

Is the benthic index AMBI impervious to seasonality and data transformations while evaluating the ecological status of an anthropized monsoonal estuary?

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Abstract

Benthic indices are a potential measure of a system's ecological quality status. It is vital for an efficient index to distinguish the anthropogenic effects from the natural stress associated with seasonality, especially in complex ecosystems such as the tropical monsoonal estuaries. AZTI's Marine Biotic Index (AMBI), one of the most successful indices, relies on the distribution of soft-bottom macrobenthic communities into five different ecological groups (EGs) according to their sensitivity to stress. The AMBI was employed in the anthropized Ulhas estuary (India) to test its vulnerability to seasonality. Also it was endeavored in this study to test whether data transformations enhanced the efficiency of AMBI. AMBI values obtained during monsoon failed to distinguish between differently impacted stations in the major stretch of the estuary due to the uniformly dominant occurrence of *Namalycastis ouanaryensis*, an EG IV species with affinity for low salinity. The AMBI successfully evaluated the status of the estuary during the non-monsoonal seasons whereas it failed to perform well during the monsoon as it either underestimated or overestimated the ecological quality status of >55% of the stations. The results obtained by averaging the AMBI scores of the non-monsoonal seasons gave an accurate ecological status of the estuary. A good level of agreement between all the applied transformations implied that the data transformations did not improve the AMBI efficiency. Conversely, AMBI based on untransformed data indicated a stronger correlation with the contamination proxies than those derived from various data transformations.

Keywords: Multivariate analyses; Macrobenthos; Tropical estuary; Ecological group; India

1. Introduction

The assessment of ecological quality status of coastal and estuarine waters was highlighted by the European Water Framework Directive (WFD), which came into effect in December 2000 (European Union, 2000). Macrobenthic communities have been used as a biological indicator for the ecosystem evaluation owing to their predictive response to the major environmental changes (Pitacco et al., 2018). Of late, the use and development of several benthic indices that attempt to evaluate the status of ecosystem within WFD has gained a lot of interest (Diaz et al., 2004; Borja et al., 2015). Biotic indices summarize the ecological status to a number which forms the basis for management decisions and implementation of environmental monitoring programs (Borja et al., 2019). AMBI, AZTI's Marine Biotic Index (Borja et al., 2000), one of the most widely applied benthic index, was designed to study the response of soft-bottom macrobenthic assemblages to the alterations in the environmental quality of marine waters. This index was originally developed in Europe and has been formerly used in temperate waters (Borja et al., 2003; Muniz et al., 2005; Josefson et al., 2008; Gillet et al., 2015). However, AMBI was reported to be successful in evaluating the status of tropical waters (Borja and Tunberg, 2011) including the Indian waters (Sigamani et al., 2015; Feebarani et al., 2016; Mulik et al., 2017). The AMBI has been proven to be potentially effective in detecting impacts from various sources such as organic matter accumulation in bottom sediments, heavy metals, dredging processes, sand extraction, submarine outfalls, and hydrocarbons contamination etc. (Bonne et al., 2003; Borja et al., 2003; Muxika et al., 2005; Ceia et al., 2013; Borja et al., 2015; Checon et al., 2018; Borja et al., 2019). AMBI is based on the extent of macrobenthic species tolerance to disturbance (Borja et al., 2000) and depends on the benthic community succession model of Pearson and Rosenberg (1978). This index is tabulated based on the tolerance values of benthic taxa which are referred to as Ecological Groups (EG). The assignments of species to the EGs in the AMBI list has been carried out usually by investigating the response of species to anthropogenic pressures in available literature, in the absence of which the new species were assigned to the same group as that of similar genus. When no appropriate reference was available for a species that is significant for a geographical area, the EG assignment have been done through consultation with benthic experts. Lastly, species were reported as 'not assigned' when adequate information was not present to be assigned to any group. These group assignments are considered to be universally applicable to any ecosystem (Borja et al., 2019). The identification of taxa to the lowest taxonomic level, species abundance in the samples to be assessed and the

most recent EG list are the only necessities to apply AMBI (Gillet et al., 2015). Hence, this index represents a sound tool for environmental management owing to its efficiency in assessing the ecosystem health.

The tropical regions, particularly the estuaries, are influenced by strong seasonality (Dias et al., 2018). The Indian subcontinent is the recipient of heavy rainfall during the monsoon season that controls the physico-chemical and biological characteristics of the coastal and estuarine environments (Sivadas et al., 2016). The seasonal variations in macrobenthic assemblages are mostly influenced by changes in salinity, temperature, dissolved oxygen and sediment type (Alongi, 1998). Benthic communities experience seasonal alterations in abundance and biomass, mainly related to the recruitment events (Reiss and Kröncke, 2005). Therefore, these natural changes that affect the species composition are likely to influence the results of benthic indices that depend upon the relative abundances of tolerant and sensitive taxa (Chainho et al., 2007). AMBI has been reported to be insensitive to seasonal changes (Salas et al., 2004; Muxika et al., 2007; Borja and Tunberg, 2011; Hutton et al., 2015). However, there are studies that have reported the vulnerability of AMBI to seasonality particularly in estuaries (Chainho et al., 2007; Sivadas et al., 2016).

The Ulhas estuary, situated on the western coast of India (Fig 1), is exposed to various anthropogenic activities owing to its proximity to the heavily urbanized and industrialized Thane-Mumbai metropolis (Athalye et al., 2003). In addition to these pressures, the estuary is also affected by natural stress during the monsoonal rainfall. AMBI has been reported to be particularly successful in assessing the ecological quality status of estuaries both on the east and west coast of India (Khan et al., 2014; Sigamani et al., 2015; Sivadas et al., 2016; Mulik et al., 2017). A survey conducted previously in Ulhas estuary during the premonsoon (Mulik et al., 2017) investigated the applicability of various benthic indices in assessing the ecological quality status. It was concluded that AMBI worked best in presenting the estuarine ecological status. In view of this, it would be of further interest to study the effect of seasonality on the performance and robustness of AMBI in an estuary subjected to natural and anthropogenic perturbations.

In the ecological assessment studies, multivariate analyses have been often used because of their sensitivity and reliability with regard to benthic communities (Clarke and Warwick, 2001). One of the applicable features of multivariate analysis is the data transformation. Transformations are generally employed to adjust the effects of rare and abundant taxa found in

high numbers (Warwick et al., 2010). For instance, when untransformed data are used, the focus is only on the dominant taxa. Conversely, when strong transformations are used (i.e., fourth root, log transformation), it down-weights the contribution of the dominant taxa and considers all the species constituting the assemblage (Clarke and Warwick, 1994). The AMBI calculation is based on the untreated abundance data, but it has been suggested that the different transformations may enhance the performance of index by down-weighting the dominant species (Warwick et al., 2010; Muxika et al., 2012; Teixeira et al., 2012; Gillet et al., 2015). In AMBI, when there is a dominance of one species (Puente and Diaz, 2008), it biases the results towards a general assignation of ecological status. Hence, to avoid the bias in the result, it may be beneficial to transform the data to reduce the effect of dominant (*r* selected) species and give importance to rare (*k* selected) species as well. Although several authors have tested the efficacy of AMBI in assessing the ecosystem status of different locations by comparing its performance with other benthic indicators, very little (temperate region) or no (tropical region) attention has hitherto been paid to understand how data transformations influence the performance of AMBI.

The objectives of this study were to examine how seasonal patterns in subtidal macrobenthic communities affected the results obtained by AMBI and to evaluate the effect of data transformation on the performance of AMBI. In order to achieve the stated objectives, following questions were postulated 1) Is AMBI impervious to seasonality? 2) Does the choice of data transformation influence the performance of AMBI? 3) Do data transformations dictate the quantum and direction of the relationship between the AMBI scores derived and the contamination variables? The raised questions were addressed by analyzing both the biotic and abiotic data. It was expected that the answers to these questions would provide valuable information on the efficient application of this versatile benthic index (AMBI) in tropical estuaries.

2. Material and Methods

2.1 Study area

Ulhas river originates in the western slopes of Sahyadrian range in Maharashtra, India and after a westerly course of about 100 km, it joins the Arabian Sea at Vasai (19° 16' N and 72° 45' E) (Mulik et al., 2017). The estuary drains an area of about 4,637 km². It is mainly a monsoon-fed river which receives an average freshwater discharge of 146 m³.s⁻¹ (Central Water Commission; CWC, Nasik) during monsoon season. Average annual rainfall is about 2943 mm.

