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Effect of different concentration of soil extracts on the growth of *Pyrodinium bahamense* var. Compressum

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Abstract
Pyrodinium bahamense var. *compressum* (*Pbc*) the main species that caused Paralytic Shellfish Poisoning (PSP) in Sabah has been extensively studied for the toxin content. The difficulty of maintaining *Pbc* culture to reach the desired concentration is explained by its specific nutrition needs. The primary purpose of this research is to determine the effect of soil extracts in improving cell growth of *Pbc* in laboratory conditions. *Pbc* cells were grown for 22 days in F/2 media enriched with different concentrations of soil extracts (0%- 35%). The culture was maintained in a temperature-controlled room at 25±0.5 ºC. The cells were illuminated at 100 µmol $m^{-2}s^{-1}$ for 12 hours (12:12 LDC). Cell growth was measured by counting the cell numbers using Sedgewick-Rafter cell on every one-day interval. Result of the experiment indicated that the growth of *Pbc* is significantly affected ($p=0.02$) by the concentration of soil extract. The fastest growth rate (0.287 K^{-1}) with doubling rate of 0.384 d^{-1} was recorded in the culture with addition of 14% soil extract.

Keywords: Red tide, din flagellates, paralytic shellfish poisoning, f/2 media

1. Introduction
Since the first report of *Pyrodnium bahamense var compressum* (*Pbc*) blooms in 1976, HABS in Malaysia, especially in the coastal water of western Sabah give a severe impact on human safety and aquaculture industry. *Pbc* is seawater planktonic dinoflagellate (undergoes photosynthesis with golden chloroplast) associated with toxin producer (Paralytic shellfish Poisoning (PSP)) in the aquatic environment mainly in the Indo-Pacific and the tropical Atlantic oceans and quite abundant in Southeast Asia water [1]. Growth of *Pbc* highly depends on salinity, temperature, light excess as well as macro and micro-nutrient requirement. Temperature and salinity of seawater desirable for *Pbc* are varies depending on the area and location of the oceans [2, 3, 4, 5]. It has been researched that *Pbc* may have specific nutrient requirements for blooms development. Two main, macro-nutrients are required for appropriate growth of *Pbc* cell, which is: Phosphorus (P) and Nitrate (N) [5]. With little excess of these two main macro-nutrients will slow down the growth rate of *Pbc* cells or turn the cell to cyst stage until ideal condition reach [6]. Until 1982, *Pbc* cells have been subjecting of few studies due in large part of the failure to provide laboratory cultures of this species [7]. The only cited as success during that time was by Harada [8] who only able to maintain the clone from Palau for a few years. Besides, this species also can grow easily in certain of the commonly filtered seawater-based culture media such as ES-DK [9] and f/2. However, cell densities in culture media, usually less than 10,000 cells/mL, still lower as compared to cultured cells of *Alexandrium spp* [10]. Some studies found that using microelement in soil extract will enhance the cultivation of microalgae. In Another study proved that soil extracts initiate rapid cell division and initiate rapid growth in certain microalgae species. Therefore, this study was conducted to identify the effects of different percentages of soil extract in f/2 medium to the cultivation of *Pbc*.

Materials and Methods
A. Preparation of soil extract
Methods for the soil extract preparation were based on previous experiments done by Teo [11]. with slightly modification. Black soil (using for gardening) was bought from the local nursery.

The soil was dried overnight at temperature 40^0C in the oven. Dried soil was then mixed with filtered sea water with the ratio 1:2, 1kg of dried soil mixed with 2 liters of filtered sea water. After the mixture settling down for 4 hours, the particles in the mixture was removed. Soil solution was then filtered using 0.45µm filter paper and the filtered soil solution was autoclaved at temperature 120^0C for 25 minutes. Sterilized soil extract was stored in the refrigerator for further use. Seawater used during preparation of f/2 media was replaced with soil extract solution according to the different percentage of soil extract required in the media.

B. Cell cultures and growth study

The strain of *Pbc* were maintained in the lab condition using f/2 medium without silicate. The growth experiment was conducted at five varying percentage of soil extract; 7%, 14%, 21%, 28% and 35% with controlled environment of temperature 25 ±2 ^{0}C, light 100 µmol^{-2}. S^{-1} on 12h: 12h light: dark cycle and salinity 30 ppt. The culture inoculated with ~50 cell/ml in 500 mL flask containing 300 ML of Sf/2 media mixed with different percentage of soil extract solution and gently shaken every day.

