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Bacterial Contamination in Selected Commercially Important Bivalve Species and Farmed Seaweed in the Panguil Bay, Northern Mindanao

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ABSTRACT

Bivalve molluscs comprise an important artisanal invertebrate fishery in Panguil Bay in northern Mindanao. The bay also supports one of the largest seaweed farming projects in the region. Recent concerns on declining water quality in the bay due to domestic sewage and industrial effluents motivated this research into investigating the bacterial contamination of food resources from the bay. Ten selected commercially important edible bivalve species from wild population and *Kappaphycus alvarezii* (*guso*) from a seaweed farm were collected monthly for 12 months from the Lanao del Norte side of Panguil Bay for the analysis of bacterial contamination, particularly fecal coliform bacteria. Water and sediments from the habitats of these organisms were also sampled for the same microbiological analyses. Fecal coliform bacteria were noted in *Arca antiquata* (*litob-litob*), *Katylesia* sp. (*punaw*), *Meretrix meretrix* (burnay), and *Modiolus metcalfei* (*amahong*) from freshly hand-picked samples from the muddy tidal flat in certain areas in the municipalities of Lala and Baroy, Lanao del Norte. Bivalve samples from market displays such as *Anadara* sp. (*balinsala*), *Modiolus* sp. (*baluyang*), *Pharella acutidens* (tudlo-datu), and *Trisidos* sp. (*lubag-lubag*), *Anodontia edentula* (*imbaw*) and *Mercenaria* sp. (*tuway*) were also similarly contaminated. The extent of bacterial contamination from hand-picked and market samples exceeded the current acceptability standards of Philippine bivalves for human consumption as set by the Bureau of Food and Drugs (BFAD). It is strongly recommended that bivalves from Panguil Bay be depurated and cooked properly before these are served in dining tables. There is no standard for fecal coliform density in seaweeds but the values obtained in the farmed seaweed, *K. alvarezii*, was within the acceptability standard for Philippine bivalves, thus, it may be considered safe for human consumption.

Keywords: Panguil Bay, bacterial contamination, fecal coliform, edible bivalves, farmed *Kappaphycus*

INTRODUCTION

Panguil Bay in Northwestern Mindanao is known for its rich fishery resources of prawns, crabs and molluscs. Bivalves are the most abundant shellfish or mollusc in shallow coastal waters near the crowded fishing villages of Lala, Baroy, Tubod and Kolambugan in the Lanao del Norte side of the Bay. The seaweed *Kappaphycus*, which does not grow naturally in Panguil Bay, has been successfully cultured at a commercial scale in the coastal areas of Kolambugan and Tubod.

Bivalve shellfish and farmed seaweed are economically valuable sources of proteins, vitamins and minerals for coastal communities in Panguil Bay, however, the lack of proper disposal facilities by many households contributes to declining water quality in the bay. Domestic sewage, feces of human and other warm-blooded animals, normally contains pathogenic bacteria, viruses, protozoans and fungi. When discharged into coastal waters, it can cause microbial contamination of filter-feeding organisms such as bivalves. Seaweeds can also become contaminated when colloidal particles from domestic sewage become jammed or embedded into the narrow forks of branching seaweed thalli. Eating these contaminated food products raw or only partially cooked presents a serious risk to human health.

Biological pollution occurs when microorganisms enter a water body from sources such as human waste, food operations, meat packing plants and medical facilities. Coliform, fecal coliform, and non-coliform bacteria such as fecal streptococcus are the major types of bacteria in polluted waters and are generally harmless to man (Tchobanoglous, 1979). Certain pathogens most commonly found in animal feces, however, are causal agents of human diseases such as food poisoning, diarrhea, typhoid fever, hepatitis A and many more (West, 1989). Bivalves such as oysters, mussels and clams are the most significant groups of shellfish associated with gastro-enteritis because of their filter-feeding activity (West, 1985). Contamination of shellfishes by pathogenic microorganisms is not, however, confined to sewage disposal since pathogens such as *Vibrio* and *Aeromonas* thrive in natural aquatic environments (Morris and Black, 1985). Contamination of shellfish viscera by pathogenic microorganisms can present a significant health risk to people who consume them raw or lightly cooked.

