Chapter 1

Introduction

1-1-Introduction

Mangrove habitats are dynamic ecosystems of the intertidal zone in tropical and subtropical seas. Ecologically, mangroves are defined as trees that inhabit the coastal intertidal zone. A mangrove community is composed of plant species that especial adaptations allow them to survive the variable environmental stresses. Mangroves are defined by their ecology rather than their taxonomy. From a total of approximately 20 plant families containing mangrove species, only two, Pellicieraceae and Avicenniaceae, are comprised exclusively of mangroves. In the family Rhizophoraceae, for example, only four of sixteen genera live in mangrove ecosystems (Kathiresan and Bingham, 2001). Mangrove forests are most productive ecosystems around the world (Lee, 1999). They are often called as 'tidal forests', 'coastal woodlands' or 'oceanic rainforests'. These plants and their associated organisms (microbes, fungi, plants and animals), constitute the 'mangrove forest community' or 'mangal'. The mangal and its associated abiotic factors constitute the mangrove ecosystem (Fig.1-1) (Kathiresan and Bingham, 2001).

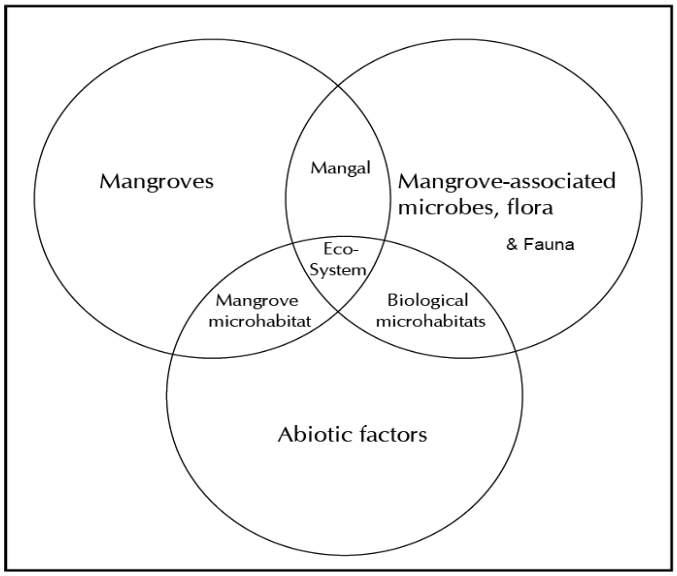

Fig. 1-1. Physical and biological components of mangrove ecosystems (Kathiresan and Bingham, 2001).

Mangroves are distributed circum-tropically, occurring in 112 countries and territories (Kathiresan and Bingham, 2001). The total mangrove area of the world has decreased from 18.8 million to 15.2 million hectares (FAO, 2005). They are distributed between 30^{o}N and 30^{o}S latitudes (Kathiresan and Bingham, 2001). Distribution of mangrove is broader along the eastern coastlines of each continental compare to the western coastlines (Fig. 1-2). This difference in mangrove distribution is due to the presence of warm and cold oceanic currents (Mauris, 2005). Mangrove ecosystems could be divided to six geographic regions.

Iranian mangroves constitute the eastern Africa region and comprise the final border of their distribution in south-east Asia (Fig. 1-2). They consist of two species *Avicennia marina* and *Rhizophora mucronata* (Chandal), with dominance of *A. marina* in almost all mangrove habitats (Safyari, 2003). Latitudinal extension of mangroves in Iranian coasts is largely restricted between 25^{o} N to 27 ^{o} 50' N. Distribution of mangroves starts from the eastern border of the Oman sea, Guatr Bay (55 ^{o} 10' E) and continues to the western side of the Persian Gulf, Bardestan mangrove swamp in Bushehr province (65 ^{o} E) (Safyari, 2003). About 13214.6 hectares of Iranian coast is covered with mangrove forests. Hormozgan province with 12485.14 hectares, Sistan-Va-Baluchestan with 405.52 hectares and Bushehr with 350.5 hectares are three major regions of their dispersion. The most intensive community of Iranian mangrove forests occurs in Gheshm Island with approximately 10000 hectares. Mangrove forests in Bushehr province consist of three major parts including Nayband Bay, Mel-Gonzeh and Bardestan. Among them, Nayband Bay constitutes more than 80% of the total mangrove area (Safyari, 2003). Bardestan mangrove swamp is located in near Bardestan town and make a small patch of mangrove trees.

