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Combining a flow-through bioassay system using *Daphnia magna* with a physicochemical analysis to evaluate the effluent toxicity of the aquaculture farm on the river

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ABSTRACT

The discharge of the effluents from rapidly grown aquaculture farms into the receiving water has led to the pollution of nearby rivers. Therefore, studying the water quality of the rivers and the impact of the aquaculture discharge on the aquatic environment is important. This study evaluated the toxicity of an aquaculture effluent with a *Daphnia magna* (*D. magna*) assay in a flow-through system by combining toxicity data and physicochemical analysis. The water samples were analyzed for physicochemical characteristics, total metals, pesticides, and for immobilization with *D. magna*. The LC_{50} post 24, 48, 72, and 96 h of exposure was adopted as the endpoint and estimated statistically by the Probit method. The results showed that all these physicochemical parameters and total metals meet Environmental Protection Agency standards for the effluents and no pesticides were detected. The LC_{50} value (mg/L) of the samples was 0.731 after 24 h while it dropped to 0.527, 0.486, and 0.367 at 48, 72, and 96 h exposure intervals, respectively. Regression analysis showed significant relationships between LC_{50} vs. concentration and time exposure. That is the higher the concentration and the longer exposure time to *D. magna*, the higher the toxicity. The present study showed that aquaculture effluent has toxicity to biota even if they apparently met standards for effluent discharge limits since aquaculture effluent is complex mixtures and its individual components cannot be routinely measured. It could be concluded combining a bioassay with a physicochemical analysis is a useful tool to monitor the quality of water bodies that protect aquatic lives.

Keywords: Daphnia magna; Flow-through bioassay; Gargar River; Probit analysis

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1. Introduction

The aquaculture industry has grown significantly in Iran but the discharge of the effluents from aquaculture farms into the receiving water has changed the use of natural resources, disturbed the natural equilibriums and increased pollution of the rivers to antibiotics, fungi and toxic chemicals such as ammonia, nitrite and nitrate and so on. Therefore, it is important to study the water quality of the receiving river and the potential impact of the aquaculture discharge on aquatic organisms [1,2].

Although there are many benefits in using chemical methods for effluents quality control, control methods alone are not sufficient to determine the toxicity of particular substance. Therefore, a correct interpretation of the toxicity of chemicals to a biological system is possible by investigating their resultant toxicity to its aquatic organisms [3,4]. In addition, the chemical properties of hazardous materials in an environmental matrix, such as wastewater, are often lacking, therefore, ecotoxicological tests is a real and practical approach to determine the toxicity of chemicals and manage them. In general, biological toxicity test or bioassay is a standard method in which the toxicity of pollutants in effluents or water bodies is evaluated [1,4–6]. Moreover, methods based on bioassay tests are relatively easy to perform with high speed, low cost, no need for the use of expensive reagents, and high precision. Bioassays generally are less time consuming [7,8]. Although, the static toxicity test can be accomplished easily and with a minimum cost; but these methods for many compounds, such as absorbent compounds, rapid biodegradable compounds, volatile compounds or insoluble materials may not be appropriate. Hence, a flow-through test system overcomes these limitations 1. Other benefits of the flow-through system are constant water quality in terms of dissolved oxygen (DO), salinity and toxic substances concentration [9,10]. That is why in the past 4 decades in many developed countries this method has been considered as an alternative method to monitor the quality of effluents [6,11]. There are lots of biomarkers or bio-indicators such as fish [12–14], algae [15,16], bacteria [17,18], and etc. for the biomonitoring of effluent toxicity and water quality. A freshwater crustacean called *Daphnia* is commonly used in water pollution control due to its short time of reproduction, high sensitivity to toxic chemicals, simplicity and low cost, and most importantly its parthenogenetic property [4,17,19]. The two main species of *Daphnia* are Magna and Pollex. Nowadays, the former is more common because of its sensitivity and ease of use in pollution monitoring. *Daphnia magna* (*D. magna*) is listed in the toxicity test as an indicator organism by the American Public Health Association (APHA), Central Insecticide Board India Guidelines (CIBIG), American Standards Testing Methods (ASTM), Food & Agriculture Organization (FAO), and Organization of European Committee for Determining (OECD) [19,20].

