Doi: 10.31276/VJSTE.61(4).82-87

Chronic effects of lead and arsenic on life history traits of *Daphnia magna*

Le-Thu Quach[1], Thi-My-Chi Vo[2], Van-Tai Nguyen[2], Thanh-Son Dao[2*]

[1]Ho Chi Minh city University of Food Industry

[2]University of Technology, Vietnam National University, Ho Chi Minh city

Received 22 August 2019; accepted 12 November 2019

Abstract:

Anthropogenic activities such as industrial production, mining, agriculture and transportation are among the main causes for the increase of trace metal concentrations in the environment, especially in water bodies. In this study, we evaluated the chronic impacts of lead (Pb) and arsenic (As) on *Daphnia magna*, a crucial organism to aquatic ecosystems, at several concentrations (0, 5, 25, 50, 150 and 250 µgl^{-1} of Pb and 0, 5, 25, 50 µgl^{-1} of As) for 21 days. The organism's life history traits, including survivorship, maturation, and reproduction, were recorded daily. In addition, the survival rate of the offspring exposed to 50 µgl^{-1} Pb was also recorded when the animals were raised in (i) a Pb-containing medium and Pb-free medium for 8 days. The results showed that As, at all the test concentrations, did not only negatively affect the survival and cause a delay the maturation, but also reduced the reproductive performance of the animal, especially at the highest concentration. Compared to the control, the survivorship and reproduction of the *D. magna* exposed to Pb at the highest concentrations (150 and 250 µgl^{-1}) declined dramatically, and it took a longer time to reach maturity. On the other hand, almost all of the recorded life history traits of the organisms exposed to 5, 25 and 50 µgl^{-1} of Pb were relatively similar to those from the control. However, Pb at the concentration of 50 µgl^{-1} had detrimental effect on survival of *Daphnia*'s F1 generation after the 8-day experiment. An abnormality of the metal-exposed *D. magna* was also observed. An impairment of the daphnids was observed upon exposures to As and Pb at concentrations within the Vietnam guideline values for surface water safety. Hence, further investigations are suggested to adjust the guidelines related to As and Pd for the protection on environmental quality and ecological health.

Keywords: **chronic effects, *Daphnia magna*, guideline, life history traits, trace metals.**

Classification number: **5.1**

Introduction

Over the past decades, trace metals have been one of the most serious chemical contaminants causing environmental pollution and potential toxicity to human health and organisms [1, 2]. Some trace metals (e.g. Cu, Cr, Mg, Mn, Zn, Ni, Fe and Co with the concentration less than 10 µgl^{-1}) are essential elements for the physiological and biochemical functions of organisms [3], yet when exceeding a certain concentration, they could cause negative effects [4]. Besides, some other trace meals such as Pb, As, Cd, Hg are not only non-essential elements for biological functions of organisms, but also potent toxins to living things [5-7]. They can adversely affect cellular organelles and components, such as cell membranes, mitochondria, lysosomes, endoplasmic reticula, nuclei, some metabolic enzymes, detoxification processes, and damage repair mechanisms [8, 9], hence trace metals interfere with critical life processes of organism. Recently, there has been an abundance of evidence of aquatic pollution caused by trace metals worldwide [10-12]. In Vietnam, due to rapid economic development, the surface water quality is under constant threat of trace metal contamination, especially with Pb and As [13, 14]. Trace metals are naturally and commonly occurring elements in the Earth's crust, however in surface water, the main sources of trace metal, particularly As and Pb, are emission from anthropogenic activities e.g. metal plating, fishing operations, battery manufacturing, fertilizers and pesticides in agriculture, and smelting of ores [4, 15].

Both Pb and As are classified as top human carcinogens [5]. Additionally, Pb and As are non-essential elements for organisms and considered a major cause of aquatic environmental pollution due to the mechanism of chronic bioaccumulation and toxicity at low concentrations [16]. The presence of Pb in the environment was shown to cause behavioural abnormalities, hearing deficits, neuromuscular weakness, and shown to impair cognitive functions in human being as well as wildlife [17]. Regarding As,

*Corresponding author: Email: dao.son@hcmut.edu.vn

exposure to this chemical might affect several different organ systems including skin, respiratory, cardiovascular, immune, genitourinary, reproductive, gastrointestinal, and nervous system [18, 19].

