Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(2): 675 – 683 (2024)
www.ejabf.journals.ekb.eg

The Acute Toxic Impact of Iron (Fe) and Lead (Pb) Individually and Their Mixture on *Daphnia magna* (Straus, 1820)

Mahassen, M. El-Deeb Ghazy[1], Madlen, M. Habashy[2], Marian, G. Nassif [2, *]

[1]Water Pollution Research Department, National Research Center, 12311 Dokki, Cairo, Egypt
[2]National Institute of Oceanography and Fisheries, NIOF, Egypt
***Corresponding Author: george.marian@hotmail.com**

ARTICLE INFO

Article History:
Received: Feb. 28, 2024
Accepted: March 23, 2024
Online: April 14, 2024

Keywords:
Toxicity,
Daphnia magna,
Heavy metals,
Fe,
Pb,
LC_{50}

ABSTRACT

One of the most crucial methods for understanding the state of any aquatic ecosystem is the use of test organisms such as fish or invertebrates. The water flea, *Daphnia magna*, is commonly used as a model species for ecotoxicological studies. In the current examination, *D. magna* neonates were exposed to concentrations of metals similar to those previously reported to occur in the ecosystem. The 48-h acute toxicity tests of $FeCl_3$, $PbCl_2$, and their mixture at different concentrations were applied to *D. magna*. The Probit analysis was used to calculate the 48-h median lethal concentration (LC_{50}) for each of Fe, Pb, and their mixture. The results revealed that Fe was more hazardous than Pb, with 48-h LC_{50} values of 6.47 and 13.14mg/ l, respectively. Furthermore, the Fe-Pb mixture had a synergetic impact on *Daphnia magna* (mixture 48-h LC_{50} was 3.9mg/ l). This study indicated that the realistic levels of Fe and Pb may trigger the lethal impacts of freshwater crustaceans, causing devastating impacts on the ecosystem and food web. These results may be valuable in elaborating water quality criteria and standards to protect the national aquatic systems that are drastically affected by anthropogenic industrial contamination. Because of the unexpected responses of multi-contaminant scenarios, there is a need for more thorough, environmentally appropriate research on the mixture impacts of the pollutants.

INTRODUCTION

In the 1940s, Anderson began using *Daphnia magna* in toxicity studies **(Anderson, 1944)**, and it has currently become a standard ecotoxicological species. Its large size, short life span, parthenogenetic reproduction, and high rate of reproduction made *D. magna* a model species for toxicity tests. Based on these data, *D. magna* is considered the most suitable species to study the toxicity of heavy metals and pharmaceuticals as well **(Mano & Shinohara, 2020; Tkaczyk *et al.*, 2021)**. Recently, **Yin *et al.* (2023)** reviewed the toxicological impacts of microplastics on *Daphnia* species. The toxicity tests on this species can be adapted to predict the implications of contaminants on the aquatic ecosystem. **Pashaei *et al.* (2023)** investigated *D.*

magna's acute exposure to caffeine, nanoplastics, triclosan, microplastics and their combinations. They discovered that when pollutant concentrations and exposure time increase, death rates increase.

As heavy metals are not biodegradable, they become hazardous at a certain level of concentration. Subsequently, they accumulate in the vital organs of the aquatic species, where they adversely affect their reproduction and growth. Consequently, they threaten human health via the food chain. As a result, heavy metals pose both ecological and health risks.

Cui *et al.* (2018) examined the impacts of 11 heavy metals on both *Daphnia magna* and *Daphnia galeata*. Their study revealed that *D. galeata* was more sensitive than *D. magna.* Moreover, they proposed *D. galeata* as a potential model for aquatic toxicity testing.

According to **Ahmed *et al*. (2019)**, iron bioaccumulation could occur via ingestion, respiration, and consumption which may lead to health risks to humans through the food web. **Shaw *et al.* (2006)** mentioned that iron and aluminum are commonly present in polluted water streams as a result of mining, steel procedures, and industrial drainage water. In their study, **Cui *et al.* (2018)** recorded the 48-h LC_{50} of Fe^{+2} on *D. galeata* to be 1.05mg/ l. However, there are very limited researches using ferric metal on *D. magna*; the current research applied the impacts of different concentrations of ferric chloride ($FeCl_3$) on *D. magna* as a new metal trend.