The estuary has immense mudflats and extensive mangroves along its intertidal zones. It is heavily stressed by a wide range of anthropic activities and receives untreated domestic sewage (511 MLD; NIO, 2018), industrial effluents (46.5 MLD; NIO, 2016), terrestrial and agricultural runoff (NIO, 2017), particularly in its middle and upper zones. Dredging activities at some locations also impact the estuary (Fernandes and Nayak, 2016).

2.2 Field sampling and laboratory analyses

The sampling survey was conducted at 12 subtidal stations (Fig.1) during the year 2013 for three consecutive seasons i.e. premonsoon; Pr (May), monsoon; M (September) and postmonsoon; Po (November). Stations were divided into four zones; Coastal (U1), Lower (U2-U3), Middle (U4-U7) and Upper (U8-U12) based on their distances along the entire stretch of the estuary. The water depth of the stations varied from 0.4 m (station U12) to 14 m (station U3). The premonsoon season in this area falls between March and May followed by the southwest monsoon from June to September and October to January forms the postmonsoon season. An industrial discharge outfall is present in the upstream of Ulhas (U10) and a combination of domestic sewage and industrial effluents reaches the estuary near station U9 through a nullah (NIO, 2016; 2018). Quadruplicate sediment samples were collected at each station using a van Veen grab of 0.04 m² bite area for studying macrobenthos. The sediment samples were then sieved through 0.5 mm mesh sieve and the organisms retained in the sieve were fixed in 5% formalin mixed with Rose Bengal. Macrobenthic organisms were sorted, counted and identified to the lowest possible taxon (most often species). The available identification keys were used for the species level identification (Day, 1967; Fauchald, 1977; Lincoln, 1979; Lyla et al., 1998). Sediment and bottom water data from the same sampling locations were collected to investigate the responses of macrobenthic communities to environmental factors. Sediment samples were taken in duplicate for the analyses of texture (combined sieving and pipette method; Buchanan, 1984), organic carbon (C_{org}) (titration method; Walkey and Black, 1934) and total petroleum hydrocarbons (PHc) (IOC-UNESCO, 1982). Bottom water samples were collected in duplicate at each station during ebb tides using a Niskin sampler. After the collection of water samples, they were immediately preserved in ice for the analysis of various physicochemical factors like salinity, dissolved oxygen (DO), nitrite-nitrogen (NO₂⁻-N), nitrate-nitrogen (NO₃⁻-N) and ammonia-nitrogen (NH₄⁺-N). DO was analysed by Winkler's method, salinity by Mohr titration method and nutrients by photometric methods following Grasshoff et al. (1999).

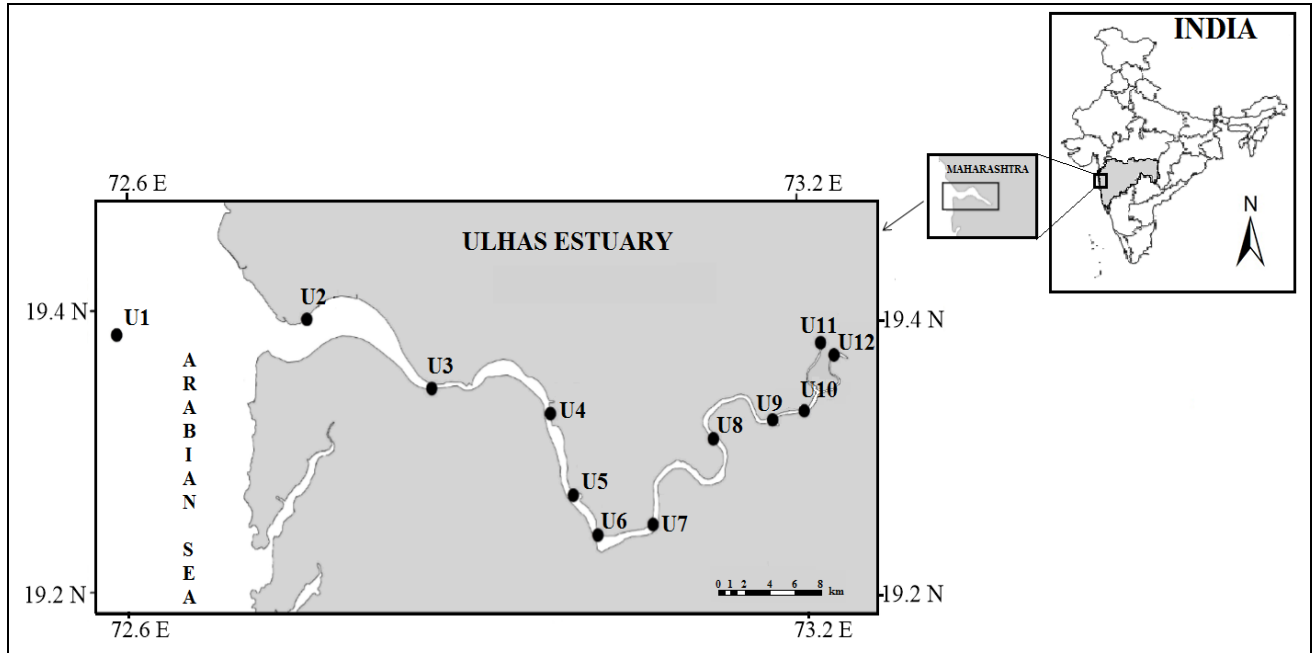


Fig. 1 Map of the study area (Ulhas estuary, northwest coast of India), including the position of sampling stations.

2.3 AMBI description

AMBI relies on the abundance-weighted average disturbance sensitivity of the macrobenthic species in a sample (Borja et al., 2000). Each species were distributed in five EGs as per their sensitivity to an increasing stress gradient (organic matter enrichment) as summarized by Grall and Glémarec (1997).

EG I: species sensitive to organic enrichment and present only under unpolluted conditions.

EG II: species indifferent to enrichment, present in low densities with non-significant variations.

EG III: species tolerant to high organic matter enrichment.

EG IV: second-order opportunistic species.

EG V: first-order opportunistic species, able to withstand high disturbance.

The index calculation was done by the software provided at AZTI's web page (<http://ambi.azti.es>; June 2017 species list), following the guidelines of Borja and Muxika (2005). The index generates a final score on a continuous scale from 1 to 6 (7 in azoic sediments) and the thresholds used to define benthic community health were <1.2 (undisturbed), 1.2-3.3

(slightly disturbed), 3.3-5 (moderately disturbed), 5-6 (heavily disturbed) and >6 (extremely disturbed) (Borja et al., 2000) which were then condensed to a high, good, moderate, poor and bad status respectively. A mean ecological status of each station was derived by averaging ecological status values for all three seasons. Further, an attempt was also made to derive the final mean ecological status of individual station by averaging values of non-monsoonal seasons (premonsoon and postmonsoon) as suggested by Dias et al. (2018).

2.4 Data treatment

Macrobenthic species abundance data from all four replicates were averaged for each station to perform univariate and multivariate analyses (Sánchez-Moyano et al., 2010) using the statistical program, PRIMER v6 (Clarke and Gorley, 2006). Univariate diversity measures such as average macrobenthic abundance (N), Shannon's index ($H' \log_2$), Margalef's richness index (d) and Pielou's evenness index (J') were calculated to monitor the environmental changes (Carvalho et al., 2011; Lv et al., 2016). The macrobenthic species abundance data was fourth root transformed to down-weight the influence of numerically dominant taxa, which were used to build a similarity matrix using the Bray-Curtis similarity coefficient. This matrix was subjected to non-metric multidimensional scaling (nMDS) ordination for analyzing the spatial distribution of macrobenthic assemblages along the estuarine gradient for each season separately. The percentage composition of five ecological groups i.e. EGI, EGII, EGIII, EGIV and EGV were superimposed as bubbles on the macrobenthic abundance MDS plot. Redundancy analysis (RDA) was carried out to explore the relationship between five ecological groups, sites and abiotic parameters using the untransformed macrobenthic abundance data. Prior to that, DCA (detrended correspondence analysis) was performed to determine whether to use CCA or RDA. In DCA, if the maximum gradient length of the axes was > 4 SD then CCA is more appropriate whereas if the maximum gradient length of the axes was < 3 SD then RDA is to be applied (Ter Braak and Smilauer, 2002). As in the DCA the maximum gradient length of the axes was 2.371 SD, RDA was performed.

