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Ecotoxicological assessment of suspended solids: The importance of biofilm and particle aggregation

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ABSTRACT

Assessment of microplastic impacts in biota is challenging due to the complex behavior of the test particles and their interactions with other particulates, including microorganisms, in the environment. To disentangle responses to microplastic exposure from those to other suspended solids, both microplastic and natural particles must be present in the test system. We evaluated how microplastic, non-plastic particles, and biofilms interacted in their effects on survivorship using acute toxicity assay with *Daphnia magna*. The animals were exposed to microplastic and kaolin at different concentrations of suspended solids (SS; 10, 100, and 1000 mg/L) with a varying microplastic contribution (%MP; 0–80%) and biofilm (presence/absence) associated with the solids. Also, we examined how these exposure parameters (SS, %MP, and *Biofilm*) affected aggregate formation that was analyzed using particle size distribution data. Under the exposure conditions, *Daphnia* mortality was primarily driven by SS concentration but ameliorated by both microplastic and biofilm. The ameliorating effects were related to increased particle aggregation in the presence of biofilm and high %MP. In addition, a weak yet significant positive effect of the biofilm on the survivorship was observed, presumably, due to microbial food supply to the daphniids in the exposure system; the bacteria were utilized at the absence of other food. Therefore, the effects of both natural and anthropogenic particulates depend on the particle behavior and aggregation in the water governed by microbial communities and physicochemical properties of the particles, which must be taken into account in the hazard assessment of plastic litter.

1. Introduction

Plastics account for an average of 75% of marine litter (Galgani et al., 2015), and the environmental impacts of plastic litter is a hot topic fueled by public concerns (Henderson and Green, 2020). Fragmentation of plastic debris takes place in the environment, with chemical and mechanical degradation generating fine-sized particles, so-called secondary microplastic (MP), the main environmental load of plastics (Andrady, 2011). However, despite the general perception of MP as hazardous waste (Henderson and Green, 2020; Rochman et al., 2013), no-effect results are common (Ogonowski et al., 2018a), and it usually remains unclear what are the mechanisms of action when the adverse effects are observed (Connors et al., 2017). Due to this conflict of information, scientists disagree on the scale and immediacy of the MP pollution problem (Backhaus and Wagner, 2020; Burns and Boxall, 2018; Gouin et al., 2019), and lack of systematic studies considering particle properties hampers read-across analysis for regulatory purposes. Possible impacts of plastic litter on the environment and human health need to be assessed with scientific rigor, including particle behavior and physicochemical properties in the analysis and establishing adequate systems for testing biological effects.

At least partly, the difficulties in the MP hazard assessment are related to the fact that plastic is a new type of environmental contaminant with yet unsettled methodology for hazard testing. Ecologically relevant assessment of the impacts of particulate matter in biota is generally challenging due to the complex behavior of the test particles and their interactions with other particulates. As an environmental contaminant, microplastic is never present without a sizeable contribution of other particulates in the water column (Nguyen et al., 2019). Various suspended solids, both natural, such as sediment, food and detritus (Ladd,

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1957), and anthropogenic, such as microplastic, cellulose and black carbon (Bubb and Lester, 1994; Jedwab, 1980; Roy et al., 1999) are always present in natural waters; moreover, their concentrations are orders of magnitude higher than those of plastic particles (Lowry et al., 2012).

At high concentrations of any suspended solids, adverse effects on biota can occur (Bilotta and Brazier, 2008; Chapman et al., 2017; Gordon and Palmer, 2015; Kjelland et al., 2015). Therefore, it is relevant to disentangle responses to microplastic exposure from those of other suspended solids present at ecologically relevant concentrations. In the aquatic environments, particulates engage in various interactions with microorganisms and macromolecules and undergo various physicochemical transformations, such as homo- and hetero-aggregation, degradation, and deposition (Alimi et al., 2018). As a result, particle size distributions are changing, which affects bioavailability, uptake, and hazard potential of the particulates (Bruinink et al., 2015; Kirk, 1991a; Zurek, 1983), including microplastic (Li et al., 2020).

Furthermore, microorganisms are an integral part of the particulate matter *in situ*, with biofilms ubiquitously present in the detritus and aggregates formed by both anthropogenic and natural particles (Rogers et al., 2020). Consequently, composition and quantities of the biofilm contribute to particle aggregation, exposure, and, thus, responses in higher consumers (Rummel et al., 2017). Hence, meaningful design for an experiment evaluating the effects of microplastic in biota should include a mixture of suspended solids with varying microplastic contribution and microorganism load if we are to understand ecologically plausible effects of exposure to microplastic pollution (Gerdes et al., 2019; Rogers et al., 2020).

Here, using a filter-feeding test organism, *Daphnia magna*, we designed an experiment to test the effects of three components ubiquitously present in a pelagic system facing plastic pollution: (1) concentration of mixed solids, (2) proportion of microplastic in the mixture, and (3) biofilm. *Daphnia* species have been extensively used as model filter-feeders in ecological studies, including investigations of their responses to suspended solids and biofilms (Kirk, 1991b), but also as test organisms in the ecotoxicology of microplastics (Anbumani and Kakkar, 2018; Gerdes et al., 2019; Kokalj et al., 2018), which facilitates interpretation of the results.

To mimic the exposure *in situ*, the daphniids were exposed to the mixtures of clay and microplastic. The mixtures had a varying proportion of the microplastic and were prepared with or without biofilm associated with the solids. The acute toxicity test design based on OECD guidelines (OECD, 2004) and modified for testing of particulates, according to Gerdes and co-workers (Gerdes et al., 2019) was employed. Based on the known effects of suspended solids in the filter feeders, including daphniids (Chapman et al., 2017; Kirk and Gilbert, 1990; Robinson and Klaine, 2008), we put forward two main questions (Q1) How the exposure parameters (i.e., total suspended solids, percentage of microplastic and presence of biofilm): affect daphniid survivorship? and (Q2) Whether the observed effects are mediated by particle aggregation in the system? Given the heterogeneous findings published so far, we had no specific hypothesis for the potential role of the microplastic when testing the effects of the microplastic contribution to the particle suspension.

2. Material and methods

2.1. Toxicity testing

2.1.1. Test suspensions and treatments

Kaolin (Sigma-Aldrich, K7375; size range 2–40 μm; Fig. S1) and polystyrene microplastic (PS; Goodfellow GmbH, artificially aged using UV-light; size range: 3–20 μm) were used to prepare the stock suspensions in M7 media (reconstituted lake water) (Loureiro et al., 2011). The PS ageing procedure is described elsewhere (Gewert et al., 2018).

Surface water was collected in a coastal bay close to Stockholm, Sweden, in May 2019; site location: N 59.371890, W 18.045810, water temperature and salinity at the collection time were 16 °C and 2 psμ, respectively. The water was transported to the Department of Environmental Science (ACES), Stockholm University, and filtered on a 5-μm polyamide sieve to remove larger plankton. The filtrate containing bacterioplankton was poured in sterile 2-L glass bottles and stored for 3 weeks at 4 °C before using it as a source of biofilm on the test particles.

The experiment used a full-factorial design and included treatments with different combinations of three factors: suspended solids concentrations (SS; three levels), percentage of microplastic in the particle mixture (%MP; five levels), and presence or absence of biofilm on the particles (*Biofilm*+ and *Biofilm*−, respectively; two levels). First, using kaolin and PS stocks, mixtures with nominal total concentrations of suspended solids (SS) corresponding to 10, 100, and 1000 mg/L where PS contributed 0% (kaolin only), 20%, 40%, 60%, and 80% by mass (%MP) were prepared in 50-mL plastic Falcon tubes; each tube was handled independently. Two identical sets of these mixtures with seven replicates for each SS × %MP combination were prepared.

