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TEMPORAL AND SPATIAL VARIATIONS AND DECAY RATES OF *E. coli* IN RIVER SEDIMENT

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Abstract: Though the Serin River is used by the community as a source of drinking water, laundry and recreation, little is known about the bacterial contamination in the sediment. Therefore, in this study, the concentration of *E. coli* in the sediment was determined at nine stations in seven sampling trips and the decay rate of *E. coli* in the river sediment was determined in the laboratory. Results of *E. coli* count in the sediment shows high variation among the trips especially at stations near human activities. The highest and second highest mean populations were recorded in the sediment near human settlements. *E. coli* counts ranged from 4.0 to 7.2 log CFU/g and between consecutive trips mean rate of change of *E. coli* in the sediment ranged from -0.2 to 0.2 log CFU/g per day. Contaminations of the tributary with animal farms varied likely due to oxidation pond discharge. Laboratory studies showed that decay rate of *E. coli* in the river sediments ranged from 0.223 to 0.435 d^{-1} and the first order decay model fitted the survival very well (R^2= 0.972-0.995) except in the sediment with lower pH and high temperature. The high count of *E. coli* in the sediment could render the water unsuitable for drinking and water contact recreation as it could be resuspended during disturbances.

KEYWORDS: River sediment, coliforms, *E. coli*, survival study

Introduction

Aquatic sediment has been reported to be a reservoir for fecal bacteria (Toothman *et al.,* 2009). According to Yan & Sadowsky (2001), enteric pathogens originated from human or animals. Sources of fecal bacteria in the river or streams include animal farms (Ling *et al.*, 2006), wild animals (Jiang *et al.,* 2007) and human including sewage treatment effluent and sewage sludge (Gerba & Smith, 2005) while the contamination could occur through point and non-point sources. Sewage treatment plant effluent, septic tank effluent, animal farm oxidation ponds effluents are point sources of contamination whereas for non-point sources, it has been reported that during storm event, bacterial loads are delivered by the storm runoff into the stream (Toothman *et al.,* 2009). Resuspension of fecal bacteria and associated pathogens in the sediment could pose a health risk to individuals involved in recreational activities and it could contaminate aquaculture products as bacteria has been detected in fish cultured in bacterial contaminated water (Sanyal *et al.,* 2011). Relatively more studies have been conducted on the bacterial quality of river water than the sediment as the bacterial quality of a river is based on the concentration in the water (USEPA, 1986). Studies conducted on sediments have shown that sediment harbored more bacteria than the water column (An *et al.*, 2002) and it could be 10 times that of the water thus the suggestion that *E. coli* concentrations in the sediment are a more reliable indicator of the sanitary status of a growout operation in shellfish aquaculture (Sonier *et al.,* 2008). Sherer *et al.* (1992) reported that fecal coliforms decay rate was lower in the presence of sediments than in the absence of sediments. Factors that affect the persistence of fecal bacteria include pH, temperature, organic matter, nutrients and sediment particle size (Maeda *et al.,* 1976; Howell *et al.,* 1996; Ling *et al.,* 2003; 2005; Hall *et al.,* 2009a).

Landuse of the Serin River includes animal farming, fish aquaculture, village settlement and a school. The river water upstream is used for bathing and laundry which is typical of the rural areas in Sarawak and the river water is also

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withdrawn for drinking purpose. Previous studies of *E. coli* in the river water of the Serin River at two tributaries and two main river stations showed bacterial contamination (Ling *et al.,* 2006). However, sediment bacterial quality and its temporal variation have not been investigated. Therefore, in the present study temporal variations of *E. coli* in the sediments of the Serin River was determined at different stations along the river with different land use and the persistence of *E. coli* in the river sediments was investigated at different temperatures in the laboratory.

Materials and Methods

Study site and sample collection

Surface sediment samples were collected from five stations along the Serin River and four stations in its tributaries in Sarawak. Seven sampling trips were made from October 2009 to March 2010. The stations along the main river were denoted as S1, S4, S6, S8 and BS and those in the four tributaries were Bukah River (SB), Penat River (ST), Pam River (SP), and Bukar River (SR) as shown in Figure 1. S1 is near to a village settlement and a school. Station BS was near Kuching-Serian Highway and a sub-urban settlement. Crops were cultivated near the Bukah River, Penat River was used for fish aquaculture, Pam River received animal farm effluent and Bukar River landuse includes villages and agriculture. Sediment samples collected from these stations were analysed for *E. coli* immediately upon arrival at the laboratory.

