

## Benthic invertebrate community in Khur-e-Mussa creeks in northwest of Persian Gulf and the application of the AMBI (AZTI's Marine Biotic Index)

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### Abstract

Benthic invertebrates are a well-established target in environmental quality status evaluations. The AMBI (AZTI's Marine Biotic Index) was developed to determine the impacts and the quality status of soft-bottom marine benthic communities. The aim of this study was investigating multivariate pattern of benthic invertebrate community and application of AMBI (AZTI's Marine Biotic Index) for determining quality of health status in Khuzestan coastal waters. Seasonal samplings were collected from eight creeks over one year study by Peterson grab sampler, beginning from October 2005. All creeks are characterized by muddy bottom. According to sensitivity to environmental stress, macrobenthic animals classified into 5 ecological groups. Among 28 identified faunal groups the most abundant groups were polychaets, molluscs, crustaceans and nematodes, respectively. Two way ANOVAs showed only seasonal significant differences in mean abundance ( $P < 0.05$ ) and Margalef richness index values ( $P < 0.05$ ), while all of studied creeks shows similar biological characters based on benthic communities. According to annual mean of AMBI value all creeks were in ecological group III with slightly polluted condition, except for Darvish that was in unpolluted category. In the present study, although the number of species initially increased, but due to appearance of dominant species such as *Capitella* sp. and nematods (as opportunist species), diversity values reduced. In general, according to AMBI and Biotic index values, the most creeks are classified into unpolluted and slightly polluted categories except for Zangy, Doragh and Patil in summer and also Zangy and Bihad in winter which was in moderate to heavily polluted category. Different types of exploitation, industrial and shipping activities in this area could result in unbalanced to polluted status in benthic animals (as the best indicators in sediment quality assessment) so these results can be acceptable.

**Keywords:** AMBI, Contaminant assessment, Khur-e-Mussa creeks, Persian Gulf

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## Introduction

Khur-e-Mussa is located at Northwest of Persian Gulf in Iranian coastal waters. Low depth and muddy sediments are characteristics of this region. Oil exporting terminals and two fishing ports are located at this region. Recently, several classification tools for establishment of marine environment quality have been developed in different ecosystems that are undertaken based upon different biotic compartments (phytoplankton, angiosperms and rocky shore communities, soft-bottom macrobenthos and fish). Marine environmental quality control is undertaken usually by means of monitoring different parameters in water, sediment and sentinel organisms (Borja et al., 2000; khodami et al., 2011; Jahani et al., 2012).

The benthic invertebrates are a well-established target in evaluations of environmental quality status. Various studies have demonstrated that the macrobenthos responds to anthropogenic and natural stress (Pearson and Rosenberg, 1978; Dauer, 1993) because macrobenthic animals, 1) are relatively sedentary and can't avoid deteriorating water/sediment quality condition, 2) have relatively long life-spans (thus, indicate and integrate water/sediment quality conditions, 3) consist of different species that exhibit different tolerances to stress, and 4) have an important role in cycling nutrients and materials, between the

underlying sediments and the overlying water column (Hily, 1984; Dauer, 1993).

