

## Biochemical responses of oysters in evaluating environmental quality of tropical Indian estuarine systems

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### Abstract

Impact of varying concentrations and counts of toxic metals and indigenous bacteria on antioxidative defense system in edible oyster, *Saccostrea cucullata*, collected from four tropical estuarine systems of Goa (west coast of India), are presented in this study. Relatively high abundance of bacteria was estimated from the oysters especially during monsoon season (June-September). Density of total and fecal coliforms were about 24 times higher in the organism than the surrounding water (average TC: 4360 and FC: 3475 MPN 100ml<sup>-1</sup>). Sedimentary Cd, Pb, and Hg concentrations varied from 0.12-0.48, 19.28-102.48, and 0.03-0.13 mg kg<sup>-1</sup> (dry wt.) while, bioaccumulation of Cd, Pb, and Hg in the oysters ranged from 5.17-10.6, 0.18-7.99, and 0.06-0.22 mg kg<sup>-1</sup> (dry wt.) respectively. Higher degree of pollution and moderate ecotoxicological risks with increasing toxicity (36-37%) was observed in two of the tropical estuaries. Biomarker response in the oysters was somewhat inconsistent with pollution load in the estuarine systems. Elevated environmental stress was observed during pre-monsoon (March-May) followed by the monsoon period. Sedimentary metals were identified as predominant inducers of antioxidative defence system in oysters from the study areas. This study suggests that, biomarker can be useful in assessing the combined effects of metal and bacterial contaminations on native oysters and in evaluating environmental quality in tropical estuarine systems.

**Keywords:** Sedimentary toxic metals; Bacteria; Antioxidant enzyme; Oxidative stress; Biomarker; Pollution in tropical estuary

### Highlights

- Ecotoxicological risks assessment of estuarine sediment could prevent adverse biological effects in marine organisms.
- Metals (especially Cd and Pb) and bacteria predominantly induce antioxidative defence system in marine organisms.
- Site specific anthropogenic activities mostly responsible for exerting environmental stresses on native organisms.
- Ecotoxicological risk elevates during the pre-monsoon season (March-May).

## 1. Introduction

Biomarkers reflect an organism's attempt to tolerate various biotic and abiotic stressors in estuarine environment. Antioxidant enzymes are naturally produced within biological systems to counterbalance the impact of stressors. They have been widely used as a potential biomarker in evaluating quality of different coastal ecosystems (Orbea et al. 2002; Cravo et al. 2009; Serafim et al. 2012). Balance between pro-oxidants and antioxidant defences play crucial role in organisms, maintaining cellular homeostasis. Environmental stress mediated cellular damages inevitably change antioxidant enzymes activities (Benedetti et al. 2015) in addition to immunomodulation (Gopalakrishnan et al. 2009). These phenomena increase vulnerability of marine organisms to stressors, leading to variety of diseases and increased mortality rate (Paul-Pont et al. 2010).

Toxic metals, such as cadmium (Cd), lead (Pb), and mercury (Hg), are well-known for exerting oxidative stress on marine organisms (Volety 2008). Wide uses of these three toxic metals in different anthropogenic activities and their release in environments cause coastal marine pollution in many developing maritime countries. The major sources of these metals in India (the fastest-growing major economy) are summarised below. In general, sources of anthropogenic Cd are nickel-cadmium (Ni-Cd) battery plant, electroplating, fertilizer, pigments, and plastics industries (ATSDR 1999). Major anthropogenic Pb sources are sewage treatment plants, lead-based paint and pipes manufacturing companies, mining industries, and combustion of coal and wood (Ansari et al. 2004; Mohammadi et al. 2008; Chakraborty et al. 2021). Burning of fossil fuel (especially coal) in thermal power plants, Chlor-alkali plants, production of chlorine, mining, and wastewater treatment plants are the major sources of Hg in coastal environments (Pirrone et al. 2010; Chakraborty 2017). It is well known that Pb, Cd and Hg are biologically non-essential and show harmful effects when accumulated in marine organisms. They do interfere with different metabolic processes due to pseudo elements behaviors. Cd has been reported to replace zinc (from carbonic anhydrase) in marine diatoms (Lane and Morel 2000). Cd suppresses haemocyte metabolism, increases proton leakage in mitochondria, and suppresses immune-related functions, resulting in decreased phagocytic activity and adhesion capacity in oysters (Sokolova 2004; Achard-Joris et al. 2006; Ivanina et al. 2014). Pb has been identified to substitute calcium (Ca) in many biological systems (Goldstein 1993). It hampers overall shell growth by ceasing biomineralization (Gifford et al. 2006), hinder protein metabolism, and disturb  $\text{Ca}^{2+}$  homeostasis in oysters (Meng et al. 2018). Methylated Hg is readily absorbed and accumulated in bivalve tissue where it is not easily excreted (Rice et al. 2014). Hg induces significant haemocyte mortality (Gagnaire et al. 2004) as well as abnormal embryogenesis, causing impaired immune functions in oysters (Beiras and His 1994).

Like toxic metals, some bacteria including *Vibrios*, impact upon all life stages of bivalves (Beaz-Hidalgo et al. 2010). It has been identified as the causative agent for the mass mortalities of Pacific oyster in Japan (Sugumar et al. 1998) and the European flat oyster in France (Thompson et al. 2003). *Vibrios* and *Aeromonas* aggravate severe mortalities in oysters by causing bacillary necrosis (Kaspar and Tamplin 1993). Bivalves are particularly susceptible to those pathogens depending upon their biology and ecology (Saulnier et al. 2010). Moreover, these pathogens can modulate toxic metals bioaccumulation in bivalves (Paul-Pont et al. 2010). Other bacteria, like coliforms, may not affect bivalve's physiology *per se* but monitoring their abundance in tropical waters is necessary for evaluating environmental quality (Bordalo et al. 2002).

Antioxidant enzymes such as superoxide dismutase (SOD), glutathione-S-transferase (GST), catalase (CAT), and metallothionein (MT) play important roles in counterbalancing oxidative stress caused by toxic metals and bacteria (Manduzio et al. 2005; Higashimoto et al. 2009; Dzoyem et al. 2014; Sarker and Oba 2018). SOD is a metal ion cofactor-requiring enzyme that catalyzes dismutation of superoxide anion into hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). CAT converts H<sub>2</sub>O<sub>2</sub> into water and oxygen. GST inactivates secondary metabolites, such as unsaturated aldehydes, epoxides, hydroperoxides and xenobiotic compounds, otherwise harmful to organisms. MT has a high affinity for metal ions and plays important roles in regulating and detoxifying toxic metals through oxygen free radical scavenging actions. Due to high specificity and ability of MT to bind metal ions, it is considered as ideal biomarker to monitor metal contamination in coastal ecosystem (Kalpaxis et al. 2004).

Bivalves have been widely used for biomonitoring purposes in marine ecosystems because of their large volume of water filtration capacity and sensitivity to oxidative pollutants (Gaspar et al. 2018; Jahan and Strezov 2019). Being sessile, they are always exposed to various toxic metals and indigenous, non-indigenous bacteria present in the environment. Since, antioxidant enzymes activity generally increases with higher pollution loading (Box et al. 2007; Jana et al. 2013; Benali et al. 2015), therefore, study of molecular biomarkers have been extensively used in bivalves to assess quality of different marine ecosystems (Kingtong et al. 2011). Along with other bivalves, oysters are well distributed marine organism and being susceptible to environmental pollution, precisely reveal cytotoxic effects of various pollutants on antioxidative defence system.

In India, most of the bivalves accumulated bacteria belong to *Aeromonas*, *Salmonella*, and *Vibrio* genera. These bacteria are normally present in soft tissue and body fluid of oysters (Silvester et al. 2017), crustacean (Parthasarathy et al. 2016) or clam (Sangeetha et al. 2017). Shellfish also accumulate *Clostridium botulinum* or *Listeria monocytogenes* from their surrounding environments (Gawade et al. 2010). Accumulation of toxic metals in oysters has been reported from different coastal

parts of India (LaxmiPriya et al. 2011; Abhilash et al. 2013; Gawade et al. 2013; Chakraborty et al. 2015, 2016). Unfortunately, all these studies have mostly focused on estimating either bacteria or toxic metals bioaccumulation in organisms, without addressing their biochemical response to prevent stresses. Scarce information is available about assessment of ecotoxicological risk of toxic metals on native organisms. In this study, an effort was made to evaluate the collective impact of metals and bacteria upon antioxidative defence system of edible oyster *Saccostrea cucullata* in different tropical estuarine systems from India. Different pollution indices were also evaluated to comprehend ecotoxicological risk probability of selective toxic metals on resident organisms.

## **2. Materials and methods**

### **2.1. Study area descriptions**

Water, sediment, and oyster (*S. cucullata*) samples were collected from four different estuaries along the Goa coast (Fig. 1) during three consecutive seasons *i.e.* pre-monsoon (March-May), monsoon (June-September), and post-monsoon (October-November) in the year 2017. The sampling locations were strategically chosen at mouth of the each estuary. The locations were Siolim (#S1), Nerul (#S2), Chicalim (#S3) and Betul (#S4), located on the bank of the river Chapora, Nerul, Zuari, and the Sal respectively (Table 1). The #S1 was selected as reference as it was reportedly less polluted (Fernandes et al. 2014). At each location, the samples were collected in triplicates during low tide. The sediment samples were processed as composite samples for further analysis. Adult oysters of consistent size range (shell length 2.7 to 3.2 cm.) were chosen for the study. The oyster samples were also processed as composite samples for further analysis. Forty-five oysters were collected from each location to generate a pooled sample and kept in a bucket of seawater collected from same location until arrived at laboratory.