Gargar River is a branch of Karun River which isolated from Karun at the beginning of Shushtar City and again joins the Karun after traveling a distance of 78 km and receiving agricultural and aquaculture effluents. The river was registered in UNESCO as a world heritage site in 2009 [21]. In addition, the study of water quality is essential in this region because of the world's cultural heritage status and related protection issues and providing water for municipal and agricultural purposes along the river. In sum, these factors make the Gargar River a good and interesting candidate for a case study. Gargar River has been contaminated by aquaculture farms located around the river due to increased use of fertilizers, including manure, nitrogen and phosphorus fertilizers, chemicals and antibiotics to control pathogenic microorganisms. Due to the low flow rate of the river and the high volume of wastewater discharged into it, in most cases, water users are forced to use water with high salinity and bad taste and smell, especially during the drought seasons. Therefore, studying the water quality of Gargar River and the impact of the aquaculture discharge into it is very valuable. On the other hand, the need to approach sustainable development and the economy of it and the central role of humans in this process is imperative that raises attention to environmental issues as an important element in sustainable development. Since the control of effluents in Iran is still based on physical and chemical parameters, there is an urgent need to develop a strategy in the country for biological effect monitoring. For that reason, this study focused on combining a flow-through bioassay system with physicochemical analysis to evaluate the toxic effects of aquaculture effluent using *D. magna* and to analyze the effect of the effluent discharges on the Gargar River. Based on this, a pilot flow-through system was constructed to conduct a continuous toxicity test. The toxicity of different water samples was assessed by the immobilization of *D. magna*. The toxic effects were quantified as LC_{50} value (i.e. the concentration (mg/L) and method are similar to that of the United States Environmental Protection Agency (EPA) [1], in which mortality is considered as an acute toxicity endpoint regarding discharges into the river.

2. Materials and methods

2.1. Study sites

Gargar is one of the tributaries of the Karun River water system in Shushtar City, Khuzestan province, southwestern Iran. The length of Gargar is 78 km with an area of 1,020 km^2 and is situated between 48′48°-20′49°E and 31′31°-40′32°N. The average monthly flow of Gargar River varies between 10 and 31 m^3/s, according to data collected in the last 10 y [6,22].

2.2. Sampling, physicochemical analysis and quality control/quality assurance

Sampling was conducted from an aquaculture effluent in the vicinity of Gargar River and from the upstream of the effluent discharge point into the river. Sampling was conducted between August and November in 2015, and physical and chemical characteristics, which including electrical conductivity (EC), pH, total dissolved solids (TDS), total Kjeldahl nitrogen (TKN), NH_3, PO_4, chemical oxygen demand (COD), biochemical oxygen demand (BOD), DO, total hardness, total metals, and pesticides were determined. The analyses were conducted immediately after arriving at the laboratory (about 2 h after sampling). EC, pH, and DO were measured using a Horiba U-10 multi-probe (Horiba,

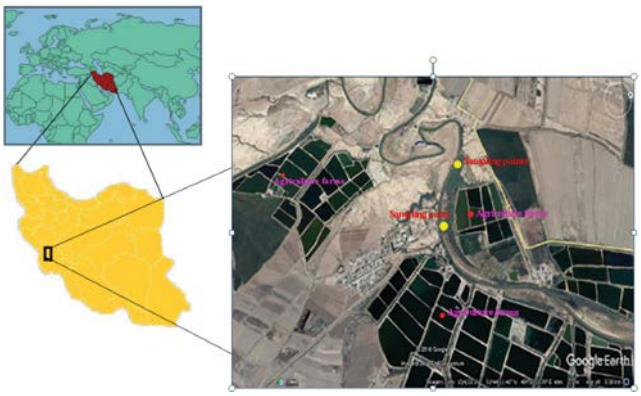

Fig. 1. Gargar River-geographical situation and sampling locations.