2.5 Abundance data transformations

AMBI uses the macrobenthic abundance data for the calculation of an index score (Borja et al., 2000). Abundance data was transformed using a set of transformations of increasing severity: square root, fourth root and $\log(x + 1)$ to understand the effect of these transformations (Teixeira et al., 2012; Gillett et al., 2015; Checon et al., 2018) on the performance of AMBI.

AMBI was calculated for each replicate of the twelve sites and three sampling periods independently using none and transformed abundance data matrices. A 3-way Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson, 2001) was applied to the similarity matrix of station replicates based on AMBI scores, in order to test the statistical significant differences for three factors: i) season (fixed, three levels), ii) station (fixed, twelve levels) and iii) transformation (fixed, four levels). The Spearman's Rank correlation coefficient (r) was estimated between abiotic variables and AMBI scores obtained from various transformations to understand if this index performed better with transformations against the proxies of pollution (nutrients, PHC, C_{org}) using Statistica 7. The Holm-Bonferroni correction method was used to correct the p-values for multiple comparisons.

3. Results

3.1 Abiotic characteristics

The spatio-temporal variability in hydro-sedimentological parameters of the estuary is presented in Figs. 2 and 3. A decreasing gradient of salinity was evident from the coastal to the upper zone of the estuary. During the monsoon, the estuary was predominantly oligohaline as the salinity ranged from 0.02-1.9 from U3 to U12 stations signifying marked monsoonal impact on the salinity regime. During premonsoon, considerably lower DO values were recorded in the middle and upper estuarine stations (U4-U10; 2.4 - 3.8 mg Γ^{-1}), whereas hypoxia was observed at U8 (1.3 mg Γ^{-1}) and U10 (0.4 mg Γ^{-1}) during postmonsoon. Higher DO content and a concomitant decrease in nutrient concentrations in the bottom water of middle and upper estuarine zones were observed during monsoon (Appendix A). Higher nutrient concentrations were detected during the non-monsoonal seasons as compared to monsoon. For instance, highest NO_2^- -N level was observed in the middle estuarine zone during both the premonsoon ($20.8 \pm 14.3 \mu\text{mol } \Gamma^{-1}$) and postmonsoon ($20.6 \pm 1.2 \mu\text{mol } \Gamma^{-1}$). NO_3^- -N concentrations were markedly elevated (4.0-128.3 $\mu\text{mol } \Gamma^{-1}$) throughout the estuary during non-monsoonal seasons except in the coastal station of premonsoon (1.9 $\mu\text{mol } \Gamma^{-1}$). Highest NH_4^+ -N values were observed in the stations (U8 and U9; 18.5-58.3 $\mu\text{mol } \Gamma^{-1}$) which were in the proximity of effluent release. The PHC-sediment values ranged from 0.5-10.5 $\mu\text{g g}^{-1}$ with relatively higher values at U8 and U9 during all sampling occasions (Fig. 2). The textural analysis (Fig. 3) revealed that the sediments were predominantly silty (57.8-85.2%) except at U3 and U4 (premonsoon) and at U12 (all seasons) where sandy substratum was noticed. Organic carbon content was $\geq 2.0\%$ at most of the stations (61%) and

presented an increasing trend from the coastal to the upper zone. Organic carbon was lower at the sandy innermost station (U12).

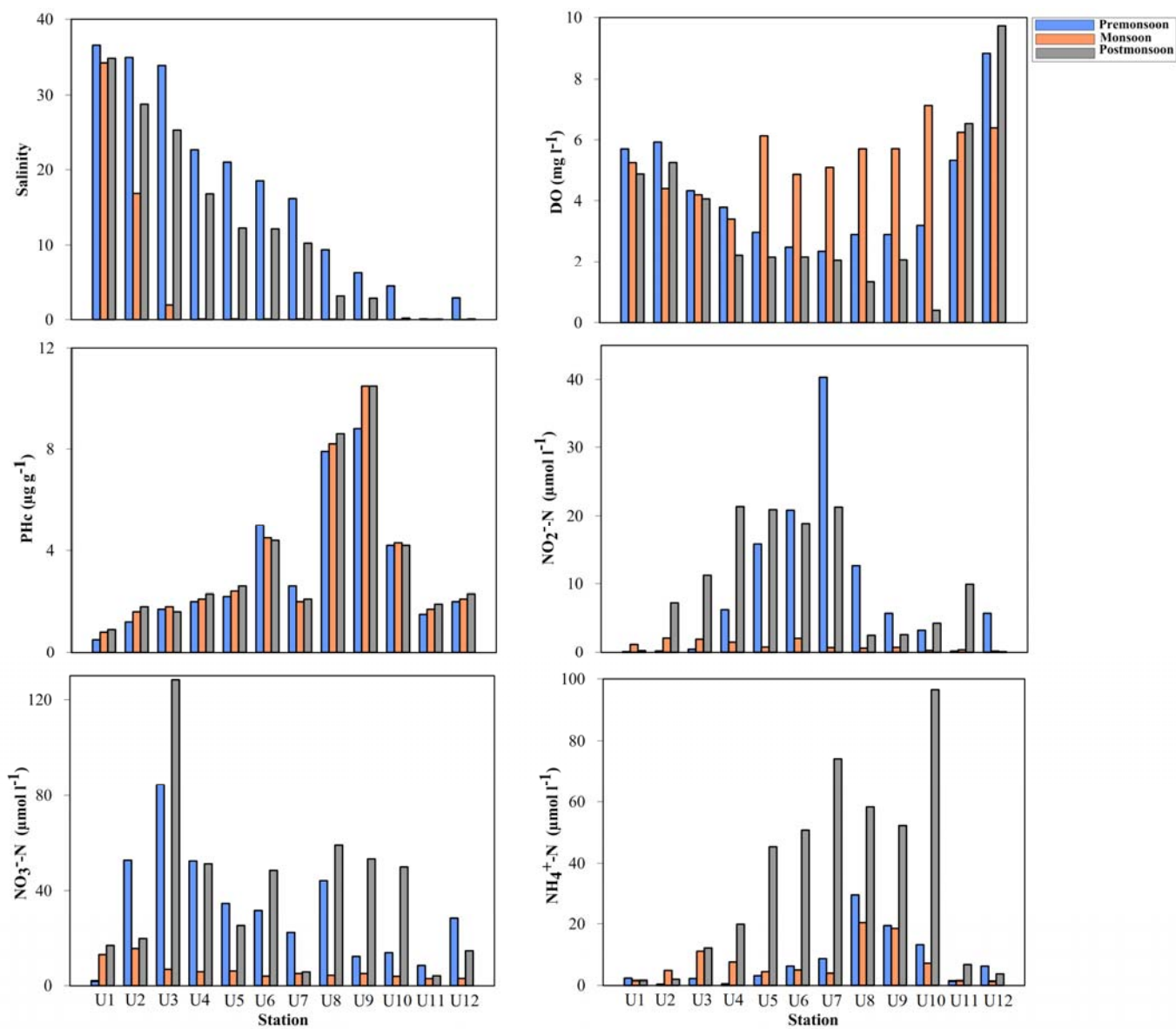


Fig. 2 Spatio-temporal distribution of abiotic variables (salinity, DO; Dissolved Oxygen, PHc-sediment, NO₂⁻-N; nitrite-nitrogen, NO₃⁻-N; nitrate-nitrogen and NH₄⁺-N; ammonia-nitrogen) in the Ulhas estuary