### **2.2. Storing of samples**

In laboratory, the oysters were carefully washed with tap water to remove mud and sand particles and kept at -20°C for antioxidant enzymes analysis. Fifteen oysters were depurated for 24hrs in filtered seawater, collected from respective locations (Sokolowski et al. 2002) and stored at -20°C for metal analysis. The sediment samples were kept at -20°C till analysed for total metal concentrations. For microbial analysis, the water, sediment, and the oyster samples were stored at 2-8°C.

### **2.3. Determination of physico-chemical parameters**

Temperature, pH, and salinity of the ambient water were recorded *in situ* by using a portable thermometer, pH meter (Model- pHep HI96107, Hanna Instruments, Italy) and digital ERMA

refractometer (Model RHS-10 ATC, Yhequipment Co. Ltd, China) respectively. The water samples were collected into respective bottles and vials and brought to the laboratory for analysis of dissolved oxygen (Winkler 1988) and total organic carbon (TOC analyzer; Shimadzu: Model- TOC LCPH).

#### **2.4. Isolation of selective indigenous bacteria in estuarine samples**

For quantification of five selective bacteria (*i.e.* *Salmonella* spp., *Vibrio* spp., *Aeromonas hydrophila*, *Clostridium botulinum*, and *Listeria monocytogenes*) from the water, sediment, and the oyster samples, standard microbiological procedures were followed as suggested by the U.S. Food and Drug Administration Bacteriological Analytical Manual (USFDA 1995). Soft tissue portion of the oysters were removed from the calcareous shells, the gills and digestive tracts were dissected out aseptically. 2gm (pooled) of the sediment and the dissected gills and digestive tracts of the oysters were immediately homogenized (1:1 w/v) with 1x PBS buffer for 5 minutes using a mortar and pestle. Serially diluted samples were spread plated on respective selective media plates in triplicates and incubated at 35-37°C for 24 hrs. Xylose-Lysine Deoxycholate agar (Himedia, Catalog No- M031F), thiosulfate-citrate-bile salts-sucrose agar (Himedia, Catalog No- M189), *Aeromonas* Isolation Medium Base (including ampicillin, 0.005 mg ml<sup>-1</sup>; Himedia, Catalog No-M884), *C. botulinum* Isolation Agar (including Cycloserine, 0.25 mg ml<sup>-1</sup>; Sulphamethoxazole, 0.076 mg ml<sup>-1</sup>; and Trimethoprim, 0.004 mg ml<sup>-1</sup>; Himedia, Catalog No- M911 with CBI supplement; Himedia, Catalog No- FD049), and *Listeria* Selective Agar (containing Amphotericin B, 0.015 mg ml<sup>-1</sup>; Nalidixic acid 0.04 mg ml<sup>-1</sup>; and acriflavin 0.05 mg ml<sup>-1</sup>; Himedia, Catalog No- M1474 with *Listeria* Selective Supplement II; Himedia, Catalog No- FD063) were used for the selective and differential isolation of *Salmonella* species (Taylor and Schelhart 1969), *Vibrio* species (Kobayashi et al. 1963), *Aeromonas hydrophila* (Pin et al. 1994), *Clostridium botulinum* (Salfinger and Tortorello 2015), and *Listeria monocytogenes* (Lovett et al. 1987) respectively. Results are reported in cfu ml<sup>-1</sup> (for the water) or cfu gm<sup>-1</sup> wet wt. (for the sediment and the oysters).

To assess the water quality of the estuaries, most probable number (MPN) of the fecal and total coliforms was determined. Conventional five-tube three-dilution procedure was adopted as recommended by the FDA Bacteriological Analytical Manual (BAM) and American Public Health Association (APHA 1970). Results are reported in MPN 100ml<sup>-1</sup> (for the water) or MPN 100gm<sup>-1</sup> (for the sediment and the oysters).

#### **2.5. Sediment collection and metal analysis**

Undisturbed surface sediments were collected using PVC corers (inner diameter 7.5cm and length 15cm). Multiple core samples were collected from each location. From each location, the top

0-4cm section of three cores were mixed to prepare a composite sample and dried using hot air oven (Model- KOMS 6FD, Make- KEMI, India) at 40°C for 48hrs. The dried samples were then sieved through 63µm nylon mesh. The fraction <63µm was chosen for digestion with acid mixture (Friedler et al. 1994). An approximate amount of 0.05gm of sediment samples were digested with 10.0ml of Suprapur HF, HNO<sub>3</sub>, and HClO<sub>4</sub> mixture (in 7:3:1 ratio) in Teflon beakers on top of a hot plate at 200°C. After the acids were evaporated to dryness, the remaining residues were re-dissolved in 2% (v/v) HNO<sub>3</sub> (Chakraborty et al. 2016).

Fifteen oysters were dissected from each location using a plastic knife; the whole soft tissue were freeze-dried and ground to a fine powder as described by Ruelas-Inzunza and Paez-Osuna (2000). 0.05gm of the powdered tissue samples were acid digested with 10.0ml of HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> mixture (1:1) on top of a hot plate at 80-100°C (Ramteke et al. 2021). After complete digestion, the crystalline pastes were re-dissolved in 2% HNO<sub>3</sub>. Total concentration of sedimentary and bioaccumulated Cd and Pb were measured by using an Electro Thermal Atomic Absorption Spectrometer (ETAAS; Model- PinAAcle 900T, Perkin Elmer, USA). Sedimentary and bioaccumulated Hg was determined by direct mercury analyzer (Model- tri-cell DMA-80, Milestone, Italy). Detection limit of the ETAAS was 0.027 µg L<sup>-1</sup> for Cd and 0.05 µg L<sup>-1</sup> for Pb. Detection limit of direct mercury analyzer was 0.02 ng. Reagents of high purity, suprapur and ultrapure acids were used throughout the analysis. Milli-Q water with 18.2 mΩ resistivity was used to prepare solutions. Each sample was analysed in triplicates to ensure precision. Certified reference materials (Channel sediment: BCR-320 R; bivalves: ERM-CE278k, mussel tissue) were used to ensure accuracy.

## **2.6. Assessment of sediment contamination**

Ecotoxicology deals with toxic effects of chemical agents on population and/or community level of organisms within a defined ecosystem. Therefore, assessment of ecotoxicological risks (through degree of sediment contamination) is particularly important to highlight and evade probable adverse impacts of pollutants on the ecosystem or on the resident organisms (Gati et al. 2016; Lim et al. 2021). To assess sediment contamination and potential ecotoxicological risk associated with observed toxic metals concentration, two sets of sediment quality guideline (SQG) were followed (Watts et al. 2017; Vetrimurugan et al. 2019). The first SQG was effect range low (ERL) and effect range median (ERM) (NOAA 2012); and the second SQG was threshold effect level (TEL) and probable effect level (PEL) (CCME 2002). Low range values (ERL or TEL) are concentration of metals below which adverse biological effects are rare. In contrast, the higher range values (ERM and PEL) are associated with frequent biological effects (Long et al. 1998).

To establish a summative indication of the overall toxic metal pollution at a particular estuary, pollution load index (PLI) was calculated (Tomllinson et al. 1980) using the sedimentary and average natural background concentration of respective metals (Turekian and Wedepohl 1961). The PLI value <1 indicates no pollution, PLI=1 indicates presence of baseline pollutants. Whereas, PLI>1 indicate pollution at particular locations (Chakravarty and Patgiri 2009). PLI was obtained by calculating the contamination factor (CF) of each metal with respect to their natural background value in the sediment. CF values for describing the contamination level are shown in Table 2 (Hakanson 1980). The contamination factor (CF) was also used to determine the contamination status of the individual sampling location.

$$CF = \frac{C_{\text{sample}}}{C_{\text{background}}} \quad (1)$$

$$PLI = \sqrt[n]{(CF_1 \times CF_2 \times CF_3 \times \dots \times CF_n)} \quad (2)$$

Where, CF= Contamination factor, n= Total number of metals, C<sub>sample</sub>= Sedimentary toxic metal concentrations, C<sub>background</sub>= Natural background value of that metal.

