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Toxicity of Surface Water Contamianted with Arthropogenic Pollutants to Micro-Crustacean, *Daphnia magna*

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Abstract: Arthropogenic pollutants are among the most serious problems to aquatic environment. They not only cause the water quality degradation but also induce severe impacts on aquatic organisms. In this study, we investigated the negative effects of surface water contaminated with pollutants from human activities collected from Xuan Huong lake, Da Lat city, on life history traits of *Daphnia magna*, over a period of 14 days. The animal was incubated in medium containing the contaminated surface water at eight different concentrations from 0.05% to 25% (v/v) in the laboratory conditions. The results showed that the contaminated surface water seriously and negatively influenced on the survival of *Daphnia*, causing the decease of 27-100% of the exposed organism population, and the higher surface water concentration the animal exposed, the higher mortality it was. The contaminated water also delayed the maturity age of the tested organisms, around 3-8 days compared to the control. The maturity postponement was concentration dependent. Besides, the fecundity of the *Daphnia* exposed to the contaminated water was inhibited or strongly reduced. Heavy metals and pesticides in the surface water should be the main factors inducing the potent toxic impacts on the test organisms. Therefore, pollutants in the effluents from human activities at Da Lat city should be effectively treated before discharged in order to protect the aquatic environment, biological resources and ecosystem.

Keywords: contaminated surface water, heavy metals, pesticides, *Daphnia magna*, life history traits

1. Introduction

Nowadays, arthropogenic pollutants in developing countries are among the most serious problems to aquatic environment. The effluents from different human activities would contain different pollutants or toxins consequently surface water contaminants including a variety of metals and xenobiotics [1, 2]. The pollutants not only cause the water quality degradation but induce negatively severe effects on aquatic organisms including zooplankton as well. Micro-crustaceans (e.g. *Daphnia*), one of the most diverse and important group of zooplankton, have the intermediate position in the freshwater food web, and play the important connector between

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primary production and other consumers [3]. The alterations of *Daphnia* in water bodies are regulated by the aquatic multi-factors.

Previously, negative effects of single purified xenobiotics (e.g. atrazine, estriol, octylphenol) or heavy metals (e.g. Cu, Cr) on micro-crustaceans were widely investigated [4-6] in which the toxins caused strong reduction of survivorship, altered the maturation and inhibited fecundity of the animals. Besides, responses of *D. magna* to treated and untreated domestic wastewater were reported including mortality increase, reproduction decrease and malformation of the off spring [7]. However, the development and reproduction of micro-crustaceans under long-term exposure to a natural mixture of wastewaters have not yet reported. Therefore, in this study, we evaluated the chronic effects of surface water contaminated with domestic wastewater, agricultural effluents and cyanobacteria from Xuan Huong lake, Da Lat city, on the life history traits of *D. magna* for 2 weeks under the laboratory conditions.

2. Materials and methods

2.1. Sample collection

Surface water (from top to 50 cm depth) was collected near Thuy Ta restaurant by Xuan Huong lake (Fig. 1), Da Lat city. The sampling site is nearly the end (or the dam) and water out let of the lake where the surface water was quite homogenized and well mixed (field observation). The surface water was brought to the laboratory in the same day and then kept at –70°C until chemical analyses and biological bioassay. At the sampling time, cyanobacterial scum was occurring and the effluent from domestic waste and agricultural activities was rushed into the lake (personal observation).

2.2. Analyses on toxic chemicals in wastewater from Xuanhuong Lake

Prior to the chemical analyses, the surface water from Xuanhuong lake was thawed then sub-samples were taken and prepared for cyanobacterial toxin, microcystin, heavy metal and herbicide/pesticide analyses. For microcystin analysis, sub-sample of field water was centrifuged at 14,000 rpm, 4°C for 15 min. Supernatant was collected for microcystin analysis by high performance liquid chromatography (HPLC, Shimadzu, Japan). Microcystins were detected by the UV detection at 238 nm [8].