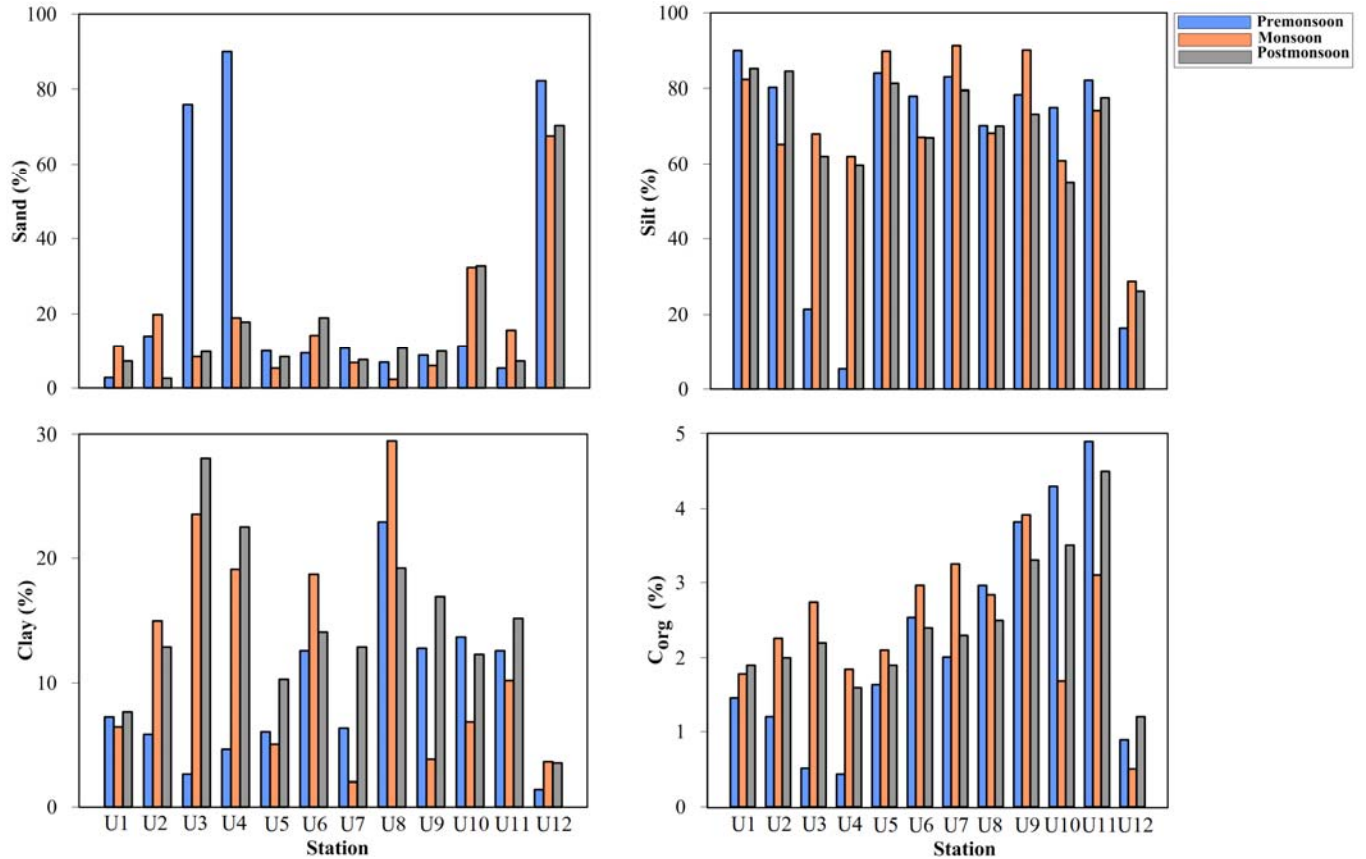


Fig. 3 Spatio-temporal distribution of sediment variables (sand%, silt%, clay% and C_{org} %) in the Ulhas estuary.

3.2 Macrobenthic community structure

A total of 86 taxa were recorded from the present study with polychaetes (83.4%) as the dominant taxa in terms of abundance. The spatio-temporal variability of diversity indices i.e. average macrobenthic abundance (N), Margalef's richness index (d), Pielou's evenness index (J), Shannon's index ($H' \log_2$) calculated for all the study sites is represented in Fig. 4. The highest mean macrobenthic density was observed in the middle estuary during premonsoon (4700 ± 4845 ind. m^{-2}). The macrobenthic diversity indices (H' , d and J) were low during monsoon in the lower and middle zone (Appendix A). The stations (U8 and U9) near the effluent release were azoic during the premonsoon. Overall, the macrobenthic abundance was dominated by *Namalycastis ouanaryensis* (43.4%), *Streblospio gynobranchiata* (27.1%) and *Namalycastis indica* (10%). The opportunistic polychaete, *S. gynobranchiata* was dominant in the middle estuary during premonsoon (87.6%) and postmonsoon (35.6%). The estuary was dominated by

N. ouanaryensis across all zones during the monsoon (Appendix B). All the identified taxa of different sensitivities were assigned to five Ecological Groups (EGs) based on the AZTI's classification (Appendix C). After assignment, 28 macrobenthic taxa (32.6%) were classified as EG I, 20 taxa (23.3%) assigned to EG II, 14 taxa (16.3%) classified as EG III, 13 taxa (15.1%) assigned to EG IV and 3 taxa (3.5%) assigned to EG V. In terms of abundance, however, EG IV and EG V dominated, due to the high densities of *N. ouanaryensis* and *S. gynobranchiata* respectively. Twelve taxa were classified according to their genus (13.9%), whereas six species (6.9%) were unassigned in the AMBI list.

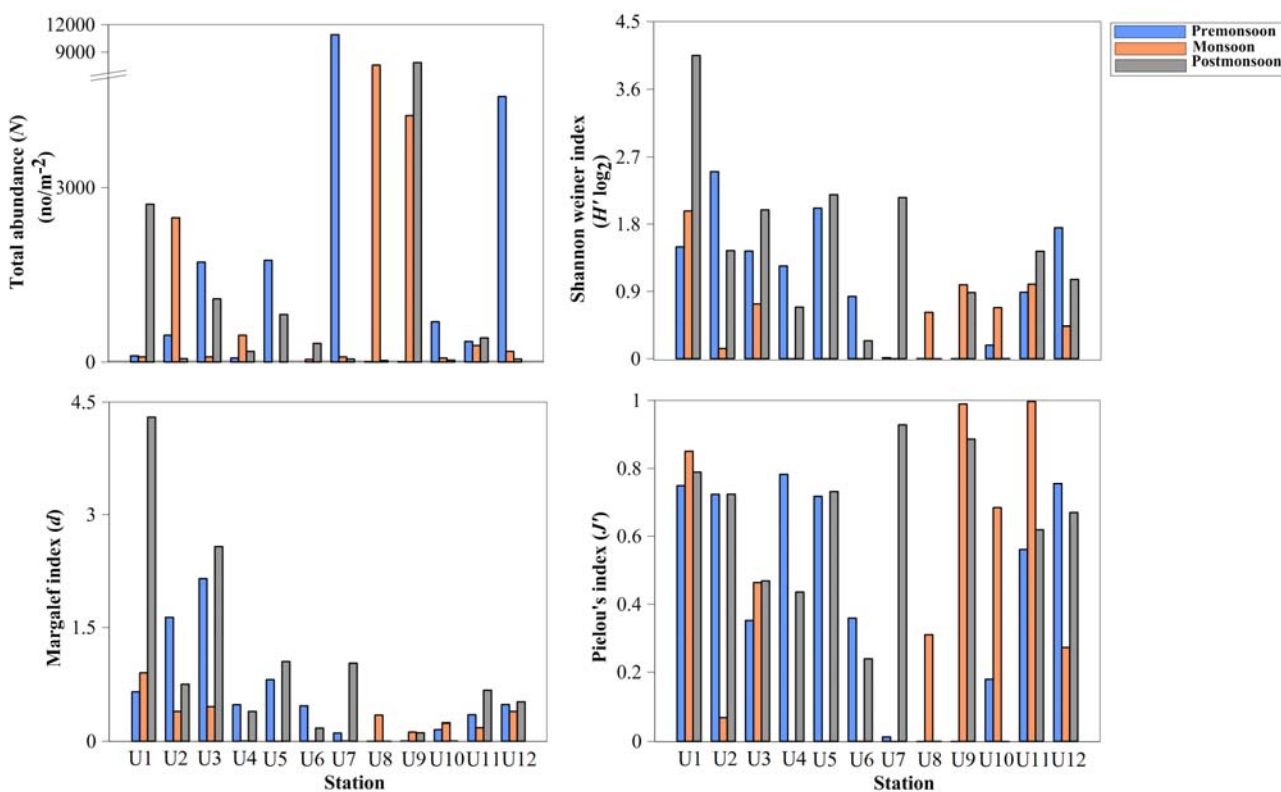


Fig. 4 Spatio-temporal distribution of the macrobenthic univariate measures (total abundance; N , Shannon Weiner index; H' , Margalef index; d and Pielou's index; J') in the Ulhas estuary.