Mean SQG quotients have been related to prediction of toxicological risks present at a particular location (Long and MacDonald 1998; Long 2000). Considering the complex nature of coastal ecosystems, these indices are often helpful to evaluate overall ecotoxicological risk probability of toxic metals to native organisms (Long and MacDonald 1998; Long et al. 1998). Mean ERM (mERM<sub>Q</sub>) and PEL quotients (mPEL<sub>Q</sub>) have been calculated as following (Long et al. 1998):

$$mERM_Q = \frac{\sum_{i=1}^n (C_i / ERM_i)}{n} \quad (3)$$

$$mPEL_Q = \frac{\sum_{i=1}^n (C_i / PEL_i)}{n} \quad (4)$$

Where C<sub>i</sub> is the sediment concentration of metal i, ERM<sub>i</sub> is the ERM value for metal i, PEL<sub>i</sub> is the PEL value for metal i and n is the number of metals. Initially, six quotient values (*i.e.* 0.1, 0.5, 0.5, 1.0, 1.5, and 2.3) were considered by Long and MacDonald (1998). Each value corresponds to certain percentage of toxicity probability to inhabitants. However, Fairey et al. (2001) used seven values (Tables 3) for better evaluation of mean SQGs that falls in the lower ranges. A mean SQGQ value <0.1 indicates 21% toxicity probability (considering mPEL<sub>Q</sub>) or 31% toxicity probability (considering mERM<sub>Q</sub>) to the resident organisms (Table 3). However, if the mean quotient value is >2.3, it would suggest 100% toxicity probability to the inhabitants. Increased percentage value indicates an increased probability towards ecotoxicity.

## 2.7. Biochemical analysis

The whole soft tissue of fifteen oysters was dissected from each location; 2gm tissue was homogenized with 1:5 (w/v) homogenization buffer (50 mM phosphate buffer, pH 7.4) using a Teflon Potter homogenizer and the homogenates were centrifuged at 10,000g for 20 min at 4°C. The supernatants were stored at -20°C for biochemical assays. The protein concentration of supernatants was determined according to Lowry et al. (1951) by using bovine serum albumin (BSA) as standard.

SOD (EC 1.15.1.1) assay was conducted by using a SOD assay kit (19160 SOD determination kit) obtained from Merck-Sigma Aldrich. SOD activity was determined by calculating its ability to inhibit the reduction of WST-1 (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2, 4-disulfophenyl)-2H-tetrazolium, monosodium salt) by superoxide anion ( $O_2^{\cdot-}$ ) which is generated by the action of Xanthine oxidase (XO). Inhibition rate % of SOD was determined at 450nm with a microplate reader (Make-BIORAD; Model- 680 XR).

CAT (EC 1.11.1.6) assay was carried out by using a CAT assay kit (CAT 100) procured from Merck-Sigma Aldrich. CAT activity ( $\mu\text{molesmin}^{-1}\text{ml}^{-1}$ ) was determined (Aebi 1974) with the help of a spectrophotometer (Make-Agilent; Model-Cary 300 UV-Vis) by calculating the decrease in absorbance ( $\lambda=520$  nm) due to  $H_2O_2$  consumption. Enzyme activity was expressed as Unit  $\text{mg}^{-1}$  of protein.

GST (EC 2.5.1.18) assay was performed by using a GST assay kit (CS 0410) supplied by Merck-Sigma Aldrich. GST activity was measured (Habig et al. 1974) by using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate. ( $\lambda=340\text{nm}$ ,  $\epsilon=9.6 \text{ mM}^{-1}\text{cm}^{-1}$ ). Enzyme specific activity ( $\mu\text{molesml}^{-1}\text{min}^{-1}$ ) was expressed as Unit  $\text{mg}^{-1}$  protein.

MT concentrations were estimated according to the method of Linde and Garcia-Vazquez (2006) by utilizing GSH as standard ( $1 \text{ mg ml}^{-1}$ ). The concentration of reduced sulphhydryl groups were evaluated by recording the absorbance at 412 nm. MT concentrations were expressed as  $\mu\text{g gm}^{-1}$  (wet wt.).

## 2.8. Statistical analyses

All results were expressed as mean  $\pm$  standard deviation (SD). Statistical analysis was carried out by using IBM<sup>®</sup> SPSS 23.0 statistical software package. A two-tailed Pearson's correlation analysis ( $p<0.05$ ) was performed to understand relationships between total organic carbon (TOC), coliforms, metals and antioxidative responses in the studied oysters. Regression analysis between antioxidant enzymes activities on coliforms and metals were performed to evaluate the influence of pollutants on antioxidant enzymes activity. Data were tested for equality of variances by Levene's test. Principal



components analysis (PCA) was performed with all the parameters to reduce the number of inter-correlated variables. The locations and seasons were converted to dummy variables and used in the PCA analysis. Varimax rotation with Kaiser Normalization was used to enhance interpretation of obtained factors. The factor coefficients were used to derive factor scores. Factors with eigenvalues greater than one were used as predictors for multiple regression analysis to eliminate multi-collinearity. One-way analysis of variance (ANOVA) was used to understand the degree of bacterial or toxic metal pollution *in situ* and subsequent antioxidative defence responses in the oysters. Mutual significance of interactions among the variables were analysed by employing a post hoc Tukey test. Post hoc Dunnett's test was also performed to compare different study locations with the reference (#S1). The acceptable level of statistical significance used throughout the study was  $p < 0.001$ , 0.01 and 0.05.

### **3. Results and discussion**

#### **3.1. Analyses of physicochemical parameters**

Seasonal variation of physico-chemical parameters (temperature, pH, salinity, dissolved oxygen, and total organic carbon) from the surface water (depth <0.5m) at the four locations (Fig.1) are represented in Table 4. Irrespective of locations, highest ambient water temperature was recorded during the pre-monsoon season (30.5-32.4°C) whereas; lowest temperature was observed during the monsoon season (26.9-28.1°C). Throughout the year (except monsoon season), pH of the water samples varied from 7.6 to 8.3 at all the location. However, during monsoon season, relatively low pH of surface water was observed at all the station. The lowest pH (6.8-7.9) was recorded at the #S4 station. Lowering of pH was probably due to influence of adjacent mangrove ecosystem. It has been documented that degradation of mangrove litter releases tannins, phenolics, and various organic acids, resulting in acidification of adjacent soil and subsequent lowering of pH of nearby water column (Laanbroek et al. 2018).

Salinity of the surface water samples remained fairly constant (except for the monsoon season) and ranged from 27 to 34 ppt at the study locations. During the monsoon, salinity was recorded significantly low at all the locations (12-16 ppt) because of high river discharge and freshwater inputs. Salinity of the four estuaries was found mesohaline (5 to 18 ppt) during the monsoon season, however varied between polyhaline (18-30 ppt) and euhaline (>30 ppt) during rest of the year.

Dissolved oxygen (DO) concentrations in surface water at all the sampling stations varied from 2.17 to 6.73 ml L<sup>-1</sup> throughout the year and represented oxic environments (>2 ml L<sup>-1</sup>). DO level was recorded highest during the monsoon season and lowest during the pre-monsoon season. Decreased in the DO concentrations during the pre-monsoon season were attributed to increased microbial activities,

owing to its metabolism and degradation of available organic matters (Le Moigne et al. 2017; Eliku and Leta 2018). It is interesting to note that, throughout the year, low concentration of D.O was observed at the #S4 in comparison to the other locations. Untreated domestic effluents (containing degradable organic compounds) from the nearby city Madgaon could be one of the reasons for lowering in the D.O concentrations at the location #S4 (Edokpayi et al. 2017). Due to degradation of mangrove litters, the #S4 was characterized by significantly higher amounts of total organic carbon (TOC) all through the year (2.73-4.92 mg L<sup>-1</sup>) followed by the #S3 (2.42-4.26 mg L<sup>-1</sup>), #S2 (1.83-3.47 mg L<sup>-1</sup>), and the #S1 (1.66-3.09 mg L<sup>-1</sup>) respectively. Regardless of locations, TOC values were found to be highest during the monsoon, probably because of high flow rate, precipitation, flushing of organic carbon from nearby soils, and agricultural lands around the sampling locations.

### 3.2. Variation of bacterial abundance in different environmental matrices

A unique seasonal variation of total bacterial count (TBC), total coliform (TC) and fecal coliform (FC); enumerated from the water, sediment, and oysters was observed in the study areas. Relatively high abundance of indigenous bacteria and coliforms were estimated during the monsoon whereas; comparatively lower abundance was recorded during the pre-monsoon at all the locations. Generally, the abundance of coliforms and bacteria (except *Vibrio* spp.) were highest in the oysters and lowest in the water samples irrespective of their spatio-temporal variations. Coliforms density in the studied oysters was about 24 and 2 times higher than the coliform densities observed in water and sediment respectively. Due to filter feeding, the oysters bioaccumulated higher amount of bacteria compared to its surroundings. In general, highest indigenous bacteria were observed in both biotic and abiotic compartments at the station #S3. Whereas, maximum nonindigenous bacteria were enumerated from the #S4.

During the monsoon season, *Salmonella* spp. was found to vary between 96-402cfu ml<sup>-1</sup> in the overlying water, 914-11.6×10<sup>3</sup>cfu gm<sup>-1</sup> (wet wt.) in the sediment and 70.1×10<sup>2</sup>-23.4×10<sup>3</sup>cfu gm<sup>-1</sup> (wet wt.) in the oysters from all the locations (Fig. 2A). Relatively low density of *Salmonella* spp. was recorded during the pre-monsoon period. It varied from 10-53cfu ml<sup>-1</sup> in the overlying water, 117-14.4×10<sup>2</sup>cfu gm<sup>-1</sup> (wet wt.) in the sediment and 893-78×10<sup>2</sup>cfu gm<sup>-1</sup> (wet wt.) in the oysters.

