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EFFECT OF SOME HEAVY METAL (Zn, Cu, Pb) POLLUTANTS AND MICROBIAL LOAD ON THE EDIBLE OYSTER (*SACCOSTREA CUCULLATA*) IN SUNDARBAN, WEST BENGAL

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ABSTRACT

Total bacterial count (TBC), total coliform (TC), total faecal coliform (TFC), *E. coli* and *Bifidobacterium* sp. in the flesh of edible oyster (*Saccostrea cucullata*), in three collecting stations of Sundarban, varied during different seasons of a year. The bacterial load was maximum in monsoon and minimum in pre monsoon. Heavy metal content in the flesh showed following sequence: Zn>Cu>Pb, with maximum values during monsoon and minimum values during pre-monsoon. Further, maximum antioxidant enzyme (Catalase and Superoxide dismutase) activities in the oyster were recorded during pre-monsoon which decreased in monsoon, while the level of lipid peroxidation (free radical) was minimum in pre-monsoon. It appears, therefore, that the antioxidant activity in oyster was inversely proportional to the heavy metal concentration in the flesh. Hence, monitoring of heavy metal content in the water of Sundarban estuary is considered vital towards the survivability of the oysters.

Key words: *Bacterial load, Heavy metals, Antioxidant enzyme, Oyster, Sundarban.*

INTRODUCTION

Oysters are bivalve molluscs which live in shallow marine and estuarine habitats in tropical and subtropical parts of the world. At least five species of oysters have been

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recorded from coastal areas of India (Anonymous, 1984). The common oyster species found in Indian Sundarban are *Saccostrea cucullata*, *Crassostrea madrasensis**, *C. gryphoides** and *C. rivularis**.

The oysters are considered to be valuable food item as they constitute rich source of many elements, essential for providing a balance diet (Nagabhushanam and Bidarkar, 1978). The edible oysters are popular as raw and processed food in some coastal areas in India, and in several South East Asian countries, Europe, USA, *etc.* Oyster flesh/meat has also got a prominent market (Santhanam *et al.,* 1990). Culturing of oysters has offered alternative livelihood for the coastal population. Oysters are filter feeders and sedentary in nature. Therefore, the health of the organism is greatly affected due to water condition of the habitat. The oysters not only bioaccumulate conservative pollutants, but also act as the site for microbial growth.

In 1909, the American Public Health Association (APHA) along with US Bureau of Chemistry (now the US Food and Drug Administration; FDA), began to evaluate the occurrence of *Bacillus coli* (now *Escherichia coli*) as a bacterial indicator in oyster meat sample. McCredy (1915) was able to quantify them in serial dilution tubes. Most probable number (MPN) method was developed for enumerating bacterial densities both from shellfish harvesting waters and oyster meats using a coliform standard. The coliform standard was further improvement of the early work using *B. coli* (now *E. coli*), and now includes both total and fecal coliform bacterial groups (Kator and Rhodes, 1994). In countries like USA, Japan, France, *etc.*, shellfishes are harvested carefully in safe waters with approved conditions. These waters are not subjected to unsafe levels of fecal contamination, and are devoid of pathogens, harmful substances and biotoxins. The approved condition of water should not exceed < 70 total coliform/100ml (MPN/100 ml water) with no more than 10% of the samples exceeding an MPN of 230 MPN/100 ml (5 tubes decimal dilution series) or 330 MPN/100 ml (3 tubes decimal dilution series) and must contain less than 14 fecal coliform/100 ml (FDA, 1997). In Europe, shellfish growing areas are classified on the basis of oyster meat coliform standard rather than a surface water standard. The Council of European Communities gave directions for production and marketing of healthy live bivalves (91/492/FFC), and as per the directive the shellfish harvesting area may be demarcated into categories A, B and C on the basis of fecal indicators in shellfish samples. Shellfish from category A can be directly taken to the market. They must meet a standard of not more than 230 *E. coli*/100 gm shellfish (or 300FC/100 gm). Shellfish from category B must be purified before being taken to the market and shellfish from category C must be placed again in clear water for two months prior to

Note: *Asterisk indicates that these three species are relegated to *Magallana bilineata, M. gryphoides* and *M. rivularis* respectively - Editor

marketing (Pommepuy, 1996). In recent past, the scientists have suggested a need for new environment monitoring procedures that focus more on the effect of contaminants rather than the level of contaminants (Lam and Gray, 2003). The scientists tried to develop methods and tools that would provide a greater sensitivity of the effects of contamination on biota in estuarine ecosystem and how they interact with pollutants (Etxeberria *et al*., 1995; Cossu *et al*., 2000). One area of interest was the activities of enzymes involved in the detoxification of xenobiotics (Shechan and Power, 1999). Activities of these enzymes can indicate the effects of contaminants in the marine environment. These indicators are termed as biomarkers (Chevre *et al*., 2003).

