Effects of dissolved organic matter and ecocorona formation on the toxicity of micro- and nanoplastic particles to *Daphnia* - A meta-analysis

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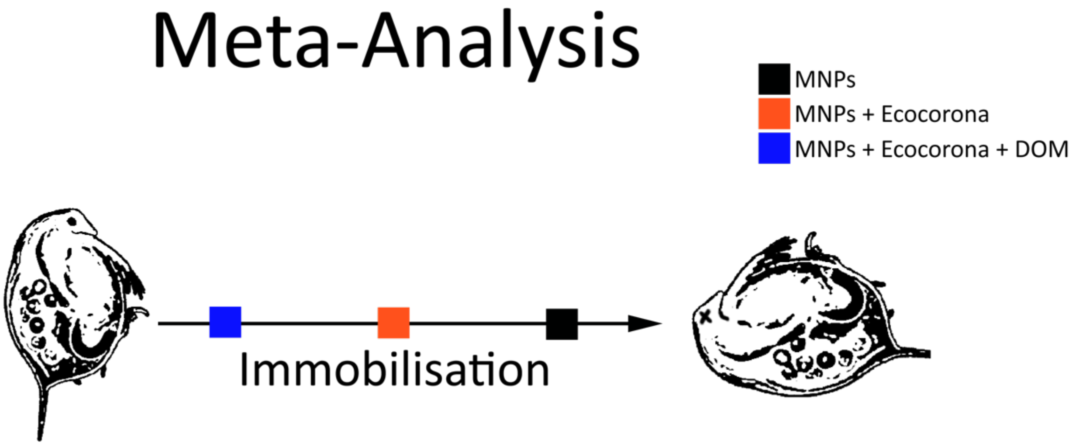
Graphical Abstract



Highlights

- Micro- and nanoplastic particles are known to increase *Daphnia* mortality.
- Dissolved organic matter and ecocorona formation may moderate these effects.
- Meta-analysis shows attenuating effects of ecocorona and DOM on microplastic toxicity.
- Moderating effects depend on DOM type and experimental setup.
- Moderating effects are stronger in co-exposure than when incubated before exposure.

1. Abstract

Significant quantities of micro- and nanoplastic particles (MNP) end up in the environment, either due to larger plastic debris breaking down or by entering directly as MNPs. Effects of MNPs on organisms have been increasingly reported in recent years, with a large number of studies conducted on water fleas of the genus *Daphnia*. Most of the available studies used pristine particles that have not been exposed to the environment or to organic substances. In natural environments, however, proteins, organic substances and, if the particles are large enough, bacteria attach to MNP, forming an ecocorona or biofilm on the particles' surface. How the formation of an ecocorona influences MNP toxicity is still uncertain. While some studies suggest that ecocorona formation can mitigate the negative effects of MNP on organisms, other studies did not find such associations. In addition, it is unclear whether the ecocorona itself is attenuating the effects of MNP or whether dissolved organic matter (DOM) affects toxicity indirectly such as by increasing *Daphnia's* resilience to stressors in general. To draw more solid conclusions about the direction and size of the mediating effect of DOM and ecocorona formation on MNP-associated immobilization in *Daphnia* spp., we synthesized evidence from the published literature and compiled 305 data points from 13 independent studies. The results of our meta-analysis show that the toxic effects of MNP are likely reduced in the presence of certain types of DOM. We observed similar mediating effects when MNP were incubated in media containing DOM before the exposure experiments, although to a lesser extent. Future studies designed to disentangle the effects of the ecocorona itself from the general effects of DOM will contribute to a deeper mechanistic understanding of MNP toxicity in nature and enhance the reliability of MNP risk assessment.

2. Introduction

Plastics are widely used in industry and are present in almost all areas of our daily lives. Plastic materials are used for food packaging, in transportation and construction, for recreation activities, clothing and personal care products, research and agriculture (Andrady & Neal, 2009). In 2021, global plastic production was estimated to be 390.7 million tones (Plastics Europe, 2022). Through inappropriate disposal, large amounts of plastic end up in the environment (Barnes et al., 2009). Over time, they break down into smaller fragments, eventually degrading into microplastic (100 nm - 5 mm) and nanoplastic (< 100 nm) particles (MNP; GESAMP, 2016). In addition, plastic particles enter the environment directly, for example in the form of intentionally added microbeads, textile fibers and tire abrasion. Once in the environment, MNPs are transported between environmental compartments, enter water bodies through surface runoff and wastewater, and accumulate in oceans, rivers and lakes (Koutnik et al., 2021; Nava et al., 2023; Petersen & Hubbart, 2021).

The number of studies investigating the potential harm of MNPs to organisms has increased substantially over the last years (Barbosa et al., 2020). MNPs are ingested by animals and can block their digestive tract, injure gut epithelia and change the gut microbiome (Qiao et al., 2019; Susanti et al., 2020). If small enough, particles can translocate into tissues and cells (Hara et al., 2020; Von Moos et al., 2012) and induce inflammatory responses and cellular damage (Solomando et al., 2020; M. Zhang et al., 2022). On an organismic level, effects on body growth, reproduction and survival have been reported, among others (Lahive et al., 2019; Sussarellu et al., 2016; Ziajahromi et al., 2017). In addition to effects elicited by the particles themselves, additives such as UV-stabilizers (Song et al., 2021) or plasticizers (Y. Yan et al., 2021) added to the polymers may leach from the particles and induce toxic responses (Zimmermann et al., 2020).Pollutants or pathogens may attach to the particle surfaces and induce toxic effects upon ingestion (vector effect; Rochman et al. 2013; Gkoutselis et al., 2021; but see also do Prado Leite et al., 2022 and Koelmans et al., 2016).