*Vibrio* spp. level was estimated highest during the monsoon and ranged 535-21.4×10<sup>2</sup>cfu ml<sup>-1</sup> in the overlying water; 94.8×10<sup>2</sup>-27×10<sup>3</sup>cfu gm<sup>-1</sup> (wet wt.) in the sediment and 51.3×10<sup>2</sup>-17.6×10<sup>3</sup>cfu gm<sup>-1</sup> (wet wt.) in the oysters from all the locations (Fig. 2B). During the pre-monsoon, lowest amount of *Vibrio* spp. was enumerated from the water (21-194 cfu ml<sup>-1</sup>), sediment (12.3×10<sup>2</sup>-95.8×10<sup>2</sup>cfu gm<sup>-1</sup> wet wt.), and the oysters (827-42.5×10<sup>2</sup>cfu gm<sup>-1</sup> wet wt.). Abundance of sedimentary *Vibrio* spp. was

relatively higher than the other samples. These bacteria are known as 'particle-lovers' and tend to attach to particulate matters that is available in plenty in the estuarine sediment (Froelich and Noble 2016), making them less available to the water column and for subsequent bioaccumulation. Johnson et al. (2012) had reported a similar observation from the coastal and estuarine waters of the USA.

During the monsoon, highest density of *Aeromonas hydrophila* was recorded and varied between 116-568cfu ml<sup>-1</sup> in the overlying water, ranged from 24.6×10<sup>2</sup>-15.7×10<sup>3</sup>cfu gm<sup>-1</sup> (wet wt.) in the sediment and 10.4×10<sup>3</sup>-44.3×10<sup>4</sup>cfu gm<sup>-1</sup> (wet wt.) in the oyster from all the locations (Fig. 2C). However, the lowest abundance of *A. hydrophila* was observed during the pre-monsoon in the water (17-82cfu ml<sup>-1</sup>), sediment (430-24.4×10<sup>2</sup>cfu gm<sup>-1</sup> wet wt.) and the oyster samples (36.5×10<sup>2</sup>-16×10<sup>3</sup>cfu gm<sup>-1</sup> wet wt.).

Maximum quantity of *Clostridium botulinum* was found in the sediment samples which varied between 415-23.4×10<sup>2</sup>cfu gm<sup>-1</sup> (wet wt.), followed by 180-870cfu gm<sup>-1</sup> (wet wt.) and 40-230cfu gm<sup>-1</sup> (wet wt.) during the monsoon, post-monsoon and the pre-monsoon respectively (Fig. 2D). *C. botulinum* was only present in the sediment samples. The bacteria is a spore forming, obligate anaerobe; ubiquitous in coastal sediments worldwide. Well mixed oxygenated estuarine water and oysters never provides a suitable condition for germination of spores. Therefore, the absence of *C. botulinum* in the water and the oyster samples is justified. *Listeria monocytogenes* was absent in all the estuarine samples throughout the year. Several studies have reported either absence (Fuchs and Surendran 1989; Manoj et al. 1991; Kamat and Nair 1994; Anthony et al. 2002) or rare presence (1.3 % of total samples) (Dhanashree et al. 2003) of the species in raw fish and shellfish from India. As suggested by Parihar et al. (2008), *L. monocytogenes* is considered as psychrotrophic bacteria and thus could be less common from tropical waters.

Highest fecal coliform (FC) load was recorded during the monsoon in all the samples collected from the #S4, succeeded by the #S3, #S2, and the #S1 respectively (Fig. 3A). However, the lowest fecal coliform density was recorded during the pre-monsoon season. Like fecal coliforms, the maximum quantity of total coliform was observed in the monsoonal samples collected from the #S4, ensued by the #S3, #S2, and the #S1 respectively (Fig. 3B). Similar to FC, during the pre-monsoon, lowest amount of TC was estimated in the samples collected from the #S4, after that the #S3, #S2, and the #S1 respectively.

The density of coliforms in the tropical waters has long been used as indicator to ensure the environmental quality. Elevated bacterial abundance at the #S3 and the #S4 throughout the year indicated towards an unsuitable habitat available for the naive oysters. Our observation was in

accordance with the latest report published by the Goa State Pollution Control Board (GSPCB 2018). Presence of fish landing and marketing sites, restaurants and hotels, construction activities near the sampling locations, and increased land runoff are the reasons behind the maximum coliforms density during the monsoon. Apart from this, the Zuari and the Sal River serves as discharge points for domestic effluents, sewage sludge, untreated municipal and industrial wastewater (from the nearby city Vasco-Da-Gama and Madgaon respectively). Raw or improperly treated effluents from fishing trawlers and ships anchored near the Mormugao port additionally contributed to the high coliform counts at those locations. Reports on high degree of fecal coliform bioaccumulation during the monsoon are in plenty from India (Hassan et al. 2013; Jana et al. 2014; Chinnadurai et al. 2016). Overall, all the samples from the #S3 were mostly polluted with indigenous bacteria whereas, the #S4 was typically polluted with the coliforms. Dunnett's test suggested that the #S1 had significant difference ( $p < 0.01$  and  $p < 0.001$ ) regarding bacteria with the #S3 or the #S4 (supplementary Table SM1).

### 3.3. Variation of toxic metal concentrations in the sediment and the oysters

Temporal and spatial distribution of total metal concentrations in the surface sediment and the oysters are presented in Table 5. Regardless of spatio-temporal variations, the sedimentary toxic metal concentrations were in the following descending order:  $Pb > Cd > Hg$  while, the bioaccumulated concentrations followed the hierarchical order:  $Cd > Pb > Hg$ . Furthermore, the spatio-temporal distribution showed that the #S3 had significantly higher sedimentary and bioaccumulated Pb concentration ( $76.47 \pm 3.31$ - $102.48 \pm 2.4$  and  $6.94 \pm 0.6$ - $7.99 \pm 0.5$   $mg\ kg^{-1}$  dry wt. respectively) throughout the year. This increased Pb concentration at the #S3 might be attributed to unregulated use of anti-fouling paints by different shipbuilding yards, road runoff, combustion of fossil fuel by boats and subsequent atmospheric deposition, ferry, ships and heavy traffic loads in the vicinity of the sampling location.

Spatial variation of sedimentary and bioaccumulated Cd and Hg concentrations were highest at the #S4 (Cd:  $2.1 \pm 0.18$ - $3.4 \pm 0.25$  and  $9.4 \pm 0.52$ - $10.6 \pm 0.5$   $mg\ kg^{-1}$  dry wt. respectively; Hg:  $0.11 \pm 0.02$ - $0.13 \pm 0.03$  and  $0.15 \pm 0.02$ - $0.22 \pm 0.02$   $mg\ kg^{-1}$  dry wt. respectively) across the seasons. It is important to note that the #S4 (Sal estuary) is surrounded by densely populated areas, This estuary has been indiscriminately used for releasing industrial sewage as well as untreated solid and domestic waste for years (Saha et al. 2021). Regardless of locations, maximum sedimentary and bioaccumulated Cd concentrations were recorded during the monsoon season ( $0.35 \pm 0.02$ - $3.4 \pm 0.25$  and  $6.7 \pm 0.35$ - $10.6 \pm 0.5$   $mg\ kg^{-1}$  dry wt. respectively) while minimum concentration was estimated during the post-monsoon season ( $0.12 \pm 0.01$ - $2.1 \pm 0.18$  and  $5.74 \pm 0.2$ - $9.4 \pm 0.52$   $mg\ kg^{-1}$  dry wt. respectively). Increased

agricultural runoff, sewage drainage, and industrial waste were potential sources for the enrichment of Cd during the wet season (Roberts 2014; Zhang et al. 2017; Shilla et al. 2019). Highest sedimentary Pb concentrations were estimated during the pre-monsoon ( $35.7 \pm 1.4$ - $102.48 \pm 2.4$  mg kg<sup>-1</sup> dry wt.) and lowest during the post-monsoon ( $19.28 \pm 0.33$ - $76.47 \pm 3.31$  mg kg<sup>-1</sup> dry wt.) from all the locations. During the dry season, saline water from the Arabian sea could extend up to 40 km upstream of the rivers (Shetye et al. 1987), turning the lower estuaries to an extension of the sea. Increased salinity affects flocculation and coagulation of suspended particulate pollutants (like lead) and favours quick settling near the source of discharge (Chakraborty et al. 2017). Temporal variation of bioaccumulated Pb concentrations were negligible and remained somewhat constant. Seasonal variation of sedimentary and bioaccumulated Hg concentrations were minimal in the locations. Sedimentary Hg concentrations were maximum during the pre-monsoon whereas, minimum concentrations were estimated during the post-monsoon. Dunnett's test confirmed that the #S1 had less difference with the #S2 or the #S3 (except Pb) regarding sedimentary or bioaccumulated metal concentrations (supplementary table SM2). Due to the random seasonal fluctuations of bioaccumulated Pb and Hg concentrations, specific temporal pattern could not be assigned for those metals. Growth rate of the oysters and differences in nutritional availability changes in coastal ecosystems depending upon seasons and variation in source and degree of bioavailable metal fraction could be responsible for the random patterns in metal bioaccumulation (Pourang et al. 2010; Chalghmi et al. 2016) during different seasons.