Microcrustaceans (e.g. *D. magna*) play an important role in aquatic ecosystems as they are vital intermediate trophic level organisms responsible for the passage of matter and energy between primary producers and top predators [20]. Hence, in order to assess the ecological risk of Pb and As, a large number of studies have been completed on the chronic and semi-chronic effects of Pb and As on the life history traits of zooplankton. Many studies [21-24] indicated that there were various detrimental impacts on the health of test organisms when they were exposed to trace metals. In surface water, As and Pb could reach concentrations of 0.64 mgl^{-1} (see in the study of Frisbie, et al. (2002) [25]) and 6.3 mgl^{-1} (see in the study of Vuković, et al. (2011) [26]), respectively. For environmental and ecological safety in Vietnam, the As and Pb concentrations should not exceed 50 μgl^{-1} [27]. However, the question of whether there is any the impact of these trace metals on *D. magna* at low concentrations during a long-term exposure and across generations has not been addressed. Therefore, this study aims to evaluate the chronic effects of Pb and As at concentrations ranging from 5-250 μgl^{-1} on the life history traits and the next generation of *D. magna*.

Materials and methods

Materials

D. magna was obtained from MicroBio Test (Belgium) and has been cultured in a medium called ISO [28] for many generations under laboratory conditions (temperature of 25±1^{0}C and a photoperiod of 14 h light: 10 h dark at a light intensity of around 1000 Lux) [29]. Both the stock chemicals $Pb(NO_3)_2$ and $As(NO_3)_3$ at a concentration of 1000 mgl^{-1} were purchased from Merck (Germany) and stored at the temperature of -4^{0}C prior to the experiment.

Experimental set up

The chronic experiments were performed according to the guideline of APHA (2012), US. EPA (2002) and Dao, et al. (2017) [29-31] with minor adjustments. Briefly, prior to the experiment, 50 healthy female *Daphnia* were collected and incubated in the 1 l glass beaker containing 800 ml of ISO medium and fed with a mixture of green alga (*Chlorella* sp.) and YTC (yeast, cerrophyl and trout chow digestion). Afterwards, offspring less than 24 hours old were randomly selected for the chronic experiments.

In the first test, 2 neonates were incubated together in one 50 ml polypropylene cup containing 40 ml ISO medium and exposed to either Pb at a concentration of 5, 25, 50, 150, and 250 μgl^{-1} or As at a concentration of 5, 25, and 50 μgl^{-1}. Moreover, a control was prepared by culturing the offspring in the same way as mentioned above in a metal-free medium. There were 15 replicates (n=15) in each treatment and the test organisms were cultured under the conditions as mentioned above. The food (a mixture of *Chlorella* sp. and YTC) and medium were completely renewed three times per week. During the experimental period of 21 days, the life history traits of *D. magna*, such as survivorship, maturation, and reproductive performance, were monitored daily and carefully recorded.

The offspring of *D. magna* (<24 hours in age) in the control and 50 μgl^{-1} of Pb exposure were collected and used for the second test. Briefly, the offspring from the control were continued to culture in the control medium. Meanwhile, the neonates from 50 μgl^{-1} of Pb exposure were separated into 2 groups: i) those continuing to culture in the control medium and ii) those continuing to be exposed to 50 μgl^{-1} of Pb. In each treatment with the offspring, 30 offspring were used and incubated in 15 polypropylene cups (n=15), hence two offspring were placed in one cup. This experiment was run under the same laboratory conditions as mentioned above. The test terminated after 8 days of incubation and the survival of organisms was recorded.

Data analyses

Sigma Plot version 12.0 was used for the data processing. The Kruskal-Wallis test was applied for calculating the statistically significant difference of the maturation of *D. magna* between the control and trace metal exposures.

