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Removal of colonial and filamentous harmful cyanobacteria by diatomite filter from raw freshwater and potential use in watering domestic animals.

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ABSTRACT

Growth of harmful cyanobacteria in freshwater causes several poisoning episodes of livestock, wild and domestic animals. Conventional surface drinking water treatment systems which utilize coagulation, flocculation, sedimentation, filtration and disinfection, are inadequate and require great financial means or application in watering domestic animals. The filtration of raw freshwater by diatomite is very simple without use of chemical products. The diatomites are largely abundant in nature and at low cost. Locally natural diatomite, dried and calcined diatomite were tested to verify their efficiency to removal cyanobacteria from raw freshwater. The results obtained shows that filtration by calcined diatomite improves efficiency to remove cyanobacteria and decreases the level of microcystin in the filtrate solutions.

KEYWORD: Cyanobacteria, diatomite, raw freshwater, watering animals

INTRODUCTION

Cyanobacteria are a morphologically diverse group of photosynthetic prokaryotes that occupy a wide range of niches, from freshwater to hydrothermal vents, from desert rocks to Antarctic lakes. Thy have been reported in freshwater lakes, basins, rivers, irrigation channels, brackish and sea waters, salty lakes, as pelagic or benthic organisms. Several cyanobacteria species produce toxins as secondary metabolites, which can impact on ecosystems, animal and human health (Chorus and Bartram;1999).

The most common toxic cyanobacteria in freshwater are *Microcystis spp*., *Cylindrospermopsis raciborskii*, *Planktothrix* (*syn. Oscillatoria*) *rubescens*, *Synechococcus spp*., *agardhii*, *Gloeotrichia spp*., *Anabaena spp*., *Lyngbya spp*., *Aphanizomenon spp*., *Nostoc spp*., some *Oscillatoria spp*., *Schizothrix spp*. and *Synechocystis spp*. Toxicity cannot be excluded for further species and genera (WHO , 2003).

Several poisoning episodes of livestock, wild and domestic animals have been associated with the occurrence of cyanobacteria blooms in surface waters used for drinking (Stewart et al. 2008). Animals, especially caws and sheep can be exposed to extremely high levels of toxins in the presence of scums accumulating by lake or river side.. In addition it has been reported that animals of different species seem to drink preferentially waters contaminated by high cyanobacteria density rather than clean ones (Cold et al. 1992). Cyanobacteria have the ability to form a great variety of several secondary metabolites, which exhibit various types of biological or biochemical activities and some of them have been identified as potent toxins (cyanotoxins). The cyanotoxins are a diverse group of compounds, both from the chemical and the toxicological points of view. In terms of their toxicological target, cyanobacteria toxins are hepatotoxins, neurotoxins, cytotoxins, dermatotoxins and irritant toxins (Wiegand and Pflugmacher, 2005). Microcystins are the most frequently occurring and widespread of the cyanotoxins.. About 70 structural analogues of microcystin have been identified (Rinehart et al., 1994; Sivonen and Jones (1999). Due to these adverse health effects, the World Health Organization established a provisional guideline of 1µg/L for microcystin-LR in drinking water (WHO, 1998). Kurmayer et al. (2002) found a relationship to colony size: 42 to 73% of the large colonies (>500 µ m) belonged to the microcystin-producing genotype, compared to only 10 to 15% of the small colonies (< 500 µm).

Conventional surface drinking water treatment utilizes coagulation, flocculation, sedimentation, filtration and disinfection as basic methods. However, conventional treatment may need to be optimized for cyanotoxin removal, relating to the form of the toxin to be removed (intra- or extracellular), the background water matrix, and possible dissolved toxin release during the treatment process (Falconer.2005). Microcystin removal from water by osmoses reverse is > 90%, but coagulation/flocculation was not effective (Wannemacher et al.1993).; studies have shown removal levels ranging from 0 to 49%. However, addition of powdered activated carbon to the clarification process can increase removal levels to 90% or more, depending on the carbon dose, type of carbon, toxin level and organic matrix (Yoo et al., 1995).In agreement with earlier findings that alum flocculation, filtration, and chlorination are ineffective in the removal of cyanobacteria toxins (

Vuori et al. 1992). Physicochemical water treatment processes have been shown to cause cell lysis and toxin release (James and Fawell, 1991).