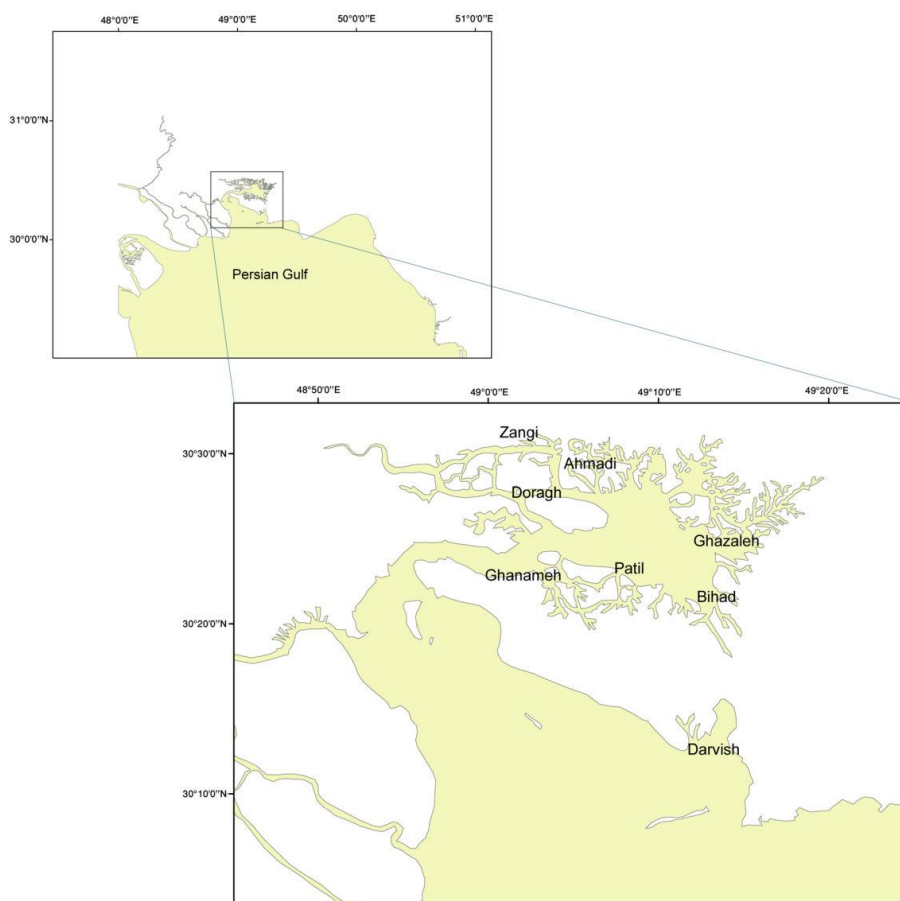
The AMBI (AZTI's Marine Biotic Index) was developed to determine the impacts and the quality status in soft-bottom marine benthic communities (Borja et al., 2000). Subsequently, it has been applied under different impact sources, demonstrating its usefulness in detecting specific localized impacts as well as diffuse pollution (Borja et al., 2003a). The principle advantage of this tool is that it is less influenced by cyclic natural variability of the ecosystem, that changes in impact sources (Muxika et al., 2003).

The aim of this study is to evaluate ecological quality in tidal creeks of Khur-e-Mussa area (Mahshahr creeks), in Khuzestan coastal waters by using AMBI index to determine the spatial sediment contaminant which induced by possible pollution sources in this area: riverine inputs, shipping and fisheries activity, several outfalls and discharging of many types of industries.

## Materials and methods

### *Study area and sampling:*

Several creeks located in Khur-e-Mussa in the Northern Persian Gulf (49° to 49° 20' E and 30° 15' to 30° 32' N). The sampling procedures were done in eight creeks (Ahmady, Ghazaleh, Doragh, Patil, Darvish, Zangy, Bihad and Ghannam) which are depicted in Figure 1.



**Figure 1- Studied area and sampling sites (Ahmady, Ghazaleh, Doragh, Patil, Darvish, Zangy, Bihad and Ghannam) in Khur-e-Mussa creeks, Northwest of Persian Gulf (2005-2006)**

Samples of surface sediment were taken from each creek in different seasons (4 times during one year study). Each sample consists of 3 subsamples. The samples were collected by Peterson grab sampler over a one year study from October 2005 to September 2006. Mean values of replications were calculated for following analysis. Thus 32 samples were used to determine organic matter and macrobenthic communities study. Table 1 illustrates date and depth of sampling. All samples were collected during high tide. Total organic matter of the investigated sediments was determined by the loss on ignition method (Holme and McIntyre,

1984). For macrobenthic study, sediments were immediately passed through a 0.5 mm sieve; benthic organisms were removed from the sieve. The identification was done by stereomicroscope. Margalef richness index ( $R_1 = (S-1)/\ln(n)$ , where S is the number of taxa, and n is the number of individuals) (Jorgensen, 2005), abundance (number of individuals per  $m^2$ ), biomass (dry weight,  $g/m^2$ ) (shell correction were applied) and diversity (Shannon Wiener  $H'$ ) ( $H' = -\sum((n_i/n)\ln(n_i/n))$ , where n is the number of individuals and  $n_i$  is number of individuals of taxon  $i$ ) (Jorgensen, 2005) were calculated.

**Table 1: Sampling date and depth (meter) for each studied station.**

Station/Time	October 2005	March 2005	May 2006	August 2006
Ahmady	12	15	15	4
Ghazaleh	16	9.5	9	8
Doragh	8.5	20	14.5	7.5
Patil	14	31	10	5
Darvish	13	16	12	7
Zangy	12	20	8	7
Bihad	17	31	5	12
Ghannam	13	21	15	18

