Responses of the Micro-Crustacean, *Daphnia magna*, across Five Generations Continuously Exposed to Di-2-Ethylhexyl Phthalate in Mekong River Water

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Abstract

Plastic pollution has been considered as an emerging environmental problem, and is among the ecological and human health concerns. Detrimental impacts of plastic pollution on living things are closely related to the plastic additives added onto the polymer during plastic manufacture. Di-2-ethylhexyl phthalate (DEHP) is one of the most common plasticizers and is usually found in water environment worldwide. Plastic additives can cause many negative effects on aquatic organisms such as fish and zooplankton. This study aimed to assess the chronic effects of DEHP on the life history traits of an ecotoxicological model micro-crustacean, Daphnia magna, across five generations (F0–F4). We used the natural water from Mekong River in Vietnam as the medium for the D. magna incubation in laboratory conditions. The concentrations of trace elements (e.g., metals and pesticides) in the natural water were under detection levels of equipment or very low which was sufficient for D. magna to grow well. The results showed that the body length was the main endpoint of the organisms inhibited by DEHP across all generations. DEHP adversely impacted the survival and fecundity of D. magna in the fourth generation (F3) only. The adverse effects of DEHP on body length of D. magna should be the consequence of the energy cost and allocation in the exposed organisms. The survival and reproduction responses of D. magna to DEHP across five generations could be explained by (i) the severe effects of the chemical on many individuals in the organism cohort, and (ii) toxin-tolerant development in the remaining exposed organisms. Although the trace elements in natural water from Mekong River were not toxic to D. magna at very low concentrations, together with DEHP they might enhance impacts on the organism. Besides, a multigenerational exposure to DEHP would reflect clearer impacts on the organism than a single exposure. Our results could be useful for extrapolation on the influence of plasticizers on freshwater zooplankton in nature.

Keywords: *chronic effects, Daphnia magna, energy cost, life traits, plastic additives*

1. Introduction

Plastic emission and pollution are among the most serious threats to aquatic environment and ecosystem globally (Miller et al. 2020; Azevedo-Santos et al. 2021). The di-2-ethylhexyl phthalate (DEHP) is one of the basic and common additives for more than 300 million tons of plastic products annually in the world (Wang et al. 2018; Chen et al. 2020). Globally, the compound DEHP was produced at more than 2 million tons (Rowdhwal and Chen 2018). In natural conditions (e.g., high temperature), DEHP can easily release out of the plastic surface and enter the surrounding environments (Wang et al. 2018). The existence of DEHP in the environment has been reported in many countries such as Finland, Denmark, Germany, Japan, China, Thailand, Poland, Sweden, and Italy (Wowkonowicz and Kijeriska 2017). The highest concentration of DEHP in river water reached 370 µg L-1 in China (Wang et al. 2018). Recently, DEHP has become an emerging pollutant. It is known as an endocrine disrupting chemical and considered as a carcinogenic compound to invertebrates (Wang et al. 2018). However, the impacts of DEHP on aquatic invertebrates are not fully understood.

Micro-crustaceans (e.g., *Daphnia magna*) are among the key animal groups in aquatic ecosystems having important position and function in aquatic food webs (Wetzel, 2001). *Daphnia magna* is the common representative of freshwater micro-crustaceans and is widely used as a model organism for testing the pollutant toxicity (US. EPA 2002; Lampert 2006; APHA 2012). The median lethal concentrations (LC50) of DEHP to *D. magna* were largely varied between 160 and 3,310 µg L-1 (Adams et al. 1995; Brown et al. 1998; Scanlan et al. 2015; Wang et al. 2018). The life history traits of *D. magna* chronically exposed to DEHP have been reported in many studies in which the DEHP concentration up to 500 µg L-1 did not impact survival of the organism (Brown and Thompson 1982; Knowles et al. 1987; Brown et al. 1998; Seyoum and Pradhan 2019; Le et al. 2019). Seyoum and Pradhan (2019) and Le et al. (2019) noted that DEHP enhanced the reproductive capacity of *D. magna*. The authors also found that the DEHP at 390 µg L-1 inhibited the growth of *D. magna*, but the inhibition was not showed at lower concentrations of the chemical.

Generally, all previous studies of the DEHP toxicity to *D. magna* were performed in artificial medium (e.g., ISO). However, the toxicity of DEHP in the artificial environment may not be the same as that in the natural water because of different chemical properties between them. Besides, the responses of *D. magna* to DEHP across multigenerational exposure are not yet fully understood (but see Le et al. 2019). Therefore, to fill the gap, in this study we assessed the effects of DEHP dissolved in natural water from Mekong River at the concentration of 500 µg L-1 on the life history traits of *D. magna* across five generations.

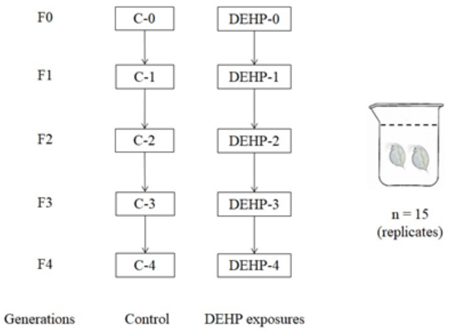

Fig. 1. Experimental setup for *D. magna* across five generations exposed to DEHP dissolved in Mekong River water
Rys. 1. Układ doświadczalny dla *D. magna* w pięciu pokoleniach poddanych działaniu DEHP rozpuszczonego w wodzie Mekongu

2. Materials and methods

2.1. Test organisms

The freshwater micro-crustacean *Daphnia magna* was purchased from Micro BioTest (Belgium) and has been maintained in ISO medium for many years. The organisms were fed with the green alga Scenedesmus sp. and YTC (US. EPA. 2002). The alga Scenedesmus sp. was cultured in a Z8 medium (Kotai 1972). The *D. magna* was raised under the temperature of 25 ± 1^{o}C, the light intensity of less than 1000 Lux, and a photo regime of 14 h light: 10 h dark (APHA 2012).

