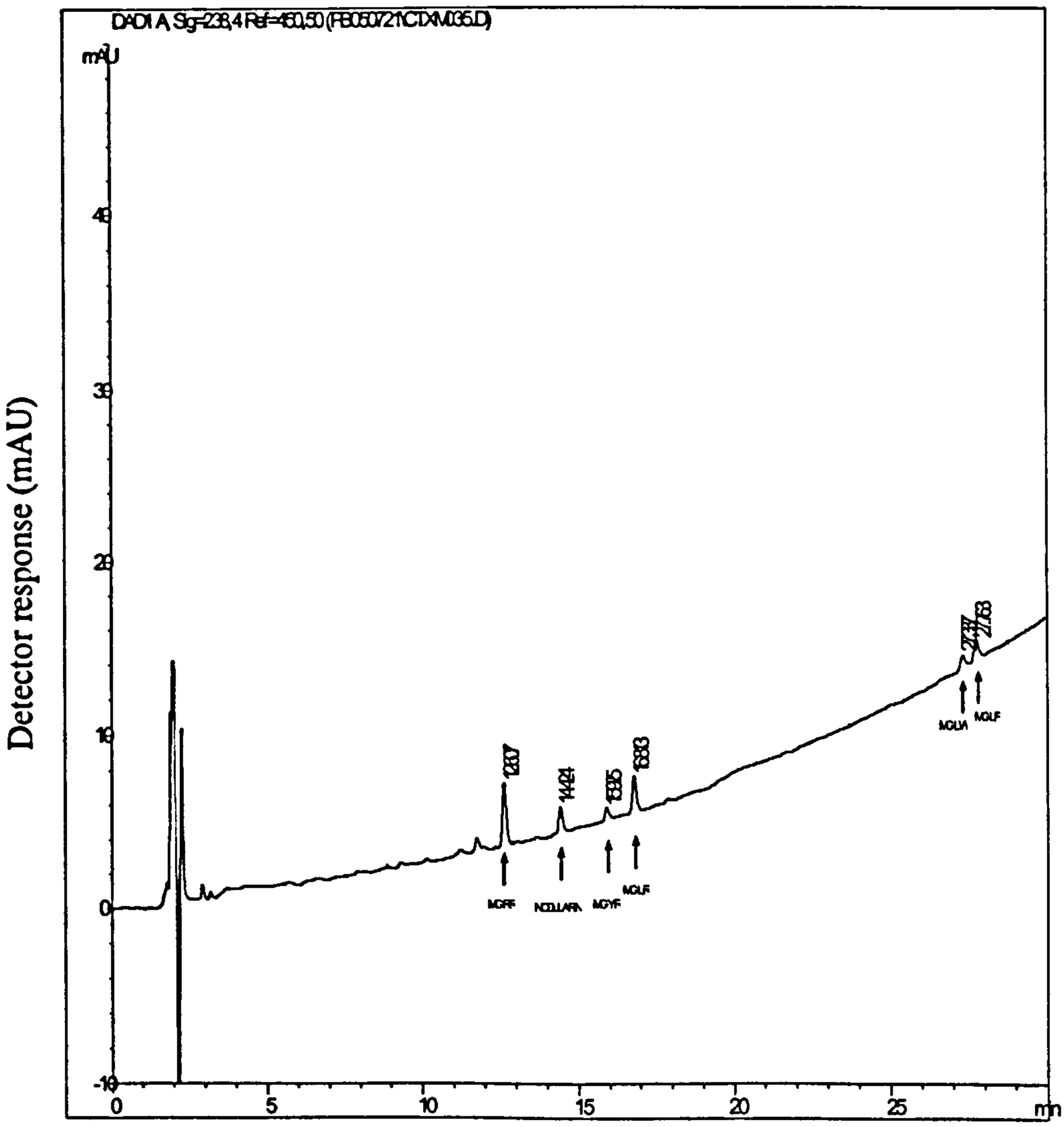

Figure 2 HPLC-UV chromatogramme of external standards of microcystins and nodularin used in analysis of *Anabaena flos-aquae* strain 1403/13B showing retention time of MC-RR at 12.607 min

Even though the quantity of microcystin-RR identified in our study is low, the mere fact that this toxin is not commonly produced by *Anabaena flos- aquae* makes the results an interesting contribution to the knowledge of cyanotoxin productivity. Experience obtained in this study will be applied to biomass and water samples collected from drinking water reservoirs in Ghana to determine the presence or other wise of microcystin and the efficiency of microcystin removal from different drinking water treatment plants in comparism with World Health Organization provisional guideline value of 1 μg/l microcystin-LR (WHO 1998).