*Biotic Index:*

BI Model is based on that first used by Glemarec & Hily (1981) and then by Hily (1984). Soft-bottom macrobenthic communities respond to environmental stress by different adaptive strategies. Salen-Picard (1983), Hily (1984) and Glemarec (1986) classified stressful environments regarding their sensitivity to increasing stress to four and then five ecological groups. These groups have been summarized by Grall & Glemarec (1997) as follow: *Group I*: Species very sensitive to organic enrichment and present under unpolluted condition, Carnivores and some deposit-feeder's polychaets. *Group II*: species indifferent to enrichment, always present in low densities with nonsignificant variations with time which included suspension feeders, less selective carnivores and scavengers. *Group III*: species tolerant to excess organic matter enrichment, these species may occur under normal condition; mainly they are suspension deposit-feeders. *Group IV*: Second order opportunistic species, mainly small polychaets, subsurface deposit-feeders. *Group V*: First order opportunistic species, mainly deposit-feeders. In this study, the taxa have been classified

according to the above ecological groups from Borja et al., (2000).

Based on the relative abundance of each ecological group, within each sample, to obtain a continuous index (AMBI) (Borja et al., 2000), where:

$$AMBI = (0 * GI) + (1.5 * GII) + (3 * GIII) + (4.5 * GIV) + (6 * GV) / 100$$

(GI, GII, GIII, GIV and GV) indicate the percentages of individuals for each ecological group. The above mentioned ecological groups (GI, GII, GIII, GIV and GV) are summarized in species not assigned to a group were not taken into account (Epibenthic fauna eliminated from calculation based on Borja (2005)). Contributions of identified taxa in AMBI index were different in each creek and it ranged between 12.7 to 100%. Pollution classification of a site which is a function of the AMBI value was obtained as a result. The distribution of these ecological groups, according to their sensitivity to pollution stress, provides a Biotic Index (BI) with eight levels, from 0 to 7 (Hily, 1984; Hily et al., 1986; Majeed, 1987). Consequently, this represents the benthic community health, represented by the entire numbers of the BI (Table 2). Table 3

shows calculated Ecological Quality Ratio (EQR), with diversity, richness and AMBI, together with the equivalence in terms of ecological status (Borja et al., 2003b).

In order to quantify our categorical variables (presence/absence) while reducing the dimensionality of the data a

Categorical Principal Analysis (CATPA) was performed (Meulman & Heiser, 2001) with the transposed original presence/absence data matrix of taxa (i.e., 32 samples (seasonal averages) \* 29 families) with Minitab 15.1.

**Table 2: summary of the AMBI and BI values (modified from Grall & Glemarec, 1997). The last column shows the proposed equivalent ecological status (Borja et al., 2003b).**

Site Pollution Classification	AMBI value	BI value	Ecological group	Benthic community health	Ecological Status
Unpolluted	$0 < \text{AMBI} \leq 0.2$	0	I	Normal	High status
	$0.2 < \text{AMBI} \leq 1.2$	1		impoverished	
Slightly polluted	$1.2 < \text{AMBI} \leq 3.3$	2	III	Unbalanced	Good status
	$3.3 < \text{AMBI} \leq 4.3$	3		Transitional to pollution	
Meanly polluted	$4.3 < \text{AMBI} \leq 4.5$	4	IV-V	Polluted	Poor status
	$4.5 < \text{AMBI} \leq 5$	4		Transitional to heavy pollution	
Heavily polluted	$5 < \text{AMBI} \leq 5.5$	5	V	Heavy polluted	Very poor
	$5.5 < \text{AMBI} \leq 6$	6			
Extremely polluted	Azoic	7	Azoic	Azoic	Bad status

**Table 3: Calculating the Ecological Quality Ratio (EQR), with diversity, richness and AMBI, together with the equivalence in terms of ecological status (Borja et al., 2003b).**

Diversity(H)	Richness*	AMBI	EQR	Ecological Status
0-1.2	0-15	5.5-7	0-0.25	Bad
1.2-2.4	15-30	4.3-5.5	0.25-0.5	Poor
2.4-3.6	30-45	3.3-4.3	0.5-0.7	Moderate
3.6-4.8	45-60	1.2-3.3	0.7-0.9	Good
>4.8	>60	0-1.2	0.9-1	High

\* = as number of species

## Results

Physical and biological parameters of benthic fauna for each studied creeks are listed in table 4.

