

Seasonal variations and relationships between environmental parameters and heavy metal concentrations in tissues of *Crassostrea* species and in its ambience from the tropical estuaries

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Abstract

This study aimed to evaluate the relationship between physico-chemical parameters and heavy metal (Cu, Ni, Pb and Cd) concentrations from sediment, seawater and its accumulation in tissues of oyster species (*Crassostreamadrasensis* and *C.gryphoides*) from the three sites (Chicalim Bay: CB, Nerul Creek: NC, Chapora Bay: ChB) along the Goa coast (India). Results showed enrichment of Cu and Ni in sediment exceeding the Effect Range Low (ERL) level. The higher concentrations of Cu and Ni in sediments and in suspended particulate matter (SPM) from all the study sites are indicative of severe contamination of estuarine and associated habitats. Moreover, particulate Ni (at all the sites), Cu (at NC and ChB), Pb (at NC) and Cd (at CB and NC) concentrations were recorded more than its total loadings in surface sediment. Concentration of Cu and Cd in oyster tissue were several folds higher than its concentration in ambience. Further, this study showed that the levels of metal in oysters and their ambient environment were higher during the monsoon season. Hence, the consumption of oysters needs to be considered carefully with respect to the health hazards posed by the elevated levels of metal contaminants in certain seasons. Present study concludes that metals associated with the particulate matter in water column is the main source of metal accumulation in oyster. It is also suggested that concentration of metal pollutants in coastal and estuarine water bodies should be monitored regularly to ensure the acceptable limits of metal concentrations.

Keywords: Anthropogenic activities, Metal pollution, Oysters, Bioaccumulation, Estuaries

Introduction

Heavy metals (also referred as trace metals) are considered as highly toxic pollutants due to their toxicity, persistence and bioaccumulation properties (Pekey 2006). These metals contaminants when released into the sea significantly affect marine ecology, and in extreme cases, lead to the destruction of whole ecosystems including sediment and water quality and living biota. Coastal and estuarine habitats (between land and sea) serve as a sink for heavy metals and thus these regions remain constantly under pressure for contaminations.

Marine environmental components such as sediments act as a final reservoir for metals (with particulate forms, various chemical species, and different complexes) with their deep effect on bioavailability and toxicity to marine organisms (Nelson and Donkin 1985). Aqueous (particulate and dissolve) phase provides a mobile medium for metal chemical reactions and metal circulation through the sediment to organism and to aquatic environment (Violante et al. 2010). It is well known that oysters significantly accumulate heavy metals from seawater from three sources: inorganic metal ions, organometallic ion complexes and metal ions accumulated by phytoplanktons.

Sediment resuspension is one of the natural processes that entrains sinking particles back into the upper water column. Suspended particulate matter (SPM) acts as a carrier phase to transport the chemical elements from seawater to the bottom sediments and vice versa. Its production, sinking and decomposition control the recycling and distribution of elements in marine waters to a large extent. Therefore, establishing a correlation of metal levels in sediment, water column and uptake in biota is essential to understand the influence of environmental metal contaminants on the food chain in estuarine environment. Further, in order to differentiate and understand the mechanisms that control bioaccumulation in marine organisms, its uptake and pathway must be separately quantified and all of the geochemical, biological, and physico-chemical conditions that a population of animals may experience need to be considered (Wang and Fisher 1999).

Sedentary bivalves are directly and continuously exposed to marine contaminants. Oyster lives near the sediment-water interface and hence get influenced by heavy metals in sediment. Bivalves, especially the oysters, have been widely used to assess the metal pollution due to their ability to accumulate metals from contaminated marine environments (O'Connor 2001). Harvesting of oysters from contaminated areas may have high concentration of metals and serve a potential risk to health of its consumer (human). India has large resources of oysters with an

annual production of about 24,000 tonnes (http://www.fao.org/fishery/countrysector/naso_india/en). However, most of the oyster beds in the coastal waters of Goa are exposed to increasing pressures of different anthropogenic activities mainly related to urban development, ship building and mining activities in the estuarine regions.

Considering the demand and local consumption of oyster, there is an urgent need to evaluate the status of metal accumulation in commercially important oyster *Crassostrea* sp. that are harvested regularly from Goa coast for human consumption. In this study we aimed to (1) estimate bioaccumulation of essential metals: copper (Cu), nickel (Ni), and non-essential metals: lead (Pb), cadmium (Cd) in the tissues of two oyster species (*C. madrasensis* and *C. gryphoides*); (2) evaluate the current status of metal concentrations in surrounding matrices (surface sediment, SPM, and surface seawater) of the oysters; (3) understand relationship between the accumulated metal in oyster tissue and in total metal content in surrounding mediums and other environmental controlling factors.

Materials and methods

Sample collection

Three different sites [Chicalim Bay (CB), NerulCreek (NC), Chapora Bay (ChB)] that are exposed to different anthropogenic activities were selected for sample collection (Fig. 1). Since the oysters beds get exposed during low tides, samples were collected at ebb tide. Monthly sampling were done for 14 months (April 2013–May 2014) at CB and NC. However at ChB, which was considered as a relatively pristine area, sampling was done once in four months covering three seasons viz. monsoon (July 2013), post-monsoon (November 2013) and pre-monsoon (March 2014). Surface water, surface sediment and oyster samples were collected from the above-mentioned sites and brought to the laboratory in an ice box for further analysis.

Climatological Data collection

The monthly average data of rainfall for study period (April 2013–May 2014) were obtained from the Autonomous Weather Station (AWS) installed at the National Institute of Oceanography (NIO), Dona Paula, Goa.

Physico-chemical parameters analysis

Surface water temperature and pH were measured on site using calibrated thermometer and a portable pH meter, respectively. Salinity was determined by a refractometer (ATAGO, S/Milli-E). The samples for dissolved oxygen (DO) were fixed on site and later analyzed according to Winkler's procedure (Parsons et al. 1984). Estimation of chlorophyll *a* (Chl*a*) was done by following the JGOFS protocol (UNESCO 1994). Nutrients such as nitrate (NO₃⁻), nitrite (NO₂⁻) and phosphate (PO₄⁻), particulate organic carbon (POC) were analyzed by using Parsons et al. (1984) method. Total suspended solids (TSS) in water samples were estimated as described by Reddy et al. (1994). All the samples were analyzed in triplicates.

For sediment texture estimation, surface sediment samples were desalinated by washing repeatedly with deionized water and dried at 45 °C. Dried sediment samples (15 g) were first disaggregated using 10 % hexametaphosphate and then treated with 30 % H₂O₂ for removal of organic matter. The samples were put through a 63 µm mesh (USA standard testing sieve, ASTM) to separate the sand fraction from the bulk sediment and were oven dried at 60 °C and weighed to calculate sand fraction percentage. The remaining mud (silt+clay) fraction from the same was analyzed by laser size particle analyzer (Malvern Mastersizer 2000). For measurement of total organic carbon (TOC), sediment samples were decalcified by using 1N HCl, washed thoroughly with deionized water, and dried at 60 °C. The total carbon (TC) was analyzed without acid treatment. The concentration of TOC and TC was measured using an elemental analyzer (Flash EA 1112 series, Thermo Fisher Scientific). All the samples were analyzed in triplicates.

Total metal determination in surface sediment

Surface (upper 2 cm) sediment layer (~100 g) in triplicate were scraped using a stainless-steel spoon and collected in metal-free polyethylene bags to avoid metal contamination. Samples were collected from all the three sites for 14 months except Chapora Bay where sediments were collected once in a four months. Samples were dried in oven at 60 °C for 72 hours and pulverized using agate mortar and pestle. Next, 0.05 g sediment powder of each sample was taken in Teflon beaker and 10 ml of HF:HNO₃:HClO₄ (7:3:1 ratio) acid mixture was added and kept for overnight soaking for reaction. Next day, mixture was heated at 180 °C until dryness. Dryness step was repeated in 5 ml of above mentioned acid mixture. Digested samples were cooled at room temperature and then diluted to 10 ml with 2 % HNO₃. Quantification of metals

was done by using Thermo X Series II Inductively-Coupled Plasma Mass Spectrometer (ICP-MS).

Total metal determination in SPM

Surface seawater (~ 5 l) was collected in polyethylene jerry cans. Before sample collections, cans were washed with detergent, soaked in 5 % nitric acid (HNO₃) for overnight, and then washed with distilled water. Seawater samples were taken carefully to avoid the floating debris. Suspended particulate matter was collected by filtering the collected seawater through acid treated 0.4 µm polycarbonate membrane filter paper. The SPM retained on filter paper was dried at 60 °C and weighed. Next, 0.05 g of SPM was taken and analyzed as described in above mentioned section 'Total metal determination in surface sediment'.

