Assessment of the Toxicity of Microplastic Fibres on Lymnaea Stagnalis

Abstract

The presence of microplastics (particles of the size smaller than 5 mm) poses a threat in aquatic ecosystems all around the world. Due to their small size, the particles are bioavailable and thus can be taken up by organisms at lower trophic levels. This can lead to a transfer of plastics to higher trophic levels until they reach humans. A big amount of those plastics is of fibrous shape shed during the washing of synthetic clothes and can enter the aquatic system via wastewater treatment plants. It is found that polyester fibres make up the biggest amount of those fibres.

To assess the toxicity of microplastic fibres on Lymnaea stagnalis snails, a laboratory toxicity experiment with a duration of 28 days was conducted. The snails were exposed to different concentrations of two different sizes of PET fibres. As endpoints mortality and growth rate of the snails' shell were measured. Snails are considered to be well suited for this kind of toxicity test because they serve as food source for many other organisms which makes them a good indicator for the health of an aquatic ecosystem. The results regarding the inhibition of the growth of the snails suggest a relation between inhibition and concentration for both size fractions tested; however, the results are not conclusive. Furthermore, no trend regarding the correlation between the mortality of the snails and the fibre concentration is visible. The results suggest that PET fibres could potentially exhibit an adverse effect towards small aquatic organisms. Nevertheless, further research is needed to determine the actual toxic effect of PET fibres on aquatic organisms.

Keywords: **ecotoxicological test, Lymnaea stagnalis, microplastic, polyester fibres**

Introduction

The aquatic ecosystem including both inland waters and oceans, but also polar regions, are threatened by the presence of microplastics [1] (plastic particles smaller than 5 mm in length) [2]. Despite a variety of sources of microplastics, there is an abundance of fibrous-shaped particles found in the marine environment [2].

Those microplastic fibres are released from textiles in washing machines [3] and usually enter the ocean after passing through wastewater treatment plants [4]. The dominant fibre for clothing on the market nowadays with still increasing numbers is polyester [3]. Different conditions influence the quantity of microplastic fibres emitted from textiles in washing machines [3]. As to how much, there are still different opinions found in literature. According to Carney Almroth et al. 2017, polyester fleece in particular, sheds the most in comparison to other knit fabrics [5]. But not only the type of fibre matters, a difference between worn clothes to new ones can also be observed [3].

Once found in the environment, due to their small size, microplastics can be easily ingested by different aquatic organisms, such as zooplankton, fish and whales [1]. The knowledge about the impact of size and concentration of fibres on aquatic organisms is essential for further development of knit techniques and additional filters in wastewater treatment plants or washing machines. Lymnaea stagnalis are the organisms of choice for an ecotoxicological test since they are key to the health of many aquatic systems because they are part of the diet of many other aquatic animals [6]. Furthermore, due to the uptake of microplastic by lower trophic organisms like snails, the particles can be transferred to higher trophic

levels [2]. Blue mussels and beer can, for example, contain microplastic fibres [5]. In order to better understand the damage caused by microplastic fibres and to find solutions preventing unacceptable negative outcomes, ecotoxicological tests like the one presented below are necessary. Especially, since most studies focus on round shaped particles in the ocean and only few approach the effect of fibrous shaped plastics [5] in freshwater systems.

The following assesses the toxicity of a concentration range of polyester fibres of two different sizes of PET fibres on Lymnaea stagnalis snails. To test the toxicity of microplastic fibres on Lymnaea stagnalis, a 28-day ecotoxicological test was performed. The toxicity is measured by ascertaining the mortality rate and the growth of the snails' shell length subjected to plastic particles during the exposure period compared to snails which are not exposed to microplastic. The endpoints mentioned above are being analysed for correlations between the concentration and/or the size of microplastic fibres.

