

SURVIVAL OF PATHOGENIC BACTERIA IN DREDGED MARINE SOILS

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Graphical abstract



Abstract

Dredging work involves a range of marine soils, varying from coarse to fine, clean to contaminated. The work includes excavation and disposal phase, may affect the marine environment through releasing of the possible contaminants. The biological hazards in marine soils were observed to identify the present of pathogenic bacteria which may have adverse effects on human health and ecosystem. Numbers of pathogen, including the indicator bacteria, *Escherichia coli* have been used to assess the level of contamination in marine environment. The effect of natural factors including salinity, pH, soils particle sizes and solar exposure were investigated. The main goal is to understand bacteria survival ability, as an approach to deal with the hazards.

Keywords: Dredged marine soils; pathogenic bacteria; environmental factors

Abstrak

Kerja-kerja pengorekan melibatkan pelbagai jenis tanah laut, berbeza-beza dari kasar kepada halus, bersih sehingga tercemar. Kerja-kerja ini termasuk fasa penggalian dan pelupusan, boleh menjejaskan alam sekitar marin melalui pengeluaran bahan cemar. Bahaya biologi dalam tanah laut diperhatikan untuk mengenal pasti kehadiran bakteria patogenik yang mungkin mempunyai kesan buruk ke atas kesihatan manusia dan ekosistem. Pelbagai patogen, termasuk bakteria penunjuk, *Escherichia coli* telah digunakan untuk menilai tahap pencemaran dalam persekitaran marin. Kesan faktor-faktor semulajadi termasuk kemasinan, pH, saiz zarah tanah dan pendedahan solar telah dikaji. Matlamat utama adalah untuk memahami keupayaan survival bakteria, sebagai pendekatan untuk berurusan dengan bahaya.

Kata kunci: Tanah kerukan laut; bakteria patogenik; faktor alam sekitar

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1.0 INTRODUCTION

Over recent years, there has been an explosive growth being largely surrounded by the sea, Malaysia has a large number of people who live along its 4675 km of coastline. Almost 60 % of the populations are located in the coastal district. The Malaysia coasts play an important role in economic activity due to the presence of ports for trade,

infrastructure for tourism and recreational activity, as well as petroleum exploitation and refining [1]. According to [2], there are a total of 60 ports in Peninsular Malaysia, 11 ports in the state of Sabah and 36 ports in the state of Sarawak. Due to the deposition of sediments in waterway, this natural process could bring various disadvantages effect to local environment, channel and navigation areas as well. Necessity of maintaining safe navigation in ports, harbours and marina then required dredging

work to be made. The process involved excavating and removal of unwanted marine soil from the bottom of harbours and waterways to another area. Besides maintaining navigation, dredging work could help in ensure safe passage of ship and allow larger vessels to travel up rivers [3]. In addition, dredging work can occur either at fresh water, brackish water or saltwater environment [4].

A growing number of human bacterial infections have been associated with recreational and commercial uses of marine resources [29]. A study by [30] had found a high number of pathogens been reported from the marine environment. Some pathogens such as *Vibrio* occur naturally in marine water while the others from fecal contamination sources. Feces in sediments which contain bacteria, viruses and protozoa can be ingested and also cause health problem such as intestinal diseases.

For instant, pathogenic bacteria find sediments more favorable as a habitat than in overlying water [5]. Previous studies have also found that sediments can contain 100 to 1000 times as many bacteria than the overlying water. *Escherichia coli* (*E. coli*) and pathogenic bacteria are associated with sediments where the properties and condition of the sediment could influence their survival and transportation [6]. Due to the risk of pathogens in the marine soils, the opportunities for the reuse of the dredged material have been decrease. In the recent study, it shows the influence of microorganism community in measuring the soil quality. Assessment of microorganism population in the dredged marine soils would give an idea about the degree of contamination occurred in that soil [7]. It is not possible for monitoring all pathogens bacteria in marine environment. For this reason, the fecal indicator bacteria, *E. coli* was used to determine the factors affecting the survival of pathogenic bacteria in dredged marine soils.

