African Journal of Biotechnology Vol. 10(86), pp. 19883-19889, 30 December, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJBX11.024
ISSN 1684–5315

Review

Evaluation of a low cost technology to manage algal toxins in rural water supplies

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Accepted 25 November, 2011

South Africa is a water scarce country with freshwater resources that are deteriorating mostly due to anthropogenic activities. Several dams in South Africa are eutrophic and present potential health risks to water consumers and users. Cyanobacteria (blue-green algae) are known to produce toxins that present a threat to human health and wildlife. In this review, a low technology method that can be applied to the management of rural water supplies that are contaminated with algal toxins such as microcystins is examined. The method uses aquatic macrophytes. The bioaccumulation potential of some aquatic macrophytes (the 'Green liver' concept) has commonly been applied in the phytoremediation of polluted water bodies. The use of aquatic macrophytes in the *in-situ* bioremediation of algal toxins can offer numerous advantages, among them; the ability to treat large areas and low costs. The main objective of this review was to assess the feasibilty of using selected species of naturally occurring aquatic macrophytes and their effectiveness in cyanotoxin elimination by using their bioaccumulation potential from raw surface water collected from rivers in Limpopo province, South Africa for the *in-situ* bioremediation of the polluted water.

Key words: microcystins, bio-accumulation, in-situ bioremediation, aquatic macrophytes, Green liver concept

INTRODUCTION

Poisoning of livestock by toxic cyanobacteria was first reported in the 19th century, and throughout the 20th century, cyanobacteria related poisonings of livestock and wildlife in all continents have been described (Stewart et al., 2008). In South Africa and other parts of the world, livestock, water fowl, wildlife and game animals have died after drinking water containing heavy blooms of blue-green algae (Masango et al., 2008). Incidents of fatal cyanobacterial poisoning in South African reservoirs are widespread and occur almost every year, but to date these poisonings have been limited to death of livestock, domestic animals and wildlife and no human fatalities have been recorded (Oberholster and Ashton, 2008).

Cases of cyanotoxin poisoning in South Africa have mainly been described from the Gauteng, Mpumalanga, Free State and Western Cape provinces (Oberholster et al., 2005). Estimated values of approximately 4.74 tonne/km^3 for Chemical Oxygen Demand and 0.73 mg/L of orthophosphate, indicate that South African freshwater resources are excessively enriched and are considered to be moderately to highly eutrophic (Oberholster and Ashton, 2008). Cyanobacterial blooms have been recorded in many, if not most of the river and reservoir systems in South Africa, because of prevailing high levels of eutrophication. This is mainly attributed to the inadequate treatment of domestic and industrial effluents that are discharged in their catchments (Du Preez and Van Baalen, 2006). The genera *Microcystis* and *Anabaena*

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are now occuring on regular basis in inland waters of South Africa (Oberholster et al., 2009a). These cyanobacteria have been implicated in the production of mcrocystins and taste and odour compounds, namely, geosmin (GSM) and 2-methylisoborneol (MIB) (Wnorowski, 1992).

In South Africa, most of the drinking water that is supplied to communities is obtained from surface water sources (rivers and reservoirs), though groundwater is important as a source in arid areas (Oberholster and Ashton, 2008). In South Africa as in many parts of the world, microcystins are a serious concern to drinking water providers both from a health and economic perspective (Gumbo et al., 2008). This is because the toxins produced by cyanobacteria are not removed by conventional water treatment processes such as flocculation, sedimentation, sand filtration and chlorination (Oberholster et al., 2005; Van Apeldoorn et al., 2007). Treatment processes that include potassium permanganate or chlorine, may release the toxins from the cyanobacteria, which may therefore reach people through water supplies (Van Apeldoorn et al., 2007). These toxins are also stable in natural and finished waters, which make them, pose a considerable threat to public health (USEPA, 2001).

Epidemiological studies in China, have linked high incidence of colorectal and liver cancer to water supplies from well, tap, river and pond drinking water with microcystin concentration within the range of 0.09 to 0.46 µg/L (Oberholster and Ashton, 2008; Oberholster et al., 2009a). A study by Fosso-Kankeu et al. (2008) showed that rural communities may be exposed to toxic microcystins at low levels but over a longer period of time. It can be postulated that chronic exposure to low levels of cyanotoxins by people that live in rural areas, and who have compromised or suppressed immune systems due to HIV/AIDS, and possibly also suffer from other communicable and poverty-related diseases such as Tuberculosis, may experience serious social and economic consequences as a result of cyanotoxins (Oberholster and Ashton, 2008).

