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Original Article

Mitigation of Cyanobacteria Isolated from Tri An Reservoir Using Local Soils

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Abstract: Cyanobacterial blooms in eutrophic waters pose serious threats to public health, water quality, and ecological management worldwide. Mitigation of the problem is essential to maintain stainable development. In this study, the effectiveness of local soils collected from the Tri An Reservoir (TAR) lakeside in the removal of cyanobacteria *Microcystis aeruginosa* was investigated. The organic matter content and clay mineral were characterized and used for the experiment. Cell density, percentage of growth inhibition, and changes in cell morphology were observed and used at endpoints of the experiment. The three soil samples named TA1, TA2, and TA3 were characterized with a high proportion of illit, montmorillonit, and kaolinite, respectively. Our results indicated that different soil compositions generated different inhibition on the growth of *M. aeruginosa*. Exposure to the soil high in kaolinite (52.1%) at a dose of 500 mg/L completely inhibited *M. Aeruginosa* growth, while the soil contained a high amount of montmorillonit (42.3%) inhibited only 38.4% of the growth of *M. aeruginosa*. The half-maximal effective concentration (EC_{50}) values of the TA1, TA2, and TA3 soil samples were 351.6 mg/L, 668.9 mg/L, 201.3 mg/L, respectively. Our results suggested that soil rich in kaolinite can be used to prevent harmful cyanobacterial blooms in inland waters.

Keywords: Clays; control cyanobacteria; growth inhibition, natural soils.

1. Introduction

Human activities have led to the nutrient enrichment in freshwater systems creating favor conditions for harmful cyanobacterial blooms (HCBs), which caused degrade water quality, disrupt food web, reduce biodiversity and produce toxic secondary metabolites [1, 2]. Microcystins (MC) produced from several toxic *Microcystis* species are among the most common toxic secondary metabolites detected

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in freshwater ecosystems [3, 4]. MCs have been reported to be toxic to a wide range of aquatic and terrestrial animals including zooplankton, shellfish, fish, cattle, domestic pets, and humans [4, 5]. The proliferation of HCBs and harmful impacts from MCs have been reported to be exacerbated due to global warming and climate change [2, 6]. Therefore, mitigating or controlling the risk of HCBs, and reducing their negative impacts from MCs, is urgent management needs for eutrophic water bodies, especially for ones using for drinking water supply or recreational sites.

During the last decade, HCBs issues associated with threats to ecosystems and human health are more severe in Vietnam [7-11]. HCBs and MCs have been documented in many lakes and reservoirs in Vietnam using for drinking purposes and recreational activities [3, 8-11]. HCBs and cyanotoxins have been found in many Vietnamese water bodies such as Huong River, Hoan Kiem Lake, Nui Coc, Hoa My, and Dau Tieng Reservoirs (3, 8-11). The Tri An Reservoir (TAR) is one of the most important drinking water sources in southern Vietnam, it supplies drinking water for millions of people in Ho Chi Minh City and nearby provinces [9, 10]. Anthropogenic activities are causing pollution of the water quality resulting in eutrophication. Cyanobacterial blooms forming by *Microcystis* spp. with MC concentration up to 640 µg/g dry weight have recently been reported from the TAR [9, 10]. Therefore, it is necessary to implement algal bloom monitoring and forecasting program, along with best practices on the mitigation or reduction of HCBs to protect the aquatic ecosystem and reduce the risk to humans.

Several methods, such as chemical, mechanical and biological techniques have been developed and applied for controlling HCBs [12-15]. Chemical methods for example using algaecide may result in toxic effects on other organisms and releasing intracellular MCs [14, 15], and engineered silver nanoparticles [12] can cause adverse effects on other aquatic organisms. Mechanical methods such as harvesting algal cells by filtering or centrifugation can reduce the problems but it is high cost and impossible for large-scale management [16]. Biological and ecological control methods such as using filter-feeding animals are safe for reducing algal cells but they need a long time to operate and in some cases are not efficient enough to treat large-scale HABs [15]. Another way to solve the problems is to use natural, non-toxic, and inexpensive clays or soils to flocculate and remove algal cells. Clays such as montmorillonite, kaolinite, and phosphatic have been reported as promising, environmentally friendly, and effective flocculants with effective loading from 0.2 to 2.5 g/L for removal efficiency of over 90% [15-17].