The water of Indian Sundarban is presently under stress due to discharge of untreated sewage and industrial wastes from the cities of Kolkata, Howrah, and Haldia port- cum-industrial complex (Mitra and Choudhury, 1993). Bivalve molluscs are useful organisms to assess the effects of contaminants in aquatic environments and are used for monitoring coastal ecosystems (Goldberg, 1975). A range of antioxidant defenses in bivalve molluscs make them appropriate organisms as biomarkers by measuring the antioxidant enzyme activities (Verlecer *et al*., 2008). A number of studies were made on antioxidant defense system enzymes in bivalves to predict their diminished health. These studies produced varied results but have shown that the enzymes can respond differently to varying levels of contaminants. It was shown that antioxidant enzyme activities decreased with increase in contamination (Box *et al*., 2007; Osman *et al.,* 2007). On the other hand, it was found that activities of this enzyme increased in organisms collected from more polluted sites (Morales-Caselles *et al*., 2008; Tsangaris *et al*., 2007). In this background, the present study has tried to highlight the effect of some heavy metal (Zn, Cu, Pb) pollutants and microbial load on the edible oyster (*Saccostrea cucullata*) in different seasons of Sundarban, West Bengal.

MATERIALS AND METHODS

Study area: The present investigation was carried out during the year of 2016 and 2017 at three different stations, namely, Namkhana, Frazergaunge and Sajnekhali. The sampling stations were selected considering the magnitude of anthropogenic pressure. Station 1. Namkhana (Lat. 21° 45´48.54´´ N and Long. 88° 13´ 52.55´´ E) is situated in the western sector of Sundarban, which is not only an important fish landing station, but also receives the wastewater from Kolkata and nearby Haldia port- cum industrial complex. Station 2. Frazergaunge (Lat. 21° 36´ 55.72´´ N, Long. 88° 12´ 33.15´´ E) is also a fish landing station and receives discharge of several hotels and tourism units located at Bakkhali. Station 3. Sajnekhali (Lat. 22° 07´ 36.21´´ N and Long. 88° 49´ 50.60´´ E) is situated in the eastern sector of Sundarban, which is noted for its

wilderness and less anthropogenic stress, owing to closeness of the mangrove forest (Fig. 1).

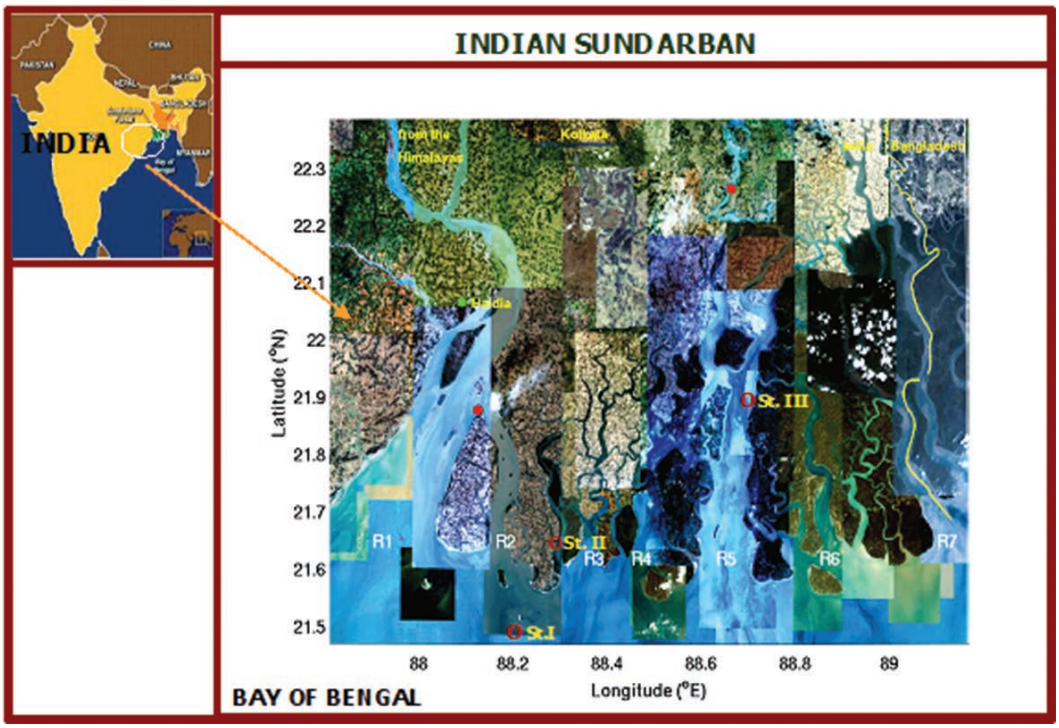

Fig. 1. Map of the study region showing the sampling stations: Namkhana (St-I), Frazergaunge (St-II) and Sajnekhali (St-III). R1 to R7 are the seven rivers of Sundarban starting from west to east, *viz*., Hooghly, Mooriganga, Saptamukhi, Thakuran, Matla, Gosaba and Harinbhanga.