The presence of fecal coliform bacteria is commonly used as an indicator to assess potential health hazards to consumers of raw shellfish, the effectiveness of depuration and cooking, and the effects of sewage disposal on the aquatic environment. Fecal coliform also indicates potential contamination by pathogenic microorganisms not only from humans but also from other warm-blooded animals. Reports on bacterial contamination of shellfishes in the Philippines, however, are few and have been confined to few species such as oysters and mussels (Gacutan, *et al.* 1986). Some works have been published on bacterial depuration to reduce high bacterial and virus levels to acceptable ones (Dore and Lees, 1995; Lee, *et al.* 2008). Likewise very little work on bacterial contamination of seaweed is available and mostly focused on "ice-ice" disease (Largo *et al*. 1995; Tisera and Naguit, 2009). This study was conducted to determine the extent of bacterial contamination, particularly of fecal coliform, on bivalves and farmed red seaweed harvested from Panguil Bay, and to recommend strategies to reduce its potential impact on public health.

MATERIALS AND METHODS

Collection site for bivalve samples

Panguil Bay is located in the northwestern part of Mindanao (between 7°51' to 8°12' N; 123°37' to 123°55' E) and is a shared resource of the provinces of Lanao del Norte, Zamboanga del Sur, and Misamis Occidental (Fig.1). Bivalve samples were collected from the littoral zones of four sites, namely, Pacita, Lala; Raw-an Pt., Baroy; Poblacion,Tubod; and Mukas,

Kolambugan (black circles in Fig. 1). Pacita, Lala and Raw-an Pt., Baroy (Fig. 2) are major gathering grounds of both traditional and commercial bivalve gatherers and are located close to fishing villages with adequate toilet facilities. Bivalve samples from these sites were handpicked by the project team while bivalves from Tubod and Mukas were obtained from market displays.

Figure 1. Map of Panguil Bay showing the sampling sites for bivalves (black circles) and farmed seaweed (green square).

Collection site for farmed Seaweeds

The source of farmed seaweed samples of *K. alvarezii* (locally called *guso*) used in this study is the commercial seaweed farm in the coastal waters of Simbuco, Kolambugan, Lanao del Norte (open circle in Fig. 1) The farm site covers about 300 hectares and is located about 100 m from shore at a depth of 3-6 m (Fig. 2). The shoreline of Simbuco near the *guso* farm site is densely populated where people mostly lived in stilt houses with inadequate toilets.

Sample collection

At least two liters of samples of selected commercially important bivalves (from shellfish gatherers and market vendors) and two kilograms of farmed seaweed thalli were obtained monthly from May 2003 to April 2004 and transported to MSU-Naawan following standard protocols in handling and transport of samples (Al-Jebouri, 1981; West, 1988; APHA, 1998). One-kg samples of water and sediment in each site were also collected.

Figure 2. Collection sites for bivalve (top panel) and seaweed (bottom panel) samples in Lanao del Norte.

Laboratory analysis

Sample processing. Random samples of 30 -100 pieces from each pack of bivalve samples were rinsed with sterile seawater and processed for analysis immediately upon arrival in the laboratory following the procedure described by Al-Jebouri (1981) and Hasegawa (1987). The bivalves were pried open and shucked using a sterilized knife. Exactly 50 g of bivalve meat were aseptically weighed in an electronic top loading balance and homogenized in a sterile blender for 2-5 minutes. The homogenate was mixed with 450 ml sterilized seawater to produce 500 ml of bivalve homogenate of 1:10 dilution. The homogenate was transferred into a 1000-ml capacity sterile beaker and was serially diluted and inoculated to appropriate medium (Fig. 3). A random sample from the two-kilogram seaweed thalli was cut into small pieces then 50g of cuttings were homogenized in precisely the same manner as the bivalve preparation. A 50-gram aliquot sample from each 1kg sediment and water samples was likewise prepared for analysis.

Total Bacterial Density (Heterotrophic Plate Count)

The bacterial densities (expressed as colony-forming units per mL or CFU^{-mL}) of the different samples of bivalves, seaweeds, water and sediments were estimated using the pour plate method following the standard APHA (1998) protocol for bacteriological analysis.