Results and discussion

Effects of Pb and As on the survivorship of D. Magna

As showed in Fig. 1, at the end of the experiment, all *D. magna* were still thriving in the control sample. Similarly, the survival rate of the organisms exposed to the lowest concentrations (5, 25 and 50 μgl^{-1}) of Pb was 100% during the 21 experimental days. However, after only 3 days of experiment, exposure to Pb at the concentrations of 150 and 250 μgl^{-1} caused a dramatic reduction of 20 and 77% of the total daphnids, respectively. By the end of the test, all *D. magna* exposed to the two highest concentrations of Pb had died (Fig. 1A). During the 8 first days, the survival of the organisms exposed to As at concentrations ranging from 5-50 μgl^{-1} remained steady at 100%, the same as those in the control sample, but this significantly decreased during the remainder of the As exposure. Finally, only 30, 63, and 17% of the total number of *D. magna* exposed to 5, 25, and 50 µg As l^{-1}, respectively, remained alive (Fig. 1B).

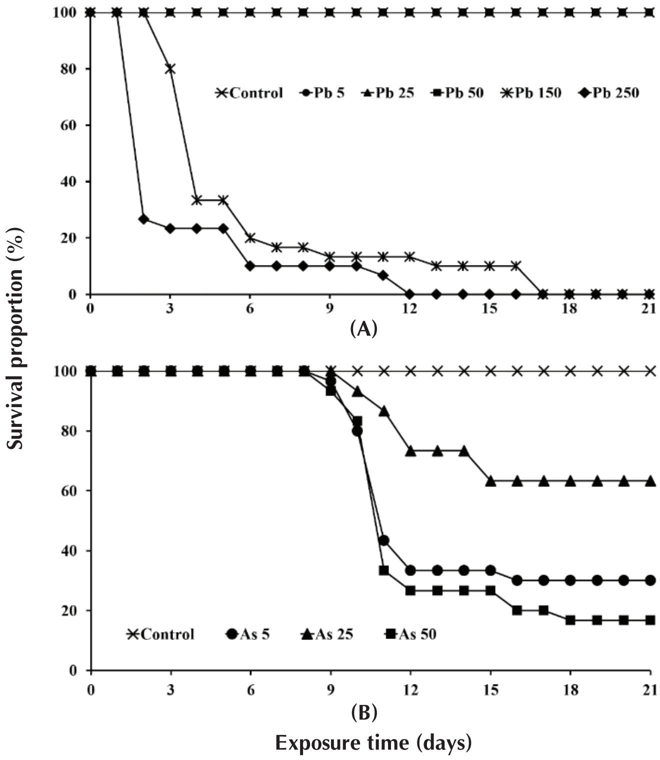

Fig. 1. Survivorship of *D. magna* from control and exposures during 3 experimental weeks (n=30 at the beginning). Samples denoted Pb 5, Pb 25, Pb 50, Pb 150, Pb 250 are animals exposed to 5, 25, 50, 150, 250 μgl^{-1} Pb, respectively, and As 5, As 25, As 50 denote animals exposed to 5, 25, 50 μgl^{-1} As, respectively.

Aquatic animals upon exposure to high metal concentrations would suffer from several impacts such as organ dysfunction, physiological imbalance, and enzyme inhibition/stimulation [32-35]. Pb is a neurotoxic chemical that can affect an organism's gills (respiratory system), cause renal dysfunction, enzymatic inhibition, and anemia [36, 37], resulting in increased energy cost for normal activity and maintenance of the animals. Besides, the metal may reduce food filtering, consequently lowering energy intake [38]. To summarize, in the presence of metals (e.g. Pb), *D. magna* would suffer an energy cost and consequently a decline in the survivorship. Additionally, our results were in line with the previous study of Regaldo, et al. (2013), although the survival of *D. magna* exposed to 30 μgl^{-1} Pb reached 100%, those exposed to this chemical at 90 and 270 μgl^{-1} declined to 10% after 15 days [21].

