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Original Article

Effect of microbial load on the condition index of the edible oyster, *Saccostrea cucullata* in the Sundarbans, India

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Abstract: The effect of microbial load on the condition Index of the edible oyster, *Saccostrea cucullata* were analysed on monthly basis during 2010 and 2011 from the three different stations (Namkhana, Frasergaunge and Sajnekhali) of Indian Sundarbans. The results showed significant variation with respect to microbial load between stations and seasons, which is reflected in the tissue of edible oyster. Significant positive correlations were observed between microbial load of the ambient environment and the tissue system of oyster. The Condition Index of the oyster species also exhibited negative correlation with the microbial load of oyster tissue, which confirms the negative stress induced by microbes on the growth and survival of the species.

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Introduction

The condition index (CI) is used to estimate the effect that different environmental factors on oyster meat quality. The growth or health status of oyster is generally represented by its condition Index value. The indices (CI) compare the dry meat weight of the animal to the interior of the carbohydrate and protein fractions, lipid and mineral contents and has been related to pollution effect (Austin et al., 1993). The CI is an inexpensive, quick, representative and responsive tool for monitoring pollution (Scott and Lawrence, 1982) and has also been used to estimate growth differences among oysters living in different environmental conditions (Austin et al., 1993; Schumacker et al., 1998). The better condition index of the oyster is a reflection of the good environmental quality of the water (Bhattacharyya et al., 2010).

A number of studies revealed that the sources of fecal pollution mainly include municipal sewage system, storm water runoff, marine and boaters, recreationalists, farm animals, pets and wild life (Glasoe and Christy, 2004). A primary concern in shellfish growing areas, which are generally located in the intertidal and shallow sub-tidal coastal zone, is contaminated from human sewage and animal wastes and the related health risk. Krishnakumari et al. (1990) observed that condition index and percentage edibility values were higher at less polluted stations along Maharastra Coast. On this background, no study in Indian Sundarbans has been conducted to evaluate the microbial population in relation to condition index of oyster.

The Indian Sundarbans is presently under stress due to discharge of untreated sewage and industrial wastes from the highly urbanized and industrialized city of Kolkata, Howrah and the newly emerging Haldia port- cum- industrial complex. According to UNEP report, 1125 million lit. Of waste water is discharged per day through Hooghly estuary. The lower stretches receive waste and wastewater load of 396 ×108 km^3 per hour along with the annual run off

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493 km^3. The total volume of sewage discharge from the city of Kolkata has been estimated 350 million tons (Mitra et al., 2009). In addition, a large number of shrimp culture farms are situated at the out skirt of the Kolkata city area (like Malancha, Minakhan block etc.) discharging huge organic load and trigger the microbial growth. In this context, it is extremely important to evaluate the impact of microbial load on the growth (CI value) of the oyster. In the present investigation for the first time, the impact of microbial load on the CI index of oyster (*Saccostrea cucullata*) was evaluated. For this study, three potent pollution indicating bacterial group viz. total bacterial count (TBC), total coliform (TC) and fecal coliform (FC) in the oyster flesh, water and sediment were measured from three different station of Sundarbans to evaluate the degree of contamination and its subsequent impact on the condition index of the oyster. The studies were conducted for two consecutive years in the three different sites of Indian Sundarbans namely Namkhana, Frasergaunge and Sajnekhali, which are exposed to different level of environmental pollution. This analytical study will have great impact to the farmer and consumer who are dependent on this important sea food.

Materials and Methods

Study area: The present investigation was carried for consecutive two years (2010-2011) in the three different stations like Namkhana, Frasergaunge and Sajnekhali of Indian Sundarbans, which are exposed to varying degree of anthropogenic pollution (Fig. 1). Station-I (Namkhana: 21°45'48.54''N, 88°13'52.55''E) is situated in the western sector of Sundarbans, where high concentration of heavy metals was observed in water and the oyster. Station-II (Frasergaunge: 21°36'55.72''N, 88°12'33.15''E) is located further from pollution sources than station I. Station-III (Sajnekhali: 22°07'36.21''N, 88°49' 50.60'' E) is situated in the eastern sector of Indian Sundarbans, which is considered to be relatively low in sources of pollution (Barua et al., 2011).