Second, for testing biofilm effects, biofilm present (*Biofilm*+) and biofilm absent (*Biofilm*−) treatment combinations were prepared using the full set of mixtures (Fig. 1). To prepare the *Biofilm* + treatments, we added 3 mL of the ambient water containing bacterioplankton to each tube in one set of the PS/kaolin mixtures described above. No bacterioplankton were added to the other set of the PS/kaolin mixtures that were assigned to the *Biofilm*− treatments. The tubes containing bacterioplankton were incubated for eight days under gentle shaking at room temperature with 16^{D}:8^{L} photoperiod.

2.1.2. Exposure experiment

We exposed *Daphnia magna* to the particle mixtures with different concentrations of suspended solids, %MP in these mixtures, and with or without biofilm; seven replicates were used for each SS × %MP × *Biofilm* combination. The exposure design was based on that described by Gerdes et al. (2019). Ten neonates (<24 h old) were placed in each tube containing a particle mixture. All tubes from both treatments were filled with M7 to the top (~57 mL) and sealed with parafilm to avoid air bubbles. All experimental

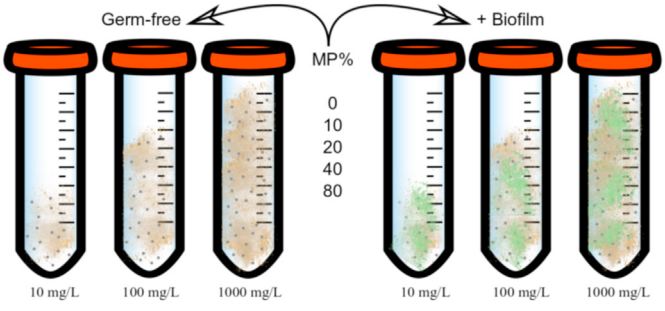

Fig. 1. Experimental design: three concentrations of suspended solids (SS; 10–1000 mg/L) were prepared using varying contributions of kaolin (20–100%) and MP (0–80%); seven replicates per treatment combination were used. First, half of the test suspensions were incubated with ambient water containing bacterioplankton (*Biofilm*+), whereas the other half were used as no biofilm treatment (*Biofilm* -). Second, daphniids were added to each tube, and their survivorship after 96 h was recorded.

animals originated from the same clone (Clone 5; The Federal Environment Agency, Berlin, Germany) cultured in M7 media at a density of ~10 ind. L−1 and fed a mixture of green algae (*Pseudokirchneriella subcapitata* and *Scenedesmus spicatus*). As a procedural control, we used daphniids incubated in the M7 media, no particles added.

All tubes were mounted on a plankton wheel, placed in a thermo-constant room at 21 °C with 16^{D}:8^{L} photoperiod. The test was terminated after 96 h, and mortality in each replicate was assayed following OECD guidelines (OECD, 2004). The daphniids which were not able to swim within 30 s after gentle agitation were considered immobilized (dead). If the control mortality was 5–10%, the mortality values observed in the treatments were adjusted for that in the control using Abbott's correction (Hoekstra, 1987); in no case control mortality was >10%.

At the termination of the experiment, all daphniids were removed from the tubes, and the test suspension of the particles from each SS × %MP × *Biofilm* combination were randomly assigned to either particle size distribution (PSD) analysis (three replicates) or biofilm quantification (three replicates); one replicate was kept as a reserve. The samples designated for PSD analysis were analyzed directly. The samples designated for biofilm quantification were collected by filtering the whole suspension on MILLIPORE HA filters (47 mm, pore size 0.45 μm); the filters were folded, placed in Eppendorf tubes, and stored at −80 °C until the analysis. To measure quantities of the biofilm that was associated with the particles retained on the filters, we used the total DNA extracted from these filters.

2.2. Particle size distribution analysis

We used a Spectrex Laser Particle Counter (Spectrex, PC-2000, Redwood city, USA) to analyze PSD in the samples. The experimental suspensions were diluted 100 to 1000-fold to achieve the particle concentrations within the linear dynamic range of the instrument; particle-free water (<10 counts/mL) was used for dilution. The size spectra were determined for the 1–100 μm range and processed with GRADISTAT software 8.0 (Blott and Pye, 2001) following the computational method of Folk and Ward (1957). As most of the size spectra deviated significantly from the normal distribution, we used a median particle size (D_{50}) as a proxy for the most typical aggregate size in suspension.

2.3. DNA extraction and quantification

Total DNA was extracted from the filter samples using 10% Chelex (Straughan and Lehman, 2000). The DNA concentrations were measured using QuantiT PicoGreen dsDNA Assay kit (ThermoFisher, USA), and fluorometric quantitation was performed with excitation and emission set at 480 nm and 530 nm, respectively, in a Tecan Ultra Spectro Fluorometer (PerkinElmer, USA). As a proxy for biofilm thickness, we used mass-specific DNA concentration, i.e., the amount of DNA in the system normalized to the nominal mass of suspended solids in the treatment combination (ng DNA/mg SS).

3. Statistical analysis

3.1. Factors affecting mass-specific DNA and median size of the aggregates

First, we confirmed that exposure to natural bacterioplankton resulted in greater biofilm abundance in *Biofilm* + treatment compared to *Biofilm*− treatment using Generalized Linear Models (GLM) with the treatment factors (SS, %MP, and Biofilm) as predictors and the mass-specific DNA concentration (DNA/SS) as the dependent variable. Second, we evaluated whether the particle aggregation was affected by the experimental factors using the same approach; the median particle size (D_{50}) was used as a dependent variable, SS and MP% as continuous predictors, and *Biofilm* as a categorical predictor. In all GLMs, the response variables were Box-Cox transformed to stabilize the distribution of the model residuals. All models initially included the interaction terms but were simplified by sequentially removing non-significant interactions. At each step, the simplified model was compared to the more complex one using the Aikaike Information Criterion (AIC). In the end, the most parsimonious model with the lowest AIC value was chosen. Centering was applied to continuous variables to facilitate the interpretation of the main effects when the interaction was significant.

3.2. PLS-SEM analysis of daphniids mortality

Partial Least Squares (PLS) which is an approach to Structural Equation Models (SEM) was used to explore a hypothetical research model by creating latent variables with single or multiple indicators and analyzing relationships between these variables using the SmartPLS 3.0 (v. 3.3.2) software (Ringle et al., 2015). Partial least squares-structural equation modeling (PLS-SEM) allows to analyze the relationships simultaneously under limited conditions, such as multivariate normality assumptions, smaller sample sizes, residual distribution, and presence of formative indicators, such as parameters of experimental design (Chin, 1998; Hair et al., 2014). This approach also allows efficient testing of mediation and moderation effects (MacKinnon et al., 2002) that were relevant for our research questions.

In our research model, we evaluated the effects of suspended material composed of kaolin and MP at different proportions and concentrations, as well as the presence of biofilm on the particle aggregation in the system and the outcome of exposure (i.e., daphniid mortality). Four latent variables (LV) were considered comprising the main components of the study system: (1) *HAZARD* reflecting a hazard potential of the suspended solids in the system and represented by *Mortality* as a single indicator; (2) *SM* (Suspended Matter; formative construct) represented by the nominal concentration of SS and %MP in the system; (3) *BIOFILM* reflecting presence/absence of the biofilm in the exposure and represented by *Biofilm* as a single indicator; and (4) *AGGREGATION* (reflective construct) represented by the indicators characterizing particle size distribution (median size [D50]) and biofilm thickness (mass-specific DNA concentration). The sample size (90 observations) satisfied both the *10-times rule* (Barclay et al., 1995) and the PLS-SEM guidelines proposed by (Hair et al., 2011).

