Preliminary Results On The Use Of Clay To Control *Pyrodinium* Bloom - A Mitigation Strategy

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ABSTRACT

The frequent and expanded occurrence of *Pyrodinium bahamense* var *compressum* blooms in the Philippines since 1983 has prompted the need to find mechanisms to control the harmful effects of these toxic dinoflagellates. A promising method now being explored is the use of powdered clay minerals which when added to the growth media is capable of flocculating with the algal cells. In this study, the efficiency of ball clay, brown bentonite, and Malampaya Sound sediments to remove *Pyrodinium* cells in seawater was tested. The addition of 1 g/L of suspended ball clay to 50 mL of cultured *Pyrodinium* cells (~1.037 x10^6 cells/L) removed 99.56% of the algal cells after 2.5 hours. Prolonging the exposure time to 5 and 24 hours showed no significant increase in flocculation. Brown bentonite and Malampaya Sound sediments showed low to moderate removal efficiency not exceeding 70% and 50%, respectively. The effect of ball clay addition on seawater chemistry showed no change in ammonia concentration but nitrate decreased after 5 and 24 hours of clay addition. Results for nitrite and phosphate were however more variable.

INTRODUCTION

Among the dinoflagellate species that are known to cause harmful algal blooms (HABs), *Pyrodinium bahamense var compressum* poses one of the most alarming effects both to man and the environment. The first toxic paralytic shellfish poisoning in the Philippines caused by this species was in 1983 in Western Samar and Leyte which resulted in losses to the green mussel industry in the area and several loss of life and numerous poisonings to the locals who ingested the infected shellfish. (Azanza, 1997; Arafiles et al., 1984).

Various control measures have been proposed to help mitigate the toxic bloom caused by *P. bahamense*. These include use of chemical compounds such as hydrogen peroxides and copper sulfate that could directly kill the algal cells and the introduction of predatory species that would consume the target phytoplankton (Boesch et al., 1997; CENR, 2000). The effectivity of these methods vary with some having negative impacts on the marine ecosystem (e.g. non species specific effect, persistent in seawater). A potentially effective control method is the use of clay minerals that could attach to the algal cells, increase their density, and force them to settle to the sediments where they will die or encyst.

Previous studies have shown that many types of clay can be effective in removing harmful algae. According

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to Sengco et al. (2000) 12 of the 25 types of clays that were tested against *Gymnodinium breve* are effective. Later studies showed that agitation could improve the cell removal efficiency of clay against specific type of algae (i.e. *Karenia brevis*, *Heterocapsa triquetra*) (Sengco et al., 2004; Archambault et al., 2003). Furthermore, prolonging the exposure period of the clay treatment may help in the removal of the algal cells from the water column (Hagstrom and Grancli, 2004; Sengco et al., 2004; Yu et al., 2003; Pierce et al., 2003). The use of various types of clay in mitigating HABs has been widely explored in China (Yu et al., 1994), South Korea (Bae et al., 1998; Na et al., 1996), Japan (Maruyama et al., 1987) and the US (Anderson et al., 2004; Beaulieu et al., 2005). In the Philippines, mitigation measures against algal blooms have not been explored. This study examines the use of clay to remove harmful algae. Although the method is not new, this is the first attempt to control blooms of *P. bahamense*. The possible effect of using clay on water chemistry (nutrients N and P) was also determined.

MATERIALS AND METHODS

Clay preparation and *Pyrodinium* cultures

The clay mineral samples used in this study were purchased from Industrial Specialties Co. Inc., a local mining company in Taytay, Rizal. Ball clay and brown bentonite materials came in powder form, and using wet sieving, grain size was determined to be less than 63μm (>99%). Particle sizes of the clay material were also determined using pipette analysis of mud method (Lewis et al., 1994). A clay stock was prepared by adding 100 g powdered clay to 1L of filtered (<0.2μm) and autoclaved seawater from Bolinao, Pangasinan. Sediments obtained from Malampaya Sound, Palawan were also tested for removal efficiency.

Strains of *P. bahamense* (04-25-95 Pbc MZ RVA) were subcultured using f/2 media (Gullard and Rhyther, 1962) on autoclaved seawater from Bolinao (Pangasinan) as base and incubated at 28±2 °C. The cultured organisms were allowed to reach mid exponential growth phase before they were harvested and tested against the clay and sediment materials. The growth stages of the batch culture were monitored by cell counting every three days until the desired experimental cell density was reached (>1000 cells/ml).

