SURVIVAL OF E. coli IN SOILS OF DIFFERENT PH & TEMPERATURE

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ABSTRACT

The survival of Escherichia coli (E. coli) in tropical soils of different pH and temperature was studied under saturated moisture content. Soils with pH 3, 4, 5, 6, and 7 were inoculated with E. coli. Soil samples (pH 3 to 7) were incubated at 20°C and 30°C in triplicates for 21 days or until all bacteria were undetectable. This study showed that decay rate of E. coli increased when the pH of soils decreased. This is no significant difference between decay rate in pH 6 and 7. E. coli survived best in pH 6 and 7. Linear decay model fit the data well in most cases. A relationship between decay rate, pH and temperature was developed. Results on this study indicate pH and temperature can effect survival of E. coli in tropical soils. Therefore, the effective management in the application and disposal of animal waste should be developed based on factors of pH and temperature.

Key words: Escherichia coli, decay rate, soil, fecal coliform.

ABSTRAK


Kata kunci: Escherichia coli, kadar kemortalan, tanah, fekal koliform.
INTRODUCTION

Farmers have been using animal waste such as feces from pig, chicken and cow as an organic fertilizer to improve fertility of the soil in the agriculture land. But, animal waste may contain various types of pathogenic organisms and contribute to agricultural non-point source pollution (Reddy et al., 1981). Usually, pathogenic organisms contained in animal waste are fecal bacteria. A fecal bacterium belongs to the genera Escherichia, Klebsiella and Enterobacter (Brock and Madigan, 1991). The fecal bacterium that is most widely studied by scientist is Escherichia coli from the genera of Escherichia.

Escherichia coli (E. coli) are the most widely studied living organism and what is known about E. coli is summarized in a recent monograph (Nathan & Richard, 1997). Escherichia is a genus from the family of Enterobacteriaceae and Escherichia coli is a species (Nathan & Richard, 1997). E. coli is a short, non-spore forming and often fimbriate Gram-negative bacillus which grow readily on simple culture media or synthetic media with glycerol or glucose as the sole carbon source (Nathan & Richard, 1997). The primary habitat of Escherichia coli is the large intestine and tissues of warm-blooded animals. However, they are able to survive outside the body of animal for a certain period of time. As a result, these fecal bacteria can cause contamination of the river and underground water through the fluid from agricultural lands if management is inefficient (Crane and Moore, 1986).

When animal waste is applied to the agricultural land, fecal bacterium such as E. coli will be released to the topsoil and it was found to be able to survive for a certain period of time. During rainy days, the bacteria that are alive will penetrate into the soil and enter groundwater system. Groundwater that contained fecal bacterium finally flows out from the soil and released into the river or lake resulting in fecal contamination of river and lake. Therefore, Escherichia coli and other fecal bacterium were used as indicators of fecal pollution in freshwater, marine and estuarine habitats (Flint, 1987).

There are many previous studies related to survival rate or die-off rate of fecal bacterium such as Escherichia coli in soil and water habitats. Temperature has the greatest effect on fecal bacterial survival. In the study of Ling et al. (2002b) on decay rate of Escherichia coli at different temperatures of 25°C, 30°C and 35°C, it was shown that the decay rate of Escherichia coli increased with the increase of temperature. Lower temperature appeared to increase the fecal bacteria survival time (Klein and Casida, 1967). Higher temperature, especially combined with dry condition will effectively increase die-off rate of fecal bacterium (Van Donsel et al., 1967). Reddy et al. (1981) found that die-off rate of pathogen microorganism and indicator organism generally double with an increase of 10°C. Freeze-thaw condition will also reduce the fecal bacterial population as noted by Calcott et al. (1976) and Kibbey et al. (1978).

Besides temperature, pH also has effect on fecal bacterial survival rate. In the study of McFeters and Stuart (1972) on the survival rate of Escherichia coli MH 3427 in water with different pH, it was shown that the optimum pH for the survival of Escherichia coli MH 3427 is between pH 5.5-7.5 and bacteria die-off rapidly when the pH of water was above or below these values. In the study of Lambert (1974), it was reported that the bacterial survival was jeopardized outside the values pH 5.8-8.4. Acidic condition in the soil or water greatly increased the die-off rate of fecal bacteria
(Kibbey et al. 1978; Cuthbert et al. 1955). Generally, neutral condition in the soil or water will extend the bacterial survival period (McFeters & Stuart, 1972).

