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Original Article

Assessing the ecological quality status of arid mangroves in the Gulf of Oman, Iran, using benthic indices of AMBI, M-AMBI, and BENTIX

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Abstract: Polychaetes are suitable indicators to evaluate the benthic ecological status and respond to natural and anthropogenic. We evaluated the ecosystem health of the mangroves of Azini and Gwadar based on benthic indices including AMBI, M-AMBI, and BENTIX using polychaete communities. The results showed that in both regions, EcoQ classifications ranged from "high" to "moderate" in BENTIX, "good" to "excellent" in AMBI, and "good" in M-AMBI. The M-AMBI was significantly correlated with sediment variables, including total organic matter (TOM), total organic carbon (TOC), and silt/clay. The result revealed a significant correlation between the biotic indices and the TOC content of sediments. According to the results, TOC can be used as a descriptor and indicator to evaluate the health of mangrove ecosystems in relation to benthic indices. In addition, it is necessary to combine several indices to assess the status of ecosystems.

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Introduction

The mangrove forests grow in estuaries and intertidal zones of the tropics and subtropics (Field et al. 1998). The mangrove ecosystem is a biologically active ecosystem with numerous ecological functions. Mangrove forests provide many benefits, including breeding grounds for fish and shellfish, birds, and other wildlife, preventing shoreline erosion, and protection during hurricanes and tidal waves (Quarto, 2005). In addition, they are responsible for providing many ecosystem goods and services, including natural barriers, carbon sequestration, and biodiversity (Duke et al. 2007). However, it has been threatened by urbanization, pollution, and overexploitation over the past decades (Alongi, 2002).

The spatial distribution patterns of polychaetes have been extensively investigated with respect to environmental variables since they constitute a dominant element of benthic communities (Tyler and Kowalewski, 2018). The abundance of polychaetes on estuarine macrofauna and their occurrence under a variety of environmental conditions make them an excellent biological model for studying estuarine ecosystems (Schüller et al., 2009). The assemblages of polychaetes display changes in standing stock as a response to variables in the environment, with the increase in organic sediment content being one of the most significant impacts of anthropogenic activity (Dauvin et al., 2016; Alvarez-Aguilar et al., 2017). Polychaetes can be used as a bioindicator of organic pollution due to their high diversity, abundance, and functional significance. They are frequently used as ecological groups to determine ecosystem quality as part of biotic indices (Borja et al., 2014).

Several indices based on benthic invertebrate communities have been proposed to evaluate environmental health status, including the AZTI Marine Biotic Index (AMBI) (Borja et al. 2000), the multivariate AMBI (M-AMBI) (Muxika et al., 2007), and the BENTIX Index (Simboura and Zenetos 2002). AMBI is based on species assigned to one of five levels of sensitivity, ranging from very

