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Acute and Chronic Toxicity of Battery Waste Leachates to *Daphnia magna*

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Abstract
In developing countries, e-waste battery treatment and management is a severe problem. Most batteries contain heavy metals, but some can contain very toxic heavy metals such as mercury that can be hazardous to the environment. The current investigation aims to evaluate both the acute and chronic effects of the leachates from two cells (named Con O and Maxell) that are commonly used in Vietnam on the micro-crustacean *Daphnia magna*. The median lethal concentrations at 24h and 48h (LC_{50}) of the *D. magna* exposed to leachate from Con O cell were 150 and 100 mg/l, respectively. The 24h- and 48h-LC_{50} values of the Maxell cell to the animals were 100 and 70 mg/l, respectively. Therefore, the toxicity of the leachates from the Maxell battery was more severe than that of Con O based on the 24h- and 48h-LC_{50} values. Moreover, the life-history traits of the *D. magna* such as survivorship, maturation or reproduction, were detrimentally impacted including mass mortality, delayed maturation and reproductive inhibition, when the animals exposed to the leachates from both batteries at the concentrations ranged from 1 to 50 mg/l over the period of 2 weeks. Overall, this study could provide useful information on the ecological and environmental risk caused by untreated batteries to the environment, or even contribute to changing a sense of civic responsibility on economically using, recycling, waste management and treatment related to cells.

Keywords: Negative effects, Battery leachates, Life history traits, *Daphnia magna*

1. Introduction

Due to the development in the energy industry, the vast battery consumption (e.g., in portable electrical or electronic devices) along with short life span has resulted in the generation of a large number of spent batteries (Meshram, Abhilash, Pandey, Mankhand, & Deveci, 2016). For instance, according to the report on hazardous household waste generation in Japan (Yasuda & Tanaka, 2006), used batteries constituted about 52-71% of the household hazardous waste. Besides, their recovery is also tricky and not economically feasible as they are used in alloys with other metals such as iron or in low concentration (Peiro, Mendez, & Ayres, 2013). In developing countries, such as Vietnam, the used batteries are not well managed and treated. The e-waste batteries (e.g. in houses, offices, entertainment services, etc.) are put into the trash then end in a landfill. This would be a sink and source of pollutants leached out of the e-waste batteries to the environment. Seriously, almost batteries contain several materials (e.g., trace metal, plastics, see Table 1) that can be extremely harmful to human health and ecosystems if they are not treated suitably. Hence, the untreated or not adequately treated wastewater from the landfill would contain leachates from batteries and enter natural water of lakes, ponds, reservoirs, rivers. Theoretically, in water bodies, the pollutant-containing leachates at certain concentrations would impact living things in ecosystems such as zooplankton among others (e.g. phytoplankton, fish, mollusks, amphibians, mammals, aquatic macrophytes).

In the aquatic ecosystem, micro-crustaceans (e.g., *Daphnia magna*) play an essential role in the matter and energy fluxes (Sterner, 2009). Hence they are among the most vulnerable organisms to toxic chemicals in water bodies. Recently, there have been numerous investigations on the single effects on animals of significant components in batteries including trace metals (e.g., Pb, Zn) and plasticizers (e.g., Tris (2-butoxyethyl) phosphate, polyethylene terephthalate) (Araujo, Pavlaki, Soares, Abessa, & Loureiro, 2019; Giraudo et al., 2017; Luciana, Ulises, Susana, Horacio, & Maria, 2014; Wagner & Oehlmann, 2009). However, there is gap information on the detrimental impacts of untreated batteries on aquatic animals, especially zooplankton. Hence this study aims to evaluate the effects of spent batteries on the life-history traits of *Daphnia magna*.

Table 1. Zinc carbon battery composition (Fisher, Wallén, Laenen, & Collins, 2006).

Components	Percentage (%)
Iron & Steel	16.8
Manganese	15.0
Lead	0.1
Zinc	19.4
Other metals	0.8
Alkali	6.0
Carbon	9.2
Paper	0.7
Plastics	4.0
Water	12.3
Other nonmetals	15.2

2. Materials and Methods

2.1 Materials

In this experiment, two leachates from the used batteries (named Con O and Maxell batteries) were prepared. In Vietnam, these two batteries are common products and widely used in houses, offices as energy supplies for clockwatch, children toys, electric remote device controllers, cassette recorder, and services and entertainments. However, these batteries are not rechargeable and got unique collected, recycled and treated in Vietnam. Consequently, the used batteries have been mainly emitted into the environment as solid waste and ended up in landfills.