2.2. Chemicals

The plastic additive Di-2-ethylhexyl phthalate (DEHP, Aldrich Sigma, purification 99.5%) was dissolved in acetone (Merck, Germany) at the concentration of 3292 mg L-1 referred as stock solution. The stock solution was kept at 4^{o}C before the test implementation. The natural water used in this study was collected from Mekong River in Vietnam. The water was settled and then filtered through a GF/A filter (Advantech, Japan) before being used as a medium to culture *D. magna*. The chemical characterization of pesticides (all below 1 µg L-1) and trace metals (mostly below 1 µg L-1, and much far below the 48h-LC50 values to *D. magna*) confirmed that the natural water from Mekong River was suitable for cultivation of *D. magna* (Dao et al. accepted manuscript).

2.3. Experimental design

The chronic experiment was conducted according to APHA (2012) with minor adjustment. At the start of the experiment, nearly 40 mother *D. magna* were randomly selected and transferred into a one-liter glass beaker containing 800 mL of filtered river water. Sixty neonates (<24 hours old) from the second and third broods of the mother *D. magna* were randomly used for the chronic experiment. The experiment was performed over five generations of *D. magna* (Fig. 1). In more detail, at the first generation (called F0) of the experiment, two organisms were cultured together in a 50 mL glass beaker containing 40 mL of filtered river water. We conducted two treatments including the exposure to DEHP and the control. In the exposure, *D. magna* was raised in the natural water containing 500 µg DEHP L-1 while the organisms in the control were just incubated in the natural water without the addition of DEHP. There were 15 replicates in each treatment (n=15). Offspring from the second and third brood of F0 *D. magna* in control and DEHP exposure were used for the next generational experiment (hereafter we called F1) and continuously raised in the same medium as their mothers were (2 neonates per beaker, 15 replicates). This process repeated until the fifth generation (F4). The test concentration of 500 µg DEHP L-1 was chosen for the present study because the highest concentration of phthalates in surface water and landfill leachate could reach up to 370 µg L-1 and 460 µg L-1, respectively (Wowkonowicz and Kijeriska 2017; Wang et al. 2018).

The organisms in each treatment were fed daily with the mixture of green alga Scenedesmus sp. and YTC (US. EPA. 2002). The chronic test was performed in the laboratory conditions (as mentioned above) and lasted for 21 days for each generation (Adema 1978). We conducted this study with data within five generations of exposures because the Covid-19 quarantine was applied in the city before we could complete the experiment on the sixth generation. The test medium (filtered river water and food) in each treatment was totally renewed three times per week. During the time of the experiment, the life history traits including the survival, reproductive performance of *D. magna* were carefully checked and recorded daily. The dead mother *D. magna* and neonates released from each beaker were counted and discarded daily. By the end of the experiment, alive *D. magna* was fixed with Lugol solution (Sournia 1978) and its body length was measured from the head top to the base of tail spine of the *D. magna* on a microscope coupled with a digital camera. The pH and dissolved oxygen (DO) of the test medium were measured and their values ranged from 7.2–7.6 (for pH), and from 7.4–7.9 mg L-1 (for DO) which met the requirements for chronic experiments with *D. magna* according to APHA (2012).

2.3. Data treatment

Sigma Plot version 12.0 was used for data analyses. The Kruskal-Wallis test was utilized to calculate the statistically significant difference ($p < 0.05$) in body length, and accumulative offspring per beaker between control and DEHP exposure.

3. Results and discussion

3.1. The effects of DEHP on the survival of Daphnia magna

In the control, the survival of *D. magna* in all six generations was from 80–93% when the experiments terminated (Fig. 2) which meets the requirement of APHA (2012) for chronic treatments with micro-crustaceans. Similarly, the survival of *D. magna* in DEHP exposures was within the range of 80–100% except the survival of DEHP-exposed *D. magna* in F3 was 53% by the end of experiment.

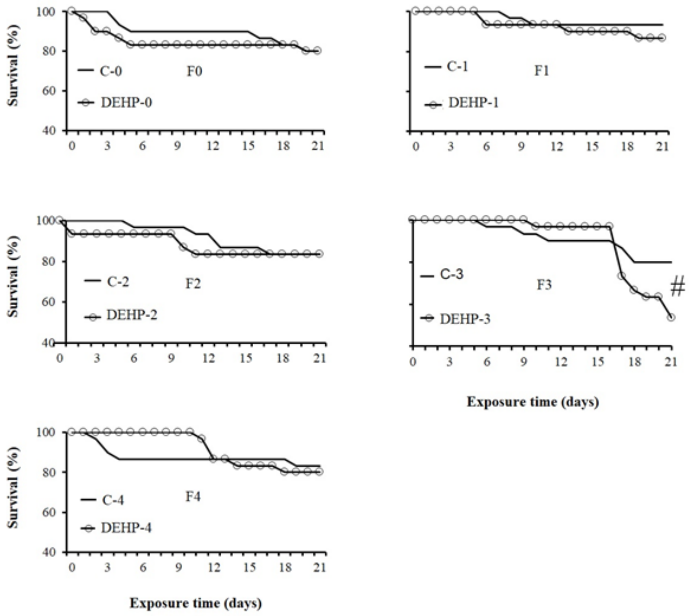

Fig. 2. The survival of *D. magna* in the control and DEHP exposure through five generations. The symbol (#) indicated the significant difference between control and DEHP exposure according to APHA (2012)

Rys. 2. Przeżywalność D. magna w próbce kontrolnej i poddanej ekspozycji na DEHP przez pięć pokoleń. Symbol (#) wskazywał na istotną różnicę między kontrolą a narażeniem na DEHP według APHA (2012)

In the first and second generational exposures (F0 and F1), the Daphnia's survival in present study is consistent with that in previous studies (Seyoum and Pradhan 2019; Le et al. 2019) in which *D. magna* was incubated in DEHP at the concentrations of 390–500 µg L-1. However, a significant decrease of *D. magna* survival (55%) in the third generation (F2) exposed to DEHP (500 µg L-1) was reported by Le et al. (2019). Therefore, the previous studies (Seyoum and Pradhan 2019; Le et al. 2019) and our study have a similar trend of effects reflecting the impact of DEHP on the *D. magna*'s survival was only profound in the later generations of exposure.