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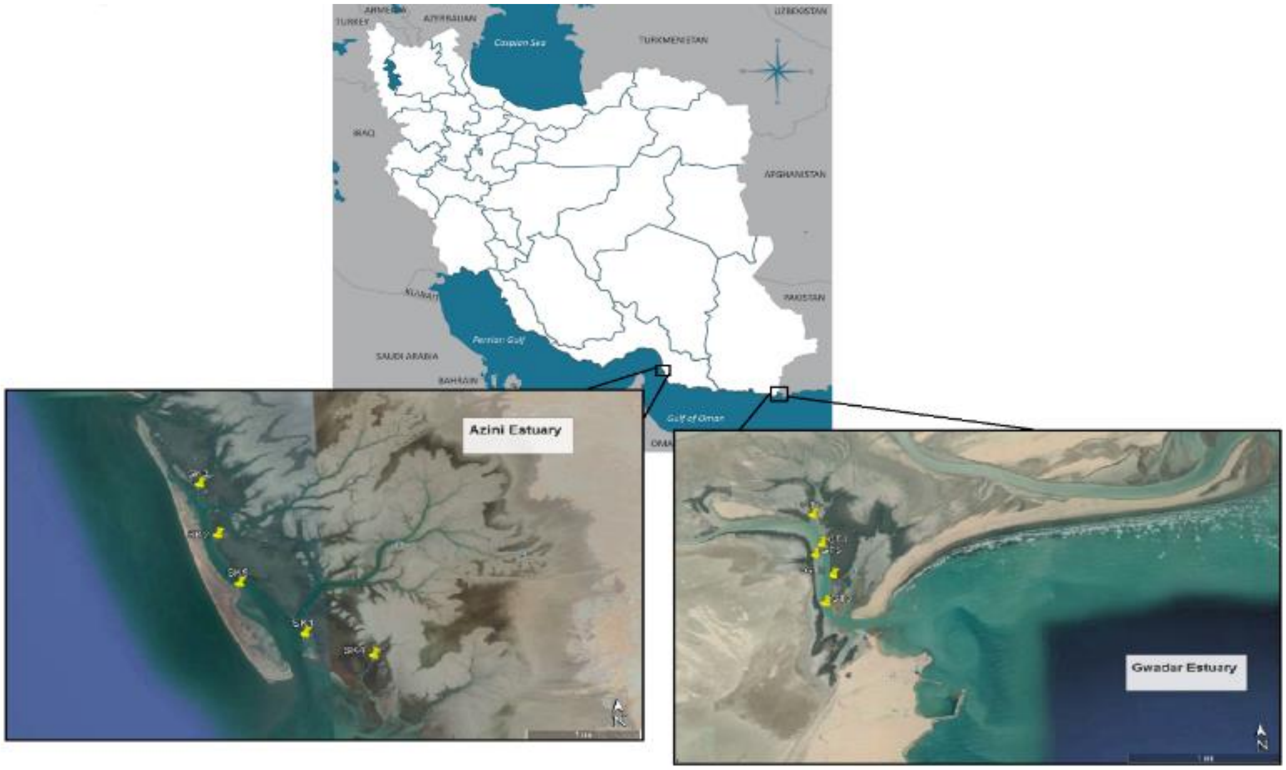

Figure 1. Positions of the sampling sites of polychaetes in the Hara Biosphere Reserve, Gulf of Oman (the Gwadar and Azini estuaries).

sensitive to opportunistic species (Borja et al., 2000). M-AMBI is a multivariate analysis that combines Shannon-Wiener diversity, species richness, and AMBI (Muxika et al., 2007). Bentix index calculates the relative contributions of tolerant and sensitive taxa based on their occurrence ratios in the benthic fauna by weighting them according to the concept of indicator groups (Simboura and Zenetos, 2002). AMBI, M-AMBI, and BENTIX indices are designed to evaluate the impact resulting from general stress factors and can not distinguish between natural and anthropogenic disturbances (Borja et al., 2003).

In order to detect and develop proper strategies for mitigating the effects of the discharge of anthropogenic chemical contaminants into marine ecosystems, we must assess and understand the ecological effects that affect habitat alteration and benthic marine communities. Hence, our study assessed the ecological quality of the Azini and Gwadar mangrove ecosystems in the Gulf of Oman using benthic quality indices including AMBI, M-AMBI, and BENTIX based on polychaete communities and determining the correlation of these indices with environmental variables was investigated.

Materials and methods

In the Iranian coastal zone, mangrove forests are distributed along 1250 km. Mangrove forests in Iran consist of two species of *Avicennia marina* and *Rhizophora mucronata*, that both species are found in the Azini estuary, but there is only *A. marina* in the Gwadar estuary. In the summer and winter of 2019, sediment samples were taken from mangrove forests in Gwadar and Azini estuaries (Fig. 1). Sediment samples were collected using a metal quadrat (25*25*25 cm) from 10 stations i.e. 5 stations for each estuary. The sediments were collected in three independent replicates for sediment variables and polychaetes. Polychaetes were sorted under a stereomicroscope and identified at the lowest taxonomic level possible. Measurements of total organic matter (TOM), total organic carbon (TOC), and the grain size composition of the sediment surface were performed using the ignition method (Heiri et al., 2001), the Walkley-Black (Walkley and Black, 1934) method,