Studies on effect of pH in survival of *Escherichia coli* were done in other countries where temperature and soil condition are different when compared with conditions in Sarawak. Tropical soils generally differ from temperate soils due to different climate conditions (tropical climate and temperate climate). In Sarawak, atmospheric temperatures vary little throughout the year and variations in soil temperatures are assumed to be even less (Andriesse, 1972). Most soils in West Sarawak are very acid, the pH measured in water suspension being commonly between pH 4 and 5.5 (Andriesse, 1972). Low pH values presented in Sarawak soils are indirectly due to the result of the generally very low contents of bivalent cations such as calcium and magnesium (Andriesse, 1972). Acidic condition on the tropical soils also due to high organic matter content. High organic matter content in tropical soils resulting in more organisms populations surviving in the soils. The metabolites released from those soil organisms such as carbon dioxide, humic acid reduced the pH of soils resulting in acidic condition soils in tropical regions.

In the studies of Ling et al. (2002a) and Ling et al. (2002b) on survival of *Escherichia coli* in tropical soil at different temperature, particle size, and moisture content, it was reported that pH factor may have resulted in the die-off rate of *Escherichia coli* in tropical soil being higher than in temperate soil. Therefore in this study, the survival rate of *Escherichia coli* in tropical soil at different pH and temperature were studied. Information obtained from this study could contribute to effective agricultural land management and reduction in the contamination of water resources by fecal bacteria. There are three main objectives in this study:

1. To investigate the survival of *Escherichia coli* in soils of different pH and temperature.
2. To determine half life and decay rate.
3. To model die-off rates as a function of temperature and pH.
MATERIALS AND METHODS

Bacteria & media

Bacterium used in this study is wild strain of *E. coli* isolated from fresh feces of pig. This bacterium is grown and cultured on LB broth (Fluka, Switzerland), nutrient agar (Oxoid, England) and Eosin methylene blue (EMB) agar (Oxoid, England). All the bacterial culture was grown for 24 hours or overnight at 37°C.

Calibration Curve

The calibration curve is a growth curve of *E. coli*. The universal bottles contained LB broth was inoculated with the fresh *E. coli* culture from nutrient agar and incubated in the incubator shaker (New Brunswick Scientific) at 37°C, 200rpm. The culture was sampled at 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360 minutes. The optical density (OD) of culture was measured using ultraviolet visible spectrophotometer (Techcomp). The OD of the blank LB broth was (without inoculum) also measured. The concentration of bacteria was determined by plating the culture on EMB agar using spread plate technique (Kasing, 2001). Calibration curve is obtained by plotting the graph of concentration (log cfu/g) versus optical density.

Soil Sample

The soil sample used in this study was taken from the hill top at 01°27.541'N/110°26.908'E near the UNIMAS campus. Location of the soil sampled was determined using Geographical Positioning System (MAGELLAN GPS ProMARKX). The soil sample was taken from top layer. The soils were air-dried for one week and sieved using 2.0mm sieve.

The soil particle size distribution was analyzed using the Pipet method (Gee & Bauder, 1986) with a 25 ml pipette which allows for nondestructive sampling of suspensions undergoing settling. Moisture content of the soil was determined by heating the soil in the oven at 105°C for 24 hours and the weight loss was measured. Organic matter content of soil was determined using Loss-On-Ignition method (Nelson & Sommers, 1996). The soil samples was igniting in the muffle furnace (Stuart Scientific Furnace, GB) at 400°C for 16 hours and the weight loss is measured. The soil pH was measured by using pH meter (Thermo Orion) in triplicate. One gram of soil was placed in test tube and added with 1 ml of distilled water. pH of the supernatant of soil is measured.
**Bacterial Analysis**

Concentration of bacteria was determined using spread plate technique. One gram of soil was sampled and transferred to the test tube. A series of dilution with the dilution factor: $10^{-1}$-$10^{-6}$ was conducted. Plate contained solid EMB agar was inoculated with 0.1 ml diluted sample and spread over surface evenly using a sterile spreader. The plate was incubated at 37°C for 24 hours or overnight to allow bacterial colonies growth. Plate contained 30-300 colonies was selected and the quantity of colonies was counted. The concentration of bacteria in this study was counted in cfu/g.