2.0 EXPERIMENTAL

The dredged marine soils sample was obtained from dredging work at marine. The marine soils was dredged at a depth of 3.5 m and packed in sampling bags and maintained at 4 °C before analysis. Figure 1 shows the sampling sites at Marina Melaka. The sample for dredged marine soils was collected from river mouth of Marina Melaka. The soils collected during dredging work were stored in the sterilizer sampling bag and put in the polystyrene box. The box was covered with an ice to keep the samples cool during transportation time. At the laboratory, the samples were kept at temperature 4 °C in the dark condition. The soils were manually mixed to prepare for the homogenous condition. Samples then mixed with the sterilize water for soils solution preparation. Once supernatant was retrieved, they were immediately cultured onto agar. Plate Count Agar, Xylose Lysine Deoxycholate,

Chromocult Coliform Agar and MacConkey agar were used to isolate the pathogenic bacteria. Subculture of *E. coli* was obtained by transfer colonies from the plate culture contain CCA to a growth media. The growth media was sterilized before being inoculate with the bacteria colonies. The fresh inoculate medium allowed for bacteria growth as normal until such time the cells are used for experiments.

Survival of *E. coli* under salinity exposure was determined in the salt solution. Microcosms were prepared with the dominated salt solution in soil, NaCl. Each microcosm consisted 50 ml of salt solution brought to appropriate salinity. Salinity of solution was expressed in parts per thousand (‰). The salinity values from 0 until 35 ‰ covering much of the range occurring from fresh until saline environment.

Microcosms with salinities 5, 10, 15, 20, 25, 30 and 35 ‰ were prepared and inoculated with 1 ml of culture consists 10^2 CFU of *E. coli*. The microcosms were prepared in triplicate and incubated at room temperature for 42 days.

For solar exposure factor, the solar simulator used was a tropical lamp (EXO Terra 26 W). The lamp emitted optimal levels of UVA and UVB radiation similar to the environment in tropical region. Based on the technical data provided by manufacturer, 10 cm distance between the lamp and the samples is a recommended distance in obtaining optimal levels of UVA and UVB radiation. The experiment was carried out using modification method by Garzio-Hardick et al. [24]. A glass box, used as reactor with dimension of 49 cm long, 25.5 cm depth and 24 cm height was contained soil column made from PVC pipe to hold the soils samples. The 20 cm soil column was divided into 5, 10, 15 and 20 cm subsamples.

The light was directly irradiated onto a surface of the samples contained in the soil column. At the 10 cm distance, the temperature of the samples was measured as high as 32 °C. Under the condition without the existence of predatory microorganisms, experiments are performed at nine hours of solar exposure and subsequent 15-hours darkness. The period of solar exposures were meant to stimulate the period of Malaysia receive solar radiation. The subsamples was then assess for the number of *E. coli* survive. Different experimental conditions were evaluated at its origin pH and salinity level. The effects of particle sizes in bacteria survivability were studied as well. For all experiments, the initial bacteria concentrations were 10^6 – 10^7 CFU/ml. Bacteria were grown overnight in the soils column to allow the acclimatization process [25].

Tryptic Soy Broth (TSB) was used as a culture medium in pH effects experiment [26]. TSB pH value in the flask was adjusted by drop-wise addition of 1M hydrochloric acid (HCl) or sodium hydroxide (NaOH) before autoclaving. The pH values obtained were 6, 7, 8 and 9. In order to measure pH effects on the survival of *E. coli*, the culture medium were inoculated with 10^6 CFU per 1 ml of bacteria. The flasks were then sealed and incubate for 5 days at

37°C. The number of *E. coli* was counted after 24 h of incubation.

Survival of *E. coli* under salinity exposure was determined in the salt solution. Microcosms were prepared with the dominated salt solution in soil, NaCl. Each microcosm consisted 50 ml of salt solution brought to appropriate salinity. Salinity of solution was expressed in parts per thousand (‰). The salinity values from 0 until 35 ‰ covering much of the range occurring from fresh until saline environment. Microcosms with salinities 5, 10, 15, 20, 25, 30 and 35 ‰ were prepared and inoculated with 1 ml of culture consists 10² CFU of *E. coli*. The microcosms were prepared in triplicate and incubated at room temperature with absence of dredged marine soils. This is to study the effect of salinity alone on the survival of *E. coli*. The number of *E. coli* was determined starting from before incubation until 42 days.