Due to their complexity, the effects of MNP cannot be easily generalized. Unlike chemicals which have distinct molecular structures and stable properties and can usually be assigned unique identifiers (e.g., CAS number), MNP are more diverse. Each single particle possesses its own set of chemical and physical properties. These properties include simple characteristics such as polymer type, size and shape, as well as more complex properties like mixtures of plastic-associated chemicals, surface structure and charge, and substances attached to the particles' surface including organic substances from the environment (ecocorona; Nasser et al., 2020), proteins (protein corona; Fadare et al., 2020; Kihara et al., 2020) and bacteria (biofilm; Barros et al., 2020; Shi et al., 2023). In addition, all these properties can change over time. This complexity makes extensive testing necessary if we want to understand how specific MNP properties relate to specific toxicity outcomes and how strong effects are to be expected in natural environments.

Water fleas of the genus *Daphnia* are important standard test organisms for aquatic systems that occur in most stagnant and slowly flowing freshwater habitats. As part of the zooplankton, *Daphnia ssp.* play an essential role in the aquatic food web. They form a link from producer plankton which contains essential fatty acids to higher trophic levels of the food web and are considered sensitive keystone species. As highly effective filter feeders they are exposed to substances and matter contained in the surrounding water. The non-selective feeding method makes them particularly vulnerable to accidental ingestion of particulate pollutants (Giovio et al., 2020). *Daphnia* spp. are easy to maintain in the lab, as they have short generation times and typically reproduce parthenogenetically, if not stressed. Consequently, *Daphnia* spp. are widely used in acute and chronic standard toxicity tests (OECD, 2004, 2012). *Daphnia magna* is the most tested aquatic invertebrate species in studies investigating the ecotoxicological effects of MNP on organisms (see ToMEx database: Hampton et al., 2022; and Brehm et al., 2023).

Several studies have shown effects of MNP on *Daphnia spp.* including a variety of endpoints and particle characteristics (for an overview see Brehm et al., 2023). For example, Eltemsah & Bøhn (2019) observed increased mortality rates, decreased growth, and stimulation of early reproduction at the expense of later reproduction in *Daphnia* exposed to polystyrene microbeads; P. Zhang et al. (2019) observed changes in levels of radical oxygen species (ROS); and Lin et al. (2019) observed changes in the daphnids' swimming activity. While earlier studies often tested only one specific type of MNP, later studies more frequently demonstrated that effects are not uniform across all types of MNP but instead depend on the MNPs' properties. Effects have been shown to depend, among others, on polymer type, particle size and shape, surface charge and the presence of additives (Lin et al., 2019; Saavedra et al., 2019; Schrank et al., 2019; Schwarzer et al., 2022; Zimmermann et al., 2020). Most studies so far have worked with pristine particles (Brehm et al., 2023), i.e., particles that have not had contact with natural environments. Only a few studies have attempted to investigate effects under more realistic circumstances. Whether effects observed in the lab are representative of true effects expected in nature is therefore still under debate (Nasser et al., 2020; Nasser & Lynch, 2016; see also Petersen et al., 2022)

In natural environments, an ecocorona forms on the particles within seconds when organic molecules attach to the MNPs' surface (e.g., Rummel et al., 2017). This ecocorona alters the particles' surface structure and charge (Natarajan et al., 2021; Shi et al., 2023; Witzmann et al., 2022), influences their behavior in the water column (Elagami et al., 2022; Fischer et al., 2022) and modifies their attachment rate to cellular surfaces (Ramsperger et al., 2020). These alterations may in consequence also affect outcomes on the organismic level, for instance mediated by changes in uptake and tissue translocation rates (Raftis & Miller, 2019; Triebskorn et al., 2019). In *Daphnia*, it has been demonstrated that the presence of ecocorona influences the uptake rates for MNPs and their retention time in the gut (Nasser & Lynch, 2016). In exposed organisms, the presence of an ecocorona has for instance been shown to alter MNP effects on mobility/survival (e.g., Fadare et al., 2019; Schür et al., 2021), feeding behavior (Nasser & Lynch, 2016) and molecular effects (e.g., Fadare et al., 2020). However, deriving a general pattern for the direction of these mediating effects of ecocorona formation is still challenging. One reason for this is that results in the published literature are not entirely consistent: while ameliorating effects of ecocorona formation were found for some MNPs (e.g., Amariei et al., 2022; Saavedra et al., 2019; Wu et al., 2019), this was not the case for other MNPs (e.g., Pochelon et al., 2021; F. Zhang et al., 2019), and also increased toxicities have been observed (Nasser & Lynch, 2016). Another factor complicating generalized conclusions is the way experiments are conducted. While ecocorona formation in some experiments was induced by co-exposing organisms to MNP and dissolved organic matter (DOM) or organic substances simultaneously (e.g., F. Zhang et al., 2019), other studies investigated mediating effects of the ecocorona alone by incubating MNP in media containing DOM prior to being transferred to the exposure medium (i.e., no additional DOM in the exposure medium; e.g.Schür et al., 2021). It is thus unclear, whether it was the ecocorona itself or the DOM in the media that led to the observed differences.