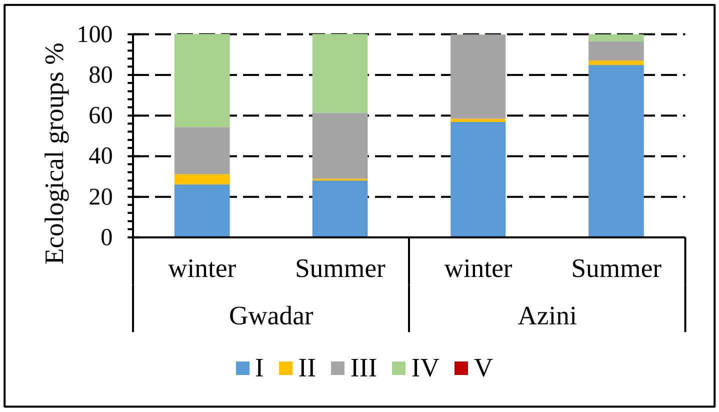

Figure 2. AMBI ecological groups (EG) for the polychaete communities in mangrove ecosystems in the Gulf of Oman (the Gwadar and Azini estuaries).

Table 1. The threshold levels of three indices for benthic ecological quality status assessment.

Biotic index	Ecological quality status (EcoQs)				
	High	Good	Moderate	Poor	Bad
AMBI	0-1.2	1.2-3.3	3.3-4.3	4.3-5.5	5.5-7
M-AMBI	0.77-1	0.53-0.77	0.39-0.53	0.2-0.39	0.0-0.2
BENTIX	4.5-6	3.5-4.5	2.5-3.5	2-2.5	0.0-2

Note: The thresholds of AMBI and M-AMBI are referred to Borja et al. (2000), Borja and Muxika (2005), Muxika et al. (2007), Li et al. (2017), and the thresholds of BENTIX is referred to Simboura and Zenetos 92002)

and the hydrometer technique (Bouyoucos, 1962), respectively.

The AMBI and M-AMBI indices were used to assess the benthic ecological status of the study areas and calculated using the AMBI program (version 6.0; available at http://ambi.azti.es). The software divides polychaetes into five ecological groups (GI + GV) based on their sensitivity to environmental stress gradients. Following the newest AMBI species list (December 2020), most of the collected species were classified into different ecological groups (EGs).

The assignment of some species, including some native species, was based on expert opinion or on the assignment of other species in the same genus (Borja et al., 2008). To improve the reliability of the results, AMBI values with more than 20% unassigned individuals were removed from the AMBI analyses but included in the M-AMBI analyses (Borja and Muxika, 2005). M-AMBI values were calculated using Factor Analysis of AMBI, Shannon diversity (H'), and species richness (S) (Muxika et al., 2007). In order to assess ecological status, it was crucial to set an appropriate M-AMBI reference condition. Therefore, in the present study, the reference conditions for the M-AMBI index proposed by Borja and Tunberg (2011) and Forchino et al. (2011), which increased the highest species diversity and richness values by 15% and decreased AMBI to half of the lowest value.

Bentix simplified the five ecological groups into two, the sensitive species group (GS), which includes GI and GII of the AMBI method, and the tolerant species group (GT), which includes GIII, GIV, and GV (Add-in v.1.0 version: (Simboura and Zenetos, 2002). Table 1 shows the different ecological quality statuses determined by AMBI, M-AMBI, and BENTIX.

To assess differences in benthic indices between regions, and seasons, we used a repeated measure PERMANOVA model (permutational multivariate analysis of variance), where 'region' and 'season' was fixed factors. Permutations of row data were unlimited and 9999 permutations were applied. Spearman correlation was done to examine the relationship between sediment parameters and benthic indices. The univariate and multivariate analyses were conducted using PRIMER Ver. 6 with the PERMANOVA add-on package and SPSS 21.0 software.

Table 2. List of polychaete species and their assigned ecological groups (EG) in mangrove ecosystems in the Gulf of Oman (the Gwadar and Azini estuaries).