Sample collection: Water, sediments and six healthy oysters (4 to 5 cm) samples were collected in each month from the intertidal zone of each sampling station. Each oyster was scrubbed, rinsed with distilled water (several times) and stored in a container, preserved in crushed ice and brought to the laboratory. The meat was aseptically extracted using a sterile knife and immediately used for the microbial analysis of oyster flesh (Cleseri et al., 1998). Water samples were collected using sterile water sampler. The sediments were collected using Peterson grab, then put into sterile polythene bags and transported to the laboratory under ice bag for bacteriological examinations (Cleseri et al., 1998).

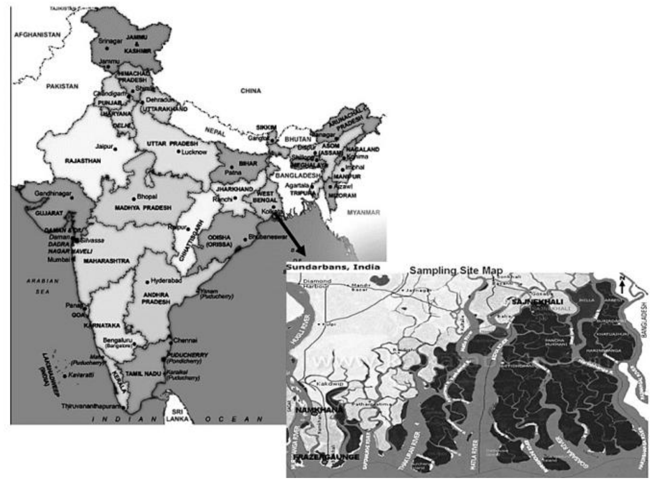

Figure 1. Map of Indian Sundarbans showing the location of sampling stations. Three stations viz., Namkhana, Frasergaunge and Sajnekhali.

Condition index measurement: Condition index (CI) of oyster is recognized as the degree of fatness or the extent to which the meat fills in the shell cavity. It was measured as the ratio of dry oyster meat (oven dried at 60ºC for 48 hrs) to the volume of the shell cavity (Abbe and Albright, 2003).

$$\text{Condition index (CI)} = \frac{\text{Dry tissue weight (g)}}{\text{Shell cavity volume (ml)}} \times 100$$

Bacteriological Analysis: Total coliform (TC), fecal coliform (FC) and total bacterial load (TBC) in the flesh of oyster, sediment and water of three different stations were enumerated. For bacterial analysis, 10 g oyster sample (meat) was blended with 90 ml of sterile 0.5% (w/v) peptone buffer (pH = 7) and different dilutions (10^{-1} to 10^{-2}) were made. For enumeration of coliform, fecal coliform, the standard