Furthermore, the type of DOM used in experiments affects the composition and thickness of the formed ecocorona and may in turn influence observed outcomes (Reilly et al., 2022; Schefer et al., 2023).

Meta-analyses are a tool for addressing exactly these kinds of questions, where the presence, direction and size of effects are unclear (Field & Gillett, 2010). By aggregating data from several studies in a quantitative way, meta-analyses aim to derive effect size estimates with reduced bias and greater precision (lower uncertainty) than estimates from single studies (Gurevitch et al., 2001; Harrison, 2011).

We performed a meta-analysis to answer the question of how the presence of DOM and ecocorona alter the effects of MNP on *Daphnia* immobilization rates. Through a systematic literature search of experimental studies, we compared the effects of MNP with ecocorona/DOM to effects of the exact same particles without ecocorona/DOM. Based on the gathered data, we discuss the strength of evidence regarding mediating effects of ecocorona formation on MNP toxicity. In addition, we investigate whether the mediating effects depend on the type of DOM and the type of experimental approach used (either co-exposure with DOM or incubation prior to exposure).

3. Materials and Methods

3.1 Literature search

We conducted a literature search for studies that investigated effects of MNP on immobilization rates (including mortality) in water fleas of the genus *Daphnia*. The aim was to compile data from studies that met all of the following inclusion criteria: (1) experimental research (excluding books and reviews) published in English, (2) investigation of alterations of MNP effects due to ecocorona formation (i.e. studies contained at least one MNP treatment with ecocorona and one treatment with the same particle properties without ecocorona), (3) testing of water fleas of the genus *Daphnia*, and (4) absence of additional stressors during exposure (e.g., chemicals). The final search in December on Web of Science (WoS) and PubMed using the search string "((micro* OR nano*) AND (plastic* OR particle*) OR microplastic OR nanoplastic) AND Daphnia AND (eco-corona OR ecocorona OR biofilm OR humic acid OR DOC OR DOM OR fulvic acid OR lake water OR protein corona OR protein-corona OR proteincorona OR incub*)”. In parallel, we searched for review articles addressing effects of MNPs on *Daphnia* or freshwater organisms as additional sources of literature. After removing duplicates, all titles and abstracts were screened. Studies that clearly did not follow the inclusion criteria were removed. All remaining studies were subjected to full text screening and only those studies that met all selection criteria were kept for data extraction (see full text screening list in the supplementary online material).

3.2 Data extraction

The extracted data consists of immobilization and mortality measurements, information on added DOM, characteristics of the used MNP, information on the test organisms and experimental parameters. As studies frequently did not distinguish between immobilization (absence of movement after agitation; OECD, 2004) and mortality (absence of heartbeat in addition to the absence of movement), we will refer to both as immobilization.

We extracted immobilization rates (i.e., the number of immobile/mobile individuals or the proportion of immobile individuals) for both the MNP treatment with ecocorona/DOM and the control treatment without ecocorona/DOM. Whenever possible, we extracted the rates directly from the text, data tables or raw data files provided in the supplementary online material. In cases where the data was instead presented in figures, the rates were extracted from the plots using the R package *metaDigitise* (Pick et al., 2019). If daphnids were observed repeatedly over time, immobilization rates were extracted for the latest time point (one of either 24, 48, 72 or 96 hours). If none of these time points were measured, we used the latest reported time point in the study. Immobilization rates for all other time points were neglected. Additionally, we extracted the number of replicates (i.e., the number of independent test vessels) and the number of individuals per replicate (i.e., the number of daphnids per test vessel). These numbers were used to calculate the number of immobile and mobile individuals if immobilization rates were reported as proportions.

The pairing of treatments using identical experimental setups and particles that differed only in the presence or absence of an ecocorona/DOM allowed us to directly control for confounding by other MNP properties, different experimental parameters, and characteristics of the test organisms. As the main explanatory variables, we thus only noted (1) the type of DOM added in experiments (*DOM_type*; e.g., humic or fulvic acid, different types of lake water, etc.), (2) whether DOM was added during exposure (*DOM_conditioned*: no) or whether the particles had instead been incubated in DOM-containing media prior to their use in experiments (i.e. they were removed from the DOM-containing media prior to transfer to the exposure vessels without DOM; *DOM_incubated*: yes).

For completeness and to enable extended use of the data in the future, we extracted the following additional information from the studies if available: particle concentration (in mg per ml and particles per ml), MNP properties including polymer type, particle size (mean ± standard deviation), particle shape (spherical, fragment, fiber), the particles' chemical surface modification (e.g. carboxylation, amination) and surface charge (either positive or negative); characteristics of the test organisms including species, clone and age at the start of exposure; experimental conditions including temperature, pH of the test medium, whether food was provided during exposure and its concentration, and the concentration of DOM added during MNP incubation or during exposure.