Sample collection: Oyster samples were collected from the intertidal zone of the selected sampling stations for enzymatic assay, free radicals, heavy metals, and carrying out microbial load analysis in terms of total coliform (TC), total fecal coliform (TFC) and total bacterial count (TBC) in the oyster flesh.

Bacteriological analysis: For bacterial analysis, (TC and TFC), a minimum of 100 g oyster sample (meat and liquor) was aseptically shucked into a sterile, tarred blender jar using sterile shucking equipment, an equal weight of sterile 0.5% peptone solution was added to the blender jar, and the content was blended at high speed for 90 to 120 seconds. Immediately after blending, 20 g of this mixture was aseptically added to 80 ml of dilution water resulting in a 1/10 dilution of the original sample. A 1/100 dilution was prepared by aseptically adding 10 ml of the 1/10 dilution into 90 ml of dilution water. The standard MPN procedure (using LTB/EC) was performed with these dilutions with 10 and 1 ml aliquots inoculated (5 test tube) from the 1/10 dilution and 1 ml aliquots from the 1/100 dilution, (APHA, 1970). The result was reported as MPN index per 100 g basis. The enumeration of total bacterial count was done by standard plate counting method.

Biochemical analysis: The biochemical analysis was done on tissue samples pooled from individual oysters. The sample (average length of 8.0 cm) was collected from the cultured site at monthly intervals. They were washed with double distilled water and processed for biochemical analysis. For enzymatic assay, samples were homogenized in a cold buffer solution containing 0.2 M KH_2PO_4 and 0.2 M K_2HPO_4. Catalase (CAT) activity was evaluated by the standard process of Beers and Sizer (1952). The activity of superoxide dismutase (SOD) was measured following Marklund *et al*. (1974). Lipid peroxidase level (LPO) was evaluated by thiobarbituric acid (TBA) reaction (Ohkawa *et al.*, 1979).

Heavy metals analysis: The samples were washed with metal free double distilled water and dried in an oven at 105 degree centigrade for 5-6 hours. The flesh tissue freed from shell was grinded to powder in mortar and stored in an acid washed polythene bag. Analysis of biologically available metals was done after re-drying the sample from which 1 g was taken and digested with 0.5(N) HCL. The resulting solution was then stored in a polythene container for analysis. The solution was finally aspirated in the flame atomic absorption Spectrophotometer for determination of metal concentration.

RESULTS

Monthly variation of total bacteria, coliform and fecal coliform; monthly variation of *E. coli* and *Bifidobacterium* sp.; monthly variation of heavy metals (ppm) and monthly variation of different stress induced enzymes and lipid peroxidation in oyster flesh in three different study stations of Sundarban are presented in Tables 1 to 4.

Table 1. Monthly variation of total bacteria, coliform and fecal coliform in oyster flesh at three different stations of Sundarban (Mean ± Standard Deviation).

Months	TBC of flesh (cfux10^8/g)			TC of flesh (MPN×10^4/100g)			FC of flesh (MPN×10^4/100g)		
	Station I	Station II	Station III	Station I	Station II	Station III	Station I	Station II	Station III
1st year sampling-2016									
Jan	1.6±0.34	2.3±0.45	1.8±0.32	0.91±0.26	0.24±0.03	1.60±0.64	0.54±0.08	0.24±0.03	1.10±0.06
Feb	1.8±0.01	2.4±0.04	1.9±0.01	0.74±0.86	0.64±0.03	0.54±0.03	0.35±0.06	0.35±0.06	0.24±0.03
Mar	1.1±0.01	1.2±0.01	1.0±0.01	0.91±0.26	0.46±0.05	0.91±0.02	0.35±0.06	0.26±0.06	0.35±0.62
Apr	1.4±0.01	1.8±0.01	1.4±0.01	1.61±0.64	2.50±0.01	1.54±0.37	1.60±0.64	2.31±0.01	1.35±0.06
May	1.5±0.02	1.1±0.01	1.3±0.03	0.54±0.37	0.41±0.02	0.28±0.06	0.54±0.37	0.21±0.02	0.14±0.02
Jun	1.3±0.01	0.9±0.01	1.0±0.01	0.26±0.55	0.91±0.02	0.35±0.03	0.16±0.05	0.54±0.08	0.24±0.03
Jul	2.1±0.01	2.8±0.02	2.2±0.02	4.41±0.35	2.40±0.03	1.81±0.04	1.71±0.03	2.40±0.03	1.60±0.06
Aug	8.9±0.03	2.0±0.04	4.8±0.05	18.0±0.86	9.11±0.26	9.11±0.76	18.0±0.86	5.40±0.08	3.50±0.62
Sep	7.0±0.01	5.0±0.01	2.2±0.01	16.0±0.86	16.0±0.86	3.51±0.70	3.50±0.62	9.10±0.76	3.45±0.62
Oct	5.5±0.33	7.4±0.20	4.0±0.11	5.41±0.86	9.10±0.26	3.51±0.70	3.40±0.62	2.81±0.62	2.40±0.35
Nov	4.5±0.03	2.0±0.04	2.2±0.01	9.10±0.86	1.60±0.64	1.67±0.64	5.41±0.86	1.60±0.64	1.22±0.02
Dec	2.8±0.10	4.9±0.33	1.5±0.01	5.41±0.86	1.60±0.64	0.92±0.02	2.44±0.86	1.01±0.64	1.22±0.02

