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River health assessment using macroinvertebrates and water quality parameters: A case of the Shanbeh-Bazar River, Anzali Wetland, Iran

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Abstract

The study of benthic macroinvertebrate communities is one of the most common methods for assessing the effects of stresses on the biological conditions of aquatic ecosystems and water quality monitoring. This research was conducted to study the changes in the macroinvertebrate communities in the Shanbeh-Bazar River, Anzali International wetland and the effects of environmental factors. Sampling was done seasonally from spring to winter in 2017 from the. Bio-indicators, MDS, PCA, correlation and ANOSIM were used in this study. A total of 3963 macroinvertebrate belonging to 3 phyla, 9 orders of 17 families were identified**.** The two families of Chironomidae and Tubificidae were the most abundant, and the dominant group of the Shanbeh-Bazar River. The bio-indicators analysis varied from medium pollution to heavy pollution at different stations. MDS plot showed that sampling stations were clearly separated into 3 groups. ANOSIM and PCA analysis were shown that the human activities and urban wastewater effects on benthic communities such as increasing the resistant families (Tubificidae). The increasing of agricultural activities and the destruction of marginal forests at Stations 3 and 4 were caused the abundance of Chironomidae and Tubificidae increased. Since the Shanbeh-Bazar River has urban disturbance, especially wastewater inputs, agriculture, fishing and recreational boats, macroinvertebrate diversity decreased with increasing contaminants and stresses, led to an increase in resistant species. With the increase of agricultural and the destruction of marginal forests, the amount of organic matter increased in the water body. Anthropogenic activities are indicated to have the most negative effects on the river ecosystem. Thus, it was concluded that the river health of Shanbeh-Bazar River is within unacceptable range.

Keywords: Macroinvertebrates, Bioindicator, Environmental factors, Land use, Shanbeh-Bazar River, Anzali Wetland

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Introduction

The study of macrobenthos invertebrate communities is one of the most common methods for assessing the effects of stresses on the biological conditions of aquatic ecosystems and water quality monitoring (Muniz *et al*., 2005). The inconsistency of the heterogeneous communities that appear to disrupt the structure of the population and its ecological relationships can disrupt the ecosystem's food webs and damage the higher levels of the food chain (Fries and Bowles, 2002). Quantitative characterization of these changes by the biodiversity indices of benthic macroinvertebrate communities provides suitable information to researchers and managers (Lydy *et al*., 2000). Several indicators are used based on the evaluation of physical and chemical indicators to determine the water quality. Due to the limited information available in terms of time and place, current aquatic ecologists use aquatic organisms as water quality indicators to determine the perimeter quality of rivers and their responses to environmental conditions (Kenney *et al*., 2009). The study of waters bodies, the identification of the pollution of rivers is not sufficient due to the usual methods of measuring the physical and chemical parameters of water since it only provides information at sampling time (Gholizadeh *et al*., 2012a).

Therefore, macroinvertebrate communities are one of the suitable methods for determining and evaluating the health and impact of human activities on reducing the quality of rivers (Gholizadeh *et al*., 2012b). Macroinvertebrates as biological indicators reflect conditions in their living environment and play a significant role in the diet of fish in the rivers, and the sea. Therefore, determination of their diversity and their seasonal variations will have a significant role in determining the final natural production potential of the river and classification of rivers in terms of pollution and final judgment on this ecosystem (Guill *et al*., 2003). Macroinvertebrate are different in terms of resistance to pollution and oxygen depletion and in some species, this difference is more obvious so that some species can survive in clean water and free from any pollution, while some of these organisms can survive in high-contaminated waters (Yokoyama *et al*., 2007).

Ghane *et al.* (2006) carried out a biological evaluation of the Chafrud River (leading to the Anzali International Wetland) using the macrobenthos population structures which pollutant stations were divided into different groups. Jalili *et al*. (2011) investigated the community structure of macrobenthos of the southwestern part of the Anzali Wetland and its correlation with organic matter. Mirzajani *et al*. (2008) investigated the size of sediment particles, organic materials and some affective physicochemical parameters on the biodiversity of macroinvertebrate communities in the Shanbeh-Bazar

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River located in Anzali International Lagoon to the Caspian Sea. Due to the Increasing of the urban population in Bandar Anzali and the impact of human activities such as urban wastewater, agricultural wastewater, and shipping activities on the aquatic environment and macroinvertebrate of this area, the present study seems to be necessary.Macroinvertebrates were used in this study to determine the effects of human activities on environmental conditions in the Shanbeh-Bazar River.

The objectives of the research were to (i) study on the benthic macroinvertebrates for water quality assessment in the Shanbeh-Bazar River; (ii) evaluate the changes of water quality in the Shanbeh-Bazar River.

Materials and methods

Study area

Anzali Wetland is located in the southwest of the Caspian Sea coast in Guilan Province, 49^{o} 28´ N and 37^{o} 29´ E. Anzali Wetland was surrounded from north part to Anzali, from south part to the city of Someh Sara, from east part to to Pir Bazaar and from west part to to the Kapur Chal and Abkenar in Anzali is limited. Stations along the river have been selected using a variety of land use, including agriculture, urban areas, and port area. (Fig. 1).

