



Evaluation of Toxicity of Nirouchlor Corporation Wastewater on *Poecilia reticulata*

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Received: 03/08/2016

Accepted: 20/09/2016

Published: 06/10/2016

Abstract

The main aim of this study is to determine of Nirouchlor wastewater's LD₅₀. Two set of experiments were conducted to determine LD₅₀ of the wastewater for contact times of 48 and 96 hours. First, three aquariums were prepared, each containing 15 pieces of *Poecilia reticulata*, 10 liters of water having three different concentrations of wastewater. The *Poecilia reticulata* was preserved in aquariums for 48 hours. Then, the number of dead fishes for each wastewater concentration was counted, and mortality percentage was calculated for each wastewater concentration. Second, the same procedure was used for 96 hours. The results show that LD₅₀ for contact times of 48 and 96 hours are equal of 0.48 and 0.3 mg/L, respectively. Comparing these results with LD₅₀ of other chemicals shows that in case of releasing of Nirouchlor wastewater into the environment without enough treatment, it can kill a large number of aquatic creatures. It is elaborated that the Nirouchlor wastewater is very toxic for aquatic creatures. The results of this study can be useful to find out which dangers will threaten the environment in the case of releasing Nirouchlor wastewater without appropriate treatment.

Keywords: LD₅₀, *Poecilia reticulata*, Wastewater toxicity

1 Introduction

In the science of toxicology, the median lethal dose is called LD₅₀. LD₅₀ is a particular dose of substance which is enough to kill 50 percent of a tested population members during a certain time. LD₅₀ is frequently used to indicate how acute a chemical toxicity is. Lower value of LD₅₀ means higher toxicity of substance. LD₅₀ test was introduced by Trevan (1927) [1]. Several animals such as mouse or fish are typically used to determine LD₅₀ [2]. Although in order for animals to relieve the pain other methods without animal tests have been introduced by the US Food and Drug Administration, LD₅₀ is still widely used [3, 4].

In this study LD₅₀ of Nirouchlor wastewater was determined. Nirouchlor is a corporation which was established in 1997 and produce chlorine and its derivatives. This corporation is located in Isfahan, Iran. The wastewater is completely treated before releasing into the environment. Since products of Nirouchlor contains high concentration of chlorine and its derivatives and also trace amount of sodium chloride, arsenic, cadmium, chromium, mercury, nickel, lead, antimony, selenium and iron [5], it

can be implied that in the wastewater of this corporation such chemicals can be found too. As several of the above mentioned chemicals are very hazardous for human and animal health, it is of the most important to know negative effects of this wastewater. The results of this study can be useful to find out which dangers will threaten the environment in the case of releasing of Nirouchlor wastewater into the environment without treatment.

Many researchers study about toxicity of different chemicals on living creatures [2, 6-9]. As instant Vajargah et al. (2014) studied on the acute toxicity of Butachlor to Caspian Kutum [2]. Butachlor is an herbicide of the acetanilide class, which is used widely in agricultural fields. *Rutilus frisii Kutum* which is a type of Caspian Kutum was used for this investigation. Vajargah et al. (2014) concluded that LD₅₀ of Butachlor for 96 hours contact time with *Rutilus frisii Kutum* is 0.258 mg/L [2]. Also there are other researches on wastewater toxicity such as investigation of Ursinos and Padilla (1992) which illustrated that LD₅₀ of olive mill wastewater toxicity is 87000 mg/L [10]. Although several studies have been carried out on determination of various chemicals toxicity, it has been found no investigation of LD₅₀ on this particular wastewater in the literature. Therefore, the aim of this study is to determine LD₅₀ of Nirouchlor corporation wastewater by using a special fish species named as *Poecilia reticulata* which is known as Guppy. This fish is the most popular species for aquarium and that is why it can be found all

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over the world. Guppies are used as a model organism in the field of ecology, evolution, and behavioral studies.