Dissolved (< 0.4 µm) metal determination in surface seawater

Filtered surface seawater samples (400 ml) were collected in acid washed polyethylene bottles and pH was adjusted to 2.5 using HNO₃. The dissolved metal content in seawater was determined by following the method of Brewer et al. (1969). Briefly, the metals were preconcentrated from seawater using ammonium pyrrolidinedithiocarbamate (APDC) as a chelating agent and after extraction taken in methyl isobutyl ketone (MIBK) as solvent. The organic extract was further treated with ultra-pure concentrated nitric acid and metals converted to inorganic form. After that, inorganic form of study metals were aspirated in Graphite Furnace Atomic Absorption Spectrometer (GF-AAS).

Total metal determination in oyster tissue

To avoid the size difference effect on analysis, only 6–7 cm length of oysters were collected. A total of 1240 oysters were collected in 14 months (20 oysters for each species and site except Chapora Bay, where sampling was done once in four month). In the laboratory, oysters were cleaned with sea water to remove adhering sediment. Subsequently, oysters were shucked and whole soft tissue was cleaned with deionized water to remove impurities and stored at -20 °C until further analysis. Tissues were dried at 60 °C till constant weight. Portion of dried tissue samples were grinded by using agate mortar and pestle. The 0.5 g of dried tissue powder was soaked overnight in 5 ml of 65 % HNO₃. Reaction mixture was heated at 80 °C till dryness and re-dissolved in 3 ml of 65 % HNO₃ for achieving the complete digestion of the tissue. The digest

was allowed to cool at room temperature. The digested clear solution was then filtered through Whatmann No. 42 filter paper and diluted to 10 ml with 2 % HNO₃ (Cheung and Wong, 1992). Metals (Cu, Ni, Pb, and Cd) determination in tissue of both the oyster species were made by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).

Utmost care was taken at every step of sample processing to avoid contamination. All reagents used in the analysis were suprapure grade. Appropriate blanks and standards were also prepared for each of the metal analysis. Marine sediment, MAG-1, was used as a certified reference material to check the data quality of sediment and SPM samples. Whereas, Fish protein certified reference material for trace metals (DORM-4) was used to ensure the quality of oyster tissue results. Appropriate dilutions of samples were made depending on the sensitivity of detection in these samples. Precision measured as percent relative standard deviation ($\% \text{ RSD} = \text{standard deviation} / \text{average} \times 100$) of triplicate values were <10 %. Accuracy of Cu, Ni, Pb and Cd analyses are expressed in recovery percentage [$\text{Recovery \%} = (\text{Measured value of standard} / \text{certified value of the standard}) \times 100$] (see supplementary material Table S1).

Statistical analysis

The basic statistics such as the average were calculated for all measured parameters using Microsoft Office Excel 2007. Pearson correlation (r values) test was performed on untransformed data to find out the degree of association among the metal (Cu, Ni, Pb and Cd) concentrations in oyster tissue, metal concentrations in their ambience [sediment, seawater (particulate and dissolve phase)] and the other measured physico-chemical (water temperature, pH, Salinity, DO, Chl_a, NO₃⁻, NO₂⁻, PO₄⁻, POC, TSS, sand, silt, clay, TOC and TC) parameters using STATISTICA 8 (StatSoft Inc., USA) software.

To study the seasonal variation, statistical analysis were carried out on four seasons (an average of 4 months were grouped in each season) such as Mon (monsoon: June–September 2013), PostM (post-monsoon: October 2013–January 2014), PreM2 (pre-monsoon 2: February–May 2014), except PreM1 (pre-monsoon 1: April–May 2013) at CB and NC. In case of ChB, statistical test was performed on three season representative months: monsoon (July 2013), post-monsoon (November 2013), and pre-monsoon (March 2014). All assumptions of normality and homogeneity of variance were tested using Kolmogorov–Smirnov test and Levene’s test respectively. However, when data failed to meet normality, permutational multivariate analysis

of variance (PERMANOVA) test was used on metal values in sediment, particulate and dissolved phase in seawater. PERMANOVA was performed on untransformed data to find out the significance level in spatio-temporal variations in the samples using PRIMER 6 (Primer-E Ltd., Plymouth, UK) software.

Redundancy Analysis (RDA) were performed to determine the relationship between metals concentration in oyster tissues and other measured variables. To reduce the overcrowding in ordination diagram, measured variables (other than metal concentrations in both oyster species) were divided into three different groups. First, water parameters (water temperature, pH, salinity, DO, Chl a , NO $_3^-$ +NO $_2^-$, PO $_4^-$, POC, TSS); second, sediment parameters (sand, silt, clay, TOC, TC) and sediment metals (Cu, Ni, Pb, Cd); and third, metal (Cu, Ni, Pb, Cd) content in particulate and dissolve phase. To determine the variability within each of these data sets, Detrended Correspondence Analysis (DCA) was performed to find out the length of gradient of the dataset. The longest gradient was found to be less than 4.0 indicating a linear distribution of metal accumulated oysters and justifying the use of a linear method RDA (Leps and Smilauer 2003). Monte Carlo permutation test was performed to determine whether the relation between biological and environmental variables was significant. For this multivariate analysis, CANOCO 4.5 for windows (terBraak and Smilauer 2002) software was used. For all the statistical analyses, significant level at $p < 0.05$ were considered.

Results

Environmental parameters

During the study period, high average rainfall was recorded in monsoonal months (June-October) which tapered off in post-monsoon. (monthly variation presented in supplementary material Fig. S1). Results of monthly variations in physico-chemical properties of surface seawater, and surface sediment were determined at the three sampling sites are presented in supplementary material fig.S2 and fig.S3 (a and b) respectively. In general, DO concentrations showed inverse relation with temperature, pH and salinity. Nutrients (NO $_3^-$, NO $_2^-$ and PO $_4^-$), POC and TSS found to be high at CB followed by NC and ChB, and with respect to seasons, it was high in Monsoon followed by post-monsoon and pre-monsoon. In sediment texture analysis, sand fraction was dominated over the silt and clay fraction at all the studied sites during study period.

Metal concentrations in sediment, SPM, seawater (dissolved) and oyster tissue

The trend of metals accumulation in the tissue of *C. madrasensis* and *C. gryphoides* was in the following orders: Cu > Cd > Ni > Pb. The observed metals concentration in the sediment (Cu > Ni > Pb > Cd) and in the SPM (Ni > Cu > Pb > Cd) was different from that of concentration in tissues at CB and NC. However, a reverse order of metal concentration was observed in sediment and SPM at ChB site. In the dissolve phase, metals were in the following ranking: Cu > Pb > Ni > Cd at CB, Cu > Ni > Pb > Cd at NC and Cu > Ni > Cd > Pb at ChB.

Metal concentrations in surface sediment

Concentrations of Cu varied from 55.3 to 1182 mg/kg at CB sediment and ~31 to 76.2 mg/kg at NC and ChB sediment. It is important to point out here that the average value of Cu content (211.8 mg/kg) in CB sediment was much higher than sediment from NC (47.9 mg/kg) and ChB (54.5 mg/kg). Moreover, Cu enrichment in sediment were observed in monsoon period at all the three sites (Fig.2). Whereas Ni content range between 34.3 and 76.6 mg/kg, 25.4 and 67.4 mg/kg, 52.7 and 61.9 mg/kg at CB, NC and ChB, respectively. The seasonal average concentration of Ni in sediment reduced progressively from monsoon to pre-monsoon at CB and NC (Fig.3). Average Pb content (5.11 mg/kg) in CB sediment was higher (with no much seasonal variations) than at NC (1.23 mg/kg) and at ChB (2.77 mg/kg). On seasonal scale sediment of NC and ChB showed higher Pb content in monsoon and lower in non-monsoon (Fig.4). Small variation was observed in average concentrations of Cd in surface sediment at CB (0.39 mg/kg), NC (0.30 mg/kg) and ChB (0.37 mg/kg) with highest concentration in monsoonal months (Fig.5).