Methodology

Test set-up

In order to assess the toxicity of textile microplastic fibres shedding during the washing of polyester clothes towards Lymnaea stagnalis snails, an ecotoxicological test for a period of 28 days is performed. The key points of the course of actions taken include the application of PET fibre stock solutions with different concentration and the renewing of the solutions each week. At the end of the exposure period, the endpoints of the experiment are measured. The snails are kept in glass beakers of 500 ml at 20 °C with a light-dark cycle of 16/8 hours with five snails per beaker, to ensure close-to-nature conditions. Throughout the duration of the test, the snails are fed with biofilm and microplastic fibres of different sizes and concentrations to test the relevance of size and concentration respectively. To keep the concentration of the fibres and the amount of food at the stable throughout the duration of the test, the content of the beakers is renewed every seven days. Additionally, apart from the different concentrations there is one control that does not contain any fibres and serves as reference for the endpoints of the toxicity test. Each combination of size and concentration as well as the controls are tested with five replicas to ensure the significance and reproducibility of the results. This sums up to 25 snails per concentration and 225 snails in total.

At the end of the exposure period, the endpoints are measured and analysed to make a conclusion about the toxicity of microplastic fibres on Lymnaea stagnalis snails. These endpoints are the mortality and the length of the snails' shell. The mortality after 28 days of each concentration can then be compared to the control and the two sizes of PET fibres to see if there is a correlation between concentration and mortality or size and mortality. At the beginning of the exposure period and at the end the length of each snail is measured. Therefore, the growth of the snails can then as well be compared between the different concentrations and sizes and the control. This comparison provides information on the possible inhibition of shell growth caused by microplastic fibres due to the decreased intake of nutrients that is expected.

Test substance

The test substance consists of PET fibres in different concentrations and sizes homogenized in Borgmann medium. PET is chosen for this experiment because it accounts for the biggest amount of synthetic fibre on the market and the demand still increases. The test concentrations are c1 = 50 mg/L, c2 = 25 mg/L, c3 = 10 mg/L and c4 = 1 mg/L plus a control without any microplastic fibres. The test sizes are 150 µm to 500 µm and 5 µm - 50 µm. Those two size fractions are chosen because they are well differentiable and could provide a good analysis of the data regarding different results of the two size fractions. Every concentration of each size and the control are tested with five replicas to obtain significant and reproducible results.

The fibres originate from white polyester clothes are marked with fluorescent dyes for a better detectability under the microscope. Two women fleece jackets in size XL made of 100 % polyester from Engelbert Strauss are washed with a front loader washing machine with the easy-care program, which is often used for domestic washing at 40 °C with 1200 rounds per minute for 90 minutes. Furthermore, the clothes are washed without any detergents or conditioners to ensure that all effects observed arise only from the microplastics. The effluent of the washing machine is filtered through a filter cascade consisting of meshes with different sizes to separate the fibres into the required sizes.

Test organism

For this toxicity test Lymnaea stagnalis snails are used. The snails in the experiment were raised in the laboratory of the Institute of Limnology, TU Dresden from batches of eggs. The culture medium (Borgmann medium) was exchanged twice a week. For further nutrients, cucumber slices were added to the water. The snails were kept in a water tank connected to a pump for the oxygen supply. At the beginning of the experiment, the snails were three to four months old.

The size of the 225 snails used in this experiment ranges from 0.3 cm to 1.3 cm with an average value of 0.6 cm at the time of application. A Vernier calliper was used to measure the size from the apex of the shell to the top with an accuracy of ± 0.05 mm.

Test conditions

The snails were kept in beakers filled with a mixture of biofilm and culture medium. As culture medium, Borgmann medium is used. This mixture provides perfect conditions for Lymnaea stagnalis to live, with enough nutrients to ensure the validity of the toxicity test and to simulate conditions found in naturals environments.

The biofilm is collected from rocks in the Gauernitzbach, Dresden. With brushes, the aufwuchs is scraped of the rocks of the stream and is then filtered through a 250 µm mesh to remove particles and small animals that would feed on the biofilm.

