Central Asian Journal of Environmental Science and Technology Innovation **1**(1): 43-52 (2020)

RESEARCH PAPER

The effect of Salmonidae farms on the changes of the macrobenthos society of the Haraz River (Gazanak to Niyak regions of Iran)

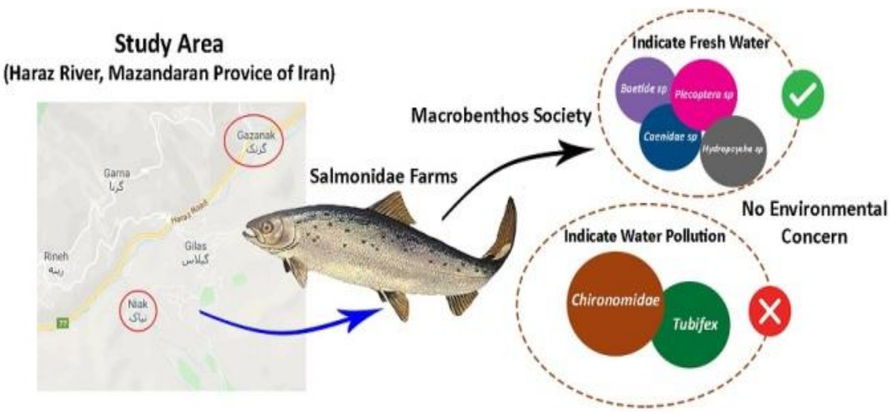
Mohsen Toughani [1,*], Hamed Manuchehri [2], Abdolghaffar Ebadi [3]

[1] Department of Natural Resources Engineering, Faculty of Agriculture, Babol Branch, Islamic Azad University, Babol, Iran
[2] Department of Fisheries, Faculty of Agriculture, Babol Branch, Islamic Azad University, Babol, Iran
[3] Department of Agriculture, Jouybar Branch, Islamic Azad University, Jouybar, Iran

Highlights

- The present study evaluates the impact of contaminants on the biological conditions of Haraz River.
- Rivers are vital sources of freshwater supply for agricultural, industrial, urban and drinking purposes.
- Of the environmental challenges of the Haraz River is aquaculture and farms wastewater impact on water quality.

Graphical Abstract



Article Info

Receive Date: 15 June 2019
Revise Date: 30 August 2019
Accept Date: 20 September
Available online: 10 January 2020

Keywords:
Biological Indices
Fish Farms Wastewater
Haraz River
Large Invertebrates

Abstract

The present study aims to investigate large communities of macrobenthic inoculants to evaluate the impact of contaminants on the biological conditions of Haraz River ecosystem. For this purpose, two Niyak and Gazanak stations were studied during October to December. The frequency of benthos samples from *Baetis* sp. (40.31%), *Caenis* sp. (17.37%), *Scopura* sp. (9.04%), *Hydropcyche* sp. (16.03%), *Chironominae* sp.(13.80%) and *Tubifex* sp. (3.45%) were counted. In order to investigate the relationships between quantitative traits, Pearson correlation coefficient analysis was performed using SAS software version 4.9 and mean comparison using Duncan's multiple range Test. Error level for all attributes was considered 0.05. Results show an increase in the relative pollution of station 1 (Niyak) towards station 6 (Gazanak). On this basis, the highest and lowest rate of pollution was observed at G2 and N1 stations, respectively. Based on the Pearson correlation coefficient, benthos of the *Choronomidae* and *Tubifex* family, which indicate water pollution, showed direct relationship with all water qualitative factors, such as BOD, COD, DO, EC, Temp, pH, nitrate, phosphate, carbonate, ammonium, TDS and chlorine, except DO. While, other benthoses that are usually indicators of fresh water, are related to all factors, except DO. Therefore, the macrobenthic pollution of the Haraz River in the studied area, does not have environmental concerns.

10.22034/CAJESTI.2020.01.05

E-ISSN: 2717-0519
P-ISSN: 2717-4034

* Corresponding author: M.toghani@baboliau.ac.ir (M. Toughani)

1. Introduction

Rivers are vital sources of freshwater supply for agricultural, industrial, urban, and drinking purposes. Investigation the Rivers recognize the health of the river ecosystem and reflect the impact of human activities on these ecosystems. The rivers were divided into two categories: "permanent" and "seasonal" in water change throughout the year. In humid climates, where there are high rainfall and low evaporation, rivers are permanent. These rivers are supplied with snow and ice in heights or groundwater when there is no rainfall. In contrast, in dry stations with low rainfall and high evaporation, rivers are more temporary and seasonal (Jahani et al., 2012; Johansen et al., 2018; Neofitou et al., 2010; Verdonschot et al., 2013).