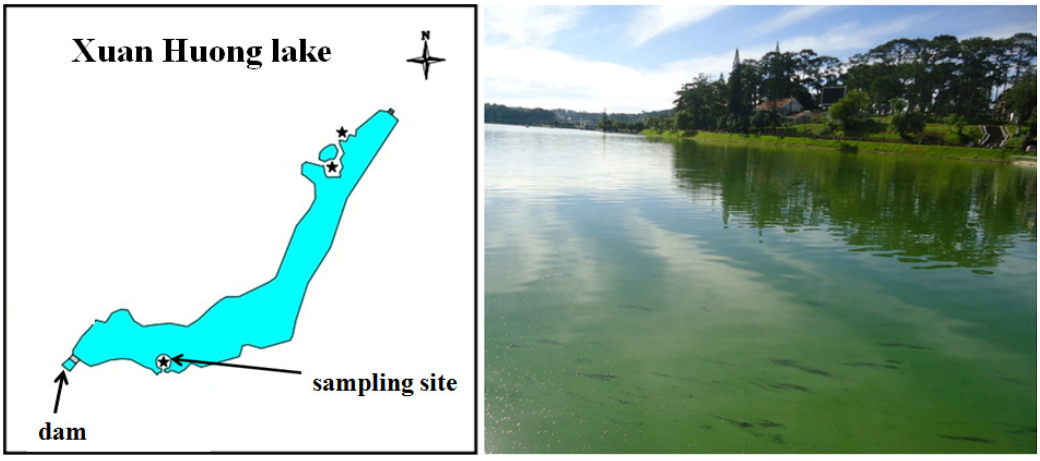

Fig.1. Xuan Huong lake (left) with sampling site (the longer arrow and right).

Pesticides/ herbicides from the surface water were analyzed by adding 100 mL water sample into 250 mL glass then extracted by the solvent (n-hexane and dichromethane). The extracted sample was de-hydrated with Na_2SO_4 and dried by nitrogen. Sample was then diluted in n-hexane and analyzed with a gas chromatography-EDC 7890ALIGENT, (column DB-5.625 length 30 m, diameter 0.25mm, film 0.25µm). Water sample for heavy metal analysis was firstly passed a 0.45 µm filter (Sartorius, Germany) and acidified with saturated HNO_3 [9]. The heavy metals were characterized under an inductively coupled plasma/ mass spectrometry (ICP/MS-9000 Shimadzu, Japan).

2.3. Experimental organisms and experimental set up

The test organism was *D. magna*, obtained from Micro BioTest Inc. (Belgium) and has been maintained in the laboratory of the Institute of Tropical Biology for many years. The *Daphnia* was raised in COMBO medium [10] and fed with green alga *Chlorella* sp. three times a week. The alga was cultivated in COMBO medium with continuous aeration. Both culturing of *Chlorella* and *Daphnia* incubation were maintained at 20 ± 1^0C and a photoperiod of 14h light: 10h dark at a light intensity of around 1000 Lux [11].

Prior to the experiments, twenty female *D. magna* were incubated in a 1000 mL beaker and fed with *Chlorella* sp. for 2-3 weeks. Offspring from the second to third clutch of these *D. magna* were used for experiments. *Daphnia* was incubated in mixture of surface water from Xuan Huong lake and COMBO medium at the ratios of 0 (control), 0.05, 0.1, 0.2, 0.4, 0.8, 1, 5 and 25% (v/v). In each treatment, 15 neonates less than 24 h old were randomly selected for each chronic exposure [11] and individually raised in 50 mL beaker containing 20 mL of medium (either COMBO or mixtures of surface water and COMBO as above). The animals were fed with *Chlorella* sp. at the concentration of 2 mg C L^{-1} per day. The medium was totally renewed 3 times every week. During incubations, animals were observed daily for their survival, maturation and reproduction [11]. The experiments lasted for 2 weeks. The pH (Metrohm 744) and dissolved oxygen concentration (WTW Oxi197) in the medium were measured at the start and every 2 days of experiment to confirm the suitable environmental conditions for the animals' living.

2.4. Statistical analysis

Kruskal-Wallis test (Sigma Plot, version 12) was applied to determine the significant difference of *Daphnia'*s maturation from control and surface water exposures.