First, a solution of Borgmann medium and biofilm is prepared. Then, 500 ml of the solution is filled in each of the 55 beakers and left to settle for four days. Afterwards, the supernatant liquid is poured off, leaving the biofilm stuck to the bottom. The solution of different sizes and concentrations of fibres was prepared in 500 ml of Borgmann medium and then poured into the beakers that contained a thin layer of biofilm. The new mixture has time to settle for another day till the snails are inserted. This is important because Lymnaea stagnalis are grazers and feed on materials settled to the bottom. The same procedure is repeated every seven days to keep the concentration of the fibres stable and to provide the snails with enough food.

To prevent contamination from air and to prevent the snails from escaping, each beaker is covered with a net. The beakers containing the snails are then placed in a plant growth chamber that ensures close-to-nature conditions with a light-dark cycle of 16/8 hours at 20 °C ±1 °C. To provide the snails with enough oxygen, each beaker is connected to an oxygen pump.

Every week three parameters are measured to make sure that each beaker provides the same conditions for the snails. Those parameters include the conductivity (κ), pH-value and the oxygen level (c(O2)). All parameters lie within the range found in the natural habitat [7].

Results

Length

The effect of polyester fibres on the growth of Lymnaea stagnalis is determined by analysing the average inhibition of shell growth of the common pond snails of each concentration. Firstly, the difference of the average size of each beaker at T0 and T28 is calculated. This gives the average growth of the snails

in each beaker. In order to get the growth rate, the average growth of the individuals in each beaker is divided by the number of exposure days (28). The same is applied to the controls. At this point, the growth rate of each replica can be divided by the average growth rate of all controls which gives the response. The counterparts of these percentages determine the inhibition of growth of each concentration.

Even though the graphs for both size fractions show a linear trend the data is not conclusive. The trend is shown for the bigger size fraction in Figure 1; however, the smaller size fraction shows similar results with a slightly lower slope.

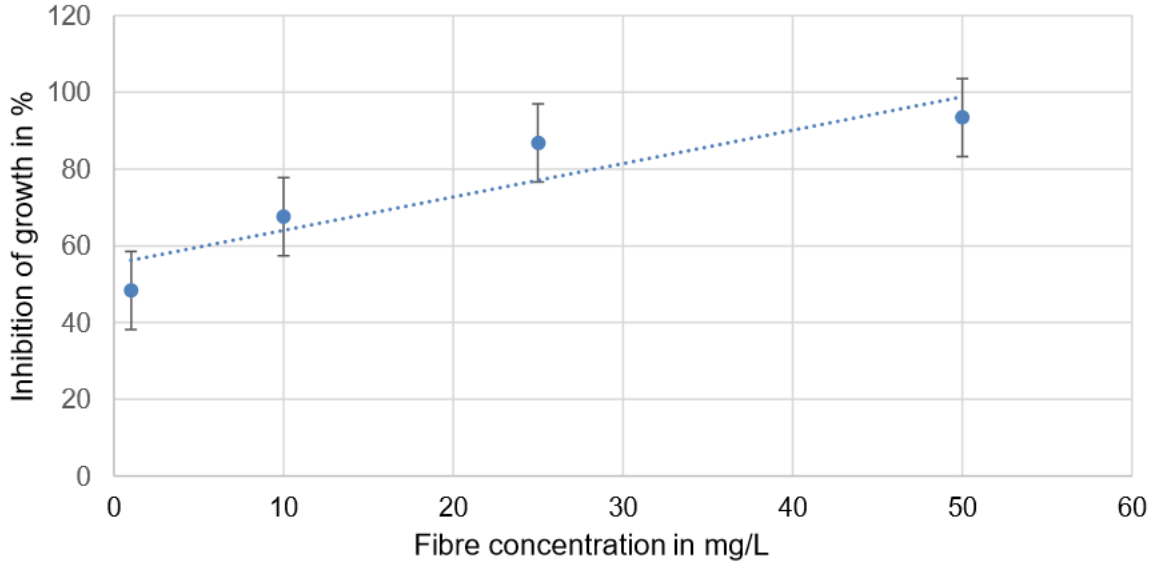

Figure 1: Inhibition of the growth of the snails exposed to the size fraction of PET fibres 150 – 500 µm. Each dot represents the average shell inhibition of the snails tested in each concentration (n=5) Error bars represent standard deviations of the data.