The Haraz River is the most comprehensive, steepest, and beautiful in the Mazandaran Province, originating from the Alborz Mountains' northern slopes, and Damavand Mountain stretched southern the basin. The studied area basin is about 48000 km^2 and 185 km in length. The water supply volume of this River is 940 million km^3. The highest and lowest water seasons of the year are the spring with an average discharge of 464 and the winter season with 132 million km3, respectively. The average slope of the Haraz River was high, and the water in this River is steep and eroded. These conditions cause pollutants with the least change and self-purification from the upstream catchment, easily transported to the downstream stations. Of the environmental challenges of this River are aquaculture and farms wastewater and its impact on water quality and the population of large benthic invertebrates (macrobenthos) (Guilpart et al., 2012; Klunder et al., 2018; Soofiani et al., 2012).

Benthos is one of the essential elements of continental water ecosystems, which can be used as an indicator of water pollution because it responds quickly to environmental change (Camargo et al., 2011; Keeley et al., 2012; Milošević et al., 2018). The distribution of benthos depends on the substrate, amount, and composition of the sediment's organic matter. Various studies were done on quantitative and qualitative changes of benthos in rivers and lakes. Results show that benthos as an indicator of water pollution from different sources can provide reliable data. Indeed, due to the severity of the contamination, species composition changes at some stations. Organisms that prefer clean water (Trichoptera & Plecoptera) were replaced by organisms resistant to water pollution (Tubificidae & Chironomidae).

Chironomidae are the most abundant and diverse insects in most freshwater ecosystems globally expanded. This family, which includes a group of metamorphosed insects in the three stages of early life (eggs, larvae, and pupae) are generally aquatic and mature to aerobic mosquitoes that fly close to the water surface. These larvae are widely involved in the diet of animal groups, especially fish. Chironomidae larvae play a vital role in the aquatic community's food chain, and they are carnivores that feed on various foods. Chironomidae larvae have been extensively identified and studied in Europe and the US more than elsewhere in the world (Shadrin et al., 2017). Below the typical characteristics of some environmental indicator macrobenthos given for more clarity.

Tubifex: Most of them are scattered around the UK and Ireland. On each side of his body, there is tough, rough hair that helps his burrow. This worm has many varieties, but it is challenging to distinguish them. Because genitals commonly used to identify species were absorbed after mating, the worm changes with the change of water salinity. The body will reach more than 20 cm in length. It lives in sediments on the margins of lakes and rivers. This worm is bisexual and has a complex genital system. Tubifex worm hosts the parasite Myxobolus cerebralis, affecting salmon and similar fish (Kang et al., 2018).

Plecoptera: They are usually insects with a medium to small body size and somewhat flat. These insects are commonly found around the rivers or rocky shores of lakes. Plecoptera has a simple metamorphosis, and the nymphal stages go through the water. Nymphs of Plecoptera are commonly found beneath rocks in the water or rocks on the shores of lakes, and that is why these insects are called rock flies. Simultaneously, everywhere the water flows with higher nutrients, Plecoptera's nymphs are also found (Ceneviva-Bastos et al., 2017).

Ephemeroptera: or one-day-old insects with small to medium size have a long and soft body. Most adults are found near streams of water. In adults, the anterior wing has a large, triangular shape and numerous rings. The back wings are small and round. The nutrients used by these insects include aquatic organisms and little organic matter in the water. One day after they are winged, they once again peeled off, which were unique among insects. One-day, adults usually fly in large numbers from the surface of lakes and ponds and sometimes gather in large numbers along the River. For this reason, larvae and adults make the food chain of most freshwater fish. Caneaidae and Bateidae are two important families of Ephemeroptera's order (Ceneviva-Bastos et al., 2017).