Species	AMBI groups	BENTIX groups
Aricidea fragilis	I	I
Armandia intermedia	I	I
Armandia sp.	I	I
Capitella aberranta	V	II
Ctenodrilus sp.	NA	NA
Glycinde sp.	II	I
Heteromastus sp.1	IV	II
Heteromastus sp.2	IV	II
Heteromastus sp.3	IV	II
Heteromastus sp.4	IV	II
Leonnates indicus	III	II
Lepidonotus purpureus	II	I
Lepidonotus sp.	II	I
Levinsenia gracilis	III	II
Linopherus hirsuta	IV	II
Marphysa sanguinea	II	I
Marphysa sp.	II	I
Mediomastus warrenae	NA	NA
Melinna monoceroides	III	II
Namalycastis sp.	NA	NA
Neanthes glandicincta	I	I
Paleaequor sp.	I	I
Perinereis horsti	III	II
Perinereis nuntia	III	II
Phyllodoce sp.	II	I
Prionospio sp.	NA	NA
Questa riseri	II	I
Scolelepis sp.	III	II
Scolelepis squamata	III	II
Sigambra sundarbanensis	IV	II
Simplisetia erythraeensis	III	II
Syllis gracilis	II	I
Tylonereis bogoyawlensky	III	II

*NA: Not assigned

Results

The collected species were categorized into five ecological groups to calculate the AMBI and M-AMBI indices (Fig. 2, Table 2). The highest number of species was in the ecological group (I) in Azini (84.9% of the total species in Azini). The lowest number of species were ecological groups (V) in the Gwadar. During two sampling seasons, ecological groups (I), (III), and (IV) included the most dominant species in the study regions.

The lowest and highest values of the AMBI index in all three regions were 0.8±0.09 and 3.2±0.5, respectively, at Azini and Gwadar in summer. According to AMBI, Gwadar, and Azini were of good to high status (Fig. 3), and the results showed a significant difference between regions ($P<0.05$). The AMBI index revealed that summer has better ecological quality, but there was no significant difference between seasons in both areas ($P>0.05$) (Table 3). M-AMBI showed that the ecological status of the study regions was good status and it ranged from 0.59±0.08 to 0.52±0.13 (Fig. 4). No significant difference was found between the two regions and seasons ($P>0.05$) (Table 3).

Two sensitive and tolerant ecological groups were used to calculate the BENTIX index (Fig. 5). The most sensitive species were found in Azini during summer (GS, 86.6%). The most tolerant species were observed in Gwadar during winter (GT, 67%).

Table 3. The results of PERMANOVA for comparing AMBI, M-AMBI and BENTIX across regions and seasons in mangrove ecosystems in the Gulf of Oman (the Gwadar and Azini estuaries). Factors: region (levels: Gwadar and Azini) and season (levels: winter and summer).

AMBI	df	Ms	Pseudo-F	P
Region	2	8638.4	2.0915	0.048
Season	1	6560.5	1.8017	0.259
Region × Season	1	5128.5	1.1307	0.438
Residual	62	2304.8		
Total	71			
M-AMBI	df	Ms	Pseudo-F	P
Region	2	1055.4	0.81601	0.567
Season	1	309.17	0.41735	0.142
Region × Season	1	150.01	0.2025	0.795
Residual	62	803		
Total	71			
BENTIX	df	Ms	Pseudo-F	P
Region	2	2825.3	1.3381	0.037
Season	1	787.02	0.60774	0.561
Region × Season	1	834.85	0.64468	0.519
Residual	62	582.58		
Total	71			

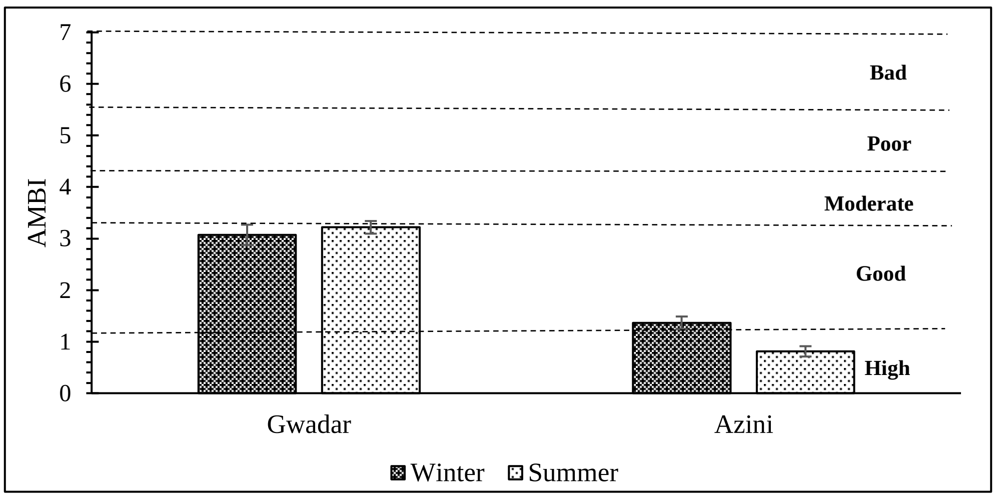

Figure 3. The AMBI values of polychaete communities in mangrove ecosystems in the Gulf of Oman (the Gwadar and Azini estuaries).