Lead is frequently associated with suspended matter in aquatic ecosystems, where it can enter any aquatic environment through runoff from streets, and industrial wastewater discharges. Lead is commonly found in the environment which has neurotoxic effects and once detected may be irreversible **(Andrade *et al.*, 2015)**. **Gooley *et al.* (2000)** and **Widiastuti *et al.* (2019)** pointed out that lead is a non-biodegradable heavy metal and extremely harmful to aquatic animals. Although its toxicity has already been established, its full ecotoxicological potential is still not fully described. **Paul *et al.* (2019)** investigated the impact of lead nitrate on common carp (*Cyprinus carpio*), and they concluded that the prolonged exposure to significantly high amounts of $Pb(NO_3)_2$ has major consequences for fish health. They found that 350mg/ l dose had critical issues on *C. carpio* histology.

Heys *et al.* (2016) mentioned that heavy metal interactions mostly increase the mortality rates in larvae, renal damage, embryonic toxicity, and sperm toxicity; however, some cases cause a decrease in expected toxicity. In nature, there are complex mixtures of chemicals that affect living organisms. Most researchers who aim to evaluate the environmental risk of chemical mixtures assume that the effect of mixtures of the chemicals can be forecasted just by the summation of the individual element toxicities. In some cases, the chemical reactions might change the toxicity of some mixtures, making them different from what would be predicted.

Since a concentration addition model is used in many experimental methods to predict mixture toxicity, interactions may have led to a considerable underestimation of the risk. This is particularly problematic, especially when the environmental mixtures are taken into account, which are frequently quite complex and composed of unidentified chemicals. Unfortunately, failure to forecast the negative impacts of the chemicals when released to the ecosystem, will lead to unpredicted detrimental impacts on the ecosystem. It is possible to observe a toxic effect, even though each separate compound is below its limit value. **Mebane *et al.* (2012)** conducted acute toxicity tests of Cd, Pb, and Zn, solely and in mixtures, on fish and invertebrates in the South Fork Coeur d'Alene River watershed, USA. In metal mixtures, the toxicities of the three metals were found to be less than additive according to a concentration-addition basis.

Although some researches have been conducted on the toxicity of certain heavy metals, the researches on Fe and Pb are very rare. Therefore, it is crucial to recognize and assess the potential undesirable effect of Fe and Pb on the aquatic ecosystem. Moreover, the impacts of Fe and Pb mixtures have not been studied before; therefore, their lethal impacts on crustaceans may be of an ecological interest. That is why this study is considered a novel trend in ecotoxicological studies in Egypt. Thus, the goals of this study were to determine the acute toxicity of Fe and Pb mixtures to *Daphnia magna* for 48-h of exposure and to compare these values with the impacts of each metal solely. In addition, The study investigated the probability of the presence of an interactive effect of this mixture (synergetic or antagonistic effect) or if these metals are confirmed to be standard toxicological prediction models (additive or independent action).

MATERIALS AND METHODS

Test species

The freshwater *D. magna* was successfully cultivated in synthetic media in the Laboratory of Hydrobiology at the National Research Center **(Fayed & Ghazy, 2000)**. Regularly, females were transferred to 1-L glass beakers, in which synthetic freshwater medium, with a pH range of 7.9- 8.3, total hardness of 90mg/ L as $CaCO_3$, alkalinity of 34mg/ L as CaCO3, and conductivity of 260µmhos/ cm. This process was renewed 3 times a week. *D. magna* were supplied with *Scenedesmus obliquus* (14x10^7 coenobia/ ml) three times/ week **(Ghazy, 1997)**. To keep the solution in a good condition, the algal culture was renovated every week. Throughout the experimental period, test animals were maintained at 22± 2°C with 16 hours of light per day.

Procedures

Ten neonates below 24 hours were tested in synthetic freshwater media in 250ml glass beakers for both the control group and all treatments. A heater thermostat kept the medium temperature at 22± 2ºC.

Acute toxicity experiment

In triplicates, groups of 10< 24h- old daphnid neonates were placed in 250ml beakers with 100ml medium and exposed to experimental circumstances for 48 hours. There was no food added during the test. After 48 hours, the number of live daphnids were recorded. The control was performed concurrently.