Caenidae and Baetidae: Insects often of small to medium size, with an elongated and very soft body, 3 mm long, little head, and short tentacles. Compound eyes in males are large and prominent and are often divided into two parts by other voids. The upper part of the compound eye in the Baetidae family is referred to as the turbinate eye. These eyes are often more enormous and in contact with together but are distant in females. In the Caenidae family, the compound eyes are small and separated in both sexes. In these insects, there are three simple grown eyes. In adults, the mouth and digestive system have been partially destroyed, so they cannot feed (Merritt et al., 2017). This study's goal was to assess Salmonidae Farms on the Changes of the Macrobenthos Society of the Haraz River from Gazanak to Niyak Range.

2. Materials and Methods

To study and identify river macrobenthos, samples were taken from three stations in Gaznak (G1, G2, and G3) and Niyak (N1, N2, and N3) three times in autumn (October, November, and December) in 2018 (Fig. 1). The sampling stations are selected based on the station's availability and location and features such as vegetation, water flow rate, river slope, pollutant entry, rock density, and rubble. The distance between the sampling point to the sewage site is about 35 meters due to the high water velocity to provide a complete analysis of sewage contamination trends on the surrounding benthic population. The altitude, longitude, and latitude of the stations are determined by GPS, as shown in Table 1 (Johannessen et al., 1994; Stojanović et al., 2017).

Table 1. Geographic Characteristics of Sampling Stations in the Haraz River from Niyak to Gaznak, 2018.

Stations		Length	Width	Height
Niyak	N1	35°.53'.33" N	52°.12'.40" E	1615
	N2	35°.53'.44" N	52°.12'.46" E	1599
	N3	35°.53'.46" N	52°.12'.51" E	1601
Gazanak	G1	35°.53'.48" N	52°.12'.54" E	1606
	G2	35°.53'.54" N	52°.13'.00" E	1595
	G3	35°.53'.56" N	52°.13'.04" E	1595

The distance between the sampling points is base on the fact that N1 stations before the first farm and G1 before the second farm were approximately 600 m to each farm's second station location (sewage disposal site). A sampling of all rock and rubble samples within the quadrate sampler with dimensions done 1×1 m (Valdemarsen et al., 2015). It is attempting to roughly equal the amount of fertilizer in places with the same rock and rubble distribution for high accuracy in counting and identifying benthic samples. After collection, the specimens were transferred to the laboratory and identified and imaged using validated identification keys (Figs. 2 and 3).

3. Results and Discussion

Based on experiments and using benthos identification keys, 2 categories, 5 orders, 6 families and 6 genera were identified. The classes included Insecta and *Clitellata*, the orders included *Diptera*, *Ephemeroptera*, *Plecoptera*, *Trichoptera* and *Haplotaxida*. From *Ephemeroptera*, two families *Baetidae* and *Caenidae*, from *Diptera* family *Chironomida*, from Plecoptera family Scopuridae, from Trichuptera family Hydropcychidae and from *Haplotaxidae* family *Tubificidae*. *Ephemeroptera* Order with *Plecoptera* and *Trichoptera* Clean Water Indicators, and Family *Chironomidae* and *Tubificidae* are indicators of polluted waters (Table 2) (Fig. 4).

Fig. 1. Sampling stations in the two study areas Niyak and Gaznak (7.1.2.2019- Google Earth Pro).

Fig. 2. Niyak station (Haraz River Region).

Fig. 3. Gazanak station (Haraz River Region).

Abundance percentage of resistant species at G2 and G3 stations, above and at other stations, mainly N1, the rate of species with less resistance to infection was high. These results show that the input water to the N1 station on the first farm is suitable for culturing cold-water fish. At the same time, the outflow water from the farm, especially the G2 station, from the second farm is heavily polluting. The high pollution load causes a decrease in oxygen, which is not suitable for fish breeding. Benthos identified at each station also show similar results. The *Tubificidae* and *Chironomidae* families directly correlate with water pollution, increasing families' number (Camargo, 2019). While *Ephemeroptera*, *Plecoptera,* and *Trichoptera* families have the opposite relationship with infection. So, the increase in pollution results in a decrease in these families' numbers, especially the *Ephemeroptera* family (Wilding and Nickell, 2013).

Table 2. Species identified in Haraz River (Niyak to Gaznak) in autumn.