Persistence Studies

The *E. coli* persistence studies were carried out according to Ling *et al.* (2005). Since the temperature of the river water ranged between 24.9 to 26.9 °C, *E. coli* persistence studies were investigated at 25 and 30 °C. The sediment samples used for the survival were obtained from BS, SB and ST. Fifty grams of sediment samples were weighed, placed in a 250 ml conical flask and 25.5 ml of sterile distilled water was added. Then, the mixture was inoculated with 5 ml of *E. coli* suspension that was obtained from pure culture of *E. coli* strain ATCC 25922. In order to exclude light penetration, the conical flask was wrapped with aluminum foil (Ling *et al.,* 2005). The conical flask was incubated in an incubator. One gram of sediment was sampled for *E. coli* enumeration and the experiment was conducted for a period of 14 days.

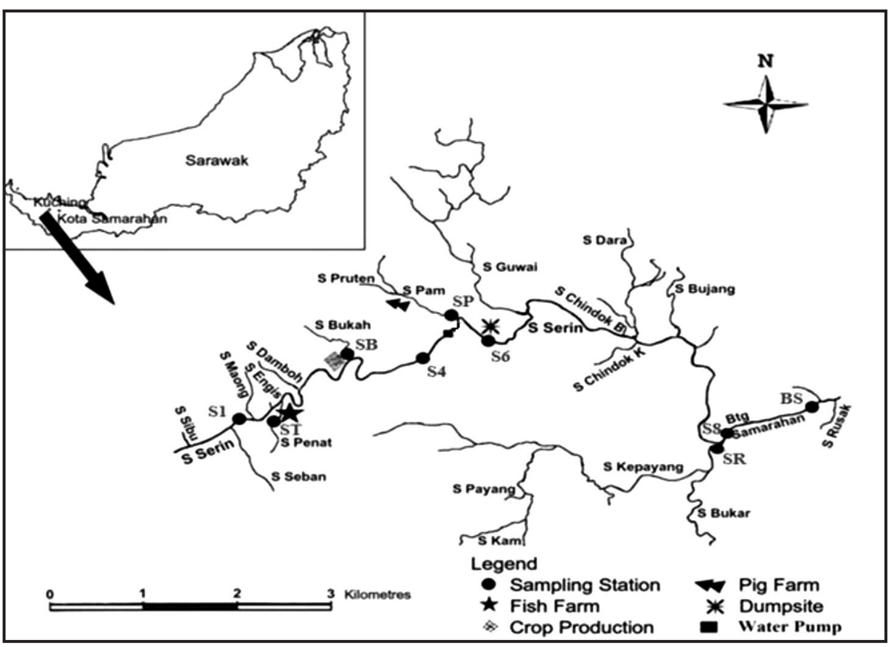

Figure 1. The nine sampling stations on the Serin River and its tributaries.

Bacterial enumeration

The spread plate technique was applied to determine the concentration of *E. coli* as recommended by APHA (1998). Nine milliliters of saline solution (0.8%, Merck) was added to one gram of the sediment. Three serial dilutions (10^{-1}-10^{-3}) and duplicate set of dilutions were prepared for each sample. Subsequently, 0.1 ml of the diluted sample was pipetted and spread onto the Eosin Methylene Blue (EMB) agar and a pre-sterilized glass rod hockey stick was used to spread the water sample evenly. Triplicate plates were prepared. The plates were incubated in an inverted position for 24 hours at 37°C. The colonies of *E.coli* grown on the EMB agar were counted manually.

Organic matter and particle size analysis

Sediments collected for persistence studies were analysed for organic matter and particle size distribution. Organic matters of the sediments for *E. coli* persistence were determined by using the loss-on-ignition method (LOI) according to Nelson & Sommers (1996). Three grams of air-dried sediment were placed in a pre-weighed crucible and dried at 105 ˚C in an oven for 24 hours. After cooling and weighing, the sediment was ignited at 400 ˚C. The percent decrease in weight was taken as organic matter content. The soil was analysed based on the primary particle distribution of silt, clay and sand by using the pipette method according to Gee & Bauder (1986). Air-dried sediment (25 g) undergo pretreatments with 100 ml of hydrogen peroxide to remove the organic matter followed by 10 ml of sodium hexametaphosphate to disperse the soil particles before the pipette method was applied. Each fraction was dried and weighed and the percent of silt, clay and sand were computed.