Clay flocculation with *Pyrodinium*

The clay removal experiment commenced when *P. bahamense* cell density reached >1000 cells/ml at its mid exponential growth phase. Eighteen (18) autoclaved 50-ml Erlenmeyer flasks were filled to the 50 ml mark with the cultured *P. bahamense*. Initial cell count was determined in each flask before addition of the clay. Prepared clay slurries with concentrations of 0.25, 0.5, 0.75, 1 and 2 g/L were added to the flasks. Autoclaved seawater was used as control. Upon clay addition, each flask was gently swirled 10 times to disperse and homogenize the clay throughout the culture media. The flasks were then placed in a well-lighted area in the laboratory and arranged following a completely randomized block design. The exposure periods of the experiment were 2.5, 5 and 24 hours. The cell counting procedure involved pipetting 5 ml of sample from the upper ~2cm layer of the flask content and putting the sample in a 20-ml scintillation vial. Samples were preserved by adding 0.02 ml of Lugol's solution and the cells later counted using a Sedgewick-Rafter chamber with grid viewed under a light microscope. The removal efficiency of the clay and sediments was determined using the formula:

Removal efficiency = 1- final cell count / initial cell count x 100%

The results obtained were statistically analyzed using repeated measures design of ANOVA for variation in exposure period, and one-way ANOVA for variation in treatment. Levene's test for homogeneity of variances was utilized to meet the ANOVA assumption that the variances are nearly equal. When data is not homogenous, log transformation was used. Statistical results were determined using the SAS program.

Effect of clay on water chemistry

The effect of clay on water chemistry was determined on the clay material that had the highest removal efficiency. Seawater from Bolinao, Pangasinan was filtered using cellulose ester membrane filter (0.2 μm) and transferred to nine 500 ml Erlenmeyer flasks. The prepared clay stock was added dropwise to seawater to obtain a final clay concentration of 0, 0.5 and 1 g/L (all in triplicate). A control was added to the set up. 125 ml of aliquot was taken prior to and after 5 and 24 hours of clay addition. Samples were filtered and later analyzed for ammonia, nitrate, nitrite and phosphate using methods described by Strickland and Parsons (1972) using a Shimadzu UV-VIS spectrophotometer. The data obtained was statistically analyzed using the same methods as in the clay flocculation experiment.

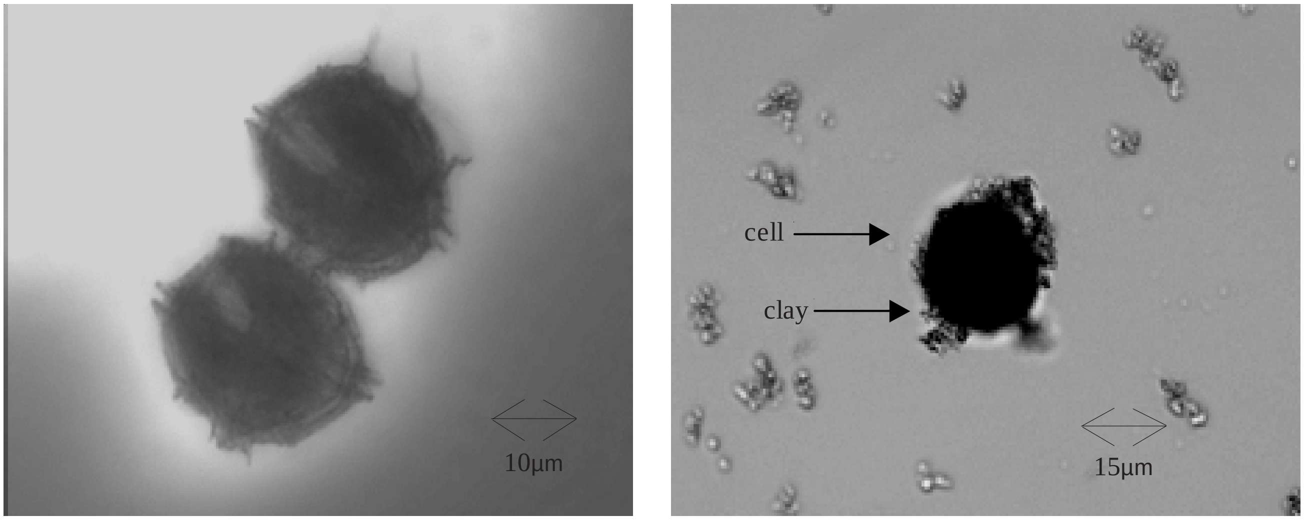
RESULTS AND DISCUSSION

Removal efficiency

Flocculation of *Pyrodinium bahamense* with clay material is shown in Figure 1. The removal efficiency of brown bentonite, ball clay and Malampaya sediment with increasing clay slurry concentration is shown in Figure 2. Highest removal efficiency was 99.9% with ball clay, 69.3% with brown bentonite, and 48.4% with Malampaya sediments.