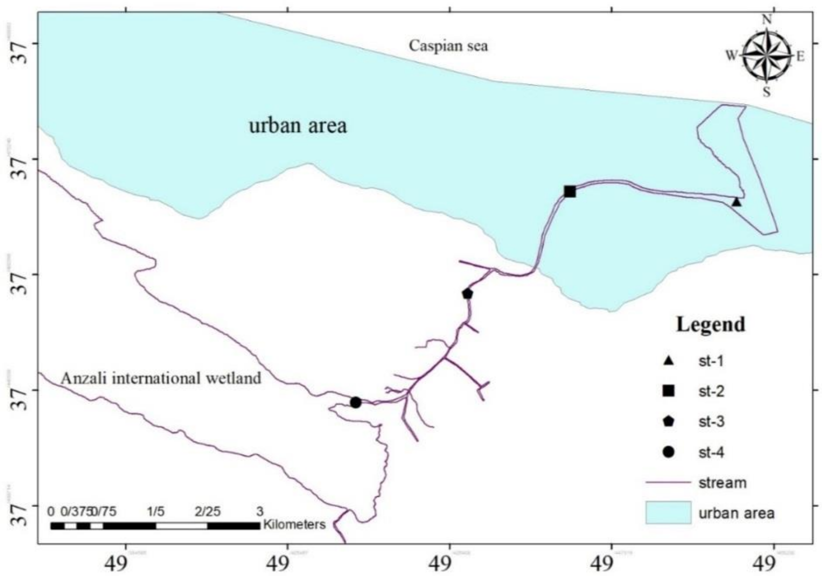

Figure 1: Map of the study area, showing location of the sampling stations in Shanbeh-Bazar River, Iran.(ref)???

Sampling was done seasonally from spring 2017 to winter 2018 of from the Shanbeh-Bazar River (from the Anzali International Wetland to Caspian Sea) in 3 replications from each station.

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Table 1: Geographical location of study stations.

Station	Region	N	E
st-1	Anzali breakwater	93' 46° 37	38' 46°49
st-2	Urban area	14 ' 47° 37	05' 45° 49
st-3	Agricultural area	60 ' 45° 37	91 ' 42° 49
st-4	Anzali Wetland	48 ' 44° 37	05 ' 42° 49

Sampling method

Sampling stations were selected for monitoring and evaluation from four different region according to different land use. This increased the probability of diversity in current river section and biological parameters among the sampling stations and, therefore, leaded to more accurate and inclusive study of changes biological and environmental parameters. Besides macroinvertebrates sampling, water temperature (◦C), dissolved oxygen (mg L^{-1}), electrical conductivity (mos cm^{-1}), turbidity (NTU) and pH were measured using portable digital water-checker model (HACH sensionTM 156-378 multiparameter meter). Nitrate, Nitrite, Ammonia and phosphate were measured following APHA (1998) methods in the laboratory in Gonbad Kavous University.

Sediment particles and macroinvertebrate samples were collected. Macroinvertebrate were collected using a hand-operated 25×25 cm Van Veen grab was lowered into the seabed to grab the benthic sample. Each sample collection was made in triplicate. The macroinvertebratein the sediment were passed through a 0.5 mm sieve. The samples were then preserved in 10% formalin diluted in seawater. The individuals were identified to the lowest possible taxonomic level using the available keys of Needham (1976); Quigley (1986), and Richard and Merritt Kenneth (2008), Samples were kept in pre-labeled polyethylene bags and return to the laboratory for further analysis. For sediment texture analysis, the samples were dried at 100°C for 24h, powdered, and sieved through a 0.5mm sieve. Grain size was analyzed following a hydrometer method described by Bouyoucos (1962). Organic matter was determined by loss on ignition (LOI) method in a Muffle furnace. The furnace was calibrated before every use with a reference digital thermometer that can measure at least 500°C (Abrantes *et al.,* 1999). Sediment particle sizes were measured on samples treated with hydrogen peroxide to remove organic matter, followed by wet sieving through a 0.063-mm mesh using distilled water with 10% sodium hexametaphosphate as dispersant. An aliquot of the <0.063-mm fraction was dried and weighed to measure silt and clay content. The >0.063-mm fraction was dried at 60°C, then separated with stacked, graded sieves, into >1.0 mm, 0.5−1.0 mm, 0.25−0.50 mm, 0.125−0.250 mm, and 0.063−0.125 mm fractions, for 6 min on a sieve shaker. Each size fraction was weighed (Wentworth, 1992) (Table 2).

Table 2: Classification of sediment size according to Wentworth 1992.

Sieve size	Sediment classification
1000 μm	Very coarse sand
425 μm	Medium sand
250 μm	Fine sand
125 μm	Very fine sand
63 μm	Silt and clay

Data analysis

The biological indices of Shannon-Wiener (H ’), Simpson (1−D), Margalef and Menhinick's richness index and Pielou were calculated in terms of abundance using the PAST software package (Paleontological Statistics v.2.17) (Hammer *et al*., 2001). The water quality assessment for a range of bio-index values were presented in table 3.

Table 3: Ranking of bio-index values using benthic macroinvertebrates

	H’	1 – DS	Ranking
1	> 3.25	> 0.90	Very light pollution
2	2.20 – 3.25	0.65 – 0.90	Light pollution
3	1.40 – 2.20	0.40 – 0.65	Low moderate pollution
4	0.80 – 1.40	0.25 – 0.40	High moderate pollution
5	0.10 – 0.80	0.10 – 0.25	Heavy pollution
6	< 0.10	< 0.10	Very heavy pollution

Notes: H’ (Shannon-Wiener Diversity Index); Ds (Simpson Dominance Index).

