Toxic effects of fluoxetine-loaded onto virgin or aged polypropylene, polyamide and polyvinyl chloride microparticles on *Daphnia magna*.

MOURA, D.S., PESTANA, C.J., MOFFAT, C.F., GKOULEMANI, N., HUI, J., IRVINE, J.T.S. and LAWTON, L.A.

2025

Journal of Hazardous Materials 489 (2025) 137645

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Toxic effects of fluoxetine-loaded onto virgin or aged polypropylene, polyamide and polyvinyl chloride microparticles on *Daphnia magna*

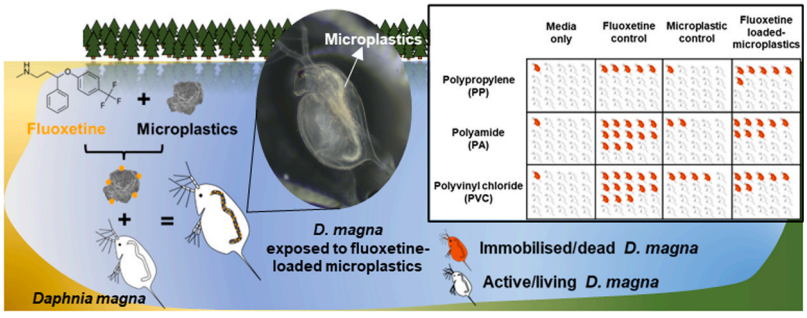
Diana S. Moura [a,*], Carlos J. Pestana [a], Colin F. Moffat [a], Nikoletta Gkoulemani [b], Jianing Hui [b], John T.S. Irvine [b], Linda A. Lawton [a]

[a] School of Pharmacy and Life Sciences, Robert Gordon University, Aberdeen AB10 7GJ, UK
[b] School of Chemistry, University of St Andrews, North Haugh, St Andrews, Scotland KY16 9ST, UK

HIGHLIGHTS

- Toxicity of fluoxetine loaded microplastics on *Daphnia magna* was investigated.
- Fluoxetine-loaded microplastics showed hazardous effects on *D. magna*.
- Microplastics alone had an insignificant impact on *D. magna* survival.
- Fluoxetine-loaded microplastic toxicity was condition (virgin and aged) and polymer-type dependent.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Microplastics
Food-web
Toxicity
Plastic pollution
Freshwater

ABSTRACT

There is an increasing recognition that microplastics can act as a vector for micropollutants when co-occurring in the environment and that pollutant-loaded microplastics can become integral to food-webs. To evaluate whether fluoxetine-loaded microplastics can act as a vector for fluoxetine to enter the food chain, a toxicity assay with *Daphnia magna* neonates was performed. This study evaluated the fluoxetine availability when adsorbed onto virgin or aged polypropylene, polyamide, and polyvinyl chloride (PVC). Results demonstrated that fluoxetine-loaded microplastics displayed toxic effects for all microplastic types, with varying toxicity depending on plastic type and weathering. *D. magna* ingested microplastics in all experiments that microplastics were present, but survival rates were not significantly affected by microplastics alone. Neonate mortality did not correlate with the adsorption/desorption capacity of the microplastics. Fluoxetine showed the highest adsorption on virgin and aged polypropylene (83–98 %), followed by aged polyamide (25–68 %) and PVC (38–90 %). While negligible desorption occurred with polypropylene, polyamide and PVC exhibited up to 20 % desorption. However, higher mortality was observed with fluoxetine-loaded virgin polypropylene (30 %), polyamide (40 %), and PVC (35 %) compared to aged particles (0–10 %). The results indicate that microplastic can enter the food-chain and act as a vector for pollutants, exhibiting hazardous effects to wildlife.

* Corresponding author.
E-mail address: d.souza-moura1@rgu.ac.uk (D.S. Moura).

https://doi.org/10.1016/j.jhazmat.2025.137645
Received 13 December 2024; Received in revised form 29 January 2025; Accepted 15 February 2025
Available online 17 February 2025

1. Introduction

Microplastics are widely detected in the environment. The ingestion of plastic particles by wildlife occurs when they are present in prey species or co-occur in the environment. A variety of animals across all trophic levels in the food-web have been shown to ingest microplastics. For instance, five groups of zooplankton, copepods, chaetognaths, jellyfish, shrimp, and fish larvae, collected from the northern South China Sea were found to have ingested microplastics, mainly polyester [1]. Microplastics were found in the gastrointestinal tract of three fish species (317 individuals) from the South-Eastern Black Sea coast of Turkey [2]. Microplastics were also found in the stomach and intestines of stranded, bycaught, or mercy-killed seals found at the coast of the federal state of Schleswig-Holstein in Germany [3]. Although the ingestion of microplastics is now widely acknowledged, the impact of the ingestion of microplastics on wildlife is poorly understood. Studies have investigated how the ingestion of microplastic effects food uptake, mobility, and survival of various organisms [4,5]. However, the study of vertebrates for scientific purposes poses difficulties, including ethical issues. For this reason, the zooplankton *Daphnia* spp. (crustacea) has been widely used in the research field to evaluate how compounds can impact wildlife [6–8]. *Daphnia* spp., more specifically *D. magna,* is the most commonly used organism in the study and control of water quality and is also a sensitive freshwater organism. *D. magna* is classified as a water quality indicator organism by international bodies such as European Union's Water Framework Directive, United States Environmental Protection Agency and the wider scientific community [9,10]. *D. magna* was found to be sensitive and useful for detecting water quality changes and how these alter the function of keystone organisms [11]. The ingestion of plastic particles seems to have differing effects on *D. magna* survival. For instance, polyethylene (PE) fragments of approximately 17 μm and 35 μm exhibited chronic toxicity to *D. magna* [12]. However, Canniff and Hoang [13] showed that despite the fact that *D. magna* were able to ingest PE microbeads of between 63 and 75 μm diameter, there was no effect on survival and reproduction [13]. Studies have been conducted using a single type of non-weathered particles, including polystyrene (PS) beads, to evaluate the toxic effects of microplastics on aquatic organisms [14]. However, perfectly spherically-shaped, weathered and non-weathered microplastics only represent approximately 14 % of the microplastic type reported in the freshwater environment [15]. While plastic beads may lack environmental relevance on the basis of the type and shape of microplastics found in water systems, studies using such beads also potentially underestimate the toxicity of microplastics on *D. magna*. Studies have reported greater toxicity of fragments of plastic to aquatic organisms such as *D. magna* when compared to plastic beads [12,16]. Furthermore, when in the environment, microplastics undergo degradation processes such as photo- and thermal-oxidation [17]. Therefore, weathered microplastics are representative of a proportion of the microplastics found in the environment. Allied to this, microplastics are one of many pollutants found in freshwater systems. For instance, pharmaceuticals have been widely detected in freshwater ecosystems across the world [18]. The antidepressant fluoxetine is in the top 20 North American compounds found in freshwater systems, causing concern as it was found to induce mussel spawning [19]. Furthermore, fluoxetine was also included in the list of pharmaceuticals most commonly detected in rivers across the world [18]. At the same time, there is an increasing recognition that microplastics can act as a vector for micropollutants when both occur in the same environment. In a previous study conducted by the authors, the antidepressant fluoxetine demonstrated significant adsorption onto microplastics when in a mixture of five pharmaceuticals (ibuprofen, venlafaxine, fluoxetine, ofloxacin, and carbamazepine) with greater amounts adsorbed onto artificially aged microplastics [20]. The findings of this study highlighted the potential of microplastics, specifically particles that have been aged, to be a vector for hydrophobic and positively charged micropollutants, such as fluoxetine. However, adsorption results alone do not address our lack of understanding with respect to whether or not microplastics are a vector or a sink for micropollutants when they co-occur. Furthermore, it is undetermined whether this accumulation of micropollutants onto microplastics can result in harmful effects to wildlife. For this reason, a toxicity test is crucial for eliciting a better understanding of the interaction between chemicals adsorbed to microplastics and aquatic organisms. The current study investigated how the interaction of fluoxetine with microplastics in freshwater can impact co-existing organisms. To achieve this, an acute toxicity test of fluoxetine-loaded microplastics, using the water quality indicator *D. magna*, was performed. Three widely reported microplastic types, in both their virgin and weathered forms, were tested individually and when loaded with fluoxetine to assess if there were differences in toxicity depending on polymer type and weathering. To better understand the factors influencing the toxicity of micropollutant-loaded microplastics on *D. magna* neonates, the availability of fluoxetine when adsorbed onto the virgin and artificially aged microplastics was investigated. Therefore, adsorption/desorption kinetics and isotherms were studied under freshwater conditions.

2. Materials and methods

2.1. Microplastics

Virgin microparticles of polypropylene (PP, median particle size (D_{50}) 8 μm), polyamide (PA, D_{50} 33 μm), and polyvinyl chloride (PVC, $D_{50\ (1)}$ 0.11 μm, $D_{50\ (2)}$ 1.3 μm)) were purchased from Shanghai Guanbu Electromechanical Technology Co. Ltd., China (Table 1). PP and PVC were used as received, while PA was sieved using 20 μm and 45 μm sieves due to the wide range of sizes in the purchased material [19]. The commercially acquired virgin microplastics were aged using the SUNTEST XLS+ Xenon Arc weathering testing unit (ATLAS AMETEK Electronics Instrument Group, USA) equipped with a 1700 W Xenon Arc lamp operated at 60 W m^{-2} (300–400 nm) intensity, which irradiates a similar intensity of sunlight to that of summer (~ 1000 W m^{-2} total irradiation) [20].

A detailed characterisation was carried out of both the virgin and artificially aged microplastics used in this study (Table 1) [20,21]. The characterisation included analyses to confirm the polymer material (Fourier Transform infrared spectroscopy), particle size distribution (laser diffraction particle size analysis), surface morphology (scanning electron microscopy), calorimetric characteristics (differential scanning calorimetry), surface area (N_2-Brunauer, Emmett and Teller adsorption-desorption surface area analysis), and surface charge (zeta potential measurement).

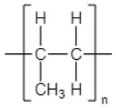
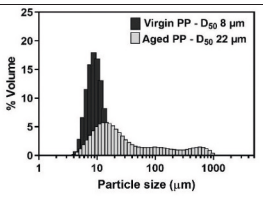
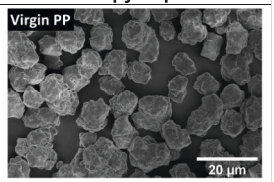
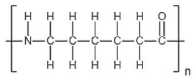
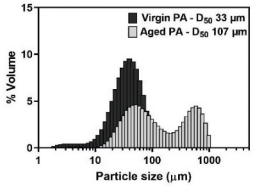
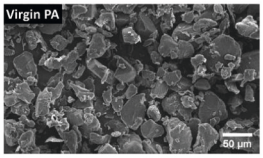
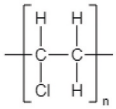
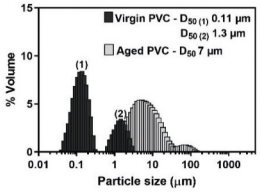
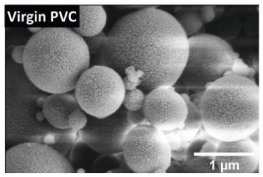
2.2. Chemicals

Artificial freshwater (AFW) was used as the experimental media for the adsorption/desorption and toxicity investigation. The AFW for the adsorption/desorption evaluations was prepared by mixing ultrapure water (18.2 MΩ) with sodium azide (NaN_3) (0.02 %; w/v, 200 mg L^{-1}) which was used as a microbial inhibitor. The AFW included $CaCl_2.2H_2O$ (58.5 mg L^{-1}), $MgSO_4.7H_2O$ (24.7 mg L^{-1}), $NaHCO_3$ (12.0 mg L^{-1}), and KCl (1.2 mg L^{-1}) in accordance with Akkanen and Kukkonen [22], and NaN_3 (200.0 mg L^{-1}). The pH of the AFW was pH 7.0.

For the toxicity tests, AFW was prepared with ultrapure water (18.2 MΩ) and the addition of $CaCl_2.2H_2O$ (294 mg L^{-1}), $MgSO_4.7H_2O$ (123.25 mg L^{-1}), $NaHCO_3$ (64.75 mg L^{-1}), and KCl (5.75 mg L^{-1}) according to the Daphtoxkit F (Microbiotest, BE) manual procedure. The pH of the media was pH 8. This media was used for all aspects of the toxicity tests, including the hatching of the ephippia, preparation of solutions, and the preparation of fluoxetine-loaded microplastics.

Fluoxetine hydrochloride (CAS 56296-78-7) was acquired from Tokyo Chemical Industry (UK) as HPLC grade, greater than 97 % purity. Stock solutions at concentration of 100 μg mL^{-1} (10 mg in 100 mL)

Table 1
Details of the virgin microplastics used in all evaluations, including chemical structure, glass transition temperature (Tg) and degree of crystallinity (XC) measured by the differential scanning calorimetry (DSC) analysis, N2 adsorption-desorption surface area (*SBET*), particle size distribution and median particle size (D_{50}) measured by laser diffraction particle size analysis. For more details see [20,21]. Note: there was no visual differences between the virgin and aged microplastics, therefore only the virgin scanning electron microscopy of the microplastics are shown.

Plastic	Chemical structure	T_g (°C)	X_C (%)	S_{BET} ($m^2\ g^{-1}$)	Particle size distribution	Scanning electron microscopy of plastics
Polypropylene (PP)		Virgin: < 0 Aged: < 0 (Rubbery)	Virgin: 32 Aged: 30 (Semi-crystalline)	Virgin: 52.2 Aged: 49.3		
Polyamide (PA)[α]		Virgin: 91 Aged: 91 (Glassy)	Virgin: 9 Aged: 10 (Semi-crystalline)	Virgin: 0.6 Aged: 0.23		
Polyvinyl Chloride (PVC)		Virgin: 84 Aged: 84 (Glassy)	Virgin: 0 Aged: 0 (Amorphous)	Virgin: 4.3 Aged: 2.89		

[α] Polyamide 6 (nylon 6).

were prepared using AFW (for adsorption/desorption determination, 0.02 % w/v NaN_3 was added). No organic solvents were used to avoid cosolvent interference on the adsorption evaluation. Prior to use, the stock solution was stored at 4 °C in the dark.

2.3. Toxicity test organisms and test parameters

A toxicity evaluation with *D. magna* neonates (less than 24 h old) was performed using a Daphtoxkit F (Microbiotest, BE) according to the standard operating procedure of the kit with adaptations noted. The neonates were obtained by hatching ephippia (dormant egg) for 72 h at 20 °C under continuous illumination of approximately 6000 lux (Fig. S1 and S2). The neonates were fed with spirulina, 2 h prior to experimentation, to avoid mortality from starvation during the investigation (Fig. S3 and S4). During the 48 h exposure, the neonates were kept in the dark at 20 °C. The PS multiwell plate (30 wells, 3 × 3 × 3 cm each) provided with the kit was replaced by vials, in order to avoid adsorption of fluoxetine to the plastic plate (Fig. S5). The immobilisation and mortality of the neonates were assessed under a dissecting microscope after 24 h and 48 h exposure (Fig. S6 and S7). The neonates which were not able to swim after gentle agitation of the liquid for 15 seconds were considered immobile, even if they could still move their antennae. To avoid repetition and prolonged descriptions, both immobilised and dead neonates were considered as 'dead' in the current study. For details see Supplementary information (S1.2 and S1.3).