The metals used in the toxicity tests were Fe as ferric chloride ($FeCl_3$) and Pb as lead chloride ($PbCl_2$), with a purity of 99%. The concentrations of Fe tested were: 2, 4, 6, 8, 10, 12, and 14mg/ l, whereas the Pb concentrations were: 2, 4, 8, 12, 16, 20, and 24mg/ l for 48hr acute tests. For the Fe-Pb mixture experiment, series of concentrations equivalent to LC_1, LC_3, LC_7, LC_{10}, and LC_{15}, deduced from regression lines of both Fe and Pb toxicity tests on *D. magna* for 48h in a static system, were applied.

Statistical analysis

Probit analysis was applied for the 48-h LC_{50}s acute tests on *D. magna* using the SPSS program.

In toxicological investigations, mixed toxic effects are commonly analyzed using toxic units (TUs). TU is the chemical concentration of a mixture of toxins divided by its single toxic concentration for the endpoint measured **(Ishaque *et al.*, 2006)**. A linear additive index was applied to estimate the combined toxic impact of a mixture of Fe and Pb **(Marking, 1977)**, as expressed in the equation:

$$S = \frac{Am}{Ai} + \frac{Bm}{Bi} \quad \text{.................. Eq. (1)}$$

Where, A and B are the test metals;

i is the median lethal concentration (LC_{50}) of an individual metal;

m represents the median lethal concentration (LC_{50})of the metal mixture;

S represents the summation of the effective contributions, and can be modified using Equation 2 or 3. The additive index (AI) was applied to predict the type of combined effect that occurred. AI can be calculated using Equations 2 and 3.

$$AI = \left(\frac{1}{S}\right) - 1 \qquad (S<1) \text{ .... Eq. (2)}$$

$$AI = (-1)XS + 1 \qquad (S \geq 1) \text{ .... Eq. (3)}$$

If the cumulative toxic impact is additive, AI will be 0. If AI> 0; a synergistic impact is indicated. An antagonistic impact is indicated when AI< 0.

RESULTS AND DISCUSSION

Neonates of *D. magna* were exposed to different concentrations of Fe (2, 4, 6, 8, 10, 12, and 14mg/ l), and the calculated 48-h LC_{50} by Probit analysis was 6.47mg/ l (Table 1). **Santos-Medrano and Rico-Martínez (2013)** investigated the lethal impacts of some heavy metals on *Asplanchna brigthwellii* and *Brachionus calyciflorus*. They revealed that *B. calyciflorus* was more sensitive than *A. brigthwellii*, and Fe was the least toxic metal. The 24-h LC_{50} values for Fe were estimated at 0.35mg/ l for *A. brigthwellii* and 0.23mg/ l for *B. calyciflorus*. **Cui *et al.* (2018)** recorded the 48-h LC_{50} of Fe^{+2} on *D. galeata* as 1.05mg/ l. Hence, these results indicated that *A. brigthwellii*, *B. calyciflorus*, and *D. galeata* are more sensitive than *D. magna*.

In another experiment, the neonates were exposed to 2, 4, 8, 12, 16, 20 and 24mg/ l of Pb. As illustrated in Table (1), the 48-h LC_{50} was 13.14mg/ l. No mortality rate was observed in the control group, and individuals did not exhibit any abnormal behavior. These results disagree with those of **Le Blanc (1982)**, who recorded the 48-h LC_{50} for lead nitrate to *D. magna* as 0.15mg/ L. Furthermore, **Altında *et al.* (2008)** investigated the acute toxicity test of lead nitrate on *D. magna*, and they found the 24-h EC_{50} as 0.44mg/ l. This could be attributed to the higher tolerance of *D. magna* over time. In addition, **Theegala *et al.* (2007)** pointed out that, the 48h-LC_{50} of lead nitrate on *D. pulex* was 4mg/ l. **Bodar *et al.* (1989)** concluded that, early life stages of *D. magna* are more tolerant to heavy metals than adults, and that high concentrations of lead nitrate (1, 10, and 25mg/ L) had no evident impacts on the mortality rates of early life stages. **Gordillo *et al.* (1998)** found the 24h LC50 for lead nitrate as 4.92mg/ L for *D. magna*. According to **Santos-Medrano and Rico-Martínez (2013)**, the 24-h Pb LC_{50} values were 0.318 and 0.248mg/ l on *A. brigthwellii* and *B. calyciflorus*, respectively. Thus, these results revealed that rotifers (*A. brigthwellii* and *B. calyciflorus*) are more sensitive than the cladoceran *D. magna*. Although **Hernández-Flores *et al.* (2020)** concluded that Fe was the least toxic metal on *Euchlanis dilatata*, it seems that Fe has a higher toxicity effect on *D. magna* than Pb in the current acute toxicity exposure.

