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Acute and Chronic Toxic Effects of a Waste Stabilization Pond Wastewater on *Daphnia magna*

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Abstract: This work aimed at evaluating the performance of a wastewater stabilization pond at El-Mofti village (Kafr El-Sheikh Governorate) through determining survival, growth and reproduction rates of *Daphnia magna* in different concentrations of raw wastewater as well as the effluents from the different steps of the treatment system. The LC50 of the raw wastewater for *D. magna* was found to be 21% of the wastewater using the 48h acute toxicity test, while, the effluents from the anaerobic, facultative and maturation steps showed less toxicity than the raw water being 25, 36 and 40% respectively. Reduction in toxicity could be due to several biological and physical factors taking place during the passage of water through the treatment system. Based on this work the wastewater stabilization pond under investigation requires adjustment of detention time such that more assimilation and settlement of toxicants can be achieved. Chronic toxicity test was applied using sublethal concentrations equivalent to LC10, LC15, LC20, LC25, LC30, LC35 and LC40, as deduced from the regression line of the data obtained from the acute test. The percent survival of the test organism at different concentrations of the wastewater at the 21st day exposure period ranged between 90% in the lowest concentration and 60% in the highest concentration, compared to 100% in the control. The mean length of the adult female in the control exceeded the length of those exposed to the wastewater. The average weight of the adult female in the control was 341 µg and decreased gradually with the increase in wastewater concentration to reach 228 µg. The time of onset of reproduction was 7days in the control while it took 13 days in the highest wastewater concentration. The results indicated thus that the raw wastewater had adverse effects on survival, growth and reproduction of the test organism; although water of the drain after mixing with the pond effluent did not show acute toxicity on daphnids indicating that the rate of dilution with drain water was sufficient to remove toxic effect of the effluent.

Key words: Acute and chronic toxicity, *Daphnia magna*, waste stabilization pond, influent, effluent, wastewater treatment, performance evaluation.

INTRODUCTION

We use a wide variety of chemicals including human and veterinary drugs, diagnostic agents, cosmetics, preservatives and detergents (Halling-Sorenson *et al.*, 1998). Antibiotics were found in the aquatic system with a concentration generally within the microgram per liter range (Yang *et al*., 2005; Yang and Carlson, 2003; Kim *et al*., 2007). There are increasing concerns about the potential environmental effects that may occur from such “emerging contaminants” (Schnoebelen *et al*., 2006).

Sewage treatment is a pressing global need being of environmental as well as human health concerns. In areas where piped sewerage systems are viable, waste stabilization pond (WSP) systems are one possible method for the treatment of wastewater. The relative simplicity and low operating cost of the WSP make it the preferred technology for handling, treatment and disposal of domestic waste for small communities (Agunwamba and Ogarekpe, 2010).

Treatment of wastewater occurs as constituents are removed by sedimentation or removed by biological and chemical processes (Crites *et al*., 2006; Nelsona *et al*., 2004). Stabilization ponds are well adapted to low socio-economic conditions in developing countries (Kouraa *et al*., 2002; Alcalde *et al*., 2002). High efficiencies of WSP have been reported with respect to removal of intestinal nematode (Lakshmarayana and Abelulapa, 1972; Feachem ,1986; Saqqar and Pescod 1992), organic compounds and faecal bacteria (Mara ,1976). In a pond system the three steps: anaerobic, facultative and maturation ponds are used for wastewater reclamation via natural purification processes.

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Discharging wastewaters without proper treatment can have adverse effects on receiving water bodies; even if concentrations of toxic chemicals in the effluents as determined by chemical analysis pass water quality criteria because chemical analysis alone is difficult to assess ecological effects of toxic chemicals in wastewater (Burgess *et al*., 1995; Rosa *et al*., 2001).

