Evaluation of Water Quality of the Karun River in Ahvaz, Iran Based on Biological Indicators (Daphnia and Crustaceans)

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ABSTRACT
Given the fact that the Karun River, as the most important source of water supply in Khuzestan province, has recently been affected by various pollutants such as sewage, monitoring its water quality is a necessity. This study aimed to determine the water quality of the Karun River based on biological indicators (Daphnia-crustaceans).
In each season of the year, water samples were obtained from three stations at three different points along the river. After measuring the physical and chemical parameters, the samples were left to settle for 24 hours in the laboratory. Then 1 cc of the sediment was placed on the slide to study the diversity and frequency of crustaceans. Data were analyzed using SPSS version 21.
In the present study, the frequency and variety of crustacean species isolated from the samples decreased from the initial station of the river to the final station. In addition, 27 species of Protozoa were isolated from three stations, of which Daphnia and Rotifer species with an average of 2.166 and 4.833 and standard deviation of 1.984 and 5.166, respectively had the highest frequency compared to other species. The results of the Pearson correlation showed that there is a direct and statistically significant relationship between dissolved oxygen (DO), Turbidity, Daphnia, and Rotifers.
Based on the results of this study, it can be stated that the entry of effluents into river water causes a significant reduction in water quality, which in turn reduces the diversity and frequency of crustaceans living in the water. This is possibly due to an increase in sewage discharge from Ahvaz city to Karun river.

Keywords: Biological Indicators, Water Quality, Crustaceans, Daphnia, the Karun River, Iran

INTRODUCTION
Rivers are vital sources of fresh water for agriculture, drinking, and industry, and their pollution can affect the environmental quality of other parts of nature. River pollution is one of the most important environmental issues that has received much attention today [1]. River water pollution can in fact be considered an indicator of human-induced environmental pollution because rivers are the only sources that travel a long way through cities, villages as well as industrial and agricultural areas [2]. As far as pollution is concerned, every river has a certain capacity for self-purification, and if the entry of pollutants into a river goes beyond that capacity, then the environmental problems in it will become sustainable this river will be permanent [3].
In the past, controlling the quality of aquatic environments and creating meaningful measurements of water health and quality focused more on Ecotoxicological, physical, and chemical variables, and less attention used to be paid to biological variables. However, today biological criteria are considered as important components. Water quality is considered important because biological communities reflect the ecological quality and show the effects of various stressors. In this respect, biological assessment (monitoring) is one of the practical and cost-effective methods to determine the ecological health of water and decide whether human activities reduce water quality. Because it can provide a comprehensive assessment of water health at all times [4]. Running water ecologists use aquatic organisms as water quality indicators to determine the environmental quality of streams and rivers and consider their reactions to environmental conditions [5].
Benthic invertebrates are often regarded as suitable biological indicators due to the following reasons. Compared with fish, they have less mobility, and movement and tend to avoid the influence of pollutants. The life cycle of benthic invertebrates is longer than that of algae and bacteria, and they are more sensitive to water quality. They also have a wide range of tolerance to pollutants among other species and using different strategies they change their

nutrition and energy compared to higher beings. Unlike fish that move away from contaminated sites, some benthic invertebrates are found in damaged or poisonous habitats. Finally, these biological indicators are easily collected using sampling and analysis methods and respond relatively slowly to changes in environmental factors in the ecosystem [6].

Increased water consumption, reduced natural water reserves, environmental pollution, and increased human activities needing water have made the assessment of water resource quality an important issue in recent years. Under such circumstances, monitoring water quality changes, especially those in drinking water, at regular intervals seems necessary. Traditional methods of water quality monitoring include sampling water and transferring it to the laboratory and performing various physical and chemical tests which are both time-consuming and expensive. Furthermore, these methods often fail to detect non-point contaminants, while the biological monitoring method, using biological variables to assess water quality, is much more appropriate. In this respect, it seems that large benthic invertebrates are among the most suitable candidates to be used as biological indicators [7].