Mixtures of different concentrations of Fe and Pb were added to the neonates' media. Five different sublethal concentrations were prepared as follows: the concentration that kills 1% of neonates LC_1 of both Fe and Pb (0.38 and 1.06mg/ l, respectively), LC_3 of both of Fe and Pb (0.66 and 1.72mg/ l, respectively), LC_7 of Fe and Pb (1.08 and 2.66mg/ l, respectively), LC_{10} of Fe and Pb (1.37 and 3.28mg/ l, respectively), and LC_{15} of Fe and Pb (1.84 and 4.28, respectively). As shown in Table (1), the calculated median lethal concentration (48-h LC_{50}) of Fe and Pb mixture was 3.9mg/ l. Therefore, according to **Marking (1977)**, S= 0.89 (S< 1) and

AI= 0.12 (AI> 0), and that means adding Fe to Pb in the ecosystem has a synergistic effect on *D. magna* mortality rate. Fig. (1) illustrates the 48-h LC_{50} regression line of Fe-Pb mixture. Similar results were reported by **Piri and Ordag (1993)**, who examined the impact of titanium dioxide and nanoparticles mixture on *D. magna* and noticed that the mortality rate of pollution mixture was three times higher compared to the toxicity rate of each pollutant solely. **Chen *et al.* (2015)** agreed with this result as they recorded a synergetic effect of galaxolide (HHCB) and Pb mixture on *D. magna*. They also mentioned the significant impact of the absence and existence of suspended matters on the mixture's toxic effect. Consequently, while the concentration of one metal may not be hazardous, the existence of other chemicals may increase the toxicity percentage. **Gholami *et al.* (2013)** confirmed the same output, which revealed that toxicity rate and mortality rate had grown in a mixture of Cd and Cu compared to the effect of each metal individually.

Table 1. The 48-h lethal doses of Fe, Pb, and their mixture on *D. magna*

Metal	48-h LC_{10}	48-h LC_{50}	48-h LC_{90}
Fe	1.37	6.47	30.57
Pb	3.28	13.14	52.52
Fe and Pb Mixture	1.64	3.9	9.44

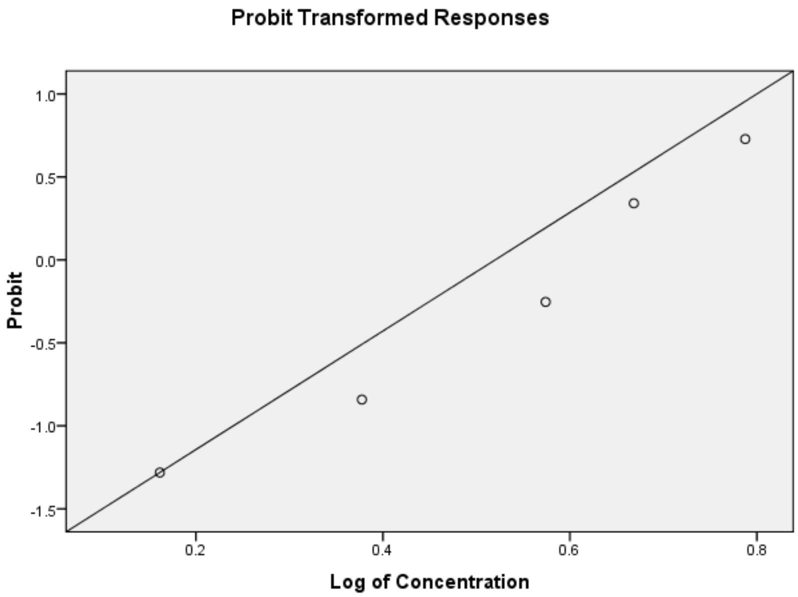

Fig. 1. The 48h-lethal concentration (LC_{50}) regression line of Fe-Pb mixture to *Daphnia magna*

CONCLUSION

The conducted study concludes that Fe, Pb, and their mixture can pose hazards to the aquatic ecosystem. Although Fe is more toxic to *D. magna* than Pb, the Fe-Pb mixture has more mortality impacts than each metal solely. The mortality rate in the mixture is three times higher than the Pb alone and almost two times higher compared to Fe's lethal concentration. Hence, more ecotoxicological studies of different metals are recommended in order to update heavy metals' standard limits. Further researches on mixture toxicology are needed to observe the synergetic and antagonistic impacts of various metals on each other.