The results of MDS plot displayed a clear segregation of most stressed azoic stations (Pr8 and Pr9) from other stations during premonsoon (Fig. 5a). The sensitive groups of EG I and EG II were abundant at the less impacted uppermost (Pr12) and lower estuarine station (Pr3), while the opportunistic species belonging to EG IV and EG V were dominant at the stressed upper (Pr11) and middle (Pr7) estuarine stations respectively. The EG III species were present in high percentage at Pr4. In monsoon, EG I and EG II species were found only at coastal (M1) and

lower (M2, M3) stations, EG III species were limited to the upper estuarine stations (M11 and M12), while EG V species were restricted to M8 and M12 (Fig. 5b). The sensitive Ecological Groups that showed drastic decline during monsoon reappeared during postmonsoon. For instance, the middle estuary was characterized by the presence of EG IV alone during monsoon while multiple EGs were re-established during postmonsoon (Fig. 5c). Species comprising EG IV were present throughout the estuary during all the sampling periods except at Pr4.

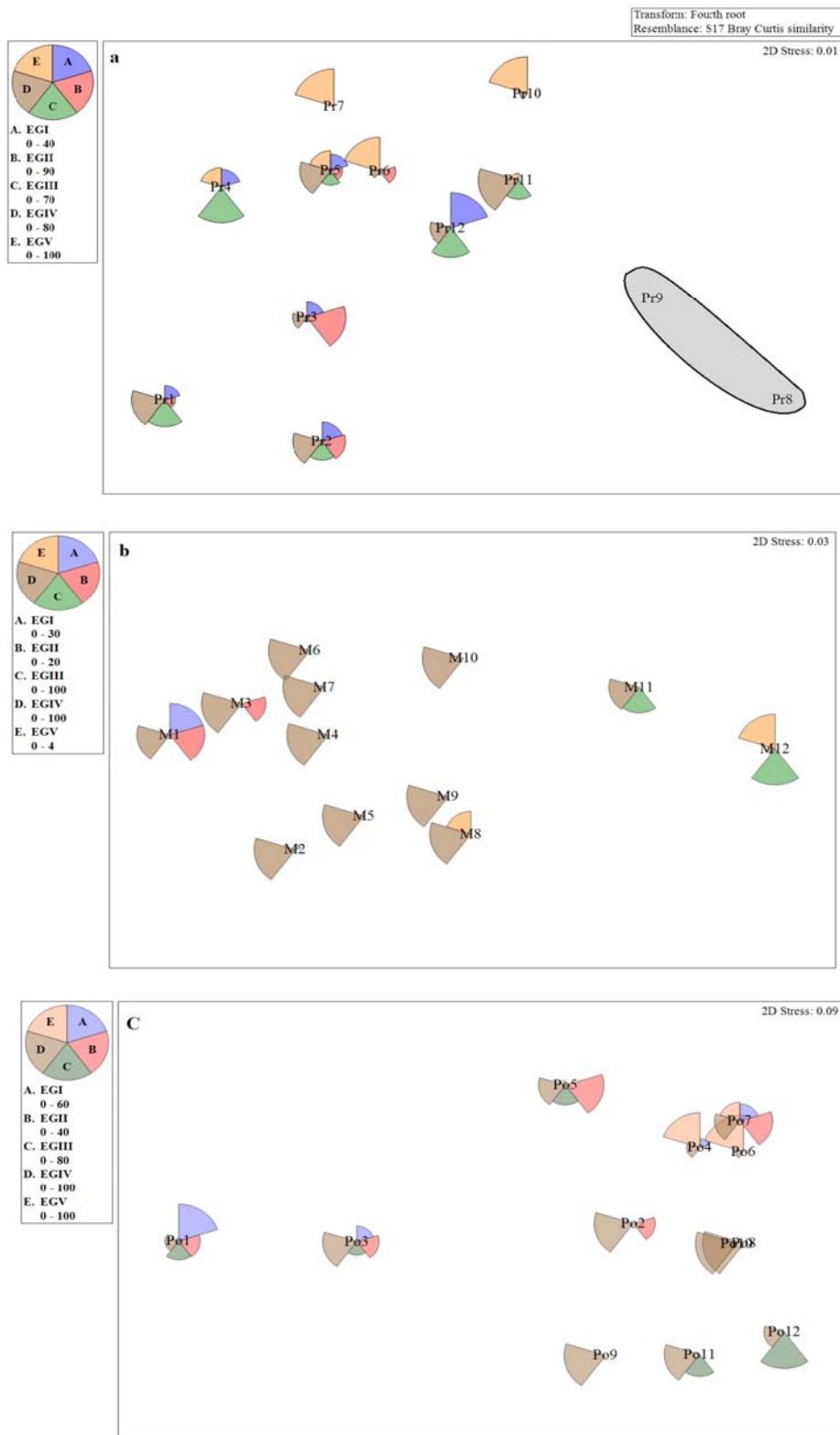


Fig. 5 Non-metric multidimensional scaling ordination of the fourth root transformed macrobenthos abundance data using Bray-Curtis similarities of Ulhas estuary with percentage compositions of Ecological Groups (EG I, EG II, EG III, EG IV and EG V) superimposed as bubbles. Stations are labeled in accordance with season i.e. a) Pr; Premonsoon, b) M; Monsoon and c) Po; Postmonsoon). Shaded region indicates the azoic zone.

3.3 Performance of AMBI

Overall, the ecological status derived from the AMBI indicated that the areas with moderate to extreme disturbances were found mainly at the middle (U4-U7) and upper (U8-U11) estuarine stations. The coastal (U1), lower (U2, U3) and upper (U12) stations were undisturbed to moderately disturbed, while the middle (U4-U7) and upper (U8-U11) estuarine stations were moderately to extremely disturbed. In terms of temporal variability, 8 of the 12 sampled stations showed variations in their ecological status over the study period (Table 1). Most of the changes occurred among adjacent categories such as moderate and poor status (U4, U6 and U7), good and moderate status (U2 and U3), while station U1 fluctuated between high, good and moderate status. Sites with poor macrobenthic condition (U8 and U9) changed from bad (premonsoon) to moderate (monsoon) and further to poor/bad (postmonsoon). The stations with no temporal changes were classified as good (U12), moderate (U5 and U11) and poor (U10) ecological status. During monsoon season, 83.3% of stations showed similar ecological status (moderate) indicating no spatial trends. The mean index value of each station achieved by merging all seasons either underestimated (U2-U3) or overestimated (U7-U9) the ecological status between the adjacent categories. Conversely, 83.3 % of stations showed accurate ecological status on averaging only premonsoon and postmonsoon seasons.

Stations	Pr	M	Po	Mean	Mean
				All seasons	Pr and Po
1	2.83	3.63	1.13	2.53	1.98
2	3.11	4.4	4.31	3.94	3.71
3	2.51	4.25	3.87	3.54	3.19
4	4	4.5	5.49	4.66	4.74
5	3.51	4.5	3.35	3.79	3.46
6	5.37	4.5	5.93	5.27	5.65
7	6	4.5	3.56	4.69	4.78
8	7	4.57	5.75	5.77	6.38
9	7	4.5	6.38	5.96	6.69
10	5.96	5.13	5.4	5.50	5.68
11	4.35	3.98	4.22	4.18	4.29
12	2.29	3.19	3.25	2.91	2.77

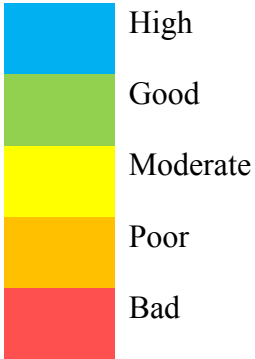


Table 1: Ecological Quality Status (EQS) and AMBI value for each station during the three seasons (Pr=Premonsoon, M=Monsoon, Po=Postmonsoon).