A principal component analysis (PCA) based on a correlation matrix among samples was used to analyze the physico-chemical data. PCA was accomplished using CANOCO v.4.5 software (TerBraak and Smilauer, 2002). Differences in environmental variables were surveyed among the 4 stations using one-way ANOVA at a significance level of $p<0.05$. To survey variation in community structure among the 4 stations, one -way ANOSIM was employed based on the Bray-Curtis matrix obtained from log(x + 1) transformed abundance data, using the software PRIMER 5.0 (Clarke and Warwick, 2014).

Results

A total of 3963 macroinvertebrate belonging to 3 phyla, 9 orders of 17 families were identified in the Shanbeh-Bazar River during the study period (Table 4). The insect class with 4 orders and 8 families was the most diverse, but the most abundance belonged to Oligochaeta. The two families of Chironomidae and Tubificidae had the highest frequency and distribution and were the dominant fauna of the benthic invertebrates of the Shanbeh-Bazar River. Station 4 has the most diversity and abundance of benthic invertebrates compared to other stations during sampling seasons in Anzali wetland (Table 5).

Oligochaeta was the most abundant in the spring (91%), summer (73%), and autumn (72%). The highest amount of Diptera (88.44%) was observed in winter and the lowest was in summer (2.41%) (Fig. 2).

Table 4: Macroinvertebrates observed in Shanbeh-Bazar River.

phylum	Class	Order	Family	Species
Annelida	Clitellata	Oligocheata	Naididae Tubificidae Lumbriculidae	
	Polychaeta	Phyllodocida	Nereidae	*Hediste diversicolor*
		Spionida	Spionidae	*Streblospio* sp.
		Sabellida	Sabellidae	*Fabricia sabella*
Arthropoda	Insecta	Diptera	Chironomidae Ceratopogonidae Psycodidae Chaoboridae	*Chironomus* sp. *Culicoides* sp.
		Odonata	Coenagriidae Gomphidae	*Coenagrion* sp.
		Ephemeroptera	Baetidae	
		Trichoptera	Leptoceridae	
	Malacostraca	Amphipoda	Gammaridae	*Gammarus* sp
Mollusca	Gastropoda		Lymnaeidae Valvateadae	*Radix lagotis* *Valvata cristata*

Table 5: Density (ind/m^2) at sampling stations of Shanbeh-Bazar River.

family	*sb1*	*sb2*	*sb3*	*sb4*	*st1*	*st2*	*st3*	*st4*	*sp1*	*sp2*	*sp3*	*sp4*	*sz1*	*sz2*	*sz3*	*sz4*
Lumbriculidae	128	1440	1408	1504	-	-	-	192	-	-	-	48	-	-	-	-
Tubificidae	128	13456	5904	9776	32	48	112	512	1504	208	1232	3888	32	128	368	64
Naididae	-	-	-	-	-	64	16	-	-	-	-	16	96	160	272	-
Chironomidae	-	-	1056	2016	-	-	-	32	32	192	1456	208	-	32	4240	9168
Coenagriidae	-	-	16	-	-	-	-	-	-	32	-	80	-	-	-	-
Ceratopogoidae	-	-	32	32	-	-	-	-	-	-	-	-	-	-	-	32
Nereidae	32	-	-	-	48	-	-	-	-	-	-	-	-	-	-	-
Psychodidae Pupa	-	-	-	-	-	-	-	-	-	32	-	-	-	16	-	-
Chir.Pupa	-	-	16	16	-	-	-	-	-	-	48	-	-	-	48	128
Psychodidae	-	-	32	-	-	-	-	-	-	32	-	-	-	32	-	-
Gammaridae	-	-	48	-	-	-	-	-	-	-	-	-	-	-	32	208
Spionidae	-	-	-	-	64	32	32	128	16	16	-	-	-	-	-	-
Sabellidae	-	-	32	-	-	-	-	16	-	-	-	32	-	-	32	48
Lymnaeidae	-	-	-	-	-	-	-	-	-	-	-	432	-	-	64	128
Valvatidae	-	-	-	-	-	-	-	-	-	-	-	32	-	-	16	48
Beatidae	-	-	-	-	-	-	-	-	-	-	-	-	-	16	16	-
Chaoboridae	-	-	-	-	-	-	-	-	-	-	16	-	-	48	-	-
Leptoceridae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16
Gomphidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16

sb (spring), st (summer), sp (autumn), sz (winter).

In the results of sampling in all seasons, the highest level of species richness index was in the second station of the winter season (1.84) and the lowest was in the second station of the spring season (0.14). The highest level of Evenness index was observed in the first and second stations (0.96) in the summer and the lowest was in the first station (0.94) in the autumn.

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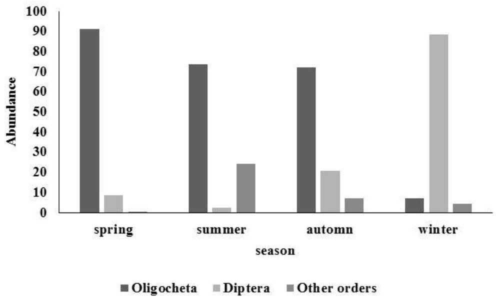

Figure 2: abundance of macroinvertebrate identified on Shanbeh-Bazar River.