3.3 Statistical analysis

As a measure of effect size, we calculated log risk ratios (log (RR)) for immobilization. A multiple mixed meta regression model without intercept was fit to the data including the two factors *DOM_type* and *DOM_conditioned* (either yes or no) as moderators and a random intercept for the publication identifier (*Publication ID*):

Moderator effects, i.e., the effects of the two fixed factors, were investigated by fitting reduced models including only one of the moderators at a time and comparing these reduced models to the full model through likelihood ratio tests. In addition, the variance component attributed to *Publication ID* (sigma squared) was checked using profile likelihood plots to ensure that the component was successfully identified, indicated by the curve peaking at the maximum likelihood estimate. To see whether the two factors in the model adequately accounted for the heterogeneity among data points, a test for residual heterogeneity was conducted and the variance attributable to among-sample heterogeneity rather than sampling variance (I^2) was calculated.

Based on the full model, an orchard plot was generated to visualize the average marginal effect estimates for each combination of moderator levels. In contrast to forest plots, orchard plots include individual effect size estimates, confidence intervals (CI) and 95% prediction intervals.

To investigate potential publication bias visually, standard errors were plotted against residuals of the full model in a funnel plot. Selective publishing in funnel plots is indicated by a clear shift of data to higher effect sizes in studies with high standard errors, suggesting that studies with low power are preferentially published when observed effects are large.

All analyses were done in R version 4.3.1 (R Core Team 2023). The *metafor* package version 4.2 (Viechtbauer, 2010) was used for effect size calculation, fitting meta regression models and investigating publication bias. The *orchaRd 2.0* package (Nakagawa et al., 2023) was used for creating the orchard plot and calculating I^2.

4. Results

4.1 Literature search and data extraction

The literature search resulted in 955 publications, of which 925 failed to meet at least one of the selection criteria. After full text screening of the remaining 30 publications, 17 studies were excluded either because they did not fulfill all the selection criteria or data extraction was not possible. The remaining 13 publications were used for data extraction. In total, we extracted 305 data points (Fig. 1). We grouped the types of DOM used in the studies into seven main categories: metabolites excreted from *Daphnia* (2 studies, 21 data points), humic acid (4 studies, 43 data points), fulvic acid (2 studies, 24 data points), commercially bought natural organic matter (1 study, 6 data points), stream water (3 studies, 34 data points), lake water (3 studies, 128 data points) and wastewater (4 studies, 49 data points). In seven of the studies, the MNPs were incubated in the respective DOM type prior to exposure (105 data points). In the other six studies, DOM was added during exposure or exposure was conducted in water sampled from natural environments (200 data points). All included studies tested the species *Daphnia magna* (for a full overview of experimental parameters covered in the studies see raw data in the supplementary online material).