2.4. Experimental design of the toxicity test

Three controls and two treatments each containing a total of 20 neonates (5 neonates in each vial) were evaluated. Each test vial contained 10 mL of experimental medium. The vials containing the neonates were placed in an incubator (MaxQ 6000, Thermofisher scientific, UK) at 20 °C in the dark for 48 h without agitation. The investigation consisted of a protocol control (media only), fluoxetine control (1 µg mL^{-1}), virgin or aged microplastic control (100 µg mL^{-1} plastic), fluoxetine-loaded microplastics at two concentrations of plastic (50 µg mL^{-1} or 100 µg mL^{-1} plastic, Fig. 1). The concentration of the fluoxetine control was chosen in accordance with the reported EC_{50} of fluoxetine (0.82 µg mL^{-1}) to *D. magna* (Fig. 1, [23]). The current study was conducted in two batches. In the first batch, the effect of fluoxetine loaded onto virgin PP on *D. magna* neonates' survival was investigated. In the second batch, identical conditions were applied to investigate the effect of fluoxetine-loaded onto aged PP, virgin and aged PA and virgin and aged PVC on neonates' survival.

Fluoxetine-loaded PP microparticles were prepared by adding virgin and aged PP microparticles (2 g L^{-1} plastic) to medium containing fluoxetine (100 µg mL^{-1}) in AFW. For virgin and aged PA and PVC, the particles were pre exposed to 15 µg mL^{-1} of fluoxetine (2 g L^{-1} plastic). The concentration of fluoxetine loaded onto the microplastics was chosen based on the results of the adsorption isotherms to ensure the saturation of the particles with fluoxetine. Particles of PP showed a greater adsorption capacity for fluoxetine when compared to PA and PVC. Therefore, a greater concentration of fluoxetine was used to saturate the particles of PP compared to PA and PVC. The fluoxetine-loading of the microplastics and the control were horizontally shaken at 200 rpm and 25 °C using a MaxQ 6000 orbital shaker (Thermofisher scientific, UK) for 24 h. After 24 h, the microplastics were filtered using a GF/F filter (Fisher scientific, UK). The microplastic control was treated exactly as the 100 µg mL^{-1} plastic fluoxetine-loaded microplastic treatment.

The software ImageJ (National Institutes of Health, US) was used to measure the neonates and to add a reference scale in the pictures. See Supplementary information (S1.4) for more information about the images captured from the neonates throughout the investigation.

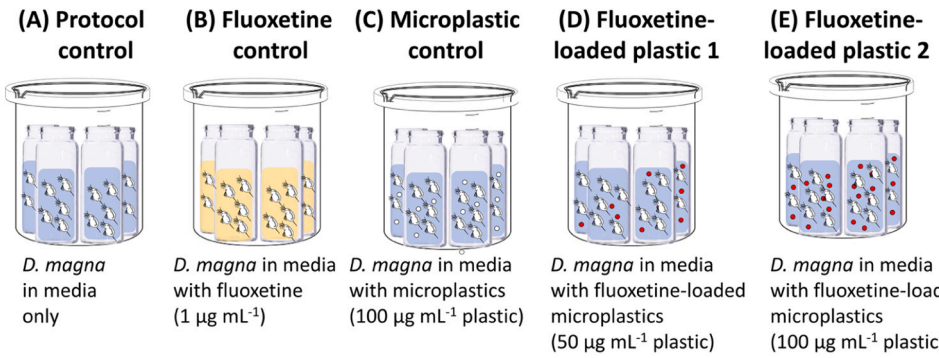


Fig. 1. Three controls and two treatments were evaluated, a) protocol control (media only), b) fluoxetine control (fluoxetine, 1 μg mL^{-1}), microplastic control (100 μg mL^{-1} plastic), fluoxetine-loaded microplastics (50 μg mL^{-1} plastic, fluoxetine-loaded plastic 1) and fluoxetine-loaded microplastics (100 μg mL^{-1} plastic, fluoxetine-loaded plastic 2). For fluoxetine-loaded plastic 1 and 2, fluoxetine was loaded onto virgin PP (37 μg mg^{-1} adsorbed), aged PP (35 μg mg^{-1} adsorbed), virgin PA (3.5 μg mg^{-1} adsorbed), aged PA (4.5 μg mg^{-1} adsorbed), virgin PVC (3.3 μg mg^{-1} adsorbed), and aged PVC (4 μg mg^{-1} adsorbed). White dots: microplastics not exposed to fluoxetine. Red dots: fluoxetine-loaded microplastics.

2.5. Adsorption/desorption kinetics and isotherm of fluoxetine on/from virgin and aged polypropylene, polyamide, and polyvinyl chloride

Adsorption/desorption kinetics and isotherms of fluoxetine for virgin and aged particles of PP, PA, and PVC were performed under the same conditions [20]. For both, samples were continuously horizontally agitated on a MaxQ 6000 orbital shaker (Thermo Scientific, UK) at 200 rpm and 25 °C in the dark. The adsorption kinetics and isotherms were performed in conical flasks (50 mL), containing either 50 mg of microplastics or no microplastics (control) in 25 mL of AFW+ 0.02 % w/v NaN_3 (equivalent to 2 g L^{-1} microparticles). The desorption kinetics and isotherms were performed straight after the adsorption kinetics and isotherm evaluation. The samples used for the adsorption experiments were filtered using 47 mm GF/F filters (Fisher scientific, UK), folded and placed in an empty conical flask. Although the control did not contain microplastics, the control solution with fluoxetine was also filtered to evaluate whether fluoxetine was adsorbed by the filter, potentially impacting the desorption results. As in the adsorption evaluation, the filter either without (control) or with microplastics was placed into a 50 mL conical flask which was filled with 25 mL AFW + 0.02 % w/v NaN_3 (2 g L^{-1}). Samples (200 μL) were removed and filtered using a microcentrifuge tube filter (2 mL spin-X tubes made of PP, cellulose acetate filter, 0.22 μm pore size, Corning USA). The filtered samples (100 μL) were then analysed by high-performance liquid chromatography (HPLC) with photodiode array (PDA) detection. All samples (prior to filtration and filtered samples) were removed using a microlitre glass syringe (250 μL) with a stainless-steel needle (Hamilton, UK) to avoid contact of the fluoxetine solution with laboratory plastics. Throughout the investigation, contact with laboratory plastics was eliminated except for the microcentrifugation filtration device which could not be avoided. All treatments and controls were conducted in triplicate.

For the kinetics evaluation, fluoxetine (50 μg mL^{-1}) was placed in contact with virgin and aged PP. For virgin and aged PA and PVC, the initial concentration of fluoxetine was 5 μg mL^{-1}. Different concentrations were used because of the greater adsorption of fluoxetine by PP previously determined [20] compared to PA and PVC. Samples of the control solution (without microplastics) or the test solution with microplastics were taken at 0.5, 1, 2, 4, 6, 10, 24, and 48 h contact. Controls containing fluoxetine without microplastic particles was also prepared and analysed at each sampling point. After 48 h, the solution containing microplastics was filtered using a GF/F filter (Fisher scientific, UK) to recover the fluoxetine-loaded microplastics and placed in fresh media. Samples of the control solution (filter without microplastics) or the test solution with microplastics were taken at 0.5, 1, 2, 4, 6, 10, 24, and 48 h contact (Fig. S8). The experimental data was applied to pseudo-first order, pseudo-second order and intraparticle diffusion to evaluate the diffusion rates (Table S1).

For the isotherm determination, fluoxetine at initial concentrations of 1.0, 2.5, 5.0, 10.0, and 15.0 μg mL^{-1} were placed in contact with virgin and aged particles of PA and PVC. Due to the adsorption potential of PP [20], fluoxetine at initial concentrations 15, 25, 50, 75, and 100 μg mL^{-1} were placed in contact with virgin and aged particles of PP (2 g L^{-1} microparticles). After 24 h contact, the equilibrium time according to the kinetics results, the fluoxetine concentration was evaluated in the samples, the solution containing microplastics was therefore filtered using a GF/F filter (Fisher scientific, UK) to recover the fluoxetine-loaded microplastics and placed in fresh media. After 24 h, the concentration of fluoxetine was evaluated in the samples (Fig. S8). The experimental data was applied to Freundlich, Langmuir, and Linear models to evaluate the mechanism of interaction (Table S2).

In the current study, the concentration of fluoxetine in the solution was measured by HPLC-PDA. See Supplementary information for more details of the fluoxetine analysis (S1.1). Controls revealed that the loss of fluoxetine through this step was approximately 11 ± 4 %. The limit of detection and limit of quantification of fluoxetine using the method described in the Supplementary information (S1.1) was 0.01 μg mL^{-1} and 0.05 μg mL^{-1}, respectively.

3. Results and discussion

3.1. Effect of fluoxetine-loaded microplastics on the survival of D. magna neonates

The acute toxic effects of fluoxetine loaded onto virgin and artificially aged microplastics (PP, PA, or PVC) on *D. magna* neonates was evaluated. Results indicated that microplastics can both be a vector or a sink for micropollutants such as fluoxetine depending on the microplastic type and whether or not the plastics has been weathered. The mortality of the neonates exposed to fluoxetine-loaded microplastics was variable. Immobilised neonates (neonates that could not actively swim) were designated as being 'dead'. All six microplastic types (PP, PA, or PVC, virgin or aged) that had been pre-exposed to fluoxetine showed toxic effects to the *D. magna* neonates, dominated by the virgin particles (Fig. 2). On the other hand, those exposed to the microplastic itself did not show significant ($p > 0.05$) mortality of *D. magna* neonates.

Fluoxetine when loaded on microplastics appeared to have a delayed effect on the toxicity being evidenced by the death of an increased number of neonates (e.g., Fig. 2C) compared to when neonates were exposed to fluoxetine in solution (fluoxetine control). For both fluoxetine controls (1 μg mL^{-1}), the same number of *D. magna* neonates were 'dead' after 24 h and 48 h exposure to fluoxetine (Fig. 2A and B-F).

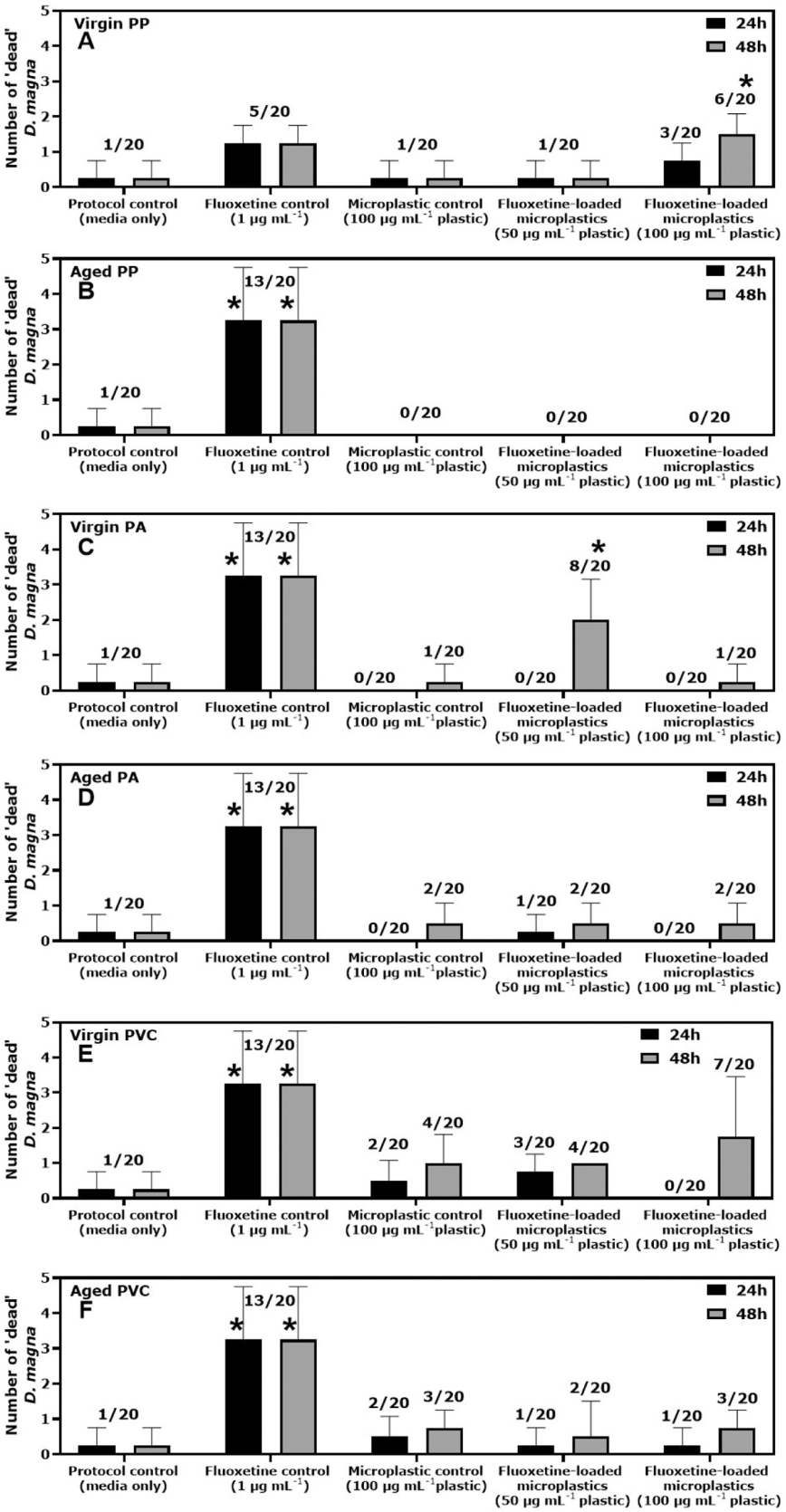


Fig. 2. Summary of the average number of 'dead' *D. magna* neonates across the 4 vials (5 neonates in each vial, totalling 20 neonates per procedure) after 24 h and 48 h. Total number of 'dead' organisms out of twenty is presented on the top of the bars. *significant difference between the treatment and the control, *t*-test $p < 0.05$. n = 4, errors bars = 1 standard deviation (SD). Note: the current study was performed in two batches. The first batch evaluated the survival of *D. magna* exposed to fluoxetine-loaded virgin PP (A) while the second batch investigated the survival of *D. magna* exposed to fluoxetine-loaded aged PP (B), virgin PA (C), aged PA (D), virgin PVC (E), and aged PVC (F).