The preparing process for the battery leachates was similar to the natural digestion of those in the aquatic environment. Briefly, before the test, five cells of each type (either Con O or Maxell batteries) were weighed and separately placed in the 1-litre plastic bottle containing 250 ml of distilled water. The average weight of one Con O and one Maxell batteries was 14.21 g and 17.35 g, respectively. Afterwards, these bottles were placed outdoor under the sunlight over a period of 14 days. Therefore, the mother solutions of leachates from Con O and Maxell were around 280,000 mg/l and 340,000 mg/l, respectively. Finally, the leachates from the two kinds of electrochemical cells or mother solutions were utilized to test the toxicity to the micro-crustacean (*D. magna*).

Daphnia magna was obtained from Micro Biotest Company (Belgium) and has been maintained in the ISO medium for many generations under the laboratory conditions at the temperature of 25±1°C, the light intensity of 1,000 Lux, and photoperiod of 14 h light: 10 h dark (American Public Health Association [APHA], 2012). Besides, *D. magna* was fed with a mixture of green alga (*Chlorella* sp.) and YTC (yeast, cerrophyl and trout chow digestion) (U.S. Environmental Protection Agency [U.S. EPA], 2002).

2.2 Experimental setup

2.2.1 Acute toxicity of electrochemical cell leachates to *Daphnia magna*

Acute experiments were conducted following the guideline of APHA (2012) and the U.S. EPA (2002) with minor modifications. Firstly, fifty healthy adult female *D. magna* were randomly chosen and cultured in the 1-litre glass beaker containing 800 ml ISO medium. After one night, neonates (less than 24 h old) were randomly collected to prepare for acute experiments. The *D. magna* neonates were fed *ad libitum* with food (*Chlorella* sp. and YTC) two hours before the test. The experiment was implemented at 8 different concentrations including 0 (control), 50, 75, 100, 150, 200, 250 and 300 mg/l of each electrochemical cell leachates.

The pH values in each treatment including the control at the beginning and end of the test were measured, ranged from 6.3-7.3, that was similar with the requirement of APHA (2012) for the acute toxicity test. For each concentration, ten neonates were incubated in a 50 ml glass beaker containing 40 ml of ISO medium. There were four replicates (n = 4) in each treatment, and the test organisms were not fed during 48 h of the acute experiment. The survival of the test organisms was recorded after 24 h and 48 h of incubation. Dead organisms (defined as the heartbeat stop observed under a microscope) were counted and discarded. The survivorship of test organisms was recorded to determine the concentration of these leachates that killed 50% of the total test animals for 24 h and 48 h (24h- and 48h-LC_{50} values, respectively).

2.2.2 Chronic toxicity of electrochemical cell to *Daphnia magna*

Chronic experiments to evaluate the toxicity of leachates from batteries Con O and Maxell were conducted according to APHA (2012) and Adema (1978). The offspring (less than 24 h old) were also randomly collected and exposed to the leachate from Con O or Maxell battery at four distinct concentrations of 0 (control), 1, 5, and 50 mg/l. For each test concentration, we prepared at once 4 litres of a medium, ISO only (for the control) and ISO containing leachates (at 1, 5 and 50 mg/l of each battery, for exposures). The volume of 4 litres of the medium would be enough for one leachate-concentration test for 14 days of incubation, and to make sure that the leachate concentrations in each test would be constant.

The pH in each test solution remained between 6.2 and 7.3, which also fulfilled conditions for the validity of the chronic toxicity assessment (APHA, 2012). For each concentration, two neonates were incubated together in the 50 ml glass beaker containing 40 ml ISO medium under laboratory conditions as mentioned above, and there were ten replicates (n = 10) in each treatment. While the food (a mixture of *Chlorella* sp. and YTC) was added daily, the medium was totally renewed three times per week during 14 experimental days. The life-history traits of *D. magna,* including survivorship, maturation and reproductive performance, were carefully monitored and recorded daily.