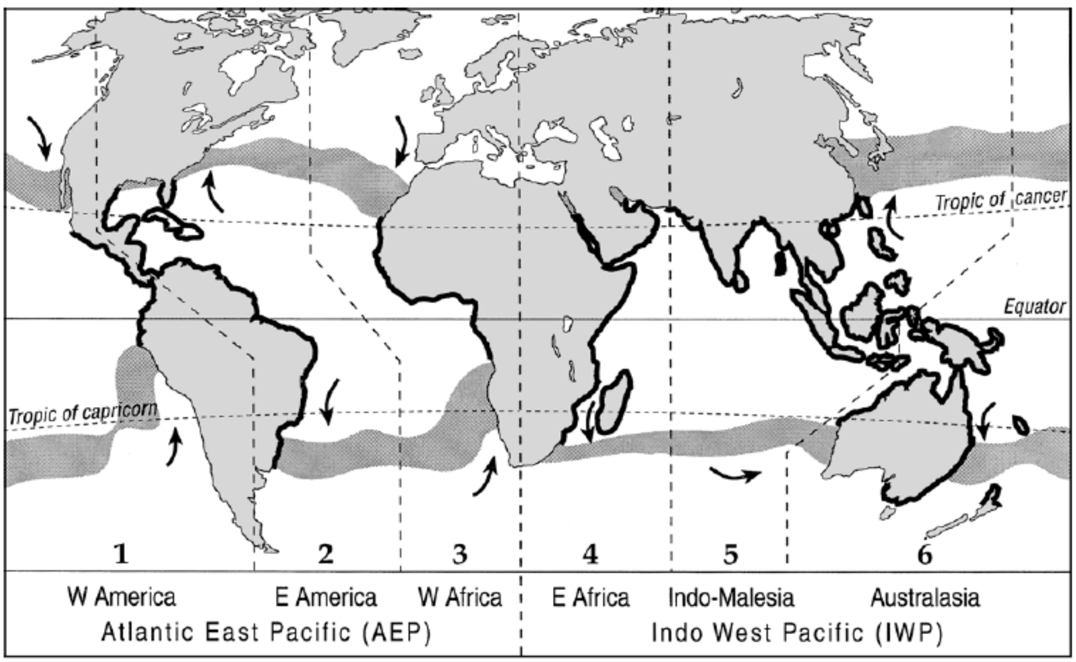

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Fig. 1-2. Global distribution of mangroves with six geographic regions (Mauris, 2005).

The muddy or sandy sediments of the mangal may serve as habitat for a variety of epibenthic, infaunal, and meiofaunal invertebrates. Mangrove sediments generally support higher densities of benthic organisms compare to non-vegetated sediments (Mauris, 2005). Mangroves are inhabited by a variety of benthic invertebrates, such as brachyuran crabs, gastropods, bivalves, hermit crabs, barnacles, sponges, tunicates, polychaetes and sipunculids. Among them, polychaets are the dominant group (Mauris, 2005).

Mangrove fauna often shows horizontal and vertical zonation. Some of them dominate in mud, some on the shrubs and leaves and the others around pneumatophore roots (Mauris, 2005). Distribution of macrobenthos is controlled by sediment grain size, salinity and ground water. Hence, the most successful benthic species in mangrove habitats are those organisms that can adapt to environmental properties of these ecosystems (Guerreiro et al., 1996).

The burrowing activities of certain benthic invertebrates have an important effect on sediment. They assist in flushing toxic substances by increasing water movement through sediment particles. In addition, their deposit and detritivory feeding enhance nutrient recycling (Kristensen et al., 2008). They also are important source of food for vertebrate predators including shallow-water fishes that enter the mangroves in the high tide (Sheaves and Molony, 2000).

Macrobenthic communities as an important part of marine ecosystems are important indices in assessment of ecological health and environmental status (Engle and Summers, 1999). Recent studies have shown strong correlations between benthic fauna and anthropogenic stresses (Bustos-Baez and Frid, 2003; Mooraki et al., 2009). Some studies recommend investigation of macrofaunal assemblages as ecological indicators to evaluate environmental status (Morrisey et al., 2003; Jorgensen et al., 2005). Due to sensitivity of macrofauna to pollution, these organisms have frequently been used to show environmental status (Gesteira and Dauvin, 2000). Living in sediment and sedentary behavior of benthic organisms, make these communities suitable to reflect long period pollutions which is accumulated in sediments (Nixon et al., 1986).