Since water treatment plants in rural areas are seldom able to produce water of acceptable quality for domestic consumption (Momba et al., 2004), and conventional water treatment technologies do not remove algal toxins (Lawton et al., 1999); carbon filtration and other forms of tertiary treatment are required (Oberholster and Ashton, 2008). These are generally known to raise the cost for water treatment and there is therefore the need for cost-effective, environmental friendly counter-measures.

CHALLENGES TO DRINKING WATER UTILITIES

One of the primary objectives of a drinking water supplier is to provide water that is safe for human consumption. According to Du Preez and Van Baalen (2006), safe drinking water is defined as ‘drinking water that does not present any significant risk to health over a lifetime of consumption, including different sensitivities that may occur between life stages.’ Due to the increasing evidence of the potential of human health effects after consuming drinking water that contains cyanotoxins, WHO issued a provisional guideline for microcystins-LR (microcystins-LR: 1 µg/l), which is the most toxic variant of microcystins (Du Preez and Van Baalen, 2006).

When cyanobacterial proliferation cannot be prevented, other barriers against human exposure are necessary (IARC, 2010). It is important to stress that dissolved cyanotoxins (dissolved organic molecules) are much more difficult and expensive to remove than the cyanotoxins within the cyanobacteria, as the cyanobacteria themselves can be effectively removed if the treatment process is optimised. It is therefore much more cost-effective and beneficial to remove cyanobacterial cells “intact” (that is, with their cyanotoxins still inside the cells) from the source water than to risk breaking the cells, thereby releasing the cyanotoxins (Du Preez and Van Baalen, 2006).

For drinking water supplies, well designed and optimised water treatment processes are the final option for the removal of cyanobacterial cells and cyanotoxins (Queensland Health, 2001). Management approaches aimed at providing safe drinking water from cyanobacteria-infested surface waters require considering the system as a whole, and using different combinations of resource management tailored to the specific locality and different treatment steps (Queensland Health, 2001). The cyanotoxins, microcystins are soluble in water and are relatively stable compounds, possibly as a result of their cyclic structure, therefore not surprising that there are not readily removed from drinking water by conventional treatment methods (Lawton and Robertson, 1999).

Several treatment options for cyanobacterial cells and dissolved toxins have been developed and adopted by waterworks in different countries and these include: slow sand filtration; coagulation; activated carbon; membrane filtration, chlorine, and ozonation (Nimptsch et al., 2008). In summary, removal of cyanobacterial cells and toxins can be broadly categorized into filtration, adsorption and oxidation (Sivonen and Jones, 1999; Queensland Health, 2001). Activated carbon; chemical oxidation; nano- and Reverse Osmosis (RO) filtration are known to be effective in removing these biotoxins, but most of these processes are very expensive and the concentrated waste stream would be extremely toxic (USEPA, 2001). In South Africa only a few water treatment plants are equipped with granular activated carbon systems, the rest make use of conventional water treatment practices that remove live cyanobacterial cells and debris but not biotoxins in solution (Oberholster et al., 2005). In rural areas the choice of water supply may be limited, depending on the stage of development of the country and in urban areas the reticulated drinking water maybe of doubtful quality. Thus the potential for injury from cyanobacteria toxins in water supplies will to some extent depend on the level of development of the country and to some extent on the socio-economic status of the family (Oberholster et al., 2005).

Since water treatment plants in rural areas are seldom able to produce water of acceptable quality for domestic consumption (Momba et al., 2004), and conventional water treatment technologies do not remove algal toxins (Lawton and Robertson, 1999); carbon filtration and other forms of tertiary treatment are required (Oberholster and Ashton, 2008). These are generally known to raise the cost for water treatment and there is therefore the need for cost-effective, environmental friendly counter-measures. The main objective of this review is to evaluate whether the cyanotoxin bioaccumulation potential of selected species of naturally occurring aquatic macrophytes can be used for the in-situ bioremediation of cyanotoxin-polluted raw surface water collected from Luvuvhu River in Limpopo Province, South Africa.

With appropriate water treatment, maximum exposure to total microcystins is probably less than 1 µg/L and average exposure generally would probably be well below this level. Not all water supplies, however, are treated by filtration or adsorption; many are untreated or simply chlorinated (WHO, 2003). According to Nimptsch et al. (2008), management and treatment of cyanobacteria and their toxins generally raise costs within the drinking water production processes. Being acceptable for developed countries, this might be a problem in countries with limited economic and technological resources. There is therefore the need for alternative, effective, and affordable water treatment options. This review will evaluate a low cost water treatment method that may be applied to water supplies that may be contaminated with cyanobacterial toxins. The methods makes use of the bioaccumulation potential of some naturally occurring aquatic plants (macrophytes).