3. Results and discussion

3.1. Micro-pollutants in the surface water from Xuan Huong lake

The cyanobacterial toxin, microcystins, was not detected in surface water from Xuan Huong lake by HPLC analysis. However, 12 heavy metals including As, Co, Cr, Cu, Fe, Mo, Mn, Ni, Pb, Sr, V and Zn were characterized in the surface water from the lake by the ICP/MS equipment. Their dissolved concentrations varied from 12 µg L^{-1} (V) to 8807 (Fe) µg L^{-1} (Table 1). Among the detected metals, As, Cr, Fe and Ni concentrations were 3 – 11 times higher than the Vietnam guideline values for irrigation (QCVN 08-2008/B1) while Cu, Pb and Zn concentrations were within the QCVN. The high concentrations of some heavy metals including those not within the QCVN (e.g. Co, Mo, Mn and Sr) could induce problems to and adverse effects on environmental quality and ecological health of Xuan Huong lake and the downstream region (e.g. Camly water fall).

Table 1. Concentrations of dissolved heavy metals (µg L^{-1}) in the water from Xuan Huong lake (XH-L) and the Vietnam guideline values 08-2008/B1 (QCVN) for irrigation. N/A: not available

Metals	As	Co	Cr	Cu	Fe	Mo	Mn	Ni	Pb	Sr	V	Zn
XH-L	145	15	276	21	8807	33	713	1101	18	318	12	71
QCVN	50	N/A	40	500	1500	N/A	N/A	100	50	N/A	N/A	1500

Table 2. Concentrations of pesticides/ herbicides (µg L^{-1}) in the water from Xuan Huong lake and the Vietnam guideline values 08-2008/B1 (QCVN) for irrigation

Chemicals	Malathion	4,4-DDE	Endosulfan-sulfate
Xuan Huong lake	17.5	15.5	4.2
QCVN	0.32	0.004 (DDT)	0.01

Three pesticides were detected in the surface water from Xuan Huong lake including Malathion, 4,4-DDE and Endosulfan-sulfate with the concentrations of 17.5, 15.5 and 4.2 µg L^{-1}, respectively (Table 2). The two chemical concentrations, Malathion and Endosulfan-sulfate were much higher than the QCVN, 54 and 400 times, respectively. Especially, the 4,4-DDE concentration (a transformed chemical of DDT) was 3000 times higher than the DDT concentration in the QCVN. Therefore, the surface water in Xuan Huong lake possessed a serious hazard to local residents and the aquatic organisms in the water body and its down stream.

3.2. Effects of surface water on survival of Daphnia magna

The pH and dissolved oxygen concentration of the test medium ranged from 6.8 – 7.0 and 6.5 – 7.1 mg L^{-1}, respectively, which were favorable for the *Daphnia*'s growth [12]. After two weeks of incubation, survival of *Daphnia* in the control slightly decreased, nearly 7% which was within the accepted range of a chronic test [9].

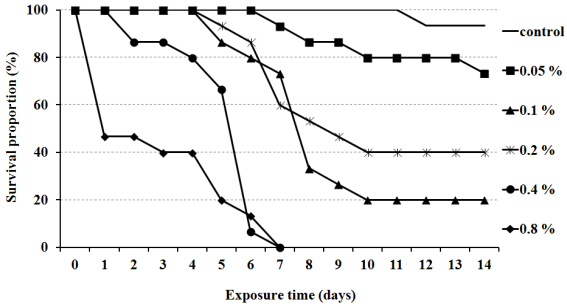

Fig.2. Survival of *Daphnia magna* during exposure time (n = 15 at the start). 0.05-0.8% indicated the concentrations of surface water from Xuan Huong lake diluted by the COMBO medium (v/v).

The survival of the test animals in the 0.05% (surface water) incubation reduced 27%. However, exposed to higher surface water concentrations (0.1 – 0.2%) the mortality of the *Daphnia* strongly increased up to 60 and 80% of the total testing animals. Seriously all *Daphnia* in the 0.4% and 0.8% died after 1 week of incubation (Fig. 2). Additionally, in the 1, 5 and 25% of surface water treatments, all the *Daphnia* died just one day after the start of the experiment (data not showed).