Phylum	Class	Order	Family	subfamily	Species
Arthropoda	Insecta	Diptera	Chironomidae	Chironominae	*Chironominae* sp.
Arthropoda	Insecta	Ephemeroptera	Baetidae	Baetis	*Baetis* sp.
Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	*Caenis* sp.
Arthropoda	Insecta	Plecoptera	Scopuridae	Scopura	*Scopura* sp.
Arthropoda	Insecta	Trichoptera	Hydropcychidae	Hydropcyche	*Hydropcyche* sp.
Annelida	Clitellata	Haplotaxida	Tubificidae	Tubifex	*Tubifex* sp.

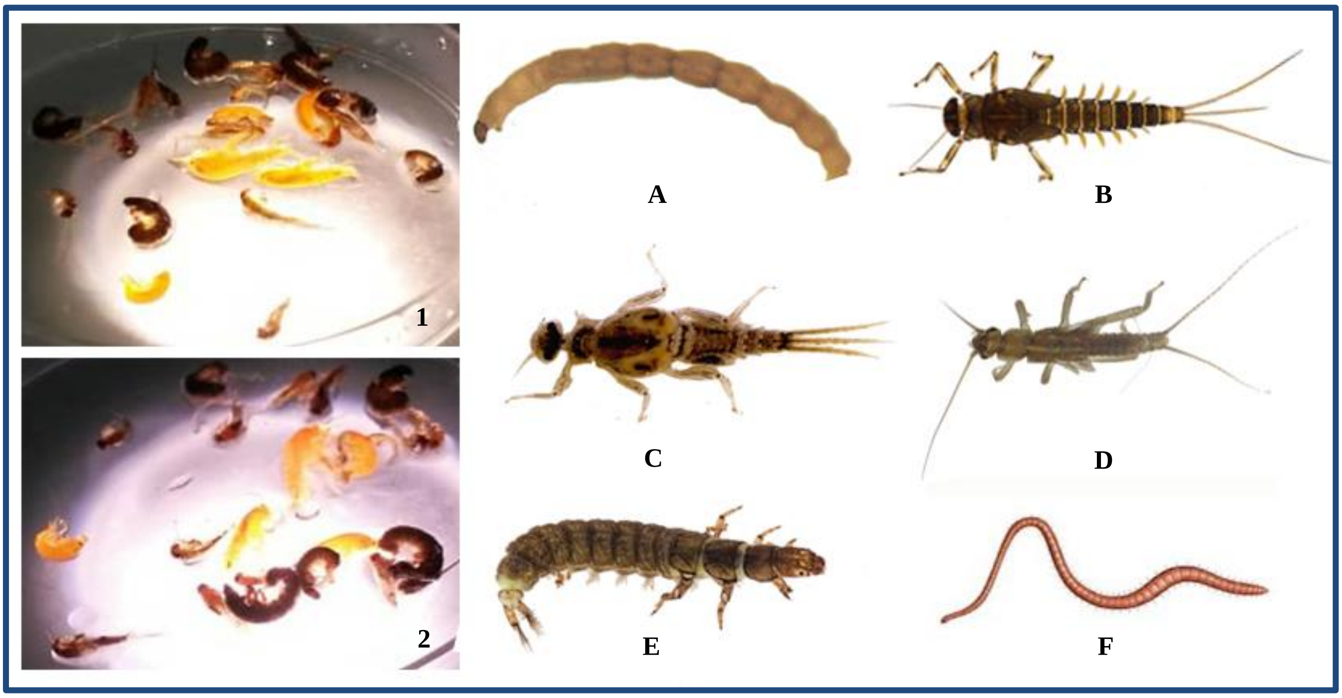


Fig. 4. Benthos species identified at stations (1 & 2), A: *Chironomidae* sp., B: *Baetis* sp., C: *Caenis* sp., D: *Scopura* sp., E: *Hydropcyche* sp., F: *Tubifex* sp.

Analysis of variance (ANOVA) compares the frequency of different types of benthos between other stations. The lunar variable in this analysis was considered as a replicate, and benthic regions were reached. Finally, the frequency of different types of benthos in each station and for each benthos of different stations was compared. Analysis of variance for the benthic frequency parameter showed that station and benthos' independent effects and station-type benthos' interaction on benthic abundance were significant (Table 3). Comparing the mean independent effect of benthos frequency on different months did not show any significant difference (Table 4). A comparison of the mean of station independent result showed that the frequency of benthos at station N1 was significantly higher than other stations (Table 4). While the lowest amount of benthos was observed at G3 and N3 stations, there was no significant difference. A comparison of the mean frequency of different types of benthos showed that *Baetidae*, *Plecoptera,* and *Hydropcyche* benthos samples were significantly higher than other benthos species (Fig. 5).