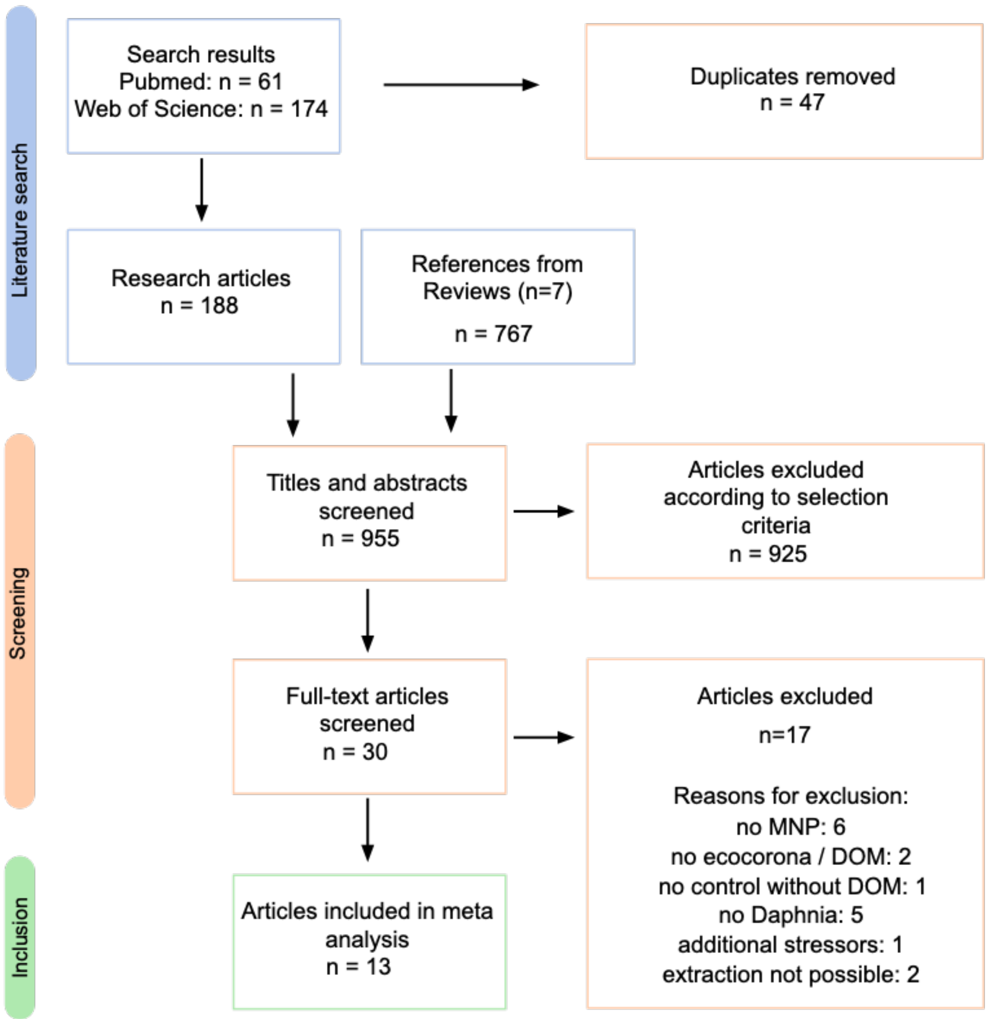


Fig. 1: PRISMA flow diagram illustrating the results from the systematic literature search and stepwise exclusion of studies not fulfilling defined selection criteria. MNP: micro- and nanoplastic particles. DOM: dissolved organic matter.

4.2 Effects of DOM type and conditioning on immobilization rates

In the full model, moderators had a significant effect on immobilization risk (test of moderators: Q_M = 17.33, df = 8, p = 0.023) indicating that at least one of them accounted for differences in effects across samples. A comparison of full and reduced models showed that DOM type was more important for the model fit (comparison of the full versus the reduced model without factor *DOM_type*: LR = 12.47, p = 0.052) than whether the particles had been conditioned prior to their use in exposure experiments (comparison of the full versus the reduced model without factor *DOM_conditioned*: LR = 0.08, p = 0.78). A reduction in immobilization risk was observed when DOM was added in the form of humic acid (log(RR): -0.58 (CI: -1.03, -0.14), RR: 0.56 (CI: 0.36, 0.87), z = -2.56, p = 0.01) or lake water during exposure (log(RR): -0.60 (CI: -1.05, -0.15), RR: 0.55 (CI: 0.35, 0.86), z = -2.62, p = 0.009), and to a lesser degree when MNP were incubated in wastewater (log(RR): -0.28 (CI: -0.88, 0.32), RR: 0.76 (CI: 0.41, 1.37), z = -0.93, p = 0.35) or stream water (log(RR): -0.21 (CI: -0.81, 0.40), RR: 0.81 (CI: 0.44, 1.49), z = -0.67, p = 0.50) prior to exposure (Fig. 2). Changes in immobilization risks due to DOM were below 15% in all other treatments.

In general, we found high variance in the data in all groups. In addition, while DOM type and experimental approach (*DOM_conditioned*) explained part of the variance in the data, the model left significant residual heterogeneity (Q_M = 680.25, df = 297, $p < 0.0001$) indicating that other factors not included in our analysis additionally contribute to the differences. In accordance with this, 97% of the variance among data points can be attributed to sample heterogeneity (I^2 = 0.97) and only 3% are estimated to result from sampling variance.

4.3 Publication bias

A visual inspection of the funnel plot (Fig. 3) did not show severe deviations from symmetry, except for a small gap in data points on the right for medium powered studies (see gap on the right at medium standard errors in Fig. 3).