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4- General Discussion

Cyanobacteria and their toxins have gained tremendous attention since the recognition in the eighteenth century of acute poisoning of domestic animals in Australia (Francis, 1978). Codd (1996) also reported that human awareness of toxic blooms existed in the twelfth century at the former Monasterium Virdis Stagni located near the eutrophic, freshwater Soulseat Loch near Stranraer in South West Scotland. As mentioned by Bartram et al., (1999) "a pet child has many names" and the organisms under discussion in this thesis, cyanobacteria, have been referred by many names: blue-greens; blue-green algae; myxophyceaens; cyanophyceans; cyanophytes; cyanobacteria; and cyanoprokaryotes (Wetzel, 1983; Bartram et al., 1999). This suggests the importance of these organisms as a subject to biologists and scientists. The unique combination of properties of both algae and bacteria which cyanobacteria exhibit have been a source of fascination and attraction for many scientists.

Incidents of acute illness attributed to cyanobacteria in water have been documented all over the world during the past century (Byth, 1980; Bourke et al. 1983; Hawkins et al. 1985, 1997; Teixera et al. 1993; Yu et al. 1995; Ueno et al. 1996; Zhou et al. 2000; Carmichael, 2001). However, information about cyanotoxin concentrations in drinking water supplies is scarce globally, and particularly in Africa. In Africa, Zilberg (1966) reported cases of human poisoning through exposure to microcystin in Harare, Zimbabwe. This was followed by Marshall (1991) who discovered, by a retrospective study, a close relationship between the number of gastrointestinal cases and the number of colonies of the cyanobacteria *Microcystis aeruginosa* and *Anabaena flos-aquae* in drinking water reservoirs. Johansson and Olsson (1998) have reported microcystin concentrations in Lake Chievero in Zimbabwe to be around 13.9 μg/l

while microcystin was also detected in the city's tap water. Recently Ndebele and Magadza (2006) obtained a microcystin concentration (MC-LR) of between 18.0 and 22.5 μg/l in cultured *Microcystis aeruginosa* cells from the same lake. Nasri et al., (2004) also reported the dominance of cyanobacteria, principally *Microcystis* sp. in the source water of Lake Oubeira which has been used for the production of drinking water for many communities in the East of Algeria.

Annadotter et. al., (2005) reported endotoxins from cyanobacteria in drinking water reservoirs in Harare, Zimbabwe. Cyanobacteria cells and elevated levels of endotoxins were detected in tap water when human subjects reported symptoms. Symptoms such as fever, malaise, muscle pains, tightness of the chest and respiratory- tract symptoms known as toxic pneumonitis occurred within 1.5 to 6 hours after a bath or shower. In a field study of 21 water bodies, the concentration of endotoxins was higher in cyanobacteria-dominated water bodies than in those dominated by eukaryotic algae. Lastly, furthermore, Mhlanga et al., (2006) have also reported cyanobacteria and cyanotoxins from Lake Chivero Zimbabwe and the presence of cyanotoxins in drinking water from the lake. These documented reports of cyanobacteria and their toxins in drinking water largely come from the East Africa. The rest of Africa including West Africa lacks an equivalent literature and therefore my study is an important source of reference for West Africa. The first paper in this thesis highlights the deficiencies in the drinking-water treatment plants of the Weija and Kpong water supply reservoirs in eliminating cyanobacteria cells in the final drinking water. The second paper reports the dominance of potentially toxic picocyanobacteria like *Aphanocapsa nubilum* and *Cyanogranis ferruginea* and also large cyanobacteria such as *Microcystis* spp. and *Cylindrospermopsis raciborskii* in the Weija, Barekese and the Owabi reservoirs, all with basic conventional water treatment facilities shown