Today, Environmental monitoring and assessment of ecological status have been developed in order to protect marine habitats against destruction (Borja, 2005). Ecological indices have been developed and used for evaluation of ecological and environmental status (see Jorgensen et al., 2005; Pinto et al, 2008, for a revision). The indices are used to assess and evaluate environmental health status, as they relate to a specific qualitative or quantitative feature of systems. Indices are useful tools in decision-making. They can be used as a good reference for management purposes and to assist researches and to guide policies and environmental programs in sustainable management.

Although, one single index can assess environmental status of a specific ecosystem, however, it is unsuitable to use for all habitats. This is because of different features and varied species' composition of different habitats (Borja and Muxika, 2005; Engle and Summers, 1999; Dauvin et al., 2006). It is recommended to use some indices together, to achieve more accurate results (Salas et al., 2006; Carvalho et al., 2006).

1-2-Statement of problem

The Persian Gulf due to its high strategic importance, suffers from various kinds of problems. High level of pollution in this region, as a result of industrial, army and transportation activities, have caused destruction of marine habitats in this region. Sheppard et al. (1992) and Price et al. (1994) mentioned that many factors are responsible for pressures on marine and coastal environments in the Persian Gulf. Among these factors, oil spill, sedimentation, destruction of habitats, discharge of sewages and agriculture fertilizers, excavations and increase of heavy metals are the most important pressures on the Persian Gulf ecosystems.

Anthropogenic and natural impacts have influenced some marine ecosystems. Among them, coastal habitats due to their vicinity to industrial and urban places have been destroyed more than offshore habitats. Bardestan mangrove swamp, as a coastal ecosystem of the Persian Gulf has not been exceptional of this adventure. Short distance of this ecosystem to the urban and industrial places has led to destruction of this habitat. Ecological and economical importance of mangrove habitats, and probable existence of threatening on them are enough and convincing reasons to study their ecological status. Comparison of several studies (Azariah et al., 1992; Bouillon et al., 2002; Chakraborty et al., 1992, Chapman et al., 2006; Daniel and Robertson, 1990; Eksisri et al., 2004, Ellison and Farnsworth, 1990, Ferraris et al., 1994; Lee, 2008; Netto and Gallucci, 2003. etc.) demonstrates that diversity, density and composition of fauna, especially benthic fauna is variable among various mangrove habitats. It seems that unique features of each habitat such as latitude, longitude, mangrove species, estuary morphology, presence or absence of fresh water flows and other variables are responsible for these differences. Performed studies in Iranian coast (Eksisri, 2004, Keshavarz, 2008 and Vazirizadeh., 1997) also confirm these variations and differences in mangrove forests of the Persian Gulf. Therefore, especial study of each mangrove habitat is necessary for comprehensive understanding of a special habitat. Obtained knowledge about ecology and biota of these habitats can be helpful in better management, protection of them against anthropogenic impacts and subsequently successful and sustainable development. Present study was carried out to make a general view of macrobenthic assemblages of Bardestan mangrove swamp and to evaluate the present environmental condition of this habitat.

1-3-Aims and objectives

Main objectives of this study were as follows:

1-To assess environmental and ecological health status of this habitat using the indices AMBI, BENTIX, Shannon-Wiener, Simpson and Berger-Parker indices.

2-To quantify the structure of macrobenthic communities through an analysis of the spatial and temporal variation in the community structure as well as the distribution patterns of dominant taxa.

3-To evaluate the applicability of AMBI index in mangrove ecosystems of Bardestan.

1-4-Hypothesis

1-Bardestan mangrove forest is impacted with anthropogenic stresses and is in bad environmental status.

2-There are five horizontal zones of macrofauna in the intertidal zone of Bardestan mangrove swamp, between the land and central canal.

3-Environmental characteristics influence density and distribution patterns of macrofauna.

4-With regard to applicability of AMBI in muddy shores and successful use of this index in other ecosystems of the Persian Gulf, this index is also applicable to evaluate environmental health of mangrove habitats.