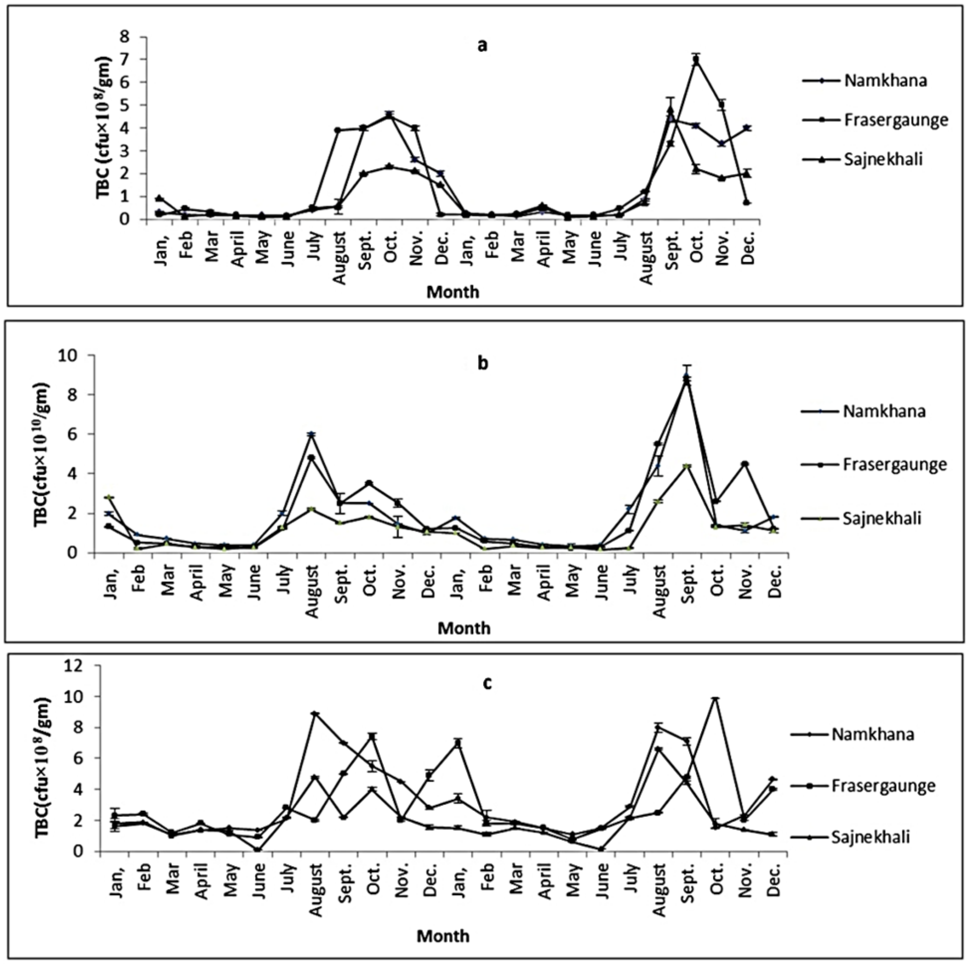

Figure 2. Variation of total bacterial count (TBC) (a) in water, (b) sediment and (c) oyster at all the sampling stations.

MPN (Most Probable Number) procedure (Cleseri et al., 1998) was adopted using LTB (lowryl tryptose broth) and EC (*Escherichia coli*) culture broth, respectively. Briefly, 10 ml of 10^{-1} dilution was added in test tube containing 10 ml volume of double strength and 1 ml of each dilution (10^{-1} and 10^{-2} dilution) was added separately in test tube containing 10 ml volume of single strength LTB broth. The total sets were incubated at 35 ± 0.5°C for 24 hrs and examined for the presence of growth accompanied by gas production. The MPN was calculated and the results were expressed as "presumptive coliform MPN/100 g." Then the positive culture was inoculated in to brilliant green lactose bile broth and the tubes were incubated at 35 ± 0.5ºC for 24 hrs and examined for growth with gas production. The MPN of total coliform (TC) was calculated and the results were expressed as "confirmed coliform MPN/100 g". To enumerate fecal coliform (FC), inocula from 24 hrs positive presumptive tubes were aseptically transferred to tubes of EC medium. These tubes were incubated at 44.0 ± 0.5°C for 24 hrs and examined for the presence of growth with gas production. The results were expressed as "fecal coliform MPN/100 g". Similarly, for total coliform and fecal coliform in the water and sediment samples were also enumerated. The total bacterial count (TBC) in all samples was done by standard plate counting method using tryptose glucose beef extract agar (TGBEA) media.

Statistical Analysis: The relationship among the parameters was analyzed by SPSS-10.0. Each sample was analyzed and data were represented as mean ±SD.

Results

The result indicated that a unique seasonal trend in all the three stations with respect to total bacterial

