Science of the Total Environment 796 (2021) 148843

Contents lists available at ScienceDirect

Science of the Total Environment

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

Fate and toxicity of engineered nanomaterials in the environment: A meta-analysis

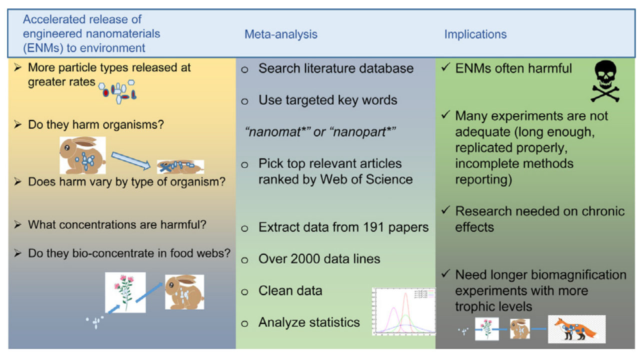
Walter K. Dodds *, James P. Guinnip, Anne E. Schechner, Peter J. Pfaff, Emma B. Smith

Division of Biology, Kansas State University, Manhattan, KS 66502, USA

HIGHLIGHTS

- Engineered nanomaterials can harm plants, heterotrophic and autotrophic microbes, and animals.
- Animals were most harmed, plants least.
- 50% lethal concentrations exceeded most environmentally relevant concentrations.
- Biodilution was more common than biomagnification.
- Most experiments were too short or too unrealistic to assess chronic environmental effects.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 April 2021
Received in revised form 9 June 2021
Accepted 30 June 2021
Available online 8 July 2021

Editor: Sergi Sabater

Keywords:
Nanomaterials
Biomagnification
Bioaccumulation
Lethal dose
Effective dose
Negative responses

ABSTRACT

The global environment annually receives thousands of tons of engineered nanomaterials (ENMs, particles less than 100 nm diameter). These particles have high active surface area, unique chemical properties, and can enter cells. Humanity uses many ENMs for their biological reactivity (e.g. microbicides), but their environmental effects are complex. We cataloged 2102 experimental results on whole organisms for 22 particle classes (mainly on Ag, Zn, Ti, and Cu) to assess biological responses, effective and lethal concentrations, and bioaccumulation of ENMs. Most responses were negative and varied significantly by particle type, functional group of organism, and type of response. Smaller particles tended to be more toxic. Aquatic organisms responded more negatively than did terrestrial organisms. Animals generally were most sensitive and plants least. Silver ENMs generally had the strongest negative effects. Effective and lethal concentrations generally exceeded modeled environmentally relevant concentrations and organisms usually did not accumulate or biomagnify to concentrations above those in their environment. However, most experiments lasted less than a week and were not field studies. Research to date is probably insufficient to understand chronic effects and long-term biomagnification. Numerous unique and untested ENMs continue to enter environments at accelerating rates, and our analysis indicates potential for negative effects. Our data suggest substantial research is still required to understand the ultimate influence of ENMs as they continue to accumulate in the environment. Around 40% of the papers with experimental data for ENMs failed with respect to reporting means, sample sizes, or experimental error, or they did not have proper experimental design (e.g. lack of true controls). We need more high-quality experiments that are more realistic (field or mesocosm), longer duration, contain a wider range of organisms, and account for complex food web structure.

Abbreviations: ANCOVA, analysis of covariance; ANOVA, analysis of variance; BAF, bioaccumulation factor; BCF, bioconcentration factors; EC_{50}, concentration where half the test subjects responded; LD_{50}, concentration where half the test subjects died respond; ED_{50}, dose where half the test subjects respond; ENM, engineered nanomaterial; LC_{50}, dose where half the test subjects die.

* Corresponding author.
E-mail address: wkdodds@ksu.edu (W.K. Dodds).

https://doi.org/10.1016/j.scitotenv.2021.148843

1. Introduction

Humans are escalating production and application of engineered nanomaterials (ENMs, particles less than 100 nm in diameter) globally because these materials have unique and useful properties for numerous applications (Hochella et al., 2019). Organisms have been exposed to particles in this size range since life evolved (Handy et al., 2008), however novel ENMs are entering the natural environment at exceptional rates. We have a modest but increasing understanding of environmental risks of these novel exposures (Owen and Handy, 2007). ENMs can enter cells, and their high surface area to volume ratio potentially increases their reactivity and toxicity relative to larger particles (Hochella et al., 2019; Jeevanandam et al., 2018). Modern societies have released other novel compounds (e.g. human synthesized pesticides) into the environment without fully understanding their influences, ultimately leading to widespread unintended negative consequences (Rubin, 2011).

