Dominant protozoan's in Karoon River Water, Ahwaz city, Iran

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ABSTRACT

Karoon River is the main source of drinking, industrial and agricultural utility in Ahwaz city. Protozoans are useful indicators for river water pollution. The aim of this study was to identify and enumerate Dominant protozoans in Karoon Water River, Ahwaz city.

In this descriptive study, we took samples from 3 points of karoon river in Ahwaz city boundaries (upstream-uptake point for water treatment plant, middle and downstream) in two seasons (dry and wet). Samples prepared and examined by optic microscope.

The results showed 6 species of mastigophora and ciliophora. Most prevalent species were Monosiga fusiformis, Bodocaudatus ، Cercomonas Longicauda ،Cercomonas Crassicauda ،Tetramitus descissus And Stalked Ciliate. The seasonal variation difference was statistically significant. Minimum concentration was in January (less than 1 cell /lit) and maximum was in October (1600 cell/ lit). The average concentration of protozoans was 129.68 cells/ lit.

Protozoans in 3 sampling stations were statistically different, where in 2 and 3 stations Bodocaudatus, Cercomonas Longicauda, Cercomonas Crassicauda, and Tetramitusdescissus were dominant. Identified protozoan species indicate pollution of Karoon River by sewage discharge.

Key words: Protozoa, Karoon, River, Ahwaz, Water

INTRODUCTION

Karoon River is the only international river that has access to oceans. The river length is about 950 km. Thus, part of the river is navigable (between Ahwaz and Khorramshahr) [1].

Plankton term first time named in 1887 by Hansen, and then in 1890 by Ernest Hekel. Suspended or floating organisms called plankton collections that are not able to change river flow. Then planktons studied by Dosart Netosurniain 1968-1965. Difference between marine and freshwater ecology was shown in 1978 by Margaluf. In 1982, Harris began extensive research on freshwater phytoplankton ecology.

Planktons, based on life type, categorized into two categories: phytoplankton (plants) and zooplankton (animal). The final product of many fish and invertebrate biomass depends on phytoplankton and zooplankton amount [2-4].

Zooplanktons are heterotrophic organisms that float in water and certain aquatic ecosystems. They are an important food source for aquatic organisms. These organisms, include protozoa, Cladocera, Copepoda, Rotifera and so on, that may be accounted as an indicator of water quality. Tucker reported that zooplanktons are rich in amino acids and essential fatty acids, Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA).

Zooplankton provided water and food such as protein, fats, carbohydrates and mineral salts, for fish's incorrect proportion. Freshwater zooplanktons are usually small in size and form, including protozoa, rotifers, and Crustacean, Cladocera, Copepoda and Ostracod. Globally, these organisms have been recognized as indicators of pollution in the aquatic environment [5].

Protozoa as indicators of water pollution have long been widely used by biologists to assess water quality. Thus, the protozoa community can provide valuable information on healthy ecosystems, because firstly protozoa are common species and widely distributed, secondly protozoa react quickly to changes in the water environment. So protozoa may show sudden changes and continuous changes in water quality parameters over a shorter time period [6].

Free-living protozoa, (e.g., Acanthamoeba, Naegleria Saccamoeba, Hartmannella, and Vexillijera) serve as hosts for Legionella to proliferate in natural and man-made freshwater environments. These protozoa multiply on biofilms and their grazing behavior is influenced by the composition and density of the biofilm [7]

Many protozoa are live, independently or swimming freely, while some species are able to form colonies. Many protozoa are food for other animals. Some of them, purifying waters carried by water filters and drains. But the strains of protozoans are pathogens that are harmful for human beings. Giardia and Cryptosporidium are currently have been identified the most important disease-causing protozoa transmitted through water, especially surface water resources. Giardia lamblia is a flagellated protozoan parasite in the intestines of humans and animals [8].

It should be noted that the amounts ingested cysts (10 cysts) can cause a chronic disease with symptoms such as: digestive disorders, diarrhea, and sometimes vomiting and in some cases may be asymptomatic, which called a carrier. In such cases the affected person (Carrier) is able to transmit disease. Unsafe drinking water is one of the main roots and the transmission of these parasites. Since Giardia cysts in water can survive with a temperature of 8 degrees Celsius for up to two months, and is resistant in conventional chlorination (more resistant than Coliforms), therefor, application of sand filters is an effective approach for treatment depend on the proper effective size (ES) and Uniformity coefficient (UC) to remove them. [8]

To overcome the above problems and to provide a tool for environmental managers and Policy makers, that needs environmental assessment and monitoring; it was decided to carry out this research.