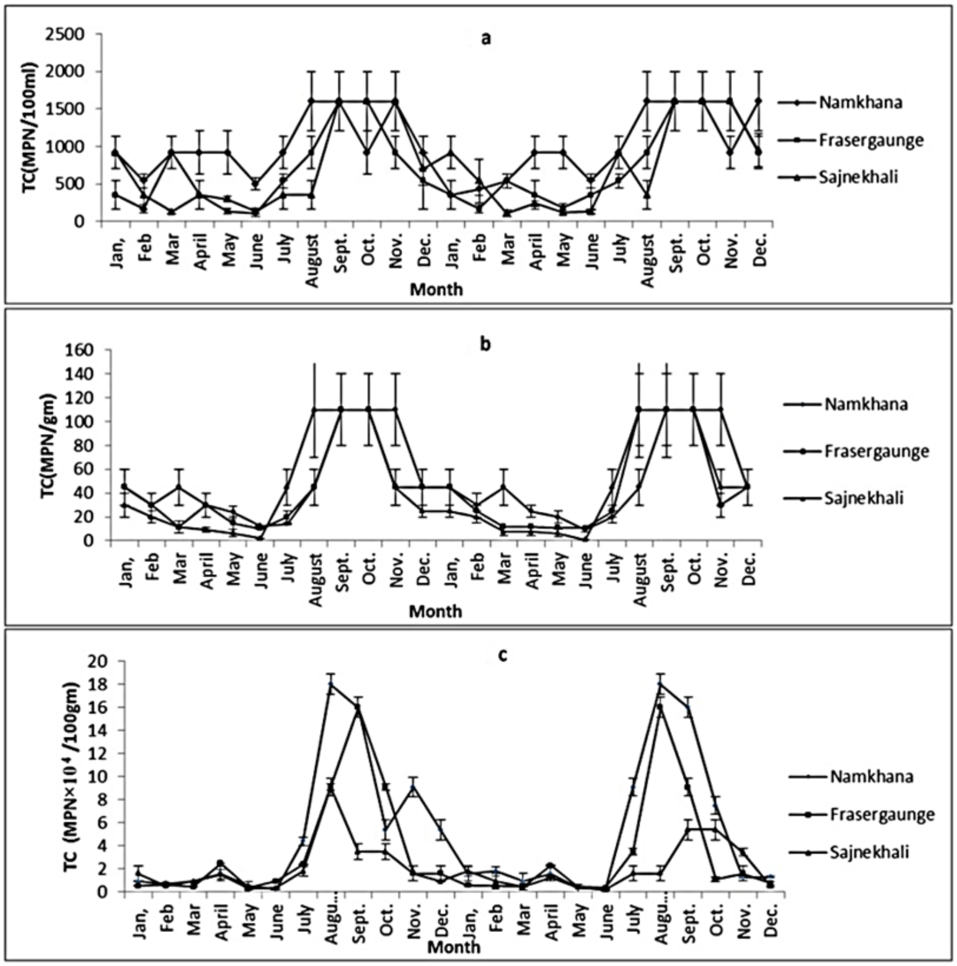

Figure 3. Variation of total coliform (TC) (a) in water, (b) sediment and (c) oyster at all the sampling stations.

count (TBC), total coliform (TC) and fecal coliform (FC) in oyster flesh, water and sediment. Relatively higher levels of total bacterial count (TBC), total coliform (TC) and fecal coliform (FC) in water, sediment and in oyster tissue were observed during the monsoon (July-October) season and lowest level during the pre-monsoon (March-June) season.

The highest and lowest quantity of TBC in water samples during monsoon and pre monsoon season recorded in Namkhana as $4.6x10^8$ cfu/ml and 1.5 $x10^7$ cfu/ml, in Frasergaunge $7x10^8$ cfu/ml and $8x10^6$ cfu/ml and in Sajnekhali $4.8x10^8$ cfu/ml and $7.8x10^6$ cfu/ml, respectively (Fig. 2a). The highest and lowest population density of total bacteria in sediment samples during monsoon and pre-monsoon season were recorded in Namkhana as $9x10^{10}$ cfu/g and $3.5x10^7$ cfu/g, in Frasergaunge $8.8x10^{10}$ cfu/g and $2.2x10^7$ cfu/g and in Sajnekhali $1.8x10^{10}$ cfu/g and $1.5x10^7$ cfu/g, respectively (Fig. 2b). In relation to the environmental sample, the quantity of TBC in oyster during monsoon and premonsoon was recorded in Namkhana as $8.9x10^8$ cfu/g and $1.1x10^8$ cfu/g, in Fresergaunge $10x10^8$ cfu/g and $8x10^7$ cfu/g and in Sajnekhali $6.6x10^8$ cfu/g and $10x10^6$ cfu/g, respectively (Fig. 2c).