Toxicity evaluation is an important parameter in wastewater quality monitoring as it provides an overview of the response of test organisms to all the compounds in the wastewater (Wang *et al*., 2003). Acute toxicity data have been used to derive water quality guidelines for regulatory measures (Sunderam *et al*. 1994). To achieve a realistic estimation of the hazard of pollutants it is necessary to know their toxic effects as has been stated by Kaza, *et al*. (2007). The main objectives of this research include: 1.determining acute and chronic toxicity of the influent of El-Mofti village (a rural area in Kafr El-Sheikh, Egypt) waste stabilization pond (WSP) using the freshwater cladoceran *Daphnia magna*, and, 2. determining acute toxicity testing of the effluents of the different treatment steps for evaluating their performance separately.

MATERIALS AND METHODS

Wastewater treatment system in El-Mofti village (Kafr El-Sheikh Governorate) was designed to serve 3000 persons. Wastewater flow is about 225 m^3/day mainly of domestic origin. This system consists of 500 primary septic tanks, each of which has a volume of approximately 1.8 m^3 with area 1.12 m^2 and depth 1.6 m) which are used as primary treatment; effluents of 500 septic tanks are collected and finally pumped to WSP. a pumping station and a wastewater stabilization pond (WSP) system having two lines in parallel, each line consists of an anaerobic pond with volume 1400 m^3 (depth 3 m and area 475 m^2), a facultative pond with volume 1500 m^3 (depth 1.5 m and area 1050 m^2) and a maturation pond with volume 850 m^3 (depth 1.4 m and area 635 m^2). The WSP effluent is discharged into El-Sabahi agricultural drain.

Sampling sites:

Samples were collected monthly from seven sites from each step of WSP and from agricultural drain during the period from February 2006 until March 2007. Samples numbers 1-4 represented: 1-the influent wastewater (effluent of all septic tanks), 2-the anaerobic pond effluent, 3-the facultative pond effluent and 4-the maturation pond effluent. Samples no.5-7 represented: 5-drain water before mixing with the treatment effluent, 6-at the mixing point and 7-drain water after 700 m downstream mixing point in the drain.

Experimental Animals and Food:

Daphnia magna a freshwater crustacean (Cladocera) strain that has been continuously reared at the Water Pollution Research Laboratory of National Research Center for more than 15 years in a synthetic freshwater medium (Fayed and Ghazy, 2000), was used as the test organism for this study. Gravid females were transferred at regular intervals to 1-L glass beakers in which the holding medium was renewed 3 times a week. The animals were fed 3 times a week with $14X10^7$ coenobia /ml of the green micro alga *Scenedesmus obliquus* (Ghazy, 1997) for rearing and for maintaining the organisms in chronic testing. The algae and daphnids were kept at a temperature 22± 2°C with a light period of 16 L: 8 D both during culturing and experimental periods.

Short Term (48h acute) test:

Forty-eight hours, acute toxicity tests (static system) were conducted with less than 24h old daphnids. The percentage of raw wastewater ranged from 20 to 45 % for samples no. 1-4 while samples no. 5 to 7 were not diluted(Table 1); the synthetic medium was used as dilution water. Ten daphnids were employed in each replicate with 100 ml of the test water introduced in 250-ml glass beaker. Three replicates were conducted per each test. Control test was run in parallel. Mortality manifested as immobility of test organisms was recorded at 48h exposure. Generally in toxicity test, death is a decisive criterion because it is easy to determine and have obvious biological and ecological significance (Nwani *et al*., 2010). Acute toxicity tests were run without food addition as recommended by the standard toxicity testing (APHA, 1989).

Long Term (21 days) chronic test:

Ten of < 24h-old daphnids were employed for each chronic toxicity testing (static-renewal system for 21 days) in 250-ml beaker containing 100 ml of test water (diluted pre-pond wastewater). The exposure concentrations 13, 14, 15, 16, 17, 18 and 19 % raw (pre-pond) wastewater were equivalent to the sublethal concentrations LC10, LC15, LC20, LC25, LC30, LC35 and LC40, respectively, as had been deduced from the regression line obtained from the static 48h acute test. Three replicates were used for each concentration as well as the control.