Nanotechnology has resulted in many useful ENMs. As a result, thousands of metric tons of ENMs (including silver (Ag), titanium (TiO_2), and zinc (ZnO)) are released to landfills, water, soil, and air each year (Keller and Lazareva, 2014). Subsequently, they can be retained, removed, or transported among trophic levels and ecosystems (Nowack et al., 2012). Several factors impede generalization about their environmental influences including 1) numerous types, sizes, and concentrations of ENMs to test, 2) various experimental testing methods, some under unnatural conditions, 3) methodological difficulties in determination of concentrations, and 4) the diversity of organisms used as test subjects (Bour et al., 2015; Hou et al., 2013; Lead et al., 2018).

Additionally, the properties of ENMs lead to ambiguity in predicting environmental effects. For example, ENMs can be more reactive than larger particles and less reactive than corresponding ions. However, they could remain in the environment longer than ionic compounds would, and move through natural environments more quickly than larger particles can. While some ENMs, such as Cu and Ag, have documented biocidal properties, even relatively inert compounds (e.g., Si, asbestos) can have negative health effects. Natural complexity of diverse and interacting organisms complicates prediction of effects as well. Thus, predictive understanding of biological effects of ENMs requires substantial data.

Two general extremes on the range of views exist with respect to ENMs: unbridled optimism of the applications of ENMs and extreme fear about the potential unknowns associated with ENM releases into the environment (Bernhardt et al., 2010). Reality probably falls between these extremes, but a movement toward the optimistic from some researchers has been proposed. Dr. Paul Westerhoff, in an invited conference presentation through the US National Science Foundation (Westerhoff, 2020), stated "Overall, the levels of engineered nanomaterials are usually extremely low (part per billion or lower), and lower than incidental or natural nanomaterials of similar composition. Occasionally, 'hotspots' in the workplace or environment were observed, but overall we concluded there are very low exposures in water. As the EHS [environmental health and safety] community saw the risks from engineered nanomaterials would likely be low, and manageable, many began focusing on exploring how unique nanoscale properties could be exploited to clean-up the environment". Similarly, Lead et al. (2018) stated "There is a developing consensus that NMs may pose a relatively low environmental risk". Simultaneously, researchers publish papers advocating for purposeful release of ENMs to solve environmental problems even though the specific ENMs are, in the same paper, demonstrated to be toxic and non-ENM solutions are available (Dodds, 2019). A recent extensive review on the influence of ENMs on microbial communities suggested even low concentrations can have sub-lethal effects on structure and function of microbial communities, particularly under realistic field conditions and chronic exposures (Wu et al., 2021).

We sought to explore if ENMs really pose a low environmental risk after our preliminary literature review found only modest systematic analyses of existing experimental data (e.g., Notter et al., 2014; Rajput et al., 2018), and most of it focused on laboratory studies (Wu et al., 2021). We take up the call made over a decade ago to use meta-analytic approaches to assess ecological effects of ENMs combining many studies (Bernhardt et al., 2010). Now, with the explosion in numbers of ENM research papers, we have enough publications on the effects of ENMs to begin to untangle the complex and diverse universe of potential environmental effects.

We assembled a large dataset on environmental effects of ENMs to explore: 1) Which ENMs are studied and how? 2) How do particle identity, size, concentration, and study duration influence toxicity (response ratios, and lethal and effective concentrations (LC_{50} and EC_{50})) in different organisms related to the role they play in the environment? 3) What are bioaccumulation (BAF), biomagnification (BMF), and bioconcentration (BCF) factors of ENMs as a function of ecosystem role and trophic level in different organisms? We paid special attention to the quality of each study and analyzed the data with established meta-analytic statistical procedures developed to avoid biases associated with combining numerous experimental results from different investigators.

2. Materials and methods

2.1. Data collection

Our general workflow consisted of multiple steps of identifying studies, assessing their relevance to our aims, harvesting the data, and approaches to data analyses (Fig. 1). We mined the peer-reviewed literature on 1 Nov 2019 using the Web of Science Core Collection Database and extracted papers by search relevance (details below and in supplementary material). We searched with terms including "nanomat*" or "nanopart*" (*indicating a search wildcard) in combination with qualifiers such as "terrestrial" or "aquatic", and we further narrowed our search to focus on microbes, aquatic or terrestrial producers, or consumers, and specific response terms (e.g., photosynthesis, growth). Each line for response ratios had at least one comparison of experimental treatment with control, and we collected ancillary information relevant to the study for all recorded experimental results. Supplementary index table S1 has detailed search terms, S2 has a list of rules for recording data and exclusion of papers, S3 has column header descriptions and metadata for the full dataset, and S4 has types of ENMs (link to full dataset here upon publication).

We collected variables to be used for response ratios, EC_{50}, ED_{50}, LC_{50}, LD_{50} (50% concentration or dose for an effect or lethality), biomagnification factors (BMF, concentration in predator versus prey, also referred to as trophic transfer factor), bioaccumulation factors (BAF, tissue concentration/environment concentration), bioconcentration factors (BCF, same as BAF but excludes dietary exposure), ENM type, size, concentration, and other experimental details. Data were reported such that negative response ratios represented harm (e.g., mortality rates were converted to survival so a decrease in survival would translate to a negative response ratio), and units were converted when needed to facilitate direct comparison.