Co., Japan). Analysis of TDS (dried at 103°C–105°C), COD (Closed Reflux Colorimetric Methods, COD Reactor, Hach, USA), total hardness (EDTA Titrimetric Method), NH_3 (Nessler Method), and total phosphorus (Ascorbic Acid Method) was also measured. Standard methods were used in all procedures [23]. Total acid extractable metals (Mo, Zn, Ni, Cd, CO, Mn, Fe, Pb, Cr, Cu, Ag, and Al) were analyzed by atomic absorption flame spectrophotometry (Varian AA240, U.S.A) in 3:1 HCl:HNO_3 digests (section 3030D) as described in standard method [23].

For the digestion of trace aluminum, polypropylene utensils were used to avoid aluminum leaching from glassware into the water. For other metals, no further pretreatment is needed.

Pesticides determination were performed using an Agilent 7890A gas chromatograph (USA), equipped with a 5975 mass selective detector and fitted with Restek Rxi®-5MS fused-silica capillary column 5% Phenyl Methyl Silox (60 m × 0.32 mm i.d. and 0.25 μm film thickness). A carrier gas, helium (purity 99.999%), was set at a flow rate of 1.0 mL min^{-1}. Other details of the methodology were described in Drăghici et al. [6]. The detection limit of the method was 1 μg/L for all pesticides, with linearity correlation coefficients > 0.995. Fig. 1 shows the position of the Gargar River and the sampling point.

Standard materials were applied to prepare the calibration curve and calibration coefficients were kept at ≥0.99. The accuracy of the analytical methods was verified against the National Institute of Standards and Technology (NIST) reference standard material SRM1640a (natural water) and SRM 3069 (Organ Chlorine Pesticides). The precision was found in a CV less than 5%. The blank and standards were used after three determinations to calibrate the instrument. The accuracy of results was ensured through repeated analysis of the sample against a reference standard.

2.3. Toxicity test procedure (bioassay)

2.3.1. Experimental animals

The organism used for the bioassay test was *D. magna* (*Cladocerans*, *Crustacea*). Preparation and cultivation of *D. magna* were performed using Standard No. 8711 in accordance with standard methods of testing water and wastewater treatment [21]. A stock of Daphnids was purchased from the microbiology laboratory, Shahid Beheshti University of Medical Science. *D. magna* were maintained in the following conditions: water temperature, 20°C; 16 h/8 h of light and dark cycle with a light intensity of *b*600 lx. The culture medium was prepared by mixing non-chlorinated tap water and deionized water 1:1 to obtain 170 mg/L $CaCO_3$. Due to the sensitivity of the organism to water hardness, the degree of water hardness for the growth of this organism was between 160 and 180 mg/L as $CaCO_3$ [1]. Although *D. magna* can survive in a wide range of pH, in this experiment, an optimum pH range was maintained between 6.8 and 7. An aquarium pump was used to achieve the desired DO in the water diluent. The daily feeding was carried out with a suspension of the green alga *Scenedesmus subspicatus* at a concentration of 2 mg cell/L supplemented with a mixture of yeast and fermented trout chow at a rate of 1.5 mL prepared food per 1,000 mL of water, three times per week [1,19,24]. The bioassay used the third generation of *D. magna* (≤24 h), which was born from parthenogenetic females, for each test because of the equal similarity of the organisms [11].

A quality assurance plan (QAP) is outlined in Section 1020A as described in the standard method [23]. As a minimum, QAPs for laboratories performing aquatic toxicity testing should provide specific guidance on data quality objectives, test procedures, sample handling, data management, internal quality control, and corrective action [23].