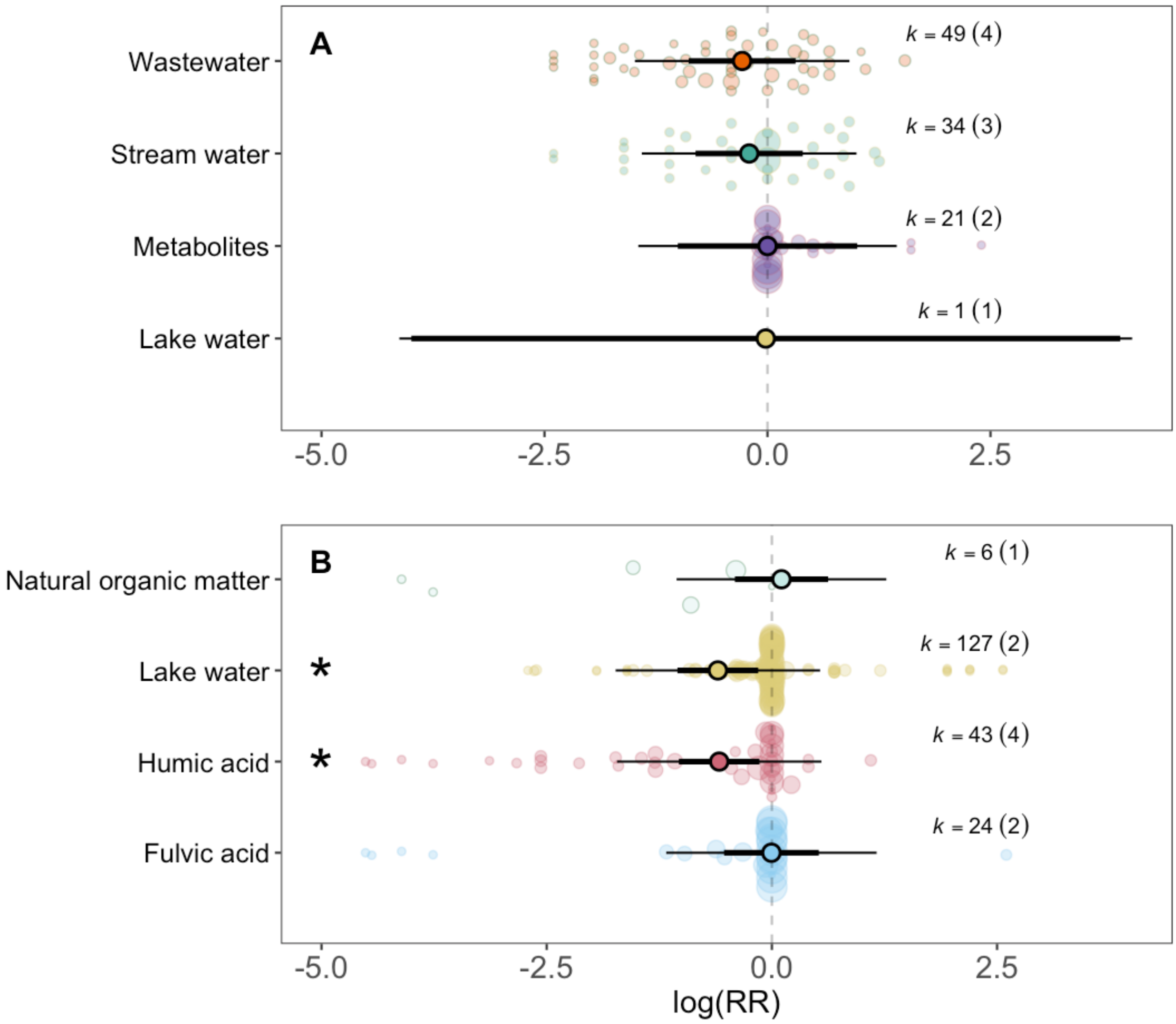


Fig. 2: Mean treatment effects of different dissolved organic matter (DOM) types from the selected studies. A: effects shown for micro- and nanoplastic particles (MNP) conditioned in DOM containing medium; B: effects of MNP with DOM added to the medium during exposure; log [RR]: Log risk ratio; thicker black lines show 95% confidence intervals; narrow lines show prediction intervals; asterisks indicate significant moderation of MNP effects ($p < 0.05$). Point sizes reflect inverse standard errors.

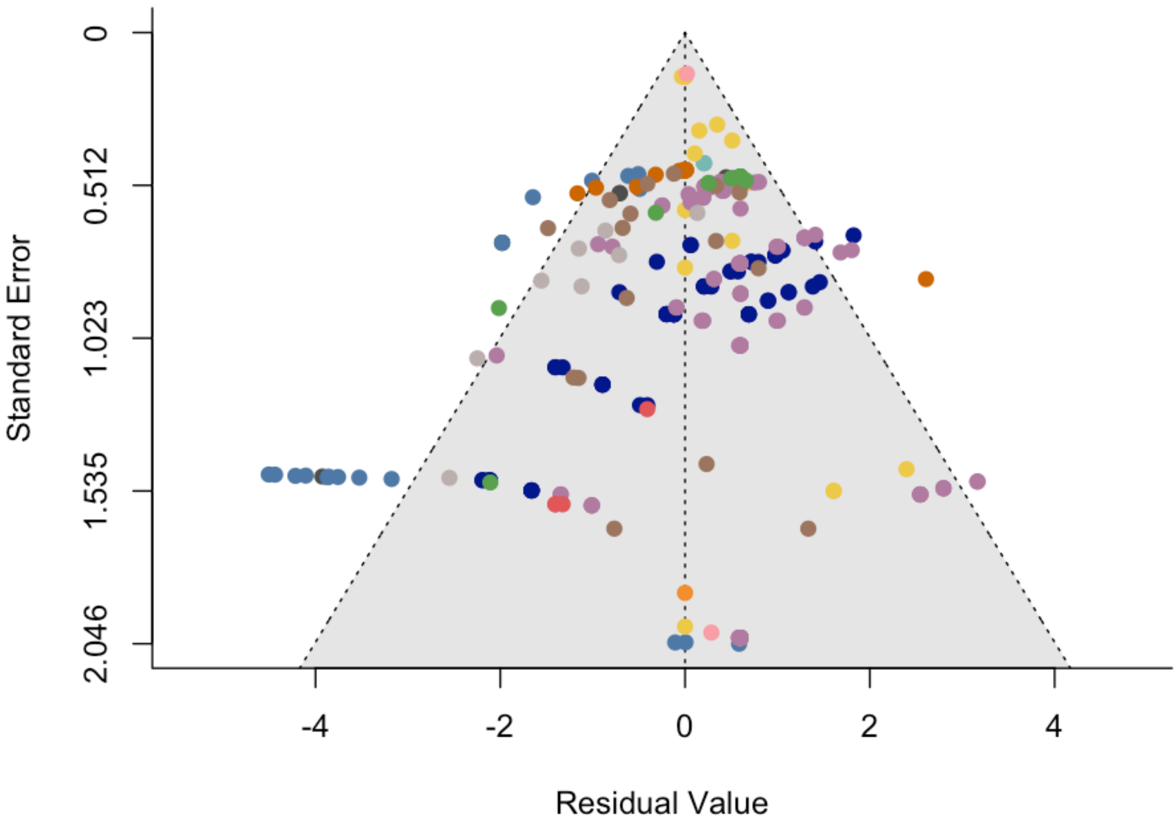


Fig. 3: Standard errors of data points from the studies included in the meta-analysis plotted against residual log (RR). Different colors represent data points extracted from different publications and the grey shaded area represents the 95 % pseudo confidence interval.