Figure 1 Location of dredging work at Marina Melaka.

3.0 RESULTS AND DISCUSSION

3.1 Identification Of Pathogens Bacteria In Dredged Marine Soils

Several genus of bacteria were founded in samples, namely *Serratia* sp., *Vibrio* sp. and *Enterobacter* sp. According to the Malaysia Biosafety Clearing House (2010), the variety of these bacteria was fall into Risk Group 2. The bacteria in this group could cause human diseases, but effective treatments are available.

Table 1 Pathogens bacteria in dredged marine soils and risk group level

Bacteria Species	Risk group level
<i>Vibrio vulnificus</i>	2
<i>Serratia marcescens</i>	2
<i>Edwardsiella tarda</i>	2
<i>Bacillus cereus</i>	2
<i>Escherichia coli</i>	2

3.2 Effect Of Pathogens Bacteria

Vibrio is a gram negative bacteria which is only found in the marine environment as it requires salt for growth. This bacterium has been isolated from water, marine soil and a variety of seafood indicating its ability to survive almost indefinitely [8]. Transmission of *Vibrio* is normally through the consumption of raw or undercooked shellfish or exposure of wounds to seawater. There are at least 12 pathogenic *Vibrio* species recognized for causing human illness. Among the isolated species in DMS, *Vibrio vulnificus* is one of the most medically significant because of the high fatality rates associated with the infection. Three major syndromes of clinical illness caused by pathogenic *Vibrio* are wound infection, gastroenteritis and septicemia. The bloodstream infections associated with *Vibrio vulnificus* may results in death within hours [9].

Serratia marcescens obtained in the DMS sample is one of the species from genus *Serratia* sp. It is the one most frequently recovered from humans [10]. A study by [11] also founded various pathogenic bacteria in sediment collected from Matang, Malaysia including *Serratia marcescens*. As a member of Enterobacteriaceae, the bacteria can be found in soil, water and plants. This species could contribute to more death than many pathogenic bacteria [11].

Edwardsiella tarda a pathogen of fish and other animal had isolated in DMS sample. The bacteria can also be found in freshwater, marine environments and in all animals living in these environments [12]. As a member of the Enterobacteriaceae family, this bacterium is an unusual human pathogen and it is associated with gastrointestinal diseases, wound infection and systematic diseases such as meningitis. It was reported that *E. tarda* has a broad host range and geographical distribution and contains virulence factor that enhance bacterial survival in hosts [13].

Bacillus cereus was founded in DMS. It was from *Bacillus* sp. species. Marine environment has been reported to be the natural reservoir for *B. cereus*, from which the water and soil may become contaminated, leading to transient colonization of the human intestine. For instance, the soil can contain between 10³ until 10⁵ per gram of *B. cereus*. Due to the widespread distribution of *B. cereus* in marine environment, this species is the important cause of food-borne illness in humans and has been related with the vomiting or diarrhea poisoning [14].

The Fecal Indicator Bacteria (FIB), *Escherichia coli* (*E. coli*) has been isolated in the DMS, indicating potential fecal contamination in the marine soils. Besides, the presence of *E. coli* suggests the presence of pathogenic bacteria in marine soils too. Potential sources for the presence of *E. coli* may come from sewage overflow, leaking septic system, fecal material from humans, seagull, and other warm-blooded animals as well [6].

3.2 Environmental Factors

3.2.1 Salinity

In coastal areas, high salinity in soil occurs because of the soil is inundated with salty seawater [15][16]. According to Xu et al. [16], soil salinity also varies with soil depth, where decreasing soil salinity was observed with increasing soil depth. The salinity impacts the bacteria by posing unwanted stress on the cell. The membranes of cell are permeable to water but not to the substances that are dissolved in water. The organisms must keep balance of water and solutes (salt that are dissolved in water) to keep their cell alive. The movement of water across a membrane due to the differences in solute concentration is called osmosis. As the cell takes much of water, it will swell and possibly burst. In contrast, if a cell loses much of water, it will dehydrate and dies. However, the pathogens bacteria found in this study are often isolated from the marine environment due to their ubiquity and capability to survive under adverse conditions [17].