Computations and Statistical Analysis

The mean daily rate of change in concentrations of *E. coli* in the sediment at each station was calculated according to Equation [1],

$$\text{Rate} = (C_i - C_j) / \Delta t \qquad [1]$$

where C_i and C_j are the *E. coli* concentrations in consecutive trips with C_j occurring later than C_i and Δt is the time interval between the two trips in days. Two-way ANOVA was used to compare the concentrations of *E. coli* among the stations studied. For the persistence study, linear regression was used to find the decay rate of *E. coli* according to the first-order decay model expressed as

$$N_t / N_0 = 10^{-kt} \qquad [2]$$

where N_t and N_0 are bacterial populations at time t (d) and time 0 respectively and k is the decay coefficient (d^{-1}). Paired *t*-test was used to compare the mean decay rate of *E. coli* at 25˚C with 30˚C. All analyses were performed using SPSS version 12.

Results and Discussion

E. coli in sediment

Table 1 shows the mean concentrations of *E. coli* in the sediment in each trip over the sampling period. In all stations, *E. coli* in the sediment showed fluctuation of one or two orders of magnitude except S8 where the count remained quite constant of about 4 log CFU/g. Furthermore, in all stations, the mean *E. coli* counts were at least 4 log CFU/g which is comparable to An *et al.,* (2002) who reported *E. coli* densities of 4.5 to 5.7 log CFU/g in lake marinas sediment. The concentration of *E. coli* was the highest (7.20 log CFU/g) on 7 and 26 October 2009 at the tributary (SP) that received animal farm effluent but not in 2010. The high counts indicate contaminations from oxidation ponds as Goh (2006) reported that *E. coli* count in oxidation ponds of two farms in the same district ranged from 4.7-6.9 log CFU/ml. Furthermore, investigations of pond sediment at six farms around the area showed *E. coli* concentrations of 6.3-8.9 log CFU/g (Goh, 2006). Therefore, outflow of such wastewater could deliver the pond sediment and suspended bacteria to the receiving stream. Tributaries SB and stations S1 and BS of the river showed *E. coli* concentrations 6 log CFU/g or more in 71% of the trips. This indicates the contributions from human settlements as there is a school and a village nearby S1, farmers settled and farmed near SB and BS is the downstream location receiving *E. coli* delivered from all tributaries and household

Table 1: Mean concentrations of *E. coli* in the sediment at the nine stations over the sampling period.

Station	7-Oct 2009	26-Oct 2009	8-Dec 2009	19-Jan 2010	1-Feb 2010	24-Feb 2010	10-Mar 2010
S1	5.96	4.00	6.60	6.10	6.63	5.62	6.10
S4	4.00	5.48	4.00	4.69	4.24	4.00	6.00
S6	4.00	4.00	6.54	6.54	5.30	4.00	6.54
S8	4.00	4.00	4.00	4.00	4.39	4.00	4.00
BS	4.00	6.50	6.35	6.30	6.28	5.48	6.52
ST	4.00	4.00	6.39	5.30	6.30	5.90	5.00
SB	6.30	4.00	6.52	6.32	6.54	4.00	6.32
SP	7.20	6.67	6.24	4.00	4.00	4.00	4.00
SR	4.00	5.30	6.20	6.00	4.39	4.00	6.00
Mean	4.83	4.88	5.87	5.47	5.34	4.56	5.61

Table 2: Mean daily change of concentrations in the sediment at each location.

	Rate of change (log CFU/g/d)					
S1	-0.103	0.200	-0.045	0.041	-0.044	0.034
S4	0.078	-0.114	0.063	-0.035	-0.010	0.143
S6	0.000	0.195	0.000	-0.095	-0.057	0.181
S8	0.000	0.000	0.000	0.030	-0.017	0.000
BS	0.132	-0.012	-0.005	-0.002	-0.035	0.074
ST	0.000	0.184	-0.099	0.077	-0.017	-0.064
SB	-0.121	0.194	-0.018	0.017	-0.110	0.166
SP	-0.028	-0.033	-0.204	0.000	0.000	0.000
SR	0.068	0.069	-0.018	-0.124	-0.017	0.143

wastewater and septic tank discharge upstream. Previous studies of wastewater discharged from households in Kuching indicated that with septic tank wastewater treatment system, *E. coli* count ranged from 4.3-5.3 log CFU/ml (Ling *et al.,* 2010). Evidence of high adsorption capacities of sediment for *E. coli* was also reported by Ling *et al.,* (2003). S8 did not show as high count likely due to the turbulence of water caused by the inflow from a large tributary. Haller *et al.,* (2009b) also reported high concentrations of 3-5 log CFU/g in the sediment at municipal wastewater treatment plant outlet pipe.