Bioaccumulation of metals depends upon environmental factors such as, temperature, salinity, pH, TOC (Mubiana and Blust 2007); source (Gupta and Singh 2011); and bioavailability of metals (Langston and Brian 1984). These factors changes seasonally, causing spatio-temporal differences in bioaccumulated metal concentrations. Interestingly, irrespective of spatio-temporal variations, the bioaccumulated Cd concentrations were fairly higher than the surroundings (average eighteen times). Bioaccumulated Cd concentration is independent of its sedimentary concentration but depend on its speciation. High concentrations of labile Cd complexes has been reported from this region by Chakraborty et al. (2016) suggesting, increased bioavailability of sedimentary Cd to native organisms. Due to analogous ionic radius (Ca 9.7 nm & Cd 9.8 nm), Cd mimics Ca ions and can enter oyster tissue by substituting Ca (Papadopoulos and Rowell 1988; Huanxin et al. 2000; Dar et al. 2018). Cadmium also has a strong affinity towards metallothionein and could be sequestered by oysters in some detoxified form (Rainbow 2002). High accumulation of Cd in oysters has also been reported earlier from various parts of Goa (Chakraborty et al. 2015; ShenaiTirodkar et al. 2016). Though sedimentary Pb concentrations were higher than Cd, bioaccumulation of Pb was comparatively less. It has been reported that, bioaccumulated Pb is rapidly excreted from biological systems due to its inert nature

(Denton and Burdon-Jones 1981). Furthermore, major proportion of sedimentary Pb are in complex formation with organic matter and/or Fe/Mn oxyhydroxide, forming thermodynamically stable complexes thus becoming partially bioavailable (Chakraborty et al. 2012, 2015) to the oysters. Also, a major portion of sedimentary Pb is present within the structure of the sediments from this region (Chakraborty et al. 2019b). Therefore, irrespective of locations, lesser amount of Pb bioaccumulation was justified in the oysters. Earlier, less amount of Pb bioaccumulation was reported in oysters by Shenoy Tirodkar et al. (2016) from this region (0.1-1.7 mg kg<sup>-1</sup>). The bioaccumulated Hg concentration was marginally higher than the sediment and agreed with our previous study from this region (Patra et al. 2019).

### 3.4. Degree of sediment contamination and adverse biological effect on the oysters

Table 6 accounts for all the sediment samples in three ranges of toxic metal concentrations where adverse biological effects are seldom (<TEL/ERL), moderate ( $\geq$ TEL/ERL and <PEL/ERM), and recurrent ( $\geq$ PEL/ERM). Considering all the toxic metals, the sediment samples from the #S4 were in moderate effect range. While considering only Pb, the sediment samples from the #S3 were in moderate effect range all around the year. For all the studied metals, none of the sediment samples were in the frequent or recurrent effect range.

The contamination factor and pollution load index are given in Table 7. CF values indicated the location #S1 and the #S2 were low (regarding Hg) to moderately contaminated (for Cd and Pb) throughout the year. Cd and Hg contamination was less at the #S3. However, this particular location was considerably contaminated by Pb across the seasons. The #S4 was highly contaminated with Cd, moderately contaminated with Pb, and less contaminated with Hg. The results suggested considerable contamination especially at the #S4 was due to the influence of external sources like industrial and agricultural runoff as well as local anthropogenic activities. Except for the #S4, PLI values from all the other locations were less than unity (<1), suggesting lesser pollution at those locations. However, PLI value at the #S4 was >1 (1.8) and suggested higher degree of toxic metal pollution.

To determine the potential biological effect of all the toxic metals combined, the mean quotient was calculated (Table 7) using two empirically derived sets of SQG's (*i.e.* ERM and PEL). According to mERM<sub>Q</sub> classifications, the sediment samples from the #S1 and the #S2 were classified as "low priority" with 31% probability of toxicity to the oysters. While, the sediment samples from the #S3 and the #S4 were classified as "medium priority" with a toxicity probability of 36-37% to the native oysters. Similarly, the mPEL<sub>Q</sub> value indicated a minimum of 29% (at the #S1 and the #S2) to a maximum of 36% (at the #S3 and the #S4) toxicity probability to the oysters. Both SQG values

suggested that ecotoxicological risk to the oysters was higher in the #S3 and the #S4 than the #S1 and the #S2.

### **3.5. Seasonal variation in antioxidative defence system of the oysters with respect to different pollutants**

In the present study, maximum antioxidant enzymes activity was observed in oysters collected during the pre-monsoon season following, the monsoon (Amutha and Subramanian 2012; Jana et al. 2013). Interestingly, seasonal variation of antioxidant enzymes activity was somewhat inconsistent with pollution loading at the locations. During the pre-monsoon season, increased temperature and pH might have influenced in increasing the antioxidant enzyme activities (Malek et al., 2004; Glippa et al. 2018). In addition, tropical estuaries are known for poor flushing, ensuring minimum net transport and subsequent increase in resident time of pollutants during this season (Chakraborty et al. 2019a; John et al. 2020). These factors altogether could have helped in increasing the enzymes activity during the season. Temporal variation of CAT activity was minimal irrespective of locations (Fig. 4A). At the #S1 and #S2, spatial variation of the enzyme was also insignificant ( $p < 0.05$ ). Oysters from the #S4 showed highest CAT activity while, the #S2 showed lowest activity throughout the year. SOD activity ranged from 70-90% across the locations during the study period (Fig. 4B). Highest SOD activity was observed during the pre-monsoon whereas; lowest activity was recorded in the post-monsoon. Location wise, highest SOD activity was estimated at the #S3 and the #S4. Increased GST activity was recorded during the pre-monsoon (Fig. 4C). Throughout the year, maximum GST activity was recorded from the #S4 while, the minimum activity was estimated at the #S1. Except for the post-monsoon, spatial variation of GST activity between the #S1 and the #S2 were insignificant ( $p < 0.05$ ). Significant spatio-temporal differences were observed for MT concentrations at the locations ( $p < 0.05$ ; Fig. 4D). Highest MT concentrations were observed during the pre-monsoon, followed by the monsoon and the post-monsoon seasons. Highest MT level was recorded from the #S4 and lowest concentrations were estimated at the #S1. MT level at the #S4 was significantly different ( $p < 0.05$ ) from the other locations throughout the year. MT concentrations does not solely rely on the environmental toxic metal contents and could differ because of differences in breeding time, age, and sex of the oysters, temperature of the ambient water and seasons (Ivanković et al. 2005). In general, spatio-temporal variation of antioxidant enzymes level at the locations could be arranged in a descending order as: #S1 < #S2 < #S3 < #S4. Results from Dunnett's test confirmed that, except MT, the #S1 was very similar to the #S2 when antioxidant enzymes were compared (supplementary table SM 3). Increased antioxidant enzymes activity at the Sal and the Zuari estuary across the year suggested towards higher environmental stress at the estuaries. The increase in environmental stress might be

attributed to higher degree of anthropogenic stress at the Sal followed by the Zuari estuary. Toxic metals (especially Cd and Hg) and bacteria (*Salmonella* spp., *Vibrio* spp., *A. hydrophila*, coliforms) showed significant positive correlation ( $p < 0.01$ ) with the antioxidant enzymes, indicating their role in inducing antioxidative defence in the oysters (Table 8). Both the estuaries receive untreated domestic effluents, sewage sludge, municipal and industrial wastewater from the nearby cities (Madgaon and Vasco-Da-Gama respectively) as well as discharge from restaurants, hotels and tourism units located upstream. These factors collectively helped in increasing overall environmental stress in those estuaries. Sediment contamination factor (CF), pollution load index (PLI), mean SQG quotient altogether indicated towards a higher degree of pollution including moderate ecotoxicological risks and increased probability of toxicity to the oysters (36-37%) at the Sal and the Zuari estuary. Overall, these results suggested that the #S1 and the #S2 were comparatively less polluted with the bacteria, but somewhat polluted with toxic metals all through the year. The #S4 was considerably polluted with the metals, coliforms and bacteria. Oxidative stress as well as ecotoxicological risk was least at the #S1 and highest at the #S4. All four antioxidant enzymes positively correlated with bioaccumulated coliforms and metals (supplementary Fig. SM1 and SM2), validating their possible use as biomarker responsive to oxidative stress exerted by metals and bacteria in field conditions.

### 3.6. Statistical analysis

Pearson's correlation matrix was applied to assess relationships between TOC, coliforms, metals and antioxidant enzymes (Table 8). A significant positive correlation was found between TOC as well as coliforms with antioxidant enzymes (except MT). The sedimentary Cd concentration had a significant positive correlation with all the antioxidant enzymes. While, the bioaccumulated Cd showed significant correlation only with CAT. Sedimentary Pb concentration showed positive correlation only with SOD. Both sedimentary and the bioaccumulated Hg showed positive correlation with all the antioxidant enzymes.