Biochemically, DEHP caused a significant reduction in the activities of catalase (CAT) and superoxide dismutase (SOD) enzymes, the antioxidant capacity and detoxifying activities in *D. magna* (Wang et al. 2018). DEHP can interfere the protein synthesis and fatty acid catabolism, consequently influence the normal functions of heart and hepatopancrea in *D. magna* (Scanlan et al. 2015; Ito et al. 2019). Hence, DEHP could indirectly affect the energy storage or cause the glycogen reduction in *D. magna* (Knowles et al. 1987). The compound DEHP could be accumulated and recalcitrant in aquatic organisms. Therefore, the DEHP-exposed *D. magna* would spend energy for biochemical adjustment and damage repair, consequently energy cost (Dao et al. 2018). The exposed organisms could withstand DEHP in the first generations by spending energy and material for survival (F0–F2; Fig. 2) and reproduction maintenance (F1, F2; Fig. 4). However, the *D. magna* could be impaired in a more generational exposure to DEHP (F3) consequently mortality increase (up to 47% of total exposed organisms).

The difference of the affected generations in the previous study (F2; Le et al. 2019) and ours (F3) could be related to the trade-off between survival and development in DEHP-exposed *D. magna*. The DEHP-exposed *D. magna* could face energy cost and material allocation to maintain its normal behaviors and fitness (e.g. survival and growth). Hence the slowdown its growth (see section 3.2) the DEHP-exposed *D. magna* could have a chance to conserve its survival rate similar to the control in F2 (Fig. 2).

Our study presented the initial evidence of *D. magna* continuously exposed to DEHP in natural river water over 5 generations. Interestingly, we found that *D. magna* was more likely to recover or even adapt in its survival to DEHP in the fifth generation (F4). Dao et al. (2018) studied the survival of a micro-crustacean Daphnia lumholtzi by exposed the organism to a cyanobacterial toxin, microcystin, across three generations and observed the tolerance of the organism in the first generation, the significant decrease of the organism in the second generation, and the acclimation of the organism in the third generation. The acclimation or recovery of microcystin-exposed mother *D. magna* was hypothesized with the formation of the genes for synthesis of detoxifying enzymes in its offspring, and thus improve the toxin tolerance in the daughters (Gustafsson et al. 2005). This helps to explain the acclimation of *D. magna* in F4 to the DEHP in present study. Further studies on the gene encoding for antioxidant enzyme and detoxification activities in *D. magna* exposed to DEHP are suggested to clarify.

3.2. The effects of DEHP on the growth of Daphnia magna

The average body length of *D. magna* in the control ranged between 3,000 and 3,610 µm. However, body length of *D. magna* in the DEHP exposure varied from 2,920–3,330 µm which was significant shorter than that in the control ($p < 0.05$; Fig. 3). Our results indicated that DEHP inhibited the growth of the organisms in all five generations.

Body size of *D. magna* is a very important endpoint of fitness because it is highly related to the time of first reproduction and clutch size of the organism (Ebert 1992). However, effects of phthalates on growth or body length of *D. magna*

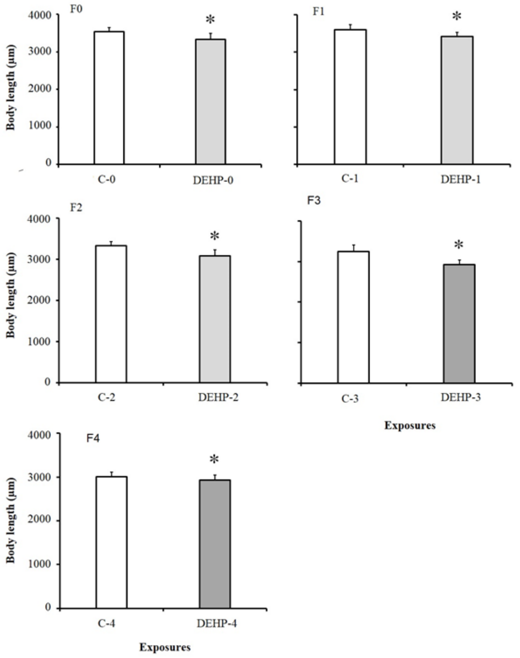

Fig. 3. The body length of *D. magna* in the control and DEHP exposure through five generations. The asterisk (*) indicated the significant difference between control and DEHP exposure by the Kruskal-Wallis test ($p < 0.05$)

Rys. 3. Długość ciała *D. magna* w próbce kontrolnej i w ekspozycji na DEHP przez pięć pokoleń. Gwiazdka (*) wskazuje istotną różnicę między próbką kontrolną a ekspozycją na DEHP w teście Kruskala-Wallisa ($p < 0,05$)

have not been studied (but see Seyoum and Pradhan 2019; Le et al. 2019). Our study firstly revealed the impact of DEHP on the growth of *D. magna* under a multigenerational exposure. The observation in present study was in line with the previous study for the F0 (Le et al. 2019). Seyoum and Pradhan (2019) also recorded a reduction of body length in *D. magna* exposed to DEHP at the concentration of 390 μg L-1 over 14 days. As mentioned above, DEHP could cause the material and energy allocation related to the fitness such as survival, growth, reproduction of *D. magna*. Generally, the DEHP-exposed *D. magna* in our study could maintain its survival at a normal rate during 21 days of incubation from F0 through F4, except F3 (Fig. 2). Therefore, DEHP-exposed organism had to face a trade-off that slowed down its growth consequently a shorter body length than the control *D. magna*.

3.3. The effects of DEHP on the reproduction of Daphnia magna

The reproduction of *D. magna* was calculated as the accumulative neonates released from each beaker in the control and the DEHP exposure during 21 days of treatment. The reproduction of *D. magna* of both control and exposure in F1, F2, and F4 was in a similar range. However, DEHP caused a significant reduction in fecundity of mother *D. magna* in the F0 ($p = 0.024$, Kruskal-Wallis test), and F3 ($p = 0.002$, Kruskal-Wallis test; Fig. 4).