5. Discussion

Our meta-analysis shows that immobilization of *Daphnia* by MNPs can be alleviated by DOM. While it has been argued that the reduction in negative effects in the presence of DOM might be attributed to the formation of an ecocorona on the MNP surface alone (e.g., Fadare et al., 2020; Junaid & Wang, 2021), our data indicate that DOM present in the media may contribute additionally to the observed mitigating effects. Furthermore, the compiled data suggest that moderating effects of DOM depend on the type of DOM used.

DOM is known to alleviate effects of various pollutants in *Daphnia* spp. For example, humic acid has been shown to attenuate negative effects of soluble substances (e.g., Oris et al., 1990; Paulauskis & Winner, 1988; Y. Zhang et al., 2019), pesticides (e.g., Day, 1991) and various particulate pollutants (e.g., Y. Zhang et al., 2019), and natural organic matter has been shown to reduce the toxicity of perfluorooctane sulfonate (PFOS; Kovacevic et al., 2019) and heavy metals (e.g., De Schamphelaere et al., 2004; Penttinen et al., 1998), among others. In addition, beneficial impacts of DOM on effects imposed by MNP and other pollutants have also been demonstrated in several other aquatic organisms. Saavedra et al. (2019) for example tested the toxicity of MNPs to the rotifer *Brachionus calyciflorus* and larvae of *Themnocephalus platyurus* and found lowered effects for ecocorona-coated compared to pristine MNP. Similarly, ameliorating effects of DOM have, among others, been found for MNP-induced oxidative stress responses (ROS production) in algae and fish (Liu et al., 2019; Natarajan et al., 2021), copper-induced mortality in freshwater mussels (Gillis et al., 2008), and for pesticide-induced mortality in the freshwater mysid shrimp *Americamysis bahia* (Mézin & Hale, 2004)

One mechanism by which DOM can reduce toxic effects is its ability to bind pollutants, alter the physico-chemical properties of particles and thus decrease their bioavailability (Kukkonen & Oikari, 1991). For instance, humic acid and, to a lesser extent, fulvic acid bind hydrophobic organic pollutants including pesticides (Chianese et al., 2020; De Paolis & Kukkonen, 1997; Landrum et al., 1985), and DOM binds pesticides and metal ions (Aiken et al., 2011; He et al., 2020; Reuter & Perdue, 1977). In contact with particulate pollutants, DOM leads to ecocorona formation (e.g., Elagami et al., 2022; Rummel et al., 2017) altering the particles' surface characteristics (Natarajan et al., 2021; Shi et al., 2023; Witzmann et al., 2022) and changing interactions with tissues and cells (Ramsperger et al., 2020). Changes in physico-chemical properties also lead to altered colloidal interactions (Witzmann et al., 2022) and altered aggregation in the test media (Aiken et al., 2011; Meng et al., 2023; M. Yan et al., 2021). Particle aggregation in turn affects the particles' transport behavior, can lead to increased sinking velocities and consequently lower the availability of particulate pollutants in the water column (Elagami et al., 2022; Karakaş et al., 2009; Petosa et al., 2010; Y. Yan et al., 2021). In addition, particle aggregation itself can alter MNP toxicity (Albanese & Chan, 2011; Meng et al., 2023; Wu et al., 2019). Furthermore, DOM attached to the particles may add nutritional value to the particles and thus partly reduce food dilution effects (Arruda et al., 1983). Nevertheless, all these effects of DOM on particle behavior and properties cannot explain sufficiently why moderating effects in our dataset were stronger in co-exposure as compared to MNP incubation setups, as these effects should show up in both setups similarly.

In general, stressed *Daphnia* are more sensitive towards additional stressors (Serra et al., 2020; Yin et al., 2011) while beneficial environments help daphnids become more resilient (Lye Koh et al., 1997; Vandenbrouck et al., 2011; for a conceptual discussion of stress addition, see Liess et al., 2016). A potential alternative explanation for the strong attenuating effects of humic acid and lake water in co-exposure experiments may be that DOM in the media generally contributes to the well-being of the daphnids, thus making them more resilient to stressors. Supplementary DOM for instance may serve as a food and nutrient source for phytoplankton, indirectly leading to better food supply for the daphnids (Thomas, 1997) or increase food supply through the microbial loop when algal food becomes limited (e.g., Hiltunen et al., 2017; McMeans et al., 2015). Second, similar to other organisms, daphnids may benefit directly from DOM in the test media via increased intestinal health, increased reproduction or increased growth induced by mild chemical stress responses (Gao et al., 2017; Steinberg et al., 2009). In contrast to these findings however, other studies have demonstrated adverse effects of natural organic matter (Wenzel et al., 2021) and humic acid (Euent et al., 2008; Saebelfeld et al., 2017) on *Daphnia* and on other invertebrate freshwater species (e.g., Timofeyev et al., 2006), indicating that the processes and mechanisms in natural waters are likely more complex and not understood well enough yet.