2.3 Data treatment

By the end of the acute test, the Probit analysis program was applied to calculate the 24h- and 48h-LC_{50}. Besides, Kruskal-Wallis test (Sigma Plot 12.0 version) was applied for calculating the statistically significant difference of the maturation of *D. magna* between the control and exposures.

3. Results and Discussion

3.1 Acute toxicity of electrochemical cell leachates to *Daphnia magna*

By the end of the acute tests, 95% of total daphnids in control were still alive well, which was within the requirement of APHA (2012). Nonetheless, the survival proportion of organisms was decreased when exposing to the leachates of both batteries at the concentrations ranged from 50 to 300 mg/l. Particularly, regarding the exposure to the leachate of Con O on *D. magna*, 24h-LC_{50} and 48h-LC_{50} values were 150 (± 20) mg/l and 100 (± 15) mg/l, respectively. Compared to Con O battery, these parameters tested the leachate of Maxell battery were lower, 100 (± 17) mg/l and 70 (± 30) mg/l, respectively (Table 2). That means the toxicity of the leachate from Maxell battery could be more severe than that from Con O to *D. magna*.

Table 2. The 24h- and 48h- lethal concentration of *Daphnia magna* exposed to electrochemical cell leachates of Con O and Maxell.

Leachates from	**24h-LC_{50}** (mg/l)	**48h-LC_{50}** (mg/l)
Con O battery	150 ± 20	100 ± 15
Maxell battery	100 ± 17	70 ± 30

Additionally, acute toxicity values on *D. magna* tested with electrochemical cell leachates in the current study seemed to be lower than those of single metals used in the production of batteries (see Table 3). That could be explained by (i) using metallic compound at low concentration in the battery production (Peiro et al., 2013), (ii) the leaching efficiencies of metals used in manufacturing battery (Aaltonen, Peng, Wilson, & Lundstrom, 2017), and (iii) antagonistic interactions between metals due to a competition effect of one metal on the biotic ligand of another metal (Jho, An, & Nam, 2011; Versieren, Smets, De Schamphelaere, Blust, & Smolders, 2014). Moreover, in this study, we did not know exactly toxicants and their concentration in the two battery leachates. Hence, in order to gain insights into the toxicity of the leachates from the electrochemical cell, further chemical analyses (on chemical composition) are suggested.

Table 3. The 48h-LC_{50} values of *Daphnia magna* exposed to metals from previous studies.

Metals	**48h-LC_{50}** (mg/l)	**References**
Zn	819 - 928	Shaw, Dempsey, Chen, Hamilton, and Folt (2006); Traudt, Ranville, Smith, and Meyer (2016)
Pb	150 - 450	Biesinger and Christensen (1972); LeBlanc (1982)

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Mn	9,300 - 10,270	Biesinger and Christensen (1972); Rathore (2001); Okamoto, Yamamuro, and Tatarazako (2015)
Fe	2,920 - 2,300	Rathore (2001); Okamoto, Yamamuro, and Tatarazako (2015)

3.2 Chronic toxicity of electrochemical cell leachates to *Daphnia magna*

3.2.1 Survivorship of *Daphnia magna*

After two weeks, 85% of total daphnids in the control still actively lived, whereas none of the organisms cultured in the medium containing both leachates at the two high concentrations (5 and 50 mg/l) could be alive. Regarding the lowest concentration (1 mg/l), exposure to the leachates from Con O and Maxell electrochemical cells caused the decrease in the survival proportion of animals, by 20% and 40 %, respectively (Figure 1). It is hypothesized that the metals in the electrochemical cells negatively affected the adsorption capacity and the metabolisms leading to the death of test animals. Similarly, Muyssen, De Schamphelaere, and Janssen (2006) also formulated the hypothesis that chronic exposure to Zn inhibited Ca uptake, resulting in *D. magna* dead as a result of hypocalcemia. Beside Zn, leachates from batteries could contain other trace metals such as Fe, Mn, Pb contributing to the change the concentration of chemicals in organisms suddenly. Almost metals have a high affinity for protein binding sites resulting in their increased toxicity when they occur at relatively high concentrations in the environment (Barata, Baird, Nogueira, Soares, & Riva, 2006). There has been numerous evidence for the synergistic effects upon simultaneous exposure to multiple trace metals on an organism, especially Fe, Mn, Pb and Zn, which are commonly main compositions in batteries (Chu & Chow, 2002; Frías-Espericueta et al., 2008; Kim et al., 2009).