Parent survival and reproduction (count of produced young) were monitored three times each week. Dry weights of daphnids survived up to the end of exposure time (21days) were recorded. The dry weight was determined after incubation at 60°C for 24h (Lovergrove, 1966) and body length was measured from the anterior-most portion of the head to the base of the caudal spine using a microscope with an eyepiece micrometer.

Data Processing:

The median lethal concentrations (LC50s) of the tested wastewaters for daphnids were calculated from the data obtained in acute toxicity bioassays after 48 h exposure, following the probit analysis method as described by Finney (1977). The terminology recommended by Sprague (1969), lethal concentration (LC) was used for survival and, as given here, represents an interpolation from three or more partial-effect concentrations.

RESULTS AND DISCUSSION

Median Lethal Concentrations:

In acute tests, neonates of *D. magna* were exposed to different dilutions of pre-pond wastewater ranging from 0 to 35% (v/v). The probit estimated 48h-LC50 for the wastewater was 21%. For the effluents of anaerobic, facultative and maturation ponds the tested dilutions ranged from 0 to 40%, 0 to 45% and 0 to 50%, respectively (Table 1) and the corresponding 48h-LC50s were found to be 25, 36 and 40% (v/v). From these results it appeared that toxicity as LC50 decreased from 21% for the influent wastewater to 40% for the final effluent of the waste stabilization pond, however this reduction in toxicity is not sufficient to safely reuse this effluent. Reduction of toxicity could be due to several biological and physical factors taking place during the passage of water through the treatment system.

These results go along with the data obtained on the changes in the levels of traditional chemical parameters and bioindicators, as a result of the wastewater treatment in the stabilization pond (Ghazy *et al*. 2008) 50.65% removal for BOD, 48.95% removal COD, 44.3% removal for TSS and 70% removal for ciliates were recorded and modification in the design of the pond to increase its efficiency was recommended. Mozaheb *et al*. (2010) evaluated stabilization ponds performance in Yazd- Iran and stated that they did not meet the lowest Iranian standards, and that they required renewing.

The Effect of Influent Wastewater on the Survival, Growth and Reproduction Rate of D. Magna:

Table (2) and Fig. (1) Show the effect of different sub-lethal concentrations of the influent wastewater on survival of *D. magna* at the end of the experiment (21 days). Generally it was found that the survival decreased with increasing the pre-pond wastewater concentration from 0% (control) to 19%. All (100%) of *D.magna* cultured in the control could survive for 21 days. While only 60% of those incubated in the highest wastewater concentration (19%) could survive for 21 days.

Figure (2) illustrate the effect of the different wastewater concentrations on growth of *D. magna* at the end of experiment. Generally it was found that the length of *D. magna* increased with increasing the period of incubation for all wastewater concentrations. At the end of experiment, the organisms in the control reached 4.363 mm, while the length of those incubated in the highest concentrations (19%) was 3.502 mm, at the end of 21 days. Also length of neonates released at 21st day decreased with increasing the wastewater concentration (Table 2).

The raw wastewater affected reproduction of females during the experiment, such that the number of progeny per female at 21 day increased with increasing the waste concentration (Table 2), the maximum number was 63 neonates/female at the highest concentration (19%). It is worth mentioning that most of the released neonates were either weak or dead. This was observed also at low and high pH levels (Ghazy *et al*.,under Publication). The least number of progeny /female was observed for those incubated in the control all of them were live and appeared in a healthy manner. The age at first reproduction was 7 days for daphnids in the control and those incubated in the wastewater concentrations 13 and 14 %, while the time to the first brood increased at higher wastewater concentrations to reach 13 days at 19%. The results indicated that the raw wastewater had adverse effects on survival, growth and reproduction of the test organism.