REFERENCES

Ahmed, A.S.S.; Rahman, M.; Sultana, S.; Babu, S.M.O.F. and Sarker, M.S.I. (2019). Bioaccumulation and heavy metal concentration in tissues of some commercial fishes from the Meghna River Estuary in Bangladesh and human health implications. *Mar. Pollut. Bull*., 145: 436 – 447. https://doi.org/10.1016/j.marpolbul.2019.06.035.

Altında, A.; Ergönül, M.B.; Yigit, S. and Baykan, Ö. (2008). The acute toxicity of lead nitrate on *Daphnia magna* Straus. *Afr. J. Biotechnol.*, 7 (23): 4298-4300. http://www.academicjournals.org/AJB.

Anderson, B.G. (1944). The toxicity thresholds of various substances found in industrial wastes as determined by the use of *Daphnia magna. J. Sewage Works*., 16: 1156.

Andrade, V.L.; Mateus, M.L.; Batoréu, M.C.; Aschner, M. and Marreilha dos Santos, A.P. (2015). Lead, arsenic and manganese metal mixture exposures: focus on biomarkers of effect, *Biol. Trace Elem. Res*., 166 (1): 13–23. doi:10.1007/s12011-015-0267-x.

Bodar, C.; Wvd Zee, A.; Voogt, P.A.; Wynne, H. and Zandee, D. I. (1989). Toxicity of heavy metals to early life stages of *Daphnia magna*. *Ecotoxicol. and Environ. Safety*.

Chen, F.; Yao, Q. and Zhou, X. (2015). The Influence of Suspended Solids on the Combined Toxicity of Galaxolide and Lead to *Daphnia magna*. *Bull. Environ. Contam. Toxicol.*, 95: 73–79. DOI 10.1007/s00128-015-1543-3.

Cui, R.; Kwak, J. I. and An, Y. (2018). Comparative study of the sensitivity of *Daphnia galeata* and *Daphnia magna* to heavy metals. *Ecotoxicol. and Environ. Safety*., 162: 63- 70. https://doi.org/10.1016/j.ecoenv.2018.06.054.

Fayed, S. E. and Ghazy, M. M. (2000). Toxicity monitoring of water supplies using *Daphnia magna* Straus. *Pol. Arch. Hydrobiol.*, 4 (2): 171-188.

Ghazy, M. M. (1997). Aquatic fauna as a water quality monitoring device. Ph.D. Thesis, Faculty of Science. Cairo Univ., pp.: 169.

Gholami, M.; Reza Fatemi, S. M.; Fallahi, M.; Sari, A. E. and Mashinchian, A. (2013). The Individual and Mixed Effect of Heavy Metals (Cu and Cd) and Detergent (LAS) on Mortality of *Daphnia Magna* (Zooplankton). *Intern. J. Ecosystem*., 3(4): 90-93.

Gooley, G. J.; Gavine, F. M.; Dalton, W.; De Silva, S. S.; Bretherton, M. and Samblebe, M. (2000). Feasibility of aquaculture in dairy manufacturing wastewater to enhance environmental performance and offset costs. *Final Report DRDC Project* No. MAF001 Marine and Freshwater Resources Institute, Snobs Creek.

Gordillo, S.; Fernandez Pereira, A. C. and Vale Parapar, J. F. (1998). Acute ecotoxicity evaluation of heavy metals with *Daphnia magna*. *Ecotoxicol. Environ. Res.*, 1(3): 3-12.

Hernández-Flores, S.; Santos-Medrano, G. E.; Rubio-Franchini, I. and Rico-Martínez, R. (2020). Evaluation of bioconcentration and toxicity of five metals in the freshwater rotifer *Euchlanis dilatata* Ehrenberg, 1832. *Environ. Sci. and Poll. Res*., 27:14058–14069. https://doi.org/10.1007/s11356-020-07958-3.

Heys, K. A.; Shore, R. F.; Pereira, M. G.; Jones, K. C. and Martin, F. L. **(**2016). Risk assessment of environmental mixture effects. *Roy. Soci. Chem. Ad*., 6: 47844-47857.