Computations of the rate of change in concentrations of *E. coli* indicates that on average the sediment *E. coli* increased as much as 0.2 log CFU/g per day at contaminated stations of S1, an upstream station and decreased as much as 0.2 log CFU/g per day at the tributary of SP. Even though on 19 January and 1 February 2010, the river was flooded, the mean concentrations in the sediment were higher than 7 and 26 October 2009 and 24 February 2010 which were non-flooding condition. This indicates that in flooded condition, the *E. coli* concentrations in the sediment increased. This is because the rainwater washed the *E. coli* from the land and flood water transport the *E. coli* from upstream where the school and village are located and from tributaries to downstream locations. Other researchers also observed the impact of rainstorms. According to Toothman *et al.,* (2009), there was a very strong 24h rainfall effect on fecal coliforms indicating recent rain drove the sediment-associated fecal coliform values. Fries *et al.,* (2008) also reported that sediment *E. coli* concentrations increased significantly following the passage of Hurricane Ophelia.

Figure 2 shows the six-month mean concentration of *E. coli* population for each station. It shows that station BS has the highest mean *E. coli* population, that is, 5.92 log CFU/g and the lowest mean *E. coli* population can be observed at Station S8, 4.06 log CFU/g. Since

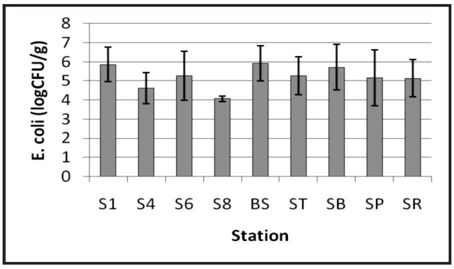

Figure 2. Mean concentrations of *E. coli* in the sediment of the nine stations.

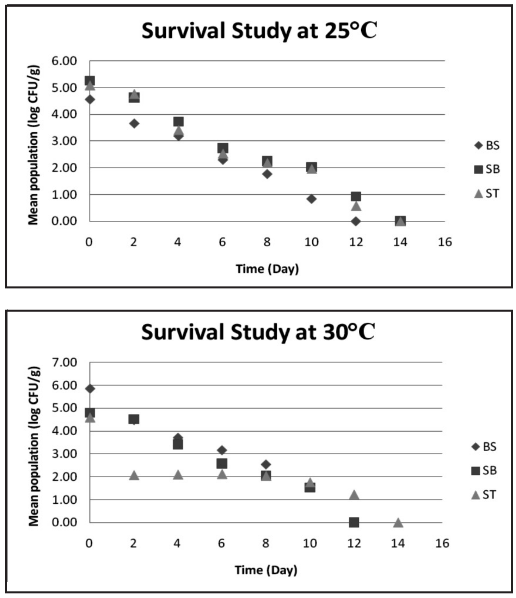

Figure 3. Population of *E. coli* in different sediments at 25˚C and 30ºC.

the station upstream of BS was S8 which showed the lowest *E. coli* mean concentration, the high population of *E. coli* at BS was likely contributed by the settlement near that station. Furthermore, S1 also showed very high mean *E. coli*, the second highest due to the settlement at the village upstream. This shows that human waste is still the main source of contamination. Two-way analysis of variance shows that the concentrations at BS and S1 were significantly higher than S8 (P=0.018, 0.025). The high *E. coli* counts in sediments show that sediment is a reservoir for *E. coli* and re-suspension of the bacteria could increase the bacteria in the water column making the water unsafe for recreational activities. An *et al.,* (2002) observed high *E. coli* densities in the water column due to resuspension as a result of boating activities.

Persistence studies

Table 3 shows the characteristics of the sediment used in the persistence study of *E. coli* and the decay rates. It shows that sediments from SB and ST were acidic with ST sediment more acidic than SB and BS. SB sediment was high in sand whereas ST sediment was high in clay. SB showed the highest organic matter.