Very high removal efficiency was obtained when ball clay was used under all exposure periods (Figure 2A). Removal efficiencies using 0.5 to 2 g/L clay concentration were not significantly different from 2.5 to 24 hours of exposure. Thus, with even a low clay concentration of 0.5 g/L, high removal efficiency can be attained with ball clay. For brown bentonite, highest removal occurred after 5 hours at 0.75 g/L clay treatment although there was no significant difference from the 0.25 to 1 g/L treatment (Figure 2B). Increasing the clay treatment to 2 g/L decreased removal efficiency after 2.5 and 5 hours exposure. The Malampaya sediments were less efficient in removing P. bahamense than clay, i.e 20% after 2.5 and 5 hours that improved to 48.42% at 2 g/L treatment after 24 hours (Figure 2C).

Swirling could simulate turbulence thereby increase the rate of collision between the cell and clay particles. According to Young-Han and Kim (2001), higher collision rate will be achieved if the clay particle size and cell size are similar. The clay-cell flocculation in low Reynolds number non turbulent flow regime can be well described by equations including hydrodynamics and interparticle forces (van der Waals force and electrostatic force). Hence the larger the size range difference, the lower the collision frequency. Approximately 88% of the ball clay used in this study fall under the 16-31 mm size fraction (Table 1). There is an overlap with the diameter of *Pyrodinium bahamense* which is about ~25-40 mm.This could explain the high removal efficiency using ball clay. Unlike ball clay, the 16-31 µm size fraction in brown bentonite comprise only 8% of the clay material (Table 1), thus the lower removal efficiency using brown



A. B.

Figure 1. Photomicrograph of *Pyrodinium bahamense* var *compressum* cell without the clay attached at 250X (A), and clay flocculated with the cell at 100X (B).