Samples of Ghazaleh and Ahmady in summer and Doragh in autumn were

Azoic so they were not contributed to index calculation. The mean of organic matter content in the sediments were high (7.80-13.95 %) and silt-clay percentage

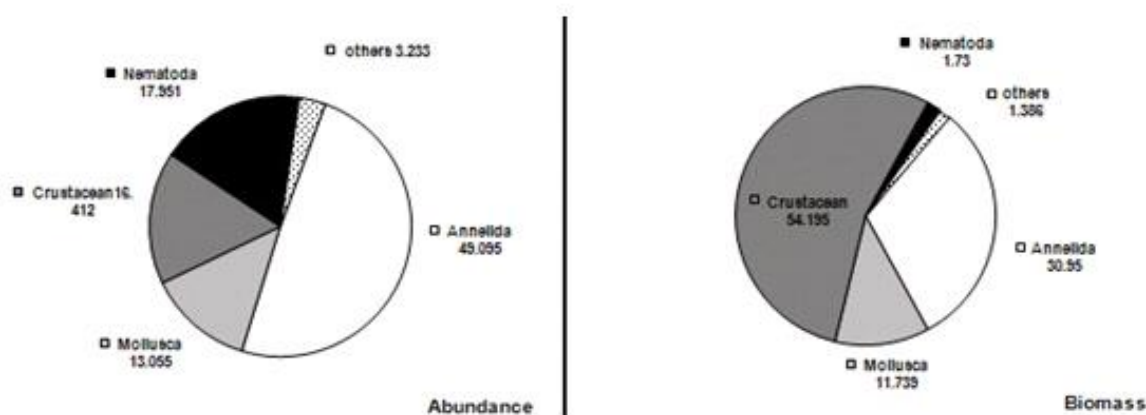
ranged between 52.12-96.88% for all studied creeks.

**Table 4: The ranges and seasonal mean of some of general characteristics of sediments sampled in Mahshahr creeks, N= 48 (2005-2006)**

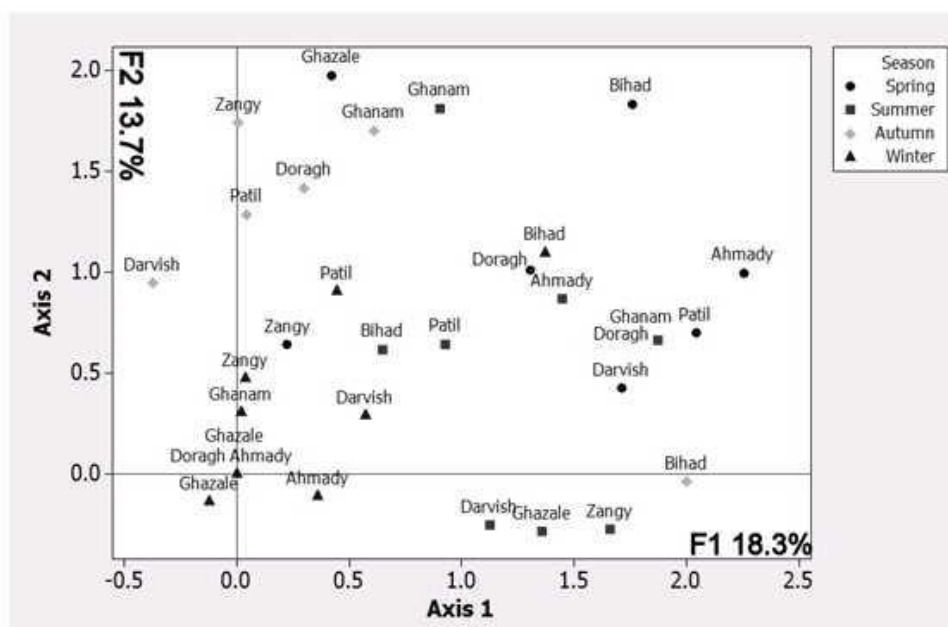
	Bihad	Patil	Doragh	Zangy	Ghazaleh	Ghannam	Ahmady	Darvish
Depth range(m)	5-31	5-31	7.5-20	7-20	9.5-16	13-21	4-15	7-16
Mean TOM (%)	13.95	7.8	8.43	13.54	13.51	8.05	11.75	7.75
Mean Fines<63 µm (%)	78.16	52.12	75.85	82.37	96.88	72.71	83.37	70.47
No. of species range	5-11	4-5	0-6	4-5	0-8	3-7	0-11	3-5
The range of Diversity Index	1.46-1.83	1.39-1.52	0-1.58	0.81-1.52	0-1.31	1.08-1.66	0-1.83	0.93-1.55
The range of Richness Index	0.75-1.24	0.48-0.67	0-0.72	0.4-0.60	0-0.82	0.35-0.85	0-1.278	0.33-0.61
The range of AMBI value	0.68-4.17	1.49-5.02	0.52- 4.96	0.49-4.44	0-2.695	0.99-2.6	0.86-1.44	0-1.2
Mean AMBI	2.495	2.25	2.185	2.54	1.21	1.70	1.23	0.74