Metal concentrations in SPM

At NC and ChB, concentration of Cu was higher in SPM than sediment. Average Cu content was 105.1 mg/kg, 76.7 mg/kg and 146.5 mg/kg at CB, NC and ChB, respectively. Highest particulate Cu was observed at ChB (range: 100.4 – 197.4 mg/kg) followed by CB (range: 77.9 – 129.2 mg/kg) and NC (range: 62.2 – 94.7 mg/kg). At CB, seasonal average of particulate Cu content was equally high in all the seasons (except in pre-monsoon 1) whereas at NC and ChB, particulate Cu level was more in monsoon and less in pre-monsoon season (Fig. 2). The observed values of Ni in SPM at CB, NC and ChB were 108.7 mg/kg, 106.8 mg/kg and 116.7 mg/kg, respectively. At all the three sites, throughout the study period, on an average particulate Ni in SPM was found to be two fold higher than total Ni loading in sediment. The recorded values of

Ni were more in monsoon (particularly in July), which decreased progressively with onset of pre-monsoonal months (Fig. 3). Average concentrations of particulate Pb at CB (3.49 mg/kg), NC (2.66 mg/kg) and ChB (2.08 mg/kg) did not show much variations. Overall, particulate Pb in surface seawaters was comparatively high at CB and ChB in monsoon and at NC in pre-monsoon. At NC, it was intriguing to find out that concentrations of Pb in SPM were much higher than its total loading in surface sediment (Fig. 4). NC waters showed a higher level of particulate Cd (avg. 0.47 mg/kg) followed by CB (avg. 0.41 mg/kg) and ChB (avg. 0.28 mg/kg) waters. Seasonally, during monsoon maximum particulate Cd level was noticed at both NC (0.69 mg/kg; July) and at CB (0.57 mg/kg; August). Minimum values were recorded in non-monsoonal period; 0.29 mg/kg (April 2013) and 0.32 mg/kg (May 2013 and February 2014) at NC and CB, respectively. Unlike ChB, values of particulate Cd was high in CB and NC waters than total Cd content in sediment (Fig. 5).

Metal concentrations in seawater (dissolved)

Monthly observations of dissolved Cu varied between 1.31 and 4.30 µg/l at CB, 0.85 and 4.59 µg/l at NC, 1.44 and 2.37 µg/l at ChB. Overall, dissolved Cu level at CB was high in monsoon and at NC and ChB it was high in non-monsoon season (Fig. 2). Average concentration of dissolved Ni content in CB, NC and ChB waters was 0.55 µg/l, 0.58 µg/l and 0.53 µg/l, respectively. Seasonal average of dissolved Ni exhibited more concentration in monsoon and less in non-monsoon at CB and ChB waters whereas opposite trend was observed at NC (Fig. 3). Dissolved Pb content ranged from 0.03 to 3.01 µg/l (avg. 0.57 µg/l) at CB, 0.04 to 2.05 µg/l (avg. 0.41 µg/l) at NC and 0.04 and 0.73 µg/l (avg. 0.28 µg/l) at ChB. On the whole, at all sites, dissolved Pb content was high in monsoon and low in non-monsoon (Fig. 4). The annual average of dissolved Cd found in ChB waters (0.24 µg/l) was higher than at CB (0.21 µg/l) and NC (0.18 µg/l). Overall, dissolved Cd in surface seawaters from all three sites were higher in non-monsoon and lower in monsoon period (Fig. 5).

Metal concentrations in oyster tissue

Concentrations of Cu in tissues of both the oysters (*C. madrasensis* and *C. gryphoides*) at all the sites were in similar ranges. In particular, average Cu content in *C. madrasensis* at CB, NC and ChB was 408.4 mg/kg, 398.1 mg/kg and 329.9 mg/kg, respectively. In case of *C. gryphoides* tissues, it was 316.7 mg/kg, 284.6 mg/kg and 526.5 mg/kg, respectively. Seasonal average

showed comparatively more Cu content in oyster tissues collected from CB and ChB during monsoon whereas at NC, concentration of Cu was higher during pre-monsoon 2(Fig.2).The average value of Ni accumulation in *C.madrasensis* tissue was found to be 0.88 mg/kg, 0.85 mg/kg and 3.11 mg/kg at CB, NC and ChB, respectively. Whereas, average Ni concentration in the tissues of *C. gryphoides* was 0.82 mg/kg, 0.94 mg/kg and 1.31 mg/kg at CB, NC and ChB, respectively. Overall, Ni accumulation in oyster tissue was high in pre-monsoon (Fig.3).Comparatively similar bioaccumulation of Pb in *C.madrasensis* and *C.gryphoides* tissues was observed at all studied sites. In tissues of *C.madrasensis*, Pb ranged from 0.10 to 0.81 mg/kg at CB, 0.15 to 1.10 mg/kg at NC and 0.24 to 0.84 mg/kg at ChB. In the case of *C. gryphoides*tissues, it varied from 0.01 mg/kg to 0.94 mg/kg at CB, 0.03 to 1.15 mg/kg at NC and 0.29 to 0.31 mg/kg at ChB. Seasonal variability in Pb accumulation in oyster tissues at all the three sites showed its high level in non-monsoon and less in monsoon period (Fig.4).The average value of Cd accumulation in *C.madrasensis* tissues was higher (43.6 mg/kg) at ChB than other two sites CB (39.7 mg/kg), NC (18.14 mg/kg). Similar results were obtained for *C. gryphoides*; average values at ChB (53.3 mg/kg) and CB (17.8 mg/kg), were higher than at NC (14.9 mg/kg). Overall, accumulation of Cd in oyster tissue at all the three sites noticed to be high in monsoon and low in pre-monsoon (Fig.5).

Statistical analysis

3.3.1. Correlation between the metals concentration in oysters and the metals concentration in sediment, SPM, seawater (dissolved) with other physico-chemical variables

Pearson correlation test was used as a first approach to explore the possible relationships between the environmental parameters and metal concentration in sediment, particulate and dissolved metal in surface seawater, and in oyster tissue at three study sites. At CB, significant ($p<0.05$) positive correlation was recorded between sediment Ni and silt ($r = 0.66$), clay ($r = 0.63$) and TOC ($r = 0.71$) (see supplementary material Table S2a). Moreover, sediment Cu and Cd showed significant correlation coefficient with each other ($r = 0.98$, $p<0.001$) and with POC ($r = 0.84$ and 0.81 , respectively $p<0.01$) in CB waters. Concentration of Cu and Cd in sediment showed significant ($p<0.05$) positive relationship with each other ($r = 0.98$), and with the Cd accumulation in both the oyster species ($r = 0.92$) (see supplementary material Table S2b). At NC, accumulation of Pb in tissues of *C. madrasensis* was positively correlated with Chla ($r = 0.89$ $p<0.05$), TSS ($r = 0.94$, $p<0.01$). Whereas, Pb content in *C. gryphoides* strongly associated

($p < 0.05$) with pH ($r = -0.86$), Chl a ($r = 0.88$) and nutrients ($\text{NO}_3^- + \text{NO}_2^-$) ($r = 0.82$) (see supplementary material Table S3a). Level of Ni, Pb and Cd in sediment showed significant positive association with TOC ($r = 0.92$, $r = 0.84$, $r = 0.82$, $p < 0.05$). Further, concentration of Pb and Cd in sediment was found positively ($p < 0.05$) correlated with their concentration in SPM (see supplementary material Table S3b). At ChB, Cd content in *C. madrasensis* had significant ($p < 0.05$) positive relationship with DO ($r = 1.00$) and negative relationship with TOC and TC ($r = -1.00$) during study period. While, Ni and Pb accumulation in *C. gryphoides* negatively correlated ($p < 0.05$) with silt ($r = -1.00$) and clay ($r = -1.00$) respectively (see supplementary material Table S4a). In addition, Cd content in *C. madrasensis* positively correlated with particulate Cu ($r = 1.00$, $p < 0.01$). While, Cu and Cd concentration was strongly correlated with each other ($r = 1.00$, $p < 0.05$) in *C. gryphoides* (see supplementary material Table S4b).

Analysis of variance

Results of PERMANOVA showed a significant variation ($p < 0.05$) in metals concentrations across all the seasons, sites, and substrates (sediment, seawater, SPM and tissue) as well as the interactions among these three main effects (see supplementary material Table S5). Since there was a significant interaction among these three factors, it has been further tested for two way PERMANOVA for sediment, SPM, water and three way PERMANOVA with two oyster species separately. A considerable significant ($p < 0.05$) site wise variations were noticed in sediment metal concentrations (see supplementary material Table S6). Metal concentrations in SPM varied spatially and temporally and with their main effect interactions (see supplementary material Table S6). Dissolved metal concentration showed only seasonal significant ($p < 0.05$) variation (see supplementary material Table S6) whereas, significant species-wise and season-wise variation was obtained in metal accumulation in both the oyster species (see supplementary material Table S6).

Multivariate analysis of metals concentration in oyster species, sediment, SPM, seawater (dissolved) with other physico-chemical variables

RDA was used to relate the pattern of metal bioaccumulation in oyster species with measured environmental variables in various seasons. The length of the arrows (environmental variables) and their orientation indicates their relative importance and approximate correlations to the axes. Based on Forward selection and Monte Carlo permutation test pH, salinity and particulate Ni

were identified as a statistically significant ($p < 0.05$) variables that best explained the variation in metal accumulation in oyster species (marginal and conditional effect) (see supplementary material Table S7). Eigen values and cumulative percentage variance of data species and of species-environment relationship for surface seawater variables, particulate and dissolve metal variables as well as sediment variables are given in supplementary material Table S8. Species scores and inter set correlation of environmental variables with axis given in Table S9. Since the large data points on single plot are not clearly evident, following three distinct RDA plots are drawn separately to make the data more understandable.