Also, in the study of diversity indices, the highest Shannon-Weiner diversity was observed in the second station in the winter (1.55) and the lowest in the first station in the autumn. The highest Simpson diversity was observed at the second station (0.76) in the winter and lowest was at the fourth station (0.13) in the winter (Table 6).

Table 6: Diversity indices of macroinvertebrate in sampling stations.

Sample	Species (S)	Abundance (N)	Margalef (R)	Pielou	Shannon (H)	Simpson (D)
sb1	3	18	0.69	0.87	0.96	0.62
sb2	2	931	0.14	0.45	0.31	0.17
sb3	9	534	1.27	0.42	0.92	0.48
sb4	5	834	0.59	0.48	0.78	0.42
st1	3	9	0.91	0.96	1.06	0.72
st2	3	9	0.91	0.96	1.06	0.72
st3	3	10	0.86	0.72	0.80	0.51
st4	5	55	0.99	0.69	1.12	0.60
sp1	3	97	0.43	0.14	0.15	0.61
sp2	5	30	1.17	0.74	1.20	0.66
sp3	4	172	0.58	0.57	0.79	0.52
sp4	8	296	1.23	0.34	0.71	0.31
sz1	2	8	0.48	0.81	0.56	0.42
sz2	7	26	1.84	0.79	1.55	0.76
sz3	9	318	1.38	0.31	0.69	0.29
sz4	10	616	1.40	0.16	0.38	0.13

According to multi-dimensional scaling (MDS), the groups of benthic invertebrates were clearly divided into 3 groups. In the first group, which is far apart from other groups, Station 4 of the winter is observable. In the second group, stations that were relatively close to each other were located around one point. In the third group, stations 2, 3 and 4 of the spring season are located slightly apart (Fig. 3).

Significant differences between physical and chemical parameters of water between different stations and seasons are shown in table 7. Temperature, pH, and NO3 had a significant difference between different seasons. Other parameters such as DO, transparency, and NO2 had a significant difference between spring and summer and between autumn and winter. EC, salinity, and TDS were significant in summer compared with other seasons.

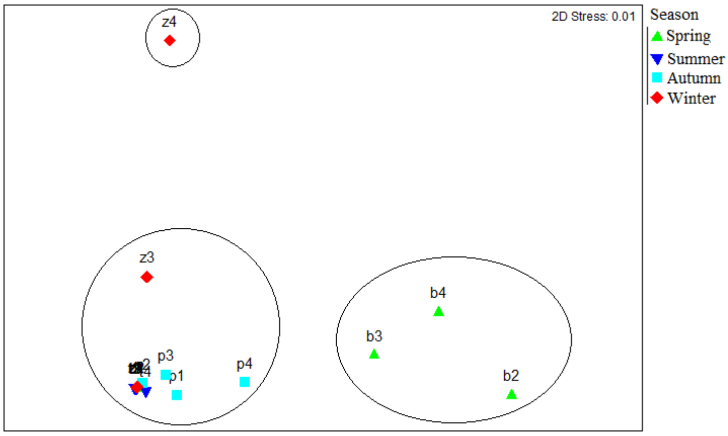

Figure 3: MDS carried out on macroinvertebrate abundance based on the Bray-Curtis similarity index in Shanbeh-Bazar River.

Table 7: Environmental variables recorded in sampled stations in Shanbeh-Bazar River.

