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TOLERANCE OF *ESCHERICHIA COLI* IN DREDGED MARINE SOILS UNDER ARTIFICIAL SOLAR EXPOSURES

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ABSTRACT

Dredging work involves a range of marine soils, varying from coarse to fine, clean to contaminated. Dredging involves excavation and disposal and both processes could affect the marine environment through release of possible contaminants. Due to the potential for transmission of diseases, this hazard becomes a major concern as the Dredged Marine Soils (DMS) have its own values for reuse or recycle purposes. To prevent the needless cost and time involved in assessing several of pathogen bacteria, the indicator bacteria, *Escherichia coli (E. coli)* been used to assess the biological contamination in marine environment. The effect of natural factors, solar exposure and depth of soil were investigated. The main goal is to understand bacteria survival ability, as an approach to deal with the hazards. Under the condition without the existence of predatory microorganisms, experiments are performed at nine hours of solar exposure. In laboratory solar exposure experiments, the data dealing with the survival of bacteria showed that the cell reduction was more pronounced when compared to the absence of solar threat. After an extended period of solar exposures, as high as 90 - 99 % of *E. coli* have being removed.

Keywords: *escherichia coli,* dredged marine soils, solar exposures, depth of soils.

INTRODUCTION

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 (Lee, 2010).

According to Khalid *et al.* (2011), there is 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 (London Convention, 1972). 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 (Manap, 2008).

Dredged materials are sediments derived from the excavation of lagoons, harbours, canals, river and marine areas (Lee *et al.*, 2010). In addition, dredging work can occur either at fresh water, brackish water or saltwater environment (Nicholas, 2011). In general, there are five types of dredged sediments, namely rock, gravel, sand, clay and silt (IADC, 2009). The sediments are classified based on the particle sizes as described in Part 2.3.1 (Manap & Voulvoulis, 2015). In situations where the sediments are dredged from contaminated areas, they are classified as toxic materials (Baruzzo *et al.*, 2006). The dredged sediments with no economic values are disposed or stored at the edge of water bodies (Barbosa and Almeida, 2001). However, a number of recent studies have investigated on the potential of dredged material as a new resource (Jin *et al.*, 2011; Baruzzo *et al.*, 2006).

The survival of *E. coli* which is the indicators of fecal contamination in marine environment has been related to several factors of its surroundings. The results of past studies suggest the involvement of environmental factors such as solar exposures; temperature, salinity, nutrients, predation, and pH are influencing the ability of *E. coli* to survive (Manjit, 2002). These factors will influence the survival by affecting their growth and death (Janelle *et al.*, 2006).

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 (Sigrid *et al.*, 2006). Solar exposures are proposed to be a factor contributing in the ability of *E. coli* to survive in marine environment. These observation suggest the extend survival of these bacteria in marine environment by adsorbed to marine soils particle to protect themselves from the solar exposures (Davies *et al.*, 1995).

According to Troussellier (1998), solar exposures impact the ability of a bacteria cell to form the colonies which explained their increasing in mortality rate after being exposed to the solar exposures.

A study by Rozen and Belkin (2001) revealed that the solar exposures inducing the bacteria DNA damaged and led to decreasing in bacteria concentration. The survival of *E. coli* was decreased with the increasing of exposure time to the solar exposures. The survival was declined with decreasing cloud cover (Hughes, 2005). During this time, high intensities of solar exposures were lower the ability of *E. coli* to survive (Chigbu *et al.*, 2005).

The time required for the survival of *E. coli* ranged from few hours to few days depend on the environmental conditions. A study by Solic *et al.* (1992) found that the survivals of *E. coli* were decreased for each 100 Watts/m² increased in solar exposures. 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 (Yukselen *et al.*, 2003)

MATERIALS AND METHODS

Materials

The sampling sites were chosen based on schedule of dredging work in East Malaysia by Malaysia Marine Department. Figure-1 shows the sampling sites at Marina Melaka. The sample for dredged marine soils was collected from river mouth of Marina Melaka. The undisturbed sample was covered with ice to keep in cool and dark during transportation time.

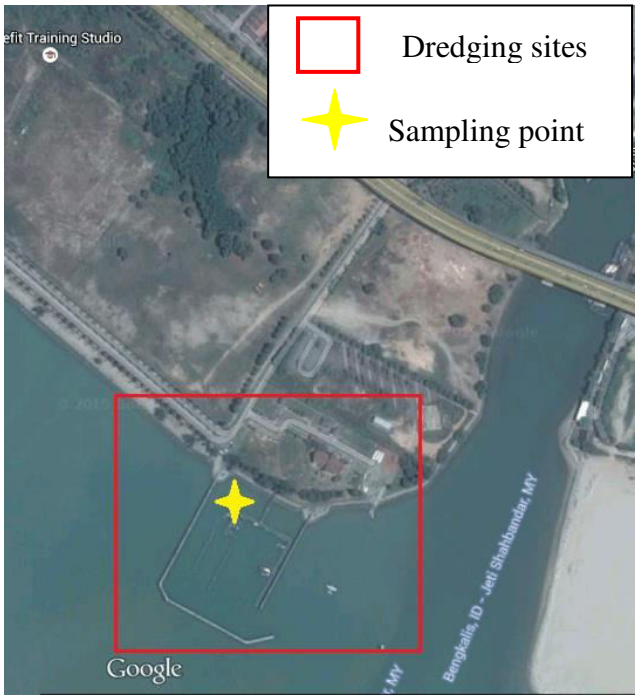

Figure-1. Location of dredging sites at Marina Melaka (Adapted from Google Earth, 2015)