In the As exposure experiments, the animals exposed to As at a moderate concentration of 25 μgl^{-1} had a higher survival rate than those exposed to 5 μgl^{-1} and 50 μgl^{-1} concentrations. This phenomenon might be explained by hormesis, a term used to refer to a biphasic dose response characterized by a low-dose stimulation and a high-dose inhibition [39]. Further investigations on the physiological and biochemical responses of *D. magna* upon exposure to As are suggested to clarify this response.

Effects of Pb and As on the maturation of D. Magna

A metal may affect an organism in more than one way [40], hence, the time it takes for animals to reach maturity can be affected by exposure to Pb and As. In the control and Pb treatments (5-50 μgl^{-1}), the maturity age of *D. magna* was around 4 days. However, exposure to Pb at the two highest concentrations, 150 and 250 μgl^{-1} Pb, the maturation of *Daphnia* was prolonged to 12 and 8 days, respectively (Fig. 2A).

There was no statistically significant difference in the age to maturation between the control and those exposed to 25 μgl^{-1} As. The animals incubated in As at a concentration of 5 and 50 μgl^{-1} delayed their maturation compared to the control, and they did not mature until the ages of approximately 5 and 6 days, respectively (Fig. 2B). Overall, exposure to trace metals caused a postponement of the maturation of the animals, especially the microcrustaceans, that were observed in several previous investigations [31, 41, 42]. Trace metals at high concentration could negatively impair the respiratory function of daphnids [33] and inhibit their sodium uptake leading to an increased energy cost for maintenance [32], which may help to explain the postponement on the maturation.

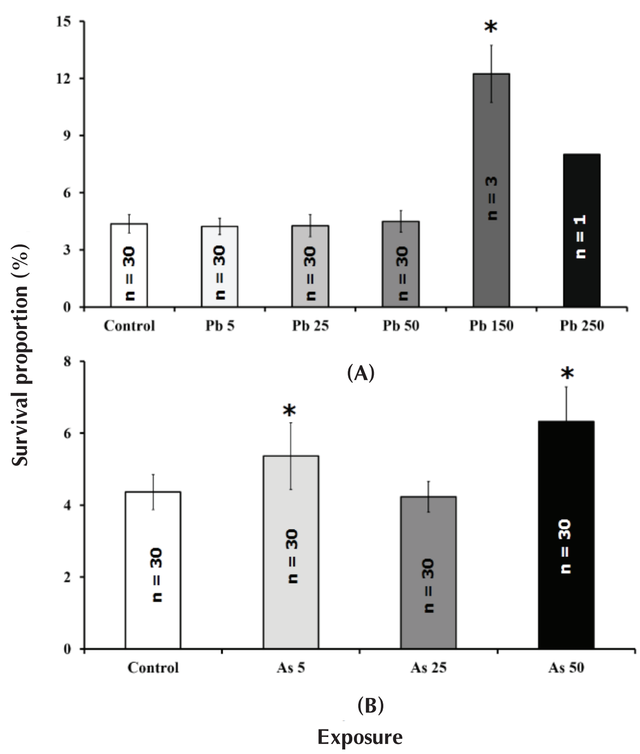

Fig. 2. Maturity age of *D. magna* (mean value ±SD) chronically exposed to Pb and As. Abbreviations given in Fig. 1. The asterisk indicates a statistically significant difference between the control and exposures by the Kruskal Wallis test (*: p≤0.001).

Effects of Pb and As on the reproduction of D. Magna

During the three weeks of incubation, the total number of offspring in the control was 3301, which was comparable to the total neonates in the exposures to Pb at low concentrations of 5, 25 and 50 µgl^{-1}. In contrast, the accumulative offspring in the 150 and 250 µgl^{-1} Pb exposures were only 42 and 5, respectively, which is much far lower than those in the control (Table 1). Regarding all the As exposures, a decline in the reproductive performance of *D. magna* was recorded. The As treatments (5, 25 and 50 µgl^{-1}) resulted in a reduction to 14, 35, and 7%, respectively, as compared to the total offspring in control (Table 1). That could be explained by the detrimental impacts on the organism mentioned above, such as the increase in mortality and postponement of the maturation. Moreover, the clutch size (the number of eggs per clutch) of the animals exposed to Pb at the highest concentration was smaller than those in the control (Fig. 3).