Water variables RDA plot (Fig. 6a) depicted negative correlation between pH and salinity, whereas DO and phosphate (moderately) correlate positively with the first axis. Axis 1 defined by positive score of Cu and Cd with *C. madrasensis* and Cu, Ni and Cd with *C. gryphoides*. Axis 2 depicts positive score of Cu on *C. madrasensis*. Sediment variables RDA plot (Fig. 6b) describes TOC and TC that are positively associated with axis 1 and sediment Ni was negatively associated with axis 2. Further, axis 1 distinct by negative score of Cd on *C. madrasensis*, Cu and Cd on *C. gryphoides*. Axis 2 characterized by positive score of Cu on *C. madrasensis*. RDA plot (Fig. 6c) shows strong and moderate correlation with particulate Cu and Ni, and particulate Pb on first axis while particulate Pb and Cd positively associated with Axis 2. Axis 1 defined by positive score of Cd on *C. madrasensis*, and Cu, Ni and Cd on *C. gryphoides*. Axis 2 is characterized by positive score of Cu on *C. madrasensis*.

Discussion

The natural environment is more complex than controlled systems, thus relationships between environmental variables and associated biota are difficult to pinpoint. Besides the natural processes, additional anthropogenic effluents coming from the industrial and mining activities contaminate the estuarine and coastal waters by enriching metal concentrations in sediments and water (Rath et al. 2009). Further, heavy metals are involved in an enormous range of chemical reactions which can also influence the biogeochemical cycle in the marine environment (Sen Gupta et al. 1978). In present study, it is noteworthy that the metal levels observed in all the measured components (surface sediment, SPM, surface seawater (dissolved) and oyster tissues) at ChB was similar in range when compared to CB and NC sites. This suggests that the reference

site is also impacted by anthropogenic activities probably due to the nearby associated major fish landing jetty and sewage disposal from land inhabitants along the bank of the river Chapora.

Metals in surface sediment

Surface sediment at all the three sites showed enrichment of Cu and Ni exceeding the Effect Range Low (ERL) value (34 and 20 mg/kg, respectively) as per Sediment Quality Guideline (SQG) (Burton 2002). In contrast, average values of Pb and Cd (in sediment were much lower than the ERL limits (46.7 and 1.2 mg/kg, respectively) (Burton 2002). Due to the absence of local and regional sediment quality guidelines for toxicity estimation of the measured heavy metal concentrations, ERL values were used for comparison. This comparison points out that the surface sediment of estuarine and coastal region of Goa is highly contaminated with Cu and Ni. The presence of ship and barge building industries, yards, workshops and other recreational anthropogenic activities and direct exposure to iron ores transportations at CB may be responsible for enrichment of metals in the surface sediment. Chipping, welding and antifouling painting works are being carried out at the ship-building unit throughout the year. Cu and Pb is being used in antifouling paints, whereas Ni is being used in welding galvanizing, and metal plating works. Furthermore, occurrence of high concentrations of metals is also most likely arising from antifouling paint residues coming from the regular use of fishing trawlers, tourist boats and sewage coming from land based activities at NC and ChB. The results of present study is consistent with earlier studies conducted in the estuarine waters of Goa (Alagarsamy 2006; Veerasingam et al. 2015; Chakraborty et al. 2015).

A significant ($p < 0.05$) site wise variations in sediment metal concentrations can be attributed to different residence time of water column over the sediments, textural difference, sediment metabolism, and characteristics of the water at these sites. It has been reported that physicochemical properties of overlying water column (Bruder et al. 2002), freshwater discharge (Forstner and Whittmann 1981), flow rates (Schoellhamer 1995) and geomorphology of the area (Viles and Spencer 1995) alter the metal distribution and speciation in estuarine sediment. The reason behind fluctuations of metal levels in CB and ChB sediment throughout the year could be due to the influence of geomorphology. The funnel shaped structure of the estuarine region (sampling site at near mouth) results in shifting of the surface sediment on regular basis under high velocity of wave, current and tidal flow. At NC, shallow and narrow basin structure results

in less tidal impact and weak tidal current. Thus, the limited recipient capacity could be responsible for the lower metal concentrations at NC.

There are various ways through which heavy metals are fixed to the sediments such as direct adsorption by inorganic particles of clays, co-precipitation with hydroxides and iron oxides, adsorption or complexation with organic matter associated with inorganic particles, and other natural mechanisms related to the physico-chemical characteristics of sediments (Bruder et al. 2002). A significant positive correlation between sediment Ni and silt, clay and TOC in CB sediments indicate that accumulation of Ni in sediments are dependent on presence of finer particles (silt and clay) which hold more organic carbon in the sediments. The positive mutual association of studied metals with TOC suggests that metal contaminants are contributed by same sources and are also associated with fine sediment particles and organic matter. Surface sediment showed higher metals concentrations in all the seasons at CB and only in monsoon period at NC and ChB. This variability can be explained by the presence of the variations in sediment texture of the sites. CB contains comparatively more clayey sediment, which has a strong capacity to hold the metals whereas NC and ChB has more of sandy sediment which has less affinity towards the metals. At NC, all the metals in sediment showed significant negative relation with salinity ($p < 0.01$) and positive relationship with $\text{NO}_3^- + \text{NO}_2^-$, PO_4^- and POC ($p < 0.05$). This explains that the study area is mainly influenced by heavy rainfall events during SW monsoon. The heavy rainfall lead to increase in terrestrial runoff and thus freshwater inputs as well as metal transfer from the terrestrial to estuarine sediments. Sediment Cu and Cd showed significant correlation coefficient with each other ($p < 0.0001$) and with POC ($p < 0.01$) in CB waters which signifies that the sediment Cu and Cd level increases with rise in POC content mainly during monsoon. This is attributed to the Cu and Cd coming along with particulate organic carbon with monsoonal land runoff to aquatic system which ultimately settles to the bottom. Aguilar et al. (2012) found that sediment characteristics and presence of organic matter have a more influence on the transport and availability of heavy metals in rainy season.

Particulate metals in surface seawater

Surface sediments often undergo resuspension into the water column due to both natural and anthropogenic processes like tides, currents, bioturbation, shipping, and dredging. SPM in the water column contains allochthonous sediment particles, organic material resuspended from the

surface sediment and autochthonous biological particles (e.g., phytoplankton) to which various metals bind (Peakall and Burger 2003). As suspended matter is well known for a metal scavenger, sediment resuspension significantly increases the risk of trace metal contaminant exposure to marine biota mainly to filter feeders (Lee et al. 2013).

Like sediment, particulate Cu and Ni also showed very high concentrations at all sites. The particulate Cu and Ni concentrations reported in present study was in agreement with the values observed by Kessarkar et al. (2013) from the same estuarine region. Earlier published results also showed the high stability of Cu and Ni with SPM enrichment, where metal source coming from industrial effluent discharges (Hamad et al. 2012; Xu et al. 2015). Furthermore, particulate metals concentrations were recorded to be high in monsoon and low in pre-monsoon. This is supported by the statistical Pearson correlation coefficient results. Metal concentrations associated with SPM at all the sites showed significant ($p < 0.05$) negative correlation with water temperature, pH, salinity and positive correlation with nutrients and POC. This attributed to the input of large amount of organic and nutrient rich terrigenous land runoff that bring metal bound suspended particles from adjoining areas into the marine water bodies during rainy period. Also, this may be associated with estuarine turbidity maximum (ETM) resulting from resuspension phenomenon lead to high SPM content in estuarine waters of Goa (Rao et al. 2011). In addition, metals associated with surface sediment get mobilized during oxidizing condition (at low pH during monsoon) and resuspension event which further bind with suspended particle available in water column. Earlier studies from present study region have also reported high concentration of Fe and Mn content (Alagarsamy 2006; Shynu et al. 2013). Therefore, presence of high Fe-Mn oxyhydroxides particles, which hold more metal content, could be the reason for the increase in the particulate metal concentrations in waters column (Chapman et al., 1998). Cadmium more likely remain in dissolve phase in overlying solution possibly due to the complexation with chlorides and other anions which can limit the sorption of Cd onto Fe oxides/hydroxides (Caetano et al. 2003; Xu et al. 2015).