The maximum density of total coliform (TC) in the water, sediment and oyster tissue was recorded in monsoon period as in Namkhana 1600 MPN/100 ml, 110 MPN/g and 180000 MPN/100 g, in Frasergaunge 1600 MPN/100 ml, 110 MPN/g and 160000 MPN/100 g and in Sajnekhali 1600 MPN/100 ml, 110MPN/g and 91000 MPN/100 g, respectively (Fig. 3). During pre-monsoon season, a comparatively low amount of TC was enumerated in Namkhana 430 MPN/100 ml, 2.4 MPN/g and 2400 MPN/100 g, in Frasergaunge 110 MPN/100 ml, 0.9 MPN/g, and 41400 MPN/100 g and in Sajnekhali 110 MPN/100 ml, 0.9 MPN/g and 2000 MPN/100 g

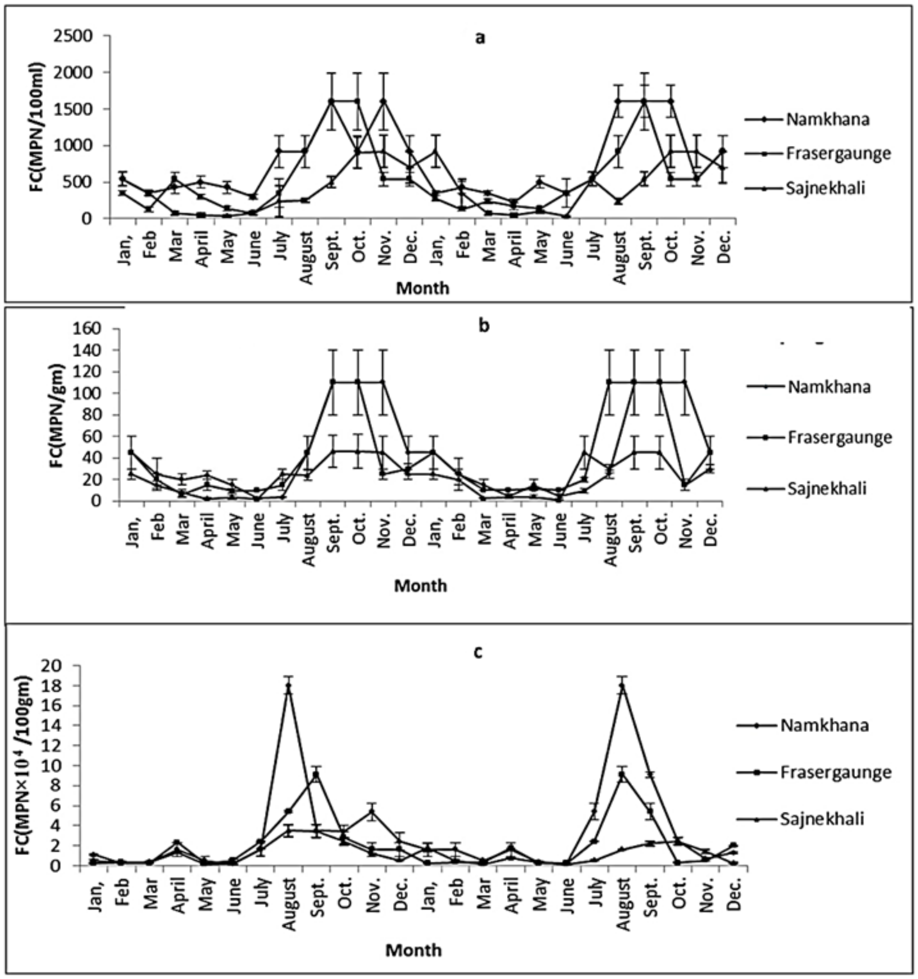

Figure 4. Variation of fecal coliform (FC) (a) in water, (b) sediment and (c) oyster at all the sampling stations.

(Fig. 3) in the water, sediment and oyster tissue, respectively.

The fecal coliform load (FC) was highest in water sample during monsoon and lowest during pre-monsoon season recorded as in Namkhana 1600 MPN/100 ml and 220 MPN/100 ml, in Frasergaunge 1600 MPN/100 ml and 80 MPN/100 ml and in Sajnekhali 920 MPN/100 ml and 33 MPN/100 ml (Fig. 4a). The highest fecal coliform (FC) in sediment samples during monsoon and pre-monsoon season were recorded as in Namkhana 110 MPN/g and 2.4 MPN/g, in Frasergaunge 110 MPN/g and 10 MPN/g and in Sajnekhali 46 MPN/g and 0.7 MPN/g (Fig. 4b). In oyster tissue, the fecal coliform (FC) recorded during monsoon and pre monsoon season recorded as in Namkhana 180000 MPN/100g and 2400 MPN/100 g, in Frasergaunge 91000 MPN/100 g and 2140 MPN/100g and in Sajnekhali 35000 MPN/100 g and 1400 MPN/100g (Fig. 4c).