to be ineffective in that they remove neither cyanobacteria nor their toxins so leading to a high risk to human health through exposure to cyanotoxins such as microcystins and cylindrospermopsin. The third paper reports the identification of microcystins LR, RR, YR and LF in drinking-water reservoirs and dissolved toxins in final drinking water, for the first time in Ghana and probably the whole of this sub region of Africa. Dissolved microcystin concentrations of 0.81μg/l and 0.57 μg/l were obtained in the final drinking water stage of the Weija and Kpong reservoirs. Even though these values are lower than the WHO recommended safe level of 1.0 μg/l for daily intake, they are environmentally significant and present a health risk to the population. Furthermore, more people in Ghana drink from open water bodies without any treatment than have access to treated piped water. Hoeger et al. (2005) cautioned that, due to tumour promoting activities of microcystin, chronic exposure of human populations to concentrations below 0.1 μg/l should be avoided. The current situation calls for close monitoring rather immediate alarm. The fourth and fifth chapters support the fact that it is not all species of cyanobacteria that produces toxins. Five known toxin producing species were cultured during the study; *Anabaena flos-aquae* (CCAP 1403/13B), *Microcystis aeruginosa* (CCAP 1450/3), *Oscillatoria limnetica* (CCAP 1459/18), *Oscillatoria agardhii* (CCAP 1459/23), *Planktothrix* sp. (CCAP 1460/13), *Lyngbya* spp (CCAP 1446/10). Only two of these species, *Planktothrix* sp (CCAP 1460/13 and *Anabaena flos-aquae* (CCAP 1403/13B) were found to produce toxins in culture. This part of the study, even though not part of its primary objectives, gave interesting results as presented in paper IV and gave an insight into the biology of cyanobacteria cultures.

Much literature about the management of drinking-water reservoirs with cyanobacteria blooms has been reviewed in the introduction of this thesis, and is

not repeated here, but so far as Ghana is concerned the way forward is the monitoring of cyanobacteria cells and toxins and integrated watershed management to reduce nutrient inputs into reservoirs. Drinking water treatment that is specifically focussed on cyanotoxins will be difficult in the light of the economic situation, as this requires intensive capital for technology and finance. Ensuring complete removal of intact cyanobacteria cells during the early stages of drinking water production, followed by effective chlorination is the way forward. Prechlorination which is the current practice in the Kpong reservoir must be discouraged because this tends to release intracellular toxins. The Barekese reservoir is currently the best of the four reservoirs studied. It had no detectable dissolved microcystin and only four intracellular samples were microcystins positive.

5- Conclusions and final considerations

A number of conclusions have been drawn in each of the five papers that make up this thesis. For final consideration I will suggest that public awareness, through communication and public participation, is the best tool to reduce the impact of cyanobacteria and their toxins on public health in Ghana. An understanding and acceptance that cyanobacterial blooms and toxins present potential hazards to human and animal health is a prerequisite for anticipating, avoiding or reducing their adverse effects. Early recognition of blooms and scums through public observation facilitates better management of blooms and associated problems and helps to reduce their impact on the community as a whole. Another form of awareness is to inform professionals, such as health workers, of the circumstances and exposure routes that leads to intoxications, and finally to inform government officials, environmental regulators, water users and water supply organizations about the potential hazards of cyanobacteria toxins and to help identify and implement appropriate policies on water access, use, consumption and treatment.

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Appendix

Appendix 1 Recipe for preparing BG11 medium

BG11

Stocks

per litre		
(1)	NaNO3	15.0 g
		per 500 ml
(2)	K2HPO4	2.0 g
(3)	MgSO4.7H2O	3.75 g
(4)	CaCl2.2H2O	1.80 g
(5)	Citric acid	0.30 g
(6)	Ammonium ferric citrate green	0.30 g
(7)	EDTANa2	0.05 g
(8)	Na2CO3	1.00 g
(9)	Trace metal solution	per litre
	H3BO3	2.86 g
	MnCl2.4H2O	1.81 g
	ZnSO4.7H2O	0.22 g
	Na2MoO4.2H2O	0.39 g
	CuSO4.5H2O	0.08 g
	Co(NO3)2.6H2O	0.05 g

Medium	Stock solution 1	100.ml
	Stock solutions 2 - 8 each	10.0 ml
	Stock solution 9	1.0 ml

Make up to 1 litre with deionised water. Adjust pH to 7.1 with 1M NaOH or HCl. For agar add *15.0 g per litre of* *Bacteriological Agar (Oxoid L11). Autoclave at 15 psi for 15 minutes.