MOTIVATION

Although, humans do not consume cyanobacteria, they may be regularly exposed to sub-lethal dosages of cyanobacteria toxins in potable water derived from contaminated dams and reservoirs (Lam et al., 1995). In Australia, elevated concentrations of microcystins were linked epidemiologically to an outbreak of human hepatoenteritis (Falconer et al., 1983). The potential damage to human health exists at the Luvuvhu River catchment as indicated by communities drawing water at Nandoni dam for various use. Since Microcystins are heat stable (Harada et al., 1996), and are not degraded by boiling, a process which is part of cooking food.

Within the Luvuvhu River catchment, due to water scarcity, most of the communities use the river water for domestic purposes (including drinking and bathing) thus exposing them to serious long term health risks if the water is contaminated with cyanotoxins.

Microcystins are known to bioaccumulate in the liver and cause liver damage. Since it is estimated that only 21% of South African households have access to piped water inside their houses (DWAF, 2004), the deemed health risk to humans is via long-term chronic exposure to low levels of cyanotoxins in water used for drinking and domestic uses. Long-term chronic exposure to low levels of cyanotoxins remains a possibility in areas that receive reliable supplies of treated drinking water since conventional water treatment processes are ineffective (not exceeding 11 to 18%) at removing cyanobacterial toxins (Oberholster and Ashton, 2008).

An important aspect that influences the toxicity of cyanobacterial blooms is the age of the victim that ingests water containing cyanobacteria. Children are more vulnerable for several reasons: they drink more water per unit of body weight; they are less likely to be able to make an informed choice of the source of their drinking water; and they are more susceptible to physiological damage that can take a considerable period of time to develop, such as environmentally induced carcinomas (Oberholster and Ashton, 2008).

The Shingwedzi and Letaba Rivers flow into the Olifants River, which is a tributary of the Limpopo. Recently a number of crocodiles have died along the Olifants River in the Kruger National Park. Their deaths and possibly deaths of other animals have been linked to the presence of cyanobacteria (Oberholster et al. 2009b).

Data from the Department of Water Affairs and Forestry (DWAF), National Eutrophication Monitoring Programme (NEMP), (2009) on impoundments in Limpopo Province, reveals the existence of various species of cyanobacteria in most of the major impoundments in the Province, presence of which varies with seasons. Some of the toxic species whose presence was confirmed include *Microcystis* and *Anabaena* which are the most common species implicated to the release of microcystins and *Cylindrospermopsis* which produces the hepatotoxic alkaloid cylindrospermopsin. A study by Makhera et al. (2011), detected microcystin-LR levels of 2 µg/l at one of the sampling sites located along Luvuvhu River, just before the confluence of Dzindi and Mvudi rivers in the month of August 2009.

In our case study (Luvuvhu River Catchment), Nandoni Dam is the major reservoir constructed along the river. Nandoni Dam is a freshwater reservoir that was completed in 2005 and the Water Treatment Works was for completed in 2009 (EWISA, 2008). The dam is constructed on the Luvuvhu River, situated between Thohoyandou and Malamulele with catchments which stretch from Makhado in the west to Malamulele in the east (Vhembe District Municipality, 2008a). The main purpose is to supply water for domestic use. The areas and communities benefiting are the urban areas of Makhado and Thohoyandou and the rural communities in the northern part of the Limpopo, from Malamulele and Lambani in the east to Sinthumule/ Kutama in the west. The total population of the Vhembe District Municipality is approximately 1.2 million people. The population of Vhembe District Municipality grew by 1.5% between 2000 and 2004, and between 1995 and 1999 (Vhembe District Municipality, 2008b). The dam also provides water supplies for irrigation of 1100 ha of farmland which were set aside for the purposes of rural development and poverty relief and alleviate water shortages in the Kruger National Park (DWAF, 2004).

Levels of algal toxins in the Luvuvhu River catchment have not yet been clearly evaluated and quantified; hence the need for studies to clearly indicate these as this will help to plan for alternative water treatment methodologies and to manage the algae blooms such that other ecosystems are not greatly impaired.

THE USE OF AQUATIC MACROPHYTES "THE GREEN LIVER CONCEPT"

Plants growing in lakes, ponds and streams are called macrophytes. These aquatic plants appear in many shapes and sizes. Some have leaves that float on the water surface, while others grow completely under water. Gerber et al. (2004), classified/defined plant types found in S.A as: free floating; floating-leaved; submerged; emergent; broad leaved; and emergent narrow leaved. Gerber et al. (2004) recognized/listed approximately 58 aquatic plant species commonly found in and around South African impoundments. Any of these macrophytes are potential candidates for in-situ bioremediation of cyanobacterial toxins found in freshwater bodies.