We created a detailed list of rules to exclude non-relevant results (Table S2). The top 80 papers for each grouping of search terms (sorted by Web of Science relevance scores) were assigned without bias to each of nine reviewers for data extraction. We identified an additional selection of 44 papers from the remaining initial search lists using targeted if-then statements to highlight papers in areas in which we were lacking data in an effort to identify papers most likely to increase sample sizes enough to ensure rigorous analyses. Thus, we examined 444 papers closely. We rejected many of these immediately because they were reviews, or while they discussed ENMs, they did not have actual experiments on ENMs.

We recorded experimental data directly from the paper when authors made results available in a table or through in-text discussion of

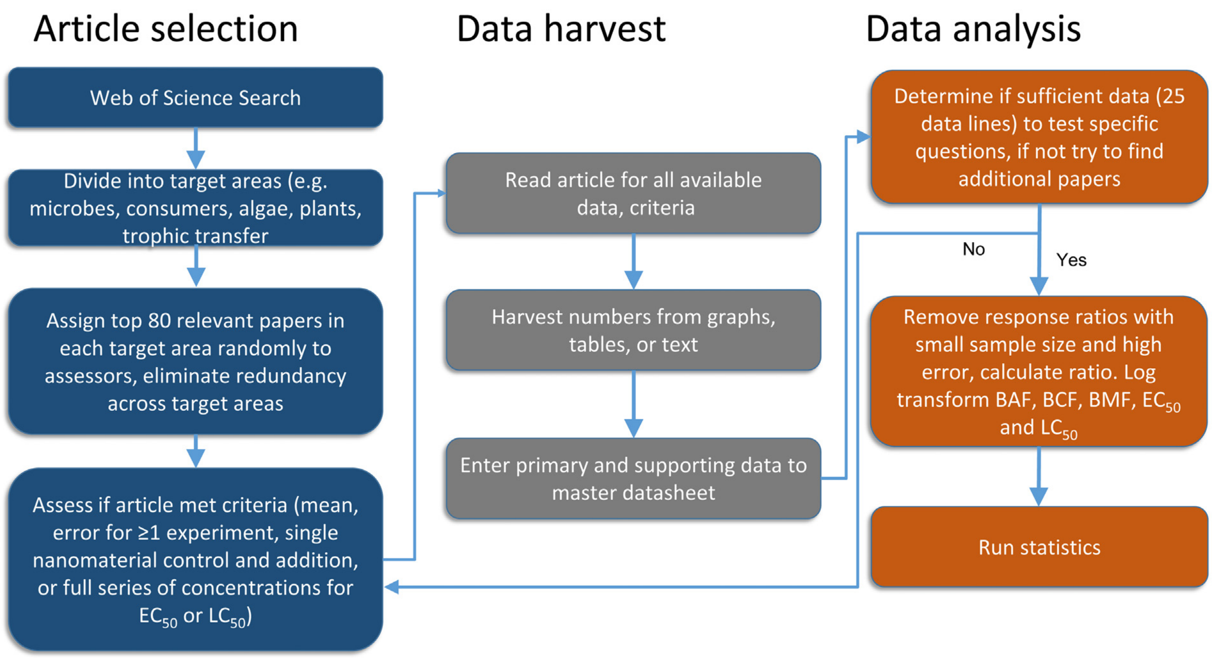


Fig. 1. Study selection, data harvest, and data analyses workflow.

results. When data were only available in the form of a graph, we extracted numeric data from images (Rohatgi, 2019).

2.2. Data analysis

We used log response ratios (Hedges et al., 1999) to quantify effect size of ENMs for each experiment, after correction for bias associated with small sample sizes in experiments (Lajeunesse, 2015). Before running statistical tests, we omitted response ratios if the experimental or control groups had a standardized mean less than three after adjustment for small sample size (Lajeunesse, 2015). We used funnel plots to assess if there was still bias (supplementary materials) and found no evidence that such bias exists (Sterne et al., 2005) Analyses were conducted in R using package *car* (Fox et al., 2012) and other base packages (RCoreTeam, 2019). ENM particle size and experiment length effects on responses were analyzed using analysis of covariance (ANCOVA). We assessed other categorical differences with ANOVA. Differences between experimental setting (e.g. laboratory or field) and ecosystem type (i.e. aquatic or terrestrial) were analyzed using ANOVA. We excluded some specific ENM types from figures when there was not a large enough sample size for proper statistical analysis. BAF, BCF, BMF, $EC_{50,}$ and LC_{50} were not normally distributed (Shapiro-Wilk test), so were log transformed prior to analyses. We recognize that multiple statistical tests can lead to false estimation of significance, but most of our results are very highly significant and application of Bonferroni corrections or similar approaches would not alter our overall conclusions.

We calculated additional BAF and BMF values by dividing the concentration of ENM in tissues by either the environmental concentration of the relevant ENM (BAF) or the concentration in the food source (BMF). We only did this for papers that did not directly report BAF or BMF values but where data were available to calculate values manually from environmental and/or organismal ENM concentration estimates.