In this study, three local clay soils collected in the TAR lakeside and characterized using scanning electron microscopy (SEM) were used for the experiment. The inhibition effect of the three clays to the bloom-forming cyanobacteria *Microcystis aeruginosa* isolated from the TAR was investigated under laboratory conditions. Cell density, changes in cell morphology, percentage of growth inhibition were calculated and used at endpoints of the experiment. The purpose of this paper is to evaluate the effectiveness of the three local soils on growth inhibition of the bloom-forming *M. aeruginosa*.

2. Material and Methods

2.1. Preparation of Clay Soils

The local clay soils were collected from the TAR lakeside. In the laboratory, the soils were dry completely and sieved through 200 µm mesh size before further use. Sub-sample prepared after sieving was examined under a scanning electron microscope (SEM) with energy dispersive X-ray analyzer (EDX) (*JSM-6480LV, JEOL, Tokyo, Japan*) at the Material science lab, Faculty of material science, University of science-VNUH, Vietnam. The organic matter content was

measured by standard methods for operating procedure soil organic carbon Walkley-Black [18]. Soil moisture content was analyzed using the gravimetric method. Clay mineral characterization was examined using a standard test for *methylene blue* index of clay [19].

2.2. Isolation and Cultivation of Microalgae

Cyanobacterial bloom samples were collected in the TAR in Sep, 2016. Under the microscope, *M. aeruginosa* (Figure 1) was found to be the dominant- and bloom-forming species in all samples. The taxonomic classification was based on the system described in Komárek and Anagnostidis [20]. *M. aeruginosa* colonies were isolated by micropipetting and washing, then transferring into cyanobacterial growth Z8 medium [21]. All cultures were grown on a 12:12 light:dark cycle at a temperature of 27 ± 1 °C under light conditions supplied by 40-W fluorescent lamps, which provided an approximate luminic intensity of 50 µmol photons/m^2/s measured by using a digital light meter (Smart Sensor AS813, Dongguan, China).

Figure 1. Cyanobacterial blooms in the Tri An Reservoir with *M. aeruginosa* colonies.

2.3. Algae Growth Inhibition Test

Pre-experiments indicated that the stock culture of M. aeruginosa can reach the exponential growth phase after 7-8 days of incubation in *Z8 medium* under the culture conditions mentioned above. To examine the effects of local soils on all growth phases of this microalgae, our experiments were observed for 10 days. The growth rate inhibition test of *M. aeruginosa* was conducted according to the methods reported by Ribeiro et al., [22]. Briefly, the microalgae starting concentration of 5×10^5 cells/mL were cultured in glass flasks with the design concentration of soils at 0, 5, 20, 80, and 200 mg/L. Three replicates were performed per concentration. The culture flasks were incubated at the same culture condition above for 10 days. All treatments were gently shaken once a day to re-suspend any settled cells. The cell number density was monitored every two days by using a Sedgewick Rafter counting champer (Graticules Optics, UK). The concentration that inhibits algal growth rate by 50% over time was determined based on the relative inhibition of growth rate as a function of the soil concentration (mg/L). The average of the specific growth rate for each period was obtained as the biomass increase after 10 days, by the following equation [22]:

$$\mu_i - j = \frac{\ln Cj - \ln Ci}{t_j - t_i}$$

where μ_{i-j} is the average specific growth rate from time i to time j, t_i is the initial time of the exposure period, t_j is the final time of exposure, C_i is the concentration of cells at time i and C_j is the concentration of cells at time j.