Previous studies revealed that DEHP could enhance the reproduction of *D. magna* at the concentration of 390 μg L-1 (Seyoum and Pradhan 2019) or not inhibited this endpoint of the organism at the concentration of 500 μg L-1 (Le et al., 2019). On the contrary, we found a much lower reproduction in *D. magna* exposed to DEHP in the first generation (F0). Without a doubt – organisms in aquatic environments could be affected by not only a single pollutant but a mixture of pollutants as well. The natural water from Mekong River used in present study had some trace elements at low levels (Dao et al. accepted manuscript) which might have side effects on *D. magna* (Le et al. 2021). The combined effects of DEHP and trace elements in natural water could cause stronger toxic effects on micro-crustacean than the solely impact of DEHP on the organism in standard medium (Dao et al. accepted manuscript). In the second (F1) and third (F2) generations, DEHP-exposed *D. magna* showed signs of reproductive recovery (Fig. 4).

In our study, in the fourth generation (F3) *D. magna* were affected again and the total number of offspring born in DEHP exposure was significantly different from the control ($p < 0.002$). this should be the consequence of the high mortality of DEHP-exposed *D. magna* in F3 (Fig. 2). The much lower number of mother *D. magna* in F3 would lead to a lower number of accumulative neonates. On the other hand, the increase of DEHP-exposed *D. magna* survival in the fifth generation (F4; Fig. 2) resulted in the increase of accumulative neonates (Fig. 4).

The present study showed the effects of DEHP in natural water from Mekong River on *D. magna* across five generations. This could be useful for the extrapolation in situ and the prediction on potential impacts of plastic additives (e.g., DEHP) on freshwater zooplankton in Mekong River, the eleventh highest river loading plastic waste into the seas globally (Lebreton et al. 2017).

Conclusions

This is the first investigation assessing the toxicity of DEHP to *D. magna* incubated in natural water from Mekong River across five generations from the best of our knowledge. High mortality (47%) was found in the DEHP exposure in the fourth generation (F3). DEHP also caused a reduction in fe-

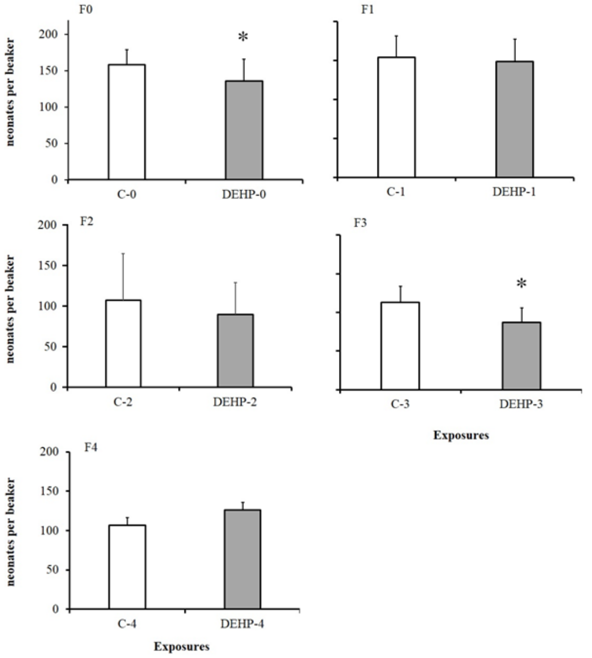

Fig. 4. The accumulative neonates from each culturing beaker in the control and DEHP exposure through five generations. The asterisk (*) indicated the significant difference between control and DEHP exposure by the Kruskal-Wallis test ($p < 0.05$)

Rys. 4. Skumulowana liczba nowych organizamów z każdej próbki hodowlanej w grupie kontrolnej i ekspozycji na DEHP przez pięć pokoleń. Gwiazdka (*) wskazuje istotną różnicę między próbką kontrolną a ekspozycją na DEHP w teście Kruskala-Wallisa ($p < 0{,}05$)

cundity of mother *D. magna* in F0 and F3. However, the body length of the animal in the DEHP exposure was significantly shorter than that of the control across five generations. The trade-off between the survival, growth and reproduction related to the material and energy allocation would be the root of the responses of *D. magna* to DEHP. The effects of DEHP in natural water from Mekong River are useful to extrapolate the toxicity of this chemical to micro-crustacean in situ, and the effects would become more severe uncomfortable environmental and biological conditions in nature.