Table 1. Accumulative offspring of *D. magna* during the 21 experimental days. Abbreviations are the same as in Fig. 1.

	Control	Pb 5	Pb 25	Pb 50	Pb 150	Pb 250	As 5	As 25	As 50
Total neonates	3301	3503	3404	3358	42	5	455	1159	246

This observation in consistent with a previous study of Enserink, et al. (1995) [22] in which the reproduction of *D. magna* was negatively influenced by Pb. In detail, exposure to Pb at a concentration of 920 µgl^{-1} resulted in the loss of large number of eggs of the female *D. magna* and a decrease in embryo size. More seriously, several abnormal responses of the organism exposed to 50 µgl^{-1} of As were recorded, such as broken and adhesive eggs and neonatal mortality in the chamber (Fig. 4). These phenomena could significantly contribute to the decrease of the number of offspring laid by the female exposed to Pb or As.

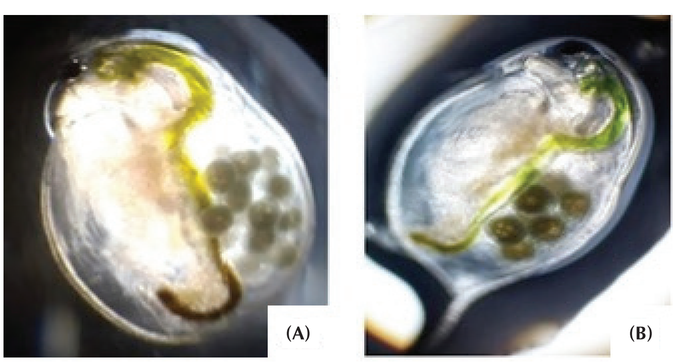

Fig. 3. The difference in the clutch size of female *D. magna* between the control (A) and exposure to 250 µg Pb l^{-1} (B).

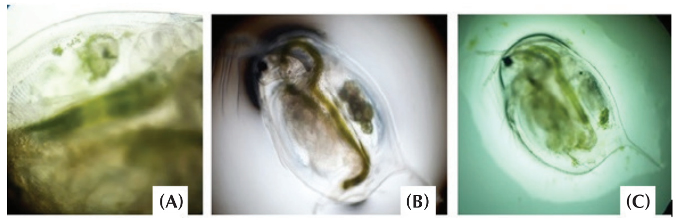

Fig. 4. Several abnormalities in *D. magna* exposed to 50 µgl^{-1} As; broken egg (A); adhesive eggs (B); death of neonate in the brood chamber of its mother (C).

Effects of Pb on the survival of the next generation of D. magna

When organisms from the mothers of the control samples were allowed to continue to rear in a metal-free medium (C/C), the survival rate was 83% (Fig. 5), which was within the requirement of the toxicity test according to APHA (2012) [29]. More seriously, the survival of the offspring of the Pb-exposed mothers incubated in both non-metal (E/C) and metal-containing medium (E/E) dramatically declined to 3 and 17%, respectively (Fig. 5). The survival rate of E/C and E/E was not significantly different but within a similar range to a chronic test according the APHA (2012). Recently, Araujo, et al. (2019) [24] showed that negative and adverse effects (e.g. malformations, Pb aggregation in neonates' dorsal region, reddish extremities, production of males, ephippia, aborted eggs, changes in egg colour) occurred through nine generations in both the temperate *D. magna* and tropical *D. similis* after long-term exposure to 50 µgl^{-1} of Pb. To our knowledge, this was the first investigation on the trans-generation effects of Pb on *D. magna* survival.