Table 1 contd.

Months	TBC of flesh (cfux10^8/g)			TC of flesh (MPN×10^4/100g)			FC of flesh (MPN×10^4/100g)		
	Station I	Station II	Station III	Station I	Station II	Station III	Station I	Station II	Station III
2nd year sampling-2017									
Jan	1.4±0.28	7.0±0.29	1.5±0.12	1.60±0.64	0.28±0.06	1.80±0.70	1.61±0.64	0.24±0.03	0.54±0.08
Feb	2.2±0.45	1.8±0.17	1.1±0.10	1.80±0.40	0.54±0.08	0.92±0.69	1.60±0.65	0.35±0.06	1.80±0.45
Mar	1.9±0.03	1.8±0.07	1.5±0.01	0.91±0.69	0.44±0.03	0.45±0.30	0.54±0.08	0.34±0.03	0.54±0.08
Apr	1.5±0.01	1.5±0.01	1.2±0.01	1.60±0.64	2.31±0.01	1.30±0.01	1.60±0.64	1.75±0.01	0.15±0.02
May	1.1±0.01	0.8±0.07	0.6±0.06	0.35±0.30	0.44±0.01	0.42±0.01	0.35±0.06	0.27±0.02	0.15±0.01
Jun	1.5±0.02	1.4±0.11	0.2±0.01	0.24±0.02	0.37±0.07	0.20±0.07	0.24±0.03	0.25±0.01	0.27±0.02
Jul	2.8±0.05	2.1±0.09	2.2±0.02	9.10±0.76	3.51±0.30	1.60±0.64	5.40±0.08	2.45±0.03	0.15±0.01
Aug	8.0±0.03	2.5±0.04	6.6±0.05	18.0±0.86	16.0±0.86	1.60±0.64	18.0±0.86	9.10±0.76	0.54±0.08
Sep	7.1±0.25	4.8±0.05	4.4±0.12	16.0±0.86	9.11±0.76	5.40±0.86	9.10±0.26	5.41±0.86	1.60±0.02
Oct	1.5±0.01	8.0±0.15	1.8±0.35	7.50±0.76	1.10±0.22	5.41±0.86	2.50±0.35	0.31±0.02	2.20±0.25
Nov	2.3±0.05	2.0±0.02	1.4±0.02	1.30±0.15	1.60±0.64	3.45±0.30	0.70±0.76	0.54±0.08	2.40±0.35
Dec	2.6±0.03	4.0±0.04	1.1±0.10	1.30±0.10	0.75±0.12	0.54±0.07	1.30±0.10	2.00±0.04	1.40±0.02

Table 2. Monthly variation of *E. coli* and *Bifidobacterium* sp. in oyster flesh at three different stations of Sundarban (Mean ± Standard Deviation).

