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Preliminary Examination of Faecal Bacterial Contamination and Water Quality in Cockles Culture Sites in Sebarang Perai, Malaysia

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A preliminary examination of faecal bacterial contamination and water quality in cockles culture sites in Sebarang Perai, Malaysia, was conducted in order to monitor the level of enteric-pathogens contamination. Comparative observations between culture sites at two neighbouring river mouths and the area between was made. Station 5 (near the open sea) had the lowest bacterial contamination as seen from the low heterotrophs count and faecal coliform count in seawater, mud and cockles as compared with that from stations near river mouths (St. 1 and St. 7). The overall results revealed that culture site near river mouth was greatly influenced by river inflow.

1 Introduction

Cockles *Anadara granosa*, one of the bivalves, is popular as a source of food in Southeast Asia, especially in Malaysia. Cockles culture is at present of considerable economic importance in Malaysia[1]. The natural distribution of *A. granosa* is largely limited to areas along the west coast of Peninsula Malaysia[2]. *A.granosa* occurs naturally in extensive brackish mud flats with fine soft brackish muds, in particular of mangrove forests, with salinity ranging from 10 to 20 ‰. It thrives best under calm conditions.

Malaysian people prefer to eat half-cooked cockles which can cause illness such as gastroenteritis, hepatitis, typoid, paratyphoid, cholera, etc. Cockles harvested from contaminated sites need to undergo the process of depuration[3,4] which is costly and also weaken the cockles. A less expensive methods is to monitor sea water, mud, and cockles at culture sites for bacterial contamination to ensure that good quality cockles which can be safely consumed are harvested. Suitable sites for cockles culture can also be selected if the bacterial load to these areas is known. A preliminary study was conducted on October, 1988 to monitor the contamination level of faecal bacteria and water quality of a cockles culture site, Seberang, Malaysia.

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2 Material and Methods

2.1 Study site

Cockles are cultured along the east Seberang Perai between Juru and Jejawi rivers. Nine stations were selected for the study (Fig.1). The distance between St.5 and the coast was about 200m.

2.2 Water analysis

Physical and chemical parameters were taken for 9 stations. *In situ* water depth, salinity, pH, dissolved oxygen, turbidity, and temperature were measured. The pH was measured with a digital pH meter (model Hanna). Dissolved oxygen and temperature was measured with a DO meter (model Hanna). Turbidity was measured by using a secchi disc. Salinity was measured by refractometer.

2.3 Bacteriological analysis

2.3.1 Sample preparation

Out of the 9 stations located, stations 1,5,7 were selected for collecting sample of seawater, muds, and cockles for bacteriological analysis. Water samples were collected with Kitahara sampler and kept in sterile bottles. Mud sample collection was conducted with mud grab and kept in a sterile plastic bag and cockles by the use of a wire scoop and kept also in plastic bag. Samples collected were kept in a styrofoam box covered with crushed ice, brought back to the laboratory (Fisheries Research Institute, Pennang, about one hour trip from the sampling site), and treated immediately for bacteriological analysis.

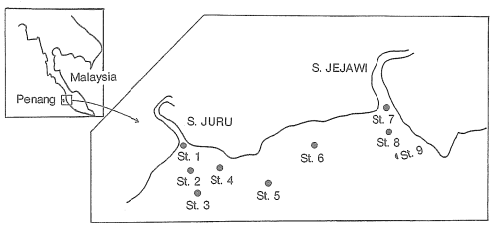

Fig. 1. Map showing sampling stations.

For seawater and mud, stepwise dilutions were prepared with 0.1% peptone water. The cockles were washed with clean freshwater to remove all the mud and swabbed with 70% alcohol. The cockles were aseptically shucked and put into sterile petri dishes. A 10 g of cockle flesh were mixed with 90 ml peptone water for 1 min by using a stomacher. Stepwise dilutions were then prepared with peptone water.

2.3.2 Total plate count (heterotrophs)

The method used for determination was Plate Count Agar (PCA) method. A 0.1 ml prepared samples was dropped onto the surface of the agar and sterile triangle glass rods was used for spreading. The agar plates were incubated at 35℃ for 36 hours. Dupricates were done for each concentration.

2.3.3 Total coliforms

The multiple tube fermentation technique was used for the enumeration of total coliforms. The media used was double and single strength of lactose sodium glutamate broth. The incubation time was 48 hrs at 37℃. Three tube run was done using 5 serial dilutions. Presence of gas in Durham tubes was used as a positive indicator.

2.3.4 Faecal coliforms

Faecal coliform inoculation samples was taken from the respective tubes used in presumptive total coliforms. Brilliant green lactose broth (BGLB) was used. The incubation temperature was maintained at 44.5℃ using the water bath for 24 hours. Presence of gas in Durham tubes was used as a positive indicator.

3 Results and Discussions

3.1 Water quality

Six parameters were measured at 9 stations to see if there were any discernible differences between the stations and also to detect possible pollution from the river inflow. The water quality of the 9 stations situated between Juru and Jejawi rivers were shown in Table 1.