A Total of 28 faunal groups of benthic animals were identified. The most abundant groups were polychaets, mollusca, crustacean and nematods (Fig. 2). Identified animals and their ecological groups are listed in Table 5. Two way ANOVAs (without replication) for different seasons and creeks showed no significant differences between creeks based on mean abundance, Richness and Shannon index value except seasonal

significant differences in mean abundance ( $P < 0.05$ ,  $f = 5.712$ ,  $df = 3, 21$ ) and Richness index values ( $P < 0.05$ ,  $f = 4.975$ ,  $df = 3, 21$ ). The CATPCA analysis of the macro invertebrates families extracted two dimensions with eigen values 0.61 for first component and 0.46 for second component that together explained 32% of total variance between all 32 samples (seasonal averages) (Fig. 3).



**Figure 2: The faunal group composition of macrobenthos based on total biomass and abundance occurring in Khur-e-Mussa creeks**



**Figure 3: Categorical principle component analysis ordination diagram of macrobenthic assemblage composition samples. Seasons are represented by different shapes.**

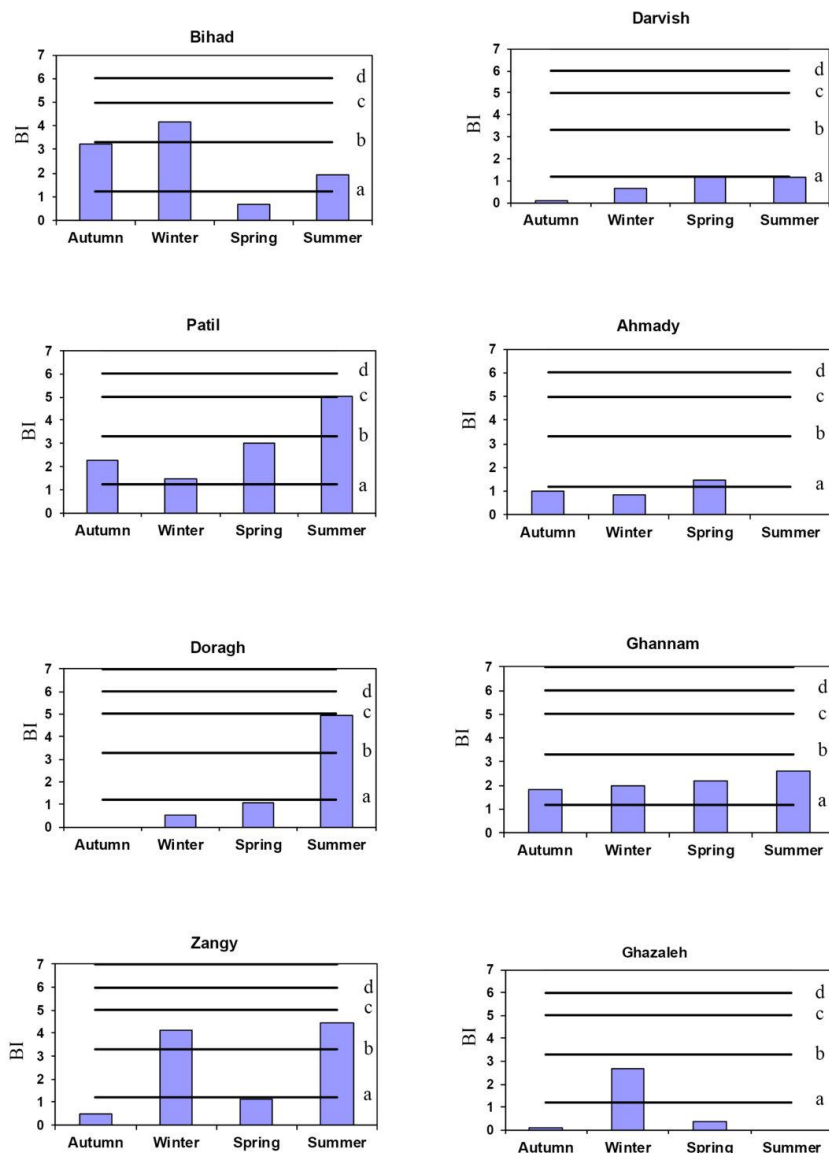
The AMBI values for all studied creeks were calculated (Table 4). Temporal variations of AMBI showed different health status in each studied creek (Fig. 4). Zangy, Doragh and Patil in summer and also Zangy and Bihad in winter showed moderate pollution. Other creeks were classified in unpolluted or slightly polluted categories, in different seasons. All creeks classified in ecological group III with slightly polluted condition, based on the annual mean values of AMBI (according to Table 3), except Darvish which is placed in unpolluted category. The average AMBI values in studied creeks were demonstrated in Figure 5. After applying the AMBI by considering its correspondence with the BI (Table 2), the results were: 2 samples with BI=0, 11 samples with BI=1, 11 samples with BI=2, 2 samples with BI=3, 2 sampled with BI=4, 1 sample with BI=5 and 3 samples