Months	Flesh *E. coli* (MPN/100g)			*Bifidobacterium* sp. (cfux10^8/g)		
	Station-I	Station-II	Station-III	Station-I	Station-II	Station-III
1st year sampling - 2016						
Jan	2400±350	750±392	290±220	0.19±0.01	0.12±0.04	0.11±0.05
Feb	3500±620	750±390	250±219	0.20±0.01	0.11±0.02	0.09±0.01
Mar	1700±390	700±392	310±175	0.71±0.01	0.50±0.01	0.03±0.01
Apr	3500±321	400±290	310±190	0.21±0.02	0.02±0.01	0.27±0.01
May	11000±500	400±210	250±28	0.15±0.05	0.12±0.02	0.03±0.01
Jun	7000±260	1100±392	200±40	0.11±0.01	0.07±0.01	0.10±0.01
Jul	12000±521	1300±390	320±55	1.21±0.01	1.20±0.01	1.50±0.01
Aug	7200±250	7500±420	700±219	19.8±0.40	13.1±0.51	6.30±0.16
Sep	3500±321	3500±395	700±190	20.1±0.41	19.2±0.75	2.20±0.08
Oct	4500±320	3800±425	750±190	13.0±0.31	12.1±0.55	1.81±0.01
Nov	3500±321	5400±290	450±290	11.5±0.21	10.2±0.43	2.11±0.08
Dec	1600±350	540±292	200±110	0.97±0.01	0.54±0.03	0.22±0.07
2nd year sampling - 2017						
Jan	5400±300	750±390	220±190	0.01±0,01	0.07±0.02	0.31±0.01
Feb	1300±220	700±420	200±155	0.11±0.04	0.15±0.01	0.31±0.01

Table 2 contd.

Months	Flesh *E. coli* (MPN/100g)			*Bifidobacterium* sp. (cfux10^8/g)		
	Station-I	Station-II	Station-III	Station-I	Station-II	Station-III
Mar	1300±220	750±160	220±55	0.22±0.01	0.08±0.02	0.03±0.01
Apr	7500±255	250±65	250±65	0.15±0.05	0.11±0.03	0.02±0.01
May	11000±500	200±190	200±40	0.13±0.03	0.12±0.03	0.22±0.01
Jun	3500±600	310±220	310±190	0.04±0.01	0.11±0.02	0.17±0.01
Jul	12000±525	750±392	380±190	1.50±0.01	2.11±0.07	2.01±0.07
Aug	7000±251	3500±390	320±190	12.4±0.21	1.15±0.05	5.31±0.15
Sep	2000±150	7500±120	900±420	19.7±0.32	12.0±0.65	8.81±0.17
Oct	1100±225	2500±219	700±390	10.9±0.21	15.9±0.75	2.81±0.06
Nov	1540±95	3500±390	310±192	1.6±0.011	1.72±0.52	2.79±0.08
Dec	1450±320	900±420	200±40	0.75±0.01	1.75±0.05	0.15±0.05

Table 3. Monthly variation of heavy metals (ppm) in oyster flesh at three different stations of Sundarban (Mean ± Standard Deviation).

Months	Zn			Cu			Pb		
	Station I	Station II	Station III	Station I	Station II	Station III	Station I	Station II	Station III
1st year sampling - 2016									
Jan	5111±7.2	4696±5.2	450.2±8.1	600.1±9.1	255.1±8.0	220.2±9.7	35.1±2.1	31.1±1.1	30.3±2.0
Feb	5151±8.0	4650±4.1	415.5±6.0	612.3±8.0	235.1±7.1	213.1±8.5	30.2±2.5	30.4±1.2	28.1±2.1
Mar	4219±4.9	4635±8.6	301.2±4.4	509.5±9.2	141.1±3.0	155.5±5.1	22.1±1.2	21.2±4.6	20.1±1.2
Apr	4228±4.9	4615±7.6	306.2±3.2	501.6±8.2	131.0±2.1	151.5±4.6	21.1±1.1	20.2±4.6	18.5±1.1
May	4230±2.9	4625±6.6	308.0±4.2	503.3±7.2	140.3±3.2	150.5±5.2	20.1±1.0	19.2±4.6	21.1±1.0
Jun	4225±4.9	4630±6.5	309.1±5.2	502.3±8.2	141.2±4.1	152.5±4.2	20.1±1.1	20.2±4.6	19.2±1.2
Jul	5615±9.6	4930±7.6	617.5±7.1	790.4±5.4	281.1±6.2	270.3±5.21	45.1±1.2	40.1±3.7	35.0±1.2
Aug	5605±8.5	4920±8.6	610.5±6.0	775.5±4.4	277.0±5.2	259.2±5.21	41.2±1.1	33.1±3.2	31.2±1.1
Sep	5405±7.3	4922±9.6	595.5±7.2	770.4±5.4	271.1±6.2	255.1±5.21	43.1±1.2	30.1±3.0	29.1±1.2
Oct	5495±8.2	4931±8.6	581.5±5.2	761.2±4.2	269.3±5.2	250.2±5.21	42.2±1.1	29.1±3.1	28.9±1.3
Nov	5101±9.2	4666±5.0	426.2±6.0	610.3±8.1	245.1±7.2	215.1±9.8	31.2±2.2	29.1±1.1	27.1±2.2
Dec	5120±6.2	4674±4.1	435.1±5.1	609.2±7.2	249.1±6.1	218.2±7.2	32.1±2.1	30.1±1.0	28.2±2.5
2nd year sampling - 2017									
Jan	5110±5.2	4689±4.2	451.2±8.1	595.1±8.1	250.1±8.0	222.2±9.7	36.1±2.1	33.1±1.1	31.3±2.0
Feb	5141±4.0	4644±4.1	411.3±6.0	605.3±8.0	234.1±7.1	215.1±8.5	32.2±2.5	32.4±1.2	30.1±2.1
Mar	4217±4.2	4632±6.6	309.2±4.1	503.5±7.2	140.1±3.0	165.5±5.1	21.1±1.2	20.2±4.6	19.1±1.2
Apr	4223±3.9	4611±7.6	305.2±3.7	501.6±7.1	129.0±2.1	150.5±4.6	19.1±1.1	19.2±4.6	19.5±1.1
May	4232±2.5	4615±6.1	306.0±4.1	502.3±6.3	136.3±3.2	152.5±5.2	20.1±1.0	17.2±4.6	21.1±1.0