MATERIALS AND METHODS

To study protozoans in the Karoon River water, Ahvaz area. Three stations were selected. Station 1 was near water treatment plant uptake (National Road Treatment Plant No. 1), the second location was the center of the city (bridge 5) and third place was downtown (Koot Abdullah). Polyethylene bottles were used for sampling. Sample volume was at least 20 Litter. Sampling performed in two seasons, wet and dry. Early fall was dry season (October) and early winter was wet season (January). Samples took one time per week from three stations for dry and wet seasons. Total samples were 16; 8 samples for dry season and 8 samples for the wet season. Samples were taken at all depths of River water, and both sides and middle of River section; 30 cm below the water surface and 30 cm above the river bottom [9].

pH and temperature were measured at the sampling site. Samples were tested for turbidity and dissolved oxygen Up to 5 hours after sampling. The water samples were concentrated by sedimentation procedure for 24 hours. After 24 hours, the supernatant water poured, and about 2 to 3 liters of sediment water samples were taken. Then, samples passed through the membrane filter with 1 micron in diameter. After passing the sample, the filter paper with forceps removed and washed in a beaker with distilled water. The above steps were repeated for each of the three samples; then sample prepared for microscopic examination. If samples preserved to examine in another day, equal volume of 10% formaldehyde solution (1/1) were added. Then obtained solution was kept in the fridge until the next step. Smears were made to obtain a drop of the sample onto a clean slide and a coverslip was placed over it. At least three slides were microscopically examined for each sample. Using an optical microscope with a magnification of 450 to 1000 times, all the slides were examined and some morphological characteristics were reported. Sedgwick–Rafter slides were used to detect and identify the type of Pathogenic and non-pathogenic protozoa. Number of protozoa per ml calculated according to the following formula:

$$No./ML = C * 1000\ mm^3 / A * D * F$$

C=number of counting protozoan
A=area of a lamella to square millimeters
D=Depth of a lamella during exposure to mm
F=by counting the number of lamella

Then the identified volume of the water column was collected which was used in the sampling density or the number of protozoa per unit volume, respectively [10-11].

SPSS software was used for statistical calculations.

RESULTS AND DISCUSSION

Identified Protozoa in the three stations were belonging to two categories; mastigophora and ciliophora. Based on 4 months consecutive sampling, in the months of September and October, the dry season, generally more species of protozoa were observed than December and January months. Among Identified species, Cercomonas Longicauda and BodoCaudatus from branch mastigophora (Flagellates) were the most common and had more density.

Stalked Ciliate species from Ciliophora were more frequent with 56.25% in station 1 (upstream) during the study period. At this station, No. 1, maximum protozoa density was 1100 cells per liter in October and minimum was less than one cell per liter in November.

In station No. 2, BodoCaudatus of branch mastigophora was more frequent with 57.32% of all protozoans. In this station maximum protozoon density was 1600cell per liter in September and maximum protozoon in January, with less than one cell per liter. In station No. 3most common species were Cercomonas Crassicauda from mastigophora branch with 43. 47%. The station had a maximum protozoon density in October with 1100 cells/lit and minimum density in January with less than one cell /lit.

The average density of the 6 protozoa species for study period as well as minimum and maximum number per volume unit calculated and are presented in Table1. Protozoa densities in Table 1 are expressed in terms of number per 20 liters of water.

In station No.1, protozoan indicators of clean water are found because Karoon River water was relatively clean and less polluted. Species found in these stations, where clusters of mastigophora and ciliophora. Identified mastigophora species are indicators of clean water. This statio0n located in

the upstream of the Karoon River; which proves water clarity from biological aspect.

In station No.2, species are found to be from mastigophora. This species is often found in fecal samples. The station located in the city center where wastewater discharges, including sewage, public and private hospitals wastewater and other public places.

In station No. 3, Species that are found from all branches of mastigophora protozoan species often are associated with contaminated water. Although some species of fecal contamination were found at this station, but there was less density than station No. 2. Presence of protozoan's indicator of polluted water proves Karoon River water pollution due to fecal pollution.