Station	Temperature (ºC)	DO (mg/L)	Depth (m)	Transparency (m)	pH	EC (µs.cm^{-1})	Salinity (ppt)	TDS (mg/L)	Hardness (mg/L)	Po4 (mg/L)	NO2 (mg/L)	NO3 (mg/L)	NH4 (mg/L)
Sb1	23^{c}	6.5^{a}	1^{a}	0.5^{b}	6.71^{b}	2820^{a}	1.33^{a}	1308^{a}	1020^{a}	0.172^{b}	0.017^{a}	3.766^{ab}	1.418^{a}
Sb2	24^{c}	6.8^{a}	1.2^{a}	0.6^{b}	6.13^{b}	554^{a}	0.27^{a}	271^{a}	360^{a}	0.201^{b}	0.015^{a}	3.595^{ab}	0.895^{a}
Sb3	23^{c}	7.12^{a}	1.1^{a}	0.45^{b}	6.62^{b}	387^{a}	0.19^{a}	190.9^{a}	310^{a}	0.077^{b}	0.032^{a}	4.794^{ab}	0.157^{a}
Sb4	23^{c}	7.37^{a}	0.6^{a}	0.45^{b}	7.36^{b}	346^{a}	0.17^{a}	172.5^{a}	290^{a}	0.042^{b}	0.01^{a}	3.253^{ab}	0.064^{a}
St1	25^{d}	6.3^{a}	1.2^{a}	0.25^{a}	7.32^{c}	6660^{b}	3.61^{b}	3530^{b}	1360^{b}	0.063^{a}	0.11^{a}	9.416^{b}	0.308^{a}
St2	26^{d}	6.58^{a}	1^{a}	0.45^{a}	7.2^{c}	6680^{b}	3.6^{b}	3490^{b}	1400^{b}	0.043^{a}	0.064^{a}	8.046^{b}	0.157^{a}
St3	27^{d}	6.12^{a}	0.4^{a}	0.45^{a}	7.35^{c}	6220^{b}	3.37^{b}	3280^{b}	1280^{b}	0.025^{a}	0.037^{a}	5.650^{b}	0.081^{a}
St4	27^{d}	7.25^{a}	0.4^{a}	0.25^{a}	7.31^{c}	5740^{b}	3.07^{b}	2990^{b}	2400^{b}	0.032^{a}	0.039^{a}	4.451^{b}	0.105^{a}
Sp1	9^{a}	6.3^{b}	0.8^{a}	0.45^{b}	5.78^{a}	1521^{a}	0.76^{a}	758^{a}	200^{a}	0.001^{a}	0.215^{b}	25.50^{c}	0.122^{a}
Sp2	8^{a}	7.1^{b}	1.2^{a}	0.6^{b}	5.52^{a}	1444^{a}	0.74^{a}	719^{a}	220^{a}	0.005^{a}	0.198^{b}	24.48^{c}	0.122^{a}
Sp3	8^{a}	11.9^{b}	1.3^{a}	0.45^{b}	6.38^{a}	1046^{a}	0.52^{a}	516^{a}	200^{a}	0.003^{a}	0.127^{b}	40.23^{c}	0.116^{a}
Sp4	7^{a}	13.47^{b}	0.55^{a}	0.5^{b}	6.72^{a}	773^{a}	0.38^{a}	377^{a}	160^{a}	0.0006^{a}	0.091^{b}	19.85^{c}	0.087^{a}
Sz1	9^{b}	9.91^{b}	0.42^{a}	0.5^{b}	7.56^{c}	963^{a}	0.48^{a}	475^{a}	620^{a}	0.007^{a}	0.289^{b}	1.198^{a}	2.336^{b}
Sz2	9^{b}	11.45^{b}	1.3^{a}	0.6^{b}	7.67^{c}	987^{a}	0.49^{a}	486^{a}	578^{a}	0.010^{a}	0.189^{b}	1.301^{a}	1.354^{b}
Sz3	8.5^{b}	11.78^{b}	1.2^{a}	0.7^{b}	7.76^{c}	848^{a}	0.41^{a}	415^{a}	514^{a}	0.013^{a}	0.184^{b}	0.565^{a}	1.348^{b}
Sz4	9^{b}	11.62^{b}	0.9^{a}	0.45^{b}	7.95^{c}	613^{a}	0.3^{a}	297a	434^{a}	0.022^{a}	0.137^{b}	0.856^{a}	0.796^{b}

b (spring), t (summer), p (autumn), z (winter)

In evaluating the amount of organic matter content obtained in all seasons, there was a significant difference between station 2 and other stations (p<0.05). The highest amount (14.88) was observed at station 1 in the winter and the lowest was (1.47) at station 1 in the summer.

Based on the size of the sediments, the substrate materials were almost different at various times and stations. At stations 1 and 2, very soft sand formed the highest level of the substrate in all seasons. The substrate types (mud and clay) showed a significant difference at stations 3 and 4 compared to other stations in all seasons (p<0.05) (Table 8).

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Table 8: Organic matter and sediment particle size at sampling stations.

Station	OM	M.S	Fine.S	V.F.S	S.C
Sb1	9.3[a]	5.98[ab]	24.71[c]	56.52[c]	12.77[b]
Sb2	3.95[b]	5.89[c]	24.84[d]	64.27[c]	4.98[a]
Sb3	10.45[a]	15.43[cd]	9.44[b]	34.98[b]	40.13[c]
Sb4	6.45[a]	0.29[a]	1.78[a]	6.52[a]	91.40[d]
St1	1.47[a]	4.74[ab]	14.71[c]	74.93[c]	5.28[b]
St2	2.55[b]	11.61[c]	24.75[d]	58.77[c]	4.86[a]
St3	8.3[a]	15.46[cd]	7.28[b]	29.76[b]	47.49[c]
St4	9.51[a]	0.2[a]	0.10[a]	2.30[a]	97.41[d]
Sp1	6.25[a]	8.82[ab]	12.41[c]	58.00[c]	20.85[b]
Sp2	2.58[b]	23.56[c]	22.66[d]	49.58[c]	4.185[a]
Sp3	7.08[a]	6.08[cd]	2.43[b]	43.64[b]	47.83[c]
Sp4	6.47[a]	3.32[a]	1.07[a]	6.64[a]	88.96[d]
Sz1	14.88[a]	6.39[ab]	7.54[c]	42.78[c]	43.28[b]
Sz2	1.77[b]	29.05[c]	18.52[d]	49.05[c]	3.35[a]
Sz3	10.21[a]	10.72[cd]	7.09[b]	31.86[b]	50.32[c]
Sz4	10.79[a]	5.88[a]	1.79[a]	8.33[a]	83.99[d]

Organic matter (OM); Medium-Sand (M.S); Fine-sand (F.S); very fine-sand (V.F.S); silt & clay (s.c). Different letters indicate significant differences (p<0.05).