The microbial load in the ambient water and sediment exhibited significant positive correlation ($P<0.01$) with that in the oyster tissue except TC and FC of sediment at Namkhana and Frasergaunge (Table 1).

The seasonal variation of condition index has been found to be relatively higher in oyster during the pre-monsoon and lowest value during the monsoon in all the three stations. The station wise order of highest CI of oyster was as Sajnekhali > Frasergaunge > Namkhana (Fig. 5). Highest CI of the oysters were 36.0 at Sajnekhali, 32.73 at Frasergaunge and 19.25 at Namkhana found during the pre-monsoon season and 10.72, 6.88 and 5.0 during the monsoon at Sajnekhali, Frasergaunge and Namkhana, respectively.

The condition index of edible oyster showed

Table 1. Inter-relation between different variables at three different stations viz., Namkhana, Frasergaunge and Sajnekhali.

Character	Namkhana		Frazergaunge		Sajnekhali	
	'r'-value	'p'-value	'r'-value	'p'-value	'r'-value	'p'-value
TBC of water × TBC of oyster.	0.436	<0.05	0.532	<0.01	0.473	<0.05
TBC of sediment × TBC of oyster.	0.813	<0.01	0.450	<0.05	0.727	<0.01
TC of water × TC of oyster.	-0.033	IS	0.531	<0.01	0.524	<0.01
FC of water × FC of oyster.	-0.066	IS	0.686	<0.01	0.607	<0.01
TC of sediment ×TC of oyster.	0.387	IS	0.376	IS	0.656	<0.01
FC of sediment × FC of oyster.	0.318	IS	0.385	IS	0.710	<0.01
TBC of oyster× CI of oyster	-0.664	<0.01	-0.454	<0.05	-0.685	<0.01
TC of oyster × CI of oyster.	-0.401	<0.05	-0.430	<0.05	-0.555	<0.01
FC of oyster × CI of oyster.	-0.386	IS	-0.405	<0.05	-0.711	<0.01

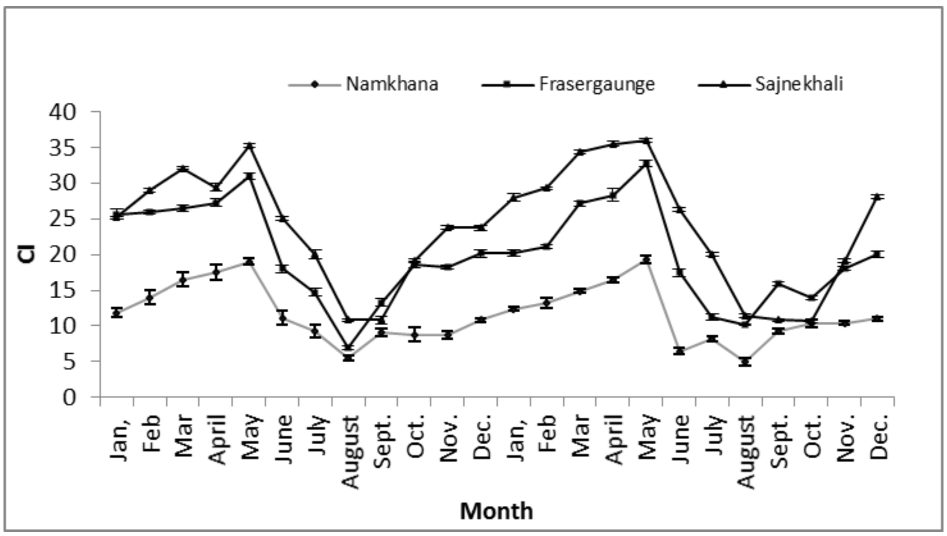

Figure 5. Variation in condition index of oyster at all the sampling stations.

significant negative correlations with total bacterial load (r = -0.664, *P*<0.01 at Station-I; r = -0.454, *P*<0.05 at Station-II and r = -0.685, *P*<0.01 at Station-III), coliform load in the oyster flesh (r = -0.401, *P*<0.05 at Station-I; r = -0.430, *P*<0.05 at Station-II and r = -0.555, *P*<0.01 at Station-III) and the condition index (CI) value showed insignificant negative correlation (r = -0.386) with fecal coliform in the oyster flesh from Station-I, while it has significant negative correlation with fecal coliform at Station-II (r = -0.405, *P*<0.05) and at Station-III (r = -0.711, *P*<0.01), respectively (Table 1).

