Iranian J Env Health Sci Eng, 2005, Vol.2, No.2, pp.1-4

Toxicity Evaluation of Wastewater Treatment Plant Effluents Using *Daphnia magna*

H Movahedian, B Bina, * GH Asghari

Dept. of Environmental Health, School of Public Health, Isfahan University of Medical Sciences, Iran

(First received 25 February 2005; accepted in revised form 13 September 2005)

ABSTRACT

Toxicity evaluation is an important parameter in wastewater quality monitoring as it provides the complete response of test organisms to all compounds in wastewater. The water flea *Daphnia magna straus* is the most commonly used zooplankton in toxicological tests. The objective of this study was to evaluate the acute toxicity of effluents from different units of Isfahan Wastewater Treatment Plant (IWTP). The samples were taken from four different physical and biological units. The acute toxicity tests were determined using *Daphnia magna*. The immobility of *Daphnia* was determined after 48h. Toxicity results showed that 48h-LC_{50} and ATU values for raw wastewater were 30% (v/v) and 3.33, respectively. It was also found that LC_{50} values after 48 h for preliminary, primary, and secondary effluents were 32%, 52% and 85% (v/v), respectively. The ATU values for these effluents were 3.1, 1.9, and 1.8, correspondingly. The efficiency levels of preliminary, primary, and secondary units for removal of toxicity were found as 6%, 38.9% and 8%, in that order. Overall, the present investigation indicated that toxicity removal by up to 50% might be achieved in IWPT. Based on the obtained results and regarding the improvement of water quality standards, coupled with public expectations in Iran, it is necessary to consider more stringent water quality policies for regular monitoring and toxicity assessment.

Keywords: *Acute toxicity,* Daphnia magna*, Wastewater*

INTRODUCTION

Treatment of domestic and industrial wastewater is crucial for protection of receiving waters. Parameters such as pH, dissolved oxygen, BOD, COD, TOC, TDS, and TSS are generally used for evaluation of effluent quality. However, these parameters can not be used for evaluation of toxicity effect on receiving waters due to some specific defects. The best way to evaluate effluent toxicity effect is to use biotoxicity test (Davis and Ford, 1992; Metcalf and Eddy, 2003). Different organisms such as fish, algae, bacteria, and other microorganisms may be used in biotoxicity tests. The water flea *Daphnia magna* is the most commonly used zooplankton in toxicological tests in wastewater treatment, due to short doubling time, high sensitivity, and simplicity; therefore, it was used as an indicator in this study (APHA, AWWA, WEF, 1992; Official Gazette, 1996; USEPA, 2000). Villegas Navaro et al. (1999) reported the use of *D. magna* as a toxicity indicator for textile industrial effluents to show that the toxicity tests combined with physico-chemical analysis are essential in the evaluation of effluent quality and also in the assessment of treatment plant efficiency in Mexico.

***Corresponding author:** Tel: +98 311 7922695, Fax: +98 311 6682509, E-mail: movahedian@hlth.mui.ac.ir

Toxicity tests were also used for evaluation of domestic and industrial wastewater effluents (Tisler and Zagorc, 1999; Villegas Navaro et al., 1999; Richard et al., 2000). It is reported that although the effluent meets all physicochemical requirements but regarding its toxicity, it may cause considerable negative effects in receiving waters. Effluent quality evaluation in Iran is based on physicochemical parameters. In this study, the toxicity removal efficiency of different units of Isfahan Wastewater Treatment Plant (IWTP) was evaluated and the validity of regular physicochemical parameters as limits for discharge to receiving waters is discussed.

MATERIALS AND METHODS

Experimental study was carried out during seven months in IWTP using *D. magna*. Materials used in this study were animal compost, garden soil and dry yeast.

Culture preparation Five g of dried animal compost was mixed with 25 g of soil garden. Then, one liter of pond water was added to the mixture. The mixture was filtered through 0.15 mm pores membrane and kept at laboratory temperature for 2 d. For the preparation of the final culture medium, one volume of filtered liquid was mixed with 6 to 8 volumes of pond water (Davis and Ford, 1992).

***Propagation and culture of* Daphnia**

Daphnia was collected from a natural park. Initially one of the isolated *Daphnia* was cultured. In the next step, the recultured daphnids were used to prepare the final culture. For this reason, 100 ml of the final culture was poured into special bottles. Then, one single *Daphnia was* added to each bottle. To support the growth of *Daphnia* during the day after the initial culture, one mg of dry yeast was added to each bottle, every other day. Identification of *Daphnia* was carried out according to US-EPA (2000).

Determination of 48h- LC_{50} Thirteen samples were taken from four different points of IWTP. Samples were taken from raw wastewater influent, and also from preliminary, primary, and secondary sedimentation tanks effluents. The samples were diluted by 3, 4.5, 7, 15, 10, 23, 34, 51, 77, and 100% (v/v). Ten daphnids were added to each dilution and the results of daphnid mortality were recorded after 48 h (LC_{50}). The results of experiments were acceptable only in cases where Daphnids in the blank tubes were observed to have a mortality rate of less than 10%. Totally, 520 samples were tested. It should be noted that temperature was checked regularly using a thermometer in the culture medium. An aerator pump was used to provide oxygen. At the end of the experiment, Acute Toxicity Unit (ATU), efficiency of each unit, and total efficiency values were determined as follows:

$$ATU = 100/LC_{50}\% \text{ (v/v)}$$

$$R = (ATUi - ATUe)/ ATUi * 100$$

Where: ATUi= influent ATU; ATUe= effluent ATU; R = efficiency.