The use of these benthic invertebrates is based on the assumption that rivers not affected by pollutants are host to more benthic taxa as well as pollutant-sensitive and non-resistant species, as opposed to rivers that are under pollution pressure where less diverse and resistant species are predominant. Thus, these biological indicators are good guides for tracking changes in an ecosystem over a long period [8-9].

Worms, mollusks, crustaceans, and larvae of aquatic insects are the main representatives of large benthic invertebrates in water resources [10-12].

The use of crustaceans in bioassay studies has a long history. It can be argued that the benefits of using crustaceans to assess the quality of effluents discharged into receiving water are greater than those of all bioassay methods. This has led to the increased development of this method compared to other bio-experimental methods. Daphnia Magna is one of the biomarkers used to assess the toxicity of metals in aquatic environments, because some of the characteristics of this species, such as rapid growth and high fertility rate, have made it a suitable indicator to measure changes in aquatic environments. Toxicological studies on Daphnia Magna have clearly shown the role of these crustaceans in relation to organic and inorganic toxins, mixed pollutants such as endorphins and drugs, adverse changes in growth and reproduction, immobility, and even mortality [13-14]. As a zooplankton, this species is present in most freshwater habitats and feeds on algae. Due to the fact that it is used by higher levels of the food chain, it plays an important role in the transfer of matter, energy, and even pollutants through food networks [15-16]. Therefore, any change in its population can induce changes in water conditions, followed by changes in high levels of the food chain and other consumers [15].

Despite the many studies that have been conducted recently on the impact of environmental factors on the distribution and biodiversity of small crustaceans in Iran [17-22]. No study using biological indicators (worms and crustaceans) has yet been conducted on the water quality of the Karun River, which is the most important source of water supply for drinking, agricultural and industrial purposes in Khuzestan province. The importance of such research lies in the fact that in addition to the quantity and amount of river inflow, water quality is also of paramount importance, the purpose of this study was to determine the water quality of the Karun River, Ahvaz, Iran based on biological indicators (crustaceans).

MATERIALS AND METHODS

The catchment area of the Karun River basin size is about 65 thousand square kilometers, and with a length of 890 km, this river is the longest in Iran [23]. The average annual rainfall of this basin is 620 mm and the height of its snow-covered part is two thousand meters above sea level. The climate of this basin is warm with dry summers and mild winters [24]. The vegetation mostly includes steppes, oak forests, and agriculture, with a sediment load of10 g/l (Yousefi, *et al*. 2016) [25]. The city of Ahvaz is one of the largest cities in Iran and hosts many polluting industries in the part where the river is located, discharging their treated or untreated sewage into the river (Neisi, *et al.* 2016)[26] (Fig. 1).

The present study is a descriptive-analytical and cross-sectional study that was conducted in 2020ona part of the Karun River basin which is host to municipal, agricultural, and livestock waste as well as hospital wastewater discharge. Based on the data obtained from a pilot study and river identification in Ahvaz, three stations were selected for field surveys and sampling. The first station was located upstream of the Karun River in Ahvaz (near the water treatment plant), the second station was located in the middle of the city (Fifth Bridge), and the third station was located downstream of the river (Kut Abdullah area).

Sampling was done during hot and cold seasons. In each season, one sample was taken from each station. At each station, water samples were collected from the sides and the middle of the river, at a depth of approximately two meters, and the samples were combined into a single sample. At the sampling site, physical and chemical parameters of water including pH, turbidity, temperature, and dissolved oxygen were measured using the relevant portable devices. 20 liters of water were collected for each station. The samples were left in the laboratory of the School of Health to settle for 24 hours. Then the deposited sediments were sampled by 15 cc centrifuge tubes, and the remaining surface water was discarded. After centrifugation, the samples taken from the sediment deposited in the bottom of the centrifuge tubes were transferred to the slide. The slide was then observed under a microscope for identification and quantification of the types of crustaceans. The slide was then observed under a microscope for the identification and quantification of the types of crustaceans [12].