Isolation of Escherichia coli

The selective media was used to isolated *E. coli* in dredged marine soils. 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. *E. coli* was isolated from the soil samples using the streak plate method. This method is rapid and simple in obtaining pure isolated colonies.

Then the plate was incubated in inverted position for 24 hours at temperature 37 ^{0}C. To avoid any contamination throughout the streaking process, the wire loop was sterilized by heating till red hot in the blue part of the Bunsen flame heat. This process was carried out before and after used the loop. After incubation period, single colony appeared on the agar was then brought with sterilized loop into pure culture namely Tryptic Soy Broth (TSB). TSB was prepared specified by manufacturer. Minimum 10 colonies were transferred into the TSB. The incubation period was 18 to 72 hours. Bacteria growth in TSB was indicated by turbidity.

Marine soils preparation

The soils used were autoclaved at 121^{0}C for 30 minutes. To confirm the sterilization of soils, the samples were isolated. No microbial growth was observed in the sterilized samples. All the experiments were conducted in triplicate. A control was set up without inoculum. It indicated there was no *E. coli* in samples.

Solar exposures effect

The experiment was carried out using modification method by Garzio-Hardick *et al.* (2010). 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. 26 soil columns were built in the reactor. The reactor holds seven rows of four lines of soil columns.

The experimental conditions was evaluated at its origin pH and salinity level. The effects time of exposures and soil depth in bacteria survivability were studied as well. In this 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 (Alkan *et al.*, 1995).

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 light was directly irradiated onto a surface of the samples contained in the soil column. At this distance, the temperature of the samples was measured as high as 32 ^{0}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.

RESULTS AND DISCUSSIONS

Generally, a result in Figure 2 agrees with recent findings that solar exposure is responsible for reducing the number of bacteria. An exposure of one hour was necessary to achieve 99 % reduction (2-log) in soils similar with the study conducted by Jaeeun *et al.* (2007). The upper 5 cm layer of marine soils in the column

demonstrated higher concentration of *E. coli* before the samples was exposed to the solar radiation.

After one hour exposure, the *E. coli* was slightly higher at 10 cm depth in the column than those counted at upper layer (5 cm).The trend was found to be similar with those in the soil profile of the present study within 3 hours of exposure. The number of *E. coli* obtained at 20 and 25 cm depth was least after 3 hours of exposures. The number of *E. coli* counted was higher at 20 and 25 cm of soil depth after 7 hours of exposure onwards. The least number of *E.coli* was counted after 9 hours of exposure in the first layer of soils (5 cm). These suggest the bacteria migrate to deeper depth of soils to survive.

A simulated solar exposure is not sufficient for the total elimination of bacteria after 9 hours of exposures. However, it is able to remove as high as 5 logs CFU. Previous study by Rubio *et al.* (2013) also reported removal as high as 5 logs CFU in water medium after 4 hours of exposures. The best survival of bacteria occurred in deeper soils. Soil depth has great effect on the survival of bacteria because it influences the solar radiation penetration (Zhang *et al.*, 2010). However, solar radiation can only penetrate to considerable depth in soils (Obernosterer *et al.*, 1999).

In a study by Arrieta *et al.* (2000), bacteria were severely affected by solar exposures stress. However, they also efficiently recover from the stress. The recovery process occur under the condition where radiation in solar has been excluded in the dark condition (Arrieta *et al.*, 2000). In the experiments on dark (in the absence of solar exposures) condition, the *E. coli* were increased after one hour being transferred compared to the number counted in the solar exposures at each soil depth layer. The experiment was meant to stimulate the night condition.

During the initial phase of the "dark" experiment, the survival of bacteria increased up to 60 % at each layer of soils. Other studies suggested that bacteria were remaining undetected under stressed conditions. As the bacteria were kept in the dark, the bacteria remained culturable. A reason for inconsistent increase in the survival rate could be that the bacteria face stresses from starvation or nutrient depletion (Gin and Goh, 2013). The numbers of bacteria counted at the end of the experiment were less compared with the initial concentration. Data from this experiment could be use in predicting the number of bacteria in another cycle of 24 hours.