3.2.2 Maturation of *Daphnia magna*

As the batteries contain dozens of harmful chemicals, especially the metal components (see Table 1), not only the survivorship but also the maturation of the organisms could be influenced upon exposure to the electrochemical cell leachates. In the control, the test organisms took around five days to reach maturity. However, there was a statistically significant difference on the maturity age between the control and the exposures at the concentrations from 1 to 5 mg/l in which the exposed animals delayed their maturation, later than 5.5 days. Seriously, the organisms in the exposures at the highest concentrations (50 mg/l) could not mature, although they still lived to the ninth-day of the experiment (Figure 2). That was in line with the result of Ramirez (2014) in which the number of days needed for releasing the first brood increased by one day for metal (Zn, and mixture of Zn and Cd) acclimated animals compared to those in the control. Additionally, trace metals (e.g., Cu, Cr) were demonstrated to cause a delay in the maturation of *D. magna* (Ghazy & Habashy, 2003; Luciana et al., 2014; Nguyen, Vo, Dao, Quang, & Dao, 2016; Tran, Do-Hong, & Dao, 2014).

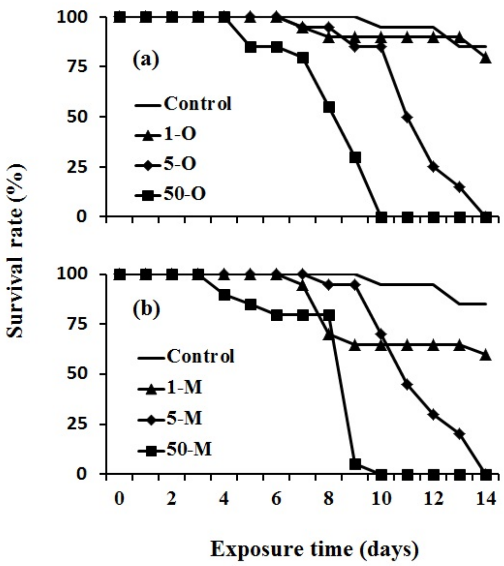

Figure 1. The survival rate of *Daphnia magna* exposed to electrochemical cell leachates of Con O (a), and Maxell batteries (b). 1-O, 5-O, and 50-O, medium containing leachate of 1, 5 and 50 mg/l, respectively, from Con O battery. 1-M, 5-M, and 50-M, medium containing leachate of 1, 5 and 50 mg/l, respectively, from Maxell battery.

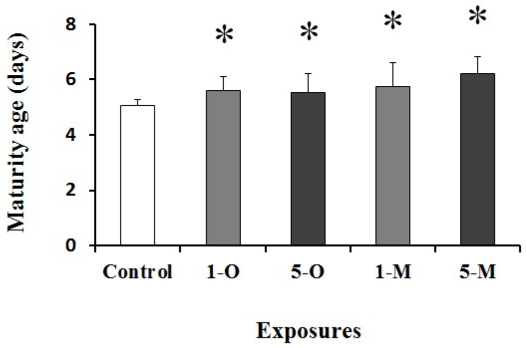

Figure 2. Maturation of *Daphnia magna* exposed to electrochemical cell leachates. The asterisk indicated the significant difference between control and exposures ($p < 0.01$, Kruskal-Wallis test). Abbreviations as in Figure 1.

3.2.3 Reproduction of *Daphnia magna*

According to Kooijman (2000), exposure to trace metals adversely affected the conversion of energy reserves and resources into the number of offspring, which was in agreement with the result in the current study. Additionally, delay in the maturation, as mentioned above, might be one of the main reasons for the reduction in the reproductive performance of *D. magna*. In particular, the total offsprings of *D. magna* in the control was 405 offsprings, whereas compared to the control, those in the exposures to both types of batteries at the lowest concentration (1 mg/l) decreased by more than 40%. Moreover, the reproductive performance of animals in the exposures to the leachates of Con O and Maxell at a concentration of 5 mg/l also reduced sharply by 68% and 74%, respectively. Regarding the organisms exposed to both leachates at the highest concentration (50 mg/l), they did not have any descendants (Table 4).

Table 4. Reproduction of *Daphnia magna* exposed to electrochemical cell leachates of Con O and Maxell.