The mortality of *Daphnia* in the surface water exposures should be related to the heavy metals and pesticides/ herbicides in the collected sample. Tran et al [6] recorded that

Cu had severe impact whereas Cr had slightly impact on survival of *D. magna*. Similarly, at the same concentrations, atrazine was much more toxic than estriol to *Daphnia* [5]. Our results showed the extremely toxic of surface water from Xuan Huong lake to *D. magna* which was in agreement with a previous investigation [13]. Cooper et al [14] evaluated the acute and long-term toxicity of Cu, Pb and Zn mixtures to a micro-crustacean, *Ceriodaphnia dubia*. The mixture of 10 µg Cu L^{-1}, 9 µg Pb L^{-1} and 101 µg Zn L^{-1} killed 65 - 100 % of the tested organisms. The combination of heavy metals, in general, could raise the toxicity (e.g. in case of Cd and As mixture) [15] or reduce the toxicity (e.g. in case of Zn and Cd mixture)[16].

In the experiments of our study, surface water was diluted with COMBO medium (surface water = 0.05 – 0.8%) hence concentrations of every metal and pesticide in the test medium should be very low, such as 0.07 – 1.16 µg As L^{-1}, 0.14 – 2.2 µg Cr L^{-1}, 0.01 – 0.17 µg Cu L^{-1}, 0.5 – 8.8 µg Ni L^{-1}, < 0.15 µg Pb L^{-1}, < 0.6 µg Zn L^{-1}, < 0.15µg Malathion L^{-1}, < 0.13 µg 4,4-DDE L^{-1}, and < 0.04 µg Endosulfan-sulfate L^{-1}. Pane et al [17] reported that Ni at the concentration of 21 µg L^{-1} did not induced adverse effects on survival, growth and reproduction of *D. magna*. In addition, the chronic investigation of Biesinger and Christensen [18] showed that the concentrations of metals (e.g. Co, Cr, As, Sn, Al, Zn) which caused negative effects on *D. magna* were between 10 and 350 µg L^{-1}. Hence it is assumed that every single heavy metal at the concentrations after dilution (as above) in our study would not be able to induce negative effects on *D. magna*. Besides, Vo et al [5] and Dao et al [4] reported that several xenobiotics (e.g. Atrazine, Estriol, Octylphonol) at the concentration of 5 µg L^{-1} did not have negative effect of survival of *D. magna*. Therefore, in the current study, there should be combined or synergistic effects of the micro-pollutants (heavy metals, pesticides) in the surface water on the test organism, *D. magna*.

3.3. Effects of surface water on the maturation of Daphnia magna

The *Daphnia* in the control reached their maturation at the age of around 4.1 days old. However, the animals exposed to surface water at the concentrations of 0.05, 0.1 and 0.2% matured at the ages of around 7.5, 11 and 12.6 days old, respectively (Fig. 3). *Daphnia* incubated in the higher surface water concentrations (0.4-25%) died before they could reach their maturation.

This result was in line with previous observations of Tran et al [6] and Vo et al [5] in which Cu (10 µg L^{-1}) and Atrazine and Estriol (5 µg L^{-1}) delayed the maturation of *D. magna*. However, Cr (50 µg L^{-1}) and Octylphenol (5 µg L^{-1}) stimulated the maturation of the animals [5, 6]. Besides, the concentrations of each metal in the exposures (0.2%) in our study were quite low (0.29 µg As L^{-1}, 0.55 µg Cr L^{-1}, 0.04 µg Cu L^{-1}2.2 µg Ni L^{-1}, 0.04 µg Pb L^{-1}, 0.14 µg Zn L^{-1}, 17 µg Fe L^{-1}) compared to those (10-20 µg Cu L^{-1}, 50-100µg Cr L^{-1}) in a previous investigation [6]. The concentrations of pesticides in the highest exposure (0.2% containing 0.035 µg Malathion L^{-1}, 0.031 µg 4,4-DDE L^{-1}, 0.00084µg Endosulfan sulfate L^{-1}) were much lower than those in previous studies (5-500µg L^{-1} of Atrazine and Estriol) [5, 6]. Therefore, the postponement of *Daphnia*'s maturation in the surface water treatments should be the consequence of the combined effects of all metals and properly pesticides in the surface water from the lake which need further investigations with purified chemicals.