Aquatic macrophytes have mainly been employed in remediation of (removal) of heavy metals; examples being Rai et al. (1995), Cardwell et al. (2002), Choo et al. (2006), Peng et al. (2008) and Mishra and Tripathi (2008). They have also been used in removal of nutrients (nitrogen and phosphorous) in nutrient rich freshwater (Hu et al., 2010). Organic pollutants can also be degraded chemically and ultimately mineralized into harmless biological compounds by plants. Numerous endogenous activities in plants give them the ability to synthesize, rearrange and detoxify the most complex array of biochemicals and biopolymers of any living organisms (Meagher, 2000). Plants remediate organic compounds by direct uptake of contaminants, followed by subsequent transformation, transport and their accumulation in a nonphytotoxic form (which does not necessarily mean nontoxic for humans) (Macek et al., 2000).

Several options such as activated carbon and ozone have been used to reduce the presence of cyanobacterial toxins in raw water as part of water treatment works in different countries but these generally increase the costs for treating driking water (Nimptsch et al., 2008; Hoeger et al., 2004). The uptake and bioaccumulation of cyanotoxin microcystin (MC-LR) in aquatic macrophytes was shown by Pfugmacher et al. (1998) and Mitrovic et al. (2005). Nimptsch et al. (2008) demonstrated the effectiveness of a water treatment system for cyanotoxin elimination via bioaccumulation in aquatic macrophytes, as a preliminary purification step before entering the water works for further processing in the People's Republic of China.

WHAT IS THE MECHANISM OF MICROCYSTIN BIOTRANSFORMATION?

The 'Green Liver Concept' is based on the fact that both mammals and plants exhibit a similar mechanism for the metabolisation of toxic compounds, for example biotransformation. In mammals, there are metalothioneins (MT) which are intracelluelar, low molecular weight, cysteine-rich proteins that have potent metal binding and redox capabilities (Coyle et al., 2002). MT-1 and MT-2, as detoxifying agents, are produced in the mammalian liver in response to the presence of toxic compounds, heavy metals, drugs and inflammatory compounds and then excreted as a by-product of metabolisation via urine and/or faeces. Plants have the ability to isolate these metabolites and export them out of cells into vacuoles or into the extracellular space and then deposit them into cell wall components such as lignins, a reservoir for storage of non-toxic compounds (Nimptsch et al., 2008). Along with strategies such as sequestration, scavenging and binding, catalytic biotransformation has evolved as an important biochemical protection mechanism against toxic chemical species. Cells possess an array of enzymes capable of biotransforming a wide range of different chemical structures and functionalities (Sheehan et al., 2001). As a main site of xenobiotics metabolism in mammals, the liver contains several families of enzymes including the cytochrome P450 monooxygenases, glutathione transfreases and UDP-glucorosyltransferases. Similar enzymes have also been detected in plants and were active against pesticides and other xenobiotics (Sandermann, 1992).

Relatively little is known about the uptake and sequestration of toxic organics in plant roots or their concentration into vacuoles (Meaghaer, 2000). According to Dietz and Schnoor (2001), once an organic chemical is taken up and translocated, it undergoes one or more phases of transformation:

(i) Phase I- Conversion: oxidation, reductions, and hydrolysis;
(ii) Phase II- Conjugation: with glutathione, sugars, amino

acids;
(iii) Phase III- Reservoir storage: Conjugates from phase II are converted to other conjugates and deposited in plant vacuoles or bound to cell wall and lignin.

The participating enzymes have similarities not only to the enzymes of normal secondary plant metabolism, but also to those of xenobiotics metabolism in mammalian liver, the most common being cytochrome P450 mono-oxygenases and glutathione transfrerases (Sivonen and Jones,1999). Phase II conjugates are sometimes termed "bound residues" because of their inability to be extracted by chemical methods. These conjugates are likely covalently bound to stable tissues in the plant (Dietz and Schnoor, 2001).

Microcystin-LR is usually present inside cyanobacterial cells and enters the surrounding water after cell lysis, although some leakage occurs from live cells (Mitrovic et al., 2005). There is some direct and indirect (elevation of glutathione S-transferase activity) evidence of glutathione and cysteine conjugates of microcystins and noduralin in animals and plants (Meriluoto and Spoof, 2005). Studies have shown that the conversion of microcystins in animal liver to a more polar compound in correlation with a depletion of the glutathione pool of the cell (Pflugmacher et al.,1998), and glutathione microcystins-LR conjugate was confirmed to be the first step in the detoxification of a cyanobacterial toxin in aquatic organisms (Pflugmacher, 2004; Pflugmacher et al., 1998). In tests using aquatic plants, increase in glutathione activities was also observed on exposure to microcystins (Mitrovic et al., 2004).