	Control	Con O cell (mg/l)			Maxell cell (mg/l)		
		1	5	50	1	5	50
Total neonates	405	209	129	0	235	105	0
Proportion to control (%)	100	52	32	0	58	26	0

4. Conclusions

To our best knowledge, there have been few investigations on the impacts of the leachates from used batteries on the organisms. In terms of acute exposure, the leachate from the Maxell battery seems to have higher toxicity to *D. magna* than the other (Con O). The 24h and 48h (LC_{50}) values of the *D. magna* exposed to leachate from Con O cell were 150 and 100 mg/l, respectively. And, the 24h- and 48h-LC_{50} values of the Maxell cell to the animals were 100 and 70 mg/l, respectively. The adversely chronic effects of the leachate from two commonly used electrochemical cells (Con O and Maxell) on the life-history traits of the micro-crustacean *D. magna* were demonstrated obviously. High mortality rate, delayed maturation and reproductive inhibition of the animals exposed to the leachates were recorded in the chronic exposures. However, to fully evaluate the ecological toxicity of the leachate from cells, further studies (e.g. a chemical component of batteries in detail, effects of the leachate on the other organisms, the fate of untreated batteries in the aquatic or soil environment) should be highly suggested. Besides, investigations on the effects of the leachates on life-history traits of multigenerations of *D. magna* are suggested.

5. References

Aaltonen, M., Peng, C., Wilson, B. P., & Lundstrom, M. (2017). Leaching of metals from spent lithium-ion batteries. *Recycling*, *2*, 20.

Adema, D. M. M. (1978). *Daphnia magna* as a test animal in acute and chronic toxicity tests. *Hydrobiologia*, *59*(2), 125-134.

American Public Health Association. (2012). *Standard methods for the examination of water and wastewater* (22nd ed.). Washington, DC: American Water Works Association, Water Environment Federation.

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

Barata, C., Baird, D. J., Nogueira, A. J. A., Soares, A. M. V. M., & Riva, M. C. (2006). Toxicity of binary mixture of metals and pyrethroid insecticides to *Daphnia magna* Straus. Implications for multi-substance risks assessment. *Aquatic Toxicology*, *78*, 1-14.

Biesinger, K. E., & Christensen, G. M. (1972). Effects of various metals on survival, growth, reproduction and metabolism of *Daphnia magna*. *Journal of the Fisheries Research Board of Canada*, *29*, 1691-1700.

Chu, K. W., & Chow, K. L. (2002). Synergistic toxicity of multiple heavy metals is revealed by a biological assay using a nematode and its transgenic derivative. *Aquatic Toxicology*, *61*, 53-64.

Fisher, K., Wallén, E., Laenen, P. P., & Collins, M. (2006). *Battery Waste Management Life Cycle Assessment*. Environmental Resources Management. Retrieved from https://www.epbaeurope.net/wp-content/uploads/2016/12/090607_2006_Oct.pdf

Frías-Espericueta, M. G, Castro-Longoria, R., Barrón-Gallardo, G. J., Osuna-López, J. I., Abad-Rosales, S. M., Páez-Osuna, F., & Voltolina, D. (2008). Histological changes and survival of *Litopenaeus vannamei* juveniles with different copper concentrations. *Aquaculture*, *278*, 97-100.

Ghazy, M. M., & Habashy, M. M. (2003). Experimental toxicity of chromium to two freshwater crustaceans: *Daphnia magna* and *Macrobrachium rosenbergii*. *Egyptian Journal of Aquatic Biology and Fisheries*, *7*(3), 49-70.

Giraudo, M., Dube, M., Lepine, M., Gagnon, P., Douville, M., & Houde, M. (2017). Multigenerational effects evaluation of the flame retardant tris (2-butoxyethyl) phosphate (TBOEP) using *Daphnia magna*. *Aquatic Toxicology*, *190*, 142-149.

Jho, E. H., An, J., & Nam, K. (2011). Extended biotic ligand model for predictions of mixture toxicity of Cd and Pb using single metal toxicity data. *Environmental Toxicology and Chemistry*, *30*(7), 1697-1703.

Kim, H. Y., Lee, C. K., Lee, J. T., Moon, C. S., Ha, S. C., Kang, S. G., … Kang, M. G. (2009). Effects of manganese exposure on dopamine and prolactin production in rat. *Neuroreport*, *20*(1), 69-73.