PERMANOVA indicated that the AMBI scores differed significantly between the seasons ($F = 11.3$; $P = 0.0001$) and stations ($F = 35.2$; $P = 0.0001$). The interactions between seasons and stations were also significant ($F = 11.8$; $P = 0.0001$). However, no significant difference between the AMBI values obtained by various data transformations was evidenced by PERMANOVA. The similarity percentage among the different data transformations was extremely high (91.6%) as represented in Fig. 6.

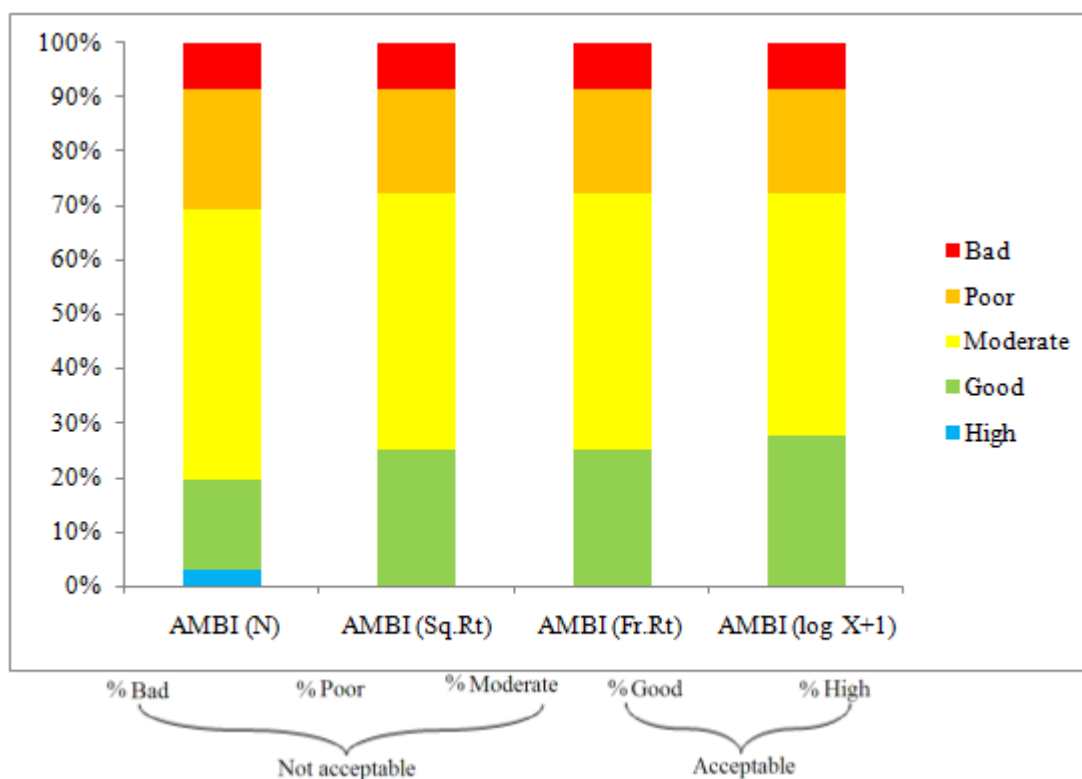


Fig. 6 Level of agreement between the ecological status obtained for N (untransformed), Sq.rt (Square root), Fr.rt (Fourth root) and $\log(x+1)$ transformed AMBI in Ulhas estuary.

3.4 Relationship between biotic and abiotic variables

AMBI scores obtained from untransformed data and three different levels of transformations indicated a significant relationship with the abiotic parameters. AMBI scores showed strong correlation with DO (except with Fourth root), $\text{NH}_4^+\text{-N}$, PHc and C_{org} (Fig. 7) while no correlation was observed with salinity. AMBI values based on different transformations were almost similarly correlated with the abiotic parameters. The highest correlation was mostly obtained with untransformed data. The relationships between the Ecological Groups and the abiotic factors were assessed using RDA. A Monte Carlo test showed a high significance for the

first and all canonical axes ($p < 0.01$). The first two axes of RDA (Fig. 8) explained 91.6% of the total variability in ecological groups and environmental factors. The first RDA axis was positively correlated with salinity and NO_2^- -N. The second axis was positively associated with DO and sand, while reverse trend was observed with NH_4^+ -N, PHc, silt, clay and C_{org} . The most sensitive species and indifferent species (EG I and EG II) were positively correlated with salinity and negatively correlated with NH_4^+ -N, PHc, silt, clay and C_{org} , while EG III species showed positive correlation with DO and sand. All the contamination variables (NH_4^+ -N, PHc and C_{org}) were in positive correlation with second-order opportunistic group (EG IV) while NO_2^- -N with the first order opportunistic species (EG V) (Fig. 8).

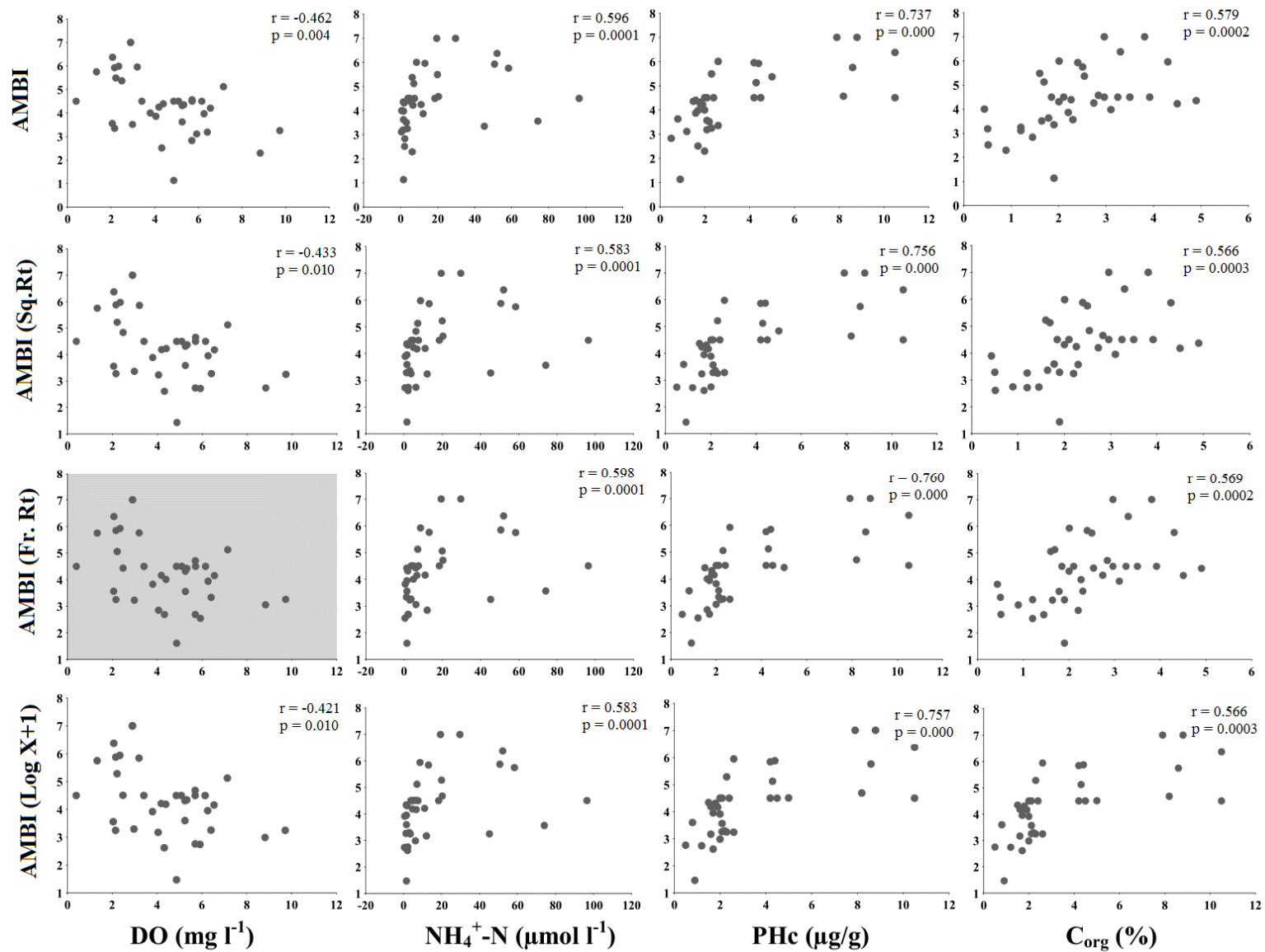


Fig. 7 Spearman's rank correlations between AMBI scores obtained by different transformations and contamination proxies. The Shaded region indicates non-significant plot.

