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Microbiological Risk Assessment of Fresh Water Aquaculture Fish: From Farm to Table

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ABSTRACT

The qualitative microbiological risk assessments of fresh water aquaculture fish from farms, markets and food premises have been carried out in six main aquaculture fish production districts in Malaysia. Three species of fish were involved in this study [red *tilapia* (*Oreachromis sp*. red hybrids), *keli* (*Clarias spp*.) and *patin* (*Pangasius sutchii*)]. About 240 fresh fish (90 red *tilapia*, 60 *keli* and 90 *patin*) were randomly collected direct from their farms (earth ponds, floating net cages and examining pools). Another 240 fish with the same ratio as farm fish samples were also randomly collected from various markets (wet markets, local markets called 'pasar tani' and night markets). The same number of samples with the same ratio of ready-to-eat fish from food premises (restaurants, food stalls and night market food stalls) was also collected. Three indicator microorganisms (TPC, coliform and faecal coliform) as well as seven pathogenic organisms (*E. coli*, *S. aureus*, *Salmonella spp*., *Campylobacter spp*., *B. cereus*, *Aeromonas spp.* and *Pseudomonas spp.*) were analyzed. The results showed that there were intermediate microbiological risk in farm and market fish samples and low microbiological risk in ready to eat fish samples.

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INTRODUCTION

Fresh water aquaculture fish have more risks to be consumed compared to sea fish especially the fish which are cultured in earth pond, at sea costal and in the river [1,2]. Many reseracher had referred the risk in fresh water aquaculture fish to biological hazards which may come from aquaculture systems [1,2,3,4]. Three most common biological hazards in fresh water aquaculture fish are parasite, bacteria and biological toxin. The prevalence of food-borne diseases that caused by bacteria were higher more compared to parasite and virus [5]. There are many listed pathogenic bacteria in fresh water fish including *E. Coli*, *Salmonella spp.*, *Campylobacter spp.*, *Vibrio spp.*, *Clostridium botulinum*, *Listeria monocytogenes*, *Shigella spp.*, *Yersinia enterolitica*, *Staphylococcus aureus*, *B. Cereus*, *Aeromonas hydrophila* and *Pseudomonas spp*[5,6,7]. Other bacteria which had been detected in fish include *Edwardsiella tarda*, *Plesiomonas shigelloides*, *Streptococcus iniae*, *Erysipelothrix rhusiopathiae*, *Leptospira interrogaus* and *mycobacterium spp*. According to Swanson [8], not all bacteria can survive in raw and processed food. Only a few dominant bacteria can grow and replicate to a certain number that can infect and cause diseases in the consumer. The survival of the bacteria depends on the intrinsic and extrinsic factors of the food.

The identification of certain hazards in food should be followed by varification step either as qualitative or quantitative analysis as reported by Asma [9]. The step is to confirm whether the hazards that exist in the food are low, intermediate or high risk. This study was conducted to verify quantitatively the existing of microbiological hazards in fresh water aquaculture fish in Malaysia and compared qualitatively the level of risk at the three different aquaculture stages (farms, markets and food premises) whether it is low, intermediate or high.

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MATERIAL AND METHODS

Fish Sampling:

About 240 fresh water aquaculture fish (90 red *tilapia*, 60 *keli* and 90 *patin*) were collected stratified randomly direct from their farms (earth ponds, floating net cages and ex-mining pools) in six main aquaculture fish production districts in Malaysia. Another 240 fresh water aquaculture fish with same proportion were also collected stratified randomly from the markets (wet markets, local morning markets call 'pasar tani' and night markets). The same number of samples with same proportion of ready to eat fresh water aquaculture fish from food premises (restaurants, food stalls and night market food stalls) also collected from the same districts. The sampling activities were performed according to the methods suggested by Health Department of Pahang [10]. The methods also been referred to FAO [11] and APHA [12]. All the samples were sent to the laboratory at cool temperature (4±1^{o}C) and the analysis were done within 24 hours.