E. coli were tested for their ability to survive at different salinity levels (Fig. 2). The concentration of *E. coli* was found to increase until day 3. At the salinity of 35 ‰, the growth rate of bacteria was higher. The lower growth rate of bacteria was found at the salinity 5 ‰. The growth rate of bacteria at salinity 35 ‰ and at 5 ‰ during day 3 was 183 % and 121 % respectively. These results was opposed to the observation by [26], where lower concentration of bacteria was observed at high salinity levels during 72 hours (3 days) of exposure, indicating no cell multiplication occurred at this level. Instead, the number of bacteria reached zero at salinity 20 ‰. The bacteria decreased at day 7 but remained high when compared to the initial concentrations. Maximum decrease of bacteria occurred in the control microcosm (0 ‰ salinity) with 43.6 % of bacteria found unable to survive. The smallest reduction of bacteria was present in 35 ‰ salinity where about 92.0 % of bacteria remained after 7 days exposed to the saline environment.

The number of bacteria continued to decrease during day 14 of experiment indicating the decay of bacteria. The results demonstrated that 35.0 – 49.0 % of bacteria could not survive at salinity range of 20 ‰ to 35 ‰. The probable reason for this high percentage of reduction in high salinity could be due to the damage of bacterial cell [27]. By day 21 of the experiment, the *E. coli* managed to survive at salinity 30 ‰ and 35 ‰ with low percentage of reduction. The bacteria probably had slow down the multiplication process; enable the longer survival of *E. coli* at high salinity. Maximum reductions were detected at lower salinity microcosms. Similar trends for bacteria survival was observed for all salinity at day 28 and 42. The bacteria concentration did not further increased.

During the last day of experiment, bacteria concentration was lower compared to the starting

concentration at all salinity level except at 25 ‰ until 35 ‰. Only 60 – 70 % of bacteria remain survive at lower salinity levels while 83 – 90 % of bacteria retain survive at high salinity levels.

Some instability observed in the number of survival bacteria is mainly due to the cell's ability to equilibrate their internal pressure relation with the salt concentration. The longer survival of *E. coli* in salinity 25 until 35 ‰ showed that it can survive in high salinity levels probably due to the presence of osmoprotectors. The bacteria may stimulate cellular protection system to slow down the effects of unstable environment condition. These responses are used to adapt to variation in salinity level as well [28]. Thus, this study showed that the *E. coli* are able to survive, even under salinity stress conditions.

3.2.2 Solar Exposures

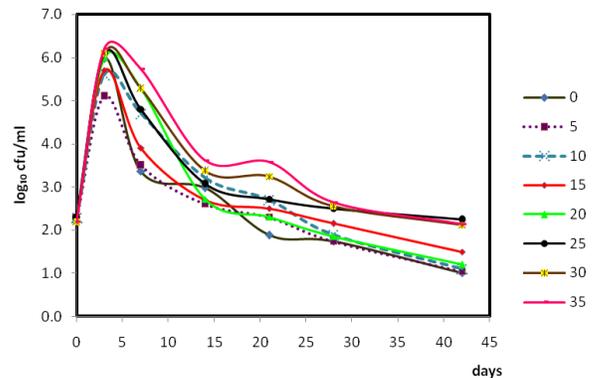


Figure 2 Survival of *E. coli* at different salinity level

The survival of bacteria was found to steadily decrease as high as 45 % in the first layer of soils until day 5 of the experiment. As can be seen (Fig. 3), the bacteria have migrated to deeper depth of soils, seeking for the protection from solar exposures. Until end of the experiment, least bacteria survived at the top layer of soil (5 – 10 cm). This result revealed that solar exposure is important for the survival of bacteria in dredged marine soils.

Solar is considered to be the most important cause of "natural disinfection" which causing direct bacteria DNA damage. Solar exposures have been shown to affect the microorganism reduction in water environment. The best reduction of microorganisms was occurred at surface water. The reduction effectiveness decreased with increasing depth of water [18]. Evidence from these studies suggested that the effect of solar exposures has been found to be one of the significant factors affecting the ability of *E. coli* to survive in marine environment. These observation was suggest the extend survival of these bacteria in marine environment by adsorbed to marine soils particle to protect themselves from the solar exposures [19].