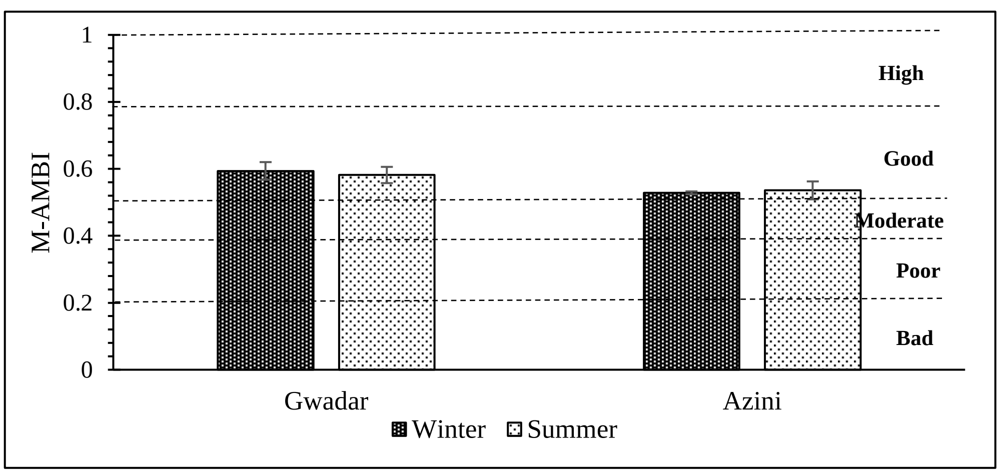

Figure 4. The M-AMBI values of polychaete communities in mangrove ecosystems in the Gulf of Oman (the Gwadar and Azini estuaries).

Table 4. Variations in environmental data (mean ± SD) between regions and seasons in mangrove ecosystems in the Gulf of Oman (the Gwadar and Azini estuaries). (TOM = Total Organic Matter; TOC = Total Organic Carbon).

Gwadar	Winter	Summer
TOM	0.51±0.09	0.71±0.06
TOC	0.91±0.3	1.23±0.4
Silt/Clay (%)	1.13±0.54	1.93±1.5
Azini	**Winter**	**Summer**
TOM	0.78±0.1	0.57±0.08
TOC	0.99±0.2	0.84±0.3
Silt/Clay (%)	1.96±0.91	1.62±0.88

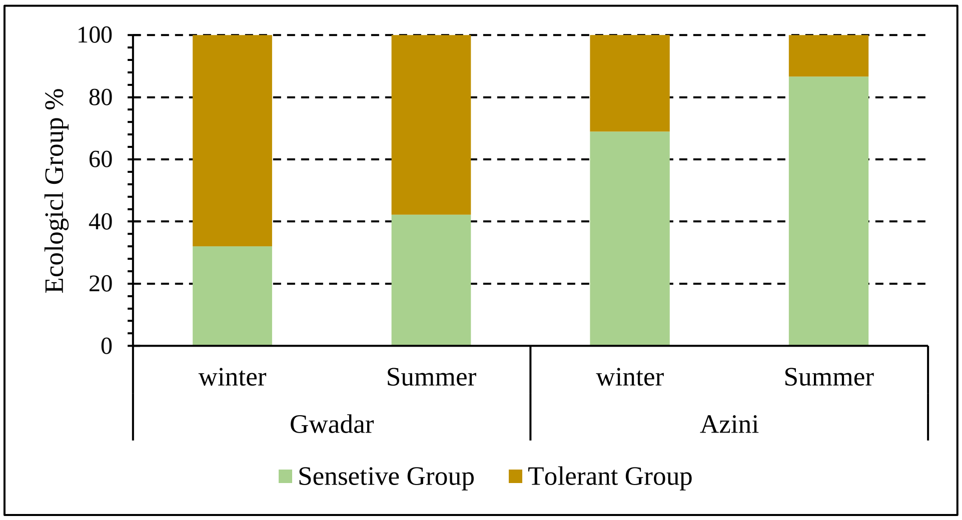

Figure 5. BENTIX ecological groups (EG) for the polychaete communities in mangrove ecosystems in the Gulf of Oman (the Gwadar and Azini estuaries).