3. Results

3.1. Types of experiments and particles

We excluded about 40% of the papers we analyzed with a complete reading because they did not report means, errors, sample sizes, ENM size or concentration used, or had no proper controls. Many of the papers without proper controls tested multiple ENMs and/or other stressors simultaneously but did not use a complete factorial experimental design that could discern individual effects and interactions. Our final dataset was composed of experimental results from 191 closely read papers, for a total of 2102 unique data lines.

The papers with adequate experimental data reported results on 22 types of ENMs, with Ag, Zn, Ti, and Cu most commonly studied (Fig. 2A). Fe, C nanotubes (CNT), Ce, Au, Ni, and Si had modest attention, and Al, carbon graphene (CG), carbon fullerenes (CF), quantum dots, Sn, Cn, Se, Mn, plastic, and Co were sparsely represented. Almost 90% were laboratory experiments, 10% were in mesocosms, and less than 1% were in field settings.

Several of the elements had different chemical forms of nanomaterials. Specific chemical forms and numbers of data points for each form are in supplementary materials. We used nested ANOVA followed by Tukey's post-hoc comparisons to assess if the form of ENM significantly influenced response ratios for ENMs with enough data to make the test (Ag or Ag_2S, graphene or graphene oxide, Cu or CuO, Fe_3O_4 or zero valent Fe). None of the comparisons were significant ($p > 0.17$). Likewise, we only had data to test differences in EC_{50} and LC_{50} for Cu or CuO, and found no significant differences ($p > 0.52$), potentially due to high variance and small sample size. We lumped results for all chemical forms by element (except carbon nanotubes, graphene, and fullerenes) in subsequent analyses.

Particle coating significantly ($p < 0.02$) influenced response ratio, but the effect of coating varied by particle type. Coated Ag particles elicited stronger responses, but Au and Ti had weaker effects with particle coating. In combination with the possibility that coating properties change over time, this led us to combine data from coated and non-coated particles for the remaining analyses.

Response ratios were generally less negative with longer experiments ($p < 2^{-16}$) and varied by trophic strategy (functional group) with a significant interaction with experiment length ($p < 2^{-5}$). Animal experiments averaged 4.3 days, aquatic producers 6.9 days, heterotrophic microbes 92 days, and terrestrial producers 55 days. Longer experiments had weaker response ratios for animals and primary producers, but not heterotrophic microbes. Particle type ($p < 0.02$) and experiment length did influence LC_{50} ($p < 0.04$), with longer experiments revealing more lethality at lower concentrations. Experiment length in the studies

