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EVALUATE THE ECOTOXICOLOGICAL RISKS OF TREATED HOSPITAL WASTEWATER USING *DAPHNIA MAGNA* BIOASSAY

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ABSTRACT

The hospital wastewater has been one of the concerned effluents so far. In Vietnam, the majority of hospital wastewater has not well treated before released into nature. In this study, wastewater samples were collected at the out let of a hospital and analyzed with thirteen of chemical and biological parameters, followed with toxicity bioassay. The *Daphnia magna* was exposed to the wastewater at the concentrations of 10; 30; 50% (v/v) for chronic test during 14 days. Adversely chronic effects were observed with the end points of survivorship and reproduction. Our results showed that most chemical and biological parameters (11/13) were within the guideline values of Vietnam National Technical Regulation on Health Care Wastewater (QCVN 28:2010/BTNMT) for discharge into nature, except pH and Nitrate. However, the wastewater reduced on survivorship and reproduction of the organism in chronic exposures. The dead neonates in brood chamber of mother daphnids were observed in 30 and 50% of wastewater exposures while the malformation of the neonate was detected in 10% of wastewater incubation. Results of this study showed that there were potentially risks to aquatic organisms from the hospital wastewater and a need for revision the National Technical Regulation on Health Care Wastewater (QCVN 28:2010/BTNMT) to protect sensitive organisms in aquatic ecosystems.

Keywords: Acute test, chronic effects, *Daphnia magna*, hospital wastewater

1. INTRODUCTION

Hospital wastewater includes macro- and micro-pollutants of wide concentration range from laboratories, research units, operation rooms, units, where medicine and nutrition solutions are prepared, and polyclinics (Akin, 2016). For macro-pollutants, pollutant concentrations of BOD (Biochemical oxygen demand), COD (chemical oxygen demand), TSS (Total Suspended Solids), NH_4, NO_3, total P are two to three fold greater contribution than unban wastewater (Verlicchi et

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al., 2012a). For micro-pollutants, which are generally present in concentrations ranging from ngL^{-1} to mgL^{-1}, such as PhCs, AOX (organic halogens adsorbable onto active carbon), volatile halogenated organic compounds and other organic compounds including acetaldehyde, ketones, alcohols acetates and phenols that are harmful to the environment has received great attention (Boillot et al., 2008). Moreover, hospital wastewaters also contain dangerous bacteria and parasites as hepatitis B, hepatitis C, and HIV (Akin, 2016). However, there is a wide variability of the characteristics of the hospital wastewater in relationship to the size of hospitals, the bed density, the number of inpatients and outpatients, the number and the type of wards, the number and types of services, the country and the seasonality (Al Aukidy et al., 2014; Verlicchi et al., 2012b). Besides, the incomplete treatment and untreatment of wastewater treatment progress also released a large amount of antibiotic (Duong et al., 2008; La et al., 2016) and radioactive elements and other toxic compounds such as Cu, Fe, Cd, Pb, Hg, Ni, Pt, Cyanide in the effluent (Amouei et al., 2015)

Vietnam National Technical Regulation on Health Care Wastewater (QCVN 28:2010/BTNMT) is a current regulation, used for guiding values of parameters in the effluent of hospital wastewater for directly discharging to receiving water. However, the ecological risks from hospital wastewater are still unknown. In European Union countries, the acute toxicity tests with aquatic organisms in 24h and 96h were applied to access the safety of the effluent of wastewater after treatment (Carraro, 2016), and the in others Asia countries such as China (National Standard of the People's Republic of China, 1998), India (Ministry of Environment & Forests, 1998) and Brazil (Carraro, 2016), they all have National Standard for hospital wastewater.