2.3.2. Exposure system

The exposure phase was conducted in a flow-through system using the EPA method [1]. The flow-through system (Fig. 2) consists of a 10 L test chamber in which the solutions were held, five glass tubes connected together to house the *D. magna* in an 8 L glass cylinder that was in turn connected to a peristaltic pump. Five glass tubes were immobilized in a cylinder and can be removed with minimal effort for washing and transporting the organisms. In addition, a sampling tap is placed at the bottom of the cylinder for water sampling whenever needed. Samples entered into the 10 L cylinder and flowed into the 5 glass tubes, and then to the test chambers through the peristaltic pump. More details are given in our previous study [5].

2.3.3. Experimental design

The experimental concentrations tested were 15%, 25%, 37%, 55%, 70% and 100% aquaculture effluent which was calculated by dividing the mean and maximum flow rate of the aquaculture effluent on Gargar flow rate. The test solution was prepared based on the volume ratios. A total of twenty *D. magna*, in 5 test groups, was transferred to the organism chamber. Neonates younger than 24 h were exposed to the solutions for 24, 48, 72 and 96 h. All observations on the mortality, DO, pH, and the temperature were recorded at these time intervals. At the end of the experiment, immobilized *Daphnia* was counted as dead organisms (if no movement was detected for 15 s after a gentle shaking of the glass cylinder). Moreover, *Daphnia* feeding was continued during the flow-through test [1]. The toxicity endpoint (LC_{50}) was determined as the concentration required

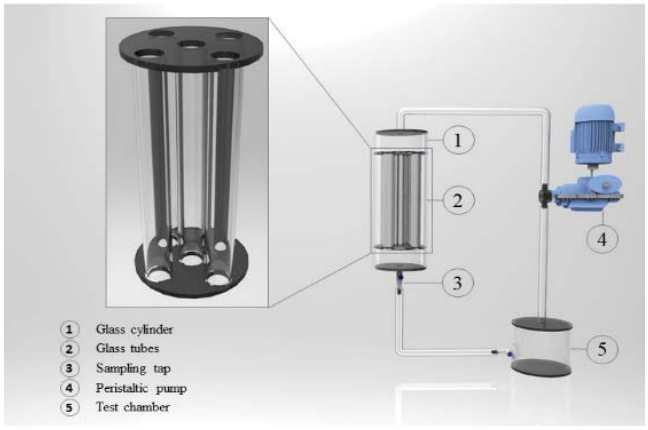

Fig. 2. Schematic of the pilot used in the flow-through.

to immobilize 50% of the Daphnids after 24, 48, 72, and 96 h exposure. A negative control group was included in which deionized water was used and the survival rate was adjusted as 100% to which the treatment groups were compared.

2.3.4. Data interpretation

Probit analysis using SPSS Statistical Package, v. 22 was used to determine LC_{50} and 95% confidence interval at the time intervals of 24, 48, 72, and 96 h. For a better interpretation of the toxicity data and for assessing the susceptibility of an organism to the toxic effluent, the $LC_{0'}$ and $LC_{100'}$ 96 h were converted into SAFE and SAR (safe application rate) coefficients. These coefficients calculate as following [5,25]:

$$\text{SAFE} = \frac{LC_0, 96\text{ h}}{LC_{100}, 96\text{ h}} \quad (1)$$

$$\text{SAR} = LC_{50} \times \text{SAFE} \quad (2)$$

The mean control survival rate greater than 90% was considered as a measure of the validity and acceptability of the test. In addition, the water quality parameters should remain within acceptable limits based on EPA standards [1,5]. Moreover, three replications were performed for each set of the volume ratio and control samples. Pearson correlation coefficient was used to determine the relationship between toxicity, concentration, and mortality. Further, data were analyzed by one-way analysis of variance (ANOVA), considering $p < 0.05$ as a significant difference.