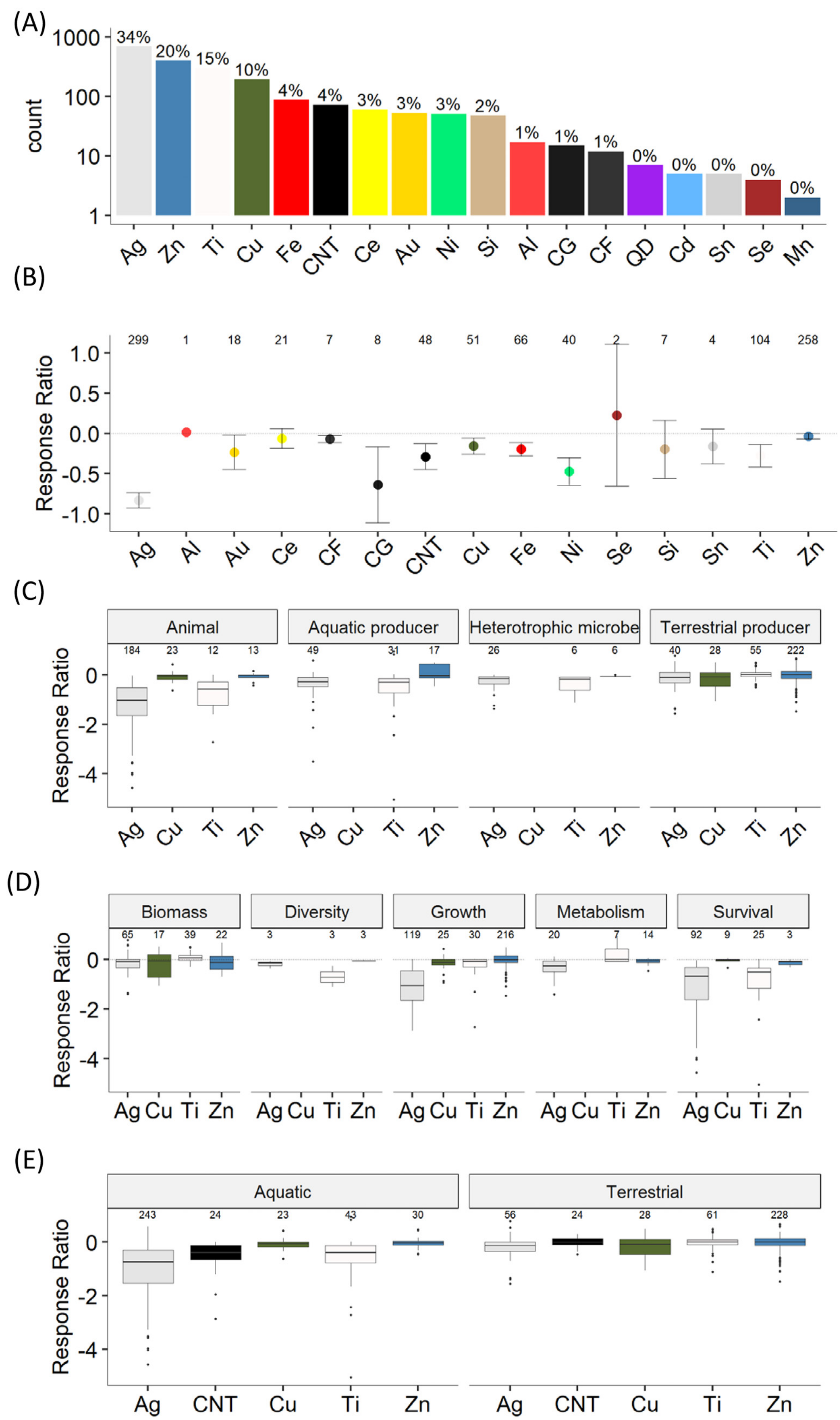


Fig. 2. Total number of data lines per type of ENM analyzed (note log scale with percentages of total data lines rounded to the nearest 1%) (A); overall response ratios for ENM particle types with means and 95% confidence intervals (B); response ratios by response group (C); response ratios by type of response ratio (D); and response ratios by habitat (E). Box plots in C–E and remaining figures have line at the median, lower and upper edges at the 25th and 75th percentiles, respectively, whiskers extending to the most extreme value no further than 1.5*Inter Quartile Range from the edges, and outlying points plotted individually with sample sizes above each bar.

we reviewed did not significantly influence EC_{50} for BCF and BMF but did for BAF ($p < 0.043$).

The interaction of ENM particle identity and size significantly influenced response ratios ($p < 0.003$, ANCOVA) with larger particles having weaker responses for Fe and Ti, and stronger responses for Ag, graphene, and Cu. LC_{50} was influenced by particle size ($p < 0.004$) with smaller particles being more lethal for Ag, Cu, Zn, and Ti. EC_{50} was highly influenced by ENM identity, size, and their interaction ($p < 3^{-6}$). Smaller particles exhibited lower EC_{50} values for Ag, graphene, Cu, Si, and Ti, but EC_{50} values were greater with larger Zn particle size. Size did not significantly correlate with BAF and BCF, but BMF was greater with smaller Ag particles and larger Ti particles ($p < 0.014$). Subsequent analyses of our data did not control for particle size.

3.2. Response ratios by organism type and response type

The type of ENM strongly influenced response ratios regardless of the covariate it was analyzed with; most responses were negative with 95% confidence intervals for 9 of 15 ENM types falling below zero, and no means were significantly above zero (Fig. 2B). Response ratios differed by organism, ENM type, and their interaction (ANOVA, $p < 2.2^{-16}$ for all effects). ENMs most strongly harmed animals, particularly via negative effects of Ag; Cu and Zn had mostly neutral influences. Terrestrial plants had the weakest responses, although on average the responses tended to be slightly negative (Fig. 2C). Response ratios were also influenced by ENM identity and response type (biomass, diversity, growth, metabolism, or survival; ANOVA $p < 2.2^{-16}$ for main effects and their interaction). Ag particles had the strongest influence on growth and survival (Fig. 2D). Response ratios of aquatic versus terrestrial organisms varied significantly by ENM type and habitat (ANOVA $p < 2.2^{-16}$ for main effects and their interaction). Responses tended to be stronger for aquatic organisms for Ag, Ti, and carbon nanotubes (Fig. 2E).

3.3. Values of LC_{50} and EC_{50}

Type of ENM influenced LC_{50} and EC_{50} (ANOVA, $p < 0.023$ and 2.2^{-16} respectively, Fig. 3A, B) when concentrations were expressed per volume of water, and EC_{50} was influenced by terrestrial or aquatic habitat, particle type, and their interaction ($p < 7^{-8}$). LC_{50} did not vary significantly by terrestrial or aquatic ecosystem. We analyzed EC_{50} for Au, Cu, and Zn to compare animals and heterotrophic microbes (Fig. 3C) with animals more sensitive than heterotrophic microbes ($p < 5^{-12}$). Fewer experiments determined LC_{50} and EC_{50} for soil and sediments (Fig. 3D–G). Heterotrophic microbes and animals had lower EC_{50} for Ag than terrestrial producers when tested in soils and sediments (Fig. 3E) though small sample size precluded statistical analysis. Relatively few studies determined LC_{50} values for soils and sediments based on concentrations per soil mass (Fig. 3F and G).