Four components were selected after PCA analysis (Fig. 5). Those components together accounted for 94.9% of the total variance of the original variables. The first component had a variance of 55.45% and was categorized by high positive loadings of indigenous bacteria and coliforms. The second component included a variance of 20.61% and was characterized by toxic metals. The third component had a variance of 13.74% and identified for positive loading of physico-chemical parameters. The fourth component only attributed to 5.05% of total variance but was considered for linear regression as the eigenvalue  $> 1$ . For linear regression, factor scores were used as independent variables and each antioxidant enzyme was used as dependent variable. In general, component 2 showed highest significance ( $p < 0.0001$ ) among all the components analysed for standardized



regression on production of antioxidant enzymes (supplementary table SM4). This analysis suggested that among all the parameters, sedimentary toxic metals were probably the main reason for the production of antioxidant enzymes in the studied oysters. Spatio-temporal variation of indigenous bacteria, coliforms, metals and the antioxidant enzymes were significantly different from each other when analysed through one-way ANOVA (supplementary Table SM5, SM6, and SM7). Post hoc Dunnett's test verified our supposition that the location #S1 was relatively pristine throughout the year.

#### **4. Conclusion**

Evaluation of antioxidant enzymes in edible oyster *S. cucullata* indicated towards the prevalence of cytotoxic contaminants at different tropical estuaries along Goa coast. Factors like toxic metal contamination and/or the presence of indigenous bacteria and coliforms could affect antioxidative defense system in *S. cucullata*. This study provided a detailed understanding of the present environmental conditions at four different estuaries, revealing that amount of Cd bioaccumulation in the oysters was substantially high (much higher than the permissible limit) in all the four estuaries.

Bioaccumulation of non-indigenous bacteria by the oysters was fairly high and suggested towards poor environmental quality especially in the Zuari and the Sal estuary. Site specific activities influenced more in those estuaries, particularly during the pre-monsoon (March-May) and the monsoon (June-September). Probability of ecotoxicological risks to native organisms was more prominent in those two estuaries. In addition to pollution load, physico-chemical factors (such as, temperature and pH) could induce antioxidant enzymes production. Our study confirms that integrated approach of evaluating biochemical responses of bioindicators against environmental stresses under field condition could precisely describe the health status in tropical estuarine systems.

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#### **Conflict of interest**

The authors declare no conflict of interest, financial or otherwise.

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**Caption and legends:**

**Fig. 1** Map of sampling locations along Goa coast.

**Fig. 2** Abundance of selective bacteria estimated from different tropical estuaries, during the pre-monsoon (PM), monsoon (M) and the post-monsoon (pM) seasons. **(A)** *Salmonella* spp., **(B)** *Vibrio* spp., **(C)** *Aeromonas hydrophila*, and **(D)** *Clostridium botulinum*

**Fig. 3** Representation of **(A)** fecal and **(B)** total coliforms from four estuaries along Goa coast, during 2017.

**Fig. 4** Antioxidant enzymes activity **(A)** Catalase, **(B)** Superoxide dismutase, **(C)** Glutathione-s-transferase and **(D)** Metallothionein concentrations estimated from the oysters.

**Fig. 5** Component plot generated by principal component analysis (PCA) -

Component 1 includes: indigenous bacteria and coliforms,

Component 2 includes: toxic metals,

Component 3 includes: physico-chemical parameters.

Table 1: Coordinates of sampling locations.

Name	Latitude	Longitude
Siolim (#S1)	15°37'50"N	73°45'30"E
Nerul (#S2)	15°30'38"N	73°46'47"E
Chicalim (#S3)	15°24'41"N	73°51'12"E
Betul (#S4)	15°9'21"N	73°57'14"E

Table 2: Ranges of contamination factor (CF) and status of contamination.

Contamination Factor (CF)	Contamination level
< 1	Low
1 ≤ to < 3	Moderate
3 ≤ to < 6	Considerable
> 6	Very high

Table 3: Percentage of. Acute biological toxicity assessed through Mean SQG quotient ranges.

SQG quotient range	Percentage of toxicity probability (for mERM <sub>Q</sub> )	Percentage of toxicity probability (for mPEL <sub>Q</sub> )
>2.3	100	100
>1.5	88	76
>1.0	78	70
>0.5	62	53
<0.5	37	36
<0.2	36	29
<0.1	31	21

Table 4: Seasonal variation\* of physico-chemical parameters in the estuaries along Goa coast, during 2017.

Locations	Seasons	Temperature ( $^{\circ}\text{C}$ )	pH	Salinity (ppt)	D.O ( $\text{ml L}^{-1}$ )	TOC ( $\text{mg L}^{-1}$ )
#S1	Pre monsoon	30.5 $\pm$ 0.2	8.2 $\pm$ 0.02	32	3.72 $\pm$ 0.12	1.66 $\pm$ 0.08
	Monsoon	27.1 $\pm$ 0.1	7.5 $\pm$ 0.07	14	6.27 $\pm$ 0.15	3.09 $\pm$ 0.22
	Post monsoon	27.0 $\pm$ 0.15	7.8 $\pm$ 0.05	29	4.19 $\pm$ 0.1	2.35 $\pm$ 0.06
#S2	Pre monsoon	31.1 $\pm$ 0.15	8.1 $\pm$ 0.05	31	2.43 $\pm$ 0.2	1.83 $\pm$ 0.1
	Monsoon	26.9 $\pm$ 0.4	7.3 $\pm$ 0.1	13	4.68 $\pm$ 0.09	3.47 $\pm$ 0.15
	Post monsoon	27.5 $\pm$ 0.1	7.7 $\pm$ 0.1	27	2.81 $\pm$ 0.19	2.82 $\pm$ 0.14
#S3	Pre monsoon	32.4 $\pm$ 0.1	8.3 $\pm$ 0.09	34	3.89 $\pm$ 0.15	2.42 $\pm$ 0.04
	Monsoon	28.1 $\pm$ 0.12	7.8 $\pm$ 0.1	16	6.73 $\pm$ 0.17	4.26 $\pm$ 0.2
	Post monsoon	29.4 $\pm$ 0.3	8.1 $\pm$ 0.02	31	4.33 $\pm$ 0.08	3.07 $\pm$ 0.09
#S4	Pre monsoon	32.1 $\pm$ 0.12	7.9 $\pm$ 0.02	30	2.17 $\pm$ 0.08	2.73 $\pm$ 0.07
	Monsoon	27.4 $\pm$ 0.15	6.8 $\pm$ 0.05	12	4.21 $\pm$ 0.24	4.92 $\pm$ 0.16
	Post monsoon	28.1 $\pm$ 0.3	7.6 $\pm$ 0.09	24	2.52 $\pm$ 0.14	3.58 $\pm$ 0.12

\*Data represented as Mean $\pm$ Standard deviation (n=3).

Table 5: Seasonal variations (Mean±SD; n=3) of total metal concentrations (mg kg<sup>-1</sup> dry wt.) in the sediment and the oysters collected from the estuaries of Goa during 2017.

Metals	Location	Pre monsoon		Monsoon		Post monsoon	
		Sediment	Oyster	Sediment	Oyster	Sediment	Oyster
Cd	#S1	0.4±0.03	6.63±0.4	0.48±0.03	6.86±0.26	0.26±0.03	5.81±0.28
	#S2	0.33±0.03	5.79±0.24	0.42±0.02	6.7±0.33	0.17±0.02	5.74±0.2
	#S3	0.28±0.02	5.17±0.2	0.35±0.02	6.7±0.35	0.12±0.01	5.78±0.23
	#S4	3.2±0.2	9.8±0.4	3.4±0.25	10.6±0.5	2.1±0.18	9.4±0.52
Pb	#S1	39.12±0.48	0.18±0.11	31.2±1.3	0.18±0.08	24.41±0.24	0.2±0.04
	#S2	35.7±1.4	0.23±0.07	27.19±2.2	0.21±0.03	19.28±0.33	0.33±0.17
	#S3	102.48±2.4	7.99±0.5	93.52±4.6	7.97±0.56	76.47±3.31	6.94±0.6
	#S4	49.2±3.57	0.49±0.13	40.7±2.4	0.39±0.25	31.21±2.4	0.43±0.12
Hg	#S1	0.043±0.01	0.067±0.02	0.037±0.01	0.068±0.01	0.031±0.01	0.061±0.01
	#S2	0.068±0.02	0.082±0.01	0.066±0.01	0.087±0.01	0.063±0.02	0.079±0.01
	#S3	0.051±0.01	0.094±0.01	0.046±0.01	0.086±0.01	0.042±0.01	0.08±0.01
	#S4	0.129±0.3	0.217±0.02	0.121±0.02	0.161±0.02	0.114±0.02	0.148±0.02

Table 6: Threshold effect level (TEL), Probable effect level (PEL), Effect range low (ERL), and Effect range median (ERM) for toxic metals\* and chance of adverse biological effect on the oysters.