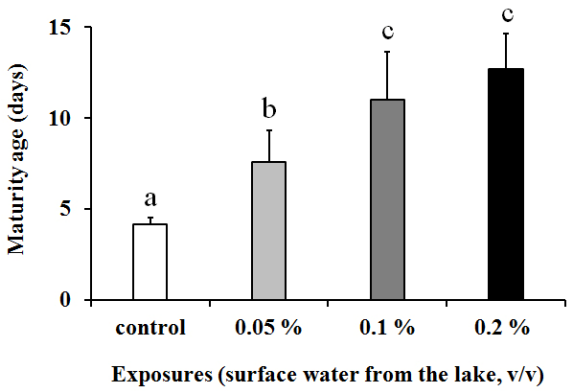

Fig. 3. Maturity age of *Daphnia magna* during exposure time. Different letters a, b and c indicated the statistically significant differences of $p < 0.05$, Kruskal-Wallis test. Abbreviations as in figure 2.

3.4. Effects of surface water on the fecundity of Daphnia magna

The number of offspring reproduced by an adult *D. magna* in control and 0.05% surface water treatment were similar and approximately 6 neonates/ adult. The fecundity of *D. magna* reduced in higher surface water treatments 0.1 and 0.2% with 4 and 3 neonates/ adult, respectively (Fig. 4). These results indicated that surface water significantly affected reproduction of *D. magna* and the adverse affect of surface water on *Daphnia*'s fecundity was concentration dependent. To our knowledge, this is the first report on the detrimental impacts of combined effluents from domestic and agricultural activities on *D. magna*.

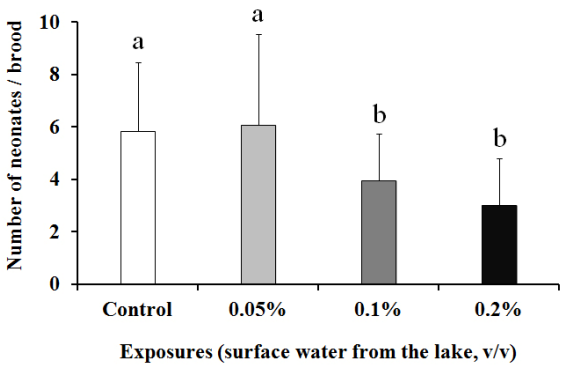

Fig.4. Fecundity of *Daphnia magna* during the experiment. Different letters a and b indicated the statistically significant differences of $p < 0.05$, Kruskal-Wallis test. Abbreviations as in figure 2.

During two weeks of experiment, all adult *Daphnia* in the control normally reproduced and the accumulative number of neonates in the control was highest, 177 offspring in total (Fig. 5). However, exposed to surface water, from 20 – 100% of adult *Daphnia* were not able to reproduce. Besides some of *Daphnia* in the surface water exposures suffered from abortion consequently the total offspring in the 0.05 and 0.1% surface water treatments were only 42 and 1, respectively. Surface water concentration from 0.2% inhibited the fecundity of the animals (Fig. 5).

Surface water from Xuan Huong lake induced decreases, concentration dependence, on the survivorship of *Daphnia*'s population and delayed the animal's maturity age consequently fecundity of the exposed *Daphnia* was strongly reduced. The negative effects on reproduction in our study were more serious than those in the study of Ngo et al [7] in which *D. magna* was exposed to domestic wastewater from Ho Chi Minh city. This should be related to the heavy metals and pesticides in surface water from Xuan Huong lake which were not present in domestic wastewater in Ho Chi Minh city [7]. Dao et al. [11] reported the abortion of