Analysis of Indicator Microorganisms:

Three parameters of indicator microorganisms were analyzed in this study, that are Total Plate Count (TPC), coliform and fecal coliform. Analysis of TPC was according to method suggested by Health Department of Pahang [10] and also been referred to Swanson [8], APHA [12], ICMSF [13], and Andrews [14]. Analysis of coliform and faecal coliform were performed according to 3 tiubs MPN methods as suggested [12]. The level of microbiological risks of TPC, coliform and faecal coliform in the fish were referred to that maximum limit allowed in the food as stated in Health Department of Pahang [10] and Food Act [15].

Analysis of Pathogenic Organisms:

Seven pathogenic organisms involving *E. coli, Salmonella spp., Campylobacter spp., S. aureus, B. cereus, Aeromonas spp*. and *Pseudomonas spp*. were analyzed in this study. Analysis of pathogenic organisms was referred to the methods suggested by Health Department of Pahang [10], APHA [12] and PHLS [15] with some modifications. The level of microbiological risks of all pathogenic organisms in the fish also been referred to the maximum limit allowed in the food [3,8, 12,14, 16].

Statistical Analysis:

All data gained from the sampling and analysis activities were statistically analyzed using Statistical Package for Social Sciences, SPSS Version 17.0 for Windows (SPSS Inc., Chicago, USA. A probability level of <0.05 will be consider as statistically significant.

RESULTS AND DISCUSSION

Analysis of Fish Samples from Farms:

The results showed about 9 (3.8%) samples from farms have low count of TPC and 231 (96.3%) samples have intermediate count of TPC. Analysis of coliform showed that about 112 (46.4%) samples from farms have low count of coliform, 82 (34.3%) samples have intermediate count of colifom and 46 (19.2%) samples have high count of coliform. Analysis of faecal coliform showed about 182 (76.2%), 31 (13.0%) and 26 (10.9%) samples have low, intermediate and high count of faecal coliform respectively (Figure 1). In average, fish samples from farms have intermediate count of TPC, intermediate count of coliform and low count of faecal coliform.

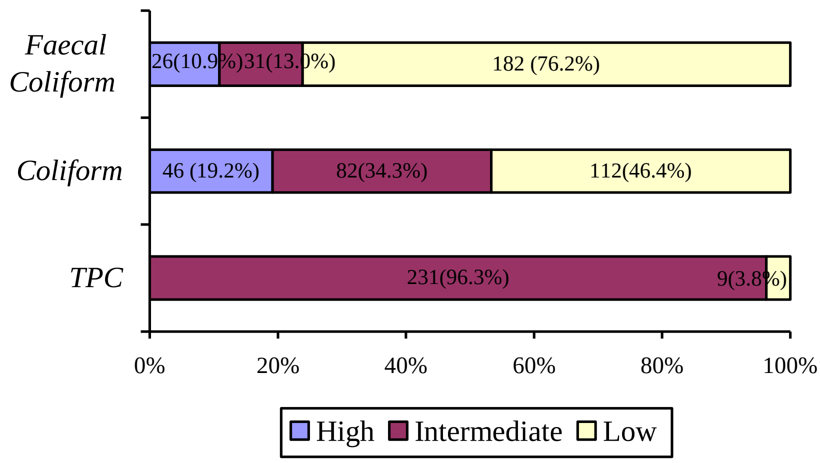

Fig. 1: The number (%) of farm fish samples according to the level of indicator organisms

The average count of log_{10}TPC, coliform and faecal coliform in farm fish samples according to the type of fish showed no significant different at P>0.05 but according to the type of farm, fish samples from earth ponds and ex-mining pools have average count of log_{10}TPC higher (P<0.05) than fish samples from river net cages (Figure 2). According to Huss [2], the water of earth pond and ex-mining pool did not flow properly and this could allowed bacteria to grow and replicate to a bigger amount. Figure 2 also show the average count of coliform and faecal coliform in the fish samples according to the type of farms where there are no significant different (P>0.05) even though it seem that the count of coliform in earth pond fish samples got highest count compared to other farm fish samples and the count of faecal coliform in river net cages fish samples was highest compared to other farm fish samples.