Figure 3 shows persistence of *E. coli* at 25 ˚C and 30 ºC in three sediments. At 25 ºC, in all the three sediments, the populations of *E. coli* were in similar decreasing trend. However, at 30 ºC, there were differences of persistence in the three sediments. In BS sediments, there was a constant decrease until it was not detectable on day 12. In SB, there was an initial lag of decrease in population likely due to high organic matter content and favorable pH. In the sediment from ST, the *E. coli* population decreased two orders of magnitude in 2 days before stabilizing for 8 days and then decreased to undetectable level on day 14. The drastic drop in population is most likely due to the acidity of the sediment from ST. According to Ling *et al.,* (2005) who studied the survival of *E. coli* in different pH and temperature, it was found that at 30 ºC in acidic soil of pH 5.2, there was a significant increase in decay rate when compared with pH of 6.4 and 7.4. After the initial drop, the population was able to stabilize for a few days before it decreased again to undetectable level. It has been reported that the survival of *E. coli* was higher in sediment that contains at least 25% of clay due to higher concentrations of organic matter and nutrients (Burton *et al.*, 1987). *E. coli* decreased until undetectable level in all sediments due to competition from other organisms such as indigenous bacteria in the unsterile sediment for the nutrients needed in order to maintain their survival and predation because it was found that fecal bacteria did not decrease as much in sterile sediment when compared to unsterile sediment (Hood & Ness, 1982; Hartz *et al.*, 2008).

At 25 ºC, the decay rates of *E. coli* in the three sediments were very similar. However, at 30 ºC it was not the case. Results show that the

Table 3: Characteristics of the sediments and mean decay rates and coefficient of determination of simple linear regression of *E. coli* populations at 25 °C and 30 °C.

Station	pH	Clay (%)	Silt (%)	Sand (%)	Organic Matter (%)	Mean decay rate (per day)		Coefficient of determination (R^2)	
						25°C	30°C	25°C	30°C
BS	7.02	22.16 (±0.05)	67.45 (±0.08)	10.39 (±0.13)	6.36 (±0.59)	0.370	0.435	0.995	0.972
SB	6.64	5.76 (±0.06)	26.92 (±0.03)	67.32 (±0.04)	7.98 (±0.27)	0.363	0.389	0.985	0.975
ST	5.08	64.98 (±0.05)	11.20 (±0.17)	23.82 (±0.13)	6.34 (±0.48)	0.365	0.223	0.973	0.733

highest mean decay rate was observed in sediment from BS (k=0.435 d^{-1}) and the lowest mean die-off rate was recorded in sediment from ST (k= 0.223 d^{-1}). In BS sediment, though pH was close to neutral which was supposed to be the best for *E. coli* survival, it did not show the lowest decay rate likely because such pH and temperature was also the most suitable for the proliferation of *E. coli* predators and other competing organisms. Paired t-test shows that there was no significant difference between decay rate at 20 and 30 °C (P=0.801). The decay rates observed in this study were close to the range (0.29-0.44 d^{-1}) reported for a wet soil at 25 and 30 °C as reported by Ling *et al.,* (2002). At 25 °C, in all the three sediments, the decay in populations of *E. coli* fitted the first order decay model better than at 30 °C (Table 3). First-order decay model was not suitable for the decay in ST sediment at 30 °C.

Conclusions

Higher *E. coli* count was frequently observed in river sediments near human settlements and animal farms compared to those farther away. Fluctuation of sediment *E. coli* concentrations between trips could be as high as two orders of magnitude. The decay study showed that *E. coli* persisted in the sediment for about two weeks. The presence of *E. coli* in high count and its persistence indicate the public health risk when the river water is used as a source of drinking water and for swimming.

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References

An Y. J., Kampbell, D. H, & Breidenbach , G. P. (2002). *Escherichia coli* & total coliforms in water and sediments at lake marinas. *Environmental Pollution* 120: 771–778.

APHA. (1998). *Standard Methods for Examination of Water and Wastewater*. 20th ed. Washington, DC: American Public Health Association.

Fries J. S., Characklis, G. W. & Noble, R. T. (2008). Sediment-water exchange of *Vibrio sp*. and fecal indicator bacteria: Implications for persistence and transport in the Neuse River Estuary, North California, USA. *Water Research* 42: 941-950.

Gee, G. W. & Bauder, J. W. (1986). Particle-size analysis. *In:* A. Klute, (ed.) *Methods of Soils Analysis Part 1- Physical and mineralogical Methods,* SSSA, Madison, Wisconsin. 383-412 pp.

Gerba, C. P., & Smith, J. E. Jr. (2006). Sources of pathogenic microorganisms and their fate during land application of wastes. *Journal of Environmental Quality* 34: 42-48.