1-5-Literature review

Several studies have been designed and performed worldwide to investigate the environmental status of marine habitats using macrobenthic communities (e.g. Lee, 1999; Fairweather, 1999; Borja et al., 2000, 2003, 2004; Netto and Galucci, 2003; Chapman, 2006; Tolhurst and Chapman, 2007; Sarvankumar et al., 2007; Bigot et al., 2008). Sarvankumar et al. (2007) carried out a survey on mangrove habitats of Gulf of Kachchh – Gujarat in the Indian Ocean. In this study, high number of macrobenthic species (62) belonged to five groups has been identified. The study showed that crustacean was dominant group with 18 species. The highest diversity of macrofauna was observed in the winter. Netto and Gallucci (2003) studied macrofauna and meiofauna in Santa Catarina Island, South Brazil. They recognized a direct relationship between detritus mass and benthos composition. Chapman (2006) has studied relationships between macrofauna and biogeochemical properties of sediments of three different mangrove habitats in Australian ports. This study showed that there was a significant correlation between sediment properties and macrofauna assemblages. They didn't find any particular and certain pattern of variation in distribution of macrobenthic communities.

Many efforts have been undertaken in Iranian coasts of the Persian Gulf to study macrobenthic communities and to assess ecological and environmental status of different coastal habitats. Shakuri (1996) investigated community structure of polychaetes in creeks of mahshahr region. He identified the total number of 69 species belong to 28 families. Shannon-wiener index indicated polluted condition for most stations of this region. Eksisri et al. (2004) studied polychaeta com□munities in Khamir and Laft harbours. Karami (2004) investigated macrobenthic community structure in subtidal zone of Zuhreh river mouth. They reported bivalvia and gastropoda as dominanat groups of macrofauna. They resulted that physical parameters including temperature and salinity had the most effects on the diversity and density of macrobenthos. Population dynamic and secondary production of fiddler crabs of Cirik mangrove swamp has been studied by Mokhtari (2006). They reported that density of fidler crabs increase in summer. Migration of this species to deeper depth of sediment (about 1-2 m depth), for wintering, leads to decrease of individual number of these organisms in the surface sediment. Dehghanmediseh (2007) studied benthic community of Khozestan's creeks. She observed low diversity of macroinvertebrates. Based on AMBI and BI indices, most creeks were classified in unpolluted (34%) or slightly polluted status. Doustshenas (2008) also studied benthic community of Khowr-e-Musa. He totally found 44 invertebrate macrofouna and reported polychaeta as dominant group. Based on B-IBI index, they resulted that more than 72 % of sites had degraded/ severely degraded condition. Macrofaunal assemblages of Khamir port was investigated by Keshavarz (2008). 33 macrobenthic species with dominance of polychaeta was reported. Macrofauna community of Bardestan mangrove swamp has been studied only once before present study, by Vazirizadeh (1997). He investigated macrofaunal communities of the coastal regions of Bushehr province, including Bardestan mangrove area. In mentioned study, low species number (22) was identified and a gradient of variation in species number and abundance of macrobenthos in different tidal levels of Bardestan mangrove swamp was observed. Diversity indices including Shannon and Margalef have indicated moderate to poor environmental status for this habitat.

The new index of present study (AMBI) has been used in various geographic regions. Applicability of this index has been evaluated in several cases worldwide (Borja et al., 2000, 2003, 2004; Salas et al., 2004; Bigot et al., 2008; Muniz et al., 2005; Muxika et al., 2005). However, a few studies investigated environmental health using AMBI index, in Iranian coastal habitats (Dehghanmediseh, 2007; Okhovat, 2009). These studies attained reasonable results. There is no published report regarding application of this index in mangrove ecosystems throughout the world (Borja, per. Comm., 2009). Therefore, present study may be the first try to apply this index in mangrove habitats.

Chapter 2

Material and Methods

2-1-Study area

Present study was carried out in Bardestan mangrove swamp close to Bardestan harbor in Bushehr province, Iran (51º 57' 28" E and 27º 50' 27" N) as is depicted in figure (2-1). The narrow mangrove forests, approximately 50–60 m upshore consisting of patches of mature *Avicennia marina* fringe each side of the estuary. The area of mangrove forest is about 2 hectares (Safyari, 2003). The central canal of the mangrove which is lined across the Persian Gulf, is the only place covered with water in the low tide. Distribution of mangroves is not the same in two sides of the canal. Most trees are located in the western side and have expanded from north to south along the canal. Only a small patch of trees is visible in south part of the eastern side, close to entrance of the estuary. The ecosystem lacks any constant waterway from land, and just seasonal runoffs affect this habitat. Industrial and urban regions surrounded the habitat and have made it so susceptible to anthropogenic impacts.