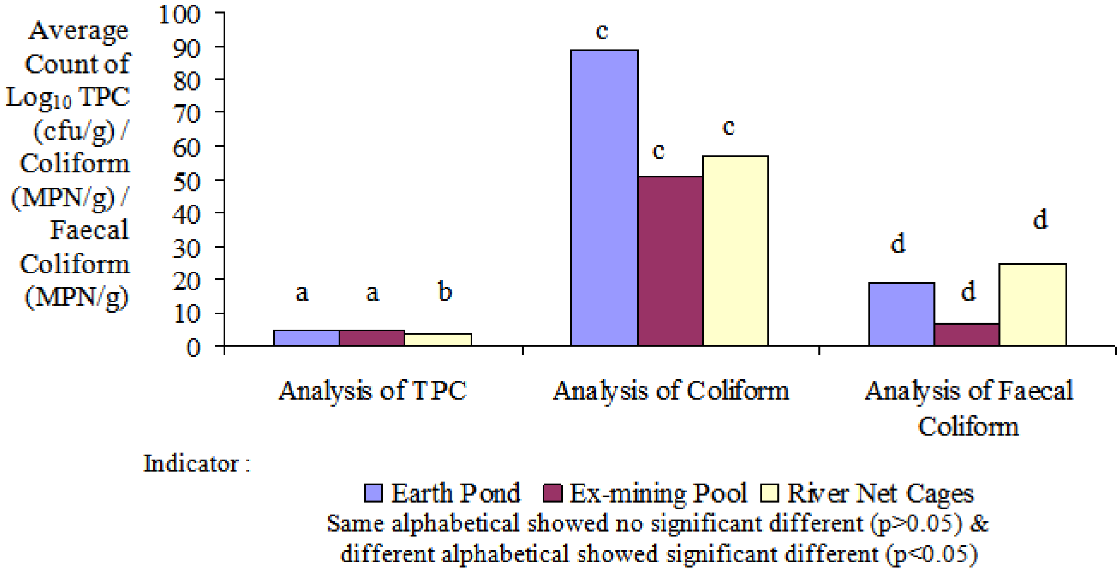

Fig. 2: Average count of Log_{10}TPC, Coliform and Faecal Coliform in farm fish samples according to the type of farm

Analysis of pathogenic organisms showed that only *Aeromonas spp*. and *Pseudomonas spp. w*ere detected in low quantity in farm fish samples and the quantity were not significant different (P>0.05) wether according to the type of fish or the type of aquaculture systems. *Aeromonas spp*. and *Pseudomonas spp*. might come from soil or water and they normally exist in small quantity [2].

Analysis of Fish Samples from Markets:

The results showed that 7 (2.9%) samples have low count of TPC, 210 (87.5%) samples have intermediate count of TPC and 23 (9.6%) samples have high count of TPC (Figure 3). Another 94 (39.2%) samples have low count of coliform and same number of 73 (30.4%) samples have intermediate and high count of coliform respectively. Analysis of faecal coliform showed that about 157 (65.4%), 62 (25.8%) and 21 (8.8%) samples have low, intermediate and high count of faecal coliform respectively. In average, fish samples from market have intermediate count of TPC, intermediate count of coliform and low count of faecal coliform.

The result also showed that there are average count of log_{10}TPC, coliform and faecal coliform in market fish samples according to the type of fish where *keli* have the lowest average count of log_{10}TPC and coliform with significant different (P<0.05) compare to *patin* but the count is not significant different (P>0.05) compared to red *tilapia* (Figure 4). This situation happened because *keli* normally were still alive when they were sold at market. Live fish normally have low count of microorganisma[2]. The average count of faecal coliform in all market fish samples according to the type of fish showed no significant different at P>0.05.