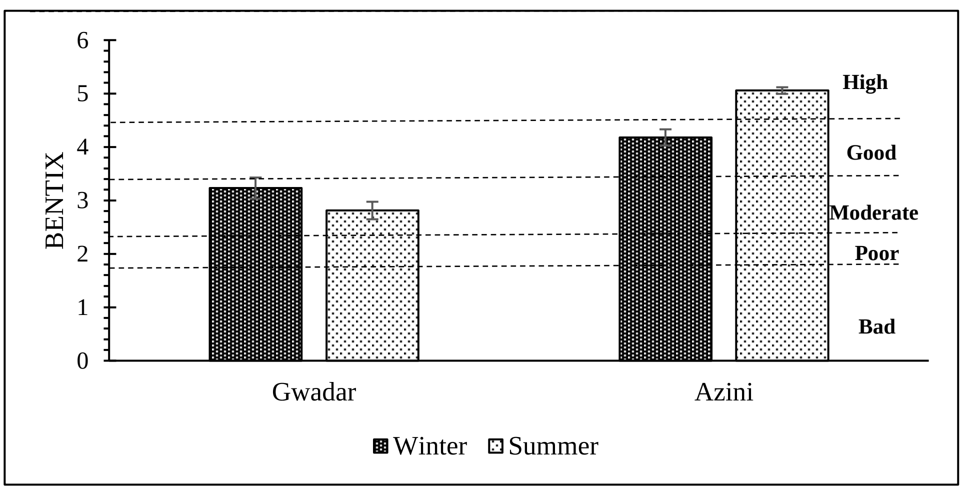

Figure 6. The BENTIX values of polychaete communities in mangrove ecosystems in the Gulf of Oman (the Gwadar and Azini estuaries).

BENTIX index values ranged from 2.8±0.32 to 5±0.67 and Gwadar and Azini had the lowest and the highest values in summer, respectively. Using the BENTIX index, two regions were moderate to high status (Fig. 6). There was a significant difference in ecological quality between Gwadar and Azini ($P<0.05$). The mean values of BENTIX did not differ significantly between winter and summer ($P>0.05$) (Table 3). Table 4 shows the physicochemical characteristics of the sediment during winter and

Table 5. Spearman correlation between biotic indices and sediment parameters in mangrove ecosystems in the Gulf of Oman (the Gwadar and Azini estuaries). (*, $P<0.05$; **, $P<0.01$).

	AMBI	M-AMBI	BENTIX	Silt/Clay	TOC
AMBI					
M-AMBI	-0.59				
BENTIX	-0.97	0.66			
Silt/Clay	-0.26	**-0.51***	0.07		
TOC	**0.61***	**-0.95****	**-0.73***	0.58	
TOM	0.46	**-0.91****	-0.46	0.36	0.75

summer from the Gwadar and Azini estuaries. The values of M-AMBI showed the best response to increasing concentrations of TOM (r = -0.91, $P<0.01$) and TOC (r = -0.95, $P<0.01$). The AMBI and BENTIX values showed a significant correlation with TOC (r = -0.61, $P<0.05$, and r = -0.73, $P<0.05$ respectively). Conversely, AMBI did not show significant correlations with TOM, and Silt/Clay (Table 5).