The obtained statistical data were analyzed at different stations and sampling times with SPSS version 21 using a one-way analysis of variance

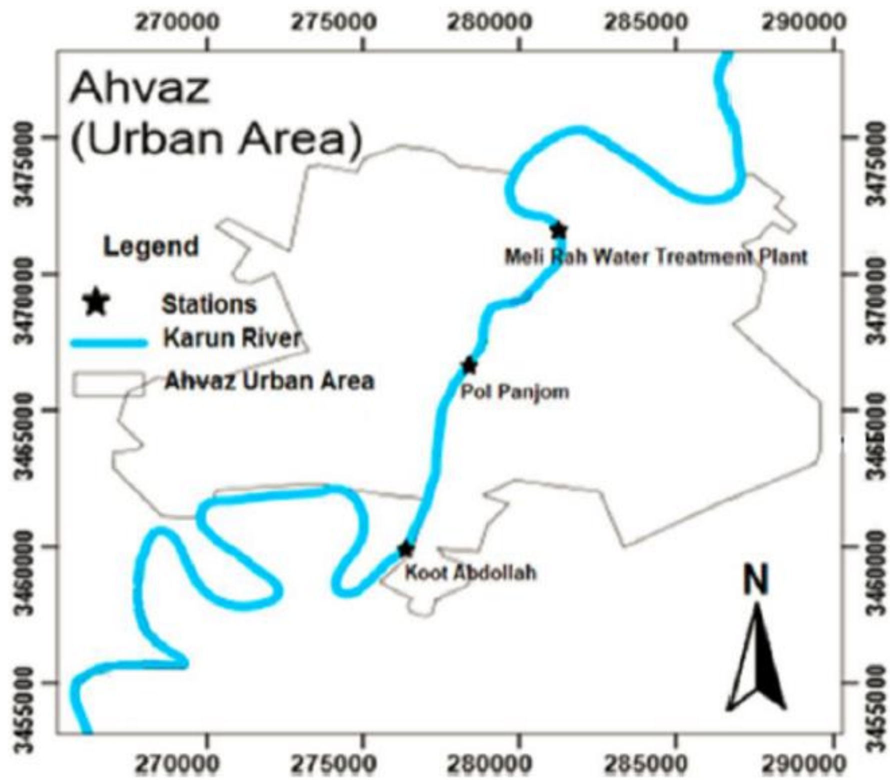

Fig. 1: The three studied stations along the Karun River

RESULTS

After sampling from the study stations at the Karun River, 27 protozoan samples were isolated and identified, which are shown in Table 1. As can be seen, the maximum number and variety of protozoa are related to Station 1whilethe minimum was obtained from Station 3 (KutAbdollah). Among benthic invertebrates identified from water samples of three stations, the Daphnia species was more prevalent than other benthic invertebrate communities in Stations 1 and 2.

Table 2 depicts the mean and standard deviation of the measured parameters along with Rotifer and Daphnia. According to Table 3, the results of the Pearson correlation showed an inverse and statistically significant relationship between Dissolved Oxygen (DO) and temperature, i.e., by increasing the temperature parameter, the amount of DO decreases ($P<0.05$). Also, the results of the Pearson correlation showed that there is a direct and statistically significant relationship between DO and Turbidity, Daphnia, and Rotifers ($P<0.05$). A direct and statistically significant relationship was also observed between Rotifers and the parameters of PH, turbidity, and temperature. These parameters had an indirect relationship with Daphnia ($P<0.05$).

According to Table 4, there is an inverse and statistically significant relationship between temperature and DO, i.e., DO decreases with an increase in temperature ($P<0.05$). There is also a direct and statistically significant relationship between temperature and pH ($P<0.05$). A statistically significant inverse relationship was found between Turbidity and DO, i.e., DO decreases by increasing turbidity ($P <0.05$). There is also a direct and statistically significant relationship among Turbidity, PH, Daphnia and Rotifers ($P<0.05$).