Percentage inhibition of growth was calculated by the equation of Ribeiro et al., (2014) [22]:

$$\%Ir = \frac{\mu_C - \mu_T}{\mu_C} \times 100$$

where $\%Ir$ is the percent inhibition in average specific growth rate; μ_C is the mean value for average specific growth rate (µ) in the control group and $\mu_T$is the average specific growth rate for the treatment replicate.

2.4. Data Analyses

The half-maximal effective concentration (EC_{50}) values after 10 days for all soil samples were calculated by non-linear regression. Kruskal-Wallis test (Sigma Plot, version 12) was applied for calculation of the significant difference of the average of the specific growth rate and percent of inhibition between control and treatments.

3. Results and Discussion

3.1. Characterization of Soils

The moisture content, organic matter content, and clay mineral characterization of the three soil samples were presented in table 1. The moisture content ranged from 2.3 to 2.8%; organic cacbon P_{OC} ranged from 0.6 to 2.3%; organic matter content P_{OM} ranged from 1.0 to 4.5%; kaolinite content was highest in the TA3 sample and ranged from 20.2 to 52.1%; montmorillonit was highest in the TA2 sample and ranged from 18.1 to 42.3%; while illit was highest in the sample TA1 and ranged from 9.1 to 48.3%. The three samples named TA1, TA2, and TA3 were characterized with high proportion of illit, montmorillonit, and kaolinite, respectively. Our results agreed well with the previous report in that the montmorillonite, kaolinite and Illit proportion were the main composition of natural clays and soils [23, 24].

Table 1. Organic matter content and clay mineral characterization of soils

No.	Parameter	Average		
		TA1	TA2	TA3
1	Moisture content (%)	2.3	2.5	2.8
2	Organic cacbon P_{OC} (%)	1.5	2.3	0.6
3	Organic matter content P_{OM} (%)	2.6	4.5	1.0
4	Kaolinite (%)	20.2	22.2	**52.1**
5	Montmorillonit (%)	18.1	**42.3**	32.5
6	Illit (%)	**48.3**	17.3	9.1
7	Particle size (µm)	2–10	2–20	2–10

The morphological of the soil samples observed under SEM were shown in Figure 2. It can be seen that the small tiny particles were the main proportion of the TA3 sample dominated with kaolinite; whereas the TA1 and TA2 samples, dominated with illit and montmorillonit, respectively, were characterized by the large particle size. The size of particles in all samples varied from 2 to around 20 µm in diameter.

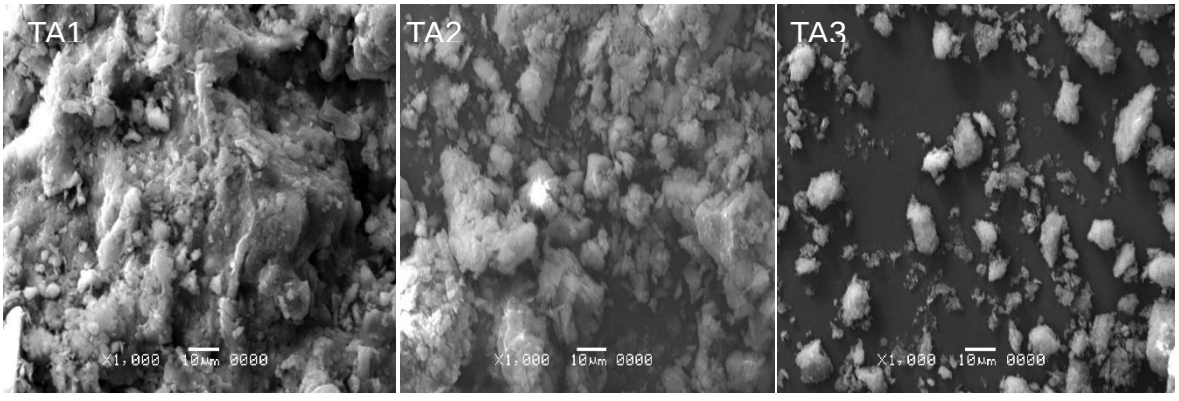

Figure 2. The SEM image of soil samples.