3. Results and discussion

3.1. Physic-chemical characteristics of water

Chemical analysis and metals of water in aquaculture effluent and Gargar River are shown in Tables 1 and 2, respectively. One of the most important parameters in the effluent entering the receiving waters is pH. There were no significant differences between the aquaculture effluent and the Gargar River in pH value with the standard. Based on the results of COD and BOD analysis, their values in the effluent

Table 1
Water chemical analysis in aquaculture effluent and Gargar River

	EC (μmohs/cm)	pH	TDS (mg/L)	NO_3 (mg/L)	NO_2 (mg/L)	TKN (mg/L)	NH_3 (mg/L)	COD (mg/L)	BOD (mg/L)	DO (mg/L)
Aquaculture effluent	3,017.1 ± 246.9	7.9 ± 0.25	1,943.3 ± 149.6	1.64 ± 0.26	0.05 ± 0.02	10.04 ± 2.52	0.87 ± 0.33	24.90 ± 2.07	3.21 ± 0.60	6.01 ± 1.02
Gargar River	3,021.4 ± 212.9	7.8 ± 0.15	1,943.1 ± 129.8	5.37 ± 0.7	0.07 ± 0.01	12.37 ± 2.72	0.15 ± 0.05	23.80 ± 1.97	2.62 ± 0.40	6.36 ± 0.78
Standard*	**	6.5–8.5	**	50	10	–	2.5	60	30	≥2

Data are expressed as a mean ± standard deviation.
*Standards are based on Iran EPA [1].
**Discharge at concentrations higher than the amount specified in the table would be allowed if chloride, sulfate, and dissolved matter concentrations of the effluent do not increase more than 10% in receiving water and in a radius of 200 m.

Table 2
Concentrations of metals in the aquaculture effluent and Gargar River

	MO	Zn	Ni	Cd	Co	Mn	Fe	Pb	Cr	Cu	Ag	Al
Aquaculture effluent (ppb)	7	3.26	<1	<1	1.52	9.1	20	20.37	2.9	4.8	2.97	115
Gargar River (ppb)	7.8	8.6	<1	<1	3.4	74.6	141.67	146.1	6	3.17	2.96	694
Standard (ppm)*	0.01	2	2	0.1	1	1	3	1	0.5	1	1	5

*Standards are based on Iran EPA [1].

and river were found to be within the discharge limits in Iran [8]. EC, TDS, and DO results indicated that they were within the standard limits of effluent discharge in Iran [8] both in aquaculture effluent and Gargar River. Moreover, nitrogen compounds including NO_3, NO_2, NH_3, and TKN were also below the standard limits [8]. On the other hand, by making a comparison between the parameters and standards, it can be inferred that all these physicochemical parameters meet Environmental Protection Agency standards for the effluents. Results shown in Table 2 reveal that the concentrations of metals in both the effluent and river water were lower than the standards. As shown in Table 3, pesticides were not detected in both the effluent and river water. (Limit of detection for all pesticide was 1 μg/L). Although aquaculture effluent and Gargar River have a good quality based on physicochemical parameters measured, the correct interpretation of the toxicity of all mixed contaminants in the water is not provided. Therefore a bioassay test can be helpful in this regard [1,5,7].

3.2. Bioassay test

In this study, the controller parameters such as pH, temperature and DO in the control group and experimental groups were maintained during the experiment period. Changes in the temperature, pH and DO in the flow-through test solution were 21.01°C ± 1.25°C, 6.8 ± 0.30, and 8.2 ± 0.26 mg/L, respectively which all were within the standards for toxicity testing on *D. magna*. Barata et al (2008) [7] in order to culture *D. magna*, considered the pH of 7.06% ± 0.1% and DO 98.2% saturated ±4.4 that were consistent with our study. Carriger et al. [12] in their test environment study in 2011, pH, temperature and DO for the cultivation of *D. magna* were 7 ± 0.3, 20°C–22.5°C, and 7.5–7.9 mg/L, respectively.

Regression analysis shows that both independent parameters (concentration and exposure time) have a direct and positive correlation with the dependent parameter (mortality). But the correlation between concentration and mortality is higher than (Pearson correlation coefficient equal 0.843, $p < 0.001$) the correlation between exposure time and mortality (Pearson correlation coefficient equal to 0.463, $p = 0.013$). This result indicates that the role of concentration on mortality rate is greater than that of the exposure time. The result of ANOVA's table (Table 4) shows that the regression with 95% reliability is significant ($p < 0.001$).