However, when the fluoxetine was loaded onto microplastics, a greater mortality was generally observed after 48 h when compared to 24 h exposure to the loaded particles. The delay in toxicity may occur because fluoxetine-loaded microplastics must be ingested, followed by chemical desorption and transport to the site of action. Alternatively, the toxicity could result from fluoxetine desorbing into the surrounding water and being directly absorbed by the neonates. Virgin loaded particles showed greater effects on the organisms compared to the artificially aged, loaded particles. Statistical analysis (student's *t*-test) showed that there was no significant difference between the mortality of fluoxetine-loaded virgin PP ($p = 0.09$, 50 and 100 μg mL^{-1} plastic, Fig. 2A), virgin PA ($p = 0.41$, 50 μg mL^{-1} plastic, Fig. 2C) and virgin PVC ($p = 0.06$, 100 μg mL^{-1} plastic, Fig. 2E) and mortality of neonates in their respective fluoxetine control. Therefore, this implies that fluoxetine in solution at the concentration used in this investigation is as toxic as fluoxetine-loaded onto the ingested microplastics. Virgin fluoxetine-loaded PP particles (6 out of 20 for the 100 μg mL^{-1} fluoxetine-loaded microplastics; Fig. 2A) and virgin fluoxetine-loaded PA (8 out of 20 for the 50 μg mL^{-1} fluoxetine-loaded microplastic; Fig. 2C) showed a significant ($p < 0.05$) toxic effect to *D. magna* when compared to the protocol control (media only). That means that, in this investigation, the lower concentrations of virgin PA fluoxetine-loaded microplastics were more toxic to *D. magna* than the higher concentrations (Fig. 2C). Microscopic analysis of the neonates after 48 h exposure showed that a lower concentration of microplastics did not result in a lower ingestion of the microplastics by the organism (Fig. 3), excluding a feed-load effect on *D. magna*. Since both microplastic concentrations (50 and 100 μg mL^{-1}) had the same amount of fluoxetine adsorbed onto the particles, the microplastic concentration investigated was not a factor regarding the toxicity of fluoxetine-loaded PA. A marked mortality (7 out of 20 neonates) was observed after 48 h exposure to virgin PVC loaded with fluoxetine. However, the number of 'dead' organisms was not significantly different from the microplastic control ($p = 0.24$) which in this particular case showed the highest mortality of all the microplastic controls (Fig. 2E). Indeed, the microplastic controls for PVC, both virgin and aged (Fig. 2 E and F respectively), contrasted with the controls for the other plastics, whether they were virgin or aged. Although both the loaded concentration and the microplastic concentration used in this study were not environmentally relevant, the toxicity of fluoxetine-loaded microplastics presented in the current study is particularly worrisome. Especially when considering the comparatively short period of time demonstrated for toxic effects to occur compared to the average reported life span for *D. magna* of 10–30 days (or even up to 100 days in a predator-free environment). Furthermore, *D. magna* is at a low trophic level in the food-web, and studies [1,24] have found that microplastics can transfer through a food-web. In a study conducted by Bao et al. [25], the shape of the microplastics and the polymer type were the main factors influencing the toxicity of the microplastics on aquatic organisms. The aging of microplastics, on the other hand, showed varied toxicity according to organism. In this study, the virgin PVC consisted of cylindrical beads (Table 1) which contrasts markedly with the other two plastics (Table 1). Virgin PVC was also the only amorphous polymer, the other two were classified as glassy. Whether or not these aspects of the polymer are relevant to the results will require further study.

During the loading of microplastics with fluoxetine, greater amounts of fluoxetine were adsorbed onto virgin PP (74 μg mL^{-1}) compared to virgin PA (7 μg mL^{-1}) and virgin PVC (6.5 μg mL^{-1}, Table 2). PP exhibits a greater adsorption capacity (Fig. 4A, [20]), which meant that in the experimental design PP was exposed to a greater fluoxetine concentration (100 μg mL^{-1}) relative to PA and PVC (15 μg mL^{-1}). This means that fluoxetine might be more available when adsorbed onto the glassy polymers like PA and PVC, than onto the rubbery polymers such as PP (Table 1). Similarly, differential fluoxetine desorption was observed when comparing virgin with aged particles for all three microplastics investigated (Table 2). The three aged microplastic types showed greater aggregation of the particles before and during the exposure to *D. magna*. This might further indicate that fluoxetine adsorbed onto the aged particles is less available relative to the virgin particles. This does not mean that aged particles are not harmful to wildlife. Aged microplastics have been demonstrated to adsorb greater amounts of chemical pollutants than their virgin equivalent when co-occurring. Furthermore, aged particles adsorb hydrophilic compounds along with hydrophobic compounds. Hydrophilic compounds might be more readily available in the dissolved form in water as against hydrophobic compounds which may be associated with organic material. A study carried out by Wagstaff and Petrie [26] observed a similar exposure risk of fluoxetine desorption from PET microplastics (maximum size 300 μm) with temperatures equivalent to the internal temperatures of warm-blooded organisms (37 °C) over that of cold-blooded organisms such as *D. magna*. (20 °C) [26]. However, at the same time, greater fluoxetine desorption was observed in gastric fluid media compared to river water. The gut temperature of *D. magna* tends to closely match the surrounding water temperature and the pH of *D. magna* guts varies between 6.0 and 7.2 [27].

The concentration of fluoxetine in the solution containing fluoxetine-loaded microplastics was determined to evaluate the amount of fluoxetine desorbed from the microplastics, which can also affect the mortality of the neonates. As expected, greater concentrations of fluoxetine were detected in the treatments from the higher concentration of microplastics. The greatest absolute desorption was observed for virgin particles of PP (0.41 ± 0.02 μg mL^{-1}) in the treatment with the higher microplastic concentration (100 μg mL^{-1} microplastics), while the lowest desorption of fluoxetine from the particles was observed for aged PVC (0.06 ± 0.01 μg mL^{-1}) in the treatment with the lower microplastic concentration (50 μg mL^{-1} microplastics, Table 2). However, considering the amount of fluoxetine adsorbed onto each of the microplastics and the plastic concentration in the treatment (50 or 100 μg mL^{-1}), proportionally, fluoxetine showed the lowest desorption when binding to PP (9–18 % desorption), while fluoxetine showed greatest desorption from PA (34–74 % desorption, Table S3). Furthermore, the concentration of fluoxetine detected in the two treatments containing fluoxetine-loaded microplastics was not directly proportional to the microplastic concentration in the treatment.

The absolute concentrations in the media of fluoxetine desorbed from the fluoxetine-loaded microplastics at the lower concentration (50 μg mL^{-1} microplastics) were similar to that observed from the treatment at the higher microplastic concentration (100 μg mL^{-1} microplastics, Table 2). That means that, proportionally, fluoxetine desorbs more from the lower concentration of microplastics when compared to the higher concentration of microplastics (Table S3). Furthermore, no correlation was observed when comparing the mortality of the neonates after 24 h or 48 h exposure with parameters such as the microplastic concentration, the concentration of fluoxetine adsorbed onto the microplastics, and the concentration of fluoxetine desorbed from the microplastics (Table S7). Interestedly, the absolute concentration of fluoxetine in solution for the aged PP (0.22 and 0.33 μg mL^{-1}) was higher when compared to any desorbed concentration of fluoxetine from the PA and PVC (0.06–0.20 μg mL^{-1}, Table 2), yet no mortalities were recorded in the aged PP exposure (Fig. 2). On the other hand, the kinetic evaluation performed in the current study demonstrated that hardly any fluoxetine desorbs from PP (considered negligible) when compared to PA and PVC (Figs. 4 and 5). That means fluoxetine might be more strongly bound to PP when compared to PA and PVC which is not affected when ingested by the neonates. Furthermore, this finding might indicate that the fluoxetine adsorbed onto microplastics allied to the ingestion of microplastics are the primary driver of mortality of neonates. This highlights the complexity of the availability of micropollutants adsorbed onto the microplastics. In the current study, availability refers to the micropollutant desorbed from the microplastics, which is readily available when exposed to wildlife either by contact or ingestion. There are several factors that can influence the observed concentration of fluoxetine in the media, even if

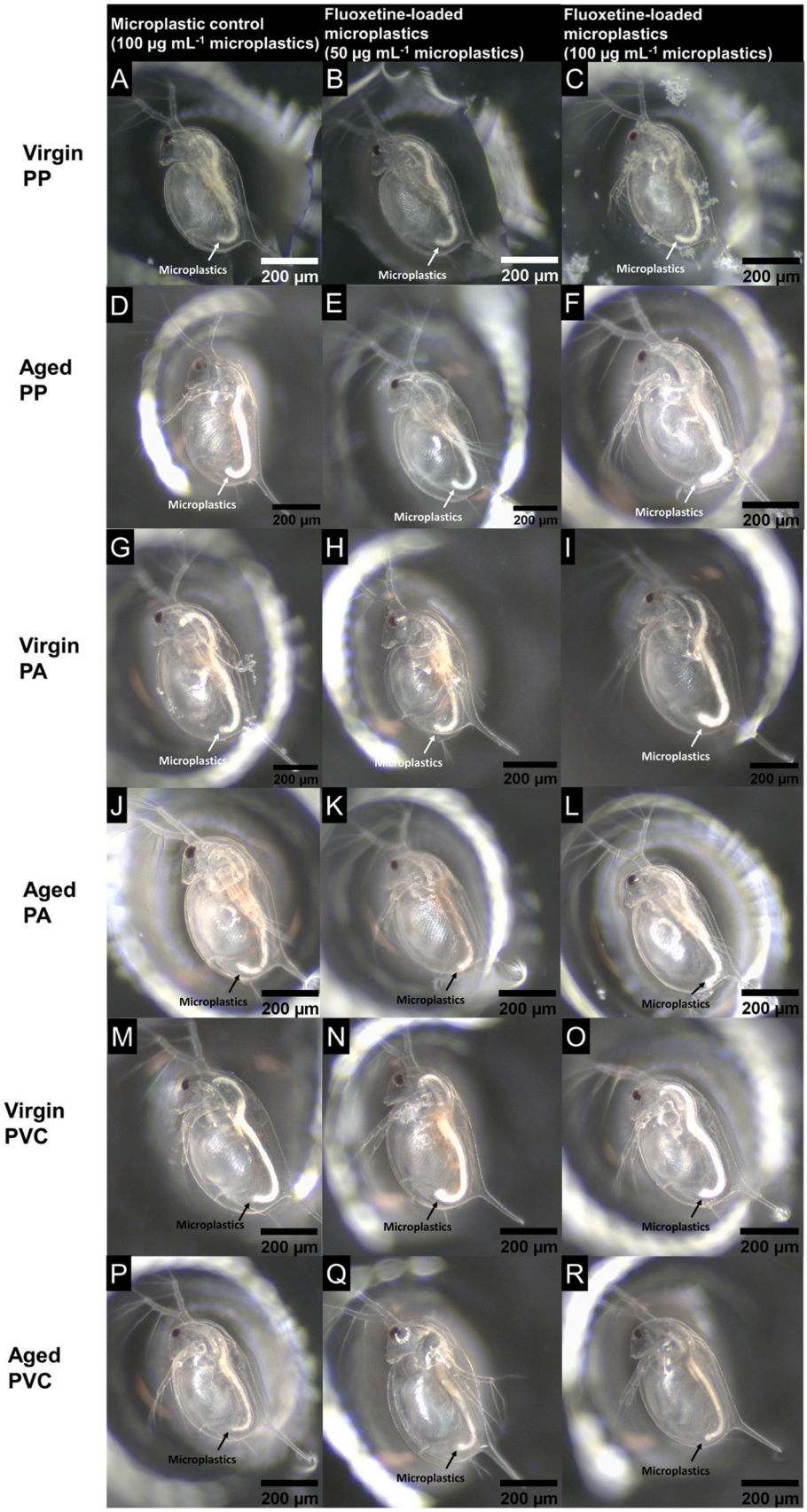


Fig. 3. *D. magna* neonates after 48 h exposure to: microplastic control (100 µg mL^{-1} microplastics, left, A – P), fluoxetine-loaded microplastics (50 µg mL^{-1}, middle, B - Q), and fluoxetine-loaded microplastics (100 µg mL^{-1}, right, C - R). The microplastics tested were virgin and aged polypropylene (PP), polyamide (PA), and polyvinyl chloride (PVC). The arrows point to microplastics in the gut of the neonates. Magnification 45x. Neither microplastics nor any particles were observed in the guts of the organisms from the protocol control (media only) or from the fluoxetine control (Fig. S9).

Table 2
Summary of the concentration and percentage adsorbed and desorbed onto and from microplastics exposed to *D. magna* neonates according to the microplastic type, microplastic weathering, and the plastic concentration. Note: the concentration of fluoxetine was evaluated in the treatments containing *D. magna* neonates at the end of the experiment after evaluation of the mortality numbers. n = 4, average ± standard deviation (SD).

Plastic	Weathering	Fluoxetine initial concentration (μg mL^{-1})	Concentration adsorbed onto microplastics (μg mL^{-1})	Plastic concentration (g L^{-1})	Concentration in the media of fluoxetine desorbed from microplastics (μg mL^{-1})
PP	Virgin	100	74.41 ± 0.5 (74 %)	50	0.34 ± 0.02
				100	0.41 ± 0.02
	Aged		67.95 ± 2.56 (68 %)	50	0.22 ± 0.02
				100	0.33 ± 0.01
PA	Virgin	15	6.92 ± 0.43 (46 %)	50	0.13 ± 0.01
				100	0.20 ± 0.01
	Aged		9.06 ± 0.56 (60 %)	50	0.11 ± 0.01
				100	0.15 ± 0.01
PVC	Virgin		6.55 ± 0.39 (44 %)	50	0.07 ± 0.01
				100	0.08 ± 0.001
	Aged		8.00 ± 0.37 (53 %)	50	0.06 ± 0.01
				100	0.07 ± 0.01

the microplastic concentrations are different. Some of these factors are the aggregation, floating and settling of the microplastics that can influence the surface contact with water, thus allowing for more or less desorption to occur. For all treatments containing fluoxetine-loaded microplastics, the concentration of fluoxetine desorbed from the microplastics was lower when compared to the fluoxetine control concentration (1 μg mL^{-1}). Microplastics were detected in the gut of the *D. magna* neonates in the treatments containing fluoxetine-loaded microplastics. That means that the presence of fluoxetine in the solution did not stop the organism from ingesting the microplastics loaded with fluoxetine.

3.2. Evaluation of the survival rates of D. magna neonates in the controls

In order to compare the toxicity of fluoxetine when in solution to when loaded onto the microplastics, a fluoxetine control (reported EC_{50}: 1 μg mL^{-1}, [23]) was included in the experimental design. A protocol control and a fluoxetine control were performed along with two concentrations of fluoxetine-loaded microplastics. Two separate investigations were conducted. The first investigated the toxicity of fluoxetine in the presence of virgin PP. Five of 20 *D. magna* neonates died when exposed to fluoxetine in solution (1 μg mL^{-1}; Fig. 2A) for 24 h. The second investigation evaluated the toxicity of fluoxetine-loaded onto aged PP, virgin and aged PA and virgin and aged PVC, the potential toxicity of the microplastics and the potential toxicity of fluoxetine (1 μg mL^{-1}; Fig. 2B–F). On this occasion, 13 neonate mortalities were observed after 24 h when exposed to fluoxetine (1 μg mL^{-1}; Fig. 2B–F) for 48 h. For both investigations, only a single organism (1 out of 20) died in the protocol control (media only, no fluoxetine or microplastics). This occurred in the first 24 h (Fig. 2A–F) of the experiment. It should be noted that in the protocol control and the fluoxetine control, there were no further deaths between 24 and 48 hours. Brooks et al. [23] reported 0.82 μg mL^{-1} as half effective concentration (EC_{50}) of fluoxetine on *D. magna*, which is consistent with the mortality of the *D. magna* neonates in the fluoxetine control in the second investigation (65 % mortality). The reported EC_{50} values of *D. magna* neonates exposed to fluoxetine in solution varies in the literature, for instance, another study showed an acute toxic effect (48 h) of fluoxetine to *D. magna* neonates at approximately 10-fold greater fluoxetine concentrations when compared to Brooks et al. [23]. Varano, Fabbri and Pasteris [28] observed an acute toxic effect of fluoxetine to *D. magna* neonates between 6.4 and 9.1 μg mL^{-1} and demonstrated a chronic effect (21 days) at a concentration of 0.23 μg mL^{-1}. The different EC_{50} values reported in the literature and the different mortality observed between the two investigations when *D. magna* neonates were exposed to identical fluoxetine concentrations, under the identical conditions, could result from changes to the protocol between the different studies reported in the literature.