The two-step approach of first evaluating the outer model (measurement models for *SM* and *AGGREGATION*) and then the inner model (structural model) was used (Ringle et al., 2015). PLS path analysis was first conducted to confirm that the model and constructs complied with specific quality criteria, namely: (1) composite constructs have good discriminant validity and reliability as indicated by average variance extracted (AVE), which reflects the overall amount of variance in the indicators accounted for by the latent construct (AVE > 0.5); composite reliability (CR; computed on standardized loadings) and Jöreskog's rhô value (rho A; computed on unstandardized loadings), which depict the degree to which the construct indicators represent the latent construct (both CR and rho A > 0.7; Chin et al., 1998); (2) reflective indicator is only retained when the factor loading is > 0.5 with $p < 0.01$ (Hair et al., 2011); and (3) construct discriminant validity is demonstrated. Consequently, PLS bootstrapping (10 000 bootstraps) was

used to compute path coefficients with t-values and exact *p* values, total effects, as well as total indirect and specific indirect effects for the outer model. The model fit was assessed by SRMR, the Standardized Root-Mean-square Residual (Ringle et al., 2015), a measure based on the differences between observed (*s*) values and the ones obtained from the model (σ) of the covariance matrix. SRMR values < 0.10 were assumed as an adequate fitting measure (Hu and Bentler, 1999). The effect size was interpreted using f^2 values, with 0.02, 0.15 and 0.35 as thresholds for weak, moderate, and strong effects, respectively (Cohen, 1977). PLS-SEM aims to maximize R^2 values of the endogenous LVs in the path model, with R^2 values of 0.19, 0.33 and 0.67 for the endogenous constructs described as respectively weak, moderate, and substantial (Chin, 1998).

The primary objectives of this analysis were to determine whether *AGGREGATION* was acting as a mediator of *SM* effects on *HAZARD* and *BIOFILM* as a mediator of *AGGREGATION* effects on *HAZARD*. Also, moderating effects of *BIOFILM* on *AGGREGATION* and *HAZARD* were tested. Consequently, the following hypotheses were put forward for this PLS-SEM model:

- H1: Exposure to high levels of suspended matter (*SM*) is hazardous and increases daphnia mortality (*HAZARD*);
- H2: High levels of suspended matter (*SM*) decrease particle aggregation (*AGGREGATION*); this is based on the observed negative relation between particle concentration and aggregation in hydrological study systems (Heath et al., 2006);
- H3: *AGGREGATION* affects *HAZARD* negatively, because with particle aggregation, the particle number concentration in the media decreases, leading to lower mortality;
- H4: *BIOFILM* increases aggregate formation (*AGGREGATION*) because it can change surface properties of the particles and increase their adhesion;
- H5: *BIOFILM* decreases *HAZARD* by providing daphniids with caloric particles (microorganisms), thus, supporting their survival.

We also tested *BIOFILM* as a moderator for the effects of *SM* on *HAZARD* and *AGGREGATION*. Following the recommendations of Hair and co-workers (Hair et al., 2017), we used the two-stage approach as it is the most versatile, and it also works when the exogenous construct (i.e., *SM*) is measured formatively. The following specific hypotheses regarding the moderating effects were put forward:

- H6: *BIOFILM* has a moderating effect on the relationship between *SM* and *HAZARD*, such that in the treatments with biofilm, the effect of suspended matter on daphnia mortality is partially alleviated compared to the treatments without biofilm;
- H7: *BIOFILM* has a moderating effect on the relationship between *SM* and *AGGREGATION*, such that in the treatments with biofilm, the effect of suspended matter on the particle aggregation is facilitated compared to the treatments without biofilm.

4. Results

4.1. Efficacy of incubation with bacterioplankton for biofilm formation

Incubation of the test mixtures with natural water containing bacterioplankton had a significant positive effect on the total DNA concentration in the system (Table 1); moreover, *Biofilm* was the most influential predictor. Also, the concentration of suspended solids (*SS*) in the system was a significant positive predictor, whereas *%MP* had no effect on the DNA concentration. No significant interaction effect was observed.

Table 1
GLM results for mass-specific DNA concentration (DNA/SS) as a function of biofilm presence, concentration of suspended solids (SS), and contribution of microplastic in the mixture (%MP). DNA/SS values were Box-Cox transformed (λ = −0.15). Significant effects are in bold.

Predictor	Level of Effect	Estimate	SE	t value	*p* value
Biofilm	*Biofilm+*	1.177	0.431	2.731	**0.008**
SS		−0.010	0.001	−20.093	**<0.0001**
%MP		0.003	0.008	0.401	0.7

Table 2
GLM results for the median particle size (D50) as a function of biofilm presence, concentration of suspended solids (SS), and contribution of microplastic in the mixture (%MP). D50 values were Box-Cox transformed (λ = 0.31), and the continuous predictors were centered. Significant effects are in bold.

Predictor	Level of effect	Estimate	SE	t value	*p* value
Biofilm	*Biofilm* +	0.031	0.011	8.7	**0.003**
SS		−0.0002	0.00003	69.2	**<0.0001**
%MP		0.002	0.0004	19.0	**<0.0001**
Biofilm × %MP	*Biofilm* +	−0.001	0.0004	13.4	**0.000**

4.2. Particle size distribution

The median particle size, D_{50}, was significantly higher in *Biofilm* + treatments and negatively affected by the suspended solid concentration (Table 2). There was a significant *Biofilm* × %MP interaction, which indicated that in the *Biofilm* + treatment, the D_{50} values increased significantly as %MP increased, whereas this was not the case in the *Biofilm*-treatment (Table 3, Fig. 2).

4.3. PLS-SEM: effects of particle aggregation and biofilm on daphnia mortality

4.3.1. Measurement model

In the formative construct *SM*, outer weights of both indicators were significant and >0.3 and rho A of the construct was >0.8 (Table 3). The multicollinearity was not an issue (VIF = 1.0). Therefore, we can conclude that each indicator of the formative construct is important in explaining the construct' domain. Notably, MP had negative weight on *SM*, whereas SS was positive, indicating the opposite effect directions. In the reflective construct *AGGREGATION*, all item loadings exceeded the recommended value of 0.5 (Chin et al., 2008). Thus, the measurement model achieved convergent validity with acceptable measurement loadings/weights and construct validity according to CR, rho A, and AVE values (Table 3). Moreover, each LV was subjectively independent of other indicators indicating discriminant validity of every LV (Table S1).

4.3.2. Structural model

All path coefficients were significant (Table 4). The model explained 62% of the variance in mortality and 57% of the variance in particle aggregation, which indicates a moderate to high level of the explanatory power (Fig. 3). The SRMR value of 0.096 indicates an acceptable fit.

In reference to the hypothesis testing, all PLS-SEM hypotheses were supported (Table 4). The exposure to the test mixtures (*SM*) was found to increase *Daphnia* mortality (*HAZARD*), with a moderate effect size. Negative effects of the particle aggregation (moderate) and biofilm presence (weak) on *HAZARD* were found; moreover, the biofilm was moderately facilitating particle aggregation in the system. Both moderation effects were negative, statistically significant, albeit weak as indicated by their low f^2 values (Table 4).

Table 3
Validity and reliability of the constructs.