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Literatura – References

1. Adams WJ, Biddinger GR, Robillard KA, Gorsuch J (1995) A summary of the acute toxicity of 14 phthalates esters to representative aquatic organisms. Environmental Toxicology and Chemistry 14(9): 1569-1574. https://doi.org/10.1002/etc.5620140916
2. Adema DMM (1978). *Daphnia magna* as a test animal in acute and chronic toxicity tests. Hydrobiologia 59: 125–134. https://doi.org/10.1007/BF00020773
3. American Public Health Association (APHA) (2012) Standard Methods for the Examination of Water and Wastewater (22nd ed.). American Water Works Association, 1496 pp.
4. Azevedo-Santos VM, Brito MFG, Manoel PS, Perroca JF, Rodrigues-Filho JL, Paschoal LRP, Gonçalves GRL, Wolf MR, Blettler MCM, Andrade MC, Nobile AB, Lima FP, Ruocco AMC, Silva CV, Perbiche-Neves G, Portinho JL, Giarrizzo T, Arcifa MS, Pelicice FM (2021) Plastic pollution: A focus on freshwater biodiversity. Ambio 50(7): 1313-1324. https://doi.org/10.1007/s13280-020-01496-5
5. Brown D, Thompson RS (1982) Phthalates and the aquatic environment: part 1. The effect of di-2-ethylhexyl phthalate (DEHP) and di-isodecyl phthalate (DIDP) on the reproduction of *Daphnia magna* and observations on their bioconcentration. Chemosphere 11: 417–426. https://doi.org/10.1016/0045-6535(82)90045-5
6. Brown D, Croudace CP, Williams NJ, Shearing JM, Johnson PA (1998) The effect of phthalate ester plasticisers tested as surfactant stabilised dispersions on the reproduction of the *Daphnia magna*. Chemosphere 36: 1367–1379. https://doi.org/10.1016/S0045-6535(97)10018-2
7. Chen H, Qin Y, Huang H, Xu W (2020) A regional difference analysis of microplastic pollution in global freshwater bodies based on a regression model. Water 12: 1889. https://doi.org/10.3390/w12071889
8. Dao TS, Vo TMC, Wiegand C, Bui BT, Dinh VK (2018) Transgenerational effects of cyanobacterial toxins on a tropical micro-crustacean Daphnia lumholtzi across three generations. Environmental Pollution 243 (B): 791-799. https://doi.org/10.1016/j.envpol.2018.09.055
9. Dao TS, Nguyen VT, Baduel C, Bui MH, Tran VT, Pham TL, Bui BT, Dinh KV. Toxicity of di-2-ethylhexyl phthalate and tris (2-butoxyethyl) phosphate to a tropical micro-crustacean (Ceriodaphnia cornuta) is higher in Mekong river water than in standard laboratory medium. Accepted manuscript in Environmental Science and Pollution Research.
10. Ebert D (1992). A food-independent maturation threshold and size at maturity in *Daphnia magna*. Limnology and Oceanography 37: 878– 881. https://doi.org/10.4319/lo.1992.37.4.0878
11. Gustafsson S, Rengefors K, Hansson LA (2005) Increased consumer fitness following transfer of toxin tolerance to offspring via maternal effects. Ecology 86(10): 2561-2567. DOI:10.1890/04-1710.
12. Ito Y, Kamijima M, Nakajima T (2019) Di(2-ethylhexyl) phthalate-induced toxicity and peroxisome proliferator-activated receptor alpha: a review. Environmental Health and Prevention Medicine 24(1): 47. https://doi.org/10.1186/s12199-019-0802-z
13. Knowles CO, McKee MJ, Palawski DU (1987) Chronic effects of di-2-ethylhexyl phthalate on biochemical composition, survival and reproduction of *Daphnia magna*. Environmental Toxicology and Chemistry 6: 201-208. https://doi.org/10.1002/etc.5620060305
14. Kotai J (1972). Instructions for preparation of modified nutrient solution Z8 for algae. Norwegian Institute for Water Research, B-11, 5pp.
15. Lampert W (2006) Daphnia: model herbivore, predator and prey. Polish Journal of Ecology 54(4): 607-620.
16. Le TPD, Nguyen VT, Vo TMC, Bui NH, Dao TS (2019) Transgenerational effects of the plasticizer di-2-ethylhexyl phthalate on survival, growth, and reproduction of *Daphnia magna*. Vietnam Journal of Science, Technology and Engineering 61(4): 64-69. https://doi.org/10.31276/VJSTE.61(4).64-69.
17. Le TPD, Nguyen VT, Bui MH, Huynh TN, Huynh AT, Tran VQ, Vo TMC, Tran T, Dao TS, (2021) Single and binary effects of di-2-ethylhexyl phthalate and trace metals (Cd, Pb) on life history traits of *Daphnia magna*. Accepted manuscript in Environmental Quality Management. DOI: 10.1002/tqem.
18. Lebreton LCM, van der Zwet J, Damsteeg JW, Slat B, Andrady A, Reisser J (2017) River plastic emissions to the world's oceans. Nature Communication 8: 15611. https://doi.org/10.1038/ncomms15611
19. Miller ME, Hamann M, Kroon FJ (2020) Bioaccumulation and biomagnification of microplastics in marine organisms: a review and meta-analysis of current data. PloS ONE 15(10): e0240792. https://doi.org/10.1371/journal.pone.0240792
20. Rowdhwal SSS, Chen J (2018) Toxic effects of di-2-ethylhexyl phthalate: an overview. BioMed Research International 2018: 1750368. https://doi.org/10.1155/2018/1750368

21. Scanlan LD, Loguinov AV, Teng Q, Antczak P, Dailey KP, Nowinski DT, Kornbluh J, Lin XX, Lachenauer E, Arai A, Douglas NK, Falciani F, Stapleton HM, Vulpe CD (2015) Gene transcription, metabolite and lipid profiling in eco-indicator *Daphnia magna* indicate diverse mechanisms of toxicity by legacy and emerging flame-retardants. Environmental Science & Technology 49: 7400-7410. https://doi.org/10.1021/acs.est.5b00977
22. Seyoum A, Pradhan A (2019) Effect of phthalates on development, reproduction, fat metabolism and lifespan in *Daphnia magna*. Science of the Total Environment 654: 969-977. https://doi.org/10.1016/j.scitotenv.2018.11.158
23. Sournia A (1978) Phytoplankton Manual UNESCO, UK, 77
24. US Environmental Protection Agency (US EPA), 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. EPA-821-R02-012, fifth ed. Office of Water, Washington, DC.
25. Wang Y, Wang T, Ban Y, Shen C, Shen Q, Chai X, Zhao W, Wei J (2018) Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme activity and gene expression in juvenile and adult *Daphnia magna*. Archives of Environmental Contamination and Toxicology 75 (1): 145-156. https://doi.org/10.1007/s00244-018-0535-9
26. Wetzel RG (2001) Limnology: Lake and River Ecosystems, 3rd ed. Academic Press, San Diego.
27. Wowkonowicz P, Kijeriska M (2017) Phthalate release in leachate from municipal landfills of central Poland. PloSONE 12(3): e0174986. https://doi.org/10.1371/journal.pone.0174986.