Up to now, studies on the effects of hospital wastewater on Cladoceran, *Daphnia magma* are limited. Emmanuel et al. (2004; 2005) noted that EC_{50} (immobilize) of untreated hospital on *D. magna* were 44; 117 TU for 24h and 71 TU for 48h (1TU = $100/EC_{50}$). For municipal wastewaters, Ra et al. (2007) found only one the effluent of wastewater treatment plant with TU was exceeded 1 (1.31) while TU values of the other four remained below 1 (0.10 – 0.80). In addition, Koçbaş and Oral (2015) reported the TU of effluent of municipal wastewater treatment plant of 3.0 and 3.4 for 24h and 48h, respectively. Although, several investigations on the chronic effects of municipal wastewater (Ghazy and Fayed, 2011; Ngo et al., 2011); industrial wastewater (Nguyen et al., 2016); pharmaceutical industry wastewater (Tišler and Zagorc-Končan, 1999) and mixture of wastewater from industry, urban, and agriculture (Parlak et al., 2010) were implemented, chronic effects on *D. magna* have not investigated.

Our study aim to evaluate the ecotoxicological risk assessment of treated hospital wastewater using *Daphnia magna* bioassay. The treated wastewater from a general hospital that has been allowed for discharging into receiving waters based on QCVN 28:2010/BTNMT was used as a

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case study. In addition, our experiment was conducted to identify the evident for proposing revisions the national current regulations.

2. MATERIALS AND METHODS

2.1 Sample collection and analysis methods

Wastewater sample was collected in May 2016 from a reservoir after treating by membrane technology, belonging to a hospital with a capacity of 500 beds, in Ho Chi Minh city, Vietnam. The wastewater sample was collected in 30 L plastic vessel and transported to the laboratory and stored at 4°C to toxicity tests in the same day.

Sub-samples of wastewater were used for quantitative analysis of thirteen parameters according to QCVN 28:2010/BTNMT according to the Vietnam guideline for water and wastewater analyses (TCVN), Standard Methods for the Examination for Water and Wastewater (SMEWW), and HACH Methods. The pH of the wastewater was measured by TCVN 6492:2011, whereas TCVN 6001–1:2008 was applied for BOD_5 determination, SMEWW 2012, 5220D for COD; SMEWW 2012, 2540D for Total suspended solids (TSS); HACH Method 8131 for Sulfur; HACH Method 8038 for NH_4^+; SMEWW 2012, 4110B for Nitrate; SMEWW 2012, 4500P, E and HACH Method 8048 or PO_4^{3-}; SMEWW 2012, 5520B and SMEWW 2012, 5520F for Oil and grease; TCVN 6187–2:1996 for Total coliform; SMEWW 2005, 9260B for *Salmonella*; SMEWW 2012, 9260E for *Shigella*; SMEWW 2012, 9260H for *Vibrio cholera*.

2.2 Toxicity experiments

2.2.1 Test organisms

The cladoceran D. magna original from Microbiotests Inc, Belgium was used for the toxicity test. The animals were raised in the ISO medium (SOP, 2007) and fed by a mixture of viable green algae Chlorella sp. and Scenedesmus sp., which were cultivated in the COMBO medium (Kilham et al., 1998) under continuous aeration. Both Daphnia and green algae were maintained at a temperature of 25 ± 1°C, 12/12 h light/dark cycle.

2.2.2 Chronic toxicity test

The testing on life history traits of *Daphnia* was conducted according to Dao et al. (2010). The experiments consisted control and wastewater exposures. The control was run in which *D. magna* raised in ISO medium without any wastewater addition. For exposures, wastewater were mixed with ISO medium to reach final concentrations of 10; 30 and 50% (v/v), hereafter referred as M10, M30, M50. For each treatment (control or wastewater exposures), fifteen neonates (<24h old) were randomly collected and individually transferred into a 50 mL beaker containing 30 mL medium. The organisms were fed with *Scenedesmus* sp. at the concentration of 1 $mgCL^{-1}$