For this reasons, the experiments on solar exposures effect had been extended for another 5 days (Figure-2). 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, 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. The result demonstrated that the dredged marine soils must be exposed for several days to allow for reduction of bacteria concentrations to acceptable levels

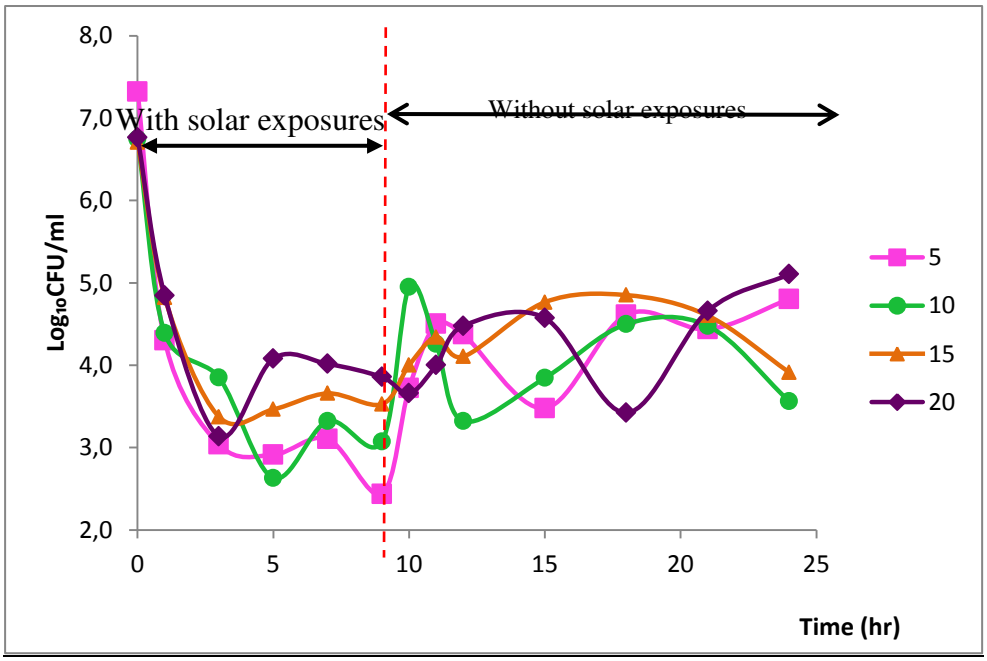

Figure-2. The survival of bacteria considering depths of soils with presence and absence of solar exposures.

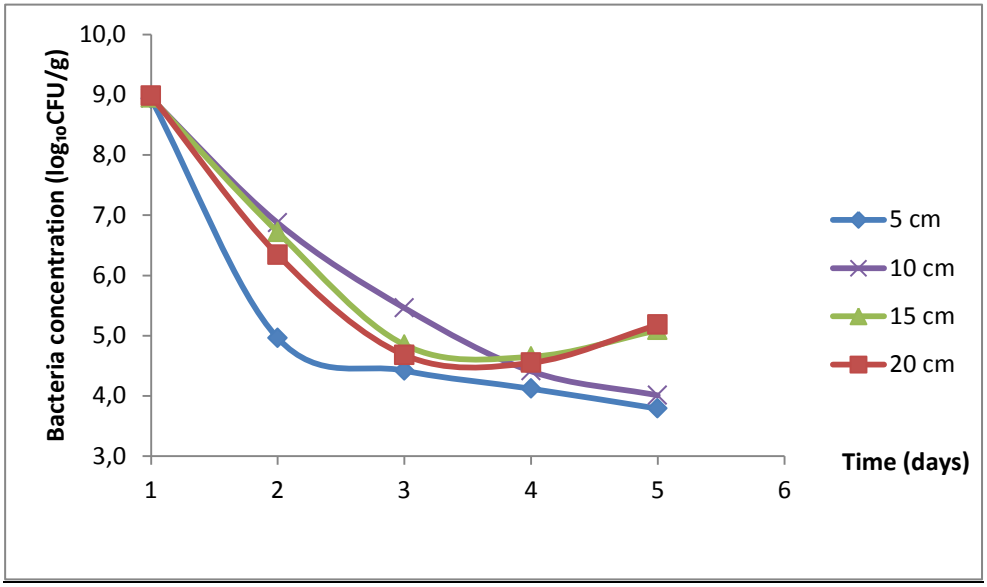

Figure-3. The survival of *E. coli* in dredged marine soils for 5 days of solar exposures.

CONCLUSIONS

In summary, this study represents an endeavor to examine and understand the factors that affect the survival of *Escherichia coli* (*E. coli*) in dredged marine soils (DMS). The study was conducted for consideration of the health risk from exposure to contaminated dredged soils. *E. coli* was used in this study due to its role in indicating the presence of fecal contamination and potential presence of pathogenic bacteria as well. Therefore, the survival of *E. coli* could indirectly point to the survival of other pathogenic bacteria in DMS.

The survival of *E. coli* does not only depend on the environmental factors but also on its adaptation capacity. The bacteria would lose their adaptation capacity where exposed to negative factors, particularly solar exposure. During these exposures, the bacterial cell would rapidly enter into a damaged state and results in death.

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