Among the types of DOM used in the screened literature, humic acid and lake water added during exposure had the strongest mitigating effects, decreasing the risk of immobilization caused by MNP by almost 50% (RR = 0.56 and 0.55, respectively). Whether these effect sizes are comparable to effects in natural environments depends among other factors on how realistic the concentrations of DOM applied in experiments were. In the studies included in our meta-analysis that reported DOM concentrations, test concentrations ranged from 1 to 50 mg L^{-1} (see raw data in the supplementary online material). Similar ranges have been reported for natural aquatic systems, spanning for instance from 0.1 to 322 mg L^{-1} in a dataset of measurements of dissolved organic carbon (DOC) from 7,500 lakes (Sobek et al., 2007; see also Thomas, 1997). It is therefore likely that attenuating effects of DOM on MNP toxicity can occur in a similar way in natural habitats.

Although the compiled dataset indicates that the mere presence of an ecocorona has lower moderating effects on immobilization risks than co-exposure with DOM, and that effect sizes differ between different DOM types, the dataset also has some important limitations. The types of DOM tested in conditioning experiments were different from the types tested in co-exposure experiments (except for one data point from an experiment using lake water-conditioned MNP). Due to this limited overlap, it is difficult to disentangle the effects of DOM type and experimental approach at this point. However, the observed patterns can serve as valuable hypotheses that can be easily validated (or disproved) in experiments or when more studies become available in the future.

Publication bias can lead to wrong effect size estimates derived from meta-analyses. Bias arises when some effects sizes are published selectively, e.g., when only significant outcomes are published or when confirmatory results are preferentially published (Sterling, 1959). A second factor that can lead to wrong effect size estimates is study (or sample) heterogeneity (Higgins & Thompson, 2002; Kenny & Judd, 2019). Significant heterogeneity indicates that the variance among data points cannot be sufficiently explained by sampling variance, but instead likely results from measured effects not being derived from a true common effect. The funnel plot from our meta-analysis shows a slight gap of data points on the side of increased immobilization risk in the presence of DOM and the full model showed significant residual heterogeneity. A likely reason for both these patterns is that the effect sizes in our dataset are moderated by additional factors not accounted for in our analysis (Ioannidis & Trikalinos, 2007). For example, it is possible that the effect of DOM on immobilization caused by MNP is further moderated by experimental temperature, food availability during exposure, different MNP concentrations, different concentrations of DOM or other experimental parameters and MNP properties. Although we accounted for confounding by pairing measurements from treatments that differed solely in DOM presence/absence while keeping all other parameters the same, we cannot rule out interaction effects. For example, it might be possible that the strength of the mediating effect of DOM on MNP toxicity differs for different polymer types, experimental temperatures, or any other parameter.

In general, we think that further research is needed to disentangle the effects of ecocorona formation and the presence of DOM in the media on MNP toxicity in

Daphnia. This includes addressing in particular the mechanisms that lead to observed differences among different approaches and DOM types. Although *Daphnia* is among the most frequently used organisms in ecotoxicological on MNP effects (Brehm et al., 2023; Hampton et al., 2022), we found only 13 studies (published until December 2022) that met our selection criteria and allowed for the extraction of effect size data. Further experiments investigating the mechanisms of MNP toxicity are thus needed to enhance our understanding of MNP effects in the presence of DOM in natural environments.

Conclusions

In the present meta-analysis, we synthesized data from 13 studies that investigated the effects of ecocorona formation and DOM on MNP induced immobilization risk in *Daphnia* spp. We showed that the mere presence of an ecocorona on the particles can moderate MNP toxicity, but the presence of DOM in the test media during exposure appears to be another important predictor for the observed attenuation of negative outcomes. Based on our results and evidence from the literature on other stressors, we hypothesize that DOM and in particular humic acid mitigates negative effects of MNP by either (1) reducing bioavailability or (2) making daphnids more resilient to stressors in general. Additional experiments are needed to challenge these hypotheses and disentangle the effects of ecocorona formation and the presence of DOM in the media, and to understand how effects of DOM on particle behavior in the medium translate into reduced effects on an organismic level. Such experiments could for example directly compare the impact of ecocorona formation during the incubation of MNP with the impact of adding the same type of DOM to the media during exposure. Comparisons could also be made regarding the attenuating effect of DOM on negative effects of different additional stressors including chemical or particulate pollutants, as well as other stressors such as heat stress.

Author contributions

Conceptualization - MMM. Data extraction - SS, EG, PK, EN, AR, SW, FB, MMM
Data validation - ALAV. Statistical analysis - PK, ALAV, MMM. Visualization - EG,

ALAV, MS, MMM. Writing - original draft - SS, EG, PK, EN, AR, SW, FB. Writing - review & editing - SS, EG, EN, AR, SW, ALAV, MS, MMM. All authors approved for the final version of the manuscript.

Open data statement

All raw data are provided in the supplemental material. All data and code are also available on github (XXto be added upon acceptanceXX) and Zenodo (XXto be added upon acceptanceXX).

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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