According to Pflugmacher (2002), it is hypothesized that after cell uptake microcystin-LR reacts from non-enzymatic binding to glutathione protein binding to protein phosphatases and enzyme-linked conjugation reaction plus transport of the conjugates via an ABC transporter into the vacuole of the plant cell. It is also postulated that microcystin-LR is taken up by the chloroplast along other similar pathways.

Several studies have demonstrated the uptake and bioaccumulation of microcystins in aquatic macrophytes (Pflugmacher, 2004; Mitrovic et al., 2005; Nimptsch et al., 2008). Using aquatic macrophytes, Mitrovic et al. (2005) demonstrated MC-LR equivalent accumulation rate of 0.05 ng/day and 0.008 ng/day by *Lemna minor* and *Chlarophora fracta* respectively. Uptake ranges between 1.0- 120 pg/g fresh weight (FW) have also been documented in aquatic macrophytes *Ceratophyllum demersum*, *Elodea Canadensis*, *Vesicularia dubyana* and *Phragmites australis* (Nimptsch et al., 2008).

A CASE STUDY OF THE APPLICATION OF AQUATIC MACROPHYTES IN IMPROVING WATER SUPPLIES

It was Nimptsch et al. (2008) who demonstrated the effectiveness of a water treatment system for cyanotoxin elimination via bioaccumulation in aquatic macrophytes, as a preliminary purification step before entering the water works for further processing. The study showed that *Myriophyllum* species, *Lemna* species, and *Hydrilla* species in series were the most efficient macrophytes achieving a reduction of over 84% of MC-LR in raw water destined for Hefei water works, Lake Chao, Anhui, People's Republic of China. In laboratory trials, the aquatic macrophytes reduced an initial MC-LR concentration of 12.1 and 9.2 µg/L to values below the WHO guidelines for drinking water of 1.0 µg/L (MC-LR) in three days.

A closer asssessment of Nimptsch et al. (2008) showed that the inflow and residence time of the water in the pond should be controlled together with the active plant biomass inorder to avoid excessively high concentrations of cyanotoxins of microcystin-LR since it is known that aquatic plants may suffer deleterious effects, such as growth reduction and lower photosynthetic rates, when exposed to high concentrations of cyanotoxins. In another study, LeBlanc et al. (2005), tested environmentally relevant concentrations ranging between 0.1 to 10 ug/l of microcystin-LR on *Lemna gibba* L. and observed no significant negative effects. In their study, Nimptsch et al. (2008), proved that *Lemna* sp, *Myriophyllum* sp., and *Phragmites* sp. are less sensitive to microcystin-LR exposure than other aquatic macrphytes. Other important factors in cyanotoxin bioaccumulation/elimination were noted to be: (1) position of macrophytes (in the mesocosm or pond system) and (2) use of impediments.

CONCLUSION

The application of the Green Liver Concept can prove to be a cheap low technology method to treat water to be used for domestic purposes, irrigation and livestock in rural and other disadvantaged areas. In the Luvuvhu River catchment, two commonly occurring plants namely *Nymphaea nouchalia* (blue water lilly) and *Persicaria decipiens* (slender knotweed) have been identified as possible candidates as these are dominant and well adapted for the environment. Whether these are effective in bioaccumulating cyanotoxins and also their sensitivity to microcystins is a subject of research. There is need to determine the environmental relevant concentrations of cyanotoxins in the catchment and test these plants under those levels. In as much as microcystins are the most important and most documented cyanotoxins, there is also need to assess the levels of other toxins such cylindrospermopsin in the catchment and also assess the ability of the identified aquatic macrophytes to bioaccumulate these and their sensitivity to these toxins also need to be assessed. On die-off of the aquatic plant, the fate of entrapped microcystins within the dead plant material is also not well understood and Nimptsch et al. (2008), recommended further studies on release rates of entrapped microcystins in aquatic plants.

ACKNOWLEDGEMENT

The authors wish to acknowledge financial support for the study came from Department of Research and Innovation, University of Venda (I437). Part of this work was orally presented at the 3rd Municipal Water Conference in Cape Town, South Africa in June, 2011 with financial assistance from NRF KIC, travel grant (UID 70618) to Dr JR Gumbo.

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