Chironomidae and *Tubifex* were less abundant than other benthos. Comparing the average effect of station and type of benthos showed that *Baetidae* frequency was higher in all stations except G2 station. While the lowest frequency at all stations was *Tubifex*. Comparison of the mean frequencies of each benthos between different stations (Fig. 6) indicated that the frequency of *Baetidae* at stations N1, N2, N3, and G1, *Caenidae* at stations N2 and N3, *Plecoptera* at stations N1, N2, N3, and G1, *Hydropcyche* at stations N1, N3, and G1, and *Tubifex* had the highest values at G2 and G3 stations (Table 5).

Table 3. Analysis of variance of Benthos trait.

Sources of changes	Degrees of freedom	Benthic abundance Ave. squares
Month	2	1.04 ns
Station	5	719.81**
Benthos type	5	133.56**
Station × Benthos	25	84.79**
Test error	70	25.89
Coefficient of variation %		61.82

ns and **, respectively, non-significant and significant at 1% probability level

Table 4. Comparison of the independent effects of station and month on the benthic parameter.

Independent effect	Level	Benthos (Mg/L) Standard error± mean
Month	October	1.31 ± 8.19 a
	November	1.74 ± 8.42 a
	December	1.34 ± 8.08 a
Station	N1	2.2 ± 20.11 a
	N2	2.3 ± 8.67 b
	N3	2.38 ± 4.5cd
	G1	2.27 ± 7.5 bc
	G2	1.15 ± 6.89 bc
	G3	1.51 ± 1.72 d
Benthos	Baetidae	2.2 ± 10.44 a
	Caenidae	1.97 ± 8.11 ab
	Plecoptera	1.13 ± 10.33 a
	Hydropcyche	1.8 ± 10.61 a
	Chironomidae	1.06 ± 4.44 a
	Tubifex	0.82 ± 5.44 bc

At least one letter means based on Duncan's test did not show a significant difference (P> 0.05)

Table 5. Comparison of mean interactions of station and month on benthic parameter.

Month	Benthos					
Station	Baetidae	Caenidae	Plecoptera	Hydropcyche	Chironomidae	Tubifex
N1	8.54±20 abA	3.84±7.67 bcCd	0.88±10.33 aBC	3.61±17 aAB	4.1±7.67 aCD	0±0 bD
N2	1.45±24.33 aA	6±14 abB	2.96±5.67 abC	1.33±1.33 bC	1.76±3.33 aC	0±0 bC
N3	0.33±26.33 aA	5.67±16.67 aB	2.33±2.33 abCD	0.67±10.33 aBC	3.18±6.33 aCD	0±0 bD
G1	1.33±27.67 aA	3.33±6.67 bcCd	1.2±8.67 aBC	2.31±15 aB	3.18±5.67 aCD	0±0 bD
G2	4.16±8 cAB	2.33±2.33 cAB	0±0 bB	0±0 bB	0.67±7.33 aAB	1.15±9 aA
G3	2.6±14.33 bcA	4.67±4.67 cBC	0±0 bC	0.67±1. 33 bC	0.58±11 aAB	0.67±1.33 abC

At least one letter means based on Duncan's test did not show a significant difference (P> 0.05)