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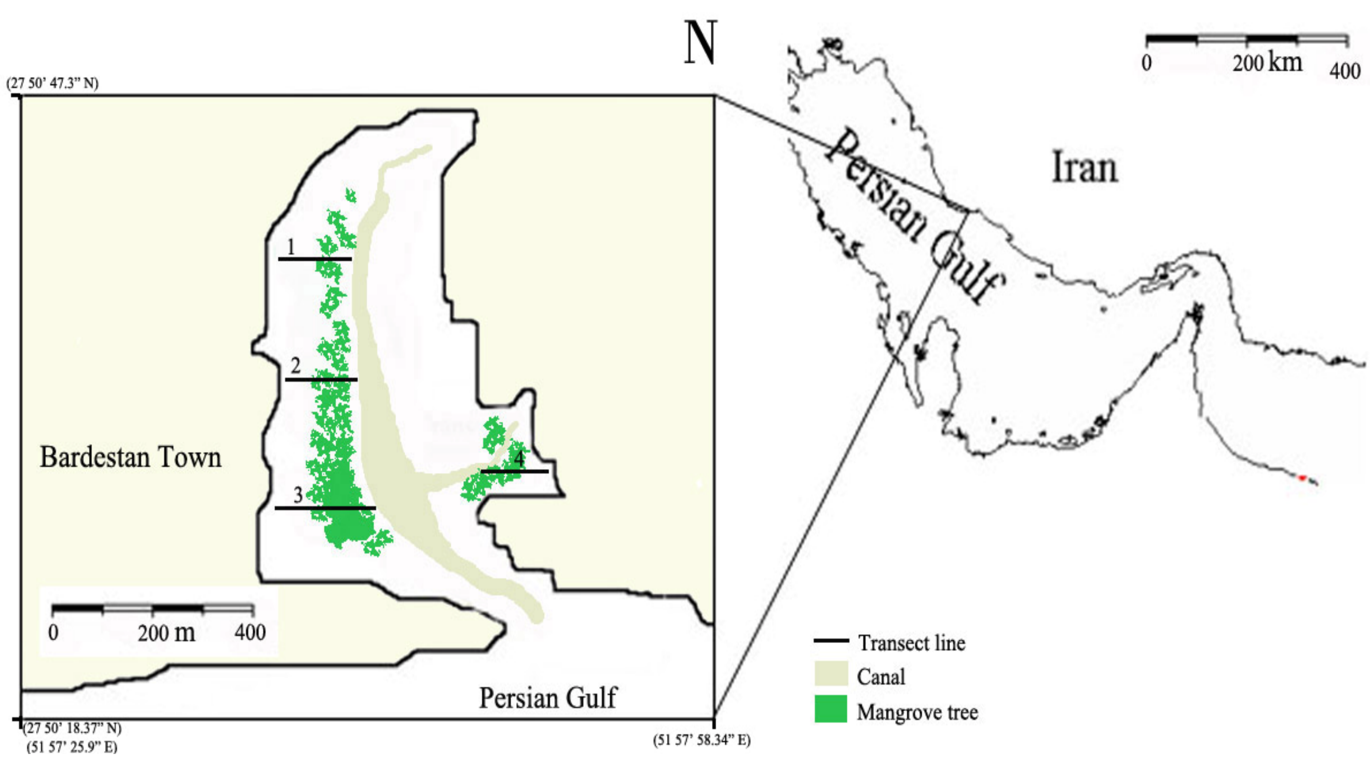

Fig. 2-1. Study area, Bardestan mangrove swamp.

2-2-Sampling design

Sampling was carried out during four seasons including summer, autumn, winter and spring. It was performed in September and November 2008 as well as February and April 2009. Tidal periods and the exact time of spring tide was determined using tide tables presented in Iran-Hydrography website (www.Iranhydrography.ir) (refer to appendix).

For covering the whole habitat including mudflats and mangrove zone, four transect lines were selected. Among them, transects 1, 2 and 3 were located in western side and transect 4 was in eastern part of mangrove swamp (Fig. 2-1). With regard to apparent horizontal zonation, each transect line was divided into five distinct stations from high tide to low tide. High tide zone (Ht) was the first station with the smooth and even substrate where was located in the highest part of the intertidal zone. It would be submerged just a few days a month, during spring tides. Halophilic terrestrial plants are occasionally visible in this station. The second station, Upper mid tide zone (Up), located in lower tidal level with rough surface and many burrows of burrower crabs. The next station was Mid mid tide zone (Mm) with several holes excavated with mudskippers from family gobiidae. Lower mid tide zone (Lm) was the fourth station and was located under the mangrove trees. The surface of this station was covered with algae and mangrove pneumatophores. The last station was the nearest one to the central canal, between mangrove forest and low tide where has a smooth surface. This is named Lower tidal zone (Lt), (Figs. 2-2 and 2-3).