Metals	Location	Maximum concentration	Minimum concentration	TEL	PEL	ERL	ERM	% samples within range of SQG's			Chance of adverse biological effect
								<TEL/ERL	TEL/ERL- PEL/ERM	>PEL/ERM	
Cd	#S1	0.48	0.26	0.68	4.21	1.2	9.6	100	-	-	Seldom
	#S2	0.42	0.17					100	-	-	Seldom
	#S3	0.35	0.12					100	-	-	Seldom
	#S4	3.4	2.1					-	100	-	Moderate
Pb	#S1	39.12	24.41	30.2	112.2	46.7	218.0	100	-	-	Seldom
	#S2	35.7	19.28					100	-	-	Seldom
	#S3	102.48	76.47					-	100	-	Moderate
	#S4	49.2	31.21					78	22	-	Seldom/Moderate
Hg	#S1	0.043	0.031	0.13	0.7	0.15	0.71	100	-	-	Seldom
	#S2	0.068	0.063					100	-	-	Seldom
	#S3	0.051	0.042					100	-	-	Seldom
	#S4	0.129	0.114					89	11	-	Seldom/Moderate

\*Values represented in mg kg<sup>-1</sup> dry wt.

Table 7: Pollution Load index (PLI)\* of metals (mg kg<sup>-1</sup> dry wt.) in the sediment samples.

Metals	Locations				Natural background concentration
	#S1	#S2	#S3	#S4	
Cd	0.38 (1.27)	0.31 (1.03)	0.25 (0.83)	2.90 (9.67)	0.3
Pb	31.58 (1.58)	27.39 (1.37)	90.82 (4.54)	40.37 (2.02)	20.0
Hg	0.04 (0.1)	0.07 (0.18)	0.05 (0.13)	0.12 (0.3)	0.4
PLI Index	0.56	0.63	0.79	1.8	
m-ERM <sub>Q</sub>	0.08	0.09	0.17	0.22	
m-PEL <sub>Q</sub>	0.14	0.14	0.31	0.41	

\*Values in parenthesis are the contamination factors (CF).

Table 8: Pearson's correlation (two tailed) for the TOC, accumulated bacteria, metals, and the antioxidant enzymes ( $p < 0.05$ ). Significant correlations are in bold. \* indicates correlation is significant at  $p < 0.01$  level.

	TOC	Sal	Vib	Aero	TC	FC	Cd	Pb	Hg	CAT	SOD	GST	MT
TOC	1												
Sal	.77	1											
Vib	.79	.94	1										
Aero	.78	.93	.97	1									
TC	.91	.73	.72	.76	1								
FC	.90	.78	.79	.83	.98	1							
Cd	<b>.55*</b>	.07	.11	.11	.57	.51	1						
Pb	.07	.51	.51	.58	.07	.19	.46	1					
Hg	<b>.53*</b>	.04	.03	.11	.5	.48	.68	.13	1				
CAT	<b>.58*</b>	<b>.51*</b>	<b>.51*</b>	<b>.54*</b>	<b>.74*</b>	<b>.75*</b>	<b>.52*</b>	.35	<b>.73*</b>	1			
SOD	<b>.55*</b>	.38	.44	<b>.51*</b>	<b>.57*</b>	<b>.6*</b>	.31	<b>.5*</b>	<b>.64*</b>	.81	1		
GST	<b>.77*</b>	<b>.66*</b>	<b>.63*</b>	<b>.67*</b>	<b>.79*</b>	<b>.8*</b>	<b>.54*</b>	.31	<b>.56*</b>	.89	.79	1	
MT	.34	.03	.04	.15	.39	.36	<b>.51*</b>	.12	<b>.84*</b>	.64	.75	.45	1