The ingestion of microplastics was observed in all experiments where they were included (Fig. 3A–R). This includes the microplastic control (100 μg mL^{-1} microplastics) and the fluoxetine-loaded microplastics at two plastic concentrations (50 μg mL^{-1} and 100 μg mL^{-1} microplastics). No apparent correlation was observed, through visual analysis, of the amount ingested by *D. magna* neonates and the concentration of microplastics in the solution (50 and 100 μg mL^{-1} microplastics, Fig. 3). Likewise, after visual inspection, no clear association was observed between the size of the particles (PVC < PP < PA size, Table 1) and the amount ingest by the neonates. The size of the microplastics did not show a correlation with the mortality of the neonates after 24 h exposure (r = -0.41) nor after 48 h exposure (r = -0.27, Table S7). However, the *D. magna* neonates appear to ingest the lowest amounts of microplastics when exposed to the lower plastic concentration of fluoxetine-loaded, aged PA (50 μg mL^{-1} microplastics, Fig. 3). The decreased ingestion might be due to aggregation of aged PA after the aging process (Table 1), which led to settling of the particles and presence of large PA agglomerates that could not be ingested by *D. magna* neonates.

Furthermore, no correlation was observed between the density of the microplastics and both the ingestion of the microplastics and the mortality of the neonates (Table S7). According to visual analysis of microplastic buoyancy, PP tended to both float on the top of, and disperse in, the water column, while virgin PA was completely dispersed in the water column. The aggregated particles of PA, however, sank to the bottom of the experimental vials. Likewise, PVC particles appeared to both sink and disperse in the water column. Among the microplastics investigated in the absence of fluoxetine, the neonates showed greatest mortality when exposed to either virgin PVC particles (four out of 20, 48 h) or aged PVC particles (three out of 20, 48 h; Fig. 2E and F respectively). None of microplastic types themselves show significant ($p > 0.05$) toxic effects on the neonates when compared to the protocol control. However, it is possible that the effect of PVC has been increased by the presence of fluoxetine (Fig. 2E). Previous studies have shown that microplastics within the size range 0.020 - 5.0 μm are commonly ingested by *D. magna*, as they represent a similar size range to their common algal food sources [6]. In a study conducted by An et al. [12], polyethylene (PE) fragments (approximately 17 μm and 35 μm) exhibited chronic toxicity to *D. magna* after 21 days exposure. While, Canniff and Hoang [13] showed that despite *D. magna* were able to ingest PE microbeads ranging in size from 63 to 75 μm, and there was no effect on survival and reproduction after 5 days. Schwarzer et al. [29] concluded that observed changes such as food uptake, mobility, and survival, were induced by microplastic ingestion and not merely by food depletion. The leachate of chemicals from microplastics can be a factor in the observed effects of microplastic on *D. magna* [30]. The impact of leachate from the particles is likely not relevant to the current study because the

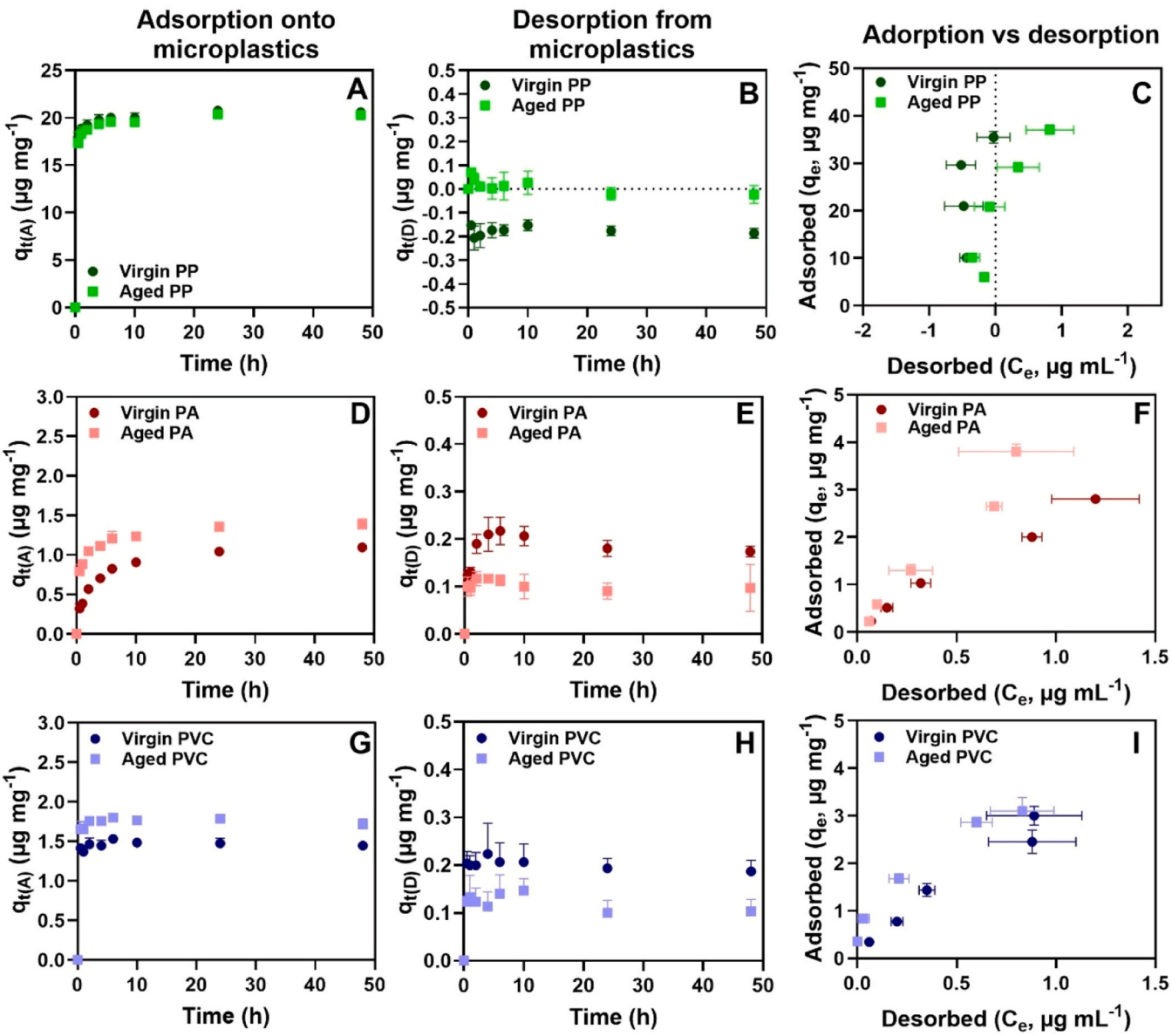


Fig. 4. Quantity of fluoxetine adsorbed ($q_{t(A)}$, left) and desorbed ($q_{t(D)}$, middle) onto/from microplastics over 48 h contact in artificial freshwater + 0.02 % (w/v) NaN_3 at an initial fluoxetine concentration of PP: 50 µg mL^{-1}, PA and PVC: 5 µg mL^{-1}. Comparison between the amount adsorbed at equilibrium (q_e) onto microplastics and the concentration desorbed at equilibrium (C_e) from the microplastics at the five different fluoxetine initial concentrations (PP: 15–100 µg mL^{-1}; PA and PVC: 1–15 µg mL^{-1}, right). The total amount adsorbed/desorbed onto/from microplastics was calculated using the Eq. (S1). n = 3, errors bars = 1 standard deviation (SD). The $q_{t(A)}$ and $q_{t(D)}$ were calculated using the Eqs. (S1) and (S2), respectively. Note: the negative values of $q_{t(D)}$ in the PP desorption samples are due to the detection of greater concentrations of fluoxetine in the control compared to the samples with microplastics. Note the different scale on the Y-axis for the PP adsorption plot compared to the PA and PVC adsorption plots. h, hour.

microplastics were previously exposed to the media, both with and without fluoxetine (see Section 2.5), which may have acted as a cleaning process. Specifically, the control microplastics and the microplastics loaded with fluoxetine were shaken in the media, without and with fluoxetine, respectively, for 24 hours before exposing them to the neonates. This procedure is expected to remove any readily available chemicals on the commercial microplastics, such as plasticisers and grafting agents, which could potentially be toxic to *D. magna*.

3.3. Adsorption/desorption of fluoxetine onto/from microplastics

Fluoxetine was brought into contact with either virgin or aged PP, PA, or PVC, and adsorption/desorption kinetics when co-occurring with these microplastics was evaluated. The adsorption of fluoxetine onto the microplastics investigated followed the order (most to least adsorbed): PP > PVC > PA. Among the microplastics investigated, PP (whether virgin or aged) showed the greatest adsorption ($q_t \sim 20$ µg mg^{-1} aged and virgin particles; Fig. 4A), followed by aged PVC ($q_{t(A)}$ 1.72 µg mg^{-1}, Fig. 4H), virgin PVC ($q_{t(A)}$ 1.45 µg mg^{-1}), aged PA ($q_{t(A)}$ 1.39 µg mg^{-1}) and virgin PA ($q_{t(A)}$ 1.09 µg mg^{-1}, Fig. 4D) over 48 h of contact (Fig. 4). The results confirmed findings from our previous publication [20], when aged particles exhibited the highest adsorption, and PP demonstrated greater adsorption than the other six microplastic types. The difference on adsorption potential among weathering and type of microplastics has been widely reported in the scientific literature [31]. The adsorption of micropollutants onto microplastics is an important indication that plastic particles can act as a vector for pollutants facilitating entry to and passage through a food-web. However, the adsorption evaluation alone lacks the information regarding the impact of the adsorbed micropollutants on aquatic life, especially when ingested. The adsorbed

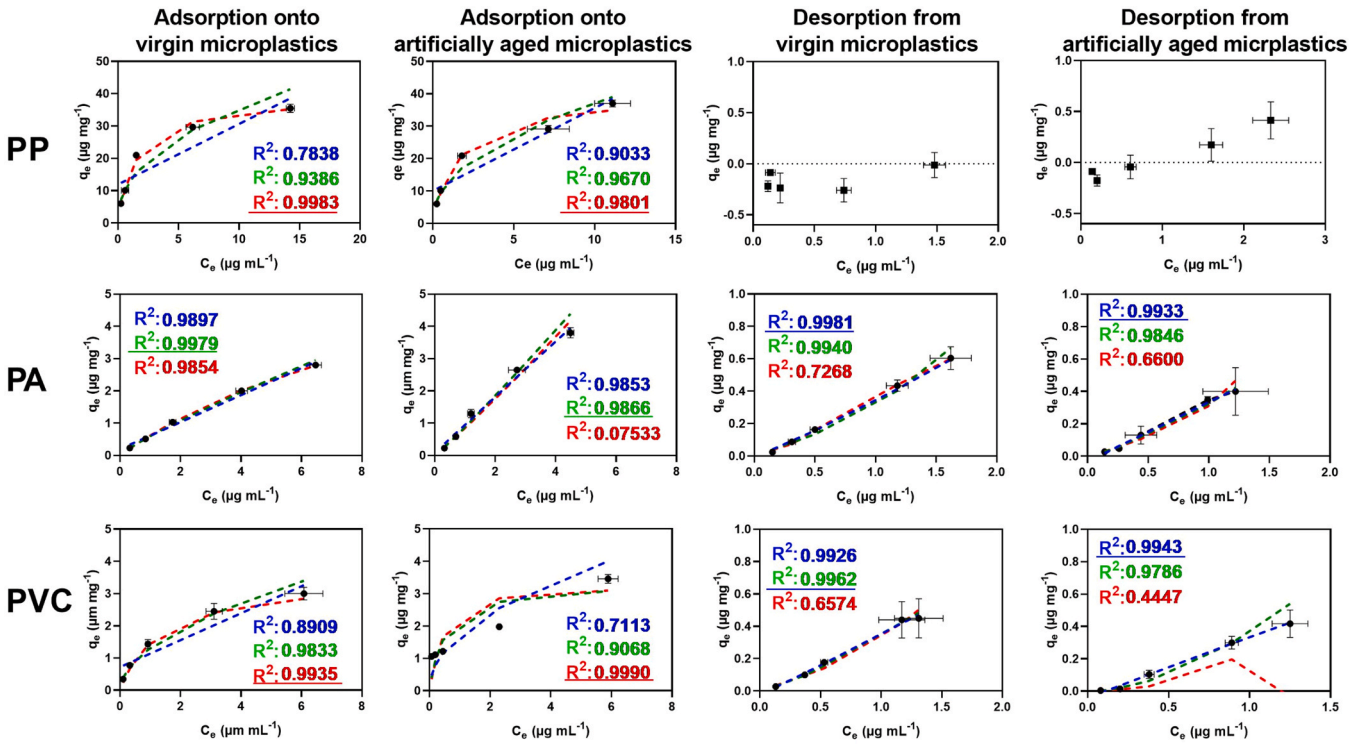


Fig. 5. Application of the experimental adsorption/desorption data of virgin and artificially aged polypropylene (PP), polyamide (PA), and polyvinyl chloride (PVC) exposed to fluoxetine (PP: 15–100 μg mL^{-1}; PA and PVC: 1–15 μg mL^{-1}) to the linear forms of the linear, Freundlich, and Langmuir models. Experimental data (black circle) compared to the fitted curves of linear (blue dashed line), Freundlich (green dashed line), and Langmuir (red dashed line) models of adsorption/desorption equilibria of fluoxetine in solution onto/from virgin and artificially aged microplastics. The R^2 that showed the greatest fit to the model is underlined. The equations are described in Table S1. Note: No desorption was observed from virgin PP and the majority of fluoxetine initial concentration (15, 25 and 50 μg mL^{-1}), therefore the experimental data could not be fitted to any isotherm model. n = 3, errors bars = 1 standard deviation (SD).

micropollutants might not be readily available when adsorbed onto the microplastics. In a similar vein, the desorption of fluoxetine from microplastics depended on the polymer type and condition (weathered or not) of the microplastics. The desorption of fluoxetine from PP was considered negligible for the methodology applied (artificial freshwater, 25 °C, pH 7, against the conditions that might be experienced when the microplastics are ingested) for the adsorption/desorption kinetics and isotherm evaluation (Fig. 4B). The negligible desorption of fluoxetine from PP (Fig. 4A–C) is attributed to the higher concentration of fluoxetine detected in the control samples when compared to the samples containing the filtered particles of PP (including the filter). The control samples for the desorption evaluation consisted of the control sample of the adsorption evaluation containing fluoxetine without microplastics that was filtered then the filter placed (without microplastics) in fresh media to assess any fluoxetine adsorption on the GF/F filter. It is important to point out that fluoxetine desorption from PP was observed in the toxicity investigation (Table 2). However, in this case, the filtered particles of fluoxetine-loaded PP (without the filter) were added into the fresh media. In the kinetics and isotherm evaluation, due to the potential interference of the fluoxetine loaded on the filter, the fluoxetine concentration in the samples with microplastics were lower when compared to the concentration detected in controls. The lack of desorption of fluoxetine from PP in the kinetics/desorption determination (Figs. 4 and 5) and the discrete desorption in the toxicity evaluation (Table 2) under freshwater conditions (pH 7 at 25 °C) does not mean that fluoxetine-loaded PP is not toxic to wildlife. An example of this is the toxic effect of fluoxetine-loaded PP on *D. magna* after 48 h exposure (Fig. 2A). Desorption of fluoxetine was detected from both PA and PVC (Figs. 4E and 4H respectively). The artificially aged particles of PA and PVC adsorbed greater amounts of fluoxetine when compared to the virgin material. However, similar adsorption of fluoxetine was observed for virgin and aged PP. For both PA and PVC, greater amounts of fluoxetine desorbed from virgin microplastics compared to the aged particles (Figs. 4E and 4H respectively). In contrast, a study conducted by Wang et al. [32] showed that the desorption of bisphenol A from PP under ultrapure conditions (18 °C) greater than from PS and PA (PP > PS > PA). This difference may be attributed to variations in the adsorption mechanism of PP, likely caused by differences in the properties of the microplastics. Same microplastic types can have differences on their surface area and size, which impact on both their adsorption and desorption behaviour [33]. This highlights the importance of a detailed characterisation of microplastics for reliable data interpretation [21].