Construct	Indicators	Loadings/outer weights and their [*p* values]	Rho A	AVE	CR
SM; Suspended Matter [formative]	SS; Suspended solids in the system, total concentration (mg/L)	0.905 [<0.0001]	1.000		
	MP; Microplastic percentage in the suspended solids (0–80%)	−0.312 [0.001]			
AGGREGATION [reflective]	D50; median size of the particle aggregate (μm)	0.830 [<0.0001]		0.691	0.817
	DNA.SS; mass-specific DNA concentration, a proxy for biofilm thickness on the particles (ng/mg)	0.832 [<0.0001]			

The mediating effects of *AGGREGATION* on the relationships between *SM* and *HAZARD* and between *BIOFILM* and *HAZARD* were significant, with path coefficients >0.1; the indirect effect of the interaction term was omitted, because of the low path coefficient. As suggested by the specific indirect effects (Table 5), in addition to the direct impact on *HAZARD* (Table 4), *SM* contributed indirectly by decreasing particle aggregation; this partial mediation was significant. *BIOFILM* also had a significant partial mediation, albeit weaker, by increasing aggregation and decreasing the hazard potential of the suspension.

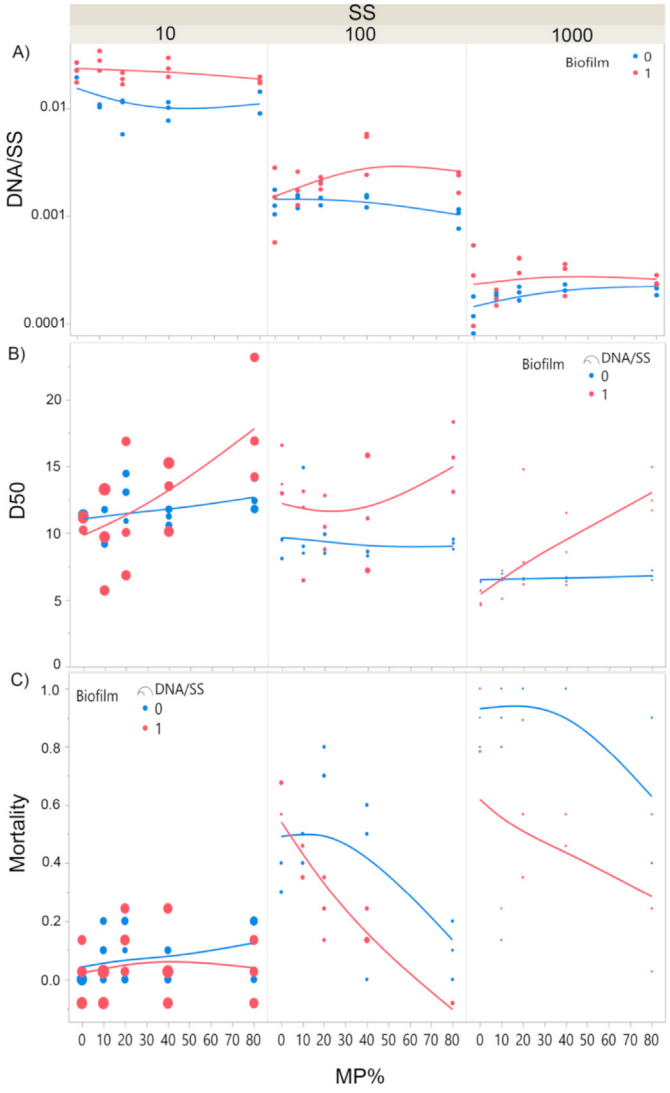

Fig. 2. Variation in (A) mass-specific DNA concentration (DNA/SS; μg/g) in the test mixture, (B) median aggregate size (D50; μm), and (C) *Daphnia magna* mortality (proportion of immobilized animals after 96-h of exposure) as a function of suspended solid concentration (SS; mg/L; shown on top), percentage of microplastic in the test mixture (%MP; *x* axis) and biofilm treatment; 0 and 1 denote *Biofilm*−and *Biofilm*+, respectively. The bubble size corresponds to DNA/SS value.

Table 4
Evaluation of the structural model based on path coefficients (β), their statistical significance, and effect size (f^2). Significant *p* values for each β are in bold.

Hypothesis	Path	β	t value	*p* value	f^2
H1	*SM* - > *HAZARD*	0.45	4.98	**0.00**	0.26
H2	*SM* - > *AGGREGATION*	−0.69	14.90	**0.00**	1.07
H3	*AGGREGATION* - > *HAZARD*	−0.37	4.07	**0.00**	0.16
H4	*BIOFILM* - > *AGGREGATION*	0.28	4.31	**0.00**	0.19
H5	*BIOFILM* - > *HAZARD*	−0.14	2.02	**0.04**	0.04
H6	*BIOFILM*SM1* - > *HAZARD*	−0.16	2.42	**0.02**	0.06
H7	*BIOFILM*SM2* - > *AGGREGATION*	−0.14	2.59	**0.01**	0.05

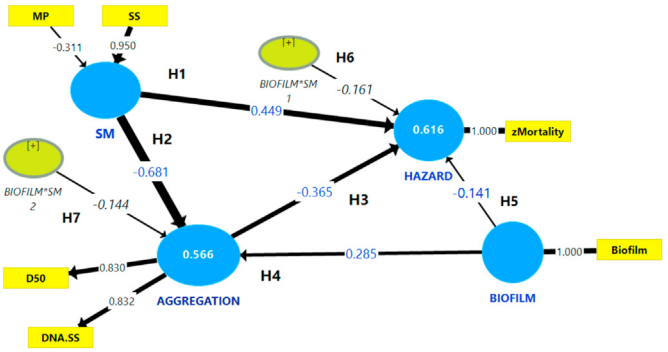

Fig. 3. The model output for PLS-SEM evaluating the effects of the mixture of suspended solids on their hazard potential. Path coefficients are shown for the inner model. Outer weights (*SM*) and loadings (*AGGREGATION*) are shown for the outer models. Coefficients in the measurement models vary between −1.0 (absolute negative correlation) and 1.0 (absolute positive correlation). The outer loading is always 1.0 in single item constructs. The numbers in the circles are adjusted R^2 values. The hypotheses (H1-SEM to H7-SEM) addressed in the model are placed by the relevant paths.

5. Discussion

Using an experimental design that included mixed solids with varying MP contribution and contrasting treatments with and without a biofilm, we found that the primary driver of mortality was the total concentration of suspended solids in the system (Table 4, Fig. 3). This observation was not particularly surprising considering many field (Hart, 1986; Zettler and Carter, 1986) and laboratory (Ogonowski et al., 2018a; Rellstab and Spaak, 2007; Zurek, 1983) studies demonstrating adverse effects of exposure to high levels of sediment and other inorganic particles on planktonic grazers, primarily daphniids. However, in addition to the moderately strong direct effect on *Daphnia* survival, the high concentrations of the suspended matter suppressed particle aggregation as revealed by the PLS-SEM analysis (Table 4, Fig. 3), which re-enforced the adverse effect of the suspension. Thus, hypotheses

Table 5
Mediation effects of *AGGREGATION* based on path coefficients (β) and their statistical significance for specific indirect effects. Significant *p* values are in bold.

Indirect Effect	β	t value	*p* value
BIOFILM - > AGGREGATION - > HAZARD	−0.104	2.971	**0.003**
*BIOFILM*SM2 - > AGGREGATION - > HAZARD*	0.052	2.048	**0.041**
SM - > AGGREGATION - > HAZARD	0.254	3.869	**0.000**

H1 and H2 were supported.