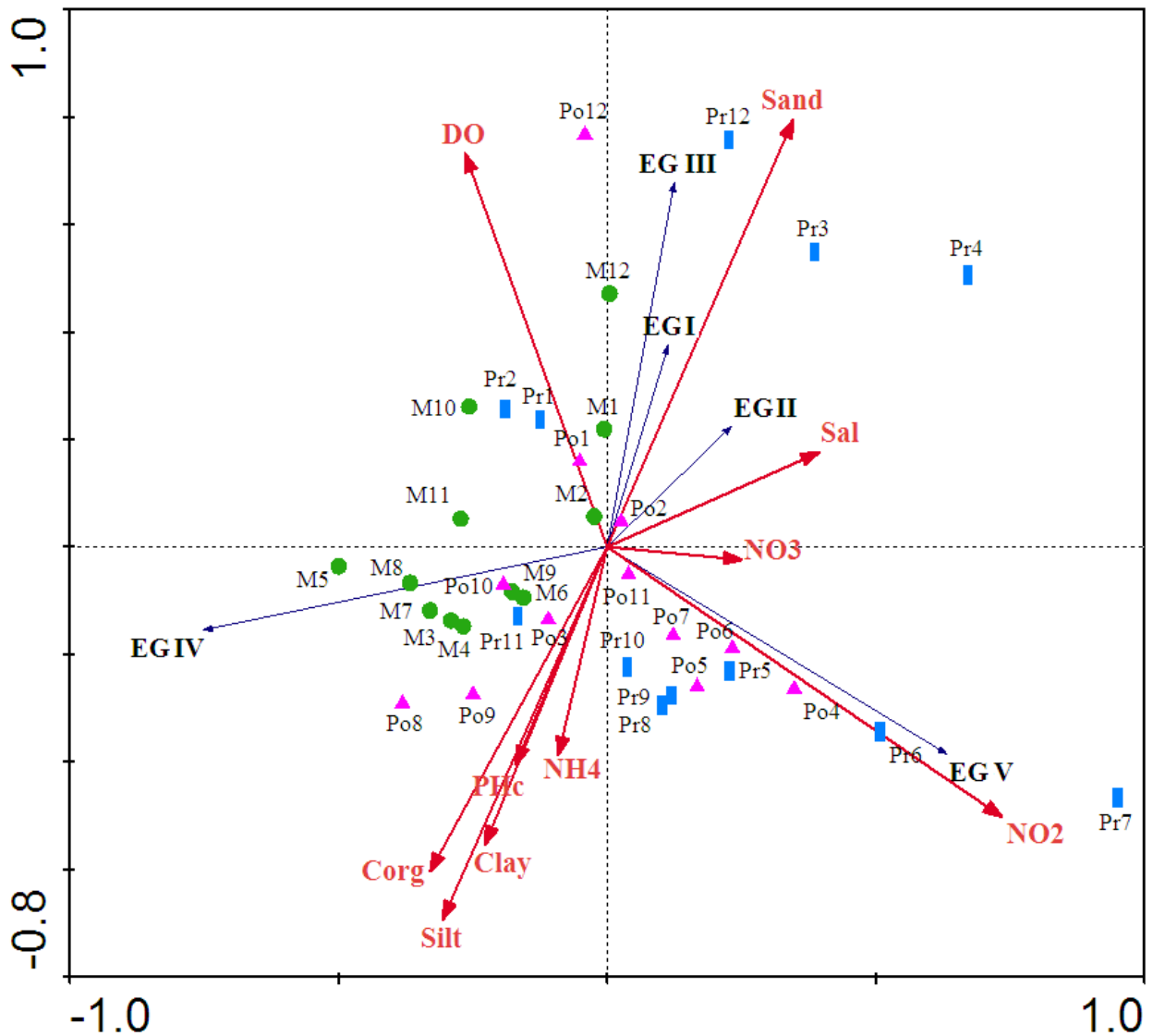


Fig. 8 Redundancy analysis triplot (RDA) representing the relationships between environmental variables, EG; Ecological Groups (I–V) and sampling sites (Pr=Premonsoon, M=Monsoon, Po=Postmonsoon, Sal=Salinity, DO=Dissolved Oxygen, PHc=Petroleum Hydrocarbon, NO_2^- -N=nitrite-nitrogen, NO_3^- -N=nitrate-nitrogen and NH_4^+ -N=ammonia-nitrogen, C_{org} =organic carbon)

4. Discussion

Temporal inconsistency is an essential subject to be addressed in monitoring programs (Paiva, 2001), and the use of benthic indices must account for this variation. AMBI has been used successfully in various environments such as coastal waters (Li et al., 2013), estuaries (Hutton et al., 2015), lagoons (Afli et al., 2008), freshwater zones (Medeiros et al., 2012), rocky

areas (Vinagre et al., 2016) etc. This uniform applicability of AMBI was reiterated by Borja et al., (2019). However, Chainho et al. (2007) indicated that the application of AMBI was difficult in systems that are naturally stressed or facing strong temporal variability such as estuaries. AMBI has been shown to be stable to natural temporal variability in temperate estuaries (Salas et al., 2004; Hutton et al., 2015). In tropical areas with heavy rainfall such as the present one, the index is reported to fluctuate temporally (Feebarani et al., 2016; Sivadas et al., 2016; Dias et al., 2018).

The current study results clearly demonstrated the temporal changes in the ecological status of most of the stations. The moderate status of the coastal station U1 during monsoon, was probably due to an export of materials and pollutants from the estuary to the coast that ultimately affected the benthic communities. The subsequent high status of this station during the postmonsoon can be attributed to the recruitment and migration of the macrobenthic fauna (Gaonkar et al., 2013). The good status observed at stations U2 and U3 were due to the substantial presence of EG I and EG II. However, this ecological status dropped to moderate status during the other seasons (monsoon and postmonsoon) when the community was replaced by EG IV (*N. ouanaryensis*). The ecological status of station U4 during the premonsoon and monsoon was moderate which turned to poor during the postmonsoon owing to the dominance of *S. gynobranchiata* (EG V). Station U5 did not show any temporal variation as *N. ouanaryensis* dominated this station during all three seasons. *S. gynobranchiata* was dominant at U6 and U7 that resulted in poor status during premonsoon; however this species was completely absent during monsoon resulting in a moderate status. Bad ecological status was observed at U8 and U9 (the nearest stations to an industrial outfall), which were recognized as the most degraded sites, indicating marked anthropogenic stress. These stations were azoic during the premonsoon and repopulated by a high abundance of opportunistic nereids (*N. ouanaryensis*; 76.3% and *N. indica*; 22.8%) during the monsoon. As a result, inconsistencies in the status were observed in this area which showed a shift from bad (azoic) to moderate status during monsoon. During postmonsoon, the poor/bad status at U8 was due to the solitary presence of *N. ouanaryensis* in low numbers while the bad status at U9 was observed due to the high abundance of *N. ouanaryensis* (69.6 %) and *N. indica* (30.4%). These stations were eutrophic with low DO, high NH_4^+ -N and PHc values owing to the weak tidal impact and poor flushing (Ram et al., 2009) resulting in the buildup of organic matter. This was reflected in the index value as the

macrobenthic community in this region was either azoic (premonsoon) or dominated by EG IV (monsoon and postmonsoon). The upper estuarine stations (U10-U12) were not influenced by the seasonality as these areas were populated by similar species assemblage irrespective of the season.