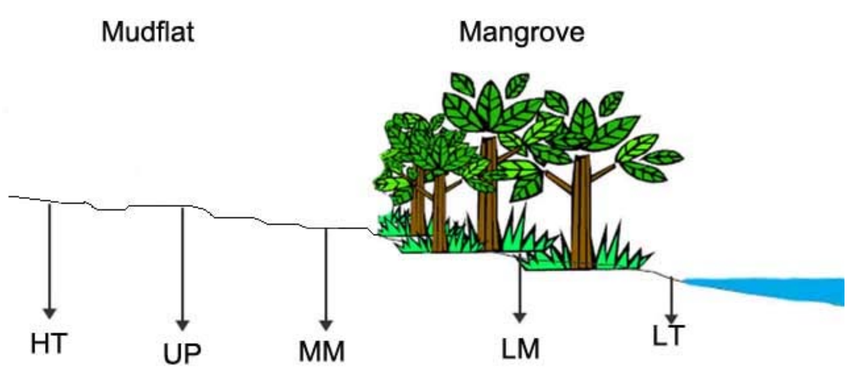

Fig. 2-2. A schematic view of stations dispersion in each transect line.

Fig. 2-3. Five sampling stations. A: Ht, B: Up, C: Mm, D: Lm and E: Lt.

In order to study macrobenthic community, three sediment samples were taken randomly using a 0.25 m^2 quadrate framework to the depth of 10 cm of sediments. For Grain Size (G.S) and Total Organic Carbon (TOC) analysis, three samples of sediment were taken from the same places using core sampler. Samples were put into plastic bags and were carried to the ecology laboratory of the Persian Gulf Research and Study Centre (PGRSC) under cold condition for further laboratory analysis.

Physiochemical factors (including salinity, pH, temperature, dissolve oxygen and redox potential) were determined using portable instruments. The factors were measured through gathered water in hand excavated holes in sediment. Salinity was determined using referactometer (Eleftheriou and Alasdair, 2005). Dissolved oxygen and temperature were measured using a portable oxygen-meter (WTW). pH-meter was used to determine pH and redox potential.

2-3-Sorting and identification of macrofauna

During laboratory work, macrobenthic fauna was isolated in several steps include sieving, staining, sorting, identification and counting. The macrofauna were separated from other materials and sediment using 0.5 mm mesh size sieve. For better separation, the specimens were stained with 0.5 $g.L^{-1}$ rose-Bengal. Sorting then was done under the stereoscope microscope. Sorted macrofauna were fixed in ethanol 70% until identification. Macrofauna were identified to species or genus level using valid identification key books such as:

- Atlas of the Persian Gulf mollusks (Hosseinzadeh et al, 2001)
- Seashells of eastern Arabia (Bosch et al, 1995)
- The invertebrates (Stochowistsch, 1992)
- Guide to shells (Emerson and Jacobson, 1974)

And some valid websites such as:

- www.sealifebase.org
- www.marinespecies.org
- www.zipcodezoo.com

2-4-Sediment analysis

2-4-1-Grain size

Grain (particle) size of sampled sediment was determined by applying sieve series of 4 mm to 63 µm (Eleftheriou and Alasdair, 2005). First, 25 g oven-dried sediment of each sample was weighted and transferred into a beaker containing 250 ml water. 10 ml of 6.2 g/l sodium hexametaphosphate $(NaPO3)_6$ was added to the aid of dispersion of clay particles. The particles were broken up with a glass rod and then were stirred mechanically for 10-15 minutes. The mixture then was allowed to soak overnight and stirred again for an additional 10–15 minutes. The dispersed sediment suspension was washed to a 63 µm sieve. The water was replaced in the basin at intervals and sieving and washing was continued the sediment until no further fines were washed out. Sediments remained on the sieve were dried again at oven in 100 C^o and then sieved through sieve series using a mechanical shaker. Finally, the remained sand in each sieve was weighted and their percentage was calculated using following equation:

Sediment % = sediment weight × 100/25

Sediment particles were divided into four grain size groups of:

Coarse sand (> 500 µm)

Medium sand (500- 250 µm)

Fine sand (250-125 µm)

Mud (< 125 µm).

(Houte-Howes et al., 2004).

2-4-2-Total Organic Carbon (TOC)

To determine TOC of sediment, Colorimetric method was used. This method is based on the oxidation of soil organic matter by dichromate-sulphuric acid mixture and measurement of intensity of green color of the chromium sulphate to give directly the amount of the oxidized carbon (Gupa, 2001).