The aggregate formation is strongly dependent on particle physicochemical and biological properties, which affect particle adhesion upon collision (Kiø rboe et al., 1990). In different systems used for particle aggregation studies, the mean aggregate size has been found to be positively affected by primary particle size and various chemicals acting as flocculants (Heath et al., 2006; Van Leussen, 1988) but negatively by shear flow (Vassileva et al., 2007) and high concentrations of suspended solids (Heath et al., 2006). As particle aggregation was a significant component involved in complex interactions with other experimental variables (Fig. 3), it is important to consider the mechanisms of these interactions in various experimental settings and recognize factors governing the aggregation processes.

We found that aggregation was a significant partial mediator of the suspended matter effect on the hazard properties of the particle suspension, thus providing support to the hypothesis H3. As it was a complementary mediation, we suggest that *Daphnia* mortality was induced by high concentrations of suspended matter both directly, by increasing energy costs for filtering and respiration (Kirk, 1991b; Kirk and Gilbert, 1990), and indirectly, by reducing aggregation and affecting particle size distribution. Although the mechanism of the aggregation effect on the animal survival (Table 4, Fig. 3) is not entirely clear, it seems plausible that higher survival was related to a reduced total particle number concentration in suspension and the decreased abundance of small-sized particles because of their allocation to the aggregates. The particle size has been suggested to be one of the primary drivers of mortality in *Daphnia* exposed to microplastics (Mattsson et al., 2017; Rehse et al., 2016) but also mineral particles (Gophen and Geller, 1984). For example, Mattson and coworkers (Mattsson et al., 2017) compared the effects of PS nanoparticles of different size and charge with various concentration on *D.magna* immobilization. Among particles tested, only amino-modified polystyrene (52−330 nm) with a diameter of 52 nm affected *Daphnia*, whereas larger particles (120−330 nm) had no effect on the animals. Furthermore, the observed effect was strongly concentration-dependent with harmful effects at higher concentrations. In daphniids and similar filter-feeders, effects of particle size on other physiological functions, such as feeding and reproduction (Rellstab and Spaak, 2007), have also been reported, with smaller particles causing more profound adverse effects due to their easy clogging to feeding appendages and carapace (Jeong et al., 2016).

As hypothesized (H5), the adverse effects of exposure were significantly suppressed by biofilm grown on the surface of the test particles (Table 5; Fig. 3), which promoted survivorship of the animals. As expected, the pre-incubation of the test mixtures with natural bacterioplankton resulted in the biofilm formation on the particle surface (Table .1). Naturally, the more suspended solids were present in the system, the thinner biofilm layers with the less biofilm material per particle mass were found, as reflected by the mass-specific DNA concentration (Fig. 2A). Notably, no effect of *%MP* on the biofilm thickness was observed (Table .1), suggesting that PS particles were colonized in a similar way as kaolin.

When the biofilm was present, we observed lower mortality compared to the treatments where the test mixture was not pre-incubated with bacterioplankton, with the difference being significant at 100 and 1000 mg/L (Fig. 2C). Moreover, the PLS-SEM analysis revealed that biofilm had both direct and indirect (i.e., mediated) effects on the hazard potential of the particle mixture. The direct effect was weak, albeit statistically significant (Tables 4 and 5), suggesting that biofilm was used by the daphniids which improved their survival rate as hypothesized in the PLS-SEM (H6). It has been shown that the impacts of microplastic in grazers are mostly related to a decreased calorie intake (Aljaibachi and Callaghan, 2018; Ogonowski et al., 2016). Therefore, a biofilm would add a nutritional value to the non-caloric particles as also suggested by (Canniff and Hoang, 2018; Schoenberg and Maccubbin, 1985).

In the PLS-SEM, there was also a complementary indirect effect of the biofilm mediated by the particle aggregation (hypothesis H4; Table 5, Fig. 3), with significantly higher aggregation in the presence of biofilm as also shown by the GLM results (Table 2; Fig. 2B). The effect was moderate and − similar to the direct effect − resulted in the improved survivorship, most likely due to the decreased abundance of smaller particles which are known to induce greater adverse effects. The increased aggregation in the presence of biofilm was expected because both living microorganisms and dissolved organics are well-known drivers of aggregate formation (McAnally and Mehta, 2000), including microplastic aggregation (Michels et al., 2018). The structure of biofilm with a sticky matrix composed of extracellular polymeric substances (EPS), such as polysaccharides, lipids, protein, and DNA, stabilizes physical bonds among the particles and thus promotes aggregation (Ajao et al., 2018; Liao et al., 2002). Thus, the biofilm was beneficial for survival via two alternative pathways, providing nutrition and rendering particle size distribution less harmful. Therefore, in natural waters, where biofilms are omnipresent and often very abundant, the adverse effects of suspended solids will be lower compared to the laboratory test systems with virgin plastic, autoclaved media, and controlled microbial levels.

The mortality was inversely related to the proportion of the microplastic in the mixture (Tables 3 and 4; Fig. 2C). The negative loading of the *%MP* indicator in the *SM* construct (Table 3; Fig. 3) indicates that the effect of the microplastic proportion in the mixture is counteracting that of suspended solid concentration in the system; the latter is also evident when examining the mortality as a function of microplastic contribution (Fig. 2C). At lower %MP, the aggregation is facilitated (Table 2, Fig. 2B), with concomitant positive effects on survival (Fig. 3). Notably, the microplastic effect on D50 was weak in the treatments without biofilm but increased greatly when the biofilm was present as indicated by significant interaction term in the GLM (Table 2, Fig. 2B) and significant moderating effect in the PLS-SEM (*BIOFILM*SM2*; Tables 3−5) (H7). Therefore, PLS-SEM mediation analysis suggests that the ameliorating effects of both biofilm and microplastic were largely related to their stimulatory effects on the particle aggregation.

The MP effect on the aggregation in the presence of biofilms could be attributed to the physicochemical properties of the material (i.e., weathered polystyrene) but also to material-specific biofilm communities. Becker and co-workers found that the EPS production by aquatic biofilm-forming bacteria increased with an increase of the substrate surface tension (Becker, 1996), and there are similar observations for soil biofilms (Zethof et al., 2020). We have also observed a higher bacterial diversity in the biofilm growing on PS compared to kaolin (Ogonowski et al., 2018b) or other polymers (McGivney et al., 2020). Although we did not measure the surface tension or contact angle of the weathered PS used here, the original pristine PS is hydrophobic, with a contact angle of 91°, while the compound contact angle of kaolin is as low

as 20° and, thus, is highly wettable (Šolc et al., 2011). Even if the contact angle of the UV-treated PS became lower after the photo-oxidation (Fox et al., 1968), the hydrophobicity of the microplastic in our experimental mixtures was still much higher compared to the kaolin. Therefore, we suggest that polystyrene as a substrate supported bacterial communities capable of higher EPS production, which translated to the positive %MP effect on the median particle size in the *Biofilm* + treatment.

Zooplankton, including daphniids, have also been reported to shift particle size distribution to larger aggregates by filtering and egestion (Filella et al., 2008). Moreover, other aquatic and soil biota can significantly stimulate formation of aggregates and stabilize their size distribution (Lehmann et al., 2019). In our experiment and modeling, we have not attempted to account for the aggregate formation induced by the test animals, because their small size (neonate body length is less than 1 mm), low density, and very high amount of the suspended solids in the test mixtures suggested a low possibility of detecting such effects. However, we detected DNA, albeit in low amounts, in all treatments that were not subjected to the pre-incubation with bacterioplankton (Fig. 2A), suggesting that daphniids have, most likely, released some of their DNA, as well as microbiota, to the surrounding media. These exudates and microorganisms might have also contributed to the particle aggregation during the exposure in the *Biofilm*-treatment. Including a daphnia-free control in the test design would allow to evaluate the effect of the test animals on particulate aggregation during the exposure.