2 Materials and Methods

In this study firstly a few samples were taken from the wastewater of Nirouchlor corporation. Six aquariums containing 10 liters of water and 15 pieces of *Poecilia reticulata* were supplied for this experiment. The experiments were performed for contact time of 48 and 96 which are explained in the following sections. Figure 1 shows *Poecilia reticulata* which is used in this study.



Figure 1: *Poecilia reticulata* which is known as Guppy

2.1 Toxicity test for contact time of 48 hours

In this part of study the effect of wastewater concentration on the *Poecilia reticulata* health for 48 hours contact time was investigated. In order to carry out this part of study three aquariums containing 10 liters of water were prepared. Air was ventilated into the aquarium by using a compressor with flow rate of 50 L/min to provide aquarium's water with enough dissolved oxygen (at least 5 mg/L). The pH of all aquarium's water were adjusted on 7. Then, 15 *Poecilia reticulata* were added to each aquarium. Next, by adding specific amount of the wastewater to each aquarium, chemical oxygen demand (COD) of each aquarium's water was adjusted on the 32, 48 and 76 mg/L. After that, the *Poecilia reticulata* were preserved into the aquariums for 48 hours. Since the best temperature for growing of *Poecilia reticulata* is around 26°C [11], by using a water heater equipped to thermostat the temperature was adjusted on 26°C during all the experiments. To avoid an increase in concentration of toxic chemicals such as nitrogen and phosphorus, 1 liter of aquariums' water was daily replaced by equivalent amount of the wastewater. After 48 hours the number of dead *Poecilia reticulata* in each aquarium was counted and the mortality percentage (PM) for each one was calculated by equation (1).

$$PM = \frac{\text{Total fishes} - \text{Dead fishes}}{\text{Total fishes}} \quad (\text{Eq. 1})$$

2-2- Toxicity test for contact time of 96 hours

The experiment shows that chemical oxygen demand (COD) of the wastewater is 800 mg/L. In this part of study effect of 96 hours contact time with different concentrations of the wastewater on the *Poecilia reticulata* health was investigated. Method of this study is exactly same as toxicity test for contact time of 48 hours but the deference is that the contact time was extended for 96 hours. After 96 hours number of dead *Poecilia reticulata* in each aquarium was counted and the mortality percentage for each one was calculated by equation (1).

2.3 Method of LD₅₀ calculation

In this study LD₅₀ was obtained for 48 and 96 hours contact time. In order to calculate LD₅₀, the mortality percentage of fishes was plotted versus wastewater concentration by using a linear regression. As a result, two linear regression equations were obtained. Then, the wastewater concentrations which can kill 50% of *Poecilia reticulata* in 48 and 96 hours were calculated by using linear regression equations.

3 Results and Discussion

The percentage of *Poecilia reticulata* mortality in different wastewater concentrations is showed in Figures 2 and 3. Also, Table 1 shows all conditions and results of the experiments carried out to determine the LD₅₀ of the wastewater.

Apparently, a rise in wastewater concentration increases the percentage of *Poecilia reticulata* mortality. Although the percentage of mortalities obtained for these two experiments carried out for contact times of 48 and 96 hours were close to each other in lower wastewater concentration, they become divergent when the concentration rises. By using the linear regression between mortality percentage and wastewater concentration Eq. 2 and 3 were obtained for contact times of 48 and 96 hours, respectively.

$$y = 0.0185x - 0.4903 \quad (\text{Eq. 2})$$

$$y = 0.0177x - 0.3122 \quad (\text{Eq. 3})$$

where x is wastewater concentration and y is percentage of *Poecilia reticulata* mortality. By taking y as 50 in both Eq. 1 and 2, the wastewater concentration that can kill 50 percent of fishes during 48 and 96 hours were calculated.