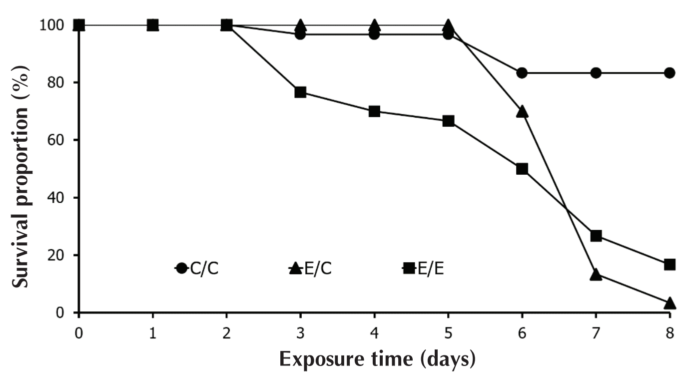

Fig. 5. Survivorship of the descendants of *D. magna* from the control and exposure to 50 µgl^{-1} of Pb during 8 experimental days (n=30 at the beginning). C/C: organisms laid in control and allowed to continue to incubate in the control medium; E/C: organisms laid in control and exposed to 50 µgl^{-1} of Pb; E/E: organisms laid in 50 µgl^{-1} of Pb and allowed to continue to culture in the same medium.

Conclusions

The results of this study proved that As and Pb at concentrations within the Vietnam guideline values for surface water safety (QCVN 08-MT:2015/BTNMT; column B1 for both Pb and As are 50 μgl^{-1}) caused several detrimental impacts on the life history traits of daphnids (e.g. survivorship, maturation, reproductive performance, and transgenerational survivorship). Therefore, more attention should be paid to the presence, distribution, and fate of trace metals in aquatic environment. Additionally, further investigations e.g. effects of a combination of more than two metals, bio-accumulation or mechanism of negative effects, are suggested in order to gain a greater understanding of the toxicity of these contaminants on aquatic organisms and ecosystems.

ACKNOWLEDGEMENTS

The authors would on like to thank Assoc. Prof. Dr. Pham Hong Nhat, University of Food Industry, for his valuable comments on this manuscript.

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] J.O. Reinhold, A.J. Hendriks, L.K. Slager, M. Ohm (1999), "Transfer of microcontaminants from sediment to chironomids, and the risk for the Pond bat Myotis dasycneme (Chiroptera) preying on them", *Aquatic Ecology*, **33(4)**, pp.363-376.

[2] P.K. Bharti (2012), *Heavy Metals in Environment*, Lambert Academic Publishing GmbH & Co. KG.

[3] D.A. Davis, D.M. Gatlin (1996), "Dietary mineral requirement of fish and marine crustaceans", *Reviews in Fisheries Science*, **4(1)**, pp.75-99.

[4] M. Jaishankar, T. Tseten, N. Anbalagan, B.B. Mathew, K.N. Beeregowda (2014), "Toxicity, mechanism and health effects of some heavy metals", *Interdisciplinary Toxicology*, **7(2)**, pp.60-72.

[5] U.S. Agency for Toxic Substances and Diseases Registry (ATSDR) (2007), *Toxicological Profile for Arsenic*, Atlanta, Georgia.

[6] P. Govind (2014), "Heavy metals causing toxicity in humans, animals and environment", *Journal of Chemical and Pharmaceutical Science*, **3(12)**, pp.172-174.

[7] W. Vincent, A. Hou, G.N. Meng (2015), "Source of lead pollution, its influence on public", *International Journal of Health, Animal Science and Food Safety*, **2(1)**, pp.18-31.

[8] K.S. Squibb, B.A. Fowler (1981), "Relationship between metal toxicity to subcellular systems and the carcinogenic response", *Environmental Health Perspectives*, **40**, pp.181-188.

[9] S. Wang, X. Shi (2001), "Molecular mechanisms of metal toxicity and carcinogenesis", *Molecullar and Cellular Biochemistry*, **222(1-2)**, pp.3-9.

[10] Z. Wang, L. Yao, G. Liu, W. Liu (2014), "Heavy metals in water, sediments and submerged macrophytes in ponds around the Dianchi Lake, China", *Ecotoxicology and Environmental Safety*, **107**, pp.200-206.

[11] M. Hassan, M.A.T.M.T. Rahman, B. Saha, A.K.I. Kamal (2015), "Status of heavy metals in water and sediment of the Meghna river, Bangladesh", *American Journal of Environmental Sciences*, **11(6)**, pp.427-439.