without any benthic animals (azoic) with BI=7. The results were obtained by comparing different biological parameters (Fig. 6), for samples with the same biotic indices. BI=7 is equivalent to an azoic site, so all the biological parameters are equal to 0 in these particular samples. The mean abundance (Fig. 6-d.) increased from 462 ind./m<sup>2</sup> (BI=0) to 1929 ind./m<sup>2</sup> (BI=4 and 5). On the other hand, the mean biomass (Fig. 6-c.) increased from 4.571 g/m<sup>2</sup> (BI=0) to 15.60 g/m<sup>2</sup> (BI=4), and then decreased to 6 g/m<sup>2</sup> (BI=5). Figure 6-b shows the mean richness of the samples rose from BI=0 to BI=3 and then sharply decreased. Diversity index values (Fig. 6-a.) shows a similar pattern to the richness index but the curve fell from BI=3. A two way ANOVA was used to compare AMBI values among different seasons and stations. The results are illustrated in Table

## 6. AMBI values only showed significant difference among seasons.

**Table 5 : list of species and taxa that have been found in all the studied creeks along the whole studied period (the assigned ecological groups (see text) also shown\*)**

Biotic taxa			Groups	
Polychaets	Syllidae	Syllis sp.	II	
	Sabellariidae	Sabella sp.	I	
	Capitellidae	Capitella sp.	V	
	Glyceridae	Glycera sp.	II	
	Spionidae	Polydora sp.	III	
	Nereidae	<i>Nereis persica</i>	III	
	Cossuridae	Cossura sp.	N.A.	
	sternapsidae	<i>Sternaspis scutat</i>	III	
	Dorvilleidae	Dorvella sp.	N.	
Bivalvia	Veneridae	<i>Irus Irus</i>	I	
		Callista sp.	I	
		<i>Paphia undolata</i>	I	
		<i>Corbula odesta</i>	I	
		<i>Dosinia alta</i>	I	
	Tellinidae	<i>Tellina capsoides</i>	I	
	Atyidae	<i>Haminoea vitrea</i>	II	
	Naticidae	<i>Natica vitelius</i>	II	
	Donacidae	Donax sp.	I	
	Acmaeidae	Acmea sp.	N.A.	
	Fasciolaridae	Fusinus sp.	N.A.	
	Collumbellidae	Coumbella sp.	I	
	Potamididae	<i>Cerithidae cingulata</i>	N.A.	
	Crustacean	Caprellidae	Caprella sp.	N.A.
		Anthuridae	<i>Apanthura sandalendis</i>	I
Squillidae		Squilla sp.	I	
Tanaidae		Tanais sp.	N.A.	
Brachiura		Schizophrys sp.	N.A.	
Nematoda	Unknown	-	N.A.	
Nemertina	Lineidae	<i>Lineus albocinctus</i>	III	
Echinodermata	Ophiuridae	<i>Ophiothella venusta</i>	II	
Antozoa	Unknown	-	I	
Bryozoa	Unknown	-	I	
Ascidian	Gorgonidae	Gorgorina sp.	I	



**Figure 4: Seasonal variation of the AMBI values (Biotic Coefficient) for each station. a:Unpolluted, b:Slightly polluted, c:meanly polluted, d:Heavily polluted (Borja et al., 2000). Azoic samples was observed in Ghazaleh and Ahmady in summer and Doragh in autumn.**