**Experimental Design**

This experiment was a factorial design with two factors: temperature and pH. Two temperatures was used in this study are 20±2°C and 30±0.5°C. Five values of pH used were pH 3, 4, 5, 6, and 7. Sampling was done every day in three weeks or until all *Escherichia coli* were undetectable. One set of beaker contained soil samples at pH 3, 4, 5, 6, and 7 without inoculums was setup as a control. The experiment was conducted in triplicate.

Fifty grams of soil samples was added into the 250 ml beakers covered with aluminum foil to avoid light entered into the soil samples. Then, 25.5 ml of sterile distilled water and 5 ml of inoculums were added to the soil to make the soil samples 100% saturated. To setup the soil samples to the desired pH: 3, 4, 5, 6, and 7, lemon juice and calcium carbonate were used. Lemon juice was used to setup the soils pH into pH 3, where the calcium carbonate was used to setup the soils pH into pH 5, 6 and 7. Soil samples with pH 4 were prepared without added any acid or alkaline. The mixtures were mixed well using a sterile glass rod and incubated at 37°C for 24 hours. Soil sampling for bacteria analysis was conducted daily wherever possible.

**Statistical Analysis**

Statistical analysis was used in this study to compare the interaction and significant difference in bacteria die-off rate between different levels of pH and temperature are general linear model and multiple comparison method (SPSS). The SPSS and Microsoft Excel software were used in this statistic analysis. The mean die-off rate for different pH and temperature were determined by using simple linear regression of bacterial log concentration (log cfu/g) versus time as measured in days.
Decay Rate and Half Life Computation

Decay rate was counted by using the first order decay model of Chick (1908):

\[ \frac{N_t}{N_0} = 10^{-kt} \]  

Where:
- \( N_t \) = number of bacteria at time \( t \)
- \( N_0 \) = number of bacteria at time 0
- \( t \) = time in days
- \( k \) = first order or die-off rate constant (d\(^{-1}\))

The equation above can be expanded as below to determine the die-off rate of \textit{Escherichia coli}:

\[ \log \frac{N_t}{N_0} = \log 10^{-kt} \]
\[ \log N_t - \log N_0 = -kt \]
\[ \log N_t = \log N_0 - kt \]  

The half life of \textit{Escherichia coli} can be determined using the equation below:

\[ \frac{1}{2} \frac{N_0}{N_0} = 10^{-kt} \]
\[ \frac{1}{2} = 10^{-kt} \]
\[ \log 0.5 = \log 10^{-kt} \]
\[ -0.301 = -kt \]
\[ t_{1/2} = 0.301/k \]

Where the \( k \) is the decay rate obtained from linear regression.
RESULTS

Graph for bacterial concentration versus optical density is shown in Figure 1. The results of the soils analysis are shown in Table 1.

![Graph showing bacterial concentration versus optical density]  

\[ \log \text{Conc.} = 5.2371 \text{OD} + 3.9748 \]

\[ R^2 = 0.7689 \]

Figure 1: Concentration (Conc.) versus optical density (OD).

Table 1: Characteristics of the soil used in this study.

<table>
<thead>
<tr>
<th>Particle Size Analysis</th>
<th>Clay (%)</th>
<th>Fine Silt (%)</th>
<th>Medium Silt (%)</th>
<th>Coarse Silt (%)</th>
<th>Sand (%)</th>
<th>pH</th>
<th>Organic Matter Content (%)</th>
<th>Bulk Density (g/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.30</td>
<td>5.33</td>
<td>13.33</td>
<td>12.56</td>
<td>62.47</td>
<td>4.23</td>
<td>6.40</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td>(±2.31)</td>
<td>(±6.11)</td>
<td>(±2.31)</td>
<td>(±2.38)</td>
<td>(±0.45)</td>
<td>(±0.02)</td>
<td>(±0.01)</td>
<td>(±0.17)</td>
</tr>
</tbody>
</table>
Graph for pH monitoring at 20°C and 30°C are shown in Figure 2 and Figure 3. Mean pH value at 20°C and 30°C for the whole experimental period is shown in Table 2.

Figure 2: pH monitoring at 20°C.

Figure 3: pH monitoring at 30°C.

Table 2: Mean pH value at 20°C and 30°C during the experiment period.