Supply * Unipath Ltd, Wade Road, Basingstoke, Hants RG24 0PW, UK

Ref Stanier et al, (1971)

Appendix 2 Recipe for preparing Jaworski's medium (JM)

Stocks		per 200 ml
(1)	Ca (NO3)2.4H2O	4.0 g
(2)	KH2PO4	2.48 g
(3)	MgSO4.7H2O	10.0 g
(4)	NaHCO3	3.18 g
(5)	EDTAFeNa	0.45 g
EDTANa2		0.45 g
(6)	H3BO3	0.496 g
	MnCl2.4H2O	0.278 g
	(NH4)6Mo7O24.4H2O	0.20 g
(7)	Cyanocobalamin	0.008 g
	Thiamine HCl	0.008 g
	Biotin	0.008 g
8)	NaNO3	16.0 g
(9)	Na2HPO4.12H2O	7.2 g

Medium	per litre
Stock solutions 1 - 9	1 ml each

Make up to 1 litre with deionised water. For agar, add 15.0 g per litre of *Bacteriological Agar (Oxoid L11). Autoclave at 15 psi for 15 minutes.

Supply * Unipath Ltd, Wade Road, Basingstoke, Hants RG24 0PW, UK

Appenix 3 Table of correlation analysis between cyanobacteria cell counts/ml nutrients and rainfall

Weija reservoir

	NO_2-N	NO_3-N	PO_4-P	*Cyanobacteria Species*
NO_2-N	1.0000			
NO_3-N	-0.4192	1.0000		
PO_4-P	0.5759	-0.5349	1.0000	
Cyanobacteria cell counts/ml	0.9997	-0.4269	0.5791	1.0000
Rainfall	0.1227	-0.5441	0.7151	0.1162

Kpong reservoir

	NO_2-N	NO_3-N	PO_4-P	*Cyanobacteria Species*
NO_2-N	1.0000			
NO_3-N	-0.2447	1.0000		
PO_4-P	-0.5167	0.2600	1.0000	
Cyanobacteria cell counts/ml	0.3743	0.6286	-0.4769	1.0000
Rainfall	0.0604	0.3787	-0.5704	0.8285

Owabi reservoir

	NO_2-N	NO_3-N	PO_4-P	*Cyanobacteria Species*
NO_2-N	1.0000			
NO_3-N	0.9084	1.0000		
PO_4-P	0.9130	0.7594	1.0000	
Cyanobacteria cell counts/m	0.8479	0.9287	0.8424	1.0000
Rainfall	0.7909	0.6541	0.6625	0.4706

Barekese reservoir

	NO_2-N	NO_3-N	PO_4-P	*Cyanobacteria Species*
NO_2-N	1.0000			
NO_3-N	-0.2557	1.0000		
PO_4-P	-0.0451	-0.2769	1.0000	
Cyanobacteria cell counts/m	-0.3008	0.5380	0.2943	1
Rainfall	0.6837	-0.3538	-0.4799	-0.8543

Appendix 4 Check-List of cyanobacteria species identified in the four drinking water reservoirs studied in Ghana

Cyanobacteria species	Weija	Kpong	Owabi	Barekese
Anabaena austro-africana	+			+
Anabaena nygaardii	+		+	+
Anabaenopsis tanganyikae	+			
Anabaenopsis ambigua	+			
Aphanocapsa nubilum	+****			
Aphanocapsa holsatica	+		+	
Chroococcus cronbergae	+	+	+	+
Coelomoron tropicalis	+	+		
Cyanogranis ferruginea	+		+****	+****
Cylindrospermopsis raciborskii	+	+	+	+
Cylindrospermopsis cuspis	+	+		
Geitlerinema unigranulatum	+	+****		
Lyngbya sp	+			
Leptolyngbya sp			+	
Microcystis aeruginosa	+			+
Microcystis wesenbergii	+			
Microcystis viridis	+			
Merismopedia tenuissima	+	+	+	+
Merismopedia punctata	+	+		+
Oscillatoria princeps				+
Planktolyngbya limnetica	+		+	
Planktolyngbya minor	+	+	+	+
Planktolyngbya circumcreta	+			
Planktothrix agardhii	+	+		
Planktothrix spp				+
Planktothrix lacustris var. solitaria	+			+
Pseudanabaena recta	+	+	+	+
Radiocystis fernandoi	+			+
Romeria elegans	+			+