Diatomite is a natural material formed from the remains of diatoms, which grew and were deposited in seas or lakes. Diatomite products are used in a variety of ways, such asreinforcing, stiffening and hardening of organic solids, reducing adhesion between solid surfaces, increasing adhesion, increasing viscosity, surfactant effects, hydrophobic effects, absorbent, catalysts and cloud seeding (Zhaolun *et al*., 2005). Diatomaceous earth, or diatomite, typically consists of 87–91% silicon dioxide (SiO2), with significant quantities of alumina and ferric oxide (Engh, 1993). The structure of diatomite is quite complex. Because there are many fine microscopic pores, cavities, and channels, it has a large specific surface area, high absorption capacity, and low density. It also has a low thermal conductivity, a relatively high melting point, chemical inertness, and small grains. Moreover, because of its relative low cost and abundance, it is utilized extensively as a filler, a filtering aid, an abrasive, an insulating material, a conventional catalyst support, and a membrane. Small, highly porous diatomite particles are useful in fabricating highly permeable microporous membrane filters (Vasconcelos et al., 2000). Diatomite was used in purification potable water, contaminated ground- and surface water, decontamination of sewage liquids and waste water. (Grešovnik, 2007).

The aim of this work is to test the efficiency of natural and calcined diatomite as filter without chemical products use to remove cyanobacteria from raw water used inwatering domestic animals .

MATERIAL AND METHODS

Sampling: The raw diatomite obtained from (Sig deposit, Algeria) under investigation was subjected to treatment under various conditions. The first sample is the natural sample (S1). The second one (S2) is treated by 0.5 N Hcl, then washed by distilled water and finally dried in drying oven at 110 ^{o}C for 5 hours. The third one (S3) is calcined diatomite at 500 ^{o}C using muffle furnace. While the fourth (S4) is calcined at 700 ^{o}C. All samples milled in a ball mill machine and sieved below 200 µm to remove all particles larger than 200 µm. ideally, these fractions contain sand, rocks, clay, and other impurities

Filtration: Raw water collected from eutrophic lake was used in this experiment. A pre-filtration was occurred on fine sand to removal microalgae and other large particular matters. To achieve filtration, 10 grams of diatomite to be tested were added in funnel and the pre-filtered water and one liter was filtered through the diatomite filter.

Quantification and identification: The quantification and identification of cyanobacteria was determined by inverted microscopic. The morphological identification of cyanobacteria was done according to (Kotare and Anagnostidis 2005 ,Komárek and Zapomelova 2008).

Microcystin determination. The determination of intracellular and the extracellular microcystin concentration in extracted samples was determined by HPLC with photo-diode array detection following the method of Lawton et al, (1994). The instrumentation for HPLC consisted of a Waters system with Model 600 solvent pump, Model 717 plus autosampler and a Model 996 photodiode array detector monitoring at 200 – 300 nm with 1.2 nm resolution. The stationary phase was a Symmetry C18 column (250 x 4.6 mm I.D., 5 µm particle size, Waters) at a flow rate of 1ml min-1 and column temperature 38 o C. The mobile phase were Milli-Q water (solvent A) and acetonitrile (solvent B) both acidified with 0.05 % v/v TFA . Sample injection volume was 50 µl. All the reagents were HPLC grade. MC-LR stock solution was prepared by mixing MC-LR standard (Sigma) with methanol, and then it was centrifuged in 2000 RPM for 15 min. MC-LR standard solutions were prepared in different concentrations by adding desired volumes of stock solution to methanol. The prepared standard solutions were 0,01, 0.1,0.2, 0.4 ,0.6, 0.8, 1.0 , 2.0 , 4.0 , 5.0, 10.,20, 40, 80 and 100 µg/L of concentrations. Before the HPLC analyses, all water samples (200 ml) were extracted by sonifiaction and filtered on GF/C filters... Each fiber was eluted by 20ml methanol and pure water 20ml separately before extraction. The methanol was evaporated to dryness under nitrogen and was reconstituted in 1ml methanol for subsequent analysis by HPLC.

RESULTS AND DISCUSSION

The identification and quantification in sample of raw water before filtration show that the *Microcystis sp* . was the predominant genus followed respectively by *Anabaena sp*., *Oscillatoria sp.*,and *Synechococcus sp.* The colonial diameter of *Microcrystals sp*. ranged from 300 to 400 µm. The length of filaments *Anabaena sp.* ranged from 100 to 200 µm. In *Oscillatoria sp.* the filament length ranged from 90 to 150 µm. The quantification of cyanobacteria was 6. 10^4 cells/ml of *Microcystis* sp, 4.10^3 cell/ml of *Anabaena sp.*, 5.10^2 cell/ml of *Oscillatoria sp.* and 1.10^2 cells / ml of *Synechococcus sp.*

Fatalities in animals have been reported following the consumption of water containing large numbers (>106 /ml) of cyanobacteria cells (Carmichael ,1992).