<table>
<thead>
<tr>
<th></th>
<th>20°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH3</td>
<td>3.19±0.20</td>
<td>3.33±0.20</td>
</tr>
<tr>
<td>pH4</td>
<td>4.45±0.30</td>
<td>4.25±0.28</td>
</tr>
<tr>
<td>pH5</td>
<td>5.19±0.20</td>
<td>5.17±0.20</td>
</tr>
<tr>
<td>pH6</td>
<td>6.43±0.23</td>
<td>6.41±0.24</td>
</tr>
<tr>
<td>pH7</td>
<td>7.36±0.21</td>
<td>7.39±0.27</td>
</tr>
</tbody>
</table>
Graph of the population of *E. coli* in different pH at 20°C is shown in Figure 4 and at 30°C is shown in Figure 5. At 20°C (Figure 4), populations of *E. coli* in pH 6 and 7 survived better than other pH and still maintained at 10^6 cfu/g after 3 weeks. Population in pH 5 decreased faster than population in pH 6 and 7 but slower than in pH 3 and 4. Population in pH 3 and 4 decreased quickly to zero in less than two weeks. At 30°C (Figure 5), populations of *E. coli* in pH 6 and 7 decreased slower than other pH and still remained at 10^5 cfu/g after 3 weeks. Population in pH 3, 4 and 5 decreased quickly to zero in less than 9 days. *E. coli* have a low decay rate in pH 7 compared with that in pH 3, 4, 5 and 6. This result showed that *E. coli* survived longer in neutral condition (pH 6-7) than in acidic condition (pH 3-5).

Populations of *E. coli* remaining in the soil in the first five days are significantly different in different temperature and also different pH. For all the pH combinations population were significantly different (P<0.0005) except the combinations of pH 4 and 5 (P=0.592) in the first 5 days.
Graph of population of *E. coli* in different temperature in pH 3 is shown in Figure 6. The graph showed that population of *E. coli* at 30°C decreased to zero in 4 days whereas at 20°C, it decreased to zero in 6 days. There is significant difference (P=0.018) in survival of *E. coli* in different temperature at pH 3.

![Figure 6: Population of *E. coli* in different temperature at pH3.](image)

Graph of population of *E. coli* in different temperature in pH 4 is shown in Figure 7. The graph showed that population of *E. coli* at 30°C decreased to zero in less than 1 week, which was faster than in 20°C (11 days, over 1 week). Survival of *E. coli* in 20 and 30°C were significantly different (P=0.005).

![Figure 7: Population of *E. coli* in different temperature at pH4.](image)
Graph of population of *E. coli* in different temperature in pH 5 is shown in Figure 8. The graph showed that population of *E. coli* in 30°C decreased to zero in 8 days, faster than population in 20°C which still remained at $10^3$ cfu/g at day 21. The survivals of *E. coli* in 20 and 30°C were significantly different (P=0.003).

Figure 8: Population of *E. coli* in different temperature at pH5.

Graph of population of *E. coli* in different temperature in pH 5 is shown in Figure 9. The graph showed that population of *E. coli* in 20 and 30°C both survived better. The survivals of *E. coli* were not significantly different (P=0.182) in different temperature (20 and 30°C) at pH 6.

Figure 9: Population of *E. coli* in different temperature at pH6.
Graph of population of *E. coli* in different temperature in pH 7 is shown in Figure 10. The graph showed that population of *E. coli* in 20 and 30°C both survived well, even after 3 weeks. Survivals of *E. coli* in 20 and 30°C were significantly different (P=0.026).

![Figure 10: Population of *E. coli* in different temperature at pH 7.](image)

Mean decay rate (k), half life (t½), and regression coefficient for *E. coli* in different pH at 20°C and 30°C are shown in Table 3. In 20°C, the highest half life of *E. coli* in pH 7 (2.99 day) and the lowest in pH 3 (0.33 day). In 30°C, the highest half life of *E. coli* in pH 7 (4.26 day) and the lowest in pH 3 (0.19 day). In 20°C, decay rates of *E. coli* were significantly different in combinations of pH 3 and 4 (P=0.003) and pH 4 and 5 (P=0.003) but no significant difference in combinations of pH 5 and 6 (P=0.528) and pH 6 and 7 (P=0.992). In 30°C, decay rates of *E. coli* were significantly different in combinations of pH 3 and 4 (P=0.001), pH 4 and 5 (P=0.008) and pH 5 and 6 (P<0.0005) but no significant difference in combination of pH 6 and 7 (P=0.670).