Results of Correlation analysis also show that among the physical parameters there is a strong and positive correlation only between BOD and NH_3 concentration and mortality (Pearson correlation coefficient for both parameters equal to 0.919, p-value = 0.01). Therefore, increased concentrations of these parameters will result in increased the effluent toxicity effect on *D. magna*'s mortality was performed using Probit analysis and the values of LC_{50} with a 95% confidence interval are given in Table 5. Although the discharged effluent parameters measured were lower than the regulations for the receiving water standards, showed toxicities in the whole effluent toxicity test in *D. magna*. In addition, the longer duration of the test contact time, the higher the biological toxic effect on *D. magna*. The LC_{50} value of the effluent was 0.731 mg/L after 24 h while it dropped to 0.527, 0.486, and 0.367 mg/L after 48, 72, and 96 h, respectively. The toxic effect of the effluent on *D. magna* increased with longer exposure times. This observation is in agreement with the study results of Freitas et al. who also used *D. magna* for the evaluation of secondary effluent before and after tertiary treatment [26]. *D. magna* has been broadly accepted as a model experimental animal in the aquatic ecotoxicity testing. Several studies have confirmed the sensitivity of this crustacean in the evaluation of effluent ecotoxicity [4,26,27]. It has been reported that *D. magna* can be a useful bio-analytical tool for the early warning indicator of the compromised quality of the water resource. The estimated mortality rates using a regression model are shown in Table 6. Consequences of the study on the flow-through acute toxicity of the aquaculture effluent on *D. magna* in Gargar River indicated that the mortality rates in six different concentrations after 24, 48, 72, and 96 h, as compared to each other were found to have a significantly higher toxic effect on *D. magna*. Because aquaculture

Table 3
Concentrations of pesticides in the aquaculture effluent and Gargar River

	Carbaryl	Lindane	Diazinon	Malathion	Endosulfan
Aquaculture effluent	ND	ND	ND	ND	ND
Gargar River	ND	ND	ND	ND	ND
Standard (mg/L)*	0.001	0.002	0.004	0.07	0.002

*Standards are based on Iran EPA [1].

Table 4
Regression analysis ANOVA[a]

Model		Sum of squares	Degree of freedom	Mean square	*F*	Sig.
1	Regression	5,588.660	2	2,794.330	156.363	0.000[b]
	Residual	446.768	25	17.871		
	Total	6,035.429	27			

[a]Dependent variable: mortality;
[b]Predictors: (constant), time exposure, concentration.

Table 5
Toxicity results of aquaculture effluent on *D. magna*

Time exposure (h)	Toxicity (mg/L)		Confidence interval	
			Low	High
24	LC10	0.327	0.165	0.434
	LC50	0.731	0.610	0.875
	LC90	1.637	1.235	3.221
48	LC10	0.338	0.236	0.406
	LC50	0.527	0.452	0.584
	LC90	0.821	0.734	0.989
72	LC10	0.355	0.232	0.422
	LC50	0.486	0.401	0.538
	LC90	0.666	0.605	0.787
96	LC10	0.235	0.136	0.300
	LC50	0.367	0.281	0.425
	LC90	0.572	0.498	0.703

effluents are complex mixtures and their individual components cannot be routinely measured, it could be concluded that by combining a bioassay with a physicochemical analysis is a helpful tool to monitor the quality of the effluents and rivers [28].

The effective amount of effluent which is the lowest mortality rate at the different time exposures was calculated with the fitted model in Probit analysis and summarized in Table 6. The lowest and highest mortality rates of *D. magna* were observed in the volume ratio of 0.15 and 1, respectively. As can be shown in Tables 5 and 6, the probability of mortality increases with effluent concentration increases. *D. magna* population is sensitive to high concentrations of effluent and long contact time, which results in a significantly high mortality rate in *D. magna*.