3.4. Bioaccumulation (BAF), bioconcentration (BCF), and biomagnification (BMF)

The longest experiment we could calculate BMF from was 42 days, and the average length of experiment was 11 days. The BAF, BCF, and BMF values varied highly significantly by particle type (Fig. 4A). Most of these values were near or less than one, with the exception of Ti for BAF and Cu for BMF. It appeared that BMF values were greater for carnivores than herbivores (Fig. 4B), though sample sizes were too small for reliable statistical analyses.

4. Discussion

4.1. Particle size and coating

We found highly variable but mostly negative organismal responses to ENMs. Responses were a function of particle type and size, experiment duration, habitat type, functional role of organisms, and response type (e.g., survival, growth, etc.). Most experiments were short laboratory studies. Particle coating led to variable effects across particles as coatings can be used to increase activity or to keep particles in solution. We note that particles can be modified as they pass through sewage treatment plants, and this modification can alter response to Ag and Ti nanomaterials (Galhano et al., 2020; Georgantzopoulou et al., 2020; Zeumer et al., 2020). Presumably, this is because wastewater treatment alters their surface properties.

Particle size can influence toxicity, and prior meta-analysis suggests that dissolved ions have about two-fold greater toxicity than nanoscale forms of Ag, ZnO, and CuO (Notter et al., 2014). Larger particles are transported less quickly from environments (Whiles and Dodds, 2002), so larger particles may have more time to interact with organisms. However, smaller particles can be more likely to enter cells (Liu et al., 2010) and have greater active surface area, increasing the probability of toxicity. Within the size range of ENMs, we could find only modest influences of particle size, and results varied with particle type. Smaller particles were generally more toxic with lower concentrations for LC_{50} and EC_{50} for most ENM types.

4.2. Applicability to real world conditions: experiment length and relevant concentrations

The reviews by Hou et al. (2013) and Geitner et al. (2020) called for better experimental standardization and consistency in data reporting. We confirm this problem, with many of the papers we examined having poor experimental design or inadequate results reporting. Subsequently, the applicability of many papers to the real world was low. However, given the large total number of publications available we were able to characterize enough experiments that had stronger design and reporting.

Our analysis was subject to several potential interferences. For example, papers published since we did our literature search could have used more advanced methods, or better control of experiments. However, when we tested year of publication against strength of response ratio or biomagnification factor we saw no significant temporal trend, indicating newer literature gave no different results than older (data not shown). We did control for bias associated with small studies as documented in our methods sections and related funnel plots in the supplemental information. Tests for homogeneity are not appropriate when large differences among studies are expected (Mengersen et al., 2013). As we assessed many different ENMs and response types, we expected large differences among studies. Thus, we present our data as non-parametric box plots in addition to results from statistical tests to allow direct visual assessment of outliers and data distributions.

Chronic exposure can intensify the influence of toxicants; however, in our analysis, longer exposures generally caused weaker responses, except for in microbial heterotrophs. Shorter experiments were probably less prone to particle aggregation and binding, leading to increased biological reactivity of ENMs, though most experiments were substantially shorter than the study organisms' lifespans. Generalization of temporal effects is difficult due to the complex nature and fate of ENMs in natural environments (Nowack et al., 2012). However, Wu et al. (2021) stressed that chronic effects are important for microbial assemblages, and that sublethal effects can have substantial long term implications for structure and function of microbial communities. Additionally these experiments often ignore the influence of multiple stressors (Wu et al., 2021). While "bottle" experiments can be informative, they may be difficult to extrapolate to the real world (Schindler, 1998).

We likely underestimated actual BMF values as the longest study in our data set measuring bioaccumulation was just over a month, and most studies were less than two weeks long. A whole-lake dosing experiment revealed 119 d turnover of Ag in Northern Pike (*Esox lucius*) tissues (Martin et al., 2018), substantially longer than most experiments