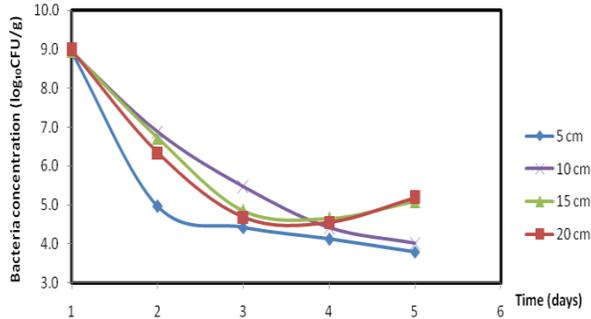


Figure 3 The survival of *E. coli* in dredged marine soils for 5 days of solar exposures.

3.2.3 pH

One of the most influential factors affecting the survival of bacteria in soils is pH [20]. As shown in Fig. 4, the survival of *E. coli* was greater at pH above 7. The maximum survival rate for *E. coli* was at pH 8. 79 % of the bacteria was survived at this pH level. Starting at pH 6 until 7, the percentage of the survival bacteria was around 70 %. While at pH 9, almost 75 % of *E. coli* was survived.

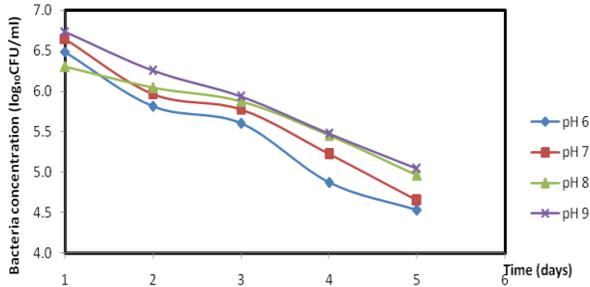


Figure 4 The survival of *E. coli* at different pH level

Excessive acidity or alkalinity makes the soil inhospitable for microbial growth. Microbial classifications based on the pH at which optimum growth occurs can be classified into three groups which are acidophiles, neutrophiles and alkalophiles. Acidophiles and alkalophiles grow best in acidic and alkaline conditions respectively. Neutrophiles refer to the microorganisms which grow best in neutral conditions. However, compare to the other factors, the effect of pH is minimal due to small variability in sediments [21].

3.2.4 Marine Soils Particle Sizes

Soil particles can vary greatly in size and are being classified according to their particle size. The size of particle is important in prolonged the survival of bacteria in the marine soils. According to Pachepsky et al. [22], after bacteria were introduced into a soil, they can either be trapped in the soil pores or adheres to the soil particles. Jaeeun et al. [23] found the irregular surface of the soil provides partial protection of bacteria against exposure to solar radiation. Therefore, once 99 % (2-log) reduction of

bacteria was achieved, no further reduction was observed regardless of the duration or intensity of solar radiation. These finding shows that marine soils not only prolong the survival time of *E. coli* but also support their growth at a same time.

4.0 CONCLUSION

In conclusion, this study represents an attempt to understand the factors that affect the presence of pathogenic bacteria in marine soils. The study was conducted for consideration of the health risk from exposure to the dredged soils. Although the DMS has been recognized as a reservoir of pathogenic bacteria, but it is unlikely to be serious hazards. The pathogenic bacteria found in DMS were categorized as Risk Group 2. Effective treatments are available. Yet the risk of infection is limited. The analysis in this study indicated that the survival of bacteria in DMS is affected by solar exposure, salinity levels, pH and particle sizes. With the presence of solar exposure, the bacteria in soils were reduced up to 99 % during the first hour of exposure. However, due to the other factors such as soil depth and particle sizes, the bacteria can survive and growth at the same time. The bacteria were also found to survive at high salinity level. The high survival rate was found to be in the salinity range of 25 to 35 %. Based on the observation in this study, decreasing salinity levels reduced the survival rates of bacteria. This is suggestive that the bacteria used in this study were intolerant to nonsaline environment as they were isolate from a saline environment. The bacteria seemed to survive best at pH 8 and 9. Approximately 75 % of the bacteria survived in this alkaline condition. As the bacteria were derived from an alkaline condition, they were more adaptive to the pH range of their natural habitat.

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