Fluoxetine readily adsorbed onto microplastics, especially microplastics with a higher surface area e.g. PP (S_{BET} 52.2 m^2 g^{-1}) and PVC (S_{BET} 4.3 m^2 g^{-1}, Table 1). The large surface area of PP suggests that, in addition to electrostatic and hydrophobic interactions, pore filling is the primary adsorption mechanism involved in its interaction with fluoxetine as observed by Moura et al. [20,34]. Similarly, the smaller particle size (D_{50} (1) 0.11 μm, D_{50} (2) 1.3 μm) and spongy surface morphology of PVC likely enhance its interaction with fluoxetine compared to the smooth surface morphology of PA particles (Table 1). Furthermore, the polar surface and higher specific surface area of PP and PVC improve their dispersion in the water column, which may explain why fluoxetine adsorption onto these virgin and aged microplastics quickly reaches a plateau (Fig. 4A and G). The majority of fluoxetine adsorbed onto virgin PP (83 ± 2 % at 30 min) and PVC (69 ± 2 % at 30 min) did so during the first 30 min of contact (Fig. 4). Likewise, within 30 min of contact, the aged particles of PP and PVC had adsorbed approximately 80 % of the available fluoxetine. Approximately 80 % of the fluoxetine desorbed from PA and PVC within 30 min when transferred to fresh media (Fig. 4E and H), reaching a plateau after 6 h. The maximum fluoxetine desorption was observed from virgin, PVC (q_e 0.19 ± 0.02 μg mg^{-1}, Fig. 4H) followed by virgin PA (q_e 0.17 ± 0.01 μg mg^{-1}, Fig. 2E). The adsorption of PA in contact with micropollutants is typically attributed to hydrogen

bond which is considered a weaker interaction than electrostatic interactions and pore filling [35]. The stronger nature of pore filling interactions might be the reason for the negligible desorption of PP when compared to less porous microplastics (PVC and PA, Table 1). Furthermore, aging process results in changes in surface properties of microplastics. This includes an increase in specific surface area and formation of surface functional groups. These can affect their adsorption behaviour and mechanism towards other pollutants [33]. In the current study, the aging of the microplastics, through light-stimulated photo- and thermal-oxidation led to a change of colour for the PVC and the formation of carbonyl function groups for PP and PVC [20]. No increase on the surface area was observed (Table 1). Carbonyl functional groups contain a carbon which has a partial positive charge, while the oxygen has a partial negative charge. This can enhance the electrostatic interactions with positively charged pollutant such as fluoxetine. However, the increased adsorption of fluoxetine on aged microplastics did not lead to either increased desorption or increased toxicity to *D. magna*.

For all three microplastic types investigated (PP, PA, and PVC), the adsorption data fitted the pseudo second order model ($R^2 > 0.999$, Table S4). The data for the desorption of fluoxetine from PA and PVC also fitted the second order model ($R^2 > 0.99$, Table S4). Fluoxetine has been shown to fit the second order model when desorbing from PET microparticles (maximum 300 μm) in river water at 20 °C. Furthermore, Wagstaff and Petrie [22] demonstrated that greater desorption of fluoxetine can occur in gastric fluid simulated media when compared to intestinal fluid and river water. As observed in the current study, Liu et al. [36] demonstrated that aging polystyrene (PS) microparticles (~ 50 μm) suppressed the desorption of pharmaceuticals. According to the authors, the reason is that the aging of the microplastics decreases the hydrophobic and π-π interactions, but at the same time, increases the electrostatic interaction between aged microplastics and pharmaceuticals, which were then less affected by the media components (gastrointestinal). The experimental data from this work and reported in the literature fitting the pseudo second order kinetics suggests chemical adsorption over a physical process (e.g., Van der Waals forces) dominates the interactions between fluoxetine and the microplastics investigated [37]. In a study conducted by Wang and Wang [38], the adsorption of pyrene, a four fused-ring polycyclic aromatic hydrocarbon (PAH), onto PE, PS, and PVC also fitted the pseudo second order model. The same was observed when the antibiotic ofloxacin was in contact with PVC microparticles [39].

In the current study, isotherms were also determined to evaluate the adsorption mechanism of fluoxetine when in contact with PP, PA, and PVC. Results demonstrated that, again the isotherm model depends on the type and the weathering of the microplastics. For the three microplastic types tested, the virgin and the artificially aged particles showed different fits to the isotherm models investigated. In general, the greater the amount adsorbed onto the microplastics, the greater the amount desorbed from the microplastics (Figs. 4F and 4I). A positive correlation (R^2 0.7868) between the amount adsorbed onto the microplastics and the amount desorbed from the microplastics was observed when the data for PA and PVC were combined (Fig. S10). The Pearson correlation (r) also demonstrated a strong correlation ($r > 0.7$) between the amount of fluoxetine loaded onto the microplastics and fluoxetine desorbed from microplastics in the toxicity investigation ($r = 0.89$). Furthermore, a strong correlation was observed between the adsorption of fluoxetine onto microplastics and the microplastics surface area (S_{BET}, $r = 1$), the degree of crystallinity (X_C, $r = 0.95$), and the surface charge of the microplastics (ζ, $r = 0.88$, Table S5, microplastic characterisation data can be found in previous publications [20,21]). On the other hand, the amount desorbed from the microplastics showed a positive strong correlation to the density ($r = 0.79$) of the microplastics and to their glass transition temperature (T_g, $r = 0.89$), while a strong negative correlation ($r < -0.7$) was observed between the amount of desorption of fluoxetine from microplastics and the S_{BET} of the microplastics (S_{BET}, $r = -0.9$) and their surface charge (ζ, $r = 0.75$, Table S5). That means that although microplastics with great porosity and surface charge, such as PP and PVC, can adsorb greater amounts of co-occurring organic compounds, especially positively charged and hydrophobic compounds, these microplastics tend to desorb lower amounts of micropollutant when compared to low porosity and surface charge microplastics such as PA.

For the microplastics evaluated, none of the isotherms fitted a linear model. That means that 'adsorption' was the key mechanism regarding the interaction of fluoxetine with microplastics, and no 'absorption' was observed. The desorption of fluoxetine from the microplastics also varied according to the type and weathering of the microplastics. However, in general, the desorption of fluoxetine fits a linear model (Fig. 5). Since no desorption was observed from virgin PP, the experimental data could not be fitted to any isotherm model. For PP and PVC, the Langmuir model showed the best fit to the experimental data for both the virgin (R^2_{PP} 0.9983; R^2_{PVC} 0.9935) and the aged particles (R^2_{PP} 0.9801; R^2_{PVC} 0.9990; Fig. 5). That means that the aging of the particles did not alter the monolayer adsorption behaviour of fluoxetine onto PP and PVC. Like PP and PVC, the aging of PA did not modify the adsorption mechanism of fluoxetine in contact with PA. However, multilayer adsorption with intramolecular interaction of fluoxetine (Freundlich model) was the adsorption mechanism of PA (R^2_{Virgin} 0.9979; R^2_{Aged} 0.9866, Table S6). Intramolecular interactions are typically weaker than the interaction between pharmaceuticals such as fluoxetine when binding to microplastics [31]. As a result, when intramolecular interactions dominate, greater desorption and, consequently, increased toxicity can be expected. However, the results in the current study showed similar toxicity for all microplastic types investigated on *D. magna* neonates, independent of the mechanism of adsorption of the microplastics. For the desorption determination, the experimental data of PA fitted the linear model for both virgin and aged particles of PA (Table S6). For PVC, two different desorption mechanisms were observed when comparing virgin and aged particles. The desorption data of virgin PVC fitted to the Freundlich model (R^2 0.9862), while the aged particles fit the linear model (R^2 0.9943, Fig. 5, Table S6).

4. Conclusions

The acute effect of fluoxetine loaded onto microplastics on *D. magna* neonate's motility and survival was evaluated. Further, the availability of fluoxetine adsorbed onto three virgin and artificially aged microplastics (PP, PA, and PVC) in freshwater was investigated. In the current study, the microplastic type and weathering of the particles were the key factors affecting the availability and acute toxicity of fluoxetine-loaded microplastics. The toxicity test highlights the complexity of the interaction of micropollutant-loaded microplastics when ingested by aquatic organisms. Although, results indicated that PP might be a sink for fluoxetine with a negligible desorption, all three microplastic types investigated when loaded with fluoxetine, including PP, had a negative impact on the *D. magna* neonate survival, particularly in the case of the virgin particles. The adsorption of a micropollutant onto microplastics can give insights on their toxicity. However, the findings of this study demonstrated that adsorption alone might not represent the true toxicity micropollutant-loaded microplastics aquatic organisms such as *D. magna*. The artificially aged microplastics showed greater adsorption of fluoxetine, however virgin microplastics demonstrated a greater toxicity when exposed to *D. magna*. Furthermore, PP demonstrated approximately 10-fold greater adsorption of fluoxetine when compared to PA and PVC, while the fluoxetine-loaded PP showed a similar toxicity to the neonates as the two other polymers when loaded with fluoxetine. This study has demonstrated that the ingestion of microplastics loaded with micropollutants is a route for micropollutants into the food-web resulting in potentially hazardous effects to wildlife. For future research, examining biochemical indices like enzymatic activity and metabolomics in *D. magna* neonates exposed to micropollutant-loaded microplastics will enhance the understanding of disruptions in key

biological pathways, such as oxidative stress and metabolism.

Environmental implication

The ingestion of microplastics by aquatic organisms is a reality. However, the effects of ingesting microplastics, especially those loaded with contaminants, are poorly understood. This study shows that fluoxetine, a widely prescribed antidepressant, not only accumulates onto the surface of the types of microplastics found in the aquatic environment, but also desorbs from these microplastics and is available under freshwater conditions. More concerning is that fluoxetine-loaded microplastics result in toxic affects when consumed by *D. magna*, a water quality indicator organism.

CRediT authorship contribution statement

J. Pestana Carlos: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. **A. Lawton Linda:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Hui Jianing:** Methodology. **T. S. Irvine John:** Methodology. **F. Moffat Colin:** Writing – review & editing, Supervision, Resources. **Gkoulemani Nikoletta:** Methodology. **S. Moura Diana:** Writing – original draft, Visualization, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank the Hydro Nation Scholar Programme funded by the Scottish Government through the Scottish Funding Council and managed by the Hydro Nation International Centre for funding this research.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2025.137645.

Data availability

Data will be made available on request.

References

[1] Sun, X., Li, Q., Zhu, M., Liang, J., Zheng, S., Zhao, Y., 2017. Ingestion of microplastics by natural zooplankton groups in the northern. Mar Pollut Bull 115, 217–224. https://doi.org/10.1016/j.marpolbul.2016.12.004.

[2] Eryaşar, A.R., Gedik, K., Mutlu, T., 2022. Ingestion of microplastics by commercial fish species from the southern Black Sea coast. Mar Pollut Bull 177, 1–8. https://doi.org/10.1016/j.marpolbul.2022.113535.

[3] Philipp, C., Unger, B., Siebert, U., 2022. Occurrence of Microplastics in Harbour Seals (Phoca vitulina) and Grey Seals (Halichoerus grypus) from German Waters. Animals 12, 1–13. https://doi.org/10.3390/ani12050551.

[4] Gong, X., Wang, Y., Huang, D., Zhang, J., 2022. Effects of microplastics of different sizes on the Chlorella vulgaris - Ganoderma lucidum co-pellets formation processes. Sci Total Environ 820, 153266. https://doi.org/10.1016/J.SCITOTENV.2022.153266.

[5] Aiguo, Z., Di, S., Chong, W., Yuliang, C., Shaolin, X., Peiqin, L., et al., 2022. Characteristics and differences of microplastics ingestion for farmed fish with different water depths, feeding habits and diets. J Environ Chem Eng 10, 107189. https://doi.org/10.1016/J.JECE.2022.107189.

[6] Horton, A.A., Vijver, M.G., Lahive, E., Spurgeon, D.J., Svendsen, C., Heutink, R., et al., 2018. Acute toxicity of organic pesticides to *Daphnia magna* is unchanged by co-exposure to polystyrene microplastics. Ecotoxicol Environ Saf 166, 26–34. https://doi.org/10.1016/J.ECOENV.2018.09.052.

[7] Lee, Y., Yoon, D.S., Lee, Y.H., Kwak, J.Il, An, Y.J., Lee, J.S., et al., 2021. Combined exposure to microplastics and zinc produces sex-specific responses in the water flea *Daphnia magna*. J Hazard Mater 420, 126652. https://doi.org/10.1016/J.JHAZMAT.2021.126652.

[8] Nkoom, M., Lu, G., Liu, J., Yang, H., Dong, H., 2019. Bioconcentration of the antiepileptic drug carbamazepine and its physiological and biochemical effects on *Daphnia magna*. Ecotoxicol Environ Saf 172, 11–18. https://doi.org/10.1016/J.ECOENV.2019.01.061.

[9] Villegas-Navarro, A., Romero González, M.C., Rosas López, E., Domínguez Aguilar, R., Sachetin Marçal, W., 1999. Evaluation of *Daphnia magna* as an indicator of toxicity and treatment efficacy of textile wastewaters. Environ Int 25, 619–624. https://doi.org/10.1016/S0160-4120(99)00034-3.

[10] Rodrigues, S., Pinto, I., Nogueira, S., C. Antunes, S., 2023. Potential indicator of reservoir water quality – current status and perspectives focused in ecotoxicological classes regarding the risk prediction. In: Massarelli, C., Campanale, C. (Eds.), Importance of monitoring correl. Of lentic lotic waters intensif. IntechOpen, p. 138.

[11] Jeong, T.Y., Simpson, M.J., 2019. *Daphnia magna* metabolic profiling as a promising water quality parameter for the biological early warning system. Water Res 166, 115033. https://doi.org/10.1016/j.watres.2019.115033.

[12] An, D., Na, J., Song, J., Jung, J., 2021. Size-dependent chronic toxicity of fragmented polyethylene microplastics to *Daphnia magna*. Chemosphere 271, 129591. https://doi.org/10.1016/j.chemosphere.2021.129591.

[13] Canniff, P.M., Hoang, T.C., 2018. Microplastic ingestion by *Daphnia magna* and its enhancement on algal growth. Sci Total Environ 633, 500–507. https://doi.org/10.1016/j.scitotenv.2018.03.176.

[14] Hampton LMT, Bouwmeester H, Brander SM, Coffin S, Cole M, Hermabessiere L, et al. Research recommendations to better understand the potential health impacts of microplastics to humans and aquatic ecosystems; n.d. ⟨https://doi.org/10.1186/s43591-022-00038-y⟩.

[15] Koelmans, A.A., Mohamed Nor, N.H., Hermsen, E., Kooi, M., Mintenig, S.M., De France, J., 2019. Microplastics in freshwaters and drinking water: critical review and assessment of data quality. Water Res 155, 410–422. https://doi.org/10.1016/j.watres.2019.02.054.

[16] Ogonowski, M., Schür, C., Jarsén, Å., Gorokhova, E., 2016. The effects of natural and anthropogenic microparticles on individual fitness in *Daphnia magna*. PLoS One 11, 1–20. https://doi.org/10.1371/journal.pone.0155063.

[17] Napper, I.E., Thompson, R.C., 2019. Marine plastic pollution: other than microplastic. In: Waste. Academic Press, pp. 425–442. https://doi.org/10.1016/B978-0-12-815060-3.00022-0.