Dissolved metals in surface seawater

Total dissolved metal concentrations varied spatio-temporally due to variation in their transport processes, current pattern, mixing patterns (Jiann et al. 2013). In the present study, dissolved metals concentration was found to be very low as compared to the metal concentrations recorded

in other components (i.e. surface sediment and SPM), however, it showed significant ($p < 0.01$) seasonal variations at all the sites. Dissolved metals in seawater tends to form complexes with inorganic and organic ligands which might be the reason for less concentration of dissolved metals in seawater (Balkis et al. 2010). Higher dissolved Cu, Ni and Pb concentrations during monsoon (except at NC where high level of Cu and Ni recorded in pre-monsoon) is due to low saline, low pH waters which increases the availability of divalent metals (bioavailable form) in seawaters. Dissolved Ni and Pb (at CB), and dissolved Pb (at NC and ChB) showed significant negative correlation with water temperature, pH, salinity and Chl a (in case of dissolve Ni) while positive correlation with nutrient loads, TSS and POC (at NC). These relationship explains the association of positively charged free ionic metals with negatively charged TSS/SPM (including clay, carbonate, oxide hydroxide of the Fe and Mn) and/or POC and its input indicates its terrigenous origin in monsoon (Dessai and Nayak 2009; Shynu et al. 2015). However, in pre-monsoon rising pH increases the possible co-adsorption or co-precipitation of dissolve metals with particulate form of Fe-Mn or with other metal binding substrate like organic matter in water column (Tait and Dipper 1998). At NC, narrow geographic structure may contribute in high availability of dissolved metals due to less mixing and dilution of dissolved metals in pre-monsoon and thus comparatively high dissolved metal content. Moreover, dissolved Cu content was higher as compare to other dissolved metals. High concentration of Cu in dissolved form may be due to either its removal from suspended matter by desorption in water column or its regeneration because of early diagenesis at the sediment and water interface (Bruland 1983).

Metals in oyster tissue

Metal concentrations in tissues of aquatic invertebrates living in the same habitat shows species specific response due to differences for metal uptake and accumulation (Rainbow 2002 and references therein). In the present study, metal accumulation in oyster tissues showed significant species-wise and season-wise variation. Overall, *C. madrasensis* accumulated more metal contaminants than *C. gryphoides* though both species were exposed to the same water conditions (temperature, salinity, pH and type of food). This may be attributed to seasonal variations in oyster physiology and metabolism (Frazier 1975). These results reveal that species of same genus and from the same site can accumulate significantly different concentrations of the same element. Similar results were observed between two oyster species *C. corteziensis* and *C. palmula* from the same lagoon in Gulf of California (Paez-Osuna and Osuna-Martinez 2015).

Oysters are normally considered as a strong net accumulator for Cu (Rainbow et al. 1990) which is used in the synthesis of haemocyanin, a blood pigment (Yap et al. 2010). In present study, oyster tissues showed enrichment of Cu which is higher than the recommended limit (32 mg/kg) set by FAO guideline (1983). Further, average seasonal values indicated maximal Cu accumulation in monsoon period in both the species at CB and ChB, and at NC in non-monsoon. The Cu enriched sediment and SPM at the study sites in monsoon could be one of the main source for Cu enrichment in tissues of oyster. It is well known that the fine suspended particles of bottom sediment and ingestion of these resuspended particles from overlying water column forms important food source of the oyster. Since high concentrations of Cu (2100–4400 mg/kg dry wt.) cause oyster's mortality (Hung and Han 1990), the excessive concentration of Cu noticed in current study indicates a higher risk to oysters along the Goa coast. These values consistent with the Cu concentrations reported from other oyster species (De Mora et al. 2004; Fuad et al. 2013; Birch et al. 2014; Bray et al. 2015). Moreover, consumption of oyster tissue as seafood with such a high concentration of Cu can lead to stunted growth, cirrhosis of the liver and jaundice in humans (Gorman 1993).

Although the sediment and particulate matter from the ambience of oyster beds are highly polluted with Ni, the concentrations of Ni detected were well below the acceptable limit (70–80 mg/kg) (USFDA 1993). This suggests that oyster has a good inequity against Ni. In addition, this explains that the Ni associated with sediment and SPM are probably not toxic to the studied species. Though the Ni concentration is far below the permissible limit, the measured levels of Ni were comparatively high in non-monsoon. This higher accumulation of metals might be attributed to the increase in surrounding surface seawater temperature during pre-monsoon season. The rise in surrounding temperature increases the metabolic activities, resulting in higher filtration rate, larger collection of suspended matter and higher uptake of heavy metals (Belivermis et al. 2016).

Likewise, the average concentration of Pb was below the recommended limit of 1.0 mg/kg(EU 2001), but few samples exhibited values >1.0 mg/kg. This indicates that oyster from study sites are contaminated by Pb to some extent during non-monsoon period. This Pb accumulation may be due to the increase in traffic load of motor vehicles, boating activities, ships, combustion of fossil fuel, and organic waste discharge. In consistence with our study, higher concentration of Pb in the soft tissues of oysters have been reported (Turkmen et al. 2005; Alfonso et al. 2013;

Vazquez-Boucard et al. 2014). Pb mainly affects the central nervous system, renal, haemopoietic, cardiovascular system of the human being (Flora et al. 2012).

Tissue of both the oyster species at all the three sites showed elevated levels of Cd concentration with exceeding acceptable limit of 0.5 mg/kg set by FAO guideline (1983). Despite very low concentrations in ambience (sediment and particulate as well as dissolve form of Cd), high accumulation of Cd in oyster tissues at studied sites during monsoon were noticed. Chakraborty et al. (2016) found high bioaccumulation of Cd in *Crassostrea* sp. even at low Cd loading in bottom sediment from the same study region. This enrichment of Cd in oyster tissues may be attributed to three different reasons. First, although heavy rainfall during monsoon period dilutes the metal concentrations in the surrounding waters, decrease in salinity (i.e. less chloride concentration) during monsoon increases the bioavailable form (free ion) of Cd to filter feeders (Rainbow 1995) and thus higher metal uptake by oysters. Second, Cd (II) mimics calcium ion Ca (II), via Ca^{2+} channels, (which is also abundant in seawater) due to its similar geochemical properties, particularly ionic radius (cf. Ca 9.7 and Cd 9.8 nm) (Huanxin et al. 2000). Third, Cd has strong affinity towards the sulfhydryl groups of protein, the metallothionein (MT) and sequestered metal in detoxified form like Cu in intracellular granules (Rainbow 2002) and less excretion, resulting in high concentration in tissue. Results of present study is further corroborated by previous studies where authors have found high concentrations of Cd in clam *Paphiamalabarica* (1.4–8.4 mg/kg) along the Goa coast (Krishna Kumari et al. 2006) and in oyster *Saccostrea cucullata* (10–40 mg/kg) along Deltaic Sunderbans (Sarkar et al. 1994). Biomonitoring studies conducted on other oyster species elsewhere also observed the high concentration of Cd in their tissue (De Mora et al. 2004; Apeti et al. 2009; Heidari et al. 2013). Cheng and Gobas (2007) have been reported that the Cd intakes of 0.43–0.71 $\mu\text{g}/\text{kg}/\text{day}$ cause a toxic effect on consumers mainly to high risk groups, including women with low iron stores, people with renal impairment, smokers and children.

Relationship between the metals concentration in oyster species, surface sediment, SPM, surface seawater and other physico-chemical variables

The bioaccumulation of heavy metals in aquatic organisms depend on their chemical properties, bioavailability, and physico-chemical factors such as pH, salinity, DO, POC, nutrients, and SPM levels (Depledge and Rainbow 1990). The negative association of metal accumulation in oyster

with pH, salinity and positive correlation with DO and nutrients demonstrates that hydrodynamics has a tendency to influence the oyster's metal uptake and its accumulation over a period of time. It is noted that pH as a major factor, and salinity as a second gradient contributing to the metal enrichment in oyster. The low pH and salinity during monsoon season generally elevates the dissolved form of metals. The low pH and salinity observed during monsoon could be the reason for higher accumulation of Cu, Cd and to some extent Ni in tissues at CB and ChB. This observation is in agreement with the results obtained by Yu et al. (2013). Moreover, since, west coast of India is characterized by upwelling event (Madhupratap et al. 2001), influence of upwelling may be another reason for Cd (than other metal) availability in oysters as suggested by Paez-Osuna and Osuna-Martinez (2015). However, samples collected in pre- and post-monsoon showed more impact of high water temperature, pH, salinity and Chl a for Ni and Pb accumulation in *C. madrasensis*. This indicates that, as mentioned above, in pre-monsoon season due to high atmospheric temperature metabolic rate of oyster increases which accelerates the metal uptake in their body (Lannig et al. 2006).

Further, the RDA diagram on sediment variables representing TOC, TC, silt and clay showed negative influence mostly on monsoonal samples. However, positive impact on most of the non-monsoonal samples such as CB PreM1, PreM2, NC PreM1 and PostM were observed. This suggests that in monsoon, metals associated with organic matter (TOC and TC) and fine particles (silt and clay) in sediment release from sediment in overlying water column and become easily bioavailable to oyster (Shynu et al. 2013; Lee et al. 2013). While in non-monsoonal period, bound metals get settled on surface of sediment when river flow velocity relaxes (Dassenakis et al. 1995) and thus become less available to oysters.