3.2. The Half-maximal Effective Concentration

The EC_{50} of the TA1, TA2, and TA3 soil samples on *M. aeruginosa* were 351.6 mg/L, 668.9 mg/L, 201.3 mg/L, respectively. The soil high in kaolinite (TA3) had the lowest EC50 value. Although the effectiveness of clays and minerals on the removal of cyanobacterial blooms was reported [16, 23, 24], little information is known about the effective concentrations of clays/minerals on the inhibition of cyanobacteria. Previous studies indicated the working loading concentration of clays in ranged from 0.1 to 2.5 g/L [23-26]. The EC_{50} value of 0.15 g Phoslock®/L of a modified clay on the growth inhibition of several phytoplankton (including green algae *Scenedesmus obliquus*, cyanobacteria *Microcystis aeruginosa* and *Anabaena* sp.) was suggested by Oosterhout and Lürling [27]. In our experiment, the EC_{50} values are higher than that of Van Oosterhout and Lürling (2013) [27], probably the unmodified clays in our experiments generated lower inhibited effects on the cyanobacteria. Future studies on improving the inhibited efficiency of natural clays and preparing the modified clays with high removal capacity *are recommended.*

3.3. Algae Growth Inhibition Test

The results of algae growth inhibition showed that local soils caused significant effects and dose-dependent increases on the growth of *M. aeruginosa*. For the soil high in illit (TA1), the dose of 500 mg/L inhibited 67.5% of the growth of *M. aeruginosa*. For the soil high in montmorillonit, the dose of 500 mg/L treatment inhibited 38.4% of the growth of *M. aeruginosa*. And for the soil containing high content of kaolinite, the *M. aeruginosa* can not grow on the exposure with 500 mg/L of soil corresponding to 121.7% inhibition on the growth of *M. aeruginosa* (Figure 3).

Our results indicated that different soil samples generated different inhibition on *M. aeruginosa*. Soils that contained a high proportion of kaolinite strongly inhibited the growth of *M. aeruginosa* when comparing with soil samples that contained a high proportion of illit or montmorillonit. Previous studies showed that the effective clay loadings were approximately in the range of 0.25-2.5 g/L [20, 22, 23], although for a few cases the using local soils and sediments modified by chitosan could be as low as 0.1 g/L [24]. In our experiment, the natural clay at a concentration of 0.5 g/L equally to 500 g/m^3 contained a high amount of kaolinite can inhibit completely the growth of of *M. aeruginosa*. This dose is compatible with those reported by other researchers [26-30] and could be applied to the natural lakes and reservoirs with cyanobacterial bloom formation.

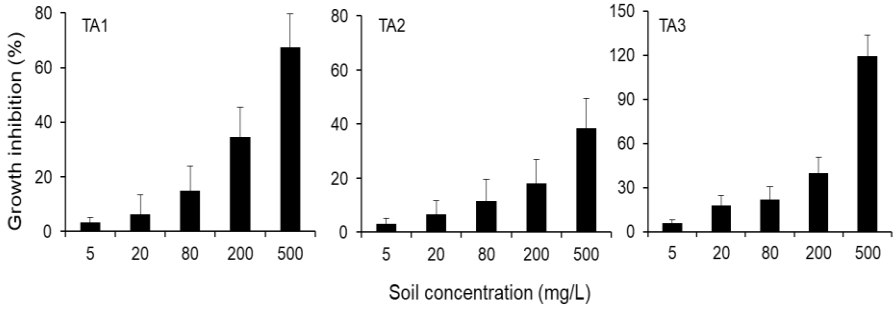

Figure 3. Growth inhibition of *M. aeruginosa* after 10 days of exposure to local soils.