Table 1: The average number of protozoa per 20 litres of water in three stations at Karun River

Species of Protozoa	Warm Season			Cold Season		
	Station 1	Station 2	Station 3	Station 1	Station 2	Station 3
Adinetagracilis	15	10	7	10	7	4
Rotrianeptunia	10	7	5	9	6	5
PlatyiasPatulus	10	7	5	9	5	4
Proalesdecipiens	15	10	7	12	10	8
Holopediumgibberum	5	4	3	5	4	0
Alonacostata	5	4	3	5	3	0
Alonellaexigua	5	4	3	4	3	1
Bythotrepheslongimanus	5	4	3	4	3	2
Candonacandida	5	4	3	4	2	0
Keratellacochlearis	10	7	5	8	0	0
Lecaneacus	5	4	3	5	4	1
Tetrasiphonhydrocora	5	4	3	4	0	0
Trichocercacylindrica	5	4	3	5	3	0
Synchaetastylata	5	4	3	4	3	1
Testudinellareflexa	10	7	5	8	5	3
Crustaceans	20	15	12	15	8	4
Polyphemuspediculus	10	7	5	8	5	3
Leptodorakindtil	4	3	3	1	4	2
Habrotrocha	4	3	2	4	3	1
Notholcalabis	4	3	2	3	0	0
Cephalodellaforficula	4	3	2	2	1	0
Proaleswernecki	15	12	10	12	9	5
Elosaworallia	5	3	2	4	2	0
Dicranophoruscaudatus	5	3	2	1	0	0
Asplanchnapriodonta	15	12	10	1	0	0
Stephanocerosfimbriatus	5	3	2	4	2	0
Daphniavuvullata	5	3	2	1	1	0
Rotifers	5	3	2	4	3	2
Daphnia	25	18	12	18	16	10

Table 2: The mean and standard deviation of measured parameters and identified protozoa

Parameter	N	Mean	SD
DO (mg/l)	30	6.683	1.435
PH	30	7.750	0.303
Temperature (℃)	30	25.10	5.691
Turbidity (NTU)	30	121.666	29.069
Oxygen (mg/l)	30	3.766	3.875
Daphnia (number)	30	2.166	1.984
Rotifers (number)	30	4.833	5.166

Table 3: Pearson coefficient correlation between dissolved oxygen and pH with other parameters

Correlation between dissolved oxygen and pH with identified protozoa		Temperature	Turbidity	Daphnia	Rotifers
DO	Pearson Coefficient	-0.553	0.696	0.612	0.627
	P Value	0.002	0.001	0.001	0.001
PH	Pearson Coefficient	0.458	0.406	-0.215	0.247
	P Value	0.011	0.026	0.255	0.188

Table 4: Pearson coefficient correlation between temperature and turbidity with other parameters

Correlation between temperature and turbidity with identified protozoa		DO	PH	Turbidity	Daphnia	Rotifers
Temperature	Pearson Coefficient	-0.553	0.458	0.192	0.261	-0.037
	P Value	0.002	0.011	0.302	0.163	0.846
Turbidity	Pearson Coefficient	-0.696	0.406	0.192	0.693	0.547
	P Value	0.001	0.026	0.302	0.001	0.002