Table 1: Minimum, maximum and average number of protozoa per 20 liters of water in 3 Stations of Karoon River

Species of protozoa	Station 1			Station 2			Station 1		
	Max.	Ave.	Min.	Max.	Ave.	Min.	Max.	Ave.	Min.
BodoCaudatus	_	_	_	*22000*	*16500*	*11000*	*22000*	*16500*	*11000*
CercomonasLongicauda	_	_	_	*32000*	*21500*	*11000*	*11000*	*	*
Cercomonas Crassicauda	_	_	_	*11000*	*	*	*22000*	*16500*	*11000*
Tetramitus Discuss	_	_	_	*11000*	*	*	*22000*	*16500*	*11000*
Monosiga Fusiformis	*22000*	*11000*	*	_	_	_	_	_	_
Stalked Ciliate	*22000*	*11000*	*	_	_	_	_	_	_

* Calculation was not possible

Large number of protozoan in dry season may be due to low river flow. In dry season due to lack of rainfall and low water flow, water turbidity is less, then there is maximum solar radiation, which penetrate the water depth. Thus, favorable conditions were provided for the growth of various species of protozoa.

In the wet season, rainfall could increase water turbidity and shine like the sun, which prevent light penetration deep in the water, so many species of protozoa reduced drastically and sometimes even reach zero.

Statistical analysis

According to Table 2, average density of six protozoa species was129. 68 cells per litter for the study period. Mean and standard deviation values for each parameter (pH, Temperature, Turbidity and identified protozoa) are shown in Table 2.

Table 2: Mean and standard deviation of measured parameters and identified protozoa

Parameter	N	Mean	Std. Deviation
DO	48	5.88	1.4214
pH	48	7.89	0.2517
Temp	48	22.33$^{\circ C}$	6.7810
Turbidity	48	96.80	65.5274
caudatus	48	252.08	375.579
Longicauda	48	125.0000	340.2127
Crassicauda	48	126.0417	283.4043
Descissus	48	91.6667	285.5255
Fusiformis	48	80.2083	277.6687
Ciliate	48	103.1250	292.7140

The Pearson correlation test was performed to examine the above relationship between parameters and other parameters at all stations and for the entire study period. The results are shown in Table 3.

Table 3: Pearson correlation test to compare relationship between dissolved oxygen and other parameters and protozoans

DO	Temp.	Turbidity	Crassicauda
Pearson Coefficient	-0.887	0.826	-0.301
Sig. (2 tailed)	0.000	0.000	0.037

There was significant correlation between dissolved oxygen, temperature, turbidity and Cercomonas Crassicauda pieces. According to Table 3, we found an inverse relationship between oxygen and temperature, and dissolved oxygen directly related to turbidity. This kind of relationship can be explained by changing seasons with starting rainfall water turbidity increases, which increase mixing and temperature drops, there for dissolved oxygen in raises the River water. Also identified species decreases with increasing dissolved oxygen. Perhaps the reason for this decrease is related to season change from warm to cool weather. It may also associate with the increase in river flow, dilution reduces protozoan population density. There was significant correlation found between pH and temperature, turbidity and Monosiga fusiformis.

Table 4 shows an inverse relationship between pH and temperature. pH and turbidity are directly related. An inverse relationship is also shown between pH and the protozoa. It may be due to the living conditions of the protozoa. This species is usually found in clean water sources on planktons. Increasing pH probably causes deaths and reduction in population to the protozoa.

Table 4: Pearson coefficient correlation between pH and other parameters

pH	Temperature	Turbidity	Fusiformis
Pearson Coefficient	-0.291	0.430	-0.296
Sig. (2-tailed)	.045	0.002	0.041

Measured temperature is correlated with dissolved oxygen, pH, turbidity and Cercomonas Crassicauda species. Table.5 shows that the temperature increases, the parameters of dissolved oxygen, pH and, turbidity dropped; because of the season change. In the warm season, high temperature is reduced dissolved oxygen. During this season, water turbidity is less. The water pH trend is acidic. In addition, we observed a direct correlation between the protozoa and temperature. This species belongs to polluted waters. Probably because of less water flow and less mixing in warm season; this species is more compatible with the conditions biologically.

Turbidity is correlated with dissolved oxygen, pH and temperature and showed in Table.6.There was a significant correlation none of the species with turbidity Cercomonas Crassicauda only species that can be said is a photo associated with a low opacity.

Because of unknown distribution of samples, Spearman analysis was conducted. The results indicate that there was significant correlation between dissolved oxygen and temperature, turbidity and Cercomonas Crassicauda.

pH with turbidity and Cercomonas Crassicauda had a significant relationship. Temperature with dissolved oxygen and turbidity had a significant relationship. Turbidity with dissolved oxygen, pH, temperature and Fusiformis Monosiga has significant correlation.