The water depth ranges from 1.5 to 2.5 m as was expected for mud flats. Consequently with the presence of strong winds, the bottom muds was stirred up resulting turbidity giving low transparency of 0.3 to 0.5 m. The pH value and temperature were almost uniform for all stations. D.O. values were generally high indicating little stress on cockles life on this area. The station having the highest D.O. values was Station 3 which was the furthest from the coast. Another possible reason for the highest D.O. values was the weather condition, i.e., winds which resulted in good mixing of the surface water. Station 1, 2, and 3 show a gradual increase in salinity as expected with increasing distance from the coast, similarly for station 7, 8, and 9. Station 5 and 6 had the highest salinity of 30 ‰ as there were areas with the least influence from the river inflow.

With regard to pollution, lower salinity values at river mouth indicated that there was possible inflow of pathogenic and non-pathogenic coliforms into these areas.

3.2 Bacteriological indicators

3.2.1 Heterotrophs

A comparison of the heterotrophic level in the seawater, mud and cockles from station 1, 5, and 7 were shown in Fig.2. The heterotrophs count in station 5 was low compared to the two other stations and station 7 had the highest heterotrophs count for seawater. This was because stations 1 and 7 were situated near the river mouths and could be contaminated by the outflow of sewage from the river. The salinity at stations 1 and 7 was between 17 to 20 ‰ which may be more suitable for the growth of heterotrophs derived from rivers.

From the three types of samples, mud had the highest value of heterotrophs since the bottom sediment would probably the area of settlement / concentration for nutrients brought in from the river.

3.2.2 Total coliforms

The total coliforms number of cockles and mud from station 5 which would have the least influence from Juru and Jejawi rivers was higher

Table 1. Water quality of the 9 stations in cockles culture sites in Sebarang Perai

Parameter	Station								
	1	2	3	4	5	6	7	8	9
Water depth (m)	1.5	2.0	2.0	2.5	2.0	1.5	2.5	2.0	2.1
Water temp. (℃)	29.4	30.5	29.9	29.5	29.1	29.1	29.3	29.5	29.7
Turbidity (m)	0.3	0.5	0.3	0.4	0.3	0.3	0.3	0.5	0.4
pH	7.51	7.51	8.11	8.01	7.83	7.94	7.95	7.98	8.14
Dissolved oxygen (%)	89.4	105.6	119.7	75.9	70.4	71.6	79.4	78.0	84.5
Salinity (‰)	24	27	29	28	30	30	27	28	29

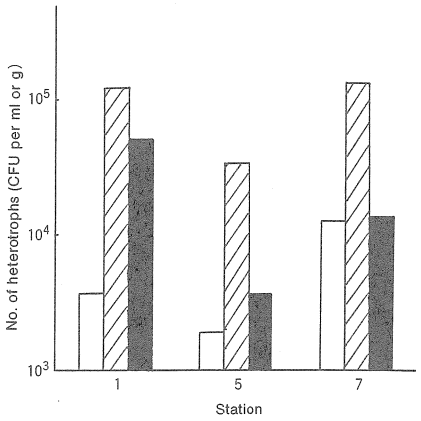

Fig. 2. Number of heterotrophs in seawater (□), mud (▨), and cockles (■) at station 1, 5, and 7 in cockles culture sites in Seberang Perai.

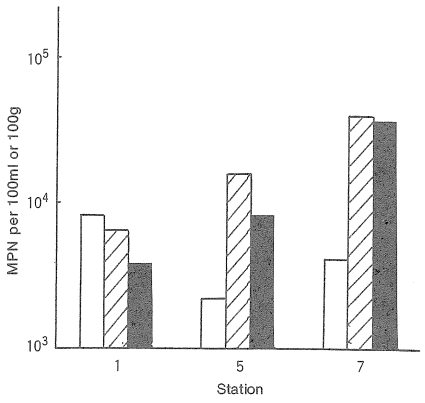

Fig. 3. Number of total coliforms in seawater (□), mud (▨), and cockles (■) at station 1, 5, and 7 in cockles culture sites in Seberang Perai.

than that from station 1 (river mouth of Juru river) but less than that from station 7 (river mouth of Jejawi river) (Fig. 3). The reason of the higher counts at station 5 was not clear. One possible reason is that there were autochtonous coliforms group which were not sewage-oriented but living in this habitat. The fact that higher counts were observed only in cockles and mud seemed to support this hypothesis. In muds and shells, sewage-oriented faecal coliforms usually makes up a low proportion of the total coliforms count, thus the test being usually less specific for sewage-oriented faecal coliforms in these samples. Total coliform number for seawater appears to reflect the influence from river whereby station 5 had the lowest number compared to station 1 and station 7.