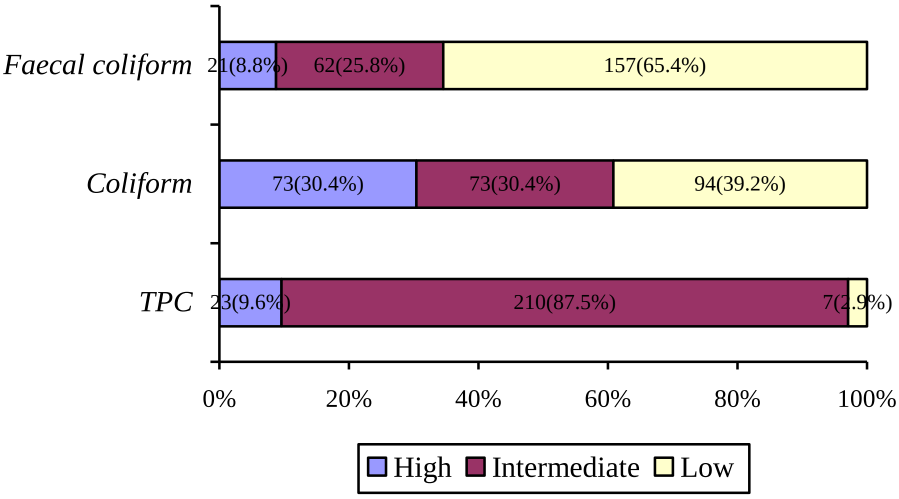

Fig. 3: The number of market fish samples according to the level of indicator organisms

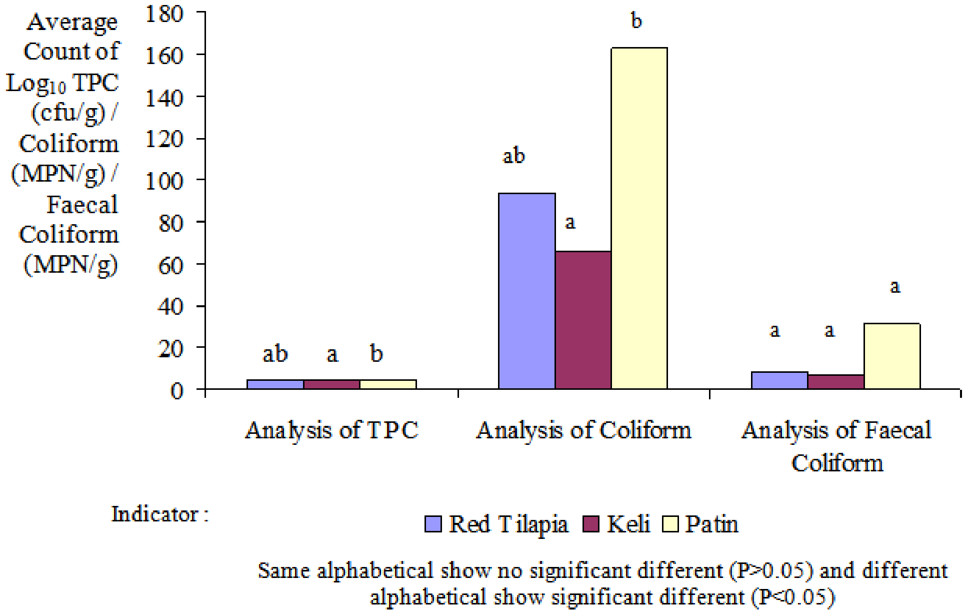

Fig. 4: Average count of Log_{10}TPC, Coliform and Faecal coliform in market fish samples according to the type of fish