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during the test. Test medium and food were renewed every two days. Besides, pH and dissolved oxygen (DO) of test media were measured with the Metrohm 744 and WTW Oxi 197 Meters, respectively, at the test initiation and termination. During experiments, the mortality of parent daphnids was recorded. Total progeny, number of neonates per female, brood size, maturity age, age to first reproduction, number of brood per female daphnid, and intrinsic rate of natural increase were measured. The number of newborn neonates was counted and discarded, daily. The test lasted for 14 days. The intrinsic rate of natural increase (r) was calculated according to the description of Villarroel et al. (2003).

$$\sum_{x=0}^{\max age} e^{-rx} l_x m_x = 1$$

where, l_x is the proportion of individuals surviving to age x, m_x number of neonates produced per surviving female at age x) and x in days. As r calculated in *D. magna* organisms after 14 days is indistinguishable from r estimated for the entire lifespan, due to the great importance of early reproduction (Van Leeuwen et al., 1985), all calculations were based on 14-day experiments.

2.3 Data mining and analysis

All chronic data of control and exposures were presented as mean ± standard deviation (SD). One way ANOVA was applied to detected the difference of survivorship and reproduction of D. magna between control and exposures. In order to test the assumption of homogeneity of variances, the Levene's test was used. Tukey HSD multiple comparison tests were used in case significant differences were detected ($p < 0.05$). If assumptions were not fulfilled, data will be transformed and test again ANOVA with transformed data before applied non-parametric Kruskal–Wallis test, followed by Multiple comparison of ranks. SPSS 22 software (IPM, USA) was used for all analysis.

3. RESULTS

3.1 Physico-chemical characteristics of hospital wastewater

The results of analysis showed that almost parameters (11/13) were within values of QCVN 28:2010/BTNMT, excepted for pH (4.3) and Nitrate (113 mgL^{-1}) (Table 1).

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Table 1. Concentrations of parameters in the effluent of wastewater and the guideline values of QCVN 28:2010/BTNMT (column B) for discharge. N/D: no detection, (-): Negative.

Parameters	Unit	Values	QCVN28:2010/BTNMT
pH		4.3	6.5-8.5
BOD_5	mgO_2L^{-1}	N/D	50
COD		17.3	100
TSS	mgL^{-1}	N/D	100
Sulfur		N/D	4
NH_4^+		1.7	10
NO_3^-		113	50
PO_4^{3-}		6.75	10
Oil and grease		N/D	20
Total coliform	MPN/100 mL	1.3×10^1	5000
Salmonella	Individual/100mL	-	-
Shigella		-	-
Vibrio cholerae		-	-

3.2 Chronic test

3.2.1 Effects on survivorship

The pH and dissolved oxygen (DO) measurement in the test media showed that pH valued from 6.5–8.0 and the dissolved oxygen was between 3.8 and 7.1 mgL^{-1}.

No deaths occurred in the M10 during 14 days test. Survival proportion of *D. magna* in both control (CT) and M30 insignificantly reduced to 93.33%. However, the reduction of survivorship in M50 was highly significant to 66.67% at the end of the experiment (Fig. 1).

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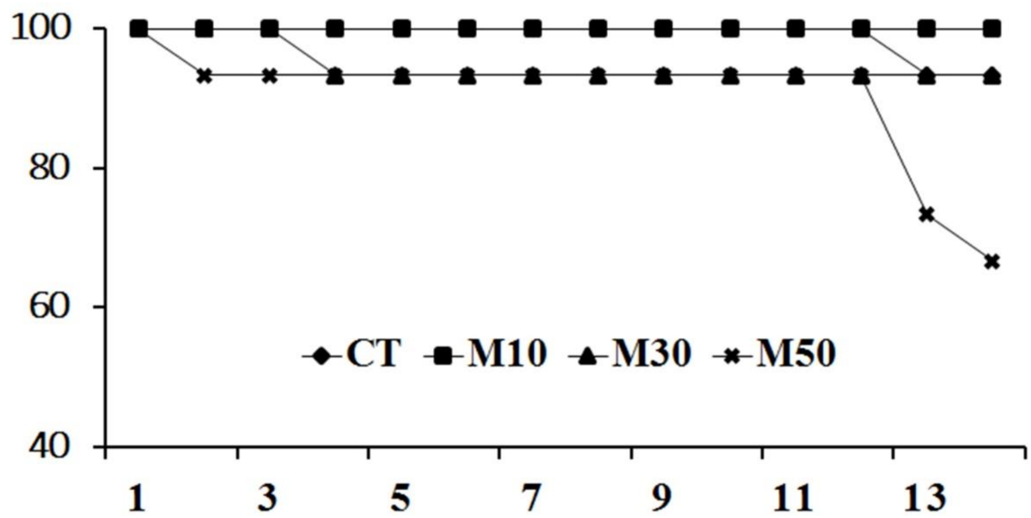