3.2.3 Faecal coliforms

Regarding faecal coliforms, results taken were almost same as in heterotrophs (Fig. 4). Faecal coliforms in station 5 was the lowest as compared to stations 1 and 7 since faecal coliforms were obviously terrestrial origin unlike total coliforms. The faecal coliform count between stations 1 and 7 were not significantly different. They would have been brought in by the rivers.

As for the distribution of faecal coliform in seawater, mud, and cockles, it would be logical for the faecal coliform to be highest in mud which is the area of sedimentation and deposition, and cockles since the cockles are mud dwelling and filter feeders. The survival of faecal coliform in seawater is usually under stress thus giving lower numbers.

From the results obtained in station 5, cockles had the highest number of faecal coliform followed by seawater and mud. The unexpectively lower number in mud was probably due to the sandy quality of muds and to the constant stirring up of the bottom muds in this area by the presence of strong winds thus making stressful condition for survival of faecal coliforms. Faecal coliforms numbers in the cockles was the highest since faecal coliform can survive better in the cockles where they were sheltered from the stressful effect on the marine environment especially from salinity. The difference in values between sea water, mud, and cockles at station 5 was large as compared to that at stations 1 and 7. This large difference was not so apparent in

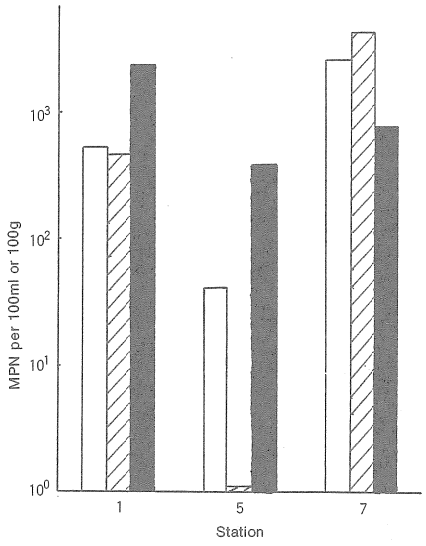

Fig. 4. Number of faecal coliforms in seawater (□), mud (▨), and cockles (■) at station 1, 5, and 7 in cockles culture sites in Seberang Perai.

stations 1 and 7 because these areas were located near the river mouth therefore having estuarine condition and also constant replenishment of freshwater and faecal coliform.

3.2.4 General discussions and conclusions

In culture sites where bivalve shelfish such as oysters, mussels, cockles, are grown in waters contaminated with sewage-derived pathogens, serious incidents of gastroenteritis in consumers of bivalve shellfish often occur, particularly when shellfish are consumed raw or lightly cooked. Since microflora of shellfish is affected by their environment-i.e. seawater and sediments, it is particularly important to know the amount and extent of microbiological pollution in seawater and sediments as well as shellfish. To determine the level of possible enteric-pathogens contamination, enumeration of faecal bacteria (not necessarily pathogens) whose presence is indicative of pollution of water by human or animal wastes is the usual recommended method[5-7].In Japan, for example, the culture sites of oysters is controlled by legislation regulating the content of coliform bacteria in seawaters (i.e. less than 70 total coliforms / 100 ml sea water for oyster culture and less than 230 faecal coliforms / 100 g oyster meat for raw eating).

A preliminary results obtained from this study revealed that faecal contamination was higher in two river mouths (St. 1 and 7) than the site between (St. 5), indicating that the culture site near to river mouth is greatly influenced by river inflow and domestic sewage flowing into rivers and coastal waters has significant effect on cockles culture. Of coures, the interpretation and applicability of the results obtained in this study is strongly limited by the lack of an overall view and detail picture of the area. Monitoring bacterial count of seawater, mud, and cockles at many other stations and in different seasons is a necessary step to achieve sanitary control of cockles culture. A guideline regarding the bacterial quality of seawater and cockles can be made only when reserch on these problems is fully conducted.

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マレーシアの食用ハイガイ生息地の大腸菌汚染と水質環境

木村　凡

東南アジア各地の浅海域では，カキ，ハイガイなど，貝類の養殖が広く行われており，これら養殖貝類に起因する食中毒が多発している。一方ハイガイ（別名，コックルス，二枚貝）は肝炎ウイルスの中間宿主となっているので，その生食は肝炎の蔓延につながっている。このたび，マレーシア政府の依頼により，食用ハイガイおよびその養殖地の食品衛生学的微生物汚染度，および水質環境の実態を把握するために基礎調査を行った。衛生指標細菌（大腸菌群，および大腸菌）による汚染度についてみると，環境水の大腸菌群数は10^3～10^4cells/100mℓ海水，ハイガイ肉の大腸菌数は10^3cells/100ｇであり，かなり高い汚染度であった。養殖地を塩分濃度からみた時，低塩分の河口域では高塩分の沖合い域に比べて，大腸菌数が環境水，供試ハイガイ，底泥のいずれにおいても高かった。したがって，河口域で採捕されたハイガイの生食は食品衛生学的に好ましくないことが示唆された。