Principal Component Analysis (PCA) using the Euclidean distance was accomplished to determine differences in environmental parameters between stations and seasons (Fig 4). According to Chatfield and Collins (1980), the components with eigenvalues more than one would be considered. From the PCA results analyzed using STATISTICA.8, the first three components were measured with eigenvalues more than one (PC1=5.88; PC2=4.61 and PC3=2.42). The total variance was calculated for PCA1 (41%) and PCA2 (20.78%) (Table 9). As a result, the first three components can be considered significant in the analysis. The First component (PC1) was the component that well characterized the connection between macroinvertebrate abundance and the other few important variables as shown in table 10 (Temperature, Dissolved oxygen, Transparency, EC, TDS, Salinity, Hardness and NO2). In the second component (PC2), sediment particle size, organic matter and depth are important parameters considered (with 25.36% of variance). Chemical variables (PO4, NO3, NH4) and pH were observed in the third component (PC3).The fluctuation of macroinvertebrate in all study locations was investigated according to the environmental parameters. The macrobenthos Chironomidae, Nereidae, Chironomidae pupae, Spionidae, Beatidae and Chaoboridae significantly correlated with the first component.

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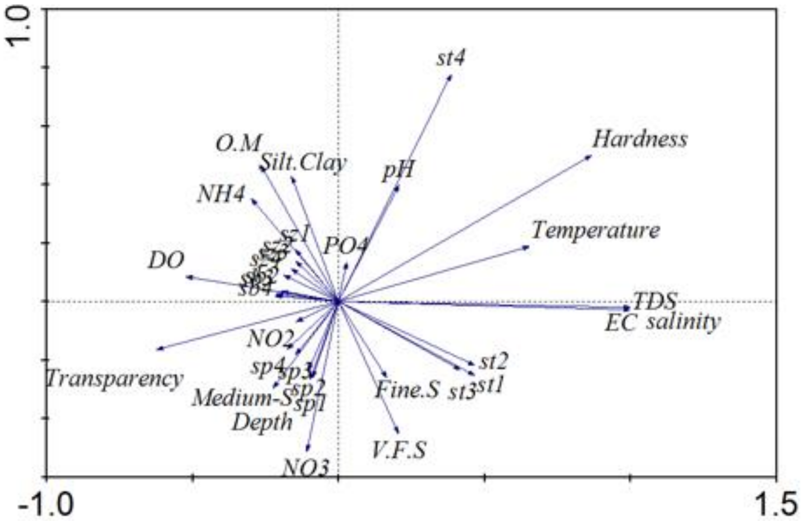

Figure 4: PCA (Principal component analysis) of the 18 environmental parameters. Vectors show the two-dimensional (PC1 and PC2) correlation structure among the environmental parameters (% of variance explained = 42.7).

Table 9: Principal Components Analysis Eigenvalues, number of components is 3 Principal Components Analysis.

NO.	Eigenvalues	Total variance%	Cumulative eigenvalue	Cumulative%
1	5.89	32.70089	5.88616	32.70089
2	4.61	25.63456	10.50038	58.33545
3	2.42	13.45257	12.92184	71.78801

Table 10: Rotated principal component loadings for 18 standardized sediment parameters and environmental factors. The three PCA factors had eigenvalues more than 1.

Variables	PC 1	PC 2	PC 3
Temperature	**-0.36**	0.01	-0.14
Dissolved oxygen	**0.29**	0.15	0.01
Depth	0.08	**-0.34**	0.01
transparency	**0.27**	-0.19	-0.19
pH	-0.04	0.25	**-0.34**
Electrical conductivity	**-0.37**	0.01	0.06
Salinity	**-0.37**	0.01	0.07
TDS	**-0.37**	0.01	0.07
hardness	**-0.34**	0.12	-0.10
Phosphate	**-0.13**	-0.16	**-0.34**
Nitrite	**0.23**	-0.06	0.04
Nitrate	0.08	-0.10	**0.56**
Ammonia	0.14	-0.04	**-0.50**
Organic matter	0.11	**0.29**	-0.24
Medium sand	0.08	**-0.27**	-0.005
Fine sand	-0.10	**-0.41**	-0.16
Very fine sand	-0.08	**-0.40**	-0.04
Silt-Clay	0.06	**0.44**	0.07

Other than these macrobenthos families, Ceratopogoidae, Psychodidae pupa, Psychodidae, Gammaridae, Sabellidae, Lymnaeidae, Valvatidae, Leptoceridae and Gomphidae significantly correlated with the second component. Lumbriculidae, Tubificidae, Naididae and Coenagriidae abundance also correlated with the third component (Table 11).

There is a clear pattern of separation of the stations in different seasons in the Shanbeh-Bazar River. The most important environmental factors in the summer are fine sand, very fine sand, temperature, salinity, EC and TDS The stations in autumn was correlated with medium sand, depth and transparency. The other stations was showed high correlated with DO, NH4, organic matter and silt-clay (Fig. 4). The ANOSIM results between each pair of stations (Table 12).

Table 11: The Spearman Rank Correlation of macroinvertebrate and three principal components in all stations.