Discussion

AMBI, M-AMBI, and BENTIX were used to assess the ecological quality of Gwadar and Azini mangroves in the Gulf of Oman. There were noteworthy differences between the results of the three biological indices. In comparison to AMBI and M-AMBI, the BENTIX showed a much lower ecological status. The indices showed different EcoQ classifications in the two regions, including "high" to "moderate" conditions in BENTIX and "high" to "good" conditions in AMBI, and "good" conditions in M-AMBI. Some studies report inconsistent classifications of EcoQs based on different benthic indices for the same study area (Simboura et al., 2014; Pelletier et al., 2018; Maghsoudlou et al., 2020; Yan et al., 2020; Xu et al., 2021; Dong et al., 2021). These differences could be due to (1) these indicators are designed to evaluate European coastal waters' ecosystems (Qiu et al., 2018; Xu et al., 2021), (2) there are different boundary limits (Simboura and Reizopoulou, 2008; Borja and Tunberg, 2011; Gamito et al., 2012; Sun et al., 2018; Ni et al., 2019), (3) in the various ecological groups, each index input is weighted differently (Simboura and Reizopoulou, 2008; Sun et al., 2018), and (4) worldwide, macrobenthos is composed differently in marine ecosystems, and they may respond differently to disturbances (Borja et al., 2000; Pelletier et al., 2018; Mulik et al., 2020; Dong et al., 2021). The Bentix classification method yields a wider high-status range (4.5-6) than the AMBI (0-1.2), which often classifies good-status sites as high-status.

In the current study, the TOC content of sediment was significantly correlated with AMBI, M-AMBI, and BENTIX indices. According to the results, the BENTIX index showed a significant negative correlation with the TOC content of the sediment. Although organic matter provides food for benthic fauna in surface sediments, excessive amounts of organic enrichment can lead to oxygen depletion as well as toxic by-products, resulting in reduced species richness, abundance, and biomass of benthic fauna that are closely associated with bottom sediments (Hyland et al., 2005). The TOC content of sediment was higher in Gwadar mangroves compared to Azini mangroves. The BENTIX index was classified the Gwadar mangroves in "moderate" status, while the Azini mangroves classified as "good" to "high" status. Therefore, the TOC content of sediment can be an important descriptor and proxy for evaluating the benthic status of mangrove ecosystems in relation to ecological indices. The M-AMBI index showed the highest correlation with TOM and TOC content of sediments. Therefore, according to the results, the AMBI, M-AMBI, and BENTIX indices can be considered suitable indicators for enriching organic matter in sediments. Some studies observed correlations between organic matter and AMBI, M-AMBI and BENTIX indices (Borja et al., 2008;

Caglar and Albayrak 2012; Umehara et al. 2019; Medeiros et al. 2021; Munari et al. 2022; Sarathy et al. 2022). However, according to Hu et al. (2018), benthic indices are not correlated with organic matter, which may explain its overestimation of benthic quality.

The Azini mangroves were dominated by sensitive species (EG I), while the Gwadar mangroves were dominated by tolerant species (EG III) and second opportunistic species (IV). Apart from the absence of industries in the coastline region, the presence of two types of trees, including *A. marina* and *R. mucronata*, may have contributed to the "high" ecological status in Azini mangroves. According to Delfan et al. (2021), the low species richness of the macrofauna may be explained by a low diversity of mangrove trees. *Avicennia marina* is the only mangrove species in Gwadar. In addition, the wastewater from shrimp farming centers may have disturbed the Gwadar mangroves.

In the current study, because of a lack of knowledge, some of the collected species, especially some native species, could not be assigned to the AMBI species list, resulting in partly affecting index evaluation results. Lastly, it is important not to ignore the characteristics of the local ecosystem when assessing its status of the local ecosystem. Hence, no one biotic index is the most suitable, and when doing ecological assessments, it is more reliable to combine the results from several indices.

Conclusion

The indices showed different EcoQ classifications in the two regions studied, including "high" to "moderate" status in BENTIX and "high" to "good" status in AMBI, and "good" status in M-AMBI. Sensitive species were dominant in the Azini mangroves, while resistant species were dominant in the Gwadar mangroves. There were significant correlations between benthic indices and the TOC content of sediments. Therefore, TOC can be a suitable descriptor and indicator to evaluate the quality of mangrove ecosystems in relation to benthic indices. Also, the highest correlation was observed between M-AMBI and sediment parameters. Our results showed that several benthic indices are needed for assessing the benthic quality of ecosystems; as well as suggest useful insights on identifying the key drivers of polychaete communities and benthic indices in mangrove ecosystems.

Acknowledgments

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