[18] Wilkinson, J.L., Boxall, A.B.A., Kolpin, D.W., Leung, K.M.Y., Lai, R.W.S., Galban-Malag, C., et al., 2022. Pharmaceutical pollution of the world's rivers. Proc Natl Acad Sci USA 119, e2113947119. https://doi.org/10.1073/PNAS.2113947119/-/DCSUPPLEMENTAL.

[19] Wang, Y., Yin, T., Kelly, B.C., Gin, K.Y.-H., 2019. Bioaccumulation behaviour of pharmaceuticals and personal care products in a constructed wetland. Chemosphere 222, 275–285. https://doi.org/10.1016/J.CHEMOSPHERE.2019.01.116.

[20] Moura, D.S., Pestana, C.J., Moffat, C.F., Gkoulemani, N., Hui, J., Irvine, J.T.S., et al., 2024. Aging microplastics enhances the adsorption of pharmaceuticals in freshwater. Sci Total Environ 912, 169467. https://doi.org/10.1016/j.scitotenv.2023.169467.

[21] Moura, D.S., Pestana, C.J., Moffat, C.F., Hui, J., Irvine, J.T.S., Lawton, L.A., 2023. Characterisation of microplastics is key for reliable data interpretation. Chemosphere 331, 138691. https://doi.org/10.1016/j.chemosphere.2023.138691.

[22] Akkanen, J., Kukkonen, J.V.K., 2003. Biotransformation and bioconcentration of pyrene in *Daphnia magna*. Aquat Toxicol 64, 53–61. https://doi.org/10.1016/S0166-445X(03)00023-7.

[23] Brooks, B.W., Turner, P.K., Stanley, J.K., Weston, J.J., Glidewell, E.A., Foran, C.M., et al., 2003. Waterborne and sediment toxicity of fluoxetine to select organisms. Chemosphere 52, 135–142. https://doi.org/10.1016/S0045-6535(03)00103-6.

[24] Nelms, S.E., Galloway, T.S., Godley, B.J., Jarvis, D.S., Lindeque, P.K., 2018. Investigating microplastic trophic transfer in marine top predators. Environ Pollut 238, 999–1007. https://doi.org/10.1016/j.envpol.2018.02.016.

[25] Bao, S., Xian, B., Yi, J., Rao, C., Xiang, D., Wu, Y., et al., 2025. Aging process potentially aggravates microplastic toxicity in aquatic organisms: evidence from a comprehensive synthesis. Sci Total Environ 958, 177992. https://doi.org/10.1016/J.SCITOTENV.2024.177992.

[26] Wagstaff, A., Petrie, B., 2022. Enhanced desorption of fluoxetine from polyethylene terephthalate microplastics in gastric fluid and sea water. Environ Chem Lett 1 (2022), 1–8. https://doi.org/10.1007/S10311-022-01405-0.

[27] Ebert, D., 2005. Introduction to Daphnia biology. In: Ecol. epidemiol. evol. parasit. daphnia. National Center for Biotechnology Information, US, pp. 1–21. ⟨https://www.ncbi.nlm.nih.gov/books/NBK2042/⟩.

[28] Varano, V., Fabbri, E., Pasteris, A., 2017. Assessing the environmental hazard of individual and combined pharmaceuticals: acute and chronic toxicity of fluoxetine and propranolol in the crustacean *Daphnia magna*. Ecotoxicology 26, 711–728. https://doi.org/10.1007/s10646-017-1803-6.

[29] Schwarzer, M., Brehm, J., Vollmer, M., Jasinski, J., Xu, C., Zainuddin, S., et al., 2022. Shape, size, and polymer dependent effects of microplastics on *Daphnia magna*. J Hazard Mater 426, 128136. https://doi.org/10.1016/J.JHAZMAT.2021.128136.

[30] Zimmermann, L., Göttlich, S., Oehlmann, J., Wagner, M., Völker, C., 2020. What are the drivers of microplastic toxicity? Comparing the toxicity of plastic chemicals and particles to *Daphnia magna*. Environ Pollut 267, 115392. https://doi.org/10.1016/J.ENVPOL.2020.115392.

[31] Atugoda, T., Vithanage, M., Wijesekara, H., Bolan, N., Sarmah, A.K., Bank, M.S., et al., 2021. Interactions between microplastics, pharmaceuticals and personal care

products: implications for vector transport. Environ Int 149, 106367. https://doi.org/10.1016/j.envint.2020.106367.
[32] Li, W., Zu, B., Li, L., Li, J., Li, J., Mei, X., 2023. Desorption of bisphenol A from microplastics under simulated gastrointestinal conditions. Front Mar Sci 10, 1195964. https://doi.org/10.3389/FMARS.2023.1195964/BIBTEX.
[33] Zhuang, S., Wang, J., 2023. Interaction between antibiotics and microplastics: recent advances and perspective. Sci Total Environ 897, 165414. https://doi.org/10.1016/J.SCITOTENV.2023.165414.
[34] Moura, D.S., Pestana, C.J., Moffat, C.F., Hui, J., Irvine, J.T.S., Edwards, C., et al., 2022. Adsorption of cyanotoxins on polypropylene and polyethylene terephthalate: microplastics as vector of eight microcystin analogues. Environ Pollut 303, 119135. https://doi.org/10.1016/j.envpol.2022.119135.
[35] Yao, L., Hui, L., Yang, Z., Chen, X., Xiao, A., 2020. Freshwater microplastics pollution: detecting and visualizing emerging trends based on Citespace II. Chemosphere 245, 125627. https://doi.org/10.1016/j.chemosphere.2019.125627.
[36] Liu, P., Lu, K., Li, J., Wu, X., Qian, L., Wang, M., et al., 2020. Effect of aging on adsorption behavior of polystyrene microplastics for pharmaceuticals: adsorption mechanism and role of aging intermediates. J Hazard Mater 384, 121193. https://doi.org/10.1016/J.JHAZMAT.2019.121193.
[37] Wagstaff, A., Lawton, L.A., Petrie, B., 2021. Polyamide microplastics in wastewater as vectors of cationic pharmaceutical drugs. Chemosphere, 132578. https://doi.org/10.1016/J.CHEMOSPHERE.2021.132578.
[38] Wang, W., Wang, J., 2018. Comparative evaluation of sorption kinetics and isotherms of pyrene onto microplastics. Chemosphere 193, 567–573. https://doi.org/10.1016/j.chemosphere.2017.11.078.
[39] Yu, F., Li, Y., Huang, G., Yang, C., Chen, C., Zhou, T., et al., 2020. Adsorption behavior of the antibiotic levofloxacin on microplastics in the presence of different heavy metals in an aqueous solution. Chemosphere 260, 127650. https://doi.org/10.1016/J.CHEMOSPHERE.2020.127650.

Supporting Information for

Toxic effects of fluoxetine-loaded onto virgin and aged polypropylene, polyamide and polyvinyl chloride microparticles on *Daphnia magna*

Diana S. Moura[a,*], Pestana, Carlos J[a], Moffat, Colin F[a], Gkoulemani, Nikoletta[b], Hui, Jianing[b], Irvine, John T. S[b] and Lawton, Linda A[a]

[a]*School of Pharmacy and Life Sciences, Robert Gordon University, Aberdeen, AB10 7GJ, UK*

[b]*School of Chemistry, University of St Andrews, North Haugh, St Andrews, Scotland, KY16 9ST, UK.*

* Corresponding Author. Tel. +44 1224 2837. E-mail address: d.souza-moura2@rgu.ac.uk

S1 Material and methods

S1.1 Quantification of fluoxetine in the solution

Analysis of the pharmaceuticals was performed using high performance liquid chromatography (HPLC; Waters Corporation, UK). The equipment included a solvent delivery system (Alliance 2695) with detection by photodiode array (PDA, Alliance 2996). The PDA scanning wavelength was set from 200 to 400 nm. Separation of fluoxetine was achieved using a Symmetry dC18 column (2.1 mm internal diameter x 150 mm; 5 µm particles size) which was maintained at 40 °C. The mobile phases were ultra-pure water (18.2 MΩ) (A) and acetonitrile (B) each containing 0.05% (v/v) trifluoroacetic acid (TFA; Fisher Scientific UK Ltd, UK). The flow rate was 0.3 mL min^{-1}. A linear gradient was used for the separation of fluoxetine. Initial mobile phase composition of 90 % (A) was reduced to 20 % (A) over 21 min. A step gradient was used to reduce from 20 % (A) to 0 % (A). This was maintained for 5 min before returning to the starting conditions. Re-equilibration of the column was achieved by further elution of the column for 9 min prior to the next injection. The total run time was 35 min, and the injection volume was 35 µL. The peak for fluoxetine (227 nm) was measured according to the maximum absorbance for this compound based on its UV absorption spectrum. The limit of detection and limit of quantification of fluoxetine using this method was 0.01 µg mL^{-1} and 0.05 µg mL^{-1}, respectively.

The concentration of fluoxetine was measured:
(1) in the fluoxetine solution prepared to load microplastics and in the solution containing fluoxetine placed in contact with microplastics (2 g L^{-1} plastics) after 24 h contact to evaluate the concentration of fluoxetine adsorbed onto microplastics;
(2) in the solution of fluoxetine control prior to experimentation and at the end of the experiment to evaluate potential fluoxetine degradation during the experiment; and
(3) in the vials containing fluoxetine-loaded microplastics (50 and 100 µg mL^{-1}) at the end of the experiment (48 h) to evaluate the concentration of fluoxetine in the media resulting from desorption from the fluoxetine-loaded microplastics.

S1.2 Data analysis

The amount of pharmaceutical adsorbed per unit mass of microplastic ($\mu g\ mg^{-1}$), was estimated using equation S1:

$$q^*_{t(A)} = \left(C_{ctrl(t)} - C_{(t)}\right)V/m \quad \text{(S1)}$$

The amount of pharmaceutical desorbed per unit mass of microplastic ($\mu g\ mg^{-1}$), was estimated using equation S2:

$$q^*_{t(D)} = \left(C_{(t)} - C_{ctrl(t)}\right)V/m \quad \text{(S2)}$$

where,

- $q_{(t)}$ is the amount of pharmaceutical adsorbed/desorbed onto/from the microplastic ($\mu g\ mg^{-1}$) at sampling time *t*
- $C_{ctrl(t)}$ is the control solution concentration of the pharmaceutical ($\mu g\ mL^{-1}$) at the sampling time *t* as determined by HPLC-PDA
- $C_{(t)}$ is the sample solution concentration of the pharmaceutical ($\mu g\ mL^{-1}$) at the sampling time *t* as determined by HPLC-PDA
- *m* is the mass of plastic added to the Erlenmeyer flask (g)
- *V* is the total volume of solution (L) in the Erlenmeyer flask

Note: * Negative values were assigned a zero value.

The percentage of pharmaceutical adsorbed at a specific sample time point (t) onto the microplastic was calculated using equation S3:

$$\%Adsorbed_{(t)} = ((C_{ctrl(t)} - C_{(t)})x100)/C_{ctrl(t)} \quad \text{(S3)}$$

where,

- $\%Adsorbed_{(t)}$ is the percent of pharmaceutical adsorbed onto the microplastic at sampling time t

The amount of pharmaceutical adsorbed and desorbed per unit mass of microplastic ($\mu g\ mg^{-1}$) at equilibrium, was estimated using equation S4:

$$q_e = \left(C_{ctrl(24\,h)} - C_e\right)V/m \quad \text{(S4)}$$

where,

- q_e is the amount of pharmaceutical adsorbed/desorbed onto/from the microplastic ($\mu g\ mg^{-1}$) at sampling time *24 h*
- $C_{ctrl(24h)}$ is the control solution concentration of the pharmaceutical ($\mu g\ mL^{-1}$) at the sampling time 24 h as determined by HPLC-PDA

- C_e is the sample solution concentration of the pharmaceutical (µg mL^{-1}) at equilibrium which consisted of the sampling time 24 h as determined by HPLC-PDA
- m is the mass of plastic added to the Erlenmeyer flask (mg)
- V is the total volume of solution (mL) in the Erlenmeyer flask

The kinetics experiments were conducted with an initial fluoxetine concentration of 5 µg mL^{-1}, the experimental data were fitted to two widely accepted kinetic models: the pseudo-first order and pseudo-second order models (Table S1).

Table S1: Kinetic models applied to the experimental data of fluoxetine with virgin and artificially aged particles of PP, PA, and PVC.

Kinetics model	Equation	Linear form	Plot
Pseudo first order	$\frac{dqt}{dt} = K_1(q_e - q_t)$	$\log(q_e - q_t) = \log q_e - \frac{K_1}{2.303}t$	$\log(q_e - q_t)\ vs\ t$
Pseudo second order	$\frac{dqt}{dt} = K_2(q_e - q_t)^2$	$\frac{1}{q_t} = \frac{1}{K_2 q_e^2} + \frac{1}{q_e}t$	$\frac{t}{q_t}\ vs\ t$
Interparticle diffusion	$qt = K_i t^{1/2} + C$	-	$q_t vs\ t^{0.5}$

where,

- q_e is the amount of compound adsorbed per mass of adsorbent at equilibrium (µg mg^{-1})
- q_t is the amount of compound adsorbed per mass of adsorbent at time t (µg mg^{-1})
- K_1, K_2, *and* K_i are the first order (L g^{-1}), second order (L g^{-1}), and intraparticle diffusion (µg g^{-1} h$^{-1/2}$) constant, respectively

Adsorption/desorption isotherm models applied to the experimental data of fluoxetine with the virgin and artificially aged particles of PP, PA, and PVC are presented in the Table S2.

Table S2: Adsorption isotherm models applied to the experimental data of virgin and artificially aged PP, PA, and PVC in contact with five different concentrations of fluoxetine (1-15 µg mL^{-1}).

Adsorption isotherm	Equation	Linear form	Plot
Freundlich model	$q_e = K_F C_e^n\ ;\ n = \frac{1}{n_F}$	$\log q_e = \log K_F + n \log C_e$	$\log q_e\ vs \log C_e$
Langmuir model	$q_e = \frac{K_L C_e}{1+\alpha_l C_e}\ ;\ Q_O = \frac{K_L}{\alpha_L}$	$\frac{C_e}{q_e} = \frac{\alpha_L}{K_L}C_e + \frac{1}{K_L}$	$\frac{C_e}{q_e}\ vs\ C_e$
Linear model	$q_e = K_p C_e$	$q_e = K_p + C_e$	$q_e\ vs\ C_e$

where,

- q_e is the amount of compound adsorbed per mass of adsorbent at equilibrium (µg mg^{-1})
- C_e is the residual adsorbate concentration in the solution at equilibrium (µg mL^{-1}) determined by HPLC-PDA
- K_F and n_F are the Freundlich constant (L g^{-1}) and exponent, respectively

- Q_0 is the maximum adsorption capacity (μg mg^{-1})
- α_L is energy of adsorption (L g^{-1})
- K_L is Langmuir constant (L g^{-1})
- K_p is Partition constant (L g^{-1})

Student's t-test was carried out to perform significance testing (Microsoft Excel). For all statistical tests, a significance level of 5% was set. A Pearson correlation matrix was performed to evaluate a correlation between variables of this study (Microsoft Excel; Table S3). A correlation coefficient (r) greater than 0.7 was considered a strong positive association, while a r lower than -0.7 was considered a strong negative association.