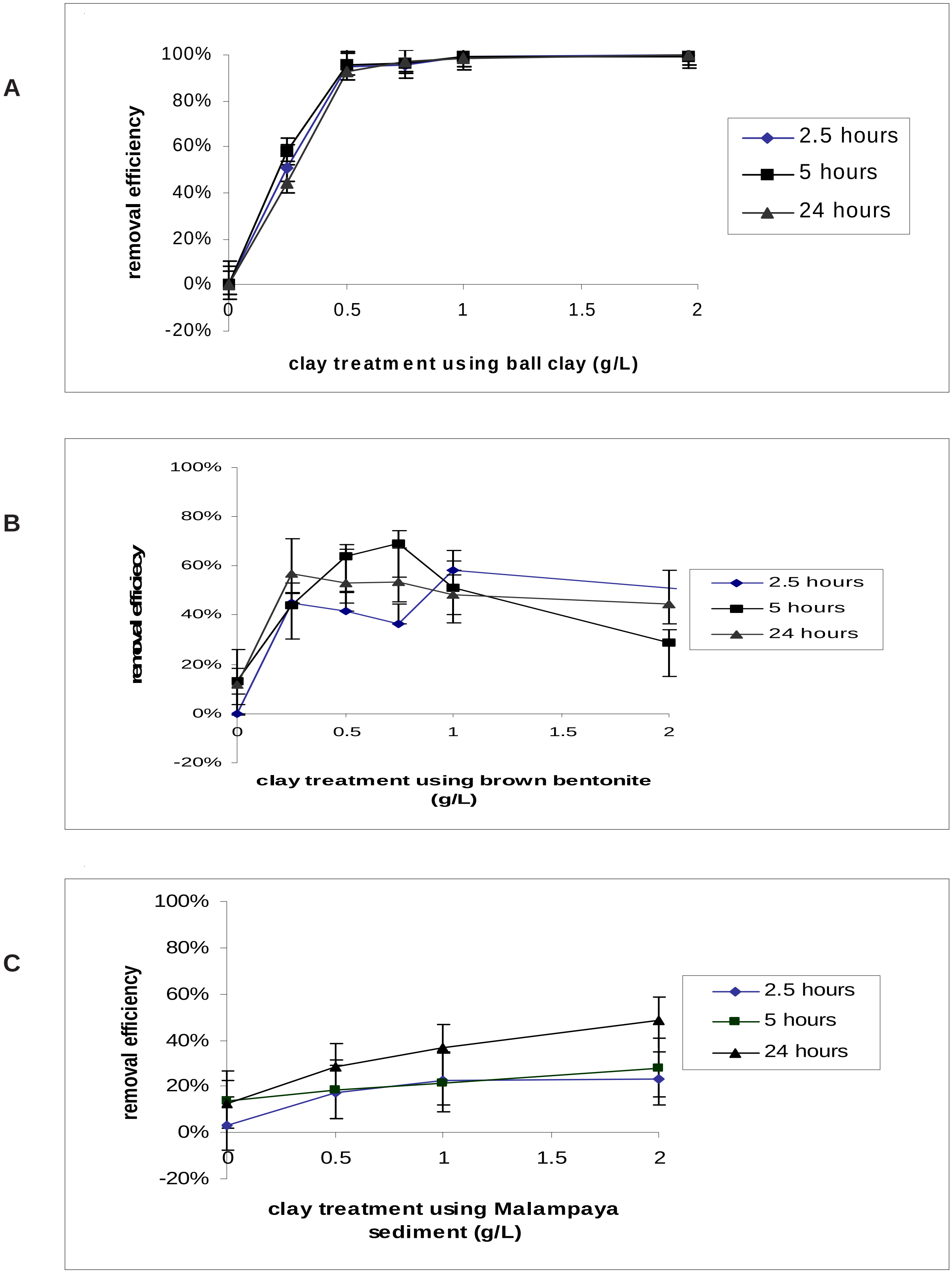


Figure 2. Removal efficiency of ball clay (A), brown bentonite (B), and Malampaya sediments (C) with increasing clay treatment.

bentonite. From visual inspection, Malampaya sediments have coarser particles, which may explain the low removal efficiency.

The relatively uniformly distributed particle sizes in brown bentonite (Table 1) could have hastened the settlement of the clay as a result of differential sedimentation. The small sized clays may have been intercepted by the denser large sizes, thus rapidly gaining size/mass for settlement and lessening the contact period between clay and the *Pyrodinium* cells. Differential sedimentation can result in increased effectiveness only when majority of clay and cells are near but not identical in sizes, as most likely the case for ball clay and *Pyrodinium* cells.

Particle Size (μm)	a) Ball clay	b) Brown bentonite
>63	0 %	14.31 %
31-63	8.6 %	2.81 %
16-31	88.17 %	8.10 %
8-16	0.2 %	15.90 %
2-8	0.16 %	31.13 %
<2	2.87 %	27.75 %

Table 1. Particle size analysis of ball clay (a) brown bentonite (b).

The results of this study suggest that even at 2 g/L, >99% removal efficiency is reached. In other studies, there was 100% removal of *Pyrmnesium parvum* and 86.6% removal of *Aureococcus anophagefferens* when 4 g/L of phosphatic clay was used (Hagstrom and Graneli, 2004; Sengco et al., 2001). As much as 10 g/L of yellow loess clay was used in the removal of 70-80% of *Cochlodinium polykrikoides* (Choi et al., 1998). According to Yu et al. (1994) 90% of *Prorocentrum minimum* was removed using 0.1 g/L of kaolin with the coagulant PACS (polyhydroxy aluminum chloride) added.Removal efficiency increased to >95% using 0.5 g/L kaolin and PACS. The concentration of ball clay slurry (2 g/L) in this study that produced the highest removal efficiency fall within the range of concentration reported by other studies.

Ball clay, brown bentonite, and the Malampaya sediments were subjected to the same exposure periods and all these materials underwent immediate reaction with the cell on the first 2.5 hours of clay addition. Prolonging the exposure of the *Pyrodinium* cells to the 24th hour increased the removal efficiency of Malampaya sediments but removal using ball clay and brown bentonite remained the same (not significantly different at 0.05 level). There is even a possibility that *Pyrodinium* cells can be removed faster than 2.5 hours. The unchanging removal efficiency with ball clay and brown bentonite after 24 hours suggests the potential of clay addition as a mitigation strategy. In a study done by Archambault et al. (2004), removal of *Karenia brevis* was 40% after 14 days. After 48 hours of clay addition, the removal efficiency of the dinoflagellate *Heterocapsa triquetra* was at 100% (Pierce et al., 2003). Further studies must be done to assess the removal efficiency of ball clay beyond 24 hours. There may also be a need to determine if flocculation is permanent or temporary and hence dependent on resuspension processes that can release *Pyrodinium* back to the water column.Temporary flocculation was observed with brown bentonite, an indication of weak attachment of the particles. Weak compaction of the bottom clay material may also result to escape or detachment of the cell.