A linear regression line not starting in point zero does not appear to be reasonable when talking about toxicity because it would suggest a jump of the toxic effect with just a minor amount of test substance. An exponential regression is far more likely with the given data; however, no statement can be made due to missing data with lower concentration. The results regarding the inhibition of growth still imply a possible effect of polyester fibres on the snails.

Mortality

The mortality of each week as well as the total sum of dead snails throughout the whole duration of the experiment seems to be totally random for both size fractions with no trend visible. The same applies for the missing snails, where no pattern is visible. The results of the 5 – 50 µm PET fibres are shown in Figure 2. The bigger size fraction shows similar results.

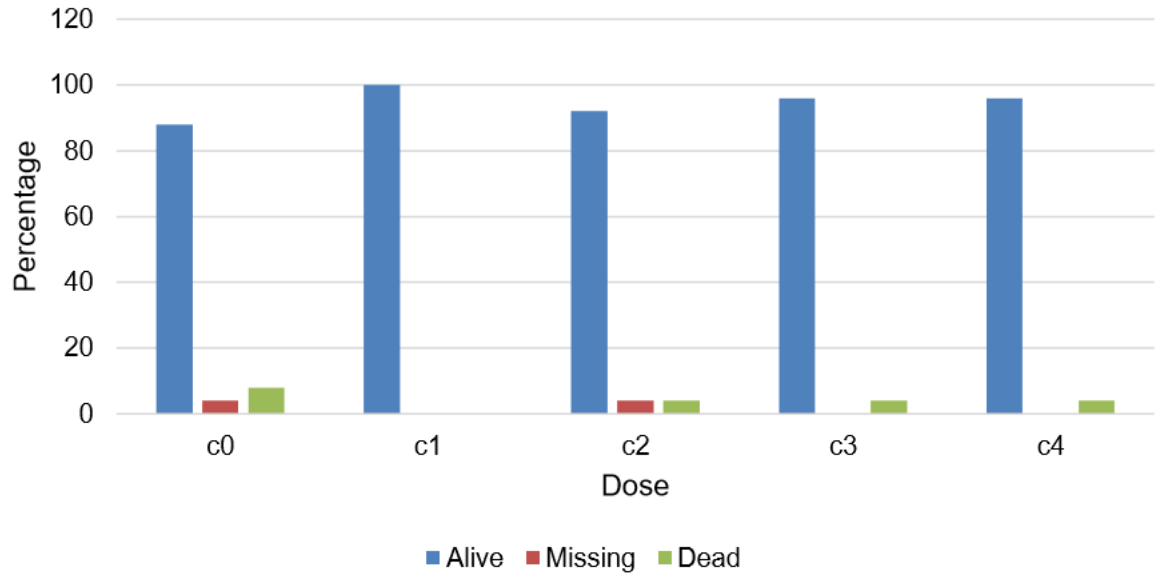

Figure 2: Percentage of the alive, dead and missing snails after the exposure period exposed to the size fraction 5 – 50 µm (T=28 days).

The analysis of the mortality of the common pond snails results in no correlation between mortality and concentration neither for PET 5 - 50 µm nor for PET 150 - 500 µm. This means that the mortality of the snails during the 28 days of exposure is statistically not affected by any of the tested concentrations of microplastic.

Conclusion

Microplastics are present in the aquatic environment with a high abundance of fibrous shapes [2]. Since polyester is the plastic type with the highest demand of all synthetic fibres [3], aquatic organisms like snails are likely to encounter those addressed particles in their natural environment. Therefore, ecotoxicological tests are needed to estimate their effect on organisms of lower trophic levels, because once up taken, microplastics can be transferred to higher levels possibly including in animals for human consumption [5].