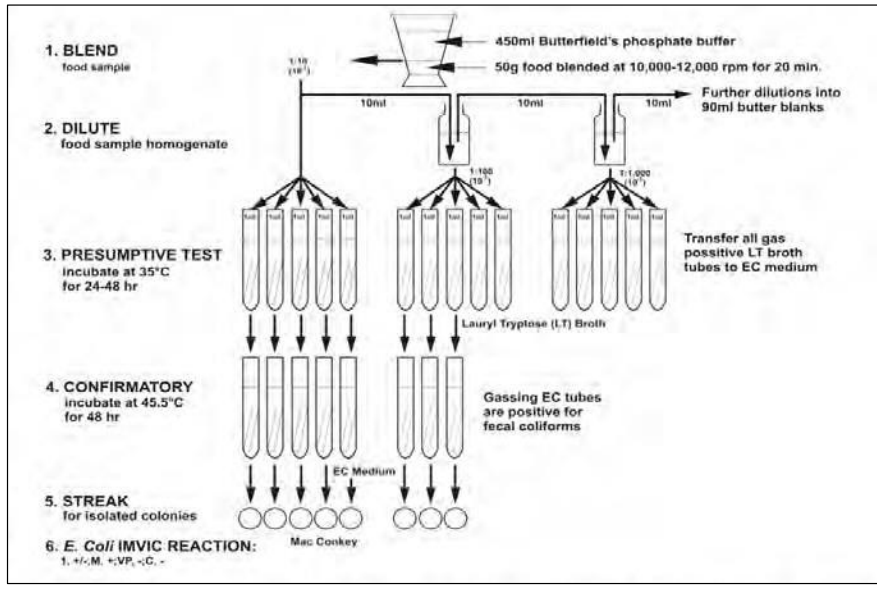

Figure 3. Flow chart of methods in sample preparation and enumeration of total and fecal coliform.

Estimation of Coliform Load

The Multiple-Tube Fermentation Technique was used to estimate the total and fecal coliform bacteria in the samples. This consisted of three tests, namely; Presumptive Test, Confirmed Test and the Completed Test following standard procedures (Refai, 1979; Hasegawa, 1987; Greenberg *et al.,* 1992; APHA, 1998) (Fig. 3). The densities of total coliform and fecal coliform bacteria were estimated based on tabulated MPN Indexes (0.1, 0.01 and 0.001) for 100 g food and other solid samples (Andrews, 1992) and 10.0, 1.0 and 0.01 for 100 mL water samples (Greenberg *et al.,* 1992; and APHA, 1998).

Depuration of Bivalves

Bacteria can be removed from bivalves in different ways, a procedure called depuration. In Panguil Bay and other Philippine fishing villages a local practice called *laming* is a practical and effective process in removing impurities from edible bivalves. The bivalves are placed in a pail or basin filled with filtered seawater or brackish water for 24-48 hrs. During this period the water is drained and replenished four to six times because bacteria accumulated by the bivalve are flushed out through the feces. A test was conducted to determine the efficiency of the said practice in removing fecal coliform and other bacteria. One can (1-liter cap) sample of each bivalve species was placed in separate basins filled with filtered seawater and provided with aeration at the wet laboratory of MSU-Naawan. The filtered seawater was changed six times for 48 hrs. Then the purified bivalves were processed for microbiological analyses to determine the bacterial load.

RESULTS AND DISCUSSION

Ten edible bivalve species from different habitat types and one red seaweed were sampled in the Lanao side of Panguil Bay (Table 1).

Table 1. List of bivalve shellfish and seaweed used in the assessment of bacterial contamination in Panguil Bay.

Local Name	English Name	Scientific Name	Collection Site	Habitat Characteristics
Litob-litob	Blood clam	*Arca antiquata*	Lala, Pacita	Muddy tidal flat
Punaw	Hen clam	*Katylesia* sp.	Lala, Pacita	Muddy tidal flat
Burnay	Surf clam	*Meretrix meretrix*	Lala, Pacita	Muddy tidal flat
Amahong	Brown mussel	*Modiolus metcalfei*	Lala, Pacita	Muddy tidal flat
Punaw	Hen clam	*Katylesia* sp.	Raw-an, Baroy	Sandy tidal flat
Burnay	Surf clam	*Meretrix meretrix*	Raw-an, Baroy	Muddy and sandy tidal flats
Balinsala	Cockle	*Anadara* sp.	Tubod market	Deep coastal waters of Tubod
Baluyang	Big Brown Mussel	*Modiolus* sp.	Tubod market	Deep coastal waters of Tubod
Tudlo-datu	*R*azor or Jacknife clam	*Pharella acutidens*	Tubod market	Muddy substrate of Tubod
Lubag-lubag	Twisted Shell; Propeller Ark	*Trisidos* sp.	Tubod market	Deep coastal waters of Tubod
Imbaw	Mangrove clam	*Anodontia edentula*	Mukas market	Muddy substrate of Kolambugan
Tuway	Mud clam	*Mercenaria* sp.	Mukas market	Muddy substrate of Kolambugan
Guso	Red Seaweed	*Kappaphycus alvarezii*	Simbuco	Deep coastal water farm site of Kolambugan