Key: + Species present in reservoir

**** Dominant species

Appenix 5a Table showing intracellular microcystin concentration obtained in the Barekese reservoir studied in the various water treatment stages

Reservoir	Station	Date	Microcystins variants	microcystin concentration (µg/l)
Barekese	Intake	10-Jan-05	0	0.00
Barekese	Flocculation	10-Jan-05	0	0.00
Barekese	Sedimentation	10-Jan-05	0	0.00
Barekese	Filtered	10-Jan-05	0	0.00
Barekese	Final	10-Jan-05	0	0.00
Barekese	Intake	10-Feb-05	0	0.00
Barekese	Sedimentation	10-Feb-05	1	1.65
Barekese	Final	10-Feb-05	1	0.45
Barekese	Flocculation	17-Mar-05	0	0.00
Barekese	Sedimentation	17-Mar-05	0	0.00
Barekese	Filtered	17-Mar-05	0	0.00
Barekese	Final	17-Mar-05	0	0.00
Barekese	Intake	12-Apr-05	1	0.46
Barekese	Flocculation	12-Apr-05	1	15.50
Barekese	Sedimentation	12-Apr-05	0	0.00
Barekese	Filtered	12-Apr-05	0	0.00
Barekese	Final	12-Apr-05	0	0.00
Barekese	Intake	26-May-05	0	0.00
Barekese	Flocculation	26-May-05	0	0.00
Barekese	Sedimentation	26-May-05	0	0.00
Barekese	Filtered	26-May-05	0	0.00
Barekese	Final	26-May-05	0	0.00
Barekese	Intake	07-Jun-05	0	0.00
Barekese	Flocculation	07-Jun-00	0	0.00
Barekese	Sedimentation	07-Jun-05	0	0.00
Barekese	Filtered	07-Jun-05	0	0.00
Barekese	Final	07-Jun-05	0	0.00

Appenix 5b Table showing intracellular microcystin concentration obtained in the Kpong reservoir studied in the various drinking water treatment stages

Reservoir	Station	Date	Microcystins variants	microcystin concentration (µg/l)
Kpong	Intake	13-Jan-05	0	0.00
Kpong	Sedimentation	13-Jan-05	0	0.00
Kpong	Filtered	13-Jan-05	0	0.00
Kpong	Final	13-Jan-05	0	0.00
Kpong	Intake	27-Jan-05	0	0.00
Kpong	Sedimentation	27-Jan-05	0	0.00
Kpong	Filtered	27-Jan-05	0	0.00
Kpong	Intake	09-Feb-05	0	0.00
Kpong	Filtered	09-Feb-05	1	0.12
Kpong	Final	09-Feb-05	0	0.00
Kpong	Intake	10-Mar-05	0	0.00
Kpong	Sedimentation	10-Mar-05	0	0.00
Kpong	Filtered	10-Mar-05	1	0.05
Kpong	Final	10-Mar-05	1	0.13
Kpong	Final	24-Mar-05	0	0.00
Kpong	Intake	08-Apr-05	1	0.43
Kpong	Sedimentation	08-Apr-05	2	0.88
Kpong	Filtered	08-Apr-05	0	0.00
Kpong	Final	08-Apr-05	3	0.46
Kpong	Intake	04-May-05	1	4.69
Kpong	Final	04-May-05	0	0.00
Kpong	Intake	18-May-05	0	0.00
Kpong	Sedimentation	18-May-05	0	0.00
Kpong	Filtered	18-May-05	0	0.00
Kpong	Final	18-May-05	0	0.00
Kpong	Sedimentation	10-Jun-05	0	0.00
Kpong	Filtered	10-Jun-05	0	0.00
Kpong	Final	10-Jun-05	0	0.00

Appenix 5c Table showing intracellular microcystin concentration obtained in the Owabi reservoir studied in the various drinking water treatment stages