RDA ordination diagram indicates that the particulate metals have more influence on the metal accumulation in oysters than metals present in dissolved form. This indicates that the bioavailable form of Cu and Cd may be associated with suspended particles at studied sites and forms a primary source of metals in the tissues of oysters. This may be attributed to the sediment texture which is made up of consolidated firm rocks with loose boulders and rocks on which sand (>79 %) are deposited. Due to high sand deposits, less metal binding ligands (e.g. silt and clay) remain available. This implies that organic matter containing more metal binding ligands is left in suspension phase in water column during natural events such as tides and waves (Eggleton

and Thomas 2004). Consequently, most metal toxicants could easily interchange and disperse through aquatic ecosystems which is finally taken up by the filter feeders such as oyster.

Conclusion

This study concludes that estuarine (and coastal) regions along the Goa coast are highly contaminated with Cu and Ni. The concentration of Cu and Cd and to some extent Pb in oyster tissue were found above the permissible limit (recommended by international authorities for safe consumption by humans) during the study period. Therefore, consumption of oysters from the study region should be restricted to avoid health hazard. Present study also concludes that metals associated with the particulate matter in water column is the main source of metal accumulation in oyster. To reduce the metal contamination at studied sites, the identification of metal source(s) is very essential. Additionally, continuous monitoring, people awareness and a stringent government policy is required to control the metal pollution along the coastal waters of Goa.

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Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

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Figures Caption

Fig. 1 Map showing sampling sites (CB = Chicalim Bay in Zuari estuary, NC = Nerul Creek in Mandovi estuary, ChB = Chapora Bay in Chapora estuary) along the Goa coast (● : monthly sampling site and ★ : seasonal sampling site)

Fig. 2 Concentration of Cu in surface sediment, SPM (suspended particulate matter), oyster (*Crassostreamadrasensis* and *Crassostreagryphoides*) tissue (mg/kg) and surface seawater (dissolved) ($\mu\text{g/l}$) measured at three study sites during April 2013–May 2014. Values are presented as an average ($n = 3$)

Fig. 3 Concentration of Ni in surface sediment, SPM (suspended particulate matter), oyster (*Crassostreamadrasensis* and *Crassostreagryphoides*) tissue, (mg/kg) and surface seawater (dissolved) ($\mu\text{g/l}$) measured at three study sites during April 2013–May 2014. Values are presented as an average ($n = 3$)

Fig. 4 Concentration of Pb in surface sediment, SPM (suspended particulate matter), oyster (*Crassostreamadrasensis* and *Crassostreagryphoides*) tissue, (mg/kg) and surface seawater (dissolved) ($\mu\text{g/l}$) measured at three study sites during April 2013–May 2014. Values are presented as an average ($n = 3$)

Fig. 5 Concentration of Cd in surface sediment, SPM (suspended particulate matter) oyster (*Crassostreamadrasensis* and *Crassostreagryphoides*) tissue, (mg/kg) and surface seawater (dissolved) ($\mu\text{g/l}$) measured at three study sites during April 2013–May 2014. Values are presented as an average ($n = 3$)

Fig. 6 Redundancy analysis ordination diagram with metals concentration in tissue of oyster species (a) in relation to water variables; (b) in relation to sedimentary variables including metals concentration in surface sediment; (c) in relation to dissolve and particulate metals concentration in surface seawater measured during four different seasons at study sites. Abbreviations: Cm = *Crassostreamadrasensis*, Cg = *Crassostreagryphoides*, DO = dissolve oxygen (mg/l), Chla = chlorophyll *a* ($\mu\text{g/l}$), NO_3^- = nitrate ($\mu\text{mol/l}$), NO_2^- = nitrite ($\mu\text{mol/l}$), PO_4^- = phosphate ($\mu\text{mol/l}$), POC = particulate organic carbon ($\mu\text{g C/l}$), TSS = total suspended solids (mg/l), sed = sediment, TC = total carbon (%), TOC = total organic carbon (%), sand, silt and clay (%), SPM = suspended particulate matter, dis = dissolve, CB = Chicalim Bay, NC = Nerul Creek, ChB = Chapora Bay, PreM1 = pre-monsoon 1, Mon = monsoon, PostM = post-monsoon, PreM2 = pre-monsoon 2. Metals concentration in sediment, SPM and oyster (*C.madrasensis* and *C.gryphoides*) tissue expressed in mg/kg, whereas in seawater (dissolved) expressed in $\mu\text{g/l}$. Numbers represents the samples such as 1: CB PreM1, 2: CB Mon, 3: CB PostM, 4: CB PreM2, 5: NC PreM1, 6: NC Mon, 7: NC PostM, 8: NC PreM2, 9: ChB Mon, 10: ChB PostM, 11: ChB PreM, yellow circle: pre-monsoonal samples, red circle: monsoonal samples, blue circle: post-monsoonal samples. Solid arrows represents the environmental variable, length of the arrows represents the relative importance of that variable, and triangle represents the metal concentration in oyster species