Bacterial Density

Bacterial densities (CFU-^{g}), total coliform and fecal coliform (MPN^{-100g} or MPN^{-100mL}) in bivalves and other samples varied with species, sampling months and sampling areas. Average bacterial density in freshly hand-picked bivalves was highest in *M. metcalfei* (720 x 10^3CFU^{-g}) from the muddy tidal flat of Pacita, Lala while *Katylesia* sp. from the sandy substratum of Raw-an Pt., Baroy had the highest bacterial density (903 x 10^3CFU^{-g}) (Table 2). Bacterial load may also vary according to body size, as in the case of *M. meretrix* where medium-sized specimens had higher bacterial count than smaller ones gathered from Lala. On the average across sites, bivalves from Raw-an Pt., Baroy had higher total bacterial accumulation where both water and sediment had higher bacterial load (Table 2).

An extremely high bacterial density was observed in *Mercenaria* sp. (*tuway*) collected from Mukas market (>6647 x 10^3CFU^{-g}) while a lower density (548 x 10^3CFU^{-g}) was obtained from a closely related mangrove clam, *Anodontia edentula* (Table 3). The bivalves obtained from Tubod market generally had high mean bacterial density with the highest value of 1,109 x 10^3CFU^{-g} obtained from *P. acutidens.* The brown mussel *Modiolus* sp., on the other hand, had a very low bacterial density (3.02 x 10^3CFU^{-g}) although its location in the deeper, offshore areas of Tubod may have reduced the level of contamination (Table 1).

Table 2. Average bacterial density (CFU^{-g} or CFU^{-ml}) and fecal coliform geometric mean (x_g) of MPN^{-100g} or MPN^{-100ml} in freshly hand-picked bivalves, seaweed, water and sediment samples collected from May 2003-April 2004.

Sampling sites/Samples	Bacterial Density ($x10^3$ CFU^{-g} or CFU^{-ml})	Fecal coliform (x_g)
Lala		
Arca antiquate	593	77
Katylesia sp.	448	178
Meretrix meretrix	512	79
Modiolus metcalfei	720	33
Water	13.9	84
Sediment	242	5.83
Raw-an Pt.		
Katylesia sp.	903	54
Meretrix meretrix (medium)	781	179
Meretrix meretrix (small)	401	24
Water	316	159
Sediment	845	23
Simbuco		
Kappaphycus alvarezii	760	4
Water	68	43

Table 3. Average bacterial density ($x10^3CFU^{-g}$ or CFU^{-ml}) and fecal coliform geometric mean(x_g) of MPN^{-100g} or MPN^{-100ml} in market collection of bivalve samples from May 2003-April 2004.

Sampling sites/Samples	Bacterial Density ($x10^3$ CFU^{-g} or CFU^{-ml})	Fecal Coliform Density (x_g)
Tubod		
Anadara sp.	930	2
Modiolus sp.	3.02	<2
Pharella acutidens	1109	85
Trisidos sp.	525	2
Mukas		
Anodontia edentula	548	34
Mercenaria sp.	>6647*	165

* - Number of colonies exceed 300 colonies per plate.

It is not within the scope of the study to differentiate the bacterial sources or determine what percentage of the bacteria comes from the normal aquatic flora and those from domestic sewage contamination. The lower bacterial density in water than that of the sediment could probably be due to the high organic matter accumulated in the sediment. The data also suggest, rather inconclusively, that muddy substrates tend to accumulate higher bacterial density than sandy substrates.

Bivalves are predominantly filter feeders. This feeding mechanism results in the natural ability to extract bacteria from the coastal waters and may be variable across species. Among the

hand-picked bivalves *Katylesia* sp., *M. meretrix*, and *M. metcalfei* had the highest bacterial load. This could not be attributed to the habitat characteristics alone because *M. meretrix*, and *M. metcalfei* are muddy substrate dwellers while *Katylesia* sp. can be found both in sandy and muddy sediments. It would be an interesting future study to determine the filtration capacity of different bivalve species and their accumulation of bacteria in the internal organs.