Reservoir	Station	Date	Microcystins variants	microcystin concentration (µg/l)
Owabi	Flocculation	10-Jan-05	1	0.96
Owabi	Sedimentation	10-Jan-05	1	0.10
Owabi	Filtered	10-Jan-05	0	0.00
Owabi	Intake	10-Feb-05	1	8.73
Owabi	Flocculation	10-Feb-05	1	6.24
Owabi	Sedimentation	10-Feb-05	1	4.02
Owabi	Filtered	10-Feb-05	1	0.38
Owabi	Final	10-Feb-05	0	0.00
Owabi	Intake	17-Mar-05	1	0.16
Owabi	Flocculation	17-Mar-05	0	0.00
Owabi	Sedimentation	17-Mar-05	1	0.13
Owabi	Filtered	17-Mar-05	0	0.00
Owabi	Final	17-Mar-05	1	0.07
Owabi	Flocculation	12-Apr-05	2	0.09
Owabi	Filtered	12-Apr-05	2	0.45
Owabi	Final	12-Apr-05	0	0.00
Owabi	Intake	26-May-05	2	1.23
Owabi	Flocculation	26-May-05	0	0.00
Owabi	Filtered	26-May-05	0	0.00
Owabi	Final	26-May-05	0	0.00
Owabi	Intake	07-Jun-05	1	0.08
Owabi	Sedimentation	07-Jun-05	0	0.00
Owabi	Filtered	07-Jun-05	0	0.00
Owabi	Final	07-Jun-05	0	0.00

Appenix 5d Table showing intracellular microcystin concentration obtained in the Weija reservoir studied in the various drinking water treatment stages

Reservoir	Station	Date	Microcystins variants	microcystin concentration (µg/l)
Weija	Intake	01-Jan-05	0	0.00
Weija	Filtered	01-Jan-05	0	0.00
Weija	Final	01-Jan-05	0	0.00
Weija	Intake	24-Jan-05	3	1.97
Weija	Flocculation	24-Jan-05	3	2.58
Weija	Sedimentation	24-Jan-05	0	0.00
Weija	Filtered	24-Jan-05	0	0.00
Weija	Final	24-Jan-05	0	0.00
Weija	Intake	09-Feb-05	0	0.00
Weija	Flocculation	09-Feb-05	2	0.31
Weija	Sedimentation	09-Feb-05	0	0.00
Weija	Filtered	09-Feb-05	0	0.00
Weija	Final	09-Feb-05	1	0.61
Weija	Intake	28-Feb-05	3	0.88
Weija	Flocculation	28-Feb-05	2	0.68
Weija	Sedimentation	28-Feb-05	0	0.00
Weija	Filtered	28-Feb-05	0	0.00
Weija	Intake	02-Mar-05	4	4.79
Weija	Flocculation	02-Mar-05	5	2.38
Weija	Sedimentation	02-Mar-05	1	0.34
Weija	Final	02-Mar-05	0	0.00
Weija	Intake	24-Mar-05	3	0.76
Weija	Flocculation	24-Mar-05	4	5.30
Weija	Sedimentation	24-Mar-05	1	0.88
Weija	Filtered	24-Mar-05	2	0.36
Weija	Final	24-Mar-05	0	0.00
Weija	Intake	06-Apr-05	1	0.25
Weija	Flocculation	06-Apr-05	0	0.00
Weija	Final	06-Apr-05	0	0.00
Weija	Intake	13-Apr-05	1	8.56
Weija	Flocculation	13-Apr-05	0	0.00
Weija	Sedimentation	13-Apr-05	0	0.00
Weija	Filtered	13-Apr-05	0	0.00
Weija	Final	13-Apr-05	0	0.00
Weija	Intake	17-May-05	1	3.99
Weija	Flocculation	17-May-05	1	10.92
Weija	Sedimentation	17-May-05	1	3.53
Weija	Filtered	17-May-05	0	0.00
Weija	Intake	28-May-05	2	0.64
Weija	Flocculation	28-May-05	1	3.53
Weija	Sedimentation	28/05/2005	1	0.44
Weija	Filtered	28/05/2005	0	0.00
Weija	Final	28/05/2005	0	0.00
Weija	Intake	04-Jun-05	1	0.28
Weija	Flocculation	04-Jun-05	1	0.32
Weija	Sedimentation	04-Jun-05	0	0.00
Weija	Filtered	04-Jun-05	0	0.00
Weija	Final	04-Jun-05	0	0.00
Weija	Intake	14-Jun-05	2	0.64
Weija	Flocculation	14-Jun-05	1	0.66
Weija	Final	14-Jun-05	0	0.00