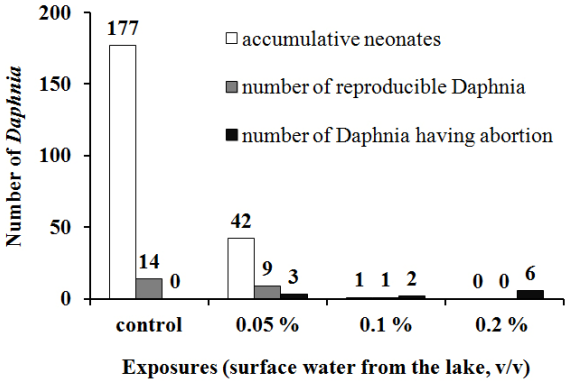

Fig. 5. Accumulative neonates, number of reproducible *Daphnia* and *Daphnia*' abortion during the experiments. Abbreviations as in figure 2.

D. magna exposed to cyanobacterial toxin, microcystins. In our study, we also observed this serious situation of the *Daphnia* although no microcystins were detected by HPLC equipment. So heavy metals and/ or pesticides would be also the root of *Daphnia*'s abortion. However, mechanisms of the abortion caused by the toxins, to the best of our knowledge, are not known which need further investigations.

5. Conclusions

Surface water from Xuan Huong lake contained a variety of pollutants, heavy metals and pesticides, of which some pollutants were extremely high in concentrations. Although highly diluted, the surface water from the lake was still seriously toxic to the micro-crustacean, *D. magna*. The surface water caused a sharp reduction of survivorship of *Daphnia*, delayed the animal's maturation, inhibited fecundity and reduced the population development of the *Daphnia*. Besides, abortion was also observed in the surface water exposures. This is, to the best of our knowledge, the first report on the chronic effects of mixed effluents from domestic and agricultural activities on *D. magna*. Study on the in situ ecological health of Xuan Huong lake is suggested.

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Độc tính của nước mặt bị nhiễm bẩn bởi các chất ô nhiễm từ hoạt động nhân sinh đối với vi giáp xác, *Daphnia magna*

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Tóm tắt: Chất ô nhiễm từ hoạt động con người là một trong những vấn đề nghiêm trọng cho môi trường nước. Các chất ô nhiễm này không chỉ làm suy giảm chất lượng nước mà còn gây ra những tác động nghiêm trọng lên thủy sinh vật. Trong nghiên cứu này, chúng tôi nghiên cứu ảnh hưởng xấu của nước mặt thu từ hồ Xuân Hương, thành phố Đà Lạt, bị nhiễm bẩn bởi chất ô nhiễm từ hoạt động nhân sinh lên các đặc điểm lịch sử vòng đời của *Daphnia magna* trong thời gian 14 ngày. Sinh vật được nuôi trong môi trường có pha nước mặt nhiễm bẩn ở 8 nồng độ khác nhau từ 0,05-25% (tỷ lệ theo thể tích) trong điều kiện phòng thí nghiệm. Kết quả cho thấy nước mặt nhiễm bẩn gây ảnh hưởng xấu và nghiêm trọng lên sức sống của *Daphnia*, giảm 27-100% quần thể sinh vật phơi nhiễm, và nồng độ nước mặt càng cao, tỷ lệ sinh vật chết càng nhiều. Nước mặt nhiễm bẩn cũng làm chậm sự thành thục của sinh vật từ 3-8 ngày so với sinh vật trong lô đối chứng. Sự trì hoãn quá trình thành thục tỷ lệ thuận với nồng độ nước mặt dùng cho thí nghiệm. Bên cạnh đó, sức sinh sản của *Daphnia* phơi nhiễm với nước mặt bị kìm hãm và suy giảm mạnh. Kim loại nặng và thuốc trừ sâu trong nước mặt được cho là nguyên nhân chính của tác động độc tính trên sinh vật thí nghiệm. Do đó, chất gây ô nhiễm trong nước mặt ở thành phố Đà Lạt nên được xử lý hiệu quả trước khi thải nhằm mục đích bảo vệ môi trường nước, tài nguyên thủy sinh vật và hệ sinh thái thủy vực.

Từ khóa: nước mặt nhiễm bẩn, kim loại nặng, thuốc trừ sâu, *Daphnia magna*, đặc điểm lịch sử vòng đời.