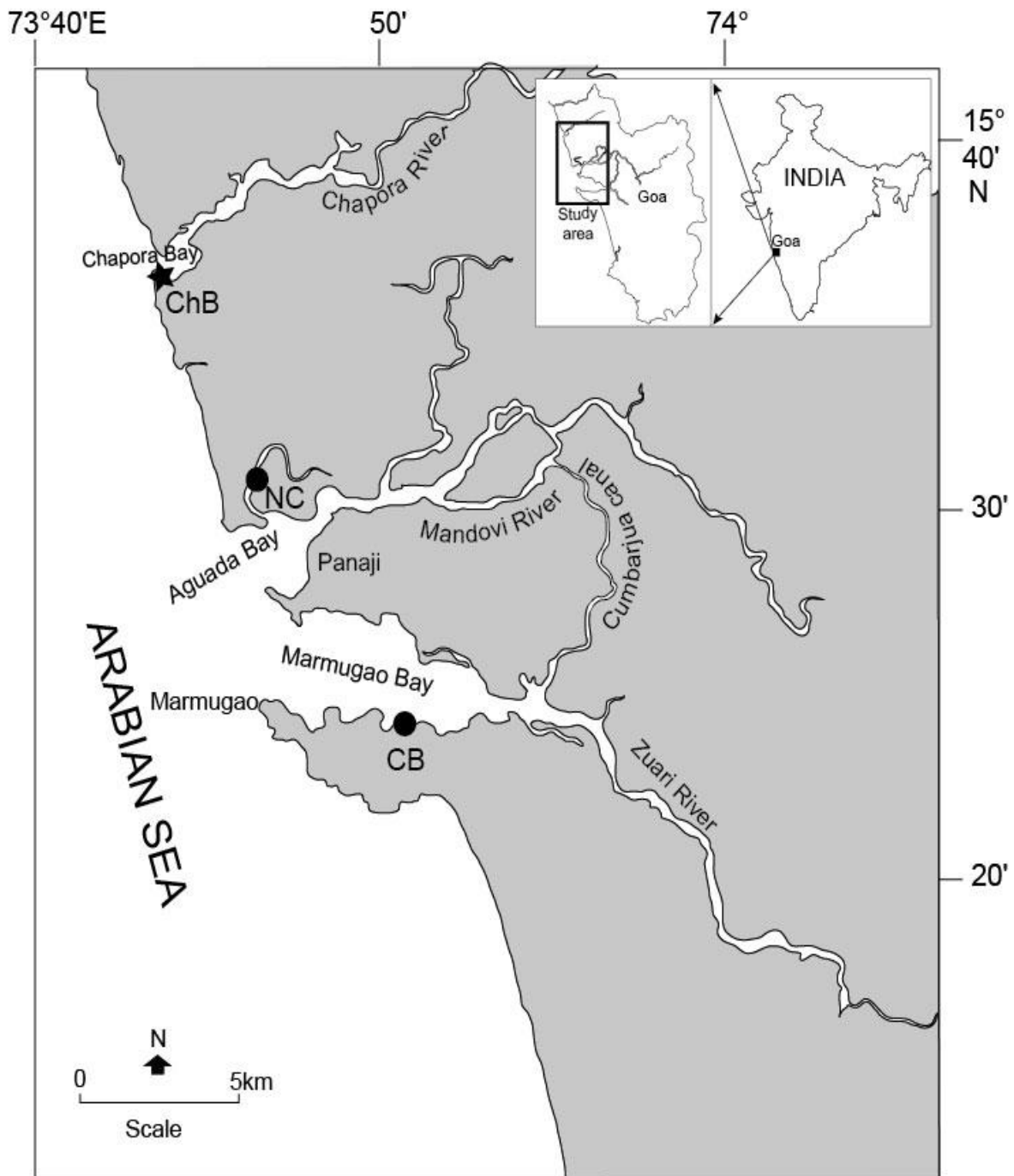


Fig. 1

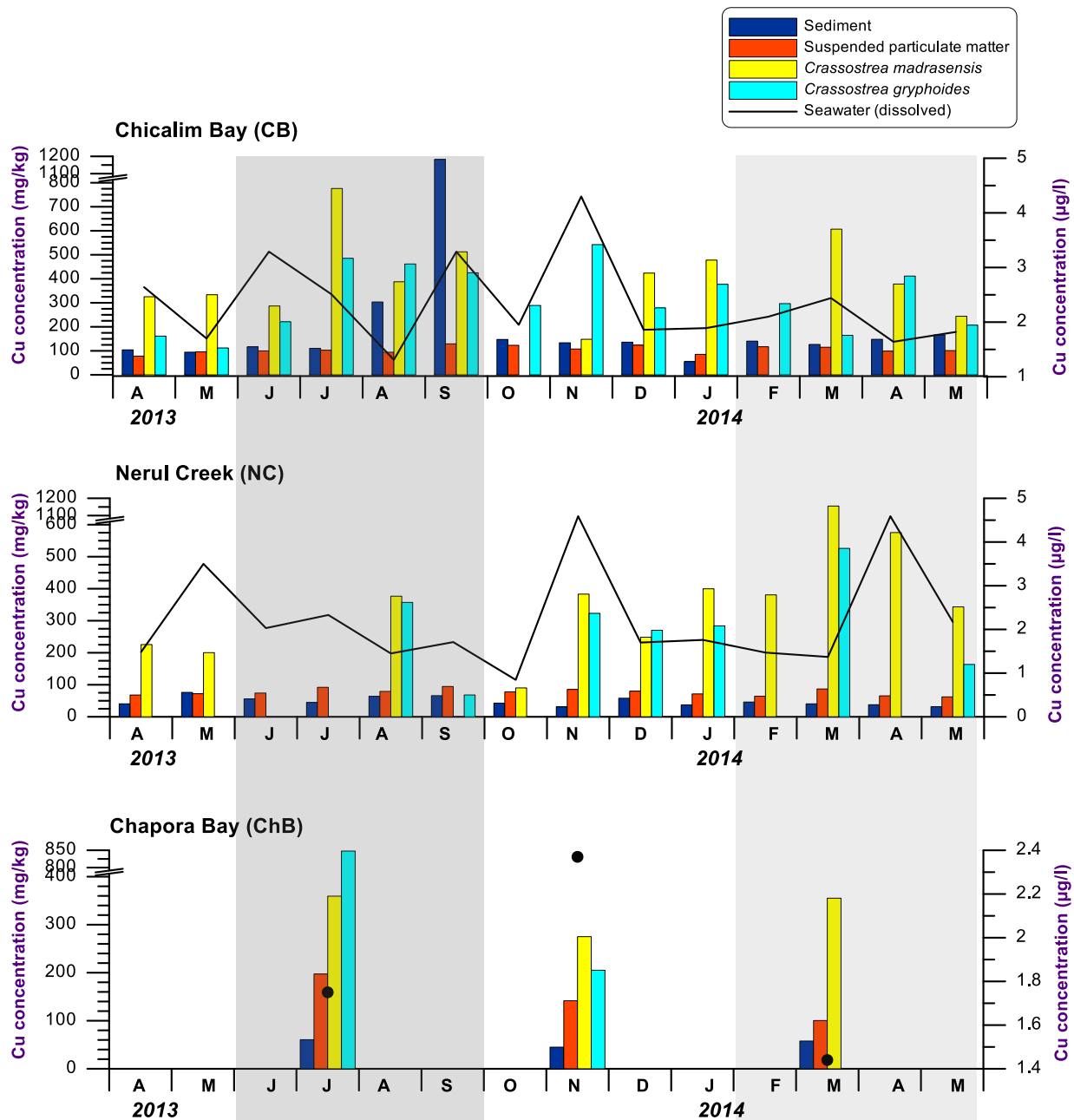


Fig. 2

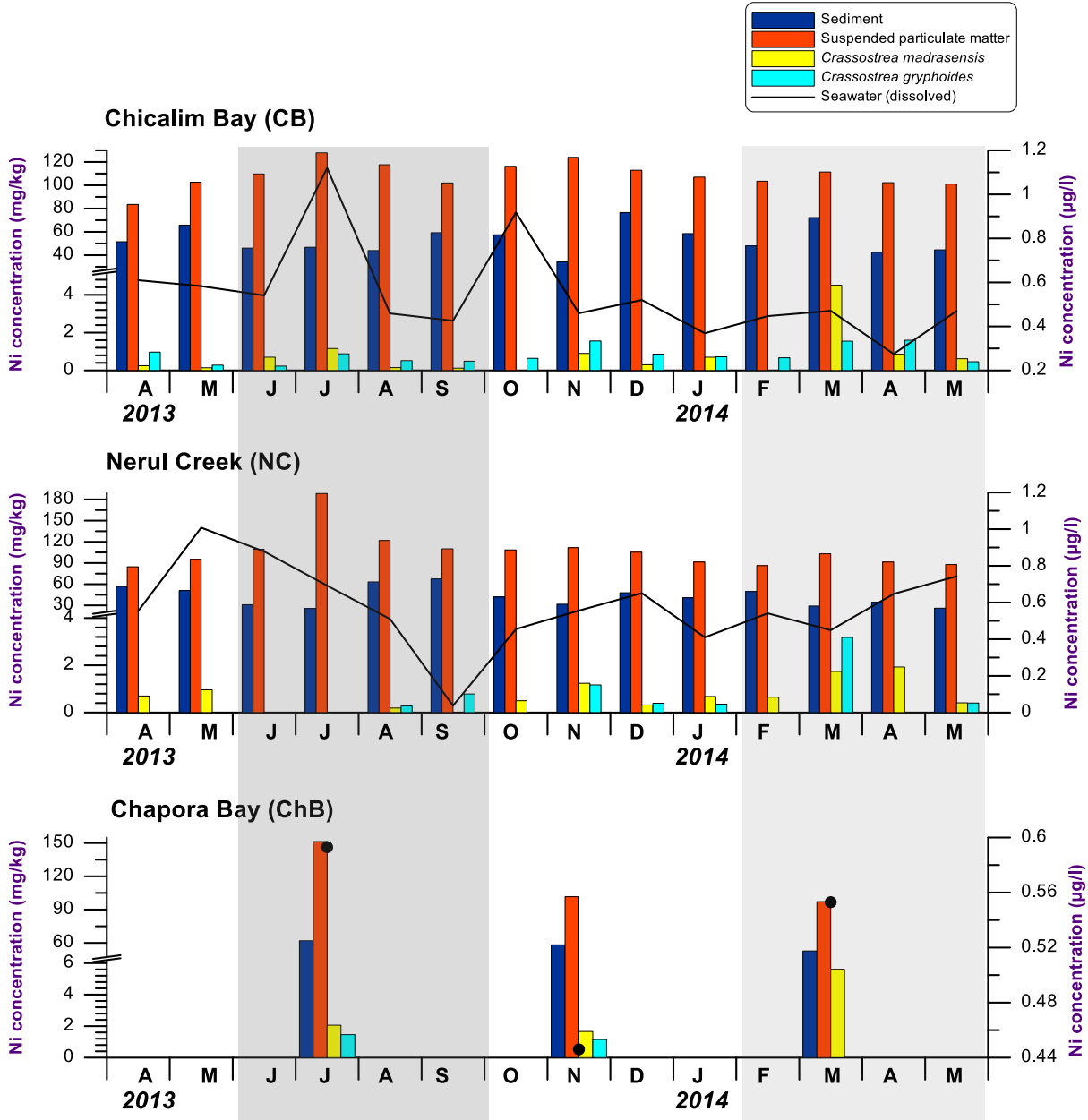


Fig. 3