Macroinvertebrates	Means	Std.Dev.	PC 1	PC 2	PC 3
Lumbriculidae	18.44	36.01	0.008	-0.01	-0.22
Tubificidae	146.06	251.81	0.108	-0.07	-0.12
Naididae	2.44	4.81	0.349	-0.06	-0.43
Chironomidae	72	151.53	0.39	0.38	-0.16
Coenagriidae	0.50	1.31	0.30	0.11	0.39
Ceratopogoidae	0.37	0.80	0.19	0.36	-0.11
Nereidae	0.31	0.87	-0.47	-0.24	-0.13
Psychodidae pupa	0.18	0.54	0.24	-0.5	0.22
Chironomidae pupea	1.00	2.12	0.4	0.34	-0.05
Psychodidae	0.18	0.54	0.14	-0.15	-0.14
Gammaridae	1.12	3.28	0.27	0.31	-0.18
Spionidae	1.12	2.15	-0.73	0.31	0.20
Sabellidae	0.62	1.02	0.34	0.50	-0.09
Lymnaeidae	2.43	6.89	0.35	0.37	0.21
Valvatidae	0.37	0.88	0.41	0.44	-0.02
Beatidae	0.12	0.34	0.37	-0.16	-0.34
Chaoboridae	0.25	0.77	0.28	-0.27	-0.04
Leptoceridae	0.06	0.25	0.22	0.314	-0.13
Gomphidae	0.06	0.25	0.22	0.31	-0.13

Table 12: Values of analysis ANOSIM (*p*<0.05%) for the stations River.

Groups	Rho	P (%)
Station 1 & 2	0.427	5.7
Station 1 & 3	0.25	14.3
Station 1 & 4	0.458	5.7
Station 2 & 3	0.281	5.7
Station 2 & 4	0.125	14.3
Station 3 & 4	0.083	28.6

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Discussion

In this study, 17 families were identified from different stations of macroinvertebrate, which 67.34% belonged to Oligochaeta, 29.77% were in the Insecta and only 2.87% belonged to other macroinvertebrate. Also, based on the number of identified benthic families, 58.93% belonged to Tubificidae, 29.05% belonged to Chironomidae and 12.02% belonged to other families which indicate the dominance and richness of these two families in comparison with other assemblages and is similar with the results obtained by other researchers (Ghane *et al.*, 2006; Richard and Merritt Kenneth, 2008). The increase in environmental pollution, including urbane, industrial, and unrefined agricultural wastewater is effective in the distribution and abundance of resistant species to contaminants such as Tubificidae in the aquatic systems (Alves *et al.,* 2006; Gholizadeh and Heydarzadeh, 2019).

Families belonging to the Diptera order, such as Chironomidae, can be present in a large number of habitats and exhibit a significant amount of abundance (Spies and Reiss, 1996; Suriano and Fonseca-Gessner, 2004). Although some species of this family are sensitive to environmental changes, most of them can tolerate various pressures in the aquatic environment. In fact, the superiority of Chironomidae is due to human interactions in the aquatic environment, such as the destruction of the forest and the introduction of used inorganic contaminants in agriculture, which are entered into the water due to the irrigation of agricultural land (Corbi *et al.*, 2010; Gholizadeh and Heydarzadeh, 2019).

Therefore, due to the increase of agricultural land and the degradation of the dominant vegetation near the sampling area at stations 3 and 4, and observing the high frequency of Chironomidae in these stations, this instance can be compatible with the present study. The reduction in the diversity and frequency of invertebrates is associated with human activities, elimination of forests and the increase of agricultural land. In general, the performance of agricultural lands is harmful to the health of the aquatic environment, which is mainly due to the destruction of marginal forests, changing their use to agricultural land and excessive use of pesticides and chemicals. These events will destroy habitats and increase the level of suspended particles, sludge, chemical contamination, and the loss of biodiversity, the removal of sensitive species, and the advent of resistant species in habitats (Suriano and Fonseca-Gessner, 2013).

In this study, significant amounts of silt and benthic plants were found in stations 3 and 4 in different seasons in agricultural areas. From station 1 to the station 4 (Anzali wetland), the substrates were softer and the amount of silt was increased, but at stations 1 and 2, the amount of soft sand was more than silt. This event is due to the increase in agricultural lands for human agriculture activities and animal

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husbandry, the destruction of coastal forests and an increase in the amount of sand which these factors increase the amount of silt in the aquatic environment (Ferreira and Casatti, 2006). The results showed that in addition to increasing the amount of contamination, the destruction of vegetation and forest around Anzali wetland and the change in the type of the substrate could be an effective reason for the removal of sensitive taxa such as Molluscs and Odanota and the replacement of stable species such as Chironomidae and Tubificidae.

The PCA results showed that there are differences between sampling stations in different seasons, which indicates water quality and different substrate types in different seasons. In the present study, the stations in the summer season had significant changes in the frequency of benthic macroinvertebrates in comparison with other seasons, so that saltwater taxa such as Sabellidae, Spionidae, and Nereida were present in this season and the most dominant families such as Chironomidae and Tubificidae had a lower frequency, and according to PCA, they had a direct correlation with temperature, TDS, EC, and Salinity. So that, with increasing temperature and evaporation, salinity, solid soluble materials and saline species were increased, and the observed effects at the four station in the summer were due to a large distance from the sea compared to other stations in this season. In autumn, the use of chemical fertilizers, rainfall after a dry summer period, washing of agricultural land by rain and entering of animal wastes around the river could be a reason for the increased level of nitrite and nitrate in this season, which according to the PCA Chart these factors had been effective on macroinvertebrate invertebrates. Some factors such as urbane wastewater, animal waste, and agricultural drainage at stations 2 and 3 around the river can increase nitrogen combinations, which can affect the abundance of benthos communities (Zhang and Huang, 2011).