The average count of log_{10}TPC, coliform and faecal coliform in market fish samples according to the type of farms also showed no significant different at P>0.05. Comparison of average count of log_{10}TPC, coliform and faecal coliform in market fish samples according to the type of market showed that the fish which were sold at night market food stalls have the highest count of log_{10}TPC, coliform and faecal coliform compared to fish from wet markets and pasar tani. Anyway, only the average count of coliform in fish sold at night market food stalls and pasar tani were significantly different (P<0.05). The existing of intermediate and high count of indicator organisms in certain food showed unsanitary condition [2, 17]. Bacteria may naturally exist in fish which were sold at market or the bacteria may contaminate the fish during the farming or during the handling process from farm to market. According to Roberts [18], the growth of bacteria may cause by unsuitable storage temperature.

The results of pathogenic organisms analysis in market fish samples showed that all samples were not detected to have pathogenic organisms or have at a very low count. Average count of *S. aureus* in market fish samples was very low which was about 0.15×10^2 cfu/g. The existing of *S. aureus* in the fish may come from unsanitary fish handling. *Aeromonas spp.* and *Pseudomonas spp.* were also detected in the market fish samples but the average count of both bacteria were very low.

Analysis of Ready to Eat Fish Samples from Food Premises:

The results of analysis of indicator organisms in ready to eat fish samples from food premises showed that a total number of 45 (18.8%) samples have low count of TPC and 194 (81.2%) samples have intermediate count of TPC. For analysis of coliform, it was showed that 113 (47.1%) samples have low count, 91 (37.9%) samples have intermediate count and 36 (15.0%) samples have high count. Another 187 (77.9%) samples have low count of faecal coliform, 30 (12.5%) samples have intermediate count of faecal coliform and 23 (9.6%) samples have high count of faecal coliform. In average, ready to eat fish samples from food premises have intermediate count of TPC, intermediate count of coliform and low count of faecal coliform (Figure 5).

The average count of log_{10}TPC in fish samples from food premises which the fish came from river net cages was the lowest and have significant different (P>0.05) compared to samples which the fish came from earth pond and ex-mining pool. On the other hand, the average count of coliform and faecal coliform in samples which the fish came from ex-mining pool were the lowest compared to the count of the fish samples from earth pond and river net cages, but all the counts were not significant different (P<0.05).

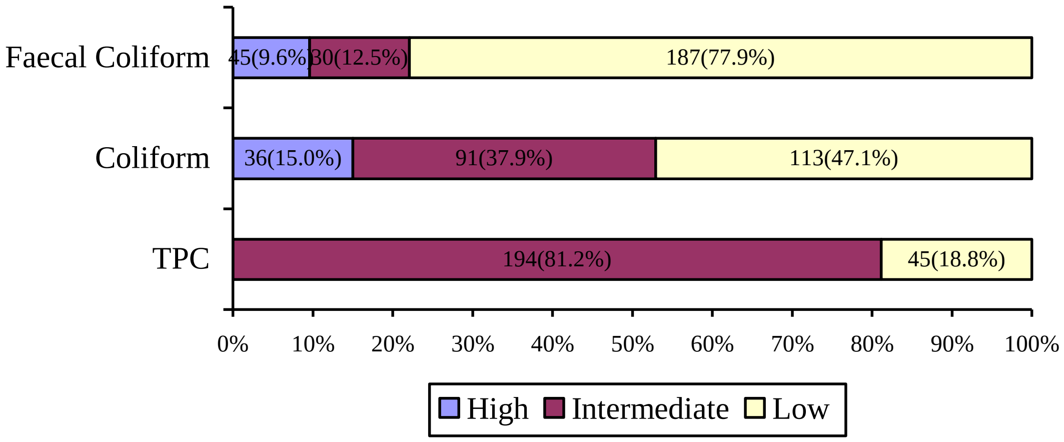

Fig. 5: The number of food premises fish samples according to the level of indicator organisms

The analysis also showed no significant different (P>0.05) in term of the average amount of log_{10}TPC, coliform and faecal coliform in all fish samples from food premises according to the type of fish and the type of food premises (Figure 6). The existing of intermediate and high count of indicator organisms in ready to eat fish samples from food premises might be from the contamination that happened during processing, storage and handling of the fish by food handles in the food premises. Teles [19] stated that good manufacturing practice during food processing can reduce microbiological contamination.