S1.3 Ecotoxicity test experimental design

To evaluate whether fluoxetine-loaded microplastics can act as a vector for fluoxetine into the food web, an ecotoxicity experiment with *Daphnia magna* neonates was performed using a Daphtoxkit F (Microbiotest, BE). The experimental media consisted of artificial freshwater (AFW), which was prepared with ultrapure water (18.2 MΩ) and the addition of $CaCl_2.2H_2O$ (294 mg L^{-1}), $MgSO_4.7H_2O$ (123.25 mg L^{-1}), $NaHCO_3$ (64.75 mg L^{-1}), and KCl (5.75 mg L^{-1}) according to the kit manual. The pH of the media was determined as 8. The media was constantly sparged with sterile air for 15 min before use for both hatching the dormant eggs and to prepare the solutions used in this experiment. The ephippia were poured into a microsieve, rinsed with tap water, and transferred to a petri dish with 15 mL pre-aerated AFW. The covered petri dish was incubated for 72 h at 20 °C under continuous illumination of approximately 6,000 lux (Figure S1). To assure the light intensity, the cool white florescence lamp irradiation was measured using a StellarNet spectrometer (BLACK-Comet C-RS-50 model, USA, Figure S2).

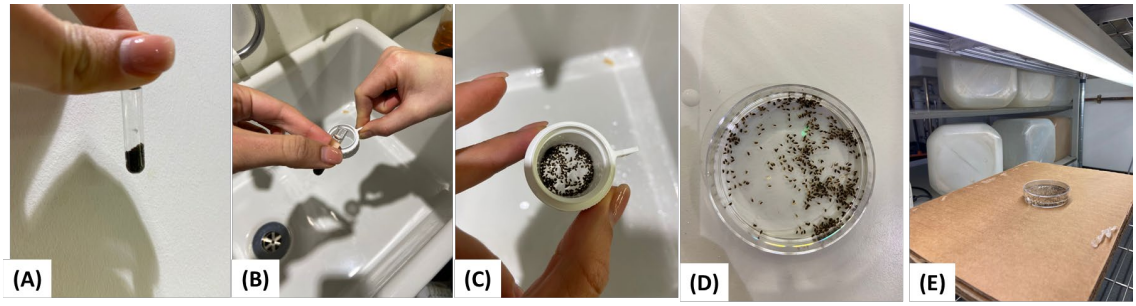


Figure S1: Hatching procedure for the Daphtoxkit (A) vial containing ephippia, (B) pouring ephippia into the microsieve, (C) rinsing the ephippia with tap water, (D) ephippia in 15 mL pre-aerated artificial freshwater pH 8, (E) hatching the ephippia for 72 h at 20 °C under 6,000 lux.

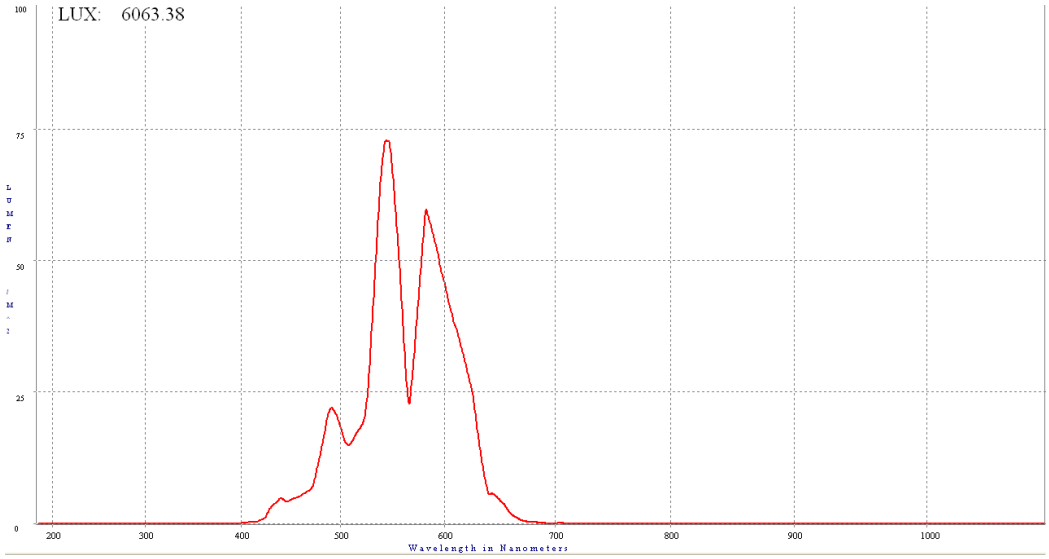


Figure S2: Light spectrum and intensity of the light measured by the StellarNet which the *D. magna* ephippia were exposed to for 72 h at 20 °C during the hatching process.

Two hours prior to collecting the neonates for the experiment, a suspension of spirulina was poured into the hatching petri dish (Figure S3 and Figure S4). This food uptake provides the neonates with an energetic reserve and precludes mortality by starvation (which would bias the test results) during the subsequent 48 h test exposure during which the organisms are not fed.

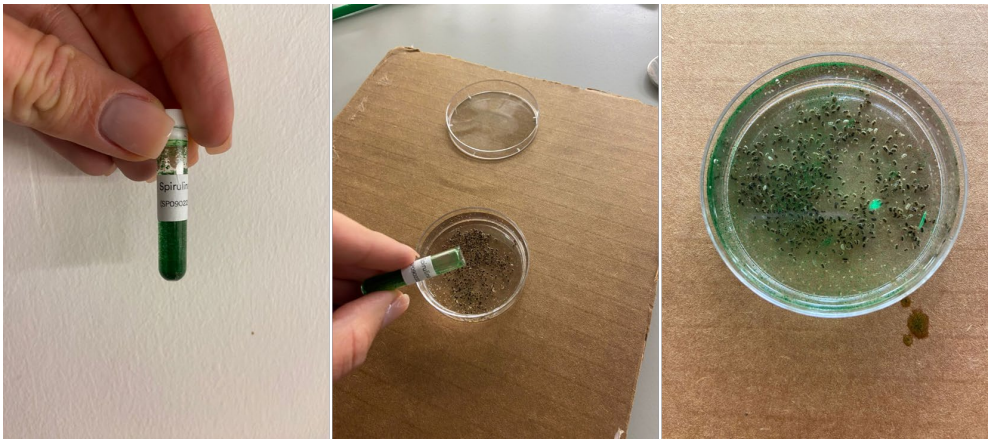


Figure S3: Pre-feeding of the *D. magna* neonates, which consisted of a vial containing spirulina powder provided by Daphtoxkit that was dissolved in the media and poured into the hatching dish two hours prior to collecting the neonates.

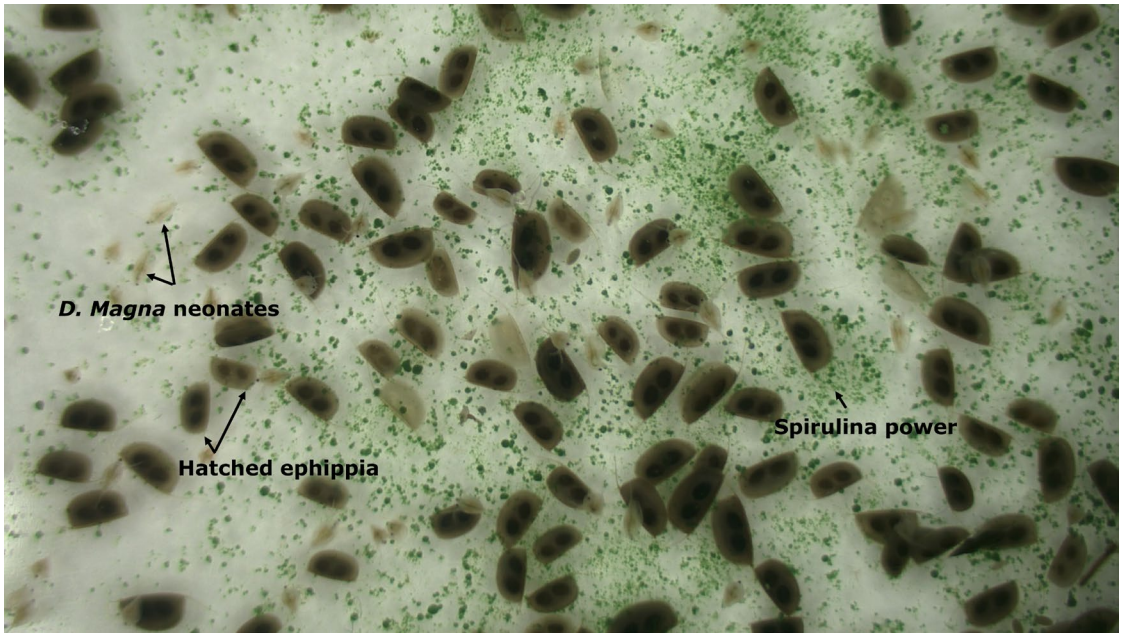


Figure S4: Hatched petri dish after 2 h pre-feeding with spirulina power. The arrows point to the neonates, the hatched ephippia, and the spirulina power (green). The blurriness of neonates was caused by the motility of the organisms.

A polystyrene (PS) multiwell plate was provided by the supplier as a neonate incubator. However, as demonstrated by this study, fluoxetine can adsorb onto the surface of plastics, which can interfere with the fluoxetine concentrations throughout the experiment. For each treatment, instead of using a PS multiwell plate, the experimental incubator consisted of four 20 mL glass vials, grouped in a 400 mL beaker covered with a PS petri dish lid to avoid cross contamination of potential volatile compounds during the experiment (Figure 5.6) and to limit evaporation.

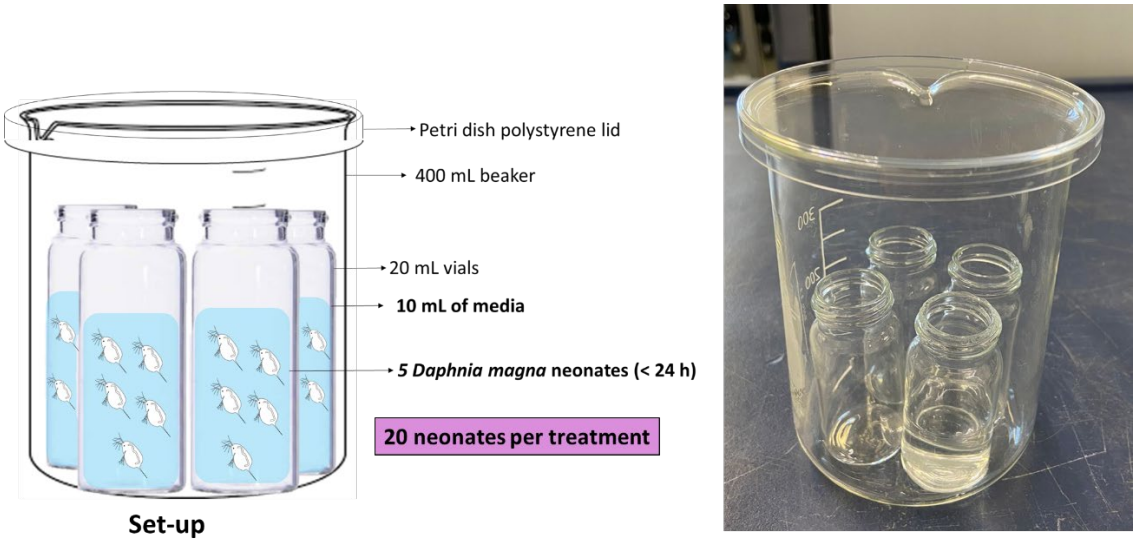


Figure S5: Adapted incubator used for each treatment consisting of four 20 mL vials grouped in a 400 mL beaker covered with a polystyrene petri dish.

S1.4 Visualisation of the neonates and capture of images

A dissecting microscope (HWF 15x, Vickers instruments, UK) was used to facilitate:

(1) transfer the neonates from the hatching petri dish to the rinse vial;

(2) transfer the neonates from the rinse vial to the four experimental vials; and

(3) counting the number of dead neonates after 24 h and 48 h exposure to the treatments (Figure S7).

The neonates which were not able to swim after gentle agitation of the liquid for 15 seconds were considered immobilized, even if they could still move their antennae. To avoid repetition and extended phrases, both immobilised and dead neonates will be considered ‘dead’ in this chapter.

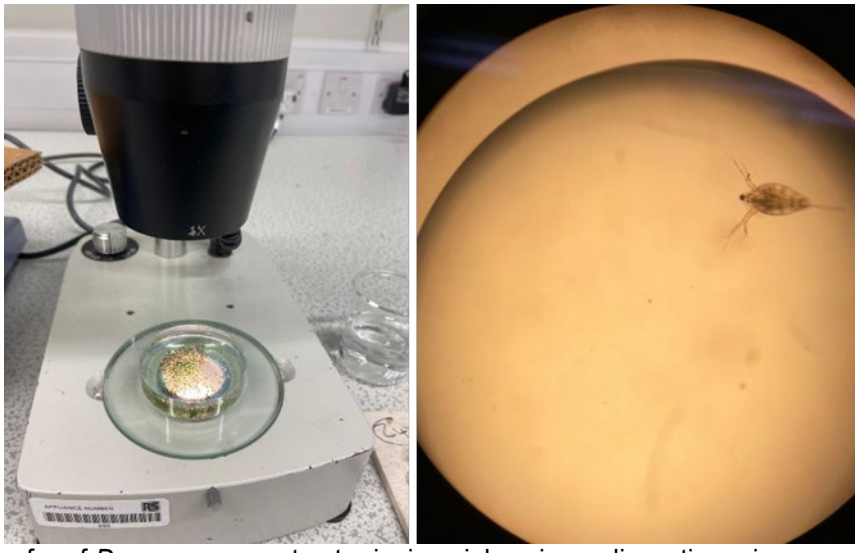


Figure S6: Transfer of *D. magna* neonates to rinsing vials using a dissection microscope (left) and an image of a neonate at magnification 15x (right).

After assessing the toxic response of the *D. magna* neonates after 48 h exposure, at least three organisms from different vials were collected using a Pasteur pipette and placed in a well plate (Thermofisher scientific, UK) to take the photographs (Figure S8). After placing the neonate in the well plate, the maximum possible amount of water was removed to immobilise the individual neonate prior to taking the photograph. From the three individuals, one was randomly selected as a representative for each of the treatments. The pictures of the *D. magna* neonates were taken using a stereo microscope (S1503 model, Sunny Instruments, Singapore) with a coupled camera (YenCam 16, Yenmay, China).

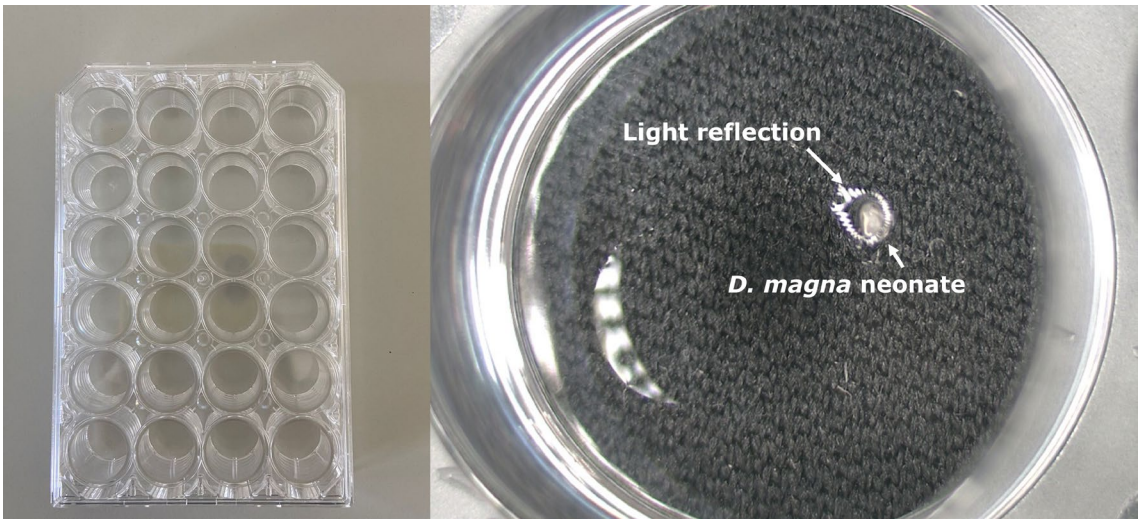


Figure S7: Well plate (left) used to take the micrograph of the neonates after 48 h exposure to the experimental treatments. A single individual was placed in each well to take the micrograph (right). A

black background was used for improved contrast with the white/clear microplastics ingested by the neonates. Magnification 10x (right).