[12] E. Antico, S. Cot, A. Ribo, I. Rodriguez-Roda, C. Fontas (2017), "Survey of heavy metal contamination in water resources in the Municipality of Torola, El Salvador, through in situ sorbent extraction", *Water*, **9(11)**, Doi: 10.3390/w9110877.

[13] T.V. Tran, D.K. Nguyen, P.B. Nguyen, N.S. Nguyen, Q.T. Dinh, P.D. Nguyen, S. Emilie, H. Seunghee (2016), "Distribution of heavy metals in surface water, suspended particulate matter, sediment and clam (*Meretrix lyrata*) from downstream of Saigon-Dongnai river, Vietnam", *Journal of Science and Technology*, **54(2A)**, pp.207-213.

[14] T.H.M. Tran, K.G. Nguyen (2018), "Metal and metalloid concentrations in soil, surface water, and vegetables and the potential ecological and human health risks in the northeastern area of Hanoi, Vietnam", *Environ. Monit. Assess.*, **190(11)**, Doi: 10.1007/s10661-018-6994-7.

[15] P. Sharma, R.S. Dubey (2005), "Lead toxicity in plants", *Brazilian Journal of Plant Physiology*, **17(1)**, pp.35-52.

[16] J. Duffus (2002), "Heavy metals - a meaningless term? (IUPAC Technical Report)", *Pure and Applied Chemistry*, **74(5)**, pp.793-807.

[17] G. Flora, D. Gupta, A. Tiwari (2012), "Toxicity of lead: a review with recent updates", *Interdisciplinary Toxicology*, **5(2)**, pp.47-58.

[18] C.O. Abernathy, Y.P. Liu, D. Longfellow, H.V. Aposhian, B. Fowler, R. Goyer, R. Menzer, T. Rossman, C. Thompson, M. Walkes (1999), "Arsenic: health effects, mechanisms of actions and research issues", *Environmental Health Perspectives*, **107(7)**, pp.593-597.

[19] T. Yoshida, H. Yamauchi, S.G. Fan (2004), "Chronic health effects in people exposed to arsenic via the drinking water: dose-response relationships in review", *Toxicology and Applied Pharmacololy*, **198(3)**, pp.243-252.

[20] R. Sterner (2009), *Role of Zooplankton in Aquatic Ecosystems*, Encyclopedia of Inland Water, pp.678-688.

[21] L. Regaldo, U. Reno, S. Gervasio, H. Troiani, A.M. Gagneten (2013), "Effect of metals on *Daphnia magna* and cladocerans representatives of the Argentinean fluvial littoral", *Environmental Biology*, **35(4)**, pp.689-697.

[22] E.L. Enserink, M.J.J. Kerkhofs, C.A.M. Baltus, J.H. Koeman (1995), "Influence of food quantit and lead exposure on maturation in *Daphnia magna* evidence for a trade-off mechanism", *Functional Ecology*, **9(2)**, pp.175-185.

[23] C.S. Theegala, A.A. Suleiman, P.A. Carriere (2007), "Toxicity and biouptake of lead and arsenic by *Daphnia pulex*", *Environmental Science and Health*, **42(1)**, pp.27-31.

[24] G.S. Araujo, M.D. Pavlaki, A.M.V.M. Soares, D.M.S. Abessa, S. Loureiro (2019), "Bioaccumulation and morphological traits in a multi-generation test with two *Daphnia* species exposed to lead", *Chemosphere*, **219**, pp.636-644.

[25] S.H. Frisbie, R. Ortega, D.M. Maynard, B. Sarkar (2002), "The concentrations of arsennic and other toxic elements in Bangladesh's drinking water", *Environmental Health Perspectives*, **110(11)**, pp.1147-1153.

[26] Z. Vuković, M. Radenković, S.J. Stanković, D. Vuković (2011), "Distribution and accumulation of heavy metals in the water and sediments of the river Sava", *Journal of the Serbian Chemical Society*, **76(5)**, pp.795-803.