Table 3 contd.

Months	Zn			Cu			Pb		
	Station I	Station II	Station III	Station I	Station II	Station III	Station I	Station II	Station III
Jun	4227±4.1	4629±6.0	311.1±4.2	501.3±7.0	139.2±4.1	150.5±4.2	18.1±1.1	21.2±4.6	20.2±1.2
Jul	5612±7.6	4927±6.6	615.5±6.1	789.4±4.4	271.1±6.2	271.3±5.21	42.1±1.2	38.1±3.7	33.0±1.2
Aug	5601±5.2	4919±7.6	613.5±4.9	775.5±4.2	267.0±5.2	258.2±5.21	41.2±1.1	33.1±3.2	30.2±1.1
Sep	5402±6.1	4921±6.6	592.5±6.2	772.4±4.0	270.1±6.2	256.1±5.21	42.1±1.2	32.1±3.0	29.1±1.2
Oct	5485±6.3	4933±5.6	580.5±4.2	762.2±4.1	269.3±5.2	251.2±5.21	40.2±1.1	30.1±3.1	27.9±1.3
Nov	5105±8.0	4656±5.0	425.2±5.0	611.3±7.1	244.1±7.2	213.1±9.8	29.2±2.2	28.1±1.1	28.5±2.2
Dec	5119±7.4	4664±4.1	425.1±5.2	607.2±6.2	242.1±6.1	212.2±7.2	30.1±2.1	30.1±1.0	27.2±2.5

Table 4. Monthly variation of different stress induced enzymes and lipid peroxidation in oyster at three different stations of Sundarban (Mean ± Standard Deviation).