In the regression model, the effect factor of effluent concentration, effect factor of exposure time and intercept were estimated at 38.84, 0.254, and –5.112, respectively. Therefore, the probable equation of *Daphnia* mortality is obtained as follows:

$$Y = \beta_0 + \left(\beta_1 X_1\right) + \left(\beta_2 X_2\right) \tag{3}$$

where Y is the probable response; β_0 is the intercept; X_1 is the independent variable number 1 (concentration); β_1 is the effect factor of independent variable number 1; X_2 is the independent variable number 2 (exposure time); β_2 is the effect factor of independent variable number 2.

So based on the data in this study the following equation is obtained.

$$Y = -5.112 + \left(38.84 \times \text{Concentration}\right) + \left(0.254 \times \text{Exposure time}\right) \tag{4}$$

The present study displayed that aquaculture effluent could be harmful to biota even if they obviously met standards for effluent discharge limits since measurement of a sufficient amount of physicochemical parameters is a very difficult task. Therefore, the physicochemical parameters alone are not a suitable indicator for the entry of effluents into the receiving waters. Current effluent discharge regulatory approaches deem exclusively on the physicochemical characterization and do not contemplate the bioassay assessment. However, the presence of highly toxic substances in the effluents requires reconsideration on the need for more specific bioassay assessments [3,29]. Because the effluent could include complex toxic, organic and inorganic pollutants, carcinogenic, and mutagenic substances which are all difficult to determine [28]. Moreover, the composition and size of SPS should be considered for the accurate estimation of toxicity for the river [2,30], but this issue was not addressed in this study.

According to the results of the Probit analysis in this study, the LC_0, LC_{50}, and LC_{100} in 96 h were 0.164, 0.367, and 0.821 (mg/L), respectively. As a result, SAFE and SAR coefficients were calculated as 0.2 and 0.073, respectively. Considering the number of SAR, it can be concluded that the effluents with this dilution can be discharged into the receiving water without any significant concern. Under this discharge condition, it is anticipated that most, if not all living organisms in the receiving water would have an adequate margin of safety against the aquaculture effluent. If there was a highly sensitive organism present in the Gargar River, then the effluent discharge may require further dilution. Results of the ecotoxicity tests provide baseline information for formulating a strategy for the discharge of aquaculture effluents into the receiving water bodies. For an application of the toxicity data in the regulation of effluent discharges and prediction of their effects on the aquatic environment both chronic and acute toxicity tests must be conducted in order to protect the aquatic life. Therefore, it can be inferred from the results of this study that the aquaculture effluent is toxic to *D. magna*. Therefore, performing only the chemical

Table 6
Mortality rate based on the fitted model

Time exposure (h)	Concentration (%)	Mortality rate (%)
24	15	6.8
	25	10.7
	37	15.4
	55	22.3
	70	28.2
	100	39.8
48	15	12.9
	25	16.8
	37	21. 5
	55	28.4
	70	34.3
	100	45.9
72	15	19.0
	25	22.9
	37	27.6
	55	34.5
	70	40.4
	100	52.0
96	15	25.1
	25	29.0
	37	33.6
	55	40.6
	70	46.5
	100	58.1

and physical analysis in the absence of bioanalysis may not be sufficient to guarantee the quality of the receiving waters and their safety to the animals in the aquatic system.

4. Conclusion

In conclusion, we have shown the aquaculture effluent posed toxicity to *D. magna* despite its physicochemical parameters tested were within the Iranian national standards. It can be inferred and proposed that the measurement of physicochemical parameters and toxicity bioassay for the monitoring of the water resource should be performed simultaneously. The discharge limits should be standardized from the perspective for the protection of biota in the freshwaters. Furthermore, the proposed integrated bioanalytical monitoring program should also consider all other possible discharges in the water body to ensure the biota are protected. Based on the results of the study and that of other experiences, we propose that ecotoxicological tests can be used for the assessment of whole effluents in Iran since bioassays can predetermine potential effluent toxicity in the shortest time possible.

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