Effect of clay addition on seawater nutrient concentration

An evaluation of the effect of clay addition on seawater nutrient content is necessary to determine any changes in water quality conditions since this may adversely affect marine organisms in the water column. There was a significant increase in phosphate concentration with increase in clay treatment (P= <0.0001 at α 0.05) and exposure periods (P= 0.0012 at α 0.05) during the 1st run with no significant difference observed in clay treatment (P= 0.2316 at α 0.05) and significant decrease (P=0.0112 at α 0.05) after 24 hours in a second run (Table 2). A significant decrease in nitrite after 24 hours was observed during the 1st and 2nd run (P= 0.0013 and P= 0.0064 at α 0.05, respectively). A significant decrease in nitrite with increasing clay treatment during the 1st run (P= 0.0780 at α 0.05) was obtained but not in the 2nd run (P= 0.0047 at α 0.05). The difference in

NUTRIENTS	First Run			Second Run		
Clay concentration	0 hours	5 hours	24 hours	0 hours	5 hours	24 hours
Phosphate						
0 g/L clay	0.08	0.07	0.09	0.23	0.2	0.14
0.5 g/L clay	0.08	0.16	1.45	0.23	0.19	0.1
1 g/L clay	0.08	1.63	1.48	0.23	0.22	0.17
Ammonia						
0 g/L clay	4.18	3.58	2.67	2.82	2.69	2.26
0.5 g/L clay	4.18	3.8	2.73	2.82	3.09	2.85
1 g/L clay	4.18	4.19	3.33	2.82	3	2.74
Nitrite						
0 g/L clay	0.05	0.09	0.09	0.07	0.07	0.06
0.5 g/L clay	0.05	0.11	0.09	0.07	0.06	0.05
1 g/L clay	0.05	0.09	0.08	0.07	0.06	0.05
Nitrate						
0 g/L clay	0.52	0.63	0.57			
0.5 g/L clay	0.52	0.5	0.46			
1 g/L clay	0.52	0.33	0.3			

Table 2. Phosphate, ammonia, nitrite, and nitrate concentrations at 0, 5 and 24 hours after addition of 0, 0.5 and 1 g/L of clay

results between the 1st and 2nd run for varying clay treatment concentrations may be due to the different seawater samples used in the two runs. Seawater during the 2nd run had higher phosphate concentration. Clay materials tend to adsorb or desorb phosphate depending on seawater phosphate levels (Lake and Macintyre, 1977).

No significant change was observed with ammonia on both runs. The decrease in nitrate concentration was determined to be significant (P= 0.0020 at α 0.05) with increase in clay treatment.However, prolonged exposure periods showed no significant change (P=0.8319 at α 0.05) in the concentration of nitrate.

Different types of clay vary in their effects on the concentration of nutrients in seawater. Anderson et al. (2004) showed that the cationic polymer-treated Kaolinite material did not release any nutrient. However, the other two clays that were tested, bentonite and Florida phosphatic clay, released nitrate and silicate and/or phosphate, respectively. Moreover, the addition of Polyaluminum chloride (a coagulant) to Kaolinite helped remove phosphate and nitrate from seawater. According to Black and Waring (1976), addition of Kaolinite and lowering the pH increased the adsorption of nitrate. In which case the nitrate attached to the clay particles are carried to the bottom upon settlement of the clay particles.

The preliminary results of this study showed a promising method to control *Pyrodinium* blooms using ball clay. It has the potential to remove more than 95% of the algal cells in the water column after 2.5 hours in controlled laboratory condition. However, a suitable technique for ball clay application in the field should be investigated. The impact of clays on benthic organisms must also be examined.

ACKNOWLEDGEMENTS

This project received support from the Philippine Council for Aquatic and Marine Research Development (PCAMRD) of the Department of Science and Technology (DOST). The authors would like to thank Ms. Estrelita Flores for her assistance and Dr. Fernando Siringan for providing the Malampaya Sound sediments.

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