Table 3: Mean decay rate (k), half life (t½) and coefficient of determination (R²) for *E. coli* in different pH at 20 and 30°C.

<table>
<thead>
<tr>
<th>pH</th>
<th>Mean decay rate, k (d⁻¹)</th>
<th>Half life, t½ (d)</th>
<th>Coefficient of determination (R²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20°C</td>
<td>30°C</td>
<td>20°C</td>
</tr>
<tr>
<td>3</td>
<td>0.97±0.28</td>
<td>1.60±0.05</td>
<td>0.33±0.08</td>
</tr>
<tr>
<td>4</td>
<td>0.57±0.03</td>
<td>1.16±0.18</td>
<td>0.53±0.02</td>
</tr>
<tr>
<td>5</td>
<td>0.17±0.04</td>
<td>0.85±0.18</td>
<td>1.89±0.56</td>
</tr>
<tr>
<td>6</td>
<td>0.10±0.01</td>
<td>0.11±0.01</td>
<td>2.97±0.32</td>
</tr>
<tr>
<td>7</td>
<td>0.10±0.01</td>
<td>0.07±0.01</td>
<td>2.99±0.35</td>
</tr>
</tbody>
</table>

† The same letters in the same column or row indicate no significant difference.
Linear regression of mean log concentration of \( E. \ coli \) in pH 3 at 20°C is shown in Figure 11. The graph showed that the coefficient of determination, \( R^2 \) was 0.85. The regression was significant (\( P=0.003 \)).

\[
p = -0.8608t + 5.1665
\]

\[
R^2 = 0.8453
\]

**Figure 11:** Fit of first order decay model of \( E. \ coli \) in pH 3 at 20°C.

Linear regression of mean log concentration of \( E. \ coli \) in pH 3 at 30°C is shown in Figure 12. The graph showed that the coefficient of determination, \( R^2 \) was 0.97, which shows the linear model fit quite well. The regression was significant (\( P=0.002 \)).

\[
p = -1.5965t + 6.3498
\]

\[
R^2 = 0.9728
\]

**Figure 12:** Fit of first order decay model of \( E. \ coli \) in pH 3 at 30°C.
Graph for linear regression of mean log concentration of *E. coli* in pH 4 at 20°C is shown in Figure 13. The graph showed that the coefficient of determination, R^2 was 0.94. The regression was significant (P<0.0005).

![Graph](image)

\[ p = -0.5733t + 7.5465 \]
\[ R^2 = 0.9377 \]

**Figure 13:** Fit of first order decay model of *E. coli* in pH 4 at 20°C.

Graph for linear regression of mean log concentration of *E. coli* in pH 4 at 30°C is shown in Figure 14. The graph showed that the coefficient of determination, R^2 was 0.99, which fit the linear model very well. The regression was significantly different (P<0.0005).

![Graph](image)

\[ p = -1.0675t + 6.5098 \]
\[ R^2 = 0.9898 \]

**Figure 14:** Fit of first order decay model of *E. coli* in pH 4 at 30°C.
Graph for linear regression of mean log concentration of *E. coli* in pH 5 at 20°C is shown in Figure 15. The graph showed that the coefficient of determination, $R^2$ was 0.86, which shows the model does not fit as well. The regression was significant. ($P<0.0005$).

![Figure 15: Fit of first order decay model of *E. coli* in pH 5 at 20°C.](image)

Graph for linear regression of mean log concentration of *E. coli* in pH 5 at 30°C is shown in Figure 16. The graph showed that the coefficient of determination, $R^2$ was 0.98. The linear model fit the data well. The regression was significant ($P<0.0005$).

![Figure 16: Fit of first order decay model of *E. coli* in pH 5 at 30°C.](image)
Graph of linear regression of mean log concentration of *E. coli* in pH 6 at 20°C is shown in Figure 17. The graph showed that the coefficient of determination, $R^2$ was 0.97. Good fit of first order decay model was observed. The regression was significant ($P<0.0005$).

![Graph](image)

**Figure 17**: Fit of first order decay model of *E. coli* in pH 6 at 20°C.

Graph for linear regression of mean log concentration of *E. coli* in pH 6 at 30°C is shown in Figure 18. The graph showed that the coefficient of determination, $R^2$ was 0.98. The regression was significant ($P<0.0005$).