Reakcje mikroskorupiaków, Daphnia magna*, w ciągu pięciu pokoleń stale narażonych na działanie ftalanu di-2-etyloheksylu w wodzie rzeki Mekong*

Zanieczyszczenie tworzywami sztucznymi zostało uznane za narastające zagrożenie środowiskowe i jest jednym z problemów ekologicznych związanych ze zdrowiem człowieka. Szkodliwy wpływ zanieczyszczenia tworzywami sztucznymi na organizmy żywe jest ściśle związany z dodatkami tworzyw sztucznych dodawanymi do polimeru podczas produkcji tworzyw sztucznych. Ftalan di-2-etyloheksylu (DEHP) jest jednym z najpowszechniejszych plastyfikatorów i zwykle występuje w środowisku wodnym na całym świecie. Dodatki do tworzyw sztucznych mogą powodować wiele negatywnych skutków dla organizmów wodnych, takich jak ryby i zooplankton. Badania przedsatwione w artykule miały na celu ocenę ciągłego wpływu DEHP na cechy historii życia modelu ekotoksykologicznego mikroskorupiaka, Daphnia magna*, w ciągu pięciu pokoleń (F0–F4). Jako pożywkę do inkubacji* D. magna *w warunkach laboratoryjnych wykorzystano naturalną wodę z rzeki Mekong w Wietnamie. Stężenia pierwiastków śladowych (np. metali i pestycydów) w naturalnej wodzie były poniżej poziomu wykrywalności sprzętu lub bardzo niskie, co było wystarczające, aby* D. magna *dobrze się rozwijała. Wyniki pokazały, że długość ciała była głównym punktem oceny rozwoju organizmów hamowanego przez DEHP we wszystkich pokoleniach. DEHP negatywnie wpłynął na przeżywalność i płodność* D. magna *jedynie w czwartym pokoleniu (F3). Negatywny wpływ DEHP na długość ciała* D. magna *powinien być konsekwencją zużycia energii i jej alokacji w narażonych organizmach. Reakcje* D. magna *na przetrwanie i reprodukcję pod wpływem DEHP w ciągu pięciu pokoleń można wytłumaczyć (i) poważnym wpływem substancji chemicznej na wiele osobników w populacji organizmów oraz (ii) rozwojem tolerancji na toksyny u pozostałych narażonych organizmów. Chociaż pierwiastki śladowe w naturalnej wodzie z Mekongu nie były toksyczne dla* D. magna *w bardzo niskich stężeniach, to razem z DEHP mogą nasilać oddziaływanie na organizm. Poza tym wielopokoleniowe narażenie na DEHP odzwierciedlałoby wyraźniejszy wpływ na organizm niż jednorazowe narażenie. Przedstawione wyniki mogą być przydatne do ekstrapolacji wpływu plastyfikatorów na zooplankton słodkowodny w przyrodzie.*

Słowa kluczowe: *skutki chroniczne,* Daphnia magna*, zużycie energii, cechy życiowe, dodatki do tworzyw sztucznych*

Assessment of Heavy Metal Pollution in the Surface Water of the Doi-Cho Dem-Ben Luc Rivers, Vietnam

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Abstract

Heavy metals are a pressing concern in terms of their pollution in aquatic ecosystems because of their persistence, environmental toxicity, bioaccumulation. Aquatic environments receive heavy metals in untreated or inadequately treated wastewater from domestic, industrial, agricultural, and navigation sources. The Doi-Cho Dem-Ben Luc Rivers play the key roles of irrigation, navigation and ecological restoration. It is crucial to ascertain the pollution status, influencing factors, ecological risks, and possible sources of heavy metals in the surface water of the Doi-Cho Dem-Ben Luc Rivers. In this study, surface water from 7 sampling sites over was collected from the Doi-Cho Dem-Ben Luc Rivers, over 7 consecutive periods from April 2019 to October 2021. Each surface sample was analyzed for 9 heavy metals including Fe, Mn, Cr, Zn, Cu, Pb, Cd, Ni, As. The sampling technique and sample treatment were done based on the Standard Methods for the Examination of Water and Wastewater. The time and space variation of heavy metal concentrations were examined to test the analysis of variance (ANOVA) and correlation among all the parameters using R statistical software.

The results suggest a spatial homogeneity of heavy metals in the surface water the studied rivers. Among all nine examined heavy metals in the studied area, the concentrations of Fe (1.00 ÷ 5.06 mg/L) and Mn (0.14 ÷ 0.28 mg/L) are the highest, and the concentrations of Cr, Cd and As are the lowest that lower limit of detection. The results suggested that the mean concentrations of Fe and Mn were above the acceptable limits of the National technical regulation on surface water quality (QCVN 08-MT: 2015/BTNMT). While the concentrations of Fe, Mn, Zn, Cu, Pb, Ni do not meet the Water quality criteria for aquatic life (United State Environmental Protection Agency). Anthropogenic activities can be the main source of heavy metals in in the surface water of the Doi-Cho Dem-Ben Luc Rivers. Among the heavy metals, a significant positive correlation was observed between Fe, Mn, Zn and Ni (0.64 ÷ 0.87), whereas Cu exhibited a significant positive correlation with Ni (0.51). While Cu and Pb showed a not too strong correlation with Fe, Mn, Zn and Ni (0.25 ÷ 0.48). The distribution of heavy metals may also be influenced by properties of heavy metals and fluctuations in water flows. The results provide guidance for controlling heavry metal pollution and protectting water sources in the Doi-Cho Dem-Ben Luc Rivers.

Keywords: *heavy metal pollution, surface water, distribution, river, Doi-Cho Dem-Ben Luc, water quality protection*

1. Introduction

The Doi-Cho Dem-Ben Luc Rivers has a length of about 30 km with a width of from 30 to 70 m and a depth of 3 ÷ 7 m. It's one of an important inland waterway that connects HCMC with Long An Province (Mekong Delta). The Doi-Cho Dem-Ben Luc rivers play an important role in irrigation, transportation and ecological restoration. Among the inorganic pollutants of river water, heavy metals are gaining importance because of their non-biodegradable nature and often accumulate at tropical levels causing harmful biological effects [1]. Heavy metals are a pressing concern in terms of their pollution in aquatic ecosystems because of their persistence, environmental toxicity, bioaccumulation [2]. Anthropogenic activities like mining, ultimate disposal of treated and untreated waste effluents containing toxic metals as well as metal chelates [3] from different industries steel plants, battery industries, thermal power plants etc. and also the indiscriminate use of heavy metal containing fertilizers and pesticides in agriculture resulted in deterioration of water quality rendering serious environmental problems posing threat on human beings [4] and sustaining aquatic biodiversity [5, 6].