The quantification of cyanobacteria in the filtrate solution filtered through the natural diatomite (S1) shows that the number of Microcystiis *sp.* decreased at 8.10^2 cell/ ml, *Anabaena sp.* at 2.10^3 cell/ ml , *Oscillatoria sp.* at 2.10^2 cell/ ml and *Synechococcus sp.* at 50 cell/ ml. For the diatomite treated by 0.5 HCl and dried at110 °C (S2), the enumeration of cyanobacteria in the filtrate solution showed that the *Microcystis spp*. was 6. 10^2 cell/ml, followed by *Anabaena sp*. 2.10^2 cell/ml and 20cell/ml of *Oscillatoria* sp . The counting of the filtrate solution filtered through the calcined diatomite at 500 °C (S3) was 50 cell / ml of *Microcystis* sp., 20 cell/ml of *Anabaena sp.* , 10 cell/ml of *Oscillatoria sp.*, 4 cell/ ml of *Synecococcus* sp. For the filtrate solution collected from the filtration by calcined diatomite at 700 °C, only 10 cell/ml of *Microcystis sp*. and 4 cell/ml of *Anabaena sp*. were detected The content of total microcystin LR in raw water reached 90 µg/L in the same sample the extracellular microcystin LR was 1.6 µg/L. The results of total and extracellular microcystin in filtrate solutions are summarized in Table. 2.

Table 1: Cyanobacteria count in filtrate solutions (cell/ ml).

Sample	*Cyanobacteria spp*.			
	Microcystis sp.	*Anabaena sp.*	*Oscillatoria sp*	*Synechococcus sp*
S1	2.10^4	2.10^3	2.10^2	50
S2	$5. 10^3$	2.10^2	20	10
S3	80	20	10	4
S4	10	4	0	0

Table.2: Content of total microcystin LR and extracellular in filtrate solutions (µg/L).

Sample	Total ***microcystin*** LR	Extracellular ***microcystin*** LR
S1	4 5.0	0.9
S2	10.5	0.7
S3	4.0	0.5
S4	0.6	0.5

Following the identification and quantification the majors groups of cyanobacteria found in raw water from this eutrophic lake were *Microcystis sp*. as the predominant genus. Carmichael (1992) observed that *Microcystis*, *Oscilatoria* and *Anabeanea* represent the genera that most frequently occur in freshwater environments. *Microcystis* is one of the most common bloom formers in freshwater systems on every continent except Antarctica. This genus can produce a suite of potentially harmful compounds (Fristachi and Sinclair, 2008). The Table.1 shows that the retention of cells increases with diatomite processing method. The highest value was observed in the diatomite calcined at 700 °C. median pore size of a calcined diatomite ranging between 2.5-5.5 µm (Ibrahi. S. and Selim A. 2010).

Lange et al. (1986) found that the grade of diatomite used affected filter performance. For the finest diatomite grade with a median particle size of 7.5 µm, turbidity reduction was close to 100%; however, for coarser grades with a median particle size of 22 µm, a 10% reduction was observed. The percent of retained cells of *Microcystis sp*. increased at 33% of initial microbial charge in raw diatomite filter (SI), this value increased to 80% in dried diatomite filter to reaching 0.01 % by calcined diatomite filter (S4). The highest retention was observed at *Oscilatoria sp.* 96% was removal by dried diatomite , 98% by calcined diatomite at 500 °C and 100% by calcined diatomite calcined at 700 °C. This is probably explained by the morphology of the filament that can reach 100 µm of long and the mucilage sheath.

The estimation of total microcystin LR and the intracellular microcystin shows that this toxin was concentered in the cells. By subtracting, the intracellular microcystin was very high (88.4µg/L) compared to extcellalar microcystin LR content (1.6 µg/L). The high percentages of dissolved MCYSTs in filtered lake water at the end of blooming season suggest that release of cyanotoxins from cells occurs during the senescence and the decomposition periods of Microcystis cells (Park et al. 1996). The results shows that the filtration by diatomite was not contributed to increase of microcystin content in all filtrates. However the use of chemical products increases the extracellular microcystin level in the freshwater treatment by lysis of cyanobacteria cells.

CONCLUSION

The diatomite is a product of choice to remove toxic cyanobacteria in raw water. Filtration through natural diatomite calcined at 700 ° C exhibits a high efficiency of filtration of all the colonial and filamentous forms of cyanobacteria. This method reduces the amount of microcystins in filtered water. This simple method can be applied in the reservoirs watering domestic animals.

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