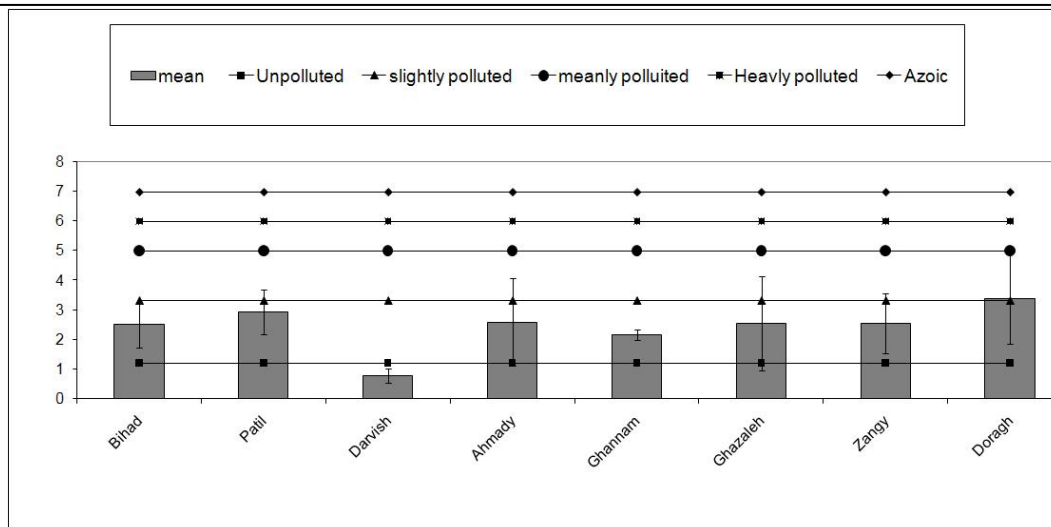


Figure 5: The average AMBI values in studied creeks (2005-2006). Azoic samples: Ghazaleh and Ahmady in summer and Doragh in autumn were omitted from data.

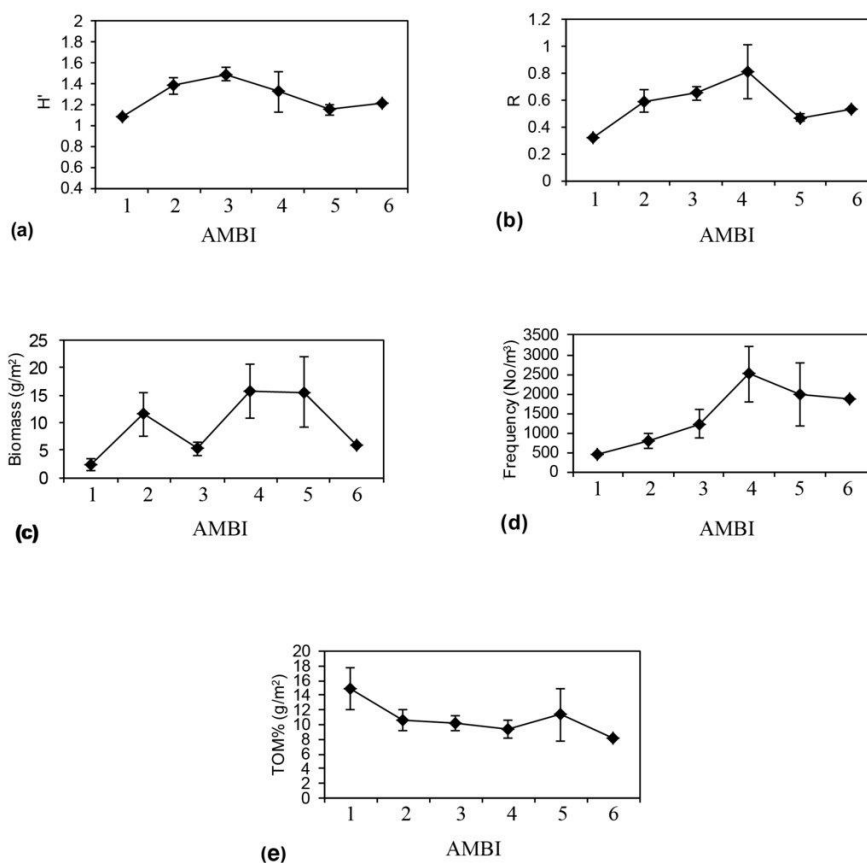


Figure 6: (a-e)- Mean values of different biological parameters obtained on samples having the same indices. a(diversity), b(richness), c(biomass), d(abundance), e(TOM).

**Table 6: Summary of ANOVA table examining AMBI value among seasons and stations**

Source of Variation	SS	df	MS	F	P-value	F crit
Stations	16.25933	7	2.322762	0.648232591	0.711827137	2.487578
Seasons	38.11235	3	12.70412	3.54544405	0.032080222	3.072467
Error	75.24768	21	3.583223			
Total	129.6194	31				

## Discussion

Khur-e-Mussa channel is a shipping channel for commercial, industrial and fishing vessels. Due to different types of exploitation, industrial sources and shipping activities, unbalanced to polluted status are expectable in benthic animals as the best indicators in sediment quality assessment. The creeks are characterized by severe water exchanges in their bottom waters and with predominantly silt-clay sediment. The results of silt-clay analysis and TOM% showed that all creeks are characterized by muddy bottom. In addition to other probable pollution sources, high rate of sedimentation may be a major factor in benthic animal's fluctuation.