S1.5 Adsorption / desorption experimental design

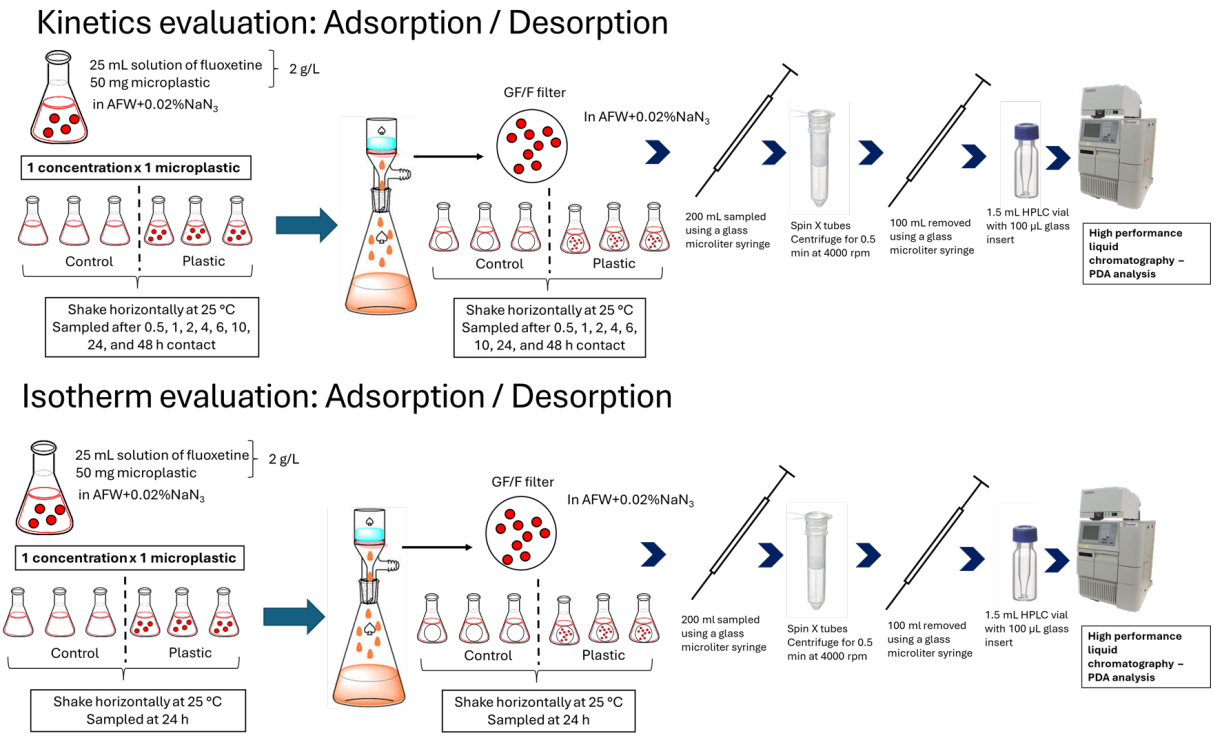


Figure S8: Adsorption / desorption experimental design of fluoxetine in contact with polypropylene (PP), polyamide (PA) and polyvinyl chloride (PVC).

S2 Results

As expected, neither microplastics nor any particles were observed in the guts of the organisms from the protocol control (media only) or from the fluoxetine control (Figure S8). Furthermore, after the 48 h of experiment, there were no remnants of spirulina in the gut of the neonates.

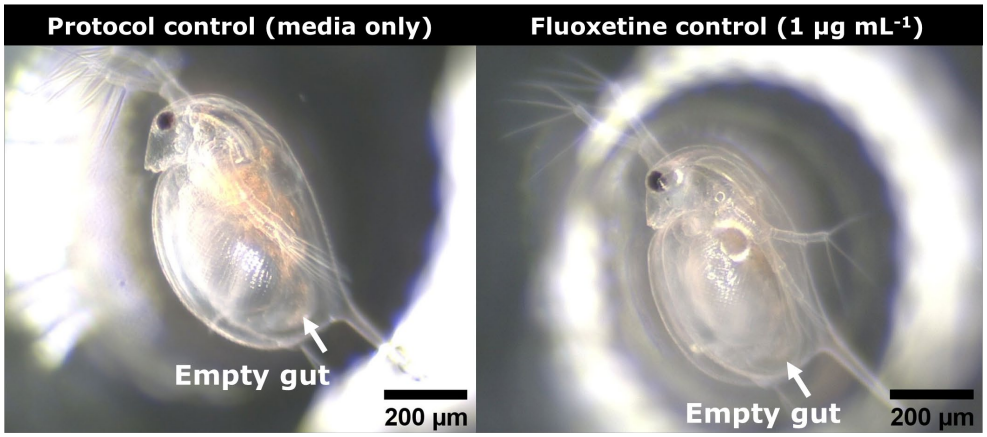


Figure S9: *D. magna* neonates after 48 h in the media only (left) and in a solution containing fluoxetine at 1 µg mL^{-1} (right). The arrows point to the empty gut of the neonates. Magnification 45x.

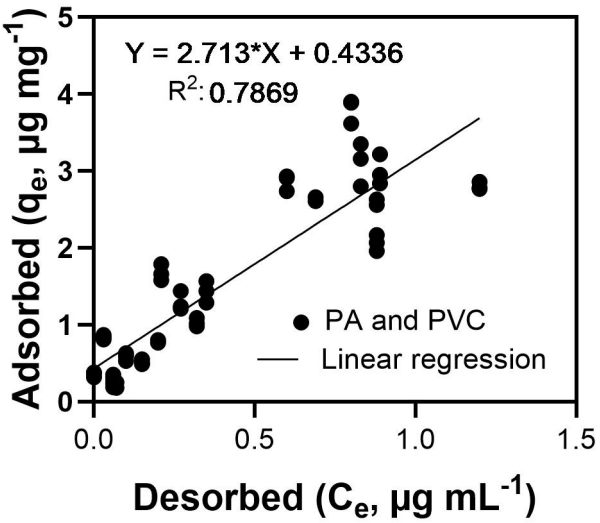


Figure S10: Comparison between the amount adsorbed at equilibrium (q_e) onto microplastics and the concentration desorbed at equilibrium (C_e) from the microplastics at the five different fluoxetine initial concentrations (PP: 15-100 µg mL^{-1}; PA and PVC: 1-15 µg mL^{-1}). n=3, errors bars = 1 SD. Note: PP was not considered for the evaluation of the correlation between adsorption vs desorption since no desorption was observed.

Table S3: Comparison between the fluoxetine concentration detected in the fluoxetine-loaded treatments of the toxicity tests with the predicted concentration considering the amount adsorbed onto microplastics was fully desorbed.

Condition of plastic	Plastic	Plastic concentration (g L^{-1})	Average amount adsorbed onto microplastics (µg mg^{-1})	Average predicted concentration in solution if 100% desorbed (ug mL^{-1})	Average concentration detected in solution (ug mL^{-1})	Detected/ Predicted (%)
Virgin	PP	50	37.20	1.86	0.34	18%
		100	37.20	3.72	0.41	11%
Aged	PP	50	35.36	1.77	0.22	12%
		100	35.36	3.54	0.33	9%

Virgin	PA	50	3.46	0.17	0.13	74%
		100	3.46	0.35	0.20	56%
Aged	PA	50	4.53	0.23	0.11	47%
		100	4.53	0.45	0.15	34%
Virgin	PVC	50	3.28	0.16	0.07	45%
		100	3.28	0.33	0.08	25%
Aged	PVC	50	4.00	0.20	0.06	28%
		100	4.00	0.40	0.07	19%

Table S4: Summary of the adsorption and desorption parameters of the pseudo first order and second order models. Desorption data was not obtained for PP as explained in the manuscript.

Adsorption				
Kinetics model	**Plastic**	**Parameters**		
Pseudo first order		**k_1 (h^{-1})**	**q_e (μg mg^{-1})**	**R^2**
	PP_{VIRGIN}	0.0561	1.88	0.9095
	PP_{AGED}	0.0367	2.03	0.7049
	PA_{VIRGIN}	0.0529	0.59	0.8785
	PA_{AGED}	0.0313	0.51	0.7894
	PVC_{VIRGIN}	0.008	0.09	0.06867
	PVC_{AGED}	0.0051	0.05	0.01391
Pseudo second order		**k_2 (mg μg^{-1} h^{-1})**	**q_e (μg mg^{-1})**	**R^2**
	PP_{VIRGIN}	0.0215	21.86	1
	PP_{AGED}	0.0166	21.63	0.9999
	PA_{VIRGIN}	0.0432	1.21	0.9992
	PA_{AGED}	0.0815	1.49	0.9995
	PVC_{VIRGIN}	6.6316	1.54	0.9999
	PVC_{AGED}	-2.0863	1.83	0.9998
Desorption				
Kinetics model	**Plastic**	**Parameters**		
Pseudo first order		**k_1 (h^{-1})**	**q_e (μg mg^{-1})**	**R^2**
	PA_{VIRGIN}	-0.0071	0.024	0.01023
	PA_{AGED}	-0.0044	0.031	0.00917
	PVC_{VIRGIN}	-0.0058	0.045	0.08023
	PVC_{AGED}	-0.0153	0.021	0.2023
Pseudo second order		**k_2 (mg μg^{-1} h^{-1})**	**q_e (μg mg^{-1})**	**R^2**
	PA_{VIRGIN}	-1.2940	0.17	0.9981
	PA_{AGED}	-1.3345	0.08	0.9948
	PVC_{VIRGIN}	-1.6178	0.18	0.9993
	PVC_{AGED}	-1.5758	0.10	0.9952

250

251

252

253

254

Table S5: Pearson correlation matrix of density of the microplastics compared to size of the microplastics, the plastic concentration, the amount of fluoxetine adsorbed onto the microplastics, the amount of fluoxetine desorbed from the microplastics, the mortality of the neonates after 24 and 48 h. A correlation coefficient (r) can vary between -1 (negative association) and 1 (positive association). The correlation coefficient (r) can vary between -1 (negative association) and 1 (positive association). A coefficient of < -0.7 (■, red), was considered a strong the negative association, meanwhile a coefficient of > 0.7 (■, green) was considered a strong positive association.

	Density	D_{50}	S_{PSA}	S_{BET}	T_g	X_C	ζ	CI	Adsorption (q_t)	Desorption (q_t)
Density	1									
D_{50}	-0.12	1								
S_{PSA}	0.55	-0.37	1							
S_{BET}	-0.84	-0.34	-0.27	1						
T_g	0.83	0.34	0.28	-1.00	1					
X_C	-0.97	-0.04	-0.47	0.94	-0.93	1				
ζ	-0.61	0.13	-0.91	0.34	-0.34	0.53	1			
CI	-0.87	-0.08	-0.64	0.85	-0.87	0.89	0.60	1		
Adsorption (q_t)	-0.86	-0.29	-0.31	1.00	-1.00	0.95	0.37	0.88	1	
Desorption (q_t)	0.79	0.18	0.44	-0.90	0.89	-0.88	-0.51	-0.75	-0.90	1

Table S6: Summary of the adsorption and desorption parameters of the linear, Freundlich, and Langmuir models. The parameters include the partition coefficient (K_p), Freundlich coefficient (K_F), Freundlich exponent (1/n), Langmuir coefficient (K_L), energy of adsorption (α_L), maximum adsorption capacity (Q_0), and the correlation coefficient (R^2).

Isotherm model	**Plastic**	**Adsorption**				**Desorption**			
		Parameters				**Parameters**			
Linear		**K_p (L g^{-1})**			**R^2**	**K_p (L g^{-1})**			**R^2**
	PP_{VIRGIN}	11.93			0.7838				
	PP_{AGED}	10.02			0.9033				
	PA_{VIRGIN}	0.19			0.9897	-0.03			0.9981
	PA_{AGED}	0.8			0.9853	-0.03			0.9933
	PVC_{VIRGIN}	0.72			0.8909	-0.03			0.9926
	PVC_{AGED}	1.03			0.7113	-0.04			0.9943
Freundlich		**K_F (L g^{-1})**	**n**		**R^2**	**K_F (L g^{-1})**	**n**		**R^2**
	PP_{VIRGIN}	13.15	0.4319		0.9386				

	PP_{AGED}	13.18	0.4513		0.9670				
	PA_{VIRGIN}	0.60	0.8516		0.9979	0.35	1.1218		0.9940
	PA_{AGED}	0.87	1.077		0.9866	0.33	1.264		0.9846
	PVC_{VIRGIN}	1.31	0.5271		0.9833	0.35	1.235		0.9962
	PVC_{AGED}	2.57	0.4759		0.9068	0.36	1.765		0.9786
Langmuir		**K_L (L g^{-1})**	**α_L (L g^{-1})**	**Q_0 (µg mg^{-1})**	**R^2**	**K_L (L g^{-1})**	**α_L (L g^{-1})**	**Q_0 (µg mg^{-1})**	**R^2**
	PP_{VIRGIN}	25.85	0.02566	38.97	0.9983				
	PP_{AGED}	25.50	0.02508	39.87	0.9801				
	PA_{VIRGIN}	0.68	0.088	7.67	0.9854	0.24	-0.258	-0.92	0.7268
	PA_{AGED}	0.83	-0.024	-34.17	0.07533	0.2	-0.377	-0.52	0.6600
	PVC_{VIRGIN}	3.04	0.739	3.48	0.9935	0.23	-0.304	-0.75	0.6574
	PVC_{AGED}	6.69	1.996	3.35	0.9990	0.05	-0.843	-0.06	0.4447

Table S7: Pearson correlation matrix of density of the microplastics compared to size of the microplastics, the plastic concentration, the amount of fluoxetine adsorbed onto the microplastics, the amount of fluoxetine desorbed from the microplastics, the mortality of the neonates after 24 and 48 h. A correlation coefficient (r) can vary between -1 (negative association) and 1 (positive association). The correlation coefficient (r) can vary between -1 (negative association) and 1 (positive association). A coefficient of < -0.7 (■, red), was considered a strong the negative association, meanwhile a coefficient of > 0.7 (■, green) was considered a strong positive association.

	Density	**Size**	**Plastic concentration**	**Amount adsorbed**	**Amount desorbed**	**Mortality (24 h)**	**Mortality (48 h)**
Density	1.00						
Size	0.01	1.00					
Plastic concentration	0.49	0.12	1.00				
Amount adsorbed	-0.24	-0.16	-0.09	1.00			
Amount desorbed	-0.84	-0.20	-0.61	0.33	1.00		
Mortality (24 h)	-0.39	-0.41	-0.50	-0.02	0.62	1.00	
Mortality (48 h)	0.08	-0.27	-0.21	-0.16	0.20	0.40	1.00