The results of pathogenic organisms analysis in ready to eat fish samples from food premises also showed that all samples have no pathogenic organisms or have very low count. Only *S. aureus* were detected in a very low count ($0.13x10^2$ cfu/g) and have no significant different (P>0.05) weather according to the type of fish, farms or food premises.

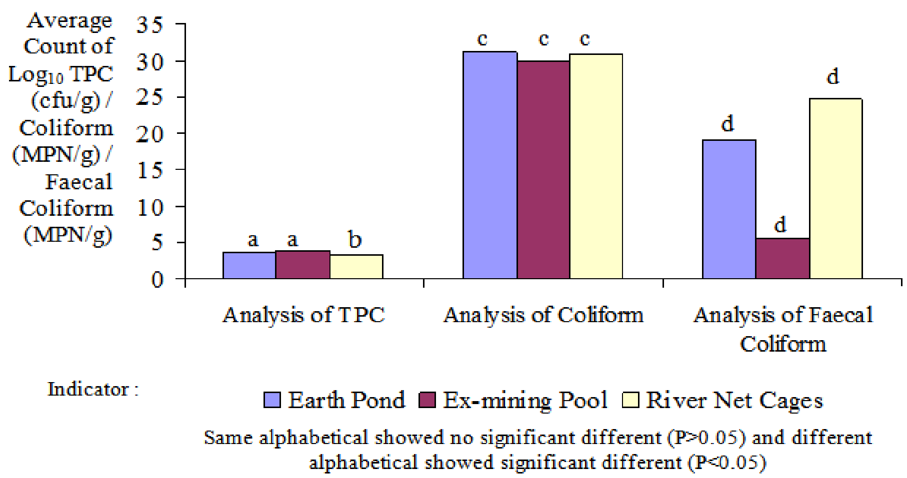

Fig. 6: Average count of Log_{10}TPC, Coliform and Faecal coliform in food premises fish samples according to the type of farm

Level of Microbiological Risk at Three Different Stages of Aquaculture System:

The comparison of the average count of $\log_{10}$ TPC, coliform and faecal coliform in the samples from the three different stages showed that the count of $\log_{10}$ TPC and coliform in samples from markets were significantly increased (P<0.05) compared to the samples from farms but the count were significantly decreased (P<0.05) in the samples from food premises. The contamination may happen at market stage through unsanitized handling and storage system but the number of bacteria may reduce at food premises stage by cooking process. There were no significant different (P<0.05) of the faecal coliform count in all samples from farms, markets and food premises.

The level of microbiological risk was qualitatively determined by combining the microbiology criteria where the risk level at farms and markets were intermediate and at food premises was low as showed in Table 1.

Table 1: Comparison of microbiological criteria and the level of microbiological risk at three different stages

	Microbiological Criteria		
Microbiological Analysis	Farms Level	Markets Level	Food Premises Level
TPC	Intermediate	Intermediate	Low
Coliform	Intermediate	Intermediate	Low
Faecal coliform	Low	Low	Low
E. coli	Low	Low	Low
Salmonella spp.	Low	Low	Low
Campylobacter spp.	Low	Low	Low
S. aureus	Low	Low	Low
B. cereus	Low	Low	Low
Aeromonas spp.	Low	Low	Low
Pseudomonas spp.	Low	Low	Low
Microbiological Risk Level	Intermediate	Intermediate	Low

Conclusion:

The overall study showed that fresh water aquaculture fish at farms and markets level have intermediate microbiological risk and ready to eat fresh water aquaculture fish at food premises have low microbiological risk level. It means that after processing, fresh water aquaculture fish especially red *tilapia*, *keli and patin* fish from three different aquaculture farms which are earth ponds, floating net cages and ex-mining pools are safe for human consumption. The fish also have potential to be further developed as alternative protein sources.