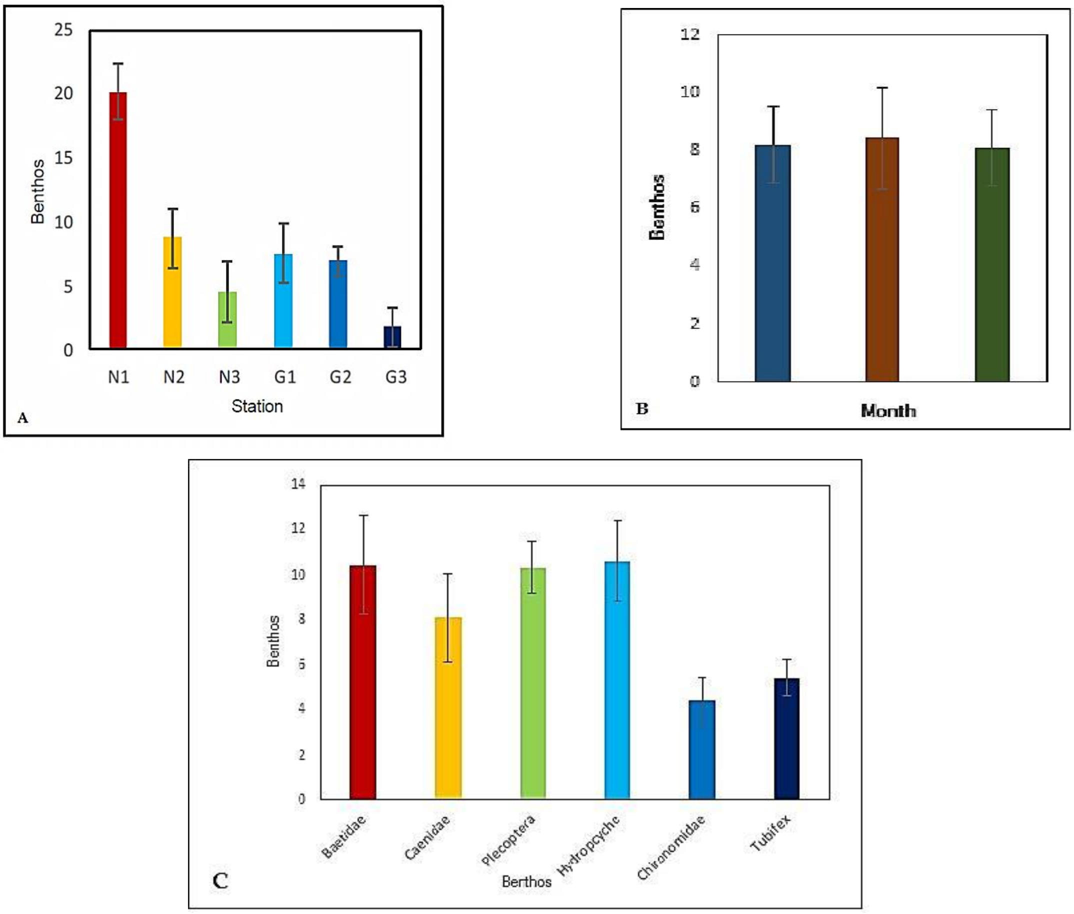
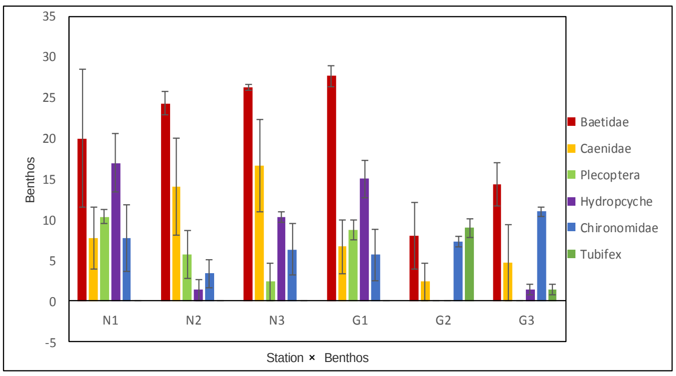


Fig. 5. Comparison of the average benthic parameter between (A): different stations; (B): extra months, and (C): the number of each sample in all stations; at least one letter means based on Duncan's test did not show a significant difference (P> 0.05).

Spearman correlation coefficients between the abundances of different types of benthos are listed in Table 6. The quantity of Baetidae benthos was positively correlated with Hydropcyche and negatively and significantly with Tubifex benthos. The frequency of Caenidae was also significantly and negatively associated with Tubifex. Plecoptera had a significant positive relationship with Hydropcyche and negative significance with Tubifex. The results also showed that Chironomidae, there is no relationship with any of the other benthos. The positive relationship between the benthos indicates that the two benthos' frequency is increasing or decreasing together. The negative correlation also indicates that the other benthos' frequency decreases with the increase of one benthos. Tubifex benthos was negatively correlated with all other benthos except Chironomidae; this indicates that this benthos is not viable along with other benthos and that its abundance decreases. (Bloodworth et al., 2019; Keeley et al., 2015; Mondy et al., 2012).