In tropical regions, the macrobenthic structure is strongly influenced by the annual rainfall that results in strong seasonality in the environmental conditions (Sivadas et al., 2016). A distinct spatio-temporal pattern in salinity was evident in the current study, as expected for most of the tropical estuaries. During monsoon, the Ulhas estuary was freshwater dominated owing to the strong influx from upstream, causing a drop in the salinity. The concentration of nutrients during this period also was observed to have reduced substantially due to dilution by increase in freshwater discharge. Monsoon along the Indian coast generally results in immense fluctuations in macrobenthic assemblage that gets mirrored in the index value (Sivadas et al., 2016; Dias et al., 2018). Average values for macrobenthic species richness, Shannon-Wiener diversity and number of taxa were lower during the monsoon in Ulhas similar to that reported in other monsoonal estuaries of the Indian coast (Sivadas et al., 2011; Feebarani et al., 2016; Dias et al., 2018).

The previous study conducted by Mulik et al. (2017) at Ulhas estuary had inferred that that the stations U1-U3 and U12 were impacted to a lesser extent, stations U4, U5 and U11 were moderately stressed, stations U6, U7 and U10 were heavily polluted and stations U8 and U9 were extremely disturbed. In general, it was observed that the ecological status of least affected stations in the estuarine mouth (U1-U3) degraded from good to moderate during monsoon. Meanwhile the ecological status of most affected stations in the middle and upper zones (U6-U10) indicated improvement from poor or bad to moderate. This could be due to the dilution of pollutants and an increase in DO concentration at these stations leading to the recolonisation of macrobenthos. The main characteristic of the fresh water dominated Ulhas estuary during monsoon was that the macrobenthic communities were entirely constituted (97.4 %) by EG IV species such as *N. ouanaryensis* and *N. indica*, which is known to proliferate in zones marked by low salinities and disturbances (Glasby, 1999). The predominantly uniform status (moderate) of the estuary during the monsoon was probably resultant of the replacement of existing diverse communities with one or two opportunistic species due to the abrupt salinity fluctuations and physical disturbances associated with increased fresh water flow (Alongi, 1990; Sivadas et al.,

2011). Thus, the monsoonal AMBI values failed to distinguish between the stations that were differently impacted in the major stretch of the estuary (U1-U9).

Several authors have concluded that AMBI could identify the degraded and healthy stations appropriately in tropical and sub-tropical estuaries (Borja and Tunberg, 2011; Valença and Santos, 2012; Mulik et al., 2017). However, other researchers (Chainho et al., 2007; Puente et al., 2008) have established a lack of sensitivity of AMBI to distinguish between polluted areas where naturally stressed community exists due to the dominance of opportunistic species. The apparent dominance of *N. ouanaryensis* throughout the estuary during the monsoon season resulted in AMBI indicating similar status for majority of the stations (83.3%). In general, inclusion of monsoonal data either underestimated (25 %) or overestimated (33.3 %) or maintained the same ecological status (41.7%) (Table 1). Kröncke and Reiss (2010) also suggested that the data collected post natural events such as cold winters should be excluded for ecological assessment studies as it can considerably lower the ecological status. Sivadas et al. (2016) and Dias et al. (2018) too have recommended the exclusion of monsoonal data for the efficient application of benthic indices in the tropical region. The present study indicated that the monsoonal perturbations affected the species diversity that in turn affected the AMBI values leading to erroneous ecological status evaluation. Thus, AMBI was proven to be vulnerable to monsoon in Ulhas estuary, thereby answering the first question.

In the current investigation, expected ecological status was obtained by taking mean of the non-monsoonal seasons (premonsoon and postmonsoon) with only two exceptions (U2 and U7) where the ecological status differed between the adjacent categories. AMBI did not show substantial temporal variations if only the non-monsoonal data were considered. Even in stations that indicated temporal variations (U2 and U7), the shift in AMBI values was between adjacent categories, as also reported by Tataranni and Lardicci (2010) and Hutton et al. (2015). In India, three seasonal sampling is recommended in a year (<http://www.moef.nic.in>) for conducting the comprehensive environmental impact assessment studies of coastal waters. However, identifying a suitable period for ecological monitoring is crucial so as to trim down the sampling efforts and consequently reduce the cost of such studies (Sivadas et al., 2016). As the magnitude of various disturbances (industrial and domestic effluents, aquaculture, water runoff, agricultural wastes, tourism etc.) also changes with seasons (Karakassis et al., 2013), it becomes imperative to determine the sampling period for application of the biotic indices in assessing the ecological

status for management purpose (Sivadas et al., 2016; Tataranni and Lardicci, 2010). From the current study, it was concluded that for an adequate assessment of ecological quality, the monsoonal data of the macrobenthic communities is to be omitted. These results also concur with the Central Pollution Control Board (CPCB) of India, which has recommended conducting ecological assessment during the non-monsoon periods to get more accurate results (<http://www.cpcbenvvis.nic.in>). The non-monsoonal seasons have more stable biological populations (Gaonkar et al., 2013) and therefore are considered as the best time for sampling.

AMBI was primarily developed for European waters and its applicability in benthic studies has been tested in several geographical zones (Muniz et al., 2005; Muxika et al., 2012; Gillet et al., 2015; Borja et al., 2019). As different regions are likely to have diverse species pools with contrasting tolerance, the performance of this index varies in different areas (Checon et al., 2018). Several authors have suggested that the performance of AMBI might get enhanced after appropriate data transformations (Warwick et al., 2010), yet, there is no agreement regarding the suitability of the type of transformation technique to be applied. For instance, square root transformation was more reliable as per Warwick et al. (2010), fourth root transformation was recommended by Teixeira et al. (2012), whereas the use of logarithmic transformation was suggested by Gillet et al. (2015). This could be due to the regional differences wherein dominant species and the distribution of abundance data influenced the outcome of data transformation (Checon et al., 2018). In a stressed estuary like Ulhas, it was reasonable to apply the data transformations so as to reduce the impact of the dominant opportunistic species. However, the index scores obtained from different transformations showed variation in the ecological status of only 16.7% of the stations. A good level of agreement was found between all the applied transformations (confirmed by PERMANOVA) suggesting that the same results could be obtained irrespective of the transformation chosen. Thus, the different data transformations did not enhance the performance of AMBI in the Ulhas estuary, thereby answering the second question. The present results contradict the conclusions of Warwick et al. (2010), who found improvement in the AMBI performance by using square root data transformation. As minimal changes were observed in the interpretation using transformed data, avoidance of transformation can be considered as ideal which was in agreement with Muxika et al. (2012) and Checon et al. (2018).

In the present study, the lower, middle and upper estuarine stations were influenced by anthropogenic factors, while the natural factors were the most controlling variables for the coastal (U1) and the uppermost (U12) station (Fig. 8). The contamination variables were negatively correlated to sensitive species and an opposite trend was found for opportunistic species. For instance, the most sensitive (EG I) and indifferent (EG II) species were found in the areas with high salinity and low contaminants, thus were categorized under high or good category. EG IV and EG V were correlated with nutrients (NO_2^- -N, NH_4^+ -N) and C_{org} which were probably due to the ecological grouping linkage with organic enrichment succession as highlighted by Checon et al. (2018). Along with C_{org} , AMBI had a strong association with the nutrients and PHc, emphasizing the efficacy of the index to identify the impacts from various sources of contamination other than organic enrichment (Muniz et al., 2005; Riera et al., 2011; Hutton et al., 2015; Borja et al., 2019). The application of various transformations did not affect the correlations between the derived AMBI values and the contamination proxies. However, AMBI values obtained with untransformed data resulted in the best correlation values. Thus, to answer the third question, transformation of data did not affect the direction of the relationship between the AMBI scores derived and the contamination variables.

5. Conclusions

Ulhas, a tropical monsoonal estuary is facing considerable stress from the anthropogenic activities besides the inherent natural seasonal variations. The stations (U8 and U9), located in the most affected stretch, had the most degraded ecological status in the estuary. AMBI was successful in evaluating the status of the estuary during the non-monsoonal seasons while it failed to perform well during the monsoon as it either underestimated or overestimated the ecological status of more than half of the stations studied. The high variability in both the biotic and abiotic parameters of the study area during the monsoon affected the AMBI performance on the temporal scale. This study recommends that the non-monsoon period is the appropriate time of the year to apply the AMBI for the successful assessment of Indian estuaries. AMBI correlated positively with the contamination parameters indicating that it was efficient in detecting the different kinds of impacts. The results revealed that data transformations had very little effect on the relationship of AMBI with contamination parameters.

Supplementary material

Appendix A

Appendix B

Appendix C

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