Months	Catalase (unit/min/mg tissue)			Superoxide dismutase (unit/min/mg tissue)			Lipid peroxidation (nmole MDA/mg tissue)		
	Station I	Station II	Station III	Station I	Station II	Station III	Station I	Station II	Station III
1st year sampling - 2016									
Jan	2413.8±240	1505.2±201	777.3±195	8.42±0.30	6.12±0.32	5.01±0.42	222.18±7.5	140.25±7.3	120.25±7.3
Feb	3717.3±249	3065.6±222	2110.5±210	14.12±0.76	11.80±0.62	9.52±0.66	212.03±7.1	137.01±5.3	127.22±5.5
Mar	15500.5±537	15005.5±525	12011.0±505	16.21±0.69	14.92±0.66	11.10±0.65	132.51±5.3	106.60±4.3	105.20±4.2
Apr	14113.9±500	13119.9±505	11800.4±501	11.20±0.35	9.12±0.31	8.42±0.42	150.90±5.5	115.25±5.3	102.15±5.3
May	14823.2±525	10553.0±512	10326.2±510	10.25±0.50	8.62±0.45	7.26±0.34	175.01±5.7	138.18±7.5	95.12±5.3
Jun	10220.2±444	6320.7±412	5402.5±402	8.90±0.55	8.72±0.50	8.11±0.31	155.19±5.5	148.52±5.4	100.02±5.2
Jul	2515.1±250	2015.3±235	1450.0±231	8.79±0.34	7.66±0.32	6.22±0.4	176.15±4.7	160.03±4.5	130.80±4.3
Aug	1712.8±230	1451.8±213	1012.5±200	7.40±0.37	7.12±0.35	6.12±0.32	210.71±4.3	175.51±5.3	132.99±7.3
Sep	1212.1±225	1002.2±212	715.2±201	8.60±0.42	7.971±0.40	6.88±0.51	214.88±5.1	182.25±7.1	153.22±76.1
Oct	1025.0±222	712.2±202	530.4±202	10.01±0.53	9.10±0.51	8.05±0.50	220.20±7.2	200.15±7.2	170.25±5.7
Nov	1920.2±235	1002.3±214	402.1±212	12.25±0.61	10.11±0.70	9.75±0.75	218.91±7.1	190.20±4.5	175.22±5.7
Dec	1713.3±228	1596.5±230	699.0±198	10.35±0.51	9.22±0.49	9.12±0.50	225.50±5.3	195.16±5.4	190.45±5.5
2nd year sampling - 2017									
Jan	2702.5±275	1789.6±245	783.2±235	8.39±0.37	6.97±0.32	5.763±0.35	222.68±7.2	138.23±6.3	138.53±6.4
Feb	4065.2±325	3965.9±301	2113.7±301	13.05±0.96	11.91±0.66	9.725±0.52	212.33±7.0	137.98±5.3	122.47±5.3
Mar	15715.3±500	15000.5±501	11948.5±500	15.24±0.75	14.o2±0.64	11.21±0.58	135.71±5.3	102.01±6.2	100.55±6.3
Apr	14012.8±445	13018.2±442	9326.2±441	10.90±0.55	8.83±0.35	8.33±0.45	156.92±5.5	126.15±5.3	95.25±5.2
May	14107.2±475	10018.0±435	6703.8±423	9.10±0.37	8.71±0.31	8.157±0.33	176.15±5.8	138.72±6.3	90.35±6.4
Jun	6210.8±225	6217.7±212	4081.5±211	8.92±0.32	7.54±0.30	6.79±0.39	179.54±5.5	145.59±5.4	105.50±5.5
Jul	2517.5±245	2317.5±234	1349.2±231	7.75±0.35	7.52±0.32	6.12±0.45	193.43±5.8	172.22±6.6	138.25±5.4
Aug	1651.8±235	1351.8±213	1030.7±210	7.52±0.32	6.91±0.30	5.98±0.32	220.75±8.2	185.84±5.2	159.25±5.3
Sep	1107.0±233	912.1±230	612.2±321	8.42±0.36	7.67±0.45	6.97±0.46	224.09±8.2	195.21±7.4	170.45±7.5
Oct	986.2±215	786.6±212	436.4±211	9.35±0.49	8.88±0.50	9.72±0.49	224.20±7.2	201.30±5.7	173.50±5.7
Nov	1700.3±225	916.9±220	455.2±213	11.96±0.52	14.42±0.45	10.71±0.55	214.51±7.1	195.25±6.5	180.25±6.4
Dec	1700.2±232	1603.3±228	669.2±224	10.18±0.61	9.20±0.55	10.76±0.54	223.50±7.2	200.20±5.6	180.26±5.3

DISCUSSION AND CONCLUSION

Total Bacterial load: The highest quantity of TBC in oyster was recorded in Namkhana followed by Frazergaunge and Sajnekhali (Table 1). Maximum microbial load in estuarine water was observed during monsoon months (July-October). The high count of microbes in water may be attributed to increased discharge and water run off from adjacent land masses. Hence, monsoon cycle played a crucial role in regulating microbial population. Huge discharges through the channels of the rivers Ganga, Damodar and Rupnarayan are received in the catchment area of Sundarban in monsoon which contributed to the increased microbial load.

The station-wise rate of microbial contamination in the oyster tissue in the study area was in following sequence: Namkhana > Frazergaunge > Sajnekhali**.** This observed variation may be attributed to the degree of anthropogenic stress. Namkhana and Frazergaunge, being the fish landing sites are constantly exposed to wastes of complex nature. In addition, the estuarine waters become contaminated with wastes released from fishing vessels, trawlers, and urban discharges from Kolkata, Howrah and Haldia. On the other hand, Sajnekhali, being a wildlife sanctuary in the eastern sector of Indian Sundarban encounters minimum environmental stress. Similar results were observed in coastal South Carolina, where scientists monitored landuse patterns and ecosystem responses in highly urbanized Murrells Inlet and relatively undeveloped North Inlet (White *et al.*, 2004). Murrells Inlet had higher incidences of *E. coli*, fewer coliform and reduced bacterial diversity due to urban influences and high sewage load in the Murrells Inlet water shed (Booth and Jackson, 1997).

Marked variation in bacterial load in the oyster flesh during monsoon and pre-monsoon seasons has been evidenced by the results of the present study. TC in oyster during monsoon and pre-monsoon, in three stations in Sundarban, showed variation as follows: Namkhana - 1,80,000 MPN/100g vs. 1,600 MPN/100g, Frazergaunge-1,60,000 MPN/100g vs. 41,400 MPN/100g, Sajnekhali - 91,000 MPN/100g vs. 2,000 MPN/100g. FC in oyster in monsoon and pre-monsoon seasons in different stations showed variations as follows: Namkhana - 1,80,000 MPN/100g vs. 2,400 MPN/100g, Frazergaunge 91,000 MPN/100g vs. 2,140 MPN/100g, Sajnekhali - 35,000 MPN/100g vs. 1,400 MPN/100g. The bacterial incidences in oyster are likely to be proportional to the bacterial concentration in coastal water. The influence of high rainfall on the bacterial counts in coastal water was shown elsewhere (Lipp and Rose, 2001). Oysters accumulate microorganisms during the process of filter feeding and total coliform count in oyster flesh varies proportionately with bacterial content of water (Strozier, 1993). The same synchronization pattern is observed for FC incidences in oyster. The TC and FC counts are indicators of environmental stress which was maximum in Namkhana followed by Frazergaunge and Sajnekhali.