Appenix 6a Table showing dissolved microcystin concentration obtained in the Weija reservoir studied in the various water treatment stages

Reservoir	Station	Date	Microcystins variants	microcystin concentration (µg/l)
Weija	Intake	01-Jan-05	0	0
Weija	Flocculation	01-Jan-05	1	0.67
Weija	Sedimentation	01-Jan-05	1	0.01
Weija	Filtered	01-Jan-05	0	0
Weija	Final	01-Jan-05	0	0
Weija	Intake	24-Jan-05	1	0.03
Weija	flocculation	24-Jan-05	1	0.52
Weija	Filtered	24-Jan-05	0	0
Weija	Final	24-Jan-05	1	0.81
Weija	Flocculation	09-Feb-05	0	0
Weija	Filtration	09-Feb-05	0	0
Weija	Final	09-Feb-05	0	0
Weija	Intake	24-Mar-05	0	0
Weija	Flocculation	24-Mar-05	0	0
Weija	Sedimentation	24-Mar-05	2	1.82
Weija	Sedimentation	06-Apr-05	0	0
Weija	Flocculation	06-Apr-05	0	0

Appenix 6b Table showing dissolved microcystin concentration obtained in the Owabi reservoir studied in the various water treatment stages

Reservoir	Station	Date	Microcystins variants	microcystin concentration (µg/l)
Owabi	Flocculation	10-Jan-05	1	0.87
Owabi	Sedimentation	10-Jan-05	1	0.32
Owabi	Final	10-Jan-05	0	0
Owabi	Final	10-Feb-05	0	0
Owabi	Intake	17-Mar-05	1	0.6
Owabi	flocculation	17-Mar-05	1	0.29
Owabi	Sedimentation	17-Mar-05	1	0.09
Owabi	Filtration	17-Mar-05	1	0.16
Owabi	Final	17-Mar-05	0	0
Owabi	Filtration	12-Apr-05	1	0.89
Owabi	Filtration	26-Mar-05	0	0
Owabi	Final	26-Mar-05	0	0

Appenix 6c Table showing dissolved microcystin concentration obtained in the Kpong reservoir studied in the various water treatment stages

Reservoir	Station	Date	Microcystins variants	microcystin concentration (µg/l)
Kpong	Intake	13-Jan-05	0	0
Kpong	Sedimentation	13-Jan-05	1	0.8
Kpong	Filtered	13-Jan-05	0	0
Kpong	Final	13-Jan-05	0	0
Kpong	Intake	27-Jan-05	0	0
Kpong	Sedimentation	27-Jan-05	0	0
Kpong	filtration	27-Jan-05	0	0
Kpong	filtration	10-Feb-05	1	0.2
Kpong	Intake	10-Mar-05	0	0
Kpong	Sedimentation	10-Mar-05	1	0.92
Kpong	Filtered	10-Mar-05	0	0
Kpong	Final	10-Mar-05	0	0
Kpong	Intake	08-Apr-05	0	0
Kpong	Sedimentation	08-Apr-05	0	0
Kpong	Intake	04-Mar-05	1	0.10
Kpong	Sedimentation	04-Mar-05	1	0.15
Kpong	Final	04-Mar-05	1	0.57

Appenix 6d Table showing dissolved microcystin concentration obtained in the Barekese reservoir studied in the various water treatment stages

Reservoir	Station	Date	Microcystins variants	microcystin concentration (µg/l)
Barekese	Intake	10-Jan-05	0	0
Barekese	Flocculation	10-Jan-05	0	0
Barekese	Sedimentation	10-Jan-05	0	0
Barekese	Filtration	10-Jan-05	0	0
Barekese	Final	10-Jan-05	0	0
Barekese	Filtration	10-Feb-05	0	0
Barekese	Intake	17-Mar-05	0	0
Barekese	Flocculation	17-Mar-05	0	0
Barekese	Sedimentation	17-Mar-05	0	0
Barekese	Filtered	17-Mar-05	0	0
Barekese	Final	17-Mar-05	0	0