Our findings suggest that microplastic hazard assessment should be conducted in the context of water quality guidelines for suspended solids. A better understanding of the effect mechanisms associated with suspended particles (e.g., composition, particle size, and shape) is need for such assessment as also advocated in the discussion on the hazard potential of suspended sediments and other particulates (Bilotta and Brazier, 2008; Chapman et al., 2017; Gordon and Palmer, 2015). In line with other studies (Burd and Jackson, 2009), our PLS-SEM results suggest that aggregation is an important parameter to consider, and particle size distribution is a crucial parameter that needs to be measured in the test system if we are to understand the effect mechanisms. Using artificially aged polystyrene, we found that particle aggregation is a function of the amount of the suspended matter (negative effect), the abundance of the biogenic material (positive effect), and − to a lesser extent − the relative abundance of microplastic (positive effect, particularly in combination with biofilm). The microplastic effect on the measured biological response may, however, differ, depending on, for example, the polymer, its degradation status, and particle size, as also shown by studies demonstrating that microplastic may reduce survivorship of the test animals (Cole et al., 2015; Martínez-Gómez et al., 2017; Ogonowski et al., 2016). In the presence of biogenic material, the particle properties are also likely to affect aggregation, with concomitant effects on the test organisms (Rogers et al., 2020; Rummel et al., 2017). Combining environmentally relevant variables in the test systems will enable a more systematic assessment of the adverse biological effects of suspended solids, both natural and anthropogenic.

6. Conclusion

Hazard assessment of microplastic is challenging due to the complex behavior of particulates and aggregate formation, which is facilitated by microorganisms present in the test systems. Using a standard test species Daphnia magna exposed to mixtures of fragmented polystyrene and clay, with and without biofilm, we have shown that both microplastic and biofilm alleviated the adverse effect of suspended solids. The ameliorating effects were related to increased particle aggregation when biofilm was present, and polystyrene contribution in the suspension was high. These findings highlight the importance of considering particle aggregation and its driving forces in effect studies and hazard evaluations of plastic litter and other persistent solid waste.

Author contributions

E.G. conceptualization the study, A.M. and M.O. designed the experiment. A.M. and S.R. conducted the experiment and collected all samples. A.M. was responsible for biofilm and particle distribution analyses and processing the data. E.G. undertook the data analysis, A.M. and M.O. contributed to statistical analysis and data synthesis. A.M. and E.G. wrote the paper. All authors commented on the manuscript and reviewed the final draft.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.116888.

References

Ajao, V., Bruning, H., Rijnaarts, H., Temmink, H., 2018. Natural flocculants from fresh and saline wastewater: comparative properties and flocculation performances. Chem. Eng. J. 349, 622−632. https://doi.org/10.1016/j.cej.2018.05.123.

Alimi, O.S., Farner Budarz, J., Hernandez, L.M., Tufenkji, N., 2018. Microplastics and nanoplastics in aquatic environments: aggregation, deposition, and enhanced contaminant transport. Environ. Sci. Technol. 52, 1704−1724. https://doi.org/10.1021/acs.est.7b05559.

Aljaibachi, R., Callaghan, A., 2018. Impact of polystyrene microplastics on *Daphnia magna* mortality and reproduction in relation to food availability. PeerJ 6, e4601. https://doi.org/10.7717/peerj.4601.

Anbumani, S., Kakkar, P., 2018. Ecotoxicological effects of microplastics on biota: a review. Environ. Sci. Pollut. Res. 25, 14373−14396. https://doi.org/10.1007/s11356-018-1999-x.

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596−1605. https://doi.org/10.1016/j.marpolbul.2011.05.030.

Backhaus, T., Wagner, M., 2020. Microplastics in the environment: much ado about nothing? A debate. Global Challenges 4, 1900022. https://doi.org/10.1002/gch2.201900022.

Barclay, D.K., Thompson, H.H., Higgins, C.M., 1995. The partial least squares (PLS) approach to causal modeling: personal computer use as an illustration. Technol. Stud. 2, 285−309.

Becker, K., 1996. Exopolysaccharide production and attachment strength of bacteria and diatoms on substrates with different surface tensions. Microb. Ecol. 32, 23−33. https://doi.org/10.1007/BF00170104.