Kooijman, S. A. L. M. (2000). *Dynamic energy and mass budgets in biological systems* (2nd ed.). Cambridge, United Kingdom: Cambridge University Press.

LeBlanc, G. A. (1982). Laboratory investigation into the development of resistance of *Daphnia magna* (Straus) to environmental pollutants. *Environmental Pollution Series A*, *27*(4), 309-322.

Luciana, R., Ulises, R., Susana, G., Horacio, T., & Maria, G. A. (2014). Effect of metals on *Daphnia magna* and cladocerans representatives of the Argentinean Fluvial Littoral. *Journal of Environmental Biology*, *35*(4), 689-697.

Meshram, P., Abhilash, Pandey, B. D., Mankhand, T. R., & Deveci, H. (2016). Acid baking of spent lithium ion batteries for selective recovery of major metals: A two-step process. *Journal of Industrial and Engineering Chemistry*, *43*, 117-126.

Muyssen, B. T. A., De Schamphelaere, K. A. C., & Janssen, C. R. (2006). Mechanisms of chronic waterborne Zn toxicity in *Daphnia magna*. *Aquatic Toxicology*, *77*(4), 393-401.

Nguyen, T. D., Vo, T. M. C, Dao, C. T., Quang, N. X., & Dao, T. S. (2016). Chronic effects of industrial wastewater on life history traits of *Daphnia magna* under the laboratory conditions. *International Journal of Agriculture and Environmental Research*, *2*(4), 1000-1012.

Okamoto, A., Yamamuro, M., & Tatarazako, N. (2015). Acute toxicity of 50 metals to *Daphnia magna*. *Journal of Applied Toxicology*, *35*(7), 824-830.

Peiro, L. T., Mendez, G. V., & Ayres, R. U. (2013). Lithium: Sources, production, uses, and recovery outlook. *The Journal of The Minerals, Metals & Materials Society*, *65*(8), 986-996.

Ramirez, D. C. S. (2014). *Tolerance of a metal adapted natural Daphnia magna population to new stressors* (Master's dissertation). Ghent University, Ghent, Belgium.

Rathore, R. S. (2001). *Studies on the use of some freshwater invertebrates as sensitive test models for the assessment of toxicity of environmental pollutants* (Doctoral dissertation). University of Lucknow, Lucknow, Uttar Pradesh, India.

Shaw, J. R., Dempsey, T. D., Chen, C. Y., Hamilton, J. W., & Folt, C. L. (2006).

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Comparative toxicity of cadmium, zinc, and mixtures of cadmium and zinc to daphnids. *Environmental Toxicology and Chemistry*, *25*(1), 182-189.

Sterner, R. W. (2009). Role of zooplankton in aquatic ecosystems. In *Encyclopedia of inland waters* (pp. 678-688). Oxford, UK: Elsevier.

Tran, P. T., Do-Hong, L. C., & Dao, T. S. (2014). Long-term impacts of copper and chromium on survivorship, maturation, fecundity and growth of *Daphnia magna*. *Vietnamese Journal of Science and Technology*, *52*, 309-315.

Traudt, E. M., Ranville, J. F., Smith, S. A., & Meyer, J. S. (2016). A test of the additivity of acute toxicity of binary-metal mixtures of Ni with Cd, Cu, and Zn to *Daphnia magna*, using the inflection point of the concentration-response curves. *Environmental Toxicology and Chemistry*, *35*(7), 1843-1851.

U.S. Environmental Protection Agency. (2002). *Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms* (5th ed.). Washington, DC: Author.

Versieren, L., Smets, E., De Schamphelaere, K., Blust, R., & Smolders, E. (2014). Mixture toxicity of copper and zinc to barley at low level effects can be described by the Biotic Ligand Model. *Plant and Soil*, *381*, 131-142.

Wagner, M., & Oehlmann, J. (2009). Endocrine disruptors in bottled mineral water: Total estrogenic burden and migration from plastic bottles. *Environmental Science and Pollution Research*, *16*(3), 278-286.

Yasuda, K., & Tanaka, M. (2006). Report on hazardous household waste generation in Japan. *Waste Management and Research*, *24*, 397-401.