ANOSIM Table showed the most similarity between Stations 3 and 4. These stations showed the least similarity with stations 1 and 2. In this study, first and second stations were distinguished from physicochemical and biological factors in comparison with the third and fourth stations. One of the main characteristics of stations 3 and 4 is the change in the aquatic plants. In general, aquatic plants play an important role in the aquatic zone and can be effective in physicochemical factors and distribution of benthos communities. Aquatic plants can also provide a suitable environment for nesting and form a colony of these macroinvertebrates (Mabidi *et al.*, 2020). However, one of the factors that can affect aquatic plants and algae is the presence of phosphate, which is used as a food to grow algae. The presence of phosphate and nitrogen at high levels indicates the interference of human and agricultural sewage pollution in water resources. Phosphates are introduced into the water through industrial

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wastewater, return water from agricultural activities, and can significantly affect the water purification phenomenon (Eivazi *et al.*, 2010). The first and second stations are located in the ports and the city, respectively, which are directly affected by urban wastewater and recreational boats. These stations also did not have any aquatic plants, and an unpleasant smell and a low level of diversity are tangible. These conditions indicate water stagnation and accumulation of contaminants in it and damage to the aquatic environment.

The decrease in the diversity of invertebrates in these stations can indicate the destruction of the aquatic environment due to the increase in the entry of urbane sewage, industrial and shipping activities. The evacuation of untreated urbane and industrial wastewater in the aquatic system increases the electrical conductivity and nutrient levels and reduces the oxygen concentration (Nedeau *et al.*, 2003), and these factors cause changes in species and density (Ogbeibu and Oribhador, 2002).

Direct entry of urban wastewater has also had a significant impact on the area that distinguishes stations 3 and 4. Changes in phosphate levels, electrical conductivity, and dissolved oxygen in the urban area indicate a source of inputs for contaminants and urban sewage in that area, which makes it different from other regions (Frizzera and Gama Alves, 2012). There were significant differences in the environmental factors of the two regions in terms of the distinction between urban and agricultural areas in the Shanbeh-Bazar River. Multidimensional scaling (MDS) shows the distribution of sampling stations in a two-dimensional space in the studied river. Sampling stations were clearly divided into 3 sections. This means that the four station in the winter was distinguished from other stations due to the observation of two rare families in this area (Gomphidae and Leptoceridae). A significant increase in the Chironomidae family in the winter samples was due to the increased sensitivity of this family to pollution compared to the Tubificidae family, and it could be due to changes and improvement of environment conditions (Thorp and Covich, 2009). The Gastropoda and Odanota were present at the stations in the fall. Also, the lumbriculidae was found in spring, which includes the third group of cluster test.

The values of Shannon-wiener and Simpson indices were homogeneous in the study area and varied in the medium to heavy range of pollution at different stations. The analysis of these indices showed that the biodiversity and stability of benthic macroinvertebrates community in urban and industrial areas decreased, which indicates the quality level of water in the Shanbeh-Bazar River and Anzali wetland. The Pielou's evenness index indicates the distribution and frequency of community members among species.
Based on this equation in a particular place or time, when the abundance of

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species varies, species diversity decreases because of species evenness and when the species populations are more balanced and less varied, diversity and evenness increase. (Ludwing and Reynolds, 1988).

According to the Margalf index, the most richness was in winter and the lowest was in the spring. One of the indicators is the total richness or a total number of identified species. . The species richness index of an ecosystem is mainly indicative of the environmental conditions in terms of its proper biological condition because favorable environmental conditions increase the presence of species (Bagheri Tavani and Jamalzade, 2014).

Anzali Lagoon is one of the most important coastal wetlands in the country, which has a great value for migratory birds and fish. This lagoon has a permanent contact with the Caspian Sea through the output of the Anzali wave breaker, but there is no available information on the connected rivers to the lagoon and the Caspian Sea. Thus, it was necessary to consider one of its important rivers. Since the Shanbeh-Bazar River has a large number of inlets from untreated urban wastewater, agriculture, fishing and recreational boats, and increasing the number of contaminants and stress, the species diversity of benthic invertebrates has decreased and has led to an increase in resistant species. With the increase of agricultural land and the destruction of marginal forests outside the urban area, the amount of organic matter that has been washed out from agricultural land has increased in the water body. Also, the amount of silt has been increased in this region. This increase has led to a reduction or elimination in sensitive species which are dependent on hard substrates. Otherwise, enhancement of aquatic plants has created a better environment than the urban area by creating a safe living environment for invertebrates, increasing oxygen, reducing the amount of contaminated chemical and suspended matters. But the increase in these plants due to the presence of chemical materials such as phosphate reduces water levels and increases substrate layers which are disturbing in the future. At Anzali lagoon station, the diversity and distribution of benthic invertebrates and the number of physicochemical contaminants showed better conditions than other stations, which could indicate its better situation than other stations in terms of pressures from human activities around the river. The contamination rate was higher in the seasons with the high flow rate such as autumn and winter. But in hot and humid (summer) season, due to the decrease in water levels and increase in evaporation, a significant increase in the number of soluble materials and salinity was occurred, which resulted in the elimination of benthic macroinvertebrates and the replacement of saline invertebrates.

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