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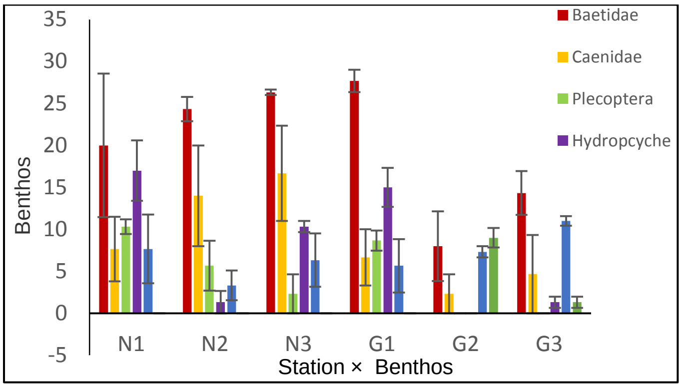

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Fig. 6. Comparison of Mean Benthos-Station Interaction (a): Frequency of benthos in each station was compared (b): Frequency of each benthos was compare between different stations. At least one letter means that Duncan's test did not show a significant difference (P> 0.05). Relationships between abundances of benthos species

Table 6. Spearman correlation coefficients between benthos types.

Benthos	Baetidae	Caenidae	Plecoptera	Hydropcyche	Chironomidae	Tubifex
Baetidae	1					
Caenidae	0.148	1				
Plecoptera	0.464	0.198	1			
Hydropcyche	0.663**	0.188	0.638**	1		
Chironomidae	-0.396	0.044	-0.168	-0.172	1	
Tubifex	-0.608**	-0.628**	-0.567*	-0.641**	0.162	1

* and ** are respectively significant at the 5% and 1% probability levels

According to the identification of macrobenthos of the Haraz River in the two regions of Niyak and Gaznak, the highest amount of benthos (all benthos) was in October, the highest number of benthic samples is for station N1 and the least for station G2 and G3. The changes of benthos in October to December were not significant, which could be due to the stability of chemicals in the water and the self-purification properties of the River (Keeley et al., 2014). At N2 and G2 stations, most of the macrobenthos samples were from Chironomidae and Tobifax families, indicating the level of contamination at these stations. Also, in N1 station, due to upstream and water quality, G1 stations are more abundant, and N3 and G3 stations have fewer examples of Ephemeroptera, Plecoptera, and Hydropcychefamilies. The existence of Chironomidae and Tubifex samples is not absolute for infected stations. This means that these samples can also be found at stations with little or no pollution. The aim was number and count samples per square meter, which at the N2 and G2 contaminated stations, was far higher than the rest of the stations. Also, several examples from stations with low contamination were found in wastewater outlets, but their numbers were down. Overall, the benthos' study can be used as an indicator or environmental or ecological indicator to measure water pollution in this part of the Haraz River. In this study, seven classes of macrobenthic were identified. The most impacts of the farm's contamination were on the three orders of Ephemeroptera, Trichoptera, and Plecoptera, known as the EPT susceptibility group, which reduced their population. In contrast, the Chironomidae family populations, which are benthic invertebrates to resistant, had increased.

5. Conclusions

Various contaminants can have short-term and long-term negative impacts on river water and sediments (Bannister et al., 2014). In the Haraz River, due to the lack of wastewater treatment, unfortunately, tens of thousands of cubic meters of these wastewaters are discharged to the River as a raw material. So far, no detailed study has been conducted on the behavior of these emerging pollutants. Consequently, the use of downstream water for sanitary and drinking applications should be performed with careful testing. Achieving a certain amount of salmon production in aquatic environments requires the consumption of nutrients in fisheries. Also, these farms' effluent causes a severe drop in water quality and even in conditions where farms run in very short distances without any biological treatment system releasing effluent into the River. Therefore, it is necessary to study the effects of fish breeding and to culture farms effluents on the water quality of this River and its impact on macrobenthos populations. According to the results, the N2 station in the Niyak area and G2 station in Gazanak area are the most important pollution sources in the Haraz River due to the receiving and input of effluents from fish farms.

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How to cite this paper:

Toughani, M., Manuchehri, H., Ebadi, A.G., 2020. The effect of Salmonidae farms on the changes of the macrobenthos society of the Haraz River (Gazanak to Niyak regions of Iran). *Cent. Asian J. Environ. Sci. Technol. Innov.* **1**(1), 43-52.