CHAPTER FIVE

DISCUSSION

5.1 Dynamics of Mean *Clostridium perfringens* Densities (MCPC) and Mean Sulphite Reducing Clostridia Densities (MBCC)

September 2007 to January 2008 was a period of higher MBCC (Fig. 4.6). The MBCC reported were above 7 x 10^3 cfu/100ml in Sungai Selangor and 3 x 10^3 cfu/100ml in Sungai Bernam. This period of higher MBCC happened when river discharge was also generally increasing during that period (Fig. 4.6 for trends and Table 4.3 for MBCC and river discharge correlations). Increasing river discharge could have brought down bigger influx of soils and sediments harbouring sulphite reducing Clostridia into the rivers and hence maintaining the higher level of MBCC. This period could also suggest the duration required by the river system to "cleanse" the excess sulphite reducing Clostridia naturally. During the five months period, MBCC in Site F and Site G revealed a bell-shape fluctuation cycle (Fig. 4.8 and Fig. 4.9). The cycle was not affected (MBCC remained high) even when river discharge dropped on 11.12.2007. In other sites, zig-zag patterns of MBCC fluctuation were instead more common. For example, a log10 difference of MBCC could happen in just three weeks' time between 17.08.2007 to 04.09.2007 in Site B and Site D1D2E (Fig. 4.10 and Fig. 4.11).

Similar to the trend of MBCC, a hike of MCPC could also happen in only two weeks' time, as shown by Site G on 27.10.2007. Interestingly the episode was followed by a CP non-detection in the following sampling (Fig. 4.5). This means that two weeks' time was enough to observe subtle changes of CP densities in Site G. Such subtle MCPC fluctuations might indicate sewage and drainage system overflows during high rainfall precipitation events or urban runoff, which in this study, were represented by high river discharge (Fig. 4.9 and Table 4.1 for river discharge level during CP non-detection in Site G). Young and Thackston (1999) stated that urban runoff commonly contained high microbial densities especially after rain episodes, with the main contribution coming from old and failing septic tanks. For sampling points that were located at the mouth of storm drains, high concentrations of sewage indicators had also been reported on days of heavy rainfall (Rees *et al*., 1998). Even in upstream area which was not affected by agricultural activities and human settlement, high levels of microorganisms were also noticed during precipitation (Kistemann *et al*., 2002).

Currently there is no mandatory standard for CP densities in river water or other types of raw water. Nevertheless, Fujioka (2001) recommended CP densities exceeding 3×10^2 cfu/100ml as indicative of faecal pollution. Bezirtzoglou *et al*. (1996) detected CP in 100-fold diluted river water samples at a frequency of 0 to 5.5% (probably equivalent to less than 5.5 cfu/100ml). The samples were taken from North-West Greece. Shehane *et al*. (2005) reported CP geometrical means of less than 1.9 CFU/100ml in Florida River. Byamukama *et al*. (2005) found the median of CP spores to be less than 2.3 log cfu/100ml (equivalent to 199.5 cfu/100ml) in water samples from streams, springs and lakes of Uganda. Therefore CP densities in rivers of this study (arithmetic means of <1 to 583 cfu/100ml, Table 4.1) were within the range of general water bodies.

5.2 Correlation Between Mean Sulphite Reducing Clostridia Densities (MBCC), Mean *Clostridium perfringens* Densities (MCPC) and River Discharge

It is important to know the correlation between MBCC, MCPC and river discharge. A positive correlation of MCPC-MBCC and MBCC-river discharge, as seen in Site B, requires effective pollution control measures to minimize the impact towards downstream or coastal area. This has been shown in some important studies (Garrido-Pérez *et al*., 2008; Touron *et al*., 2007; Kistemann *et al*., 2002). Negative MCPC-river discharge and MBCC-river discharge correlation are actually desirable as faecal pollution sources would be diluted during high river discharges. Nevertheless, negative correlations also mean that during dry season with less river discharge, sulphite reducing Clostridia and CP will probably get concentrated in the environment. They might then enter the food chain and accumulate in food products like fish and various meat products. Meanwhile, it has been reported that CP from terrestrial sources managed to colonize the guts of turnicates and sea urchins (benthic invertebrates) which were caught from marine area that reported CP counts below 300 cfu/g in the marine sediment (Edwards *et al*.,1998).

5.3 *Clostridium perfringens* And Sulphite Reducing Clostridia : Possibility of Indicating Faecal Pollution and Other Types of Pollution Simultaneously

This study has shown that sulfite reducing Clostridia and its subset, CP, can be used together to indicate faecal pollution. Such concept of genus-genus or genus-species pollution indicators has already been applied. For example, the pairs of faecal coliforms - faecal streptococci and Coliform - *E. coli* are used to indicate human or animal faecal pollution in microbial source tracking of pollution investigations (Young and Thackston, 1999; Scott *et al.*, 2002). However, those established pairs are faecal-pollution specific. If the identities of non-CP sulfite reducing Clostridia are further investigated, it could be possible to indicate faecal pollution and also other types of pollution simultaneously using CP - sulphite reducing Clostridia. This is because sulfite reducing Clostridia may also include other *Clostridium* species of public health concern. It is of the limitation of this study that concurrent *E. coli*, coliform or streptococci densities were not available, and sulphite reducing Clostridia were not identified. Thus no comparison could be made among these indicators including CP and sulphite reducing Clostridia.