Sankar et al (2018) claimed that The concentrations of different metals, like Chromium (Cr), Manganese (Mn), Cobalt (Co), Nickel (Ni), Cooper (Cu), Zink (Zn), Lead (Pb), Cadmium (Cd), Mercury (Hg) and Arsenic (As) were highly increasing in coastal areas due to the discharge of agricultural and domestic wastes; intrusion of wastes from industries like metal plating; entry of organic and inorganic chemicals; leaching of metals from solid waste; and use of metal and metal components [7]. Heavy metal concentrations in water and fish from River Yamuna, at Allahabad were found that Pb and Cu were higher than the permissible limits of WHO, that gives an indication of hazardous risk to human health. Whereas Arsenic was detected lower than the permissible limit (Kumar et al., 2014). In Vietnam, the temperature, pH, dissolved oxygen (DO), conductivity, salinity, chlorophyll-a, phaeopigments, suspended particulate matter (SPM) concentrations, grain size distributions, nutrients, dissolved and particulate organic carbon and phosphorus, and trace metal(oid) (Cr, Ni, Cu, Zn, As, Cd, Pb, Hg, and MMHg) concentrations were measured at 17 sites along the Saigon River in water (filtered and suspended matter) and sediment. This research showed that the Saigon River remains moderately contaminated albeit the city was proved to be the major contributor of metal(oid)s [8].

The Doi-Cho Dem-Ben Luc Rivers receive heavy metals in untreated or inadequately treated wastewater from domestic, industrial, agricultural, and navigation sources. It is very important to monitor and evaluate the pollution status,

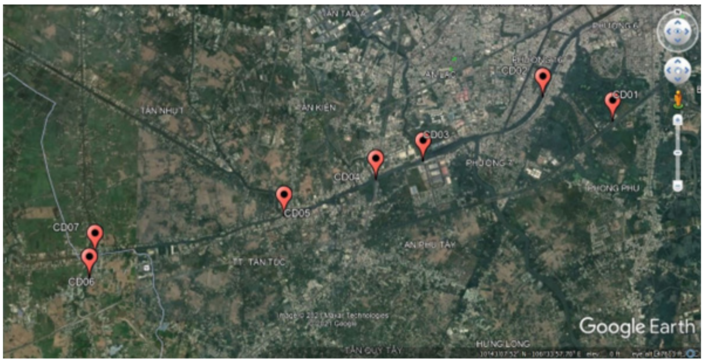

Fig. 1. Study area of heavy metal concentrations with 7 sampling sites (B1-B10)
Rys. 1. Obszar badań koncentracji metali ciężkich z 7 punktami poboru próbek (B1-B10)

Tab. 1. Coordinates and locations of the sampling sites in study area
Tab. 1. Współrzędne i położenie punktów poboru próbek na obszarze badań

Sites	**Local Names**	**Longitude (N)**	**Latitude (S)**
CD01	Ba Lon Creek flows into Doi Canal (near Residental Area 13C)	10°42'48.05"N	106°38'49.51"E
CD02	Doi Canal at 16 Ward, District 8 (near River Wharf)	10°43'06.17"N	106°37'57.22"E
CD03	Binh Dien River (near Binh Dien Market)	10°42'18.03"N	106°36'25.97"E
CD04	Binh Dien River (near Binh Dien Bridge)	10°42'04.67"N	106°35'50.39"E
CD05	Binh Dien River (near Cai Tam Bridge)	10°41'37.99"N	106°34'41.70"E
CD06	My Nhan Creek flow into Ben Luc River (near Tan Bua Ferry Station)	10°40'52.58"N	106°32'17.54"E
CD07	Ben Luc River (near Tan Bua Ferry Station)	10°41'09.70"N	106°32'21.22"E

influencing factors, ecological risks, and possible sources of heavy metals in the surface water of the Doi-Cho Dem-Ben Luc Rivers. This topic is crucial identify the levels of heavy metal pollution in the Doi-Cho Dem-Ben Luc Rivers. The results contribute to providing information and data for local authorities on heavy metal parameters as well as pollution levels of this system.

2. Materials and Methods

2.1. Study Area

Data from about 70 km^2 with length of 30 Km were used as a representative example for study areas. The water samples of heavy metal (Iron, Manganese, Chrome, Zinc, Copper, Lead, Cadmium, Nickel, and Arsenic) analysis at 7 sites were collected for 7 periods in April and October (2019, 2020); April, June and October (2021) (see Fig. 1; Tab. 1).

2.2. Sample Collection

The water samples for havey metal analyses in the field were collected according to the standards of TCVN 6663 – 1:2011 (ISO 5667 – 1:2006) Water quality – Sampling – Part 1: Guidance on the design of sampling programmes and sampling techniques; and, TCVN 6663 – 3:2008 (ISO 5667 – 3:2003) Water quality – Sampling – Part 3: Instructions for sample storage and handling [9, 10]. All samples were collected in 2.0 litre, clean polyethylene bottles, which were prewashed with 10% nitric acid and de-ionized water. Before sampling, the bottles were rinsed at least three times with water from the sampling site. Sample locations at each site were taken in the middle of the river with a depth layer of surface water of 30–40 cm [10-12]. All water samples were immediately brought to the laboratory.

2.3. Analytical Methods

The standard methods of heavy metal and their analytical methods were briefly presented in Tab. 2.

The samples were acidified with 2 mL concentrated Nitric acid to prevent precipitation of metals, reduce adsorption of the analytes onto the walls of containers and to avoid microbial activity, then water samples were stored at 2°C until the analyses. Surface water samples were filtered through milipor filtering unit using 0.45 μm Whatman filter paper. If the concentrations exceeding the calibration curve, the samples were appropriately diluted, and acid was added to measure samples. The samples for heavy metal analysis were measured in the Inductively Coupled Plasma Optimal Emission Spectrometer [13].

2.4. Data Analysis

The obtained data were subject to statistical analysis to test the analysis of variance (ANOVA) and correlation among all the parameters using R statistical software. The maps of the study area and sampling sites were applied using Google Earth.

3. Results and Discussions

3.1. Concentrations of heavy metals in river water

The concentrations of Fe, Mn, Cu, Ni, Zn, Pb, Cr, Cd and As in water surface at all seven different sites of seven periods in the Doi-Cho Dem-Ben Luc Rivers during 2019, 2020 and 2021 were detected and recorded in Tab. 3.