The bacterial load of bivalves from the local markets varied widely with opposite extreme values, with the lowest obtained from *Modiolus* sp. and the highest from *Mercenaria* sp. Differences in habitat characteristics of these two organisms could have greatly influenced the wide discrepancy in their bacterial load. *Modiolus* sp. inhabits the deeper portion of Panguil Bay, relatively far from the influence of domestic waste. *Mercenaria* sp., on the other hand, abounds in deep, muddy mangrove areas in the densely populated and sanitation-poor area of Mukas. The prop roots and pneumatophores of different mangrove species are quite effective in trapping and accumulating large volume of domestic and agricultural wastes from lowland and upland areas.

Despite the low bacterial population in Simbuco waters (68×10^3 CFU^{-g}), thalli of farmed *K. alvarezii* were found to have a high bacterial density (760×10^3 CFU^{-g}). This result suggests the ability of the seaweed to accumulate bacteria from water. In the seaweed farms of Simbuco, thalli of *K. alvarezii* are closely tied along the length of a rope (80-100 m each) hanging between two posts. The seaweed farms are adjacent each other so floating organic debris and fresh solid waste from domestic sources can become easily entangled in the mesh of thalli-laden ropes.

Fecal Coliform Density

All bivalve samples collected from different sources for this study were contaminated with fecal coliform (Table 2 and 3) and even some with *Escherichia coli*. The most contaminated bivalves from all source are *M. meretrix* (from Raw-an Pt.), *Katylesia* sp. (Pacita, Lala) and *Mercenaria* sp. (Mukas market) at 179 MPN^{-100g}, 178 MPN^{-100g} and 165 MPN^{-100g}, respectively (Fig. 4). The lowest concentration of fecal coliform was observed in *M. metcalfei* (Lala) and *M. meretrix* (Raw-an Pt). The farmed seaweed *K. alvarezii* had a very low fecal coliform content (4 MPN^{-100g}), .

The coliform bacterial composition varied with the sample group (bivalve and seaweed), sampling area and sampling month as indicated in the IMViC Test (Indole, Methyl red, Voges-Proskauer and Citrate) for preliminary differentiation of coliform and fecal coliform groups. Two coliform species (*Aeromonas* sp. and *Pseudomonas* sp.) and seven fecal coliform species (*Citrobacter freundii, Citrobacter* sp., *Enterobacter aerogenes, Escherichia coli, Escherichia hermanii, Escherichia vulneris* and *Klebsiella* sp.) were identified from the samples. Gram staining also showed that bacterial morphology varied from short rod to rod-shaped, non-spore-forming bacteria.

A. *Meretrix meretrix* **B. *Katylesia* sp.**

C. *Mercenaria* sp . **D. *Pharella acutidens***

Figure 4. Freshly handpicked (A&B) and market display (C&D) bivalves in Panguil Bay with the highest fecal coliform content.

High fecal coliform densities were generally observed in October, November, December, January, February and April. These were rainy months (except in late April) and unusual volumes of water runoff were noted in the sampling sites which must have washed away human feces and that of other warm-blooded animals and brought these to the sea. The close proximity of the bivalve beds to residential areas makes them highly vulnerable to fecal coliform. Davis et al. (1980) declared that the aquatic environment is physiologically hostile to fecal bacteria, thus, these microorganisms are not expected to grow normally in the salty coastal waters. High fecal coliform density in many bivalve species could be due to recent or continuing fecal contamination rather than bacterial reproduction or regrowth (Zaen–Ul-Abedin, undated). In contrast, water is the usually the first recipient of domestic sewage from terrestrial sources which would explain higher fecal coliform density in the water around Raw-an Pt. than some bivalves.

Depuration or Self-Purification in Bivalves

The common practice of depuration or *laming* of freshly gathered bivalves serve to reduce bacterial load through regular excretions or 'self-purification'. The experimental depuration of different bivalves for only two days reduced bacterial density between 17.64% (*M. meretrix* from Lala) to as much as 98.64% (*M. metcalfei* from Lala). Depuration also reduced total coliform and fecal coliform densities in this bivalve species by 81.25%-99.51% and

40.0%-99.51%. Mortalities occurred in some species during depuration which increased bacterial activity, but overall depuration or the local 'laming' practice can remove most of the bacterial load and fecal coliform in commercially gathered bivalves, and thus, safeguard health of the consuming public. Reduction in fecal coliform was more efficient than the removal of total bacterial load, a finding that supports expert opinion that the presence of fecal coliform in bivalves is mainly due to recontamination.