Fig. 1. Survival proportion of *D. magna* exposed to wastewater.

3.2.2 Effects on reproduction

The results of reproduction of *D. magna* exposed to the wastewater were showed in Table 2. Mean number of neonates per female in exposures was significantly higher ($p < 0.001$) than those in CT and dose-independent. Total number neonates increased strongly from 344 young ones (CT) to peak at 834 young ones (M30) and approximately 700 young ones in M10 and M50 (Table 2). The onset of first brood declined dramatically ($p < 0.001$) from 5.6 days in CT to 3.9 days in M30 and around 4 days in M10 and M50. Subsequently, mean time to first reproduction of *D. magna* in exposures were significantly shorter ($p < 0.01$) than those in CT (Table 2).

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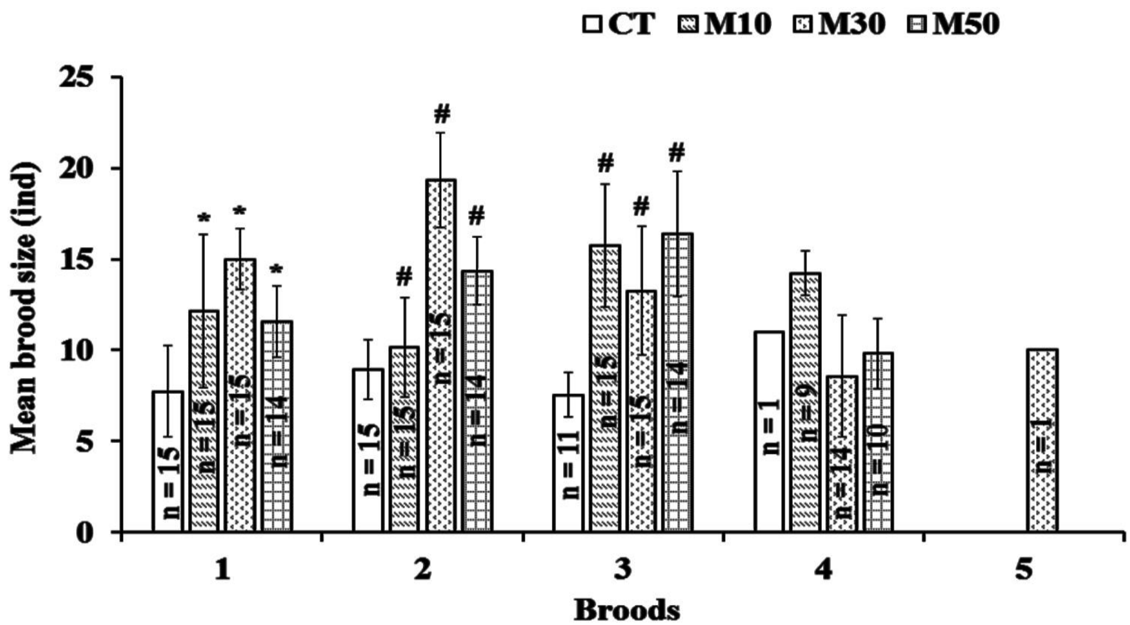

Fig. 2. The effects of wastewater on mean brood size.