Tab. 3 showed that the mean concentrations of almost heavy metals were observed in decreasing order of Fe > Mn > Cu > Ni > Zn > Pb > Cr, Cd, As whereas the concentrations of heavy metals (Mn > Cu > Ni > Zn > Pb) were within the acceptable limits of the National technical regulation on surface

Tab. 2. Parameters and methods of water quality analysis

Tab. 2. Parametry jakości wody i metody oznacznia

No.	Parameters	Unit	Methods
1	Fe	mg/L	TCVN 6177:1996
2	Mn	mg/L	SMEWW 3111B:2017
3	Cr	mg/L	TCVN 6222:2008
4	Zn	mg/L	TCVN 6193:1996
5	Cu	mg/L	SMEWW 3111B:2017
6	Pb	mg/L	SMEWW 3111B:2017
7	Cd	mg/L	SMEWW 3111B:2017
8	Ni	mg/L	SMEWW 3111B:2017
9	As	mg/L	US EPA Method 2008

Tab. 3. Concentrations of heavy metals in the surface water of the study area. Notes: ND is non detect

Tab. 3. Zawartość metali ciężkich w wodach powierzchniowych na badanym terenie. Uwaga: ND nie wykryte

Parameters (mg/L)	Sampling Sites						
	CD01	CD02	CD03	CD04	CD05	CD06	CD07
Fe (mg l^{-1})							
Range	1.01÷1.16	2.38÷3.63	2.72÷5.04	1.65÷2.11	1.21÷2.85	1.05÷1.84	1.06÷2.04
Mean	1.06	3.25	3.59	1.91	1.78	1.36	1.68
Mn (mg l^{-1})							
Range	0.14÷0.19	0.24÷0.27	0.22÷0.28	0.16÷0.23	0.16÷0.22	0.16÷0.19	0.16÷0.18
Mean	0.16	0.25	0.24	0.20	0.19	0.17	0.17
Cu (mg l^{-1})							
Range	0.029÷00.34	0.057÷0.078	0.038÷0.081	0.032÷0.065	0.026÷0.070	0.051÷0.062	0.040÷0.058
Mean	0.031	0.062	0.053	0.045	0.047	0.056	0.049
Ni (mg l^{-1})							
Range	0.029÷0.037	0.043÷0.057	0.034÷0.053	0.031÷0.043	0.025÷0.038	0.026÷0.033	0.025÷0.030
Mean	0.033	0.049	0.44	0.037	0.031	0.029	0.028
Zn (mg l^{-1})							
Range	0.026÷0.034	0.048÷0.056	0.045÷0.068	0.030÷0.041	0.026÷0.030	0.026÷0.032	0.026÷0.034
Mean	0.029	0.052	0.054	0.036	0.028	0.030	0.029
Pb (mg l^{-1})							
Range	0.008÷0.010	0.010÷0.012	0.008÷0.012	0.008÷0.010	0.008÷0.010	0.008÷0.012	0.08÷0.010
Mean	0.009	0.011	0.011	0.009	0.010	0.010	0.010
Cr (mg l^{-1})	ND	ND	ND	ND	ND	ND	ND
Cd (mg l^{-1})	ND	ND	ND	ND	ND	ND	ND
As (mg l^{-1})	ND	ND	ND	ND	ND	ND	ND

water quality for QCVN 08-MT: 2015/BTNMT at the B1 Level (water quality for irrigation and drainage purposes or other uses with similar water quality requirements) [14]. Especially, the concentrations of heavy metals such as chromium, cadmium and arsenic have not been detected in water samples in this system. While the mean concentrations of iron (Fe) were above the permissible limits of the National technical regulation on surface water quality for QCVN 08-MT: 2015/ BTNMT at the B1 Level. Moreover the mean concentrations of Fe, Mn, Cu, Ni, Zn and Pb were above concentration of the values than the permissible limits set by the water quality criteria for aquatic life [15].

3.2. Spatial in heavy metal concentrations of river water

The concentration of different metals in river water order as follow: Fe > Mn > Cu > Zn > Ni > Pb (Fig. 2). The results showed that heavy metals of iron and zinc had the highest concentrations in the sites CD2 and CD3. The next concentration indicated in the site CD4, and the lowest concentration in site CD1. The results showed that heavy metals of iron and zinc had the highest concentrations in the sites CD2 and CD3. The next concentration indicated in the site CD4, and the lowest concentration in site CD1 The results of concentrations of lead and nickel fluctuated similar to the concentrations of in manganese and copper. While the concentrations of chromium, cadmium and arsenic were the lowest that lowered the limit of detection (Fig. 2). These results were quite similar to the research of heavy metal pollution in the Lake Manzal, Egypt [16] that showed the concentration of different metals in water, plankton, and fish tissues followed the same order: Zn > Cu > Pb> Cd. The mean concentrations of metals in the water were as follow: Cu, 0.055; Zn, 0.311; Cd, 0.020; and Pb, 0.022 mg/L.

Among the six heavy metals identified above, the seasonal change was not significant. This needs to be further observed to provide a clear understanding of the characteristics of seasonal variations in heavy metal concentrations. While Tran et al. (2020) claimed that the total metal concentrations in the seawater of the Saigon – Dongnai Estuaries were higher during the rainy season than those during the dry season [17]. The increase of rainfall in the Saigon – Dongnai River Basin in the transition time mobilized both dissolved and particulate metals (Fe, Cr, Ni, and Pb) from the terrestrial environment to the aquatic environment [18]. Additionally, Raji et al. (2016) indicated that the concentrations of heavy metals that monitored at the Sokoto River in North-western Nigeria were generally higher in dry season than in the rainy season [19].

3.3. Correlation analysis

Pearson's correlation was performed on the combined data set of average values of the surface water of the studied area based on the significant levels (p=0.05). Correlation analysis was carried out for inter-metallic and intra-metallic association to understand the significance of association among the metals and the samples. The surface water exhibited a positive correlation between Fe-Mn (0.79), Fe-Zn (0.82), Fe-Cu (0.42), Fe-Pb (0.46), Fe-Ni (0.64); Mn-Zn (0.81), Mn-Cu (0.41), Mn-Pb (0.40), Mn-Ni (0.76); Zn-Cu (0.48), Zn-Pb (0.35), Zn-Ni