![Graph](image)

**Figure 18**: Fit of first order decay model of *E. coli* in pH 6 at 30°C.
Graph for linear regression of mean log concentration of *E. coli* in pH 7 at 20°C is shown in Figure 19. The graph showed that the coefficient of determination, $R^2$ was 0.95. Good fit the linear model was observed. The regression was significant ($P<0.0005$).

![Graph for first order decay model of *E. coli* in pH 7 at 20°C.](image)

**Figure 19:** Fit of first order decay model of *E. coli* in pH 7 at 20°C.

Graph for linear regression of mean log concentration of *E. coli* in pH 7 at 30°C is shown in Figure 20. The graph showed that the coefficient of determination, $R^2$ was 0.90. Good fit the linear model was observed. The regression was significant ($P<0.0005$).

![Graph for first order decay model of *E. coli* in pH 7 at 30°C.](image)

**Figure 20:** Fit of first order decay model of *E. coli* in pH 7 at 30°C.
Mean for decay rate, \( k \) of \( E. \text{coli} \) in different pH at 20°C is shown in Figure 21 and at 30°C is shown in Figure 22. The graph in Figure 21 showed that the highest decay rate in pH 3 (0.97 day\(^{-1}\)) and the lowest in pH 6 and 7 (0.10 day\(^{-1}\)). The graph in Figure 22 showed that the highest decay rate in pH 3 (1.60 day\(^{-1}\)) and the lowest in pH 7 (0.06 day\(^{-1}\)). These decay rates were significantly different at different pH (\( P<0.0005 \)) except the combination of pH 6 and 7 (\( P=0.775 \)). These decay rates also significantly different in different temperature (\( P<0.0005 \)).

At 20°C, the relationship between decay rate and pH can be expressed as:

\[
k = 5.7075e^{-0.6272pH}
\]

At 30°C, the relationship between decay rate and pH can be expressed as:

\[
k = 0.0249pH^2 - 0.6616pH + 3.3913
\]

**Figure 21:** Mean for decay rate, \( k \) of \( E. \text{coli} \) in different pH at 20°C.

**Figure 22:** Mean for decay rate, \( k \) of \( E. \text{coli} \) in different pH at 30°C.
Half life of *E. coli* in different pH at 20 and 30°C are shown in Figure 23 and 24. Graph on Figure 23 showed that half life, $t_\text{h}$ (d) for *E. coli* increased with increasing of pH. Graph on Figure 23 also showed that the highest half life ($t_\text{h}=2.99$ day) in pH 7 and the lowest ($t_\text{h}=0.33$ day) in pH 3. Graph on Figure 24 showed that half life for *E. coli* increased slowly from pH 3 to 4, than increased rapidly after pH 5. The graph on Figure 24 also showed that the highest half life ($t_\text{h}=4.26$ day) in pH 7 and the lowest ($t_\text{h}=0.19$ day) in pH 3.

Figure 23: Half life of *E. coli* in different pH at 20°C.

Figure 24: Half life of *E. coli* in different pH at 30°C.
Both temperature and pH were shown to be significant factors affecting decay rate ($P<0.0005$) by linear regression. Relationship between decay rate ($k$), temperature and pH can be expressed as:

$$k = 1.219 + 0.037T - 0.317P$$

where $k$ = Decay rate ($d^{-1}$)

$T$ = Temperature ($^\circ$C)

$P$ = pH

This relationship is valid in the temperature and pH range investigated.
DISCUSSION

The results obtained from this study indicate that survival of *E. coli* was significantly influenced by pH and temperature. From the results of mean decay rate in different temperatures, *E. coli* have a lower decay rate at 20°C compared with that in 30°C, that is, *E. coli* survive longer in lower temperature than in higher temperature. This result was similar to the result of McFeters and Stuart (1972). The observation that showed *E. coli* survived longer in low temperature also reported in previous studies. McFeters and Stuart (1972) reported that coliform bacteria survived longer in 5°C than in 25°C. Flint (1987) reported that survival of *E. coli* was also dependent upon temperature with survival at 4°C>15°C>25°C>37°C. The poor survival of *E. coli* in high temperature may probably due to the inability of *E. coli* to adapt metabolism to a high temperature condition.