Bilotta, G.S., Brazier, R.E., 2008. Understanding the influence of suspended solids on water quality and aquatic biota. Water Res. 42, 2849–2861. https://doi.org/10.1016/j.watres.2008.03.018.
Blott, S.J., Pye, K., 2001. GRADISTAT: a grain size distribution and statistics package for the analysis of unconsolidated sediments. Earth Surf. Process. Landforms 26, 1237–1248. https://doi.org/10.1002/esp.261.
Bruinink, A., Wang, J., Wick, P., 2015. Effect of particle agglomeration in nano-toxicology. Arch. Toxicol. 89, 659–675. https://doi.org/10.1007/s00204-015-1460-6.
Bubb, J.M., Lester, J.N., 1994. Anthropogenic heavy metal inputs to lowland river systems, a case study. The River Stour, U.K. Water Air Soil Pollut. 78, 279–296. https://doi.org/10.1007/BF00483037.
Burd, A.B., Jackson, G.A., 2009. Particle aggregation. Annu. Rev. Mar. Sci. 1, 65–90. https://doi.org/10.1146/annurev.marine.010908.163904.
Burns, E.E., Boxall, A.B.A., 2018. Microplastics in the aquatic environment: evidence for or against adverse impacts and major knowledge gaps. Environ. Toxicol. Chem. 37, 2776–2796. https://doi.org/10.1002/etc.4268.
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.
Chapman, P.M., Hayward, A., Faithful, J., 2017. Total suspended solids effects on freshwater lake biota other than fish. Bull. Environ. Contam. Toxicol. 99, 423–427. https://doi.org/10.1007/s00128-017-2154-y.
Chin, W.W., 1998. The partial least squares approach for structural equation modeling. Modern Methods for Business Research, Methodology for Business and Management. Lawrence Erlbaum Associates Publishers, Mahwah, NJ, US, pp. 295–336.
Chin, W.W., Peterson, R.A., Brown, S.P., 2008. Structural equation modeling in marketing: some practical reminders. J. Market. Theor. Pract. 16, 287–298. https://doi.org/10.2753/MTP1069-6679160402.
Cohen, J., 1977. In: rev (Ed.), Statistical Power Analysis for the Behavioral Sciences. Lawrence Erlbaum Associates, Inc, Hillsdale, NJ, England.
Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T.S., 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod Calanus helgolandicus. Environ. Sci. Technol. 49, 1130–1137. https://doi.org/10.1021/es504525u.
Connors, K.A., Dyer, S.D., Belanger, S.E., 2017. Advancing the quality of environmental microplastic research. Environ. Toxicol. Chem. 36, 1697–1703. https://doi.org/10.1002/etc.3829.
Filella, M., Rellstab, C., Chanudet, V., Spaak, P., 2008. Effect of the filter feeder Daphnia on the particle size distribution of inorganic colloids in freshwaters. Water Res. 42, 1919–1924. https://doi.org/10.1016/j.watres.2007.11.021.
Folk, R.L., Ward, W.C., 1957. Brazos River bar [Texas]; a study in the significance of grain size parameters. J. Sediment. Res. 27, 3–26. https://doi.org/10.1306/74D70646-2B21-11D7-8648000102C1865D.
Fox, R.B., Price, T.R., Cain, D.S., 1968. Wettability and constitution of photooxidized polystyrene and other amorphous polymers. In: Alexander, A.L. (Ed.), Interaction of Liquids at Solid Substrates, Advances in Chemistry. AMERICAN CHEMICAL SOCIETY, WASHINGTON, D. C., pp. 72–84. https://doi.org/10.1021/ba-1968-0087.ch006
Galgani, F., Hanke, G., Maes, T., 2015. Global distribution, composition and abundance of marine litter. In: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer International Publishing, Cham, pp. 29–56. https://doi.org/10.1007/978-3-319-16510-3_2.
Gerdes, Z., Hermann, M., Ogonowski, M., Gorokhova, E., 2019. A novel method for assessing microplastic effect in suspension through mixing test and reference materials. Sci. Rep. 9, 10695. https://doi.org/10.1038/s41598-019-47160-1.
Gewert, B., Plassmann, M., Sandblom, O., MacLeod, M., 2018. Identification of chain scission products released to water by plastic exposed to ultraviolet light. Environ. Sci. Technol. Lett. 5, 272–276. https://doi.org/10.1021/acs.estlett.8b00119.
Gophen, M., Geller, W., 1984. Filter mesh size and food particle uptake by <Emphasis Type="Italic">Daphnia</Emphasis>. Oecologia 64, 408–412. https://doi.org/10.1007/BF00379140.
Gordon, A.K., Palmer, C.G., 2015. Defining an exposure–response relationship for suspended kaolin clay particulates and aquatic organisms: work toward defining a water quality guideline for suspended solids. Environ. Toxicol. Chem. 34, 907–912. https://doi.org/10.1002/etc.2872.
Gouin, T., Becker, R.A., Collot, A., Davis, J.W., Howard, B., Inawaka, K., Lampi, M., Ramon, B.S., Shi, J., Hopp, P.W., 2019. Toward the development and application of an environmental risk assessment framework for microplastic. Environ. Toxicol. Chem. 38, 2087–2100. https://doi.org/10.1002/etc.4529.
Hair, J.F., Matthews, L.M., Matthews, R.L., Sarstedt, M., 2017. PLS-SEM or CB-SEM: updated guidelines on which method to use. IJMDA 1, 107. https://doi.org/10.1504/IJMDA.2017.087624.
Hair, J.F., Ringle, C.M., Sarstedt, M., 2011. PLS-SEM: indeed a silver bullet. J. Market. Theor. Pract. 19, 139–152. https://doi.org/10.2753/MTP1069-6679190202.
Hair, J.F., Sarstedt, M., Hopkins, L., G Kuppelwieser, V., 2014. Partial least squares structural equation modeling (PLS-SEM): an emerging tool in business research. Eur. Bus. Rev. 26, 106–121. https://doi.org/10.1108/EBR-10-2013-0128.
Hart, R.C., 1986. Zooplankton abundance, community structure and dynamics in relation to inorganic turbidity, and their implications for a potential fishery in subtropical Lake le Roux, South Africa. Freshw. Biol. 16, 351–371. https://doi.org/10.1111/j.1365-2427.1986.tb00976.x.
Heath, A.R., Bahri, P.A., Fawell, P.D., Farrow, J.B., 2006. Polymer flocculation of calcite: experimental results from turbulent pipe flow. AIChE J. 52, 1284–1293. https://doi.org/10.1002/aic.10729.
Henderson, L., Green, C., 2020. Making sense of microplastics? Public understandings of plastic pollution. Mar. Pollut. Bull. 152, 110908. https://doi.org/10.1016/j.marpolbul.2020.110908.
Hoekstra, J.A., 1987. Acute bioassays with control mortality. Water Air Soil Pollut. 35, 311–317. https://doi.org/10.1007/BF00290938.
Hu, L., Bentler, P.M., 1999. Cutoff criteria for fit indexes in covariance structure analysis: conventional criteria versus new alternatives. Struct. Equ. Model.: A Multidisciplinary Journal 6, 1–55. https://doi.org/10.1080/10705519909540118.
Jedwab, J., 1980. Rare anthropogenic and natural particles suspended in deep ocean waters. Earth Planet Sci. Lett. 49, 551–564. https://doi.org/10.1016/0012-821X(80)90095-3.
Jeong, C.-B., Won, E.-J., Kang, H.-M., Lee, M.-C., Hwang, D.-S., Hwang, U.-K., Zhou, B., Souissi, S., Lee, S.-J., Lee, J.-S., 2016. Microplastic size-dependent toxicity, oxidative stress induction, and p-JNK and p-p38 activation in the monogonont rotifer (Brachionus koreanus). Environ. Sci. Technol. 50, 8849–8857. https://doi.org/10.1021/acs.est.6b01441.
Kiørboe, T., Andersen, K.P., Dam, H.G., 1990. Coagulation efficiency and aggregate formation in marine phytoplankton. Mar. Biol. 107, 235–245. https://doi.org/10.1007/BF01319822.
Kirk, K.L., 1991a. Inorganic particles alter competition in grazing plankton: the role of selective feeding. Ecology 72, 915–923. https://doi.org/10.2307/1940593.
Kirk, K.L., 1991b. Suspended clay reduces Daphnia feeding rate. Freshw. Biol. 25, 357–365. https://doi.org/10.1111/j.1365-2427.1991.tb00498.x.
Kirk, K.L., Gilbert, J.J., 1990. Suspended clay and the population dynamics of planktonic rotifers and cladocerans. Ecology 71, 1741–1755. https://doi.org/10.2307/1937582.
Kjelland, M.E., Woodley, C.M., Swannack, T.M., Smith, D.L., 2015. A review of the potential effects of suspended sediment on fishes: potential dredging-related physiological, behavioral, and transgenerational implications. Environ Syst Decis 35, 334–350. https://doi.org/10.1007/s10669-015-9557-2.
Kokalj, A.J., Kunej, U., Skalar, T., 2018. Screening study of four environmentally relevant microplastic pollutants: uptake and effects on Daphnia magna and Artemia franciscana. Chemosphere 208, 522–529. https://doi.org/10.1016/j.chemosphere.2018.05.172.
Ladd, H.S., 1957. Treatise on Marine Ecology and Paleoecology. Geological Society of America. https://doi.org/10.1130/MEM67V2-p01.
Lehmann, A., Fitschen, K., Rillig, M.C., 2019. Abiotic and biotic factors influencing the effect of microplastic on soil aggregation. Soil Systems 3, 21. https://doi.org/10.3390/soilsystems3010021.
Li, S., Wang, P., Zhang, C., Zhou, X., Yin, Z., Hu, T., Hu, D., Liu, C., Zhu, L., 2020. Influence of polystyrene microplastics on the growth, photosynthetic efficiency and aggregation of freshwater microalgae Chlamydomonas reinhardtii. Sci. Total Environ. 714, 136767. https://doi.org/10.1016/j.scitotenv.2020.136767.
Liao, B.Q., Allen, D.G., Leppard, G.G., Droppo, I.G., Liss, S.N., 2002. Interparticle interactions affecting the stability of sludge flocs. J. Colloid Interface Sci. 249, 372–380. https://doi.org/10.1006/jcis.2002.8305.
Loureiro, C., Castro, B.B., Pereira, J.L., Gonçalves, F., 2011. Performance of standard media in toxicological assessments with Daphnia magna: chelators and ionic composition versus metal toxicity. Ecotoxicology 20, 139–148. https://doi.org/10.1007/s10646-010-0565-1.
Lowry, G.V., Gregory, K.B., Apte, S.C., Lead, J.R., 2012. Transformations of nanomaterials in the environment. Environ. Sci. Technol. 46, 6893–6899. https://doi.org/10.1021/es300839e.
MacKinnon, D.P., Lockwood, C.M., Hoffman, J.M., West, S.G., Sheets, V., 2002. A comparison of methods to test mediation and other intervening variable effects. Psychol. Methods 7, 83.
Martínez-Gómez, C., León, V.M., Calles, S., Gomáriz-Olcina, M., Vethaak, A.D., 2017. The adverse effects of virgin microplastics on the fertilization and larval development of sea urchins. Mar. Environ. Res. https://doi.org/10.1016/j.marenvres.2017.06.016.
Mattsson, K., Johnson, E.V., Malmendal, A., Linse, S., Hansson, L.-A., Cedervall, T., 2017. Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain. Sci. Rep. 7, 11452. https://doi.org/10.1038/s41598-017-10813-0.
McAnally, W.H., Mehta, A.J., 2000. Aggregation rate of fine sediment. J. Hydraul. Eng. 126, 883–892. https://doi.org/10.1061/(ASCE)0733-9429(2000)126:12(883).
McGivney, E., Cederholm, L., Barth, A., Hakkarainen, M., Hamacher-Barth, E., Ogonowski, M., Gorokhova, E., 2020. Rapid physicochemical changes in microplastic induced by biofilm formation. Front. Bioeng. Biotechnol. 8 https://doi.org/10.3389/fbioe.2020.00205.
Michels, J., Stippkugel, A., Lenz, M., Wirtz, K., Engel, A., 2018. Rapid aggregation of biofilm-covered microplastics with marine biogenic particles. Proc. R. Soc. B 285, 20181203. https://doi.org/10.1098/rspb.2018.1203.
Nguyen, B., Claveau-Mallet, D., Hernandez, L.M., Xu, E.G., Farner, J.M., Tufenkji, N., 2019. Separation and analysis of microplastics and nanoplastics in complex environmental samples. Acc. Chem. Res. 52, 858–866. https://doi.org/10.1021/acs.accounts.8b00602.
OECD, 2004. Test No. 202: Daphnia Sp. Acute Immobilisation Test. Organisation for Economic Co-operation and Development, Paris.
Ogonowski, M., Gerdes, Z., Gorokhova, E., 2018a. What we know and what we think we know about microplastic effects – a critical perspective. Current Opinion in Environmental Science & Health 1, 41–46. https://doi.org/10.1016/j.coesh.2017.09.001.
Ogonowski, M., Motiei, A., Ininbergs, K., Hell, E., Gerdes, Z., Udekwu, K.I., Bacsik, Z.,