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REFERENCES

[1] Reilly, A., C. Lima Dos Santos and M. Phillips, 1997. *Food safety and products from aquaculture.* Presentation Paper in Joint FAO/NACA/WHO Study Group on Food Safety Issues Associated with Products from Aquaculture. Bangkok 22-26 August.

[2] Huss, H.H., 1994. Assurance of seafood quality. FAO Fisheries Technical Paper.

[3] Garrett, E.S., M.L. Jahncke and C.A.M. Lima Dos Santos, 1997. Public, animal and environment health implications of aquaculture. Emerging Infectious Disease. 3(4): 453-457.

[4] Munir, A.H., J. Blackwell, G. Carr, F. Zhang and T.M. Jackson, 2012. Waste water irrigation and environmental health: Implication for water governance and public policy. International Journal of Hygiene and Environmental Health, 216(3): 255-269.

[5] Novotny, L., L. Dvorska, A. Lorencova, V. Beran and I. Parlik, 2004. Fish: a potential source of bacterial pathogens for human beings. Veterinarni-Medicina, 49(9): 343-353.

[6] Reilly, A. and F. Kaeferstein, 1999. Food safety and products from aquaculture. Journal of Applied Microbiology, 85(28): 249-257.

[7] Stride, M.C., A. Polkinghorne and B.F. Nowak, 2014. Chlamydial infection of fish: Diverse pathogens and emerging causes of diseases in aquaculture species. Veterinary Microbiology, 71(1-2): 258-266.

[8] Swanson, K.M.J., 1992. Colony count methods, in Vanderzont, C. & Splittstoesser, D. (eds.). *Compendium of Methods for the Microbiological Examination of Foods*, 3th Edition. Washington: Public Health Association.

[9] Asma, Z., 2003. *Risk assessment: principles and components*. Presentation Paper at Risk Assessment of Chemical Hazards in Food Seminar, Ministry of Health Malaysia, Genting Highland, 1-4 July.

[10] Health Department of Pahang, 1997. *Food Quality Control: Food Sampling and Analysis*.

[11] FAO., 1998. Simple methods for aquaculture management for fresh water fish culture: fish stocks and farm management. *FAO Trainng Series*. 21(2): 22-31.
[12] APHA, 1992. Compendium of Methods for the Microbiological Examination of Food. 3rd. Edition.
[13] ICMSF (International Commission on Microbiological Specification for Foods). 1980. *Microbiology of foods : food commodities,* Vol 2, Academic Press, New York.
[14] Andrews, L.S., A.M. Key, R.L. Martin, R. Grodner and D.L. Park, 2002. Chlorine dioxide wash of shrimp and crawfish an alternative to aqueous chlorine. Food Microbiology, 19: 261-267.
[15] Food Act., 1983. *Malaysia Act, Act 281*. Ammendment No.4/2000. Malaysia Nasional Printing Berhad, Kuala Lumpur.
[16] PHLS (Public Health Laboratory Service). 1992. *Practical food microbiology: methods for the examination of food for microorganisms of public health significance* in Roberts, D, Hooper, W & Greenwood, M. (eds.) Public Health Laboratory Service, London.
[17] Jamal, K.H., 2003. *Food Safety Risk Analysis.* Presentation Paper at Risk Assessment of Chemical Hazards in Food Seminar, Ministry of Health Malaysia, Genting Highland, 1-4 July.
[18] Roberts, C.A., 2001. *The safety information handbook*. Oryx Press, Westport USA.
[19] Teles, A.O., 2012. Nutrition and Health of Aquaculture Fish. Journal of Fish Dieseases. 35(2): 83-108.