The results showed that there are seasonal variations in macrobenthic communities in this area, but other studies made in this creeks showed similar biological characters based on benthic communities. This temporal variations could be occur due to obvious seasonal changes in physical, chemical and biological parameters in this area (according to the previous studies in Khuzestan coastal waters: Sabzalizadeh et al., 1996, Nilsaz et al., 2003, Nilsaz et al., 2005, Dehghan et al., 2006). CATPCA analysis showed clear seasonal variation in macrobenthic assemblage composition (Fig. 3). In CATPCA two cold seasons, autumn and

winter, were discriminated from warm seasons. The creeks are more similar in cold seasons and their higher differences were observed in warm seasons based on macrobenthic assemblage composition.

In the present study, although the number of species initially was increased, but due to appearance of dominant species such as *Capitella* sp., the diversity index values decreased. Seasonal mean of Shannon values in studied creeks are less than seasonal index values in Khuzestan coastal waters (Nilsaz et al., 2005).

In this study with increasing the BI, the density of *Capitella* sp. and Nematods species (as opportunist species) and some other small size polychaets (particularly in summer) and also biomasses of some fewer crustacean taxa were increased. The intermediate disturbance hypothesis predicts that any disturbance of a system will initially lead to an increase in the number of species and if the disturbance persists or increases, and then the species number falls (Gray et al., 2002). In the present study, although the number of species initially increased, but due to appearance of dominant species same as *Capitella* sp., diversity values decreased.

According to Pearson and Rosenberg model (P-R model) 1978, area subject to organic enrichment (pollution source) are generally characterized by smaller

animals. AMBI gradient (from AMBI =0 to AMBI =7), didn't match to TOM% gradient (from low to high) in this study (Fig 6-e.). But Tom% values in all creeks were upper than background values of TOM% in coastal marine sediments [(EPA 2002) and (Burone et al., 2003)]. Gray et al. (2002) investigated the effect of hypoxia and organic enrichment on the coastal marine environment they suggested that major effects on benthic fauna result from hypoxia rather than organic enrichment. The process of sedimentation itself may lead to direct effects on the benthos and sedimentation alone may lead to an increase, whether this is accompanied by an increase in organic matter loading to the sediment or not (Gray et al., 2002).

The biomass curve (Fig. 6-c.) showed the same trend with the predicted P-R model (Pearson & Rosenberg, 1978). The model suggests that the total biomass of organisms initially increased gradually as organic matter load increased above background levels, and then rose sharply to maximum level. The biomass then fallen and showed secondary peak near to, but lower than the maximal abundance. The maximum number of species coincides with the biomass peak. On the other hand abundance rises slowly at the first but then increase rapidly to a maximum (the peak of opportunists) (Gray et al., 2002).

In general, according to the AMBI and BI values, the most creeks are classified in unpolluted and slightly polluted categories except for Zangy, Doragh and Patil in summer and also Zangy and Bihad in winter that showed

moderate pollution. According to biological parameters (diversity and richness values) defined in Table 3, Ecological Quality Ratio (EQR) was calculated which used to compare results of different range of indices and determination of ecological status (Borja et al., 2003b), all creeks were classified in poor and bad status for benthic communities health but based on the mean values of AMBI and BI values, they are placed in unbalanced or transitional to polluted status. Other researches in studied region showed that the most of Khur-e-Mussa creeks are in degraded or severely degraded condition by using B-IBI index (Doustshenas et al., 2009) and AMBI (Okhovat, 2009).

Although the AMBI is particularly useful in detecting temporal and spatial impact, its robustness might be reduced when only a very low number of taxa (1 to 3) and/or individual are found in a sample (Borja & Muxika, 2005). Although this index was based on paradigm of Pearson & Rosenberg (1978), which emphasizes the influence of organic matter enrichment on benthos communities, it was shown to be useful for the assessment of other anthropogenic impacts such as physical alternations in the habitat, heavy metals, etc. (Borja et al., 2000). Furthermore, some biological characteristics of benthic animals such as life span, the length of generation time, depth of living in sediment column and trophic level in addition to fisheries and trawling activity, playing important roles in shifting and succession in macrobenthic abundance and diversity in time (Little, 2000).

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