Table 1: Conditions and results of the experiments

COD concentration (mg/L)	Total fishes	Number of dead fishes in 48 hours	Mortality percentage for 48 hours	Number of dead fishes in 96 hours	Mortality percentage for 96 hours
32	15	2	13	1	6
48	15	5	33	12	8
76	15	14	93	14	93

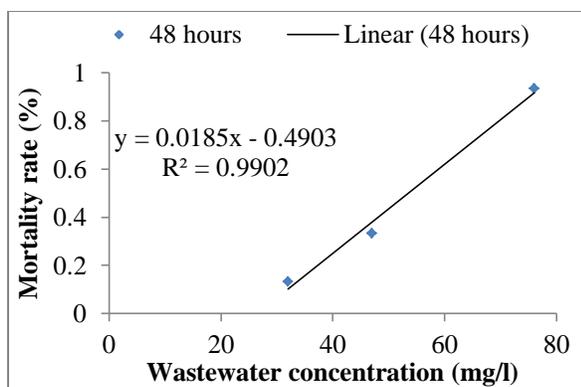


Figure 2: Variation of *Poecilia reticulata* mortality in different wastewater concentrations for contact time of 48 hours.

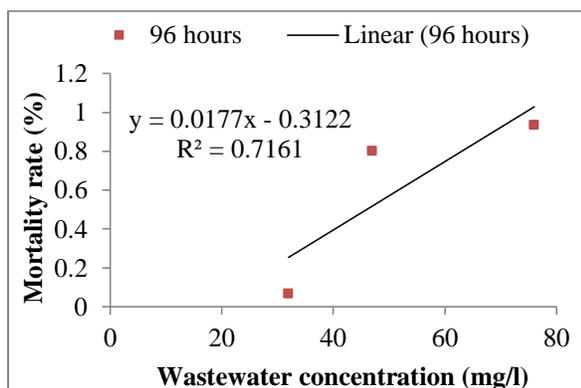


Figure 3: Variation of *Poecilia reticulata* mortality in different wastewater concentrations for contact time of 96 hours.

These concentrations are LD₅₀ for 48 and 96 hours. The results of this study shows that LD₅₀ for contact times of 48 and 96 hours are equal of 0.48 and 0.3 mg/L. Vajargah et al. (2014) concluded that LD₅₀ of butachlor for 96 hours contact time with *Rutilus frisii Kutum* is 0.258 mg/L [2]. According to our results the LD₅₀ of Nirouchlor wastewater is close to that of the butachlor, a strong herbicide of the acetanilide class with high toxicity, which is used widely in agricultural fields. A study on toxicity of olive mill wastewater shows that its LD₅₀ for contact time of 24 hours is equal to 87000 mg/L [11]. It is clearly showed that Nirouchlor wastewater is extremely toxic compared with other wastewater. Therefore, releasing of this wastewater without treatment can be very harmful for the environment.

Releasing of wastewater contaminated with chlorine into the lakes or rivers without enough treatment not only is toxic for creatures, but also lead to changing the taste and smell of water. In addition, chlorine can change the taste of coffee, tea and other beverages. Activated carbon filter or dissolving potassium metabisulfite into the wastewater are two ways to remove chlorine from the wastewater.

5. Conclusion

In this study LD₅₀ of Nirouchlor corporation wastewater was determined. Up until now, the wastewater of this corporation has been completely treated before releasing into the environment. However, there is no information about toxicity of the untreated wastewater for

living creatures, and that is why this study was carried out. Based on the results obtained in this study, LD₅₀ of this wastewater for contact times of 48 and 96 hours are equal of 0.48 and 0.3 mg/L which indicates a seriously high level of toxicity. The results of this study can be also useful to find out which dangers will threaten the environment in the case of releasing of Nirouchlor wastewater without treatment.

Acknowledgements

This article has been written from a Bachelor of Science dissertation in field of chemical engineering at Jami Institute of Technology, Isfahan, Iran. The authors of this article appreciate Jami Institute of Technology for its financial and moral support, which led to the completion of this dissertation.

Ethical issue

The authors are aware of, and comply with, best practices in publication ethics, specifically with regard to authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests and compliance with policies on research ethics. Authors adhere to publication requirements that submitted work is original and has not been published elsewhere in any language.

Competing interests

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Author contribution

It is certified that all the authors have a same contribution in the experiments and manuscript writing.

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