Small letters indicate significant difference by ANOVA, followed by Tukey's test ($^{\#}p < 0.001$); Asterisks indicate significant difference by Kruskal-Wallis test, followed by multiple comparison of ranks ($^{*}p < 0.001$). "ind": individuals, "d": days, "br": broods.

The mean number of broods per female significantly increased ($p < 0.001$) from 3.7 broods to 4.5 broods in M10 and M50 and reached to peak at 4.8 broods at M30 (Table 2). Regarding mean brood size, the first three was detected with significantly different ($p < 0.001$) between the CT (7.73; 8.93; 7.55 individuals) and M10 (12.13; 10.13; 15.73 individuals), M30 (15; 19.33; 13.27 individuals) and M50 (11.57; 14.36; 16.36 individuals) (Fig. 2). Compare to the CT, the intrinsic rate of natural increase (r) increased slightly ($p < 0.05$) with peak at M10 and following M30 (Table 2).

In spite of higher reproduction, dead neonates from daphnids' shell were observed in the exposure of M30 and M50. Whereas, the malformation of the neonate was detected in M10.

4. DISCUSSION

The records of pH and DO measurement at the test initiation and termination revealed the suitable test conditions for *Daphnia* well growing (Clare, 2002; Nebeker et al., 1992).

After 14 days of incubation, the mortality of daphnids in CT, M30 was insignificant according to guidance of EPA (1996) for chronic test. Besides, the mortality in M50 was strong (Fig. 1),

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which might be related to the high concentration of nitrate. Camargo et al (2005) reported that nitrate toxicity of mortality (LC_{50} in 120h) to several aquatic invertebrates, such as *Cheumatopsyche pettiti* and *Hydropsyche occidentalis* was from 106.5 NO_3-NL^{-1} (Early instar larvae) to 119 NO_3-NL^{-1} (Last instar larvae) and from 65.5 NO_3-NL^{-1} (Early instar larvae) to 77.2 NO_3-NL^{-1} (Last instar larvae), respectively. That maybe explained for the death of daphnids in M50 with the concentration of 113 NO_3-NL^{-1}. In many cases, hospital wastewater is considered as an effluent with a similar quality to municipal wastewater (Amouei et al., 2015; Boillot et al., 2008; Verlicchi et al., 2012a). Ghazy and Fayed (2011) found that only 60% of *D. magna* incubated in the 19% municipal wastewater (after treating by primary septic tank) could survive for 21 days.

Table 2. Reproduction of *D. magna* exposed to wastewater at several exposures in 14 days

Exposures	n	Total young (ind)	Number neonates per female (ind)	Time to maturation (d)	Time to first reproduction (d)	Number broods per female (br)	Population growth
CT	15	344	22.9 ± 3.8	5.6 ± 0.5	7.7 ± 0.5	3.7 ± 0.5	0.21 ± 0.05
M10	15	698	46.5 ± 6.1^{ccc}	$4.5 \pm 0.5^{***}$	$6.8 \pm 0.8^{**}$	4.5 ± 0.5^{ccc}	$0.27 \pm 0.01^{*}$
M30	15	834	56.3 ± 7.2^{ccc}	$3.9 \pm 0.3^{***}$	$6 \pm 0.0^{***}$	4.8 ± 0.6^{ccc}	$0.25 \pm 0.06^{*}$
M50	14	690	49.3 ± 6^{ccc}	$4 \pm 0.0^{***}$	$6.1 \pm 0.3^{***}$	4.5 ± 0.5^{ccc}	0.22 ± 0.05

Small letters indicate significant difference by ANOVA, followed by Tukey's test ($^{ccc}p < 0.001$); Asterisks indicate significant difference by Kruskal-Wallis test, followed by multiple comparison of ranks ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). "ind": individuals, "d": days, "br": broods.