Total *Bifidobacterium* sp. count in oyster tissue: The total *Bifidobacterium* sp. count in oyster flesh showed even fifty times increase during monsoon than the pre-monsoon season and decreased further in post-monsoon. Station-wise, Namkhana recorded highest count followed by Frazergaunge and Sajnekhali. This result is comparable to those of bacterial loads presented above. Some studies elsewhere indicated that higher detectable levels of *Bifidobacterium* sp. in watershed were proportional to the higher levels of human pollution sources (Cabelli *et al.*, 1983; Kator and Rhodes, 1996).

***E. coli* in oyster flesh:** Total *E. coli* counts in oyster flesh showed similar results as in cases of total bacterial count and *Bifidobacterium* sp. count as presented above (Table 2). During monsoon *E. coli* counts showed even five times increase than in pre-monsoon and then dropped in post-monsoon in the three studied stations. The fluctuation in *E. coli* counts in oyster was correlated with the load of *E. coli* in coastal waters.

Level of Catalase and Superoxide dismutase levels in oyster in relation to heavy metal concentration: The two enzymes, catalase (CAT) and superoxide dismutase (SOD) activities (unit/min/mg tissue) in oysters, of all three stations of Sundarban, exhibited their peak during premonsoon (March-June) and then declined during monsoon (July-October) (Table 4). On the contrary, heavy metal concentration in oyster flesh was maximum during monsoon, much higher than premonsoon (Table 3). The observed result indicated that enzyme activities of oyster changed inversely with the levels of metal concentration in the oyster tissue. Simply, increase in heavy metal concentration in tissues suppress enzyme activities in animal which may be due to toxic effect of metals. Seasonality has been a characteristic feature of estuarine environments. Levels of oxidative stress in oyster might have fluctuated due to seasonal change, reproductive status, growth, water temperature and nutrient availability as reported by Sheehan and Power (1999). Many biotic and abiotic factors varying with seasons can create stress onto the organism which may lead to an increase in the generation of reactive oxygen species (ROS) in tissue. To minimize these toxic products, cells also respond through different enzyme systems, which are now considered as biomarkers. The biomarker level in of organisms can fluctuate due to physiological adaptation caused by seasonality (Verlecar *et al*., 2007). The spatial variation of CAT and SOD activities has been a reflection of environmental stress which was highest in Namkhana (St-I) owing to higher pollution load caused by toxic metals. Environmental pollution monitoring studies employing biochemical biomarkers have been reported in bivalves, such as oysters and mussels (Romeo *et al.*, 2003).

Lipid Peroxidation in oyster flesh in relation to metal concentration: The Lipid peroxidation (LPO) (nmole MDA/mg) value of the oyster showed minimum count during first three months of monsoon, which rose gradually in post-monsoon and continued with a marginally lower count in pre-monsoon season. Station-wise,

the LPO values showed the following sequence at any given time: Namkhana (St-I)> Frazergunge (St-II)> Sajnekhali (St-III). The heavy metal concentration in oyster flesh (Table 3) in different stations also showed similar sequence. The higher LPO value in oyster flesh is perhaps due to stress caused by pollution due to concentration of metal and anthropogenic wastes. The higher LPO value in post-monsoon is due to the enhancement of unsaturated fatty acid in oyster tissues and lower antioxidant defenses in the animal. De Zwart *et al*. (1998) observed that higher LPO level during winter season was due to the increased level of PUFA (polyunsaturated fatty acid) in their cellular membrane.

The impact of human activities on marine environment has been a matter of concern. Anthropogenic wastes and industrial effluents released in waters do cause pollution in marine habitats. Different microbes and heavy metals affect health and physiology of many marine animals. The pollution levels vary at places depending upon the degree of contamination of wastes. The humankind rely largely upon the marine animals for food, pharmaceuticals, *etc*. Hence, health of the animals are important for human welfare. Different biomarkers are being used to assess the health of organisms. From a study in Sundarban, West Bengal, it could be concluded that oysters collected during pre-monsoon were safer for consumption.

CONFLICT OF INTEREST: No conflict

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