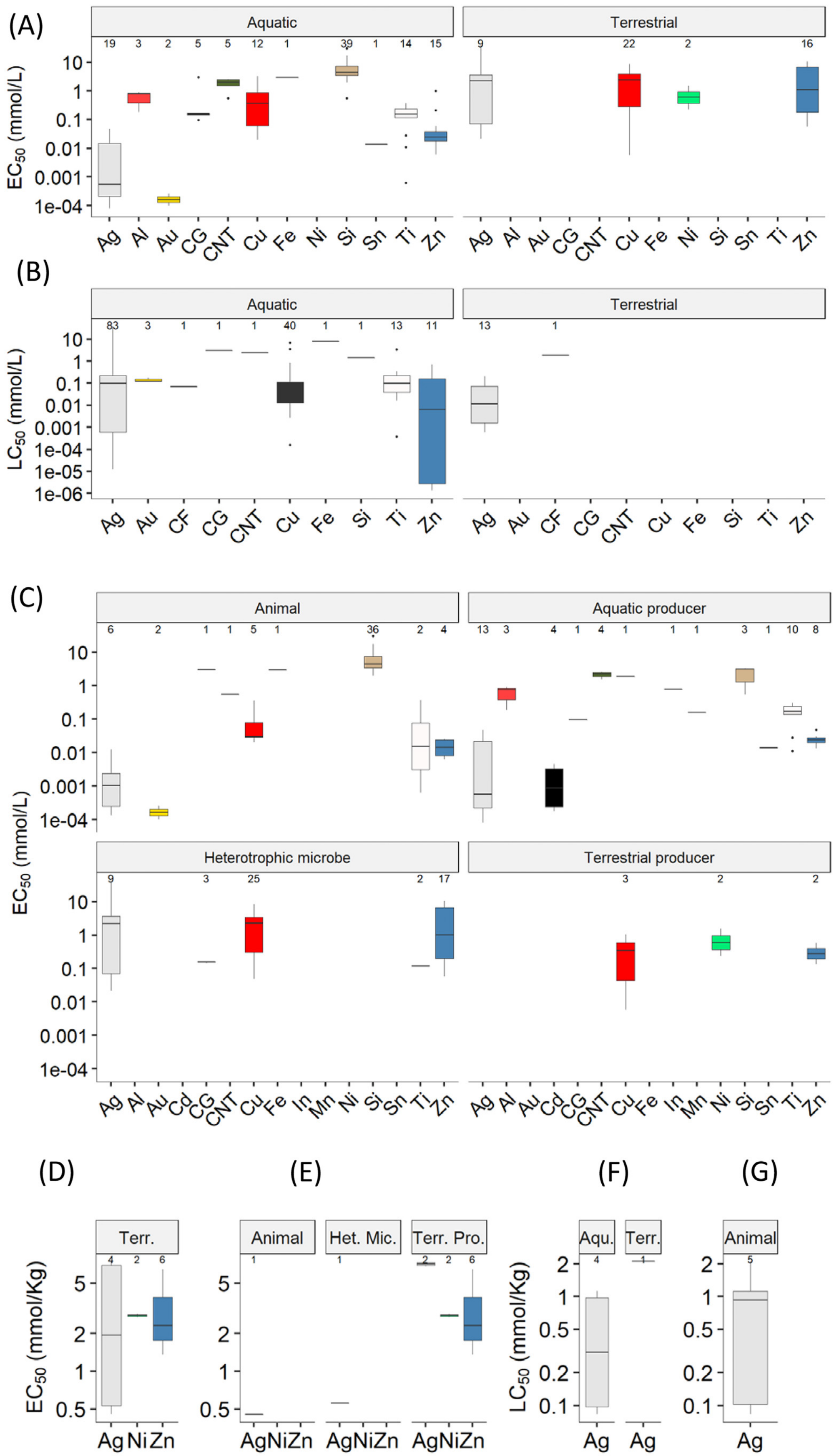


Fig. 3. EC_{50} (A) and LC_{50} (B) by particle type and ecosystem type with concentrations reported as mass per volume. (C) EC_{50} by ENM and organism type with concentrations reported as mass per volume. (D) EC_{50} for particle types for terrestrial (Terr.) experiments and by (E) functional organism type and ENM by mass per mass sediment (Het. Mic. = heterotrophic microbes, Terr. Pro. = Terrestrial Producers). (F) LC_{50} for Ag effects by aquatic (Aqu.) or terrestrial habitats and (G) for animals reported as mass per mass sediment.