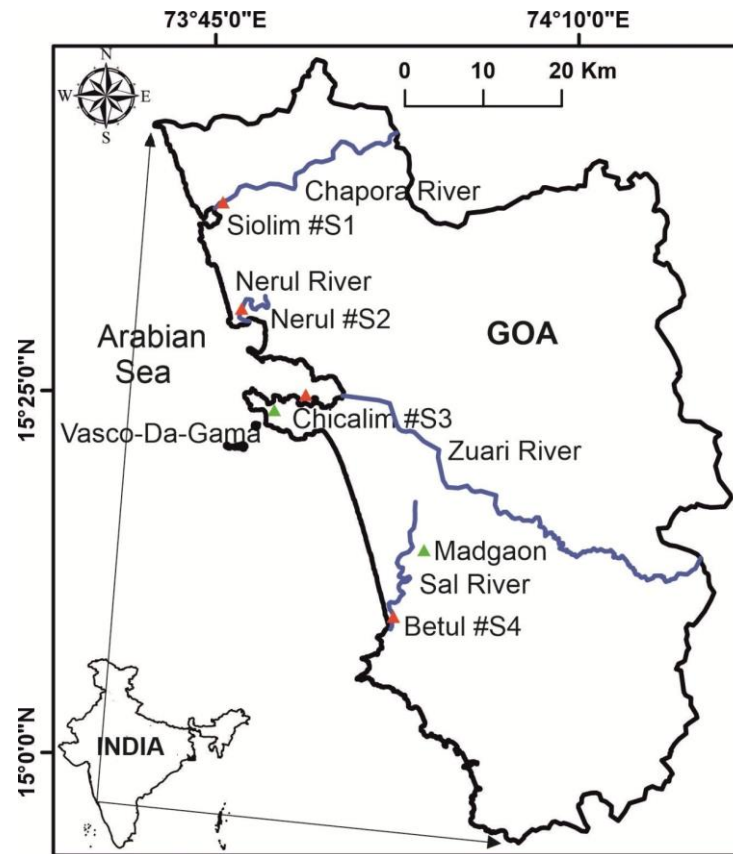


Figure 1

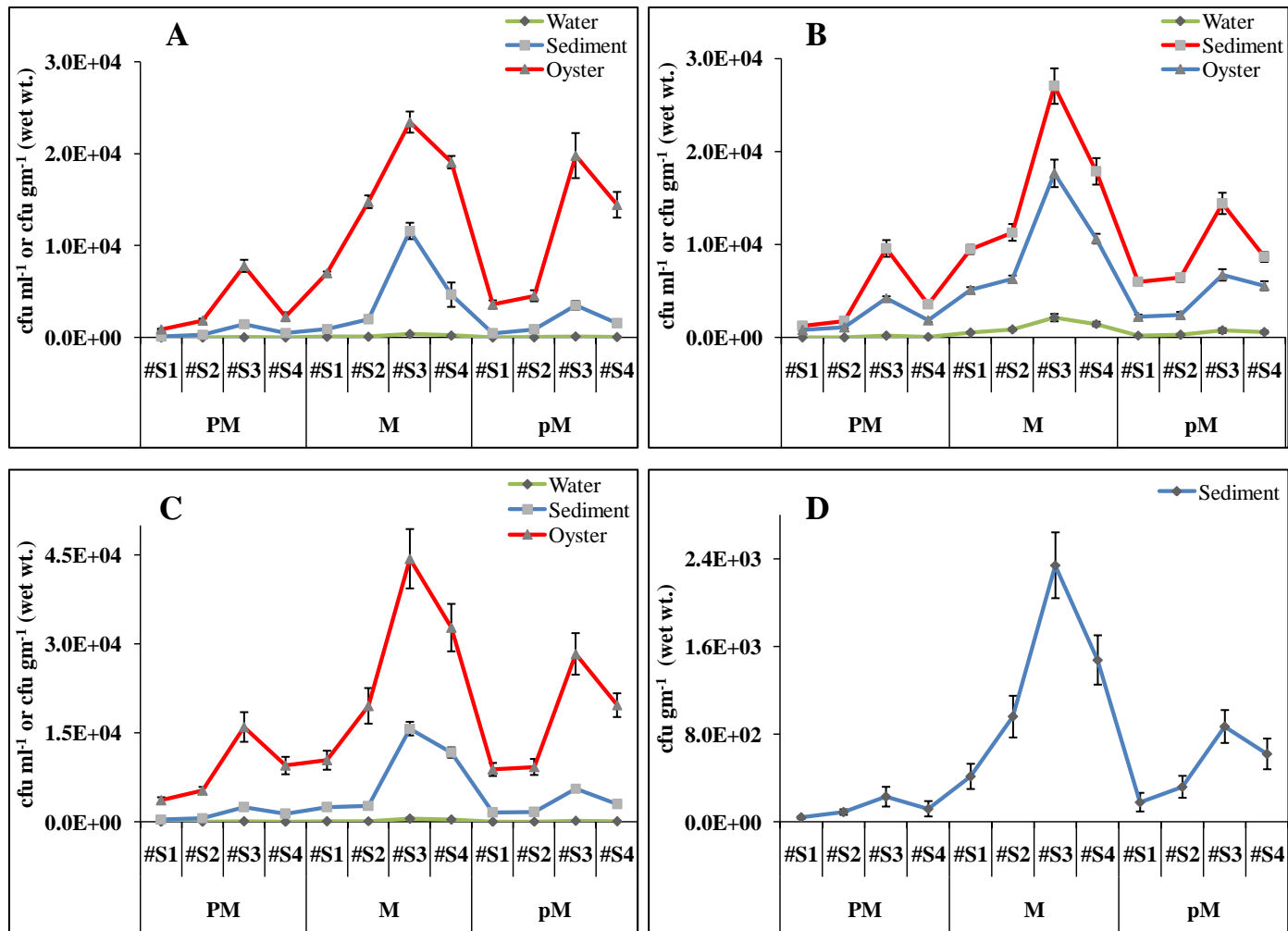
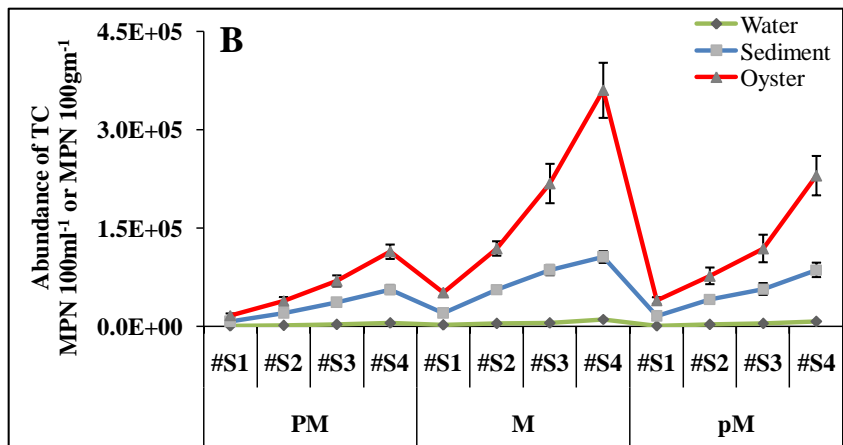
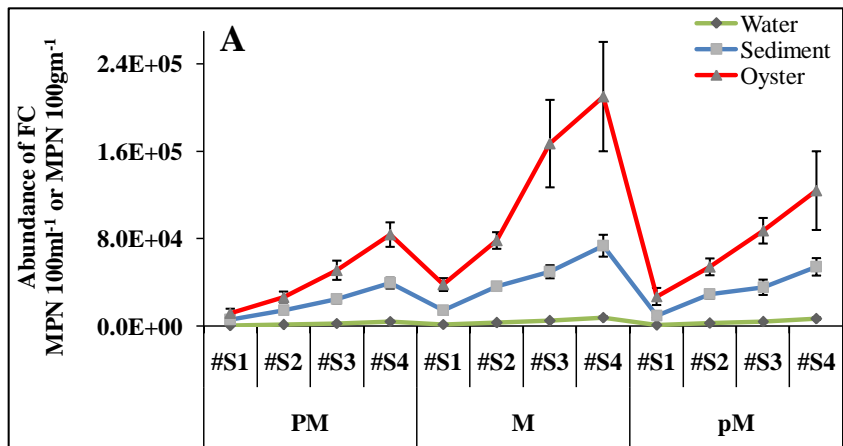
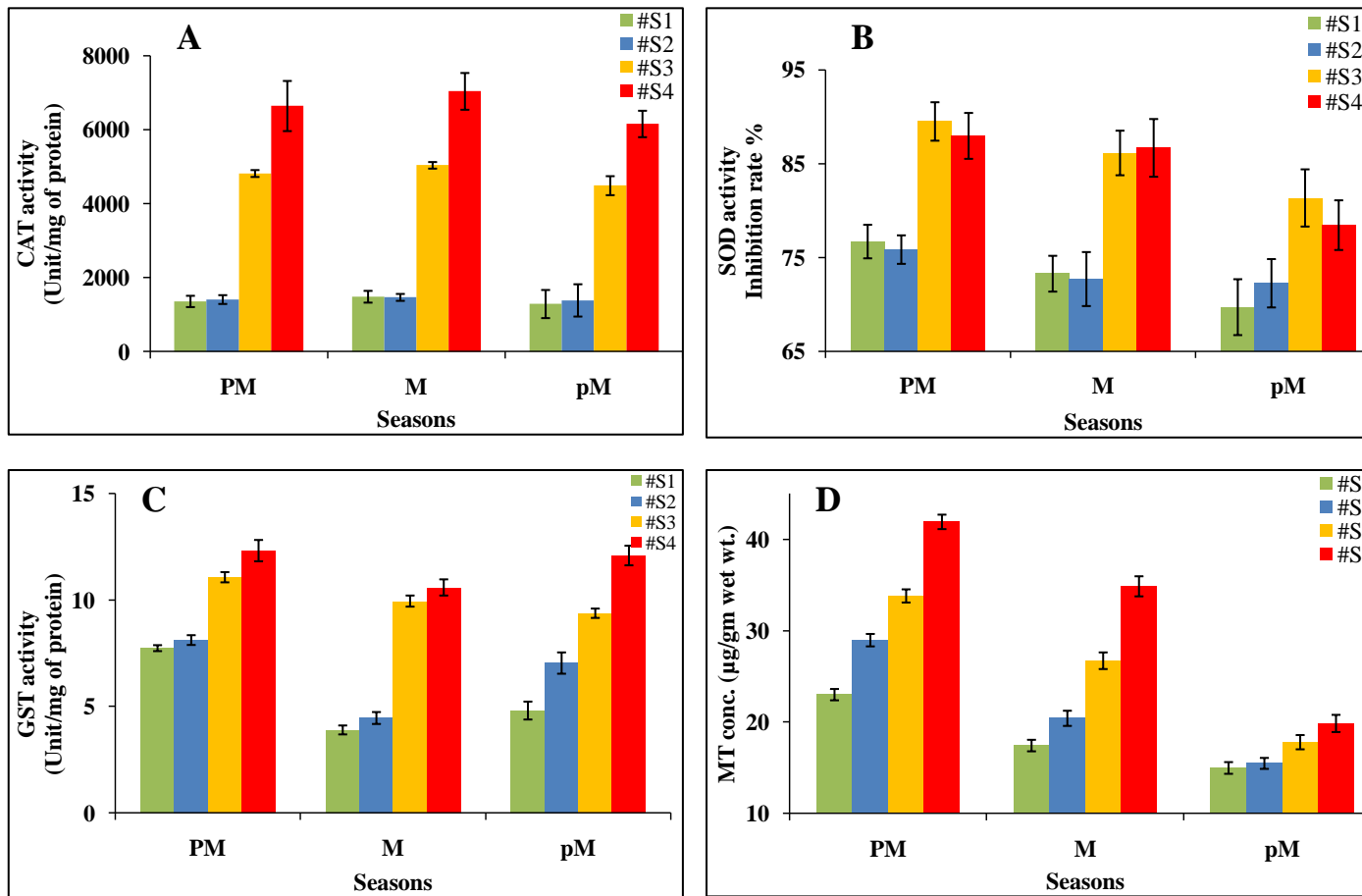


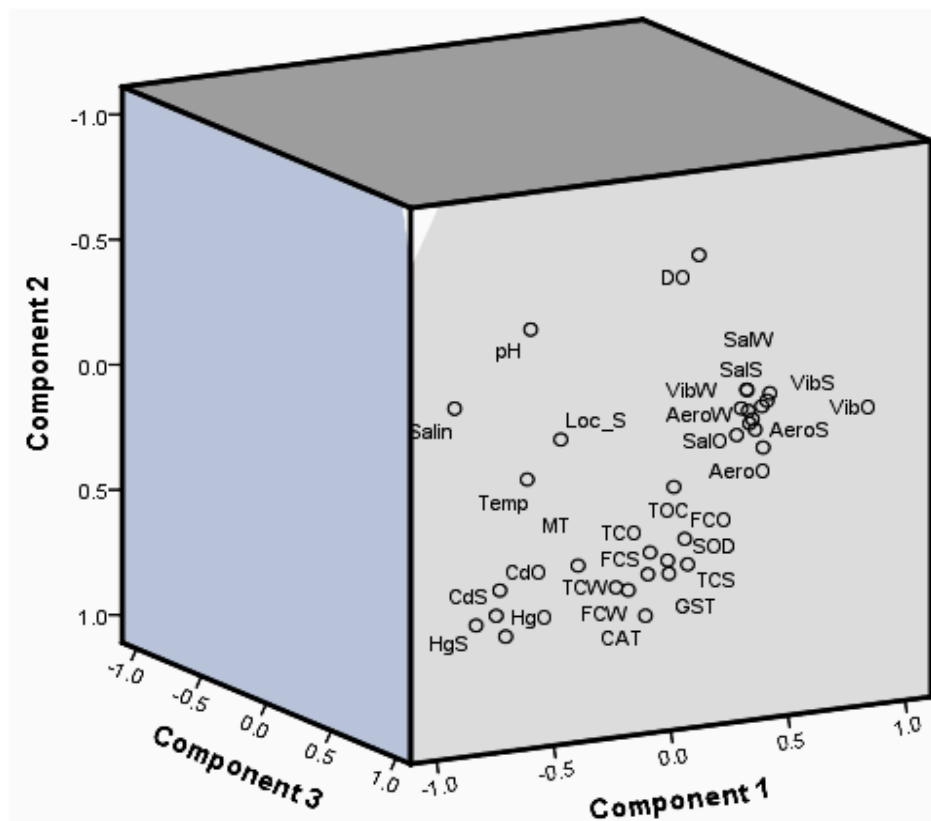
Figure 2



**Figure 3**



**Figure 4**



Component	Initial Eigenvalue	% of Variance	Cumulative % of Variance
1	17.74	55.45	55.45
2	6.61	20.61	76.1
3	4.4	13.74	89.85
4	1.62	5.05	94.9

**Figure 5**