Table 4. Comparison and percentage reduction of bacterial densities (x 10^3 CFU^{-g}); total coliform (MPN^{-100g}) and fecal coliform (MPN^{-100g} in non-depurated (ND) and depurated (D) bivalves.

Samples	Bacterial Densities			Total Coliform			Fecal Coliform		
	ND	D	%	ND	D	%	ND	D	%
M. meretrix (Lala)	567	467	17.64	1600	7.8	99.51	1600	7.8	99.51
M. meretrix (Raw-an, Muddy)	430	35.2	91.81	≥1600	32	98.00	300	32	89.33
A. antiquata (Lala)	154	71.0	53.90	≥1600	210	86.88	300	7.0	97.67
M. metcalfei (Lala)	236	3.21	98.64	1600	300	81.25	300	2.0	99.33
Katylesia sp. (Raw-an)	753	853	-13.28	≥1600	64	96.00	1600	54	96.63
M. meretrix (Raw-an, Sandy)	338	*		≥1600	17	98.94	110	7.8	92.91
Katylesia sp. (Lala)	567	1660	-192.77	≥1600	500	68.75	≥1600	300	81.25
Mercenaria sp. (Mukas)	2890	3000	-3.81	≥1600	300	81.25	500	300	40.00
Pharella acutidens (Tubod)	1640	2360	-43.90	≥1600	600	62.50	1600	500	68.75

Legend: (-) Increased bacterial density on depurated due to mussel mortality
* No analysis, >50% died during depuration process

Shellfish Standards for the Philippines

The current standards (Table 5) for bacterial load of Philippine shellfish, are 500,000 CFU^{-g} for fresh oysters and 100,000 CFU^{-g} for mussel and clams, and 20 MPN/100g for coliform load (BFAD). In the present study the densities of bacteria, coliform bacteria and fecal coliform in all the edible bivalves generally exceeded the current standards. Results generally show that coliform densities for seaweeds are low, however, a standard for bacterial and fecal coliform load for edible seaweeds is not available. If the current standards for shellfish will be adopted for seaweeds, then it is logical to say that it is safe to eat raw seaweed from Simbuco, but such an advice cannot encompass the farmed seaweed in Ozamiz City.

MANAGEMENT IMPLICATIONS AND RECOMMENDATIONS

This study provides strong evidence that the commercially important bivalves and seaweed from Panguil Bay are generally contaminated with coliform and non-coliform bacteria, in fact, exceeding the Philippine standards for safe consumption of shellfish. Bacterial contamination of seafood is chronic and pervasive in many harvesting areas and endangers public health by passing the contamination to the consumers. Microbial contamination of bivalves and seaweed is largely underestimated and undermanaged, and this oversight by the local government and the health department poses a potential public health risk. The elimination of microbial contamination from consumer seaweed and bivalves in Panguil Bay and other bays should be at the top priority agenda by government and civil society.

Table 5. Comparison of bacterial load and fecal coliform) in bivalves and BFAD (Bureau of Food and Drug) Standards.

Bivalve species	Bacterial load ($x10^3$ CFU^{-g})	BFAD Std. Bacterial load ($x10^3$ CFU^{-g})	Fecal coliform (MPN^{-100g})	BFAD Std. for coliform (MPN^{-100g})
Mussel & Clams		100		20
A. antiquata	593		77	
Anadara sp.	930		2	
A. edentula	548		34	
Katylesia sp. (Lala)	448		178	
Katylesia sp. (Raw-an)	903		54	
M. meretrix (Lala)	512		79	
M. meretrix (Raw-an muddy)	781		179	
M. meretrix (Raw-an sandy)	401		24	
M. metcalfei	720		33	
Mercenaria sp.	>6647*		165	
Modiolus sp.	3.02		<2	
Pharella acutidens	1109		85	
Trisidos sp.	525		2	

* Number of colonies exceeded 300 colonies per plate.

On the basis of these findings, it is recommended that consumers should first depurate these bivalves and properly cook them to avoid ingesting pathogenic microorganisms. Despite the low levels of bacterial contamination in farmed seaweeds it is still important to wash the thalii thoroughly with clean water to remove colloids, epiphytes and bacterial contaminants before eating them raw as salad. Further research is also needed for the improvement of public health control and the enhancement of shellfish safety.

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