The result of this study is similar to previous studies that *E. coli* survived longer in neutral condition. McFeters and Stuart (1972) reported that neutral pH environment favors extended bacterial survival. Acidic condition in soil can greatly increase decay rates (Kibbey et al., 1978; Cuthbert et al., 1955). Reddy et al. (1981) reported that several organisms including bacteria survived longer in a pH range of 6-7 and die-off rapidly under acidic conditions. The poor survival rate of *E. coli* in acidic condition may probably due to the inability of bacteria to adapt to a low pH condition. In the acidic condition, the bacterial metabolism may not function properly or failed to function resulting in rapid bacteria die-off.

Mean decay rates obtained from this study except in pH 7 at 30°C (0.07 day⁻¹) were in the range of decay rates for *E. coli* (0.15-6.39 day⁻¹) as reported by Reddy et al. (1981). From the results, mean decay rates of *E. coli* in pH 6 and pH 7 at 20°C and 30°C were not in the range (0.30-0.32 day⁻¹) based on data of McFeters and Stuart (1972). This is probably to the absence of sediment in this study. The mean decay rates obtained from this study was lower than the range reported by Reddy et al. (1981). The results obtained from this study indicated that decay rates of *E. coli* in pH 6-7 were the lowest and it agrees with the range of pH 5.5-7.5 (optimum for *E. coli* survival) as reported by McFeters and Stuart (1972). Decay rate in pH 4 at 30°C obtained from this study was higher than result obtained by Ling et al. (2002b). This is may be due to high sand and low organic matter content in this study.

The graphs for half life (day) versus pH obtained from this study showed that half life of *E. coli* increased with increasing in pH which is similar to results obtained by McFeters and Stuart (1972). The highest half life of *E. coli* (20°C: 2.99 day; 30°C: 4.26 day) that obtained from this study were different to highest half life (2.13 day) reported by McFeters and Stuart (1972). This may be due to different ability of *E. coli* to adapt in water and soil.

Populations of *E. coli* survive poorly in low pH possibly due to the actions of the acid on the bacterial cell. Acid in soil is able to chelate elements essential for growth, such as iron, which may be a possible mechanism of inhibition (Presser et al., 1997). The bacterial enzymes are possibly destroyed by hydrogen ion from acid, resulting in bacterial mechanism failure, leading to rapid bacterial die-off. Temperature in soil also can affect the enzyme activities in bacterial cell. High temperature can cause the denaturation of enzyme and then contribute to bacterial mechanism failure, which also can result in rapid bacterial die-off.
The low clay content may have contributed to the poor survival of *E. coli* in soils under acidic condition. The graphs for mean log concentration versus time (day) obtained from this study were showed that survival of *E. coli* decrease more rapidly when compared to results obtained by Ling et al. (2002a) for the first week. Ling et al. (2002a) reported that *E. coli* have low decay rate in clay loam compared with that in peat soil and silt loam. The poor survival of *E. coli* in the soil used in this study may be due to less particles surface for bacteria to adhere to when compared to clay loam. Organic matter content also may have contributed to the low survival rate of *E. coli* in soils under acidic condition. The organic matters in soil were utilized for metabolism of bacteria. Klein and Casida (1967) reported that poor survival of fecal coliform in soil with low organic matter content was due to the inability of these microorganisms to lower their metabolic requirements in a situation of lower nutrient availability.

Results were obtained from these study may not be accurate due to several reasons. Soil buffering reaction may be have caused the inaccuracy in the pH. This reaction can change the pH of soil toward its original value when acid or alkaline were added. Therefore, the adjusted pH in soils will change during experimental period (Figure 2 and 3). The incubation temperature (20°C) was too difficult to maintain during experimental period due to unbalance flow of cool air around the soil samples. These two main reasons above can affected the bacterial growth directly. Occurring of bacterial colonies clumping in spread plate agar also contributed to the inaccurate bacterial count.

**CONCLUSION**

This study showed that the survival of *E. coli* in soils of different pH and temperature was significantly different except between pH 6 and 7 as indicated by mean log bacterial concentration, mean decay rate (k) and half life (*tv*). This study also showed that decay rate of *E. coli* increased with increasing acidic condition and temperature of the soil. It is recommended that farmers should consider the factors of pH and temperature when applying or disposing animal waste in tropical soils. Farmers can adjust the acidity of the soil in agricultural land to ensure rapid die-off of *E. coli*. Further studies can be done on the effect of organic matter content on the survival of *E. coli* or other fecal bacteria in different pH of tropical soils. The duration of study also should be increased to obtain more information on survival of *E. coli* in tropical soils.

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REFERENCE