DISCUSSION

According to previous studies, increased pollution load in the river reduces water quality and leads to a reduction in the diversity and frequency of large species of benthic invertebrates [12, 18,26, 27]. In the present study, it was found that the frequency and diversity of crustacean species isolated from water samples from the area the river enters the city of Ahvaz to where it leaves the city (from Station 1 to Station 3) decreased significantly. More particularly, Station1containedthe highest diversity of protozoan species among the three stations, and some of the isolated species in previous stations could not be isolated and observed in the samples of the next stations. This decrease in the diversity of these crustaceans in the downstream areas indicates the low quality of water in these areas. In fact, the reduced diversity and frequency of crustaceans living in these areas indicate that the pollution entering Stations 2 and 3 (municipal effluents), especially station 3 (municipal and industrial effluents) reduces the quality of water and the amount of dissolved oxygen in the water. The water quality of Station 1 (near the water treatment plant), on the other hand, is at the highest level due to the lack of effluents. According to our study, the number of all crustacean spices by increasing BOD pollution and depletion of dissolved oxygen from upstream to downstream is reduced. Also, by reducing the temperature their reproduction reduces, too. the number of protozoa in winter has decreased. It is why the ambient temperature affects the replication and reproduction of aquatic spices.

Neisi *et al.* conducted a study for the identification and quantification of crustaceans and zooplanktons living in the Karun River at three stations in autumn and winter [12]. They found that the measured biochemical oxygen demand (BOD) was higher than the standard water quality index in Iranian aquatic ecosystems at all sites in winter and autumn. The most common types of zooplankton identified were Monhysterasimolis, Limnodrilus, Hoffmeister, Deroobtusa, and Rotariarotatoria, and the most common crustaceans were Daphnia Magna, Monospilusdispar, and Alonacostata. According to their results, the number and diversity of crustaceans and zooplankton decreased along the route and downstream of the river, indicating a decrease in water quality due to sewage pollution along the route, which is consistent with the results of this study.

Another study on the water quality of the Karun River was conducted by Madadinia *et al*., who reported a gradual decrease in the water quality index from the first station to the fourth station along the river route, with Kut Abdullah station having the worst quality conditions due to the accumulation of sewage, especially hospital sewage [18]. According to their results, the diversity of aquatic organisms as well as the reproduction of fish and other animal groups is reduced, and there is a possibility of aquatic vertebrate losses. The researchers concluded that parameters including BOD, temperature, and turbidity have a direct effect on the water quality index, which is consistent with our study, and that water quality is in its best condition in autumn but worst in spring.

In the present study, 27 species of protozoa were isolated from three designated stations, in which Daphnia and Rotifer species with means of 2.166 and 4.833 and standard deviations of 1.984 and 5.166, respectively, had the highest frequency compared to other species. In a study of the water quality of the Zay Aanderud River, Varnosfaderany *et al.*, found that effluent flow into this river reduces the number and frequency of crustaceans living in it, which is consistent with the results of the present study [28].

Previous studies have shown the adverse effects of pollutants on the survival of crustaceans, including Daphnia. Previous studies have shown that Daphnia species are highly resistant. These species have a higher tolerance capacity against environmental changes and concentrations of pollutants [16, 29].

CONCLUSION

Based on the results of this study, it can be stated that the entry of effluents into river water causes a significant reduction in water quality, which in turn reduces the diversity and frequency of crustaceans living in the water. Of course, Daphnia is less sensitive to the stresses of contaminants in water compared to other living organisms in aquatic ecosystems. Therefore, this hard-shelled species, as a freshwater species, can be utilized as a good indicator of metal toxicity in water, bio-indicators of bio-tests, and water quality.

ETHICAL ISSUES

This research project with the code IR.AJUMS.REC.1397.418 was approved by student research committee of Ahvaz Jundishapur university of medical sciences. The authors have observed all the ethical points in this article, including non- plagiarism, duplication, data distortion and data generation

COMPETING OF INTEREST

There is no Conflict of interest.

The authors have no conflict of interest.

AUTHORS' CONTRIBUTIONS

Abdolkazem neisi, Mena Ghanbari and Vafa Hamid participated in study design and editing the manuscript. Abdolkazem neisi and Seyedeh Leila Dehgani did the statistical analysis. Vafa Hamid and Majid Farhadi drafted the manuscript.

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