Gorokhova, E., 2018b. Evidence for selective bacterial community structuring on microplastics. Environ. Microbiol. 20, 2796–2808. https://doi.org/10.1111/1462-2920.14120.
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, e0155063. https://doi.org/10.1371/journal.pone.0155063.
Rehse, S., Kloas, W., Zarfl, C., 2016. Short-term exposure with high concentrations of pristine microplastic particles leads to immobilisation of Daphnia magna. Chemosphere 153, 91–99. https://doi.org/10.1016/j.chemosphere.2016.02.133.
Rellstab, C., Spaak, P., 2007. Starving with a full gut? Effect of suspended particles on the fitness of Daphnia hyalina. Hydrobiologia 594, 131–139. https://doi.org/10.1007/s10750-007-9089-y.
Ringle, C.M., Wende, S., Becker, J.-M., 2015. SmartPLS. SmartPLS.
Robinson, S.E., Klaine, S.J., 2008. Response of Daphnia magna to episodic exposures of several types of suspended clay. Proceedings of the 2008 South Carolina Water Resources Conference. Presented at the South Carolina Water Resources Conference. Clemson University, Charleston, USA, p. 2.
Rochman, C.M., Browne, M.A., Halpern, B.S., Hentschel, B.T., Hoh, E., Karapanagioti, H.K., Rios-Mendoza, L.M., Takada, H., Teh, S., Thompson, R.C., 2013. Policy: classify plastic waste as hazardous. Nature 494, 169–171. https://doi.org/10.1038/494169a.
Rogers, K.L., Carreres-Calabuig, J.A., Gorokhova, E., Posth, N.R., 2020. Micro-by-micro interactions: how microorganisms influence the fate of marine microplastics. Limnology and Oceanography Letters 5, 18–36. https://doi.org/10.1002/lol2.10136.
Roy, S., Gaillardet, J., Allègre, C.J., 1999. Geochemistry of dissolved and suspended loads of the Seine River, France: anthropogenic impact, carbonate and silicate weathering. Geochem. Cosmochim. Acta 63, 1277–1292. https://doi.org/10.1016/S0016-7037(99)00099-X.
Rummel, C.D., Jahnke, A., Gorokhova, E., Kühnel, D., Schmitt-Jansen, M., 2017. Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. Environmental Science & Technology Letters. https://doi.org/10.1021/acs.estlett.7b00164.
Schoenberg, S.A., Maccubbin, A.E., 1985. Relative feeding rates on free and particle-bound bacteria by freshwater macrozooplankton1. Limnol. Oceanogr. 30, 1084–1090. https://doi.org/10.4319/lo.1985.30.5.1084.
Šolc, R., Gerzabek, M.H., Lischka, H., Tunega, D., 2011. Wettability of kaolinite (001) surfaces — molecular dynamic study. Geoderma. Advances of Molecular Modeling of Biogeochemical Interfaces in Soils 169, 47–54. https://doi.org/10.1016/j.geoderma.2011.02.004.
Straughan, D.J., Lehman, N., 2000. Genetic differentiation among Oregon lake populations of the Daphnia pulex species complex. J. Hered. 91, 8–17. https://doi.org/10.1093/jhered/91.1.8.
Van Leussen, W., 1988. Aggregation of particles, settling velocity of mud flocs A review. In: Dronkers, J., van Leussen, W. (Eds.), Physical Processes in Estuaries. Springer, Berlin, Heidelberg, pp. 347–403. https://doi.org/10.1007/978-3-642-73691-9_19.
Vassileva, N.D., van den Ende, D., Mugele, F., Mellema, J., 2007. Fragmentation and erosion of two-dimensional aggregates in shear flow. Langmuir 23, 2352–2361. https://doi.org/10.1021/la0625087.
Zethof, J.H.T., Bettermann, A., Vogel, C., Babin, D., Cammeraat, E.L.H., Solé-Benet, A., Lázaro, R., Luna, L., Nesme, J., Woche, S.K., Sørensen, S.J., Smalla, K., Kalbitz, K., 2020. Prokaryotic community composition and extracellular polymeric substances affect soil microaggregation in carbonate containing semiarid grasslands. Front. Environ. Sci. 8 https://doi.org/10.3389/fenvs.2020.00051.
Zettler, E.R., Carter, J.C.H., 1986. Zooplankton community and species responses to a natural turbidity gradient in lake temiskaming, ontario–quebec. Can. J. Fish. Aquat. Sci. 43, 665–673. https://doi.org/10.1139/f86-080.
Zurek, R., 1983. Effect of suspended materials on zooplankton. II: laboratory investigations of Daphnia hyalina Leydig. Acta Hydrobiol. 24, 233–251.