C. Cells harvesting and enumeration

I Ml of culture was sampled every interval day from experimental flask. Cells were preserved in Lugols iodine and counted using Sedgwick rafter under 400 X magnifications. One-way analysis (ANOVA) was used to determine the significant differences in growth rate in different percentage of soil extract mixed with f/2 media.

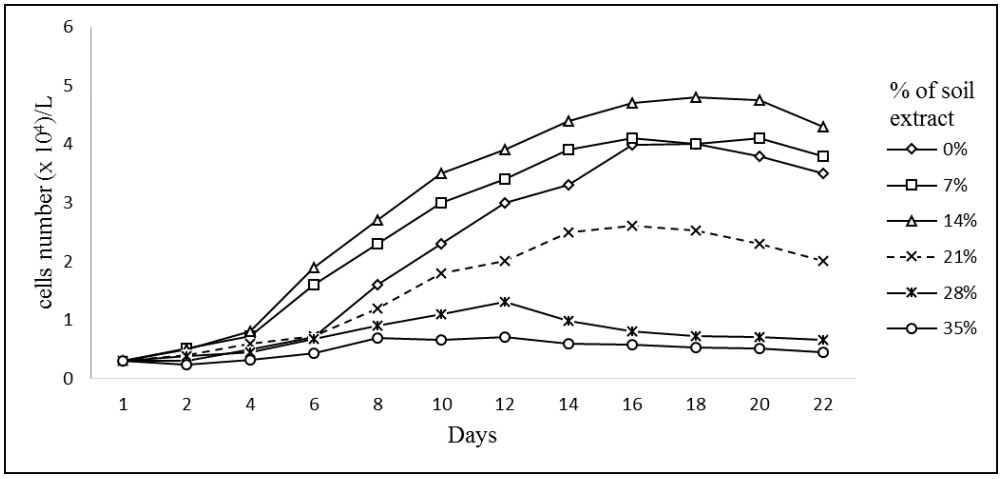

Fig 1: Growth curve of Pbc at different soil concentration mixed with f/2 culture media

Results and Discussions

Growth curve of *Pbc* clearly showed that the fastest growth rate (0.287 K^{-1}) with doubling rate of 0.384 d^{-1} division rate per day. was recorded in the f/2 culture media with addition of 14% soil extract (figure 1).

The highest cell density recorded at this concentration was 4.8 x 104 ± 487 cells/L. At high concentration (28-35%) of soil extract, minimal growth of *Pbc* was detected. One-way Anova indicates that cell density of *Pbc* cells were significantly different among all the concentration tested ($p< 0.05$). Soil extracts have numerous trace elements and vitamins which are usually introduced to culture media and essential for algal growth and production. These include iron, manganese, zinc, cobalt, copper, molybdenum, vitamin B12, thiomine, and biotin [12]. Teo [11]. found that with the presence of manganese in the soil extract is importance for the dinoflagellate growth. Manganese is an important nutrient required in the cell for catalyzing oxygen evolution in photosynthesis process [13, 14, 15]. However, at high manganese concentrations will inhibit the growth of cell due to high extracellular amounts of manganese oxides, which may disturb the nutrient uptake system in the cells. This is maybe one of the reasons at 28 and 35% of soil extract concentration, the *Pbc* growth was slow. The result of this experiment then compared with the study done by Usop [16]. Usup [16] was found that soil extract supplement did not significantly affect division rates but resulted in making longer of the exponential phase of *Pbc* cell growth. As a result, densities of *Pbc* cell reached to the maximum in when soil extract was added to the medium. Usup [16] provided strong evidence that the soil extract provided the source of selenium to the media. The highest densities of *Pbc* cells obtained in Selenium supplemented cultures was comparable to or even better than cell densities obtained in soil extract supplemented cultures. Results from the same study also found that *Pbc* could utilize selenite and organic selenide but not selenate. It is important to note, that the presence of mineral and nutrient in soil extract relies on source of soil used during extraction procedure. Not all of these essential minerals required in dinoflagellate found in every type of soil [17].

Conclusion

These findings provide a basic understanding on modified F/2 with the addition of different concentration of soil extract help the growth of *Pbc* cells. From this study found that 14% of soil extract mixed with f/2 media can increase the cell growth of *Pbc* cells. It will be important that future research investigates the exact component of minerals or nutrient presence in the soil extraction that essential for the culture of *Pbc* cells.

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