Ishaque, A.B.; Johnson, L.; Gerald, T.; Boucaud, D.; Okoh, J. and Tchounwou, P.B. (2006). Assessment of individual and combined toxicities of four non-essential metals (As, Cd, Hg and Pb) in the microtox assay. *Int. J. Environ. Res. Public Health.*, 3(1): 118–120.

Le Blanc, G. A. (1982). Laboratory investigation into the development of resistance of *Daphnia magna* to environmental pollutants. *Environ. Poll*., 27: 309-322.

Mano, H. and Shinohara, N. (2020). Acute Toxicity of Nickel to *Daphnia magna*: Validation of Bioavailability Models in Japanese Rivers. *Water Air Soil Poll*., 231: 459. https://doi.org/10.1007/s11270-020-04842-1.

Marking, L. L. (1977). Method for assessing additive toxicity of chemical mixtures. In: Mayer F. L., Hamelink J. L. (eds) *Aquatic toxicology and hazard evaluation*. 634. American *Society for Testing and Materials, Philadelphia*, PA, pp. 99–108. Doi: 10. 1520/STP32392S.

Mebane, C. A.; Dillon, F. S. and Hennessy, D. P. (2012). Acute Toxicity of Cadmium, Lead, Zinc, and their Mixturesto Stream-Resident Fish and Invertebrates. *Environ. Toxicol. Chem*., 31(6): 1334–1348. https://doi.org/10.1002/etc.1820

Pashaei, R.; Dzingelevičienė, R.; Putna-Nimane, I.; Overlinge, D.; Błaszczyk, A. and Walker, T. R. (2023). Acute toxicity of triclosan, caffeine, nanoplastics, microplastics, and their mixtures on Daphnia magna. *Marine Pollution Bulletin*, *192*: 115113.

Paul, S.; Mandal, A.; Bhattacharjee, P.; Chakraborty, S.; Paul, R. and Mukhopadhyay, B. K. (2019). Evaluation of water quality and toxicity after exposure of lead nitrate infresh water fish, major source of water pollution. *Egypt. J. Aquat. Res*., 45(4): 345–351. https://doi.org/10.1016/j.ejar.2019.09.001.

Piri, Z. M. and ordag, V. (1993). Effect of some herbicides commonly used in Iran on selenastrum capricomutum and *Daphnia magna*, (*MSc. Thesis to the University of Agricultural Sciences Hungary*).

Santos-Medrano, G. E. and Rico-Martínez, R. (2013). Lethal effects of five metals on the freshwater rotifers *Asplanchna brigthwellii* and *Brachionus calyciflorus*. *Hidrobiol*., 23 (1): 82-86. https://doi.org/10.4161/epi.24362.

Shaw, J. R.; Dempsey, T. D.; Chen, C. Y.; Hamilton, J. W. and Fol, C. L. (2006). Comparative toxicity of cadmium, zinc and mixtures of cadmium and zinc to daphnids. *Environ. Toxicol. Chem.*, 25: 182–189.

Theegala, C. S.; Suleiman, A. A. and Carriere, P. A. (2007). Toxicity and biouptake of lead and arsenic by *Daphnia pulex*. *J. Environ. Sci. Health*., 42 (1): 27-31.

Tkaczyk, A.; Bownik, A.; Dudka, J.; Kowal, K. and Ślaska, B. (2021). *Daphnia magna* model in the toxicity assessment of pharmaceuticals: A review. *Sci. The Total Environ*., 763: 143038. https://doi.org/10.1016/j.scitotenv.2020.143038

Widiastuti, I. M.; Hertika, A. M. S.; Musa, M. and Arfiati, D. (2019). Acute toxicity test and LC50 value of mercury on *tubifex tubifex*. *J. Physics*: Conf. Series 1242 (2019). doi:10.1088/1742-6596/1242/1/012040

Yin, J.; Long, Y.; Xiao, W.; Liu, D.; Tian, Q.; Li, Y.; Liu, C.; Chen, L. and Pan, Y. (2023). Ecotoxicology of microplastics in *Daphnia*: A review focusing on microplastic properties and multiscale attributes of *Daphnia*. *Ecotoxicol. and Environ. Safety*, 249: 114433. https://doi.org/10.1016/j.ecoenv.2022.114433