In the study presented no conclusive statement about the inhibition of growth of PET fibres of the size fractions 5 - 50 µm and 150 – 500 µm on Lymnaea stagnalis after 28 days of exposure can be made; however; a possible negative effect is implied but further studies with lower concentrations are necessary to support this implication. Similar studies support the hypothesis of a toxic effect. For example, the crab Carcinus maenas has been reported to show an effect after the exposure to microplastic fibres of the size 1 mm to 5 mm which made up for only 1 % of the diet during the same amount of time [8]. An inhibited body growth in relation to increasing concentrations of PET fibres was also determined during an experiment of 8 days on Ceriodaphnia dubia, testing six different concentrations with up to 1,000 µg/L of fibres with a majority of the size being 100 µm to 400 µm, although no fibres were found in the body of the organism [9]. This result suggests that the inhibition of growth might also be caused by stress induced by the presence of the particles rather than the actual uptake [9].

The mortality shows no trend which indicates there is no correlation between mortality and concentration of PET fibres. However, the results of this study come in disagreement with a different research on acute toxicity of polyester fibres on Ceriodaphnia dubia which shows an increased mortality with increasing concentrations of fibres within 48 hours of exposure, with concentration ranging between 0.125 mg/L and 4 mg/L [9].

Further research should evaluate the influence of the duration and the number of test organisms. The duration of the test as well as the number of replicas and the organisms per beaker can affect the outcome of an ecotoxicological test [10]. For instance, in case it is a matter of time-dependent effects, short duration will not show an effect, but this does not mean that there is no effect in the long run. Furthermore, lower concentrations of PET fibres need to be tested.

Reverences

[1] H. S. Auta, C. U. Emenike, and S. H. Fauziah, 'Distribution and importance of microplastik in the marine environment:of the review of the sources, fate, effects, and potential solutions.', *Environ. Int.*, vol. 102, pp. 165–176, 2017.

[2] S. L. Wright, R. C. Thompson, and T. S. Galloway, 'The physical impacts of microplastics on marine organisms: A review.', vol. 178, pp. 483–492, 2013.

[3] L. Åström, 'Shedding of synthetic microfibers from textiles', Master Thesis, University of Gothenburg, Gothenburg, 2016.

[4] K. Magnusson and C. Wahlber, 'Mikroskopiska skräppartiklar i vatten från avloppsreningsverk.', *IVL Sven. Miljöinstitutet*, vol. nr B, p. 2208, 2014.

[5] B. Carney Almroth, L. Astrom, S. Roslund, H. Petersson, M. Johansson, and N.-K. Petersson, 'Quantifying shedding of synthetic fibers from textiles; a source of microplastics released into the environment.', pp. 1191–1199, 2017.

[6] J. Amorim *et al.*, 'Lymnaea stagnalis as a freshwater model invertebrate for ecotoxicological studies.', *Sci. Total Environ.*, vol. 669, pp. 11–28, 2019.

[7] M. Masius, T. Noe, and N. Weinkauf, 'Chemische Gewässeruntersuchung und Bestimmung der chemischen Gewässergüteklasse nach Bach am Beispiel Wingelsbach', Bericht der Arbeitsgruppe Chemische Gewässeruntersuchung.

[8] T. Gordon, C. Reisser, C. Soyez, V. Quillen, and G. Le Moullac, 'Microplastics Affect Energy Balance and Gametogenesis in the Pearl Oyster Pinctada margaritifera', *Environ. Sci. Technol.*, vol. 52, pp. 5277–5286, 2018.

[9] S. Ziajahormi, K. Anupama, P. A. Neale, and F. D. L. Leusch, 'Impact of Microplastic Beads and Fibers on Waterflea (Ceriodaphnia dubia) Survival, Growth, and Reproduction: Implications of Single and Mixture Exposures', *Environ. Sci.*, vol. 51, pp. 13397–13406, 2017.

[10] T. Sparks, *Statistics in ecotoxicology.* Chichster; New York: Wiley, 2000.