To prepare samples:

1- One gram of the oven dried soil was placed in a dry 100 ml conical flask.

2- 10 ml of 1NK_2Cr_2O was added and swirled a little followed by 20 mL of concentrated sulphuric acid. The solutions was swirled again and kept for 30 minutes.

3- The flask content was carefully centrifuged to a clear state.

4- The green chromium sulphate color of the supernatant layer was read in the colorimeter (4802 UV/VIS DOUBLE BEAM SPECTROPHOTOMETER-UNICO), after adjusting the blank solution (without soil) to zero, using 660 nm filters.

In order to estimate TOC of sediment, 13 samples of anhydrous sucrose (10 to 250 mg. L^{-1}) were made and read with the colorimeter using 660 nm filter. A standard curve was drawn plotting the concentrations of carbon (in sucrose) on X axis and colorimeter reading on Y axis. This curve and its equation were used to estimate the TOC of samples using colorimeter reading.

2-5-Ecological and environmental indices

2-5-1-Shannon-Wiener

The index takes from:

H'= $-\sum p_i \log_2 p_i$

Where p_i is the proportion of individuals found in the species i in the sample. The real value of p_i is unknown, but it is estimated through the ratio N_i/N, where N_i is the number of individuals of species i and N is the total number of species (Jorgensen et al., 2005).

2-5-2-Simpson

Simpson defined his index on the probability that two individuals randomly extracted from an infinitely large community could belong to the same species. This index takes from:

D = $\sum p_i^2$

Where p_i is the individuals' proportion of the species *i*. The equation of this index for finite community is as followed:

$$D = \sum \left[n_i (n_i - 1) / N(N-1) \right]$$

Where n_i is the number of individuals in species *i* and *N* is the total number of individuals (Jorgensen et al., 2005).

2-5-3-Berger-Parker

The index expresses the relative importance of the most abundant species, and takes from:

$$D = n_{max} / N$$

Where n_{max} is the number of individuals of dominant species and N is the total number of individuals (Jorgensen et al., 2005).

2-5-4-AMBI

For development of the AMBI, the soft-bottom macrofauna is divided into five groups according to their sensitivity to an increasing stress:

(I) Species very sensitive to organic enrichment and present under unpolluted conditions.

(II) Species indifferent to enrichment, always in low densities with non-significant variations with time.

(III) Species tolerant to excess of organic matter enrichment. These species may occur under normal conditions, but their populations are stimulated by organic enrichment.

(IV) Second-order opportunist species, mainly small sized polychaetes.

(V) First-order opportunist species, essentially deposit-feeders.

This index is based on the percentages of abundance of each ecological group of one site (biotic coefficient (BC)), which is given by

$$\text{AMBI} = \left[\frac{(0 \times \%G_I) + (1.5 \times \%G_{II}) + (3 \times \%G_{III}) + (4.5 \times \%G_{IV}) + (6 \times \%G_V)}{100} \right]$$

Where G_I, G_{II}, G_{III}, G_{IV} and G_V are abundance of individuals belong to each group. The marine biotic index varies continuously from 0 (unpolluted) to 7 (extremely polluted) (Borja et al., 2002) (Fig. 2-4).