Sampling methods and analysis were performed according to standard methods (APHA, AWWA, WEF, 1992) and US-EPA (2000).

RESULTS

Table 1 shows the results obtained for the influent to and effluent from each unit, showing 48h- LC_{50} and ATU in the raw wastewater and in preliminary, primary, and secondary treatment effluents. The results were analyzed using SPSS software and Probit facility. The table also presents the highest and lowest levels for 95% confidence.

Table 1: Toxicity results obtained from IWTP

Wastewater Concentration (7.7%)	Number of organisms alive in each dilution	Average number of dead organisms after 48h contact time and 13 repetitions for each sample			
		Raw wastewater	After preliminary treatment	After primary treatment	After secondary treatment
100	10	10	10	7	6
77	10	7	7	6	4
51	10	6	5	5	3
34	10	5	5	4	2
23	10	3	3	2	1
15	10	3	3	2	1
10	10	2	2	1	0
7	10	1	1	1	0
4.5	10	1	1	1	0
3	10	1	1	0	0
0 (Blank)	10	0	0	0	0
48 h-LC_{50}		30%	32%	52.7%	85.6%
95% confidence	Lower limit	21	22	34	58
limit	Higher limit	48.5	53	108	195
48 h-LC_{50} As ATU		3.3	3.1	1.9	1.8

Table 2: Toxicity removal efficiency of different IWTP units

After preliminary treatment	After primary treatment	After secondary treatment	Total efficiency
6%	38%	8%	50%

DISCUSSION

A: Raw wastewater

As can be seen from Table 1, 48h-LC_{50} for raw wastewater as the influent of the plant was 30%(v/v) and 3.3 as ATU. The highest and lowest levels (95% confidence limit) were 48.5 and 21, respectively. Similar results were reported by Blinova (2000) which reported 48h-LC_{50} up to 34% (v/v) for raw wastewater. However, it should be noted that the quality and quantity of raw wastewater could be quite different due to culture, custom, nutrition, health education, etc. (Metcalf and Eddy, 2003). As the results of both studies were found to be in the same range of 95% confidence limit, therefore, the accuracy of the results is acceptable.

B: After preliminary treatment

Table 1 shows the 48h-LC_{50} for wastewater effluent after preliminary treatment was 32% (v/v) with the highest and lowest levels being 53 and 22, respectively. The confidence limit was 95%. The ATU was found as 3.1. The LC_{50} obtained in this step was not significant compared with that of raw wastewater. Preliminary treatment consisted of screening and grit removal. It was assumed that some of the toxic materials, which were toxic to *Daphnia*, might be adsorbed by grit and large suspended solids and, thus, removed. However, the results of this study showed that these units were not efficient in removing these materials. The toxicity removal efficiency of preliminary treatment was found to be 8% (Table 2).

C: After primary sedimentation

Table 1 also shows that the 48h-LC_{50} for wastewater after primary treatment was 52.7% (v/v) with the highest and lowest levels being 108 and 34, respectively. The ATU was found to be 1.9. The aim of primary treatment is removal of suspended solids. It was assumed that the primary sedimentation might remove 50% to 70% of suspended solids and 25% to 40% of BOD5 in the influent (Metcalf and Eddy, 1993). As suspended solids consist of organic and inorganic substances such as proteins, carbohydrates, fat, etc., the high efficiency of this unit is in the reduction of wastewater toxicity. On the whole, the present study indicated a toxicity reduction of 38% after primary sedimentation (Table 2).

D: After secondary treatment

It can be seen from Table 1 that the 48h-LC_{50} for effluent after secondary treatment was 85.6% (v/v) with the highest and lowest levels being 94% and 58%, respectively. The ATU was found to be 1.8. The results of this stage were similar to those obtained by Blinova (2000) and Richard et al. (2000), who found 48h- LC_{50} in the effluent of municipal wastewater as 84% (v/v). The main secondary treatment processes in this plant were aeration and secondary sedimentation tanks. The aim of secondary treatment is to remove soluble organics from wastewater. As it is almost impossible to mention any certain substance responsible for toxicity in wastewater, it is reasonable to declare that removal of both organic and inorganic substances in wastewater resulted in toxicity reduction. Therefore, using biotoxicity tests is an economical and technical method for direct measurement of toxicity in wastewater effluent. The Dept. of Environment of Iran (DOE) has set BOD5 and SS levels of less than 30 and 40 mg/l, respectively, as permissible limits for effluent discharge into receiving waters (DOE, 2000). Although IWTP meets this requirement, care must be taken regarding the toxicity.

The results of the present study showed that physico-chemical parameters alone were not sufficient in obtaining reliable information on treated wastewater toxicity and that toxicity tests must be performed in combination with routine analyses such as BOD and SS in order to guarantee the safety of aquatic organisms.

ACKNOWLEDGEMENTS

The authors would like to express their appreciation to the Research Deputy of Isfahan University of Medical Sciences for their financial support and help for this study.

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