Discussion

Relatively higher values of microbial load in all samples were observed in monsoon months (July to October), which may be related to storm water runoff and increased runoff from adjacent landmasses. The estuarine water budget in the Sundarban estuary is regulated by dilution caused by fresh water discharge (Mitra et al., 2009) that bring huge microbial load in the system. As a result, significant fresh water volume during monsoon in the present study area increases the microbial load in monsoon season.

The station-wise order of microbial contamination in the study area was Namkhana > Frasergaunge > Sajnekhali. This spatial variation may be attributed to the varying level of anthropogenic stress. The level of microbial load is a reflection of environmental pollution that is highest in station-I owing to fish landing and marketing activities. The lowest microbial load at station-III is reflective of the minimul pollution sources. Namkhana station receives the wastewater from kolkata and nearby

Haldia port-cum-industrial complex but Frasergaunge station receives the discharge of several hotel and tourism units located at Bakkhali. On the other hand, Sajnekhali station is situated in eastern sector of Indian Sundarbans, which is noted for its Wilderness, anthropogenic stress is minimum in this sampling station owing to reserve Mangrove forest (Jana et al., 2013). Similar results were also obtained in coastal South Carolina, where scientists have employed a variety of technique to monitor and compare land uses and ecosystem responses in highly urbanized Murrells Inlet and relatively undeveloped North Inlet (Scott et al., 1996; White et al., 2004). Murrells Inlet also had higher occurrences of *E. coli* bacteria, fewer coliform free stations, and fewer bacterial species comprising the coliform group-findings that the researchers attributed to urban influences and higher densities of on- site sewage systems in the Murrells Inlet water shed (Booth and Jackson, 1997). Subsequent analysis of Murrells Inlet by Kelsey (Kelsey et al., 2004, 2003) identified concentrations of on-site sewage systems, a rain fall events, and runoff from urban areas as key predictors of fecal coliform levels.

The microbial load in the ambient water and sediment exhibited significant positive correlation with that in the oyster tissue except TC and FC of sediment at Namkhana and Frasergaunge. As the microbes from the ambient media often get accumulated in shellfish through filter feeder activity, hence the microbial load in ambient environment is directly affected this important sea food (Mitra et al., 2007).

The lowest CI value at station-III because of the non-existence of industries and anthropogenic activities in the reserved forest area. Less pollution and anthropogenic stress may favour the maximum deposition of glycogen in oyster tissue and hence increase the CI value of oyster at station-III than other stations. Krishnakumari et al. (1990) also observed that condition index and percentage edibility values were higher at less polluted stations along Maharastra coast. Oyster condition and gonadal indices declined in areas receiving high levels of bacterial pollution, adjacent to known pollution sources such as marinas and highways (Scott, 1976).

Condition Index of oyster decreases with increase the microbial load in tissue of oyster because of markedly decline the assimilation efficiency of oyster and also oyster would expand considerable energy at high coliform concentration. Similar results were also obtained by Scott (1976) while working in Little River, SC and North Inlet, SC. He observed significant declines in oyster condition index when total coliform bacterial densities were > 70/100 ml. At these densities, the assimilation efficiency of the oyster markedly declined resulting in overproduction of gill mucous to deal effectively with the increased particulate load that generally accompanies elevated coliform bacterial densities. In the present case study, the lowest condition index values of oysters at Namkhana and also in the monsoon months coincided with high microbial load. The present result indicates an alarming situation with respect to microbial load in the oyster tissue and its inter-relationship with condition index values. Therefore proper water classification on the basis of microbial load is of extreme importance. Oyster, being an edible product needs continuous monitoring with respect to coliform load to overcome the barrier of consumer acceptability, which may otherwise poses an adverse effect on the human health.

The ability to produce, transport and market, healthy disease-free shellfish is crucial to the success of the Indian oyster industry. Under such circumstances, results of the present work may serve as baseline information for initiating oyster industry in the maritime state of West Bengal.

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