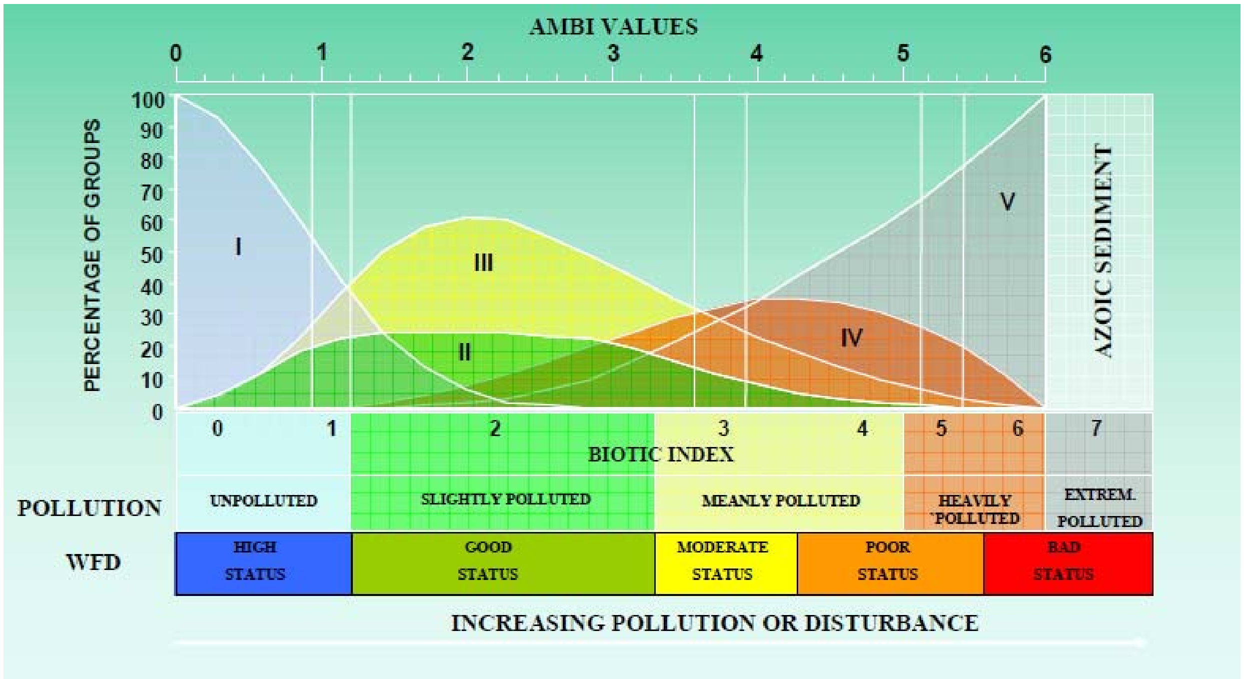

Figure 2-4. AMBI values and percentage of the different ecological group (Pinto et al, 2008).

2-5-5-BENTIX

The BENTIX index is based on AMBI (Borja et al., 2000) and reduces macrozoobenthic data in three wider ecological groups. In this index, each species receive a score from 1 to 3, which represent their ecological group (Simboura and Zenetos, 2002). The groups are described as:

Group 1 (G_I): including the species that are sensitive or indifferent to disturbances (k-strategies species).

Group 2 (G_{II}): including the species that are tolerant and may increase their densities in case of disturbances, as well as the second-order opportunistic species (r-strategies species).

Group 3 (G_{III}): including the first-order opportunistic species.

The formula that expresses this index is given by

$$\text{BENTIX} = \left[\frac{6 \times \%G_{I} + 2 \times (\%G_{II} + \%G_{III}}{100} \right]$$

In Table (2-1), classification and used color for different values of each index is shown to describe different environmental and ecological statuses.

Table 2-1. Classification of ecological and environmental status according to ranges of AMBI, BENTIX, Shannon-Wiener, Simpson and Berger-Parker. According to references: AMBI (Borja et al., 2002), BENTIX (Simboura and Zenetos, 2002), Shannon-Wiener (Jorgensen et al., 2005), Simpson and Berger-Parker.

Ecological status	**Shannon-Wiener**	**Simpson**	**Berger-**	**AMBI**	**BENTIX**	**Pollution classification**
High	>4	0-0.25	0-0.25	0-1.2	6-4.5	Unpolluted/normal
Good	3-4			1.2-3.3	4.5-3.5	Slightly polluted
Moderate	3-2	0.25-0.5	0.25-0.5	3.3-5	3.5-2.5	Moderately polluted
Poor	2-1	0.5-0.75	0.5-0.75	5-5.5	2.5-2	Heavily polluted
Bad	1-0	0.75-1	0.75-1	5.5-7	2-0	Extremely polluted/Azoic

2-6-Data analysis

Pearson's correlation test was used in order to determine the correlation between biological and physicochemical parameters as well as correlation among value of various indices. Biological parameters (i.e. abundance and species number) among different stations and different tidal levels were compared using One-Way ANOVA analysis. K-Related test (Chi-Square, Friedman Test) was used to compare biological and physiochemical parameters among different seasons or different transect lines.

The Bray–Curtis Similarity Index, based on log (x + 1) transformation, was calculated to detect the similarity between sampling stations.

"Bio diversity pro ver. 2" software was used to estimate Shannon-Wiener, Simpson and Berger-Parker indices. AMBI values were measured using "AMBI-wxp4.1" software freely available at http://www.azti.es. The statistical analyses were performed using SPSS statistical software (Ver. 14.0 for Windows) and "STATISTICA" programs.

Chapter 3

Results