ANOVA was performed to determine differences between the means of the measured parameters in the three Stations of Karoon River, the results is as follows:

There was significant difference between all Protozoa means in the three stations. But turbidity, temperature, pH and dissolved oxygen were not significantly different. (Although somewhat dissolved oxygen was significant P = 0.1)

Table 5: Pearson coefficient correlation between Temperature and other parameters

Temperature	DO	pH	Turbidity	Crassicauda
Pearson Coefficient	-0.887	-0.291	-0.922	0.292
Sig. (2-tailed)	0.000	0.045	0.000	0.044

Table 6: Pearson's coefficient correlation relationship between turbidity and other parameters and protozoans

Turbidity	DO	pH	Temperature	Crassicauda
Pearson coefficient	0.826	0.430	-0.922	-0.258
Sig. (2-tailed)	0.000	0.002	0.000	0.077

In order to determine the different stations, LSD test was used, in the majority of cases, the first station was different. This means that the first station with observed protozoan, in terms of type and number, was significantly different with second and third stations. This difference could be due the following factors:

The most important factor, that could change the type and number of protozoa, was discharged of sewage, industrial and hospital wastewater. Secondly, changes in dissolved oxygen at different stations, dissolved oxygen increased in second and third situation.

In order to determine the mean difference of measured parameters between dry and wet seasons, the statistical T Student test was performed. Results are as follows:

pH ($P \leq 0.003$), DO ($P \leq 0.000$), Temp ($P \leq 0.000$), Turbidity ($P \leq 0.000$), Crassicauda ($P \leq 0.049$).

There were significant differences of all parameters in two dry and wet seasons. But protozoa were not significantly different in the two seasons.

Reason of significant differences of turbidity, there was more rainfall in the wet season. Increasing rainfall increased water mixing, which increased dissolved oxygen in the wet season. Water temperature changed with season change. pH changes follow other three parameter changes. A significant difference in number of Cercomonas Crassicauda, which is a polluted water indicator, was due to water pollution by wastewater discharges in stations 2 and 3. Probably living conditions were compatible for Cercomonas Crassicauda because of warm water, less mixing and less water flow.

Shailendra Sharma [11] reported a direct correlation between temperature, dissolved oxygen and pH in Narmada River (India).

AliLuay, Water samples were collected on a monthly basis during January to December 2008. Results show that total number of zooplankton was 100 to 6,650 cells per litter and phytoplankton

population were from 18,773 to 269,448 cells per litter [12].

Shailendra Sharma *et al.* came to the conclusion that the most common protozoa were Arcella, Diffuzia Euglypha, Oppercularis and Oxytricain Narmadariver (India), [11].

Davies carried out a study on Harcourt Port (Nigeria) water to determine species composition, species diversity and abundance and distribution of some physicochemical parameters of surface water. The abundant protozoa were Askenasiafauriekahl and Arcella migrate. Temperature, dissolved oxygen and pH were not significantly different stations [13]. These results are similar to our study results.

Jian-GuoJiang, Yun-Fen Shen, reported identified protozoa for studied revers. These 47species were Zoomastigophora, 76 were Sarcodina and 254 were Ciliophora [14].

Aron Keve Kiss *et al*, chrysomonads typical flagellates were choanoflagellates, bicosoecids and abundant microflagellates (large chrysomonads and Collodictyon). Most abundant ciliates were oligotrichs, while Phascolodon, Urotricha, Vorticella, haptorids, Suctoria, Climacostomum and Stokesia also contributed significantly to biovolume during rapid succession processes. In October and November a second high protozoan peak occurred, with flagellate dominance, and slightly different taxonomic composition [15].

CONCLUSIONS

The results showed that 6 species of identifying protozoa belonging to mastigophoraand Ciliophora. These include: Bodocaudatus, Cercomonas Longicauda, Monosiga fusiformis, Stalked Ciliate and Cercomonas Crassicauda, Tetramitus Descissus. There were significant differences in protozoa seasonal changes between wet and dry seasons. Lowest density of protozoa was observed in January (less than one per liter), While highest density was in early October (1600cells per litter).

The protozoa density, average was 129.68 cells per liter. Type of identifying protozoa at the three stations was significantly different. Most of the species found at stations2 and 3, are included in mastigophora, BodoCaudatus, Tetramitus discuss, Cercomonas Longicauda and Cercomonas Crassicauda. These protozoa, as pollution indicators, prove water pollution due to sewage discharge into the Karoon River.

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