Goh, S. H. 2006. Modelling *Escherichia coli* Adsorption in Selected Soils in Sarawak. Unpublished. M.Sc. Thesis. Universiti Malaysia Sarawak, Malaysia. 96pp.

Haller, L., Amedegnato, E., Poté, J. & Wildi, W. (2009a). Influence of freshwater sediment characteristics on persistence of fecal indicator bacteria. *Water Air Soil Pollution* 203: 217–227.

Haller, L., Poté,, J., Loizeau, J. & Wildi, W. (2009b). Distribution and survival of faecal indicator bacteria in the sediments of the Bay of Vidy, Lake Geneva, Switzerland. *Ecological Indicators* 9(3): 540-547.

Hartz, A., Cuvelier, M., & Nowosielski, K. (2008). Survival potential of *Escherichia coli* and enterococci in subtropical beach sand: Implications for water quality managers. *Journal Environmental Quality* 37: 898-905.

Hood M. A. & Ness, G. E. (1982). Survival of *Vibrio cholerae* and *Escherichia coli* in estuarine waters and sediments. *Applied and Environmental Microbiology* 43(3): 578-584.

Howell, J. M., Coyne, M. S. & Cornelius, P. L. (1996). Effect of Sediment Particles Size and Temperature on Fecal Bacteria Mortality Rates and the Fecal Coliform/Fecal Streptococci Ratio. *Journal of Environmental Quality* 25: 1216-1220.

Jiang, S. C., Chu, W., Olson, B. H., He, J. W., Choi, S., Zhang, J., Le, J. Y. & Gedalanga, P. B. (2007). Microbial source tracking in a small southern California urban watershed indicates wild animals and growth as the source of fecal bacteria. *Applied Microbiology Biotechnology* 76: 927-934.

Ling, T. Y., Apun, K. & Mijen, A. L. (2002). Survival of an *Escherichia coli* in a Sarawak soil. *Malaysian Journal of Soil Science* 6: 43-52.

Ling, T. Y., Jong, H. J. & Apun, K. (2005). Die-off rate of *Escherichia coli* as a function of pH and temperature. *Journal of Physical Science* 16(2): 53-63.

Ling, T. Y., Layang, H. W., Then, Y.P. & Apun, K. (2006). Impacts of pig farming on the water quality of Serin River, Sarawak. *Sains Malaysiana.* 35(1): 45-50.

Ling, T.Y., Goh, S. H. & Apun, K. (2003). Adsorption of fecal coliforms, *Escherichia coli* in soils of Sarawak. *Malaysian Journal of Soil Science* 7: 57-62.

Ling, T.Y., Siew, T. F. & Nyanti, L. (2010). Quantifying pollutants from household wastewater in Kuching, Malaysia. *World Applied Sciences Journal* 8(4): 449-456.

Maeda K., Imae, Y., Shioi, J. & Oosawa, F. (1976). Effect of temperature on motility and chemotaxis of *Escherichia coli*. *Journal of Bacteriology* 127(3): 1039-1046.

Nelson, D. W. & Sommers, L. E. (1996). Total carbon, organic carbon and organic matter. *In:* D.L. Sparks (ed.) *Methods of Soil Analysis, Part 3. Chemical Methods,* SSSA, Madison, Wisconsin. 1001-1005 pp.

Roper, M. M. & Marshall, K. C. (1978). Effect of clay particle size on clay-*Escherichia coli*-bacteriophage interactions. *Journal of General Microbiology* 106: 187-189.

Sanyal, S., Basu, A. & Banerjee, S. (2011) Drug resistance profiles of coliforms from sewage exposed fish. *World Journal of Fish and Marine Sciences* 3(4): 275-282.

Sherer, B. M., Miner, J. R., Moore, J. A., & Buckhouse, J. C. (1992). Indicator bacterial survival in stream sediments. *Journal of Environmental Quality* 21(4): 591-595.

Sonier R., Mayrand, E., Boghen, A. D., Ouellette, M. & Mallet, V. (2008). Concentration of *Escherichia coli* in sediments as an indicator of the sanitary status of oyster (*Crassostrea virginica*) aquaculture sites. *Journal of Applied Ichthyology* 24: 678–684.

USEPA. (1986). *Bacteriological ambient water quality criteria for marine and fresh recreational waters.* EPA 440/5-84-002. U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, OH.

Yan, T. & Sadowsky, M. J. (2007). Determining sources of fecal bacteria in waterways. *Environmental Monitoring Assessment* 129: 97-106.