Daphnids in the exposures grew faster than those in CT. That leaded to time to maturation and time to first reproduction of daphnids in exposures were significantly shorter than those in CT. However, this short was independent upon concentration of wastewater (Table 2). We suggested that daphnids was stimulated by un-determined substances in the wastewater consequently faster growth and higher reproduction of the exposed daphnids. Scott and Crunkilton (2000), reported that the lowest observed effect concentration (LOEC), for neonate production in *Ceriodaphnia dubia* females after 7 days of exposure to nominal nitrate concentrations was from 14.1 to 113 mg NO_3-N L^{-1} (average value of 42.6 mg NO_3-N L^{-1}). That is in line with our results, where, concentration of NO_3-N was higher than guidance value (Table 1). Ngo et al. (2011) reported that the effluent of municipal wastewater caused time to mature of daphnids to be faster than those in control. Heavy metals also present in the effluent of hospital wastewater (Amouei et al., 2015; Emmanuel et al., 2005; Verlicchi, 2012a). Tran et al. (2014) reported that the low concentration of Chromium (50 µg L^{-1}) stimulated the maturation of *D. magna*. Vernberg et al. (1977) found that moderate Cadmium levels (10 and 23 mg L^{-1}) stimulated moulting frequency in *Palaemonetes pugio* species. That maybe a reason of fast mature of daphnids (Ebert, 2005). Moreover, the industrial wastewater with a diversity of concentration of heavy metals caused time to mature of *D. magna* to be faster than control (Nguyen et al., 2016).

Our results also agreed with Ghazy and Fayed (2011) as the stimulation on the average total number of neonates per female when daphnids exposed to municipal wastewater (after treating by primary septic tank), was revealed. Tisler and Zagorc-Koncan (1999) reported that wastewater of pharmaceutical industry also increased total number of neonates per female with increasing concentration of wastewater. Parlak et al. (2010) discovered that the total neonates and total neonates per daphnids increased gradually with increasing the concentration of mixture of wastewater (sources from industry, urban, and agriculture). Besides, Dave (1984) discovered the low concentration of copper stimulate the reproduction of daphnids which is supported by our results with number neonates per female, number broods per female, total young and the brood size. Moreover, the study of Kumar et al (2014) revealed that the low concentration (0.05–0.4 mgL^{-1}) of Zinc increased population growth of *Daphnia*. Further investigations are need to be performed to determine exactly what substances and/or mixture will be responsible for the effects on survivorship and reproduction of daphnia.

In our study, the death and the malformation of the neonates only recorded in wastewater exposures, the malformation and death of neonate from adult *D. magna* incubated in industrial wastewater (Nguyen et al., 2016) and municipal wastewater (Ghazy and Fayed, 2011) were record. Djekoun et al. (2015) showed that the embryos from eggs of daphnids occurred abnormally as female daphnids exposed to the several concentrations of Cadmium. Consequently, there were malformations in several embryos. The same author suggested that the endocrine disruption was a direct reason of adverse effects on embryos of daphnids. To our knowledge, we suggested that several heavy metals, such as, cadmium, copper, chromium and pharmaceuticals toxicity of may present in the wastewater and responsible for the malformations in the embryonic daphnids that need further investigated at cellular levels.

5. CONCLUSION

The adversely effect of survivorship and reproduction on daphnids were observed when exposed daphnids to different concentrations of the treated hospital wastewater. The wastewater caused death and malformations on neonates of *D. magna*. Although the treated hospital wastewater met the QCVN 28:2010/BTNMT, the potent toxicity of the wastewater was observed. Therefore, further toxicity investigations on heavy metals and pharmaceuticals in effluent of the hospital wastewater are needed to fully evaluate the toxicity of the hospital wastewater. It is necessary to revise the National Technical Regulation on Health Care Wastewater (QCVN 28:2010/BTNMT) to protect sensitive organisms in aquatic ecosystems.

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