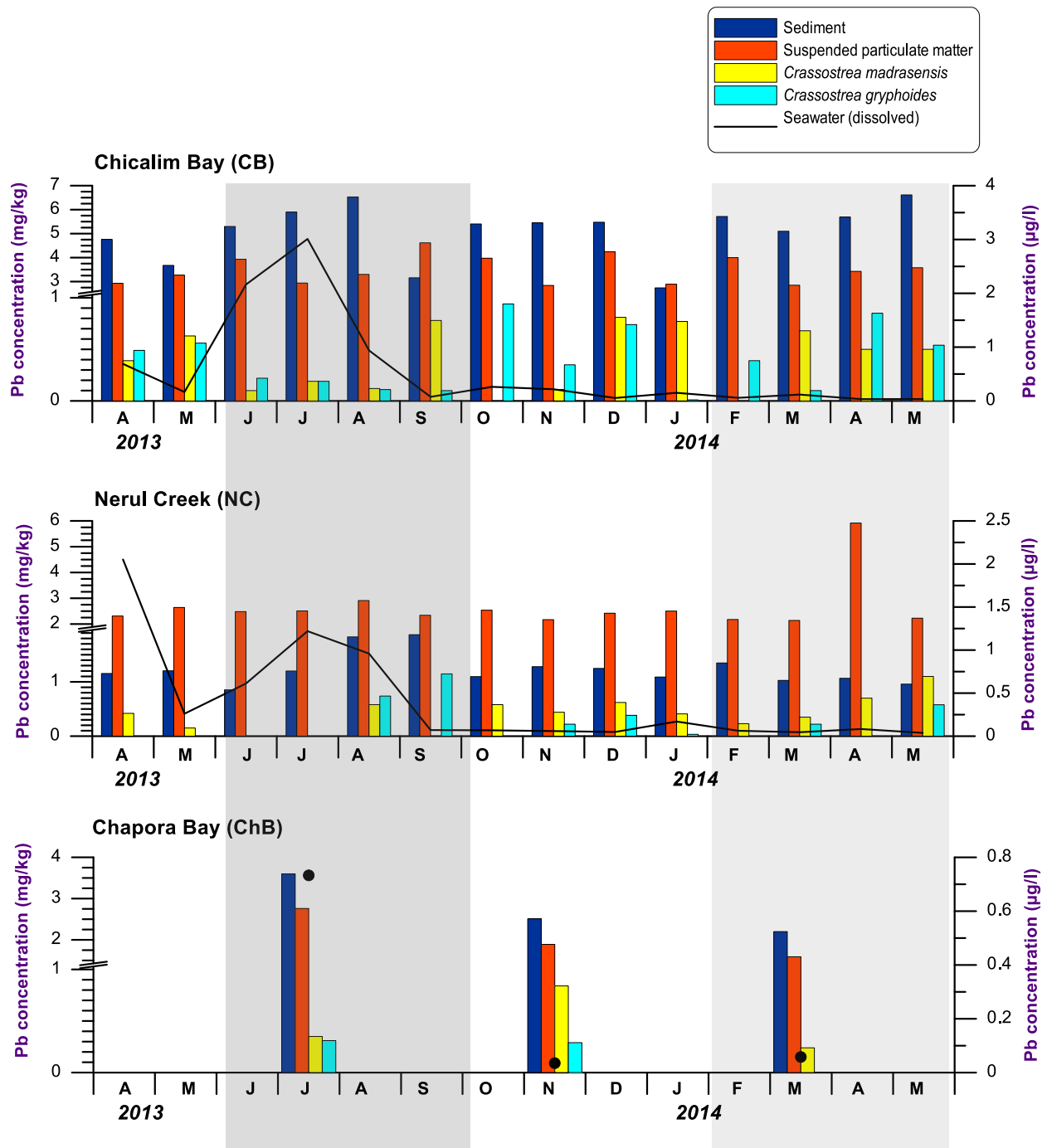


Fig. 4

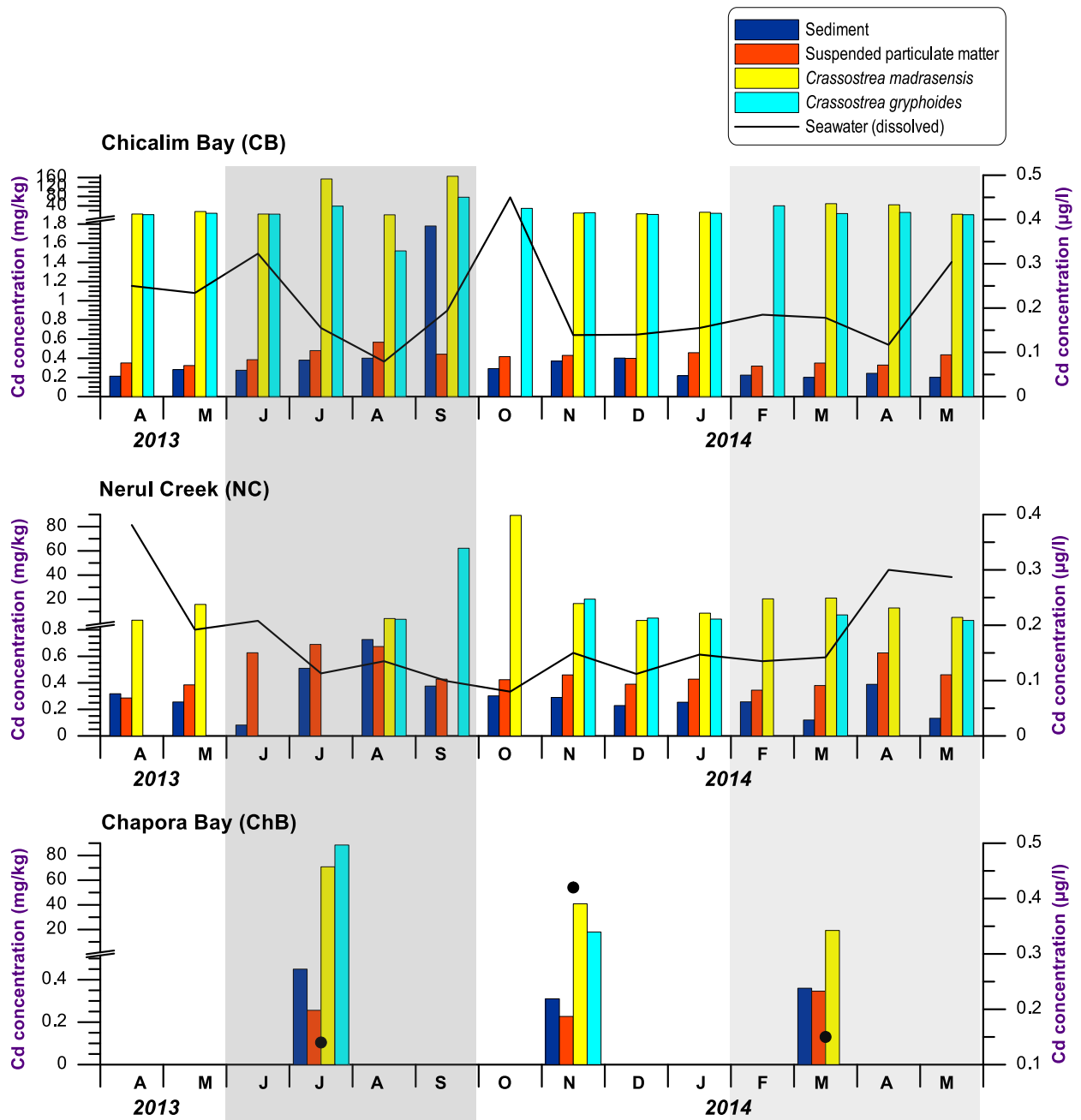


Fig. 5

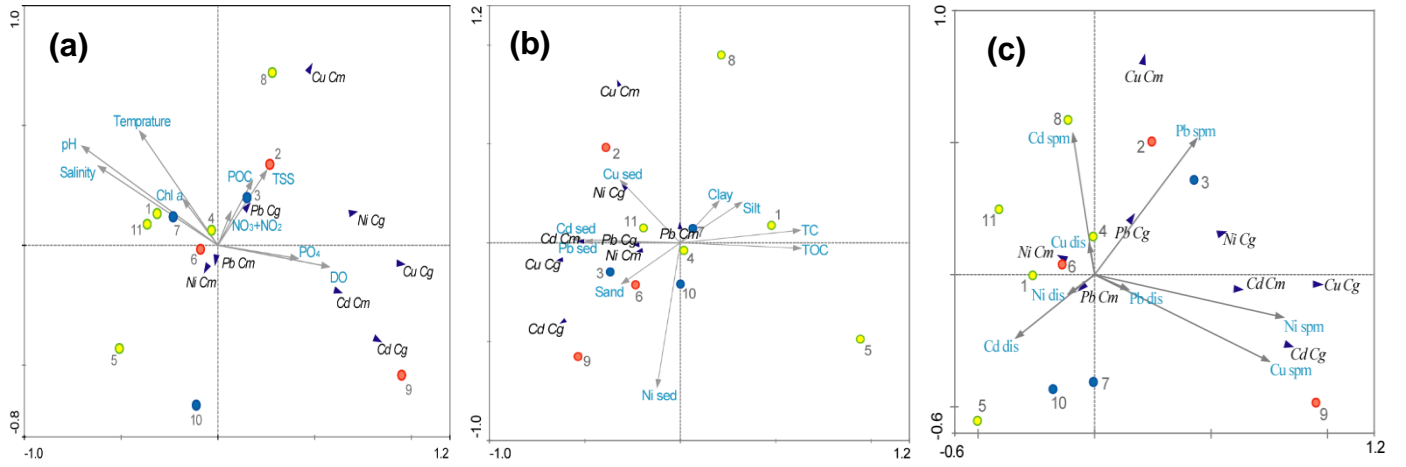


Fig.6