Previous studies showed that the cyanobacterial removal efficiencies of clays depended on clay structure and type [23, 28]. Kaolinites and montmorillonites are both layer-structured, in which kaolinites have two layers (-Al-Si-) while montmorillonites have three layers (-Si-Al-Si-), and the structure of illite is similar to the montmorillonite group with a

sandwich-type three layers structure. Natural clay minerals are electronegative and the surface negative charges of montmorillonites are stronger than those of kaolinites [28]. In contrast, zeta potentials of active cyanobacteria were positive [29]. This positive potential created the repulsive forces between clay particles and cyanobacterial cells causing the sedimentation of cyanobacterial cells. Our results showed that certain types of kaolinites had better removal efficiencies, probably, the sedimentation efficiencies in our experiment were related to factors such as particle size, shape, surface charge, and chemical compositions of clay [16, 23]. Zou et al., [24] exposed *M. aeruginosa cells to* three categories of clays/minerals including type I (talc, sepiolite, and kaolinite), type II (attapulgite, rectorite, and illite), and type III (ca-bentonite and quartz). The authors found that high efficiency for type I to flocculate *M. aeruginosa* cells in freshwaters was due to the mechanism of netting and bridging effect. In addition, previous studies indicated that increasing the surface positive charges of clay particles, and strengthening the stick together between clay particles and cyanobacterial cells are important to improve the removal efficiencies using clays [28, 30].

3.4. Morphological Change

The morphological observations of *M. aeruginosa* in the control and treatment with local soils are shown in figure 4. The results showed that *M. aeruginosa* was original shape in the control sample, but the colonies gradually began to disperse to single cells after exposure to local soils. Zou et al., [24] indicated that kaolinite particles of hard granular shape connected with the *M. aeruginosa* cells loosely, this connection appeared to be a natural property of this mineral allowing it to catch the suspended cells more effectively. It can also be seen from figure 4 that the *M. aeruginosa* cells kept their round shape in full after connected with kaolinite, indicating clay flocculation did not destroy or break the cells [24, 29]. Unlike chemical reagents such as copper sulfate, clay flocculation did not result in an acute release of toxins from the cells and can enhance the cyanobacterial cells settle to sediment where they could be biodegraded latterly by different types of bacteria [14, 27, 28].

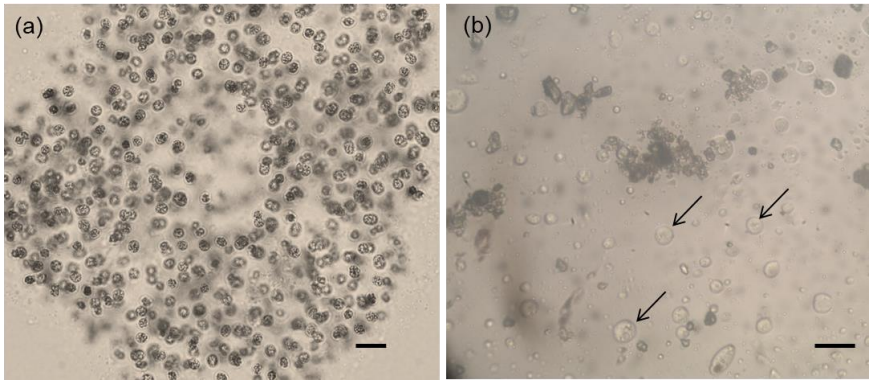

Fig. 4. Morphological of *Microcystis aeruginosa,* (a) control treatment and (b) exposure to the local soil samples (TA3). Scale bar: 10 µm.

4. Conclusion

This study investigated the effectiveness of three local soils collected from the TAR in the removal of cyanobacteria *Microcystis aeruginosa*. The three soil samples named TA1, TA2, and TA3 were characterized with a high proportion of illit, montmorillonit, and kaolinite, respectively. The results indicated that the soil rich in kaolinite at the exposure dose of 500 mg/L completely inhibited the

growth of *M. Aeruginosa.* The half-maximal effective concentration (EC_{50}) values indicated that soil samples with high proportion kaolinite and small particle size showed the most effective on growth inhibition of cyanobacteria. Morphological colonies of tested cyanobacteria gradually dispersed to single cells after exposure to soils. Our results suggested that the local soil rich in kaolinite collected from the TAR can be used to prevent harmful cyanobacterial blooms in inland waters.

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