5.4 Toxinotypes of Environmental *Clostridium perfringens*

All CP isolates of this study were found to be Type A. Similar studies about CP toxinotypes from river water samples has yet to be reported so far. Nevertheless, clinical studies references showed that CP isolates from healthy human or animals usually belonged to Type A. Beta, epsilon and iota toxin genes (Type B to Type E CP) occurred mostly in ill subjects. Gurjar *et al* (2008) reported that the prevalence of beta, epsilon and iota toxin gene among CP isolates acquired from faeces of healthy lactating cattles were less than 2.5%. Aschfalk *et al*. (2002b) found that CP isolates from faeces of apparently healthy looking sheep in Benin (a place of tropical climate) were Type A (87%) and Type C (13%). In a set of 95 Polish CP isolates collected from stools, gas gangrene, soil and animal cases over 45 years, 97% belonged to Type A while the remaining were Type C or Type E (Augustynowicz *et al*., 2000). Beta and epsilon toxin gene were absent among 48 CP isolates acquired from healthy poultry while alpha toxin gene were detected in all the isolates (Siragusa *et al*., 2006). Furthermore, wild-caught

Atlantic cod were also found to be harbouring alpha toxin gene in the absence of beta, epsilon, iota and CPE genes (Aschfalk and Müller, 2002).

Alpha toxin gene is a chromosomal gene, whereas beta, epsilon and iota toxin genes are plasmid-borne (Petit *et al*., 1999). In the study of Augustynowicz *et al* (2000), three CP isolates from soil samples were initially assigned as B, D, and C CP type, but were later and finally assigned as C, A and A CP type. They suggested that the loss of plasmids which encoded the genes, through subcultures, as the most probable reason for the changes. Therefore, the absence of beta, epsilon and iota toxin genes in this study is probably because that the CP isolates had lost their plasmidic toxin genes during the stay in river water.

5.5 Relationship between *Clostridium perfringens* Prevalence in the Environment and *Clostridium perfringens* Food Poisoning

A study on Indian meat products showed that CP occurred at 91.4%, 70.4% and 65.7% respectively in goat, poultry and buffalo meat (Singh *et al*., 2005). Meat products of herbivores and omnivores seem to have higher CP prevalence. This is probably because *Clostridium* species accumulate well in soil and livestock especially cattle and goats. During dry season, these livestocks tend to gnaw off the grass and ingest larger amount of *Clostridium* spores, which could even increase *Clostridium* related illness during that period (Hang'ombe *et al*., 2000). Similar to terrestrial livestock, elevated CP prevalence in fish can also trigger food poisoning among human (Aschfalk and Müller, 2002). Hence, seasonal and temporal variation of CP should be further explored so that the knowledge can be translated into epidemiological data for better public health management especially in hygiene of raw food product.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

Clostridium perfringens (CP) densities in downstream sampling site of Sungai Bernam (Jambatan SKC) were mostly lower than the downstream of Sungai Selangor (Rantau Panjang), meaning a lesser degree of human faecal pollution. However, sampling site at upstream of Sungai Bernam (Tanjung Malim), which is situated near sewage discharge, reported perennial CP densities.

CP densities and river discharge correlation were generally weak in all rivers. Therefore the results could not straightforwardly tell whether or not CP will get concentrated or diluted with increasing volume of river discharge. Nevertheless, curve estimation showed that the two parameters probably can still be related at certain degree (depending on study site) that might enable modeling of CP densities and river discharge in the future, although this could be more challenging compared to modeling of sulphite reducing Clostridia and river discharge.

All 142 CP isolates of this study belonged to Type A, with 5 of them also harboured the CPE toxin gene. This is probably the first report about the toxinotype of CP isolated from tropical river waters. This study also successfully designed a new pair of duplex PCR primers that can detect alpha and CPE toxin genes among the CP isolates. It has been reported that Type A is the most common toxinotype in CP food poisoning. Therefore managing the distribution of CP in the environment is important especially when the water resource or land is being used for food production and human consumption.

Subtle fluctuations of CP densities (shown by Rantau Panjang) revealed the importance of continuous monitoring for temporal besides spatial densities variations. Fortnightly sampling is largely enough to observe temporal differences. It would be better if sampling during high river discharge can be performed.

The identification of non-CP sulphite reducing Clostridia is recommended for the possibility of elucidating other types of pollution on top of human faecal pollution.