[27] Ministry of Natural Resources and Environment (2015), *National Technical Regulation QCVN 08-MT:2015/BTNMT dated December 21st, 2015 on surface water quality*.

[28] OECD guidelines for the testing of chemicals (1998), *Guideline 211: Daphnia magna Reproduction Test*.

[29] American Public Health Association, American Water Works Association and Water Environment Federation (2012), *Standard Methods for the Examination of Water and Wastewater (22nd Edition)*, Washington, DC.

[30] US Environmental Protection Agency (2002), *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (5th ed. EPA-821-R02-012)*, Washington, DC.

[31] T.S. Dao, V.N. Le, B.T. Bui, V.K. Dinh, W. Claudia, T.S. Nguyen, C.T. Dao, V.D. Nguyen, T.H. To, L.S.P. Nguyen, T.G. Vo, T.M.C. Vo (2017), "Sensity of tropical micro-crustacean (*Daphnia lumholtzi*) to trace metals tested in natural water of the Mekong river", *Science of Total Environment*, **574(1)**, pp.1360-1370.

[32] M. Grosell, C. Nielsen, A. Bianchini (2002), "Sodium turnover rate determines sensitivity to acute copper and silver exposure in freshwater animals", *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*, **133(1-2)**, pp.287-303.

[33] E.F. Pane, C. Smith, J.C. McGeer, C.M. Wood (2003), "Mechanisms of acute and chronic waterborne nickel toxicity in the freshwater cladoceran, *Daphnia magna*", *Environmental Science and Technology*, **37(19)**, pp.4382-4389.

[34] J.C. Amiard, C. Amiard-Triquet, S. Barka, J. Pellerin, P.S. Rainbow (2006), "Metallothioneins in aquatic invertebrates: their role in metal detoxification and their use as biomarkers", *Aquatic Toxicology*, **76(2)**, pp.160-202.

[35] K. Dinh Van, L. Janssen, S. Debecker, M. De Jonge, P. Lambret, V. Nilsson-Ortman, L. Beroets, R. Stocks (2013), "Susceptibility of a metal under global warming is shaped by thermal adaptation along a latitudinal gradient", *Global Change Biology*, **19(9)**, pp. 2625-2633.

[36] G.R. Reddy, M.R. Basha, C.B. Devi, A. Suresh, J.L. Baker, A. Shafeek, J. Heinz, C.S. Chetty (2003), "Lead induced effects on acetylcholinesterase activity in cerebellum and hippocampus of developing rat", *Int. J. Dev. Neurosci.*, **21(6)**, pp.347-352.

[37] S. Hernández-Flores, R. Rico-Martínez (2006), "Study of the effects of Pb and Hg toxicity using a chronic toxicity reproductive 5-day test with the freshwater rotifer *Lecane quadridentata*", *Environ. Toxicol.*, **21(5)**, pp.533-540.

[38] L. Janssens, K. Dinh Van, S. Debecker, L. Bervoets, R. Stoks (2014), "Local adaptation and the potential effects of a contaminant on predator avoidance and antipredator responses under global warming: a space-for-time substitution approach", *Evolutionary Applications*, **7(3)**, pp.421-430.

[39] M.C. Newman (2010), *Fundamentals of Ecotoxicology*, CRC Press, pp.214-218.

[40] M. Vaal, J.T. Van der Wal, J. Hermens, J. Hoekstra (1997), "Pattern analysis of the variation in the sensitive of aquatic species to toxicants", *Chemosphere*, **35(6)**, pp.1291-1309.

[41] L. Coniglio, R. Baudo (1989), "Life-tables of *Daphnia obtusa* (Kutz.) surviving exposure to toxic concentrations of chromium", *Hydrobiologia*, **188(1)**, pp.407-410.

[42] P.T. Tran, L.C. Do-Hong, T.S. Dao (2014), "Long-term impacts of copper and chromium on survivorship, maturation, fecundity and growth of *Daphnia magna*", *Vietnam J. Sci. and Tech.*, **52(3A)**, pp.309-315.