Conclusions:

The results indicated that the domestic wastewater released from septic tanks had adverse effects on survival, growth and reproduction of the test organism, *Daphnia magna,* and that the WSP caused reduction in toxicity of this wastewater. Reduction of toxicity could be due to several biological and physical factors taking place during the passage of water through the treatment system. Based on this work, the upgrading of performance of the studied waste stabilization pond requires reviewing its design parameters in order to allow better assimilation of toxicants as well as maximizing the effect of time dependent factors to get into action.

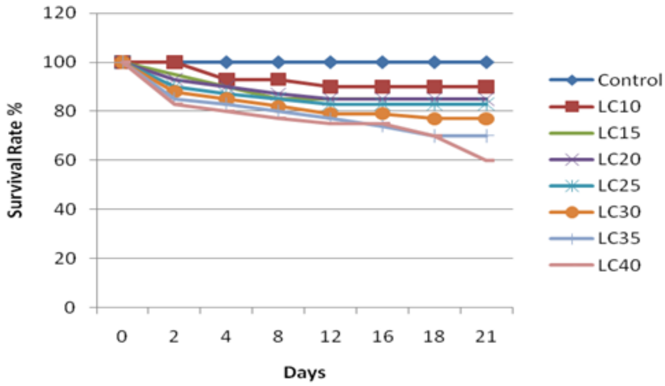

Fig. 1: Effect of different concentrations of influent of El-Mofti Waste Stabilization Pond (WSP), on survival rate of *D. magna* during the experimental period (21 days).

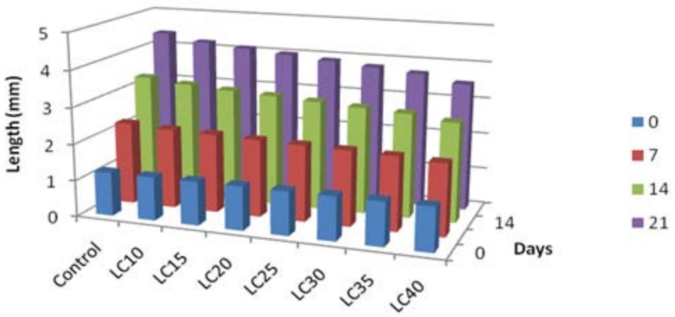

Fig. 2: Effect of different concentrations of influent of El-Mofti Waste Stabilization Pond (WSP), on growth rate of *D. magna* during the experimental period (21 days).

Table 1: Comparison between 48h- LC50s of influent, different effluents of the waste stabilization pond and drain water, to Daphnia magna in static system for 48 h

Samples No.	Wastewater Concentrations %	48h-LC50 %
1	20, 25, 30, 35	21
2	30, 35, 40	25
3	30, 35, 40, 45	36
4	35, 40, 45, 50	40

1. Influent (effluent of all septic tanks), 2. Effluent of anaerobic pond,
3. Effluent of facultative pond, 4. Effluent of maturation pond.

Table 2: Effect of influent of the waste stabilization pond on % survival, growth and reproduction rates of Daphnia magna cultured in static-renewal system for 21 days

Raw wastewater (%)	% Survival at 21st day	Time to the first brood (days)	Number of progeny per female at 21st day (mean± SD)	Mean length of adults at 21st day (mm) (mean± SD)	Mean length of neonates released at 21st day (mm) (mean± SD)	Average dry weight of adult females at 21st day (μg)
0.0(Control)	100	7	31±2	4.363±0.069	1.182±0.159	341
13 (LC10)	90	7	37±8	4.220±0.052	1.131±0.159	303
14 (LC15)	85	7	44±10	4.081±0.125	1.129±0.145	302
15 (LC20)	85	9	50±8	4.015±.156	1.047±0.145	287
16 (LC25)	83	9	58±5	3.866±.207	1.045±0.088	268
17 (LC30)	77	11	59±1	3.758±.207	1.036±0.070	251
18 (LC35)	70	12	62±17	3.813±0.158	1.008±0.045	251
19 (LC40)	60	13	63±7	3.502±.069	0.981±0.045	228

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