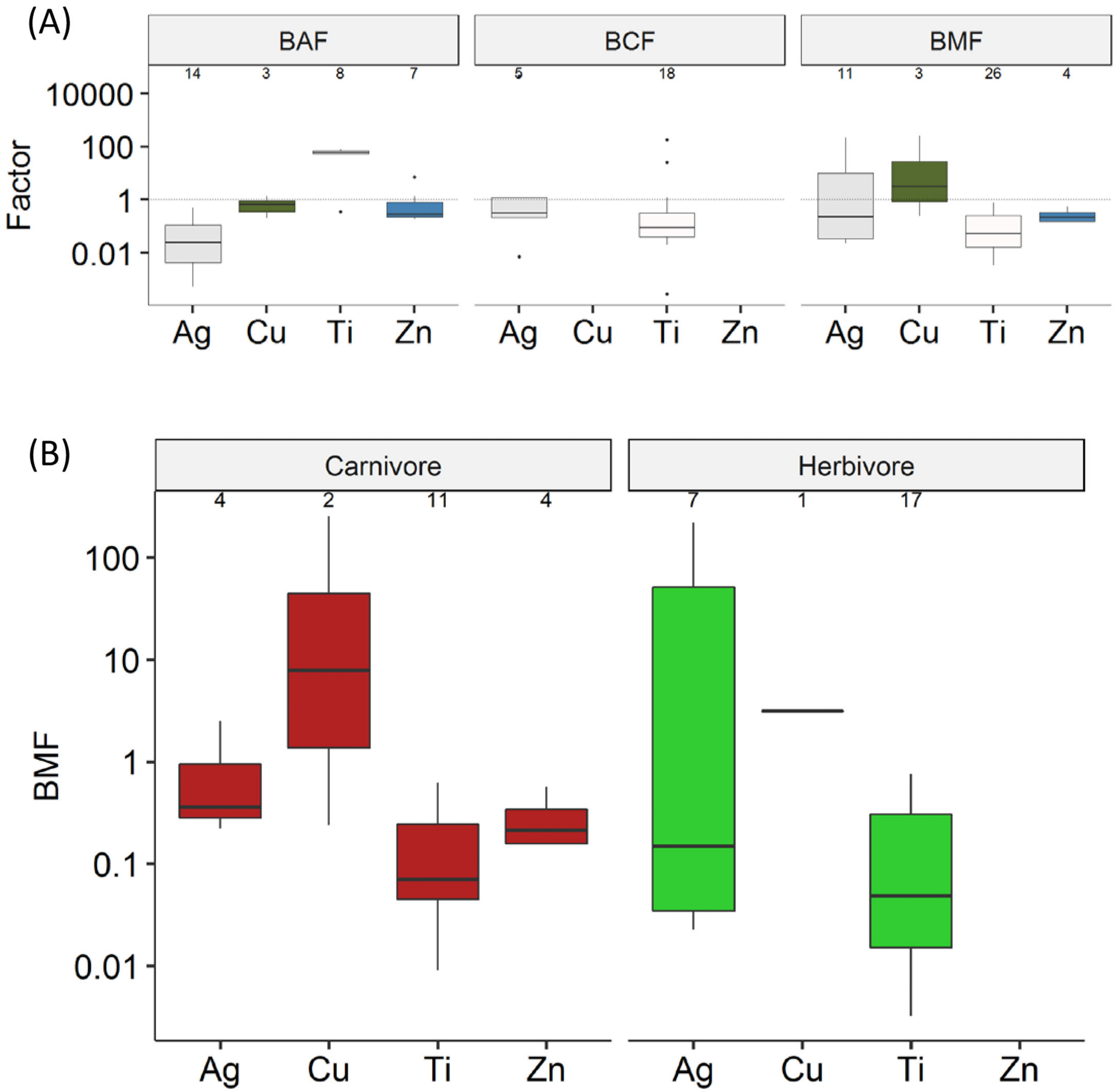


Fig. 4. (A) Bioaccumulation (BAF), bioconcentration (BCF), and biomagnification (BMF) factors by particle type, and (B) BMF seperated by carnivores and herbivores.

we analyzed. Inorganic ENMs will not degrade, suggesting the possibility of very long-term chronic effects. Most ENM experiments use ENMs in suspension, but many studied ENMs have a propensity to coagulate and settle, and should move to soils and sediments where they will concentrate and potentially re-cycle into the biological pool. Some of the ENMs can mobilize via dissolution but most will continue to accumulate in the environment with repeated releases. For example, Rearick et al. (2018) added nanoscale Ag to a lake for four months resulting in consistent increases in lake water concentrations. Additionally, biochemical processing of nanomaterials will occur in the environment, potentially changing chronic effects. In one study, the effects of Ag ENMs on microbial communities were stronger when the ENMs were subjected to pre-conditioning via simulated wastewater treatment conditions than when they were fresh (Forstner et al., 2020). Microbial communities continued to shift over a year in terrestrial and wetland mesocosm experiments dosed with $Cu(OH)_2$ nanoparticles (Carley et al., 2020). These examples suggest our data set was not adequate to estimate long-term effects and may have underestimated the potential for biomagnification.

Data suggest occurrence of ENM hotspots. Cleveland et al. (2012) placed a single black dress sock in a 366 L marine mesocosm stocked with cordgrass, sediment, and marine animals and attained a water concentration about 10 times the estimates of environmentally relevant concentrations from Gottschalk et al. (2013) within 6 h. This value was close to the lower range of EC_{50} values we found. Similarly, the upper concentrations for Ag and ZnO in soil were far lower than the EC_{50} or LC_{50} values documented here, but the Ag concentration in sediments exceeded by 10-fold those in a mesocosm with a single stuffed toy bear after 72 days.

What are environmentally relevant concentrations of ENMs? Analytical and modeling approaches found median surface water concentrations of nanoscale TiO_2 were 0.5–0.003 μmol/L, Ag from 0.004–3*10^{-5} μmol/L, ZnO from 0.003–0.0005 μmol/L, C nanotubes from 10^{-4}–10^{-5} μmol/L, fullerenes at 10^{-6} μmol/L, and CeO_2 at 0.001 μmol/L (Gottschalk et al., 2013). The EC_{50} values we found for TiO_2 were mostly greater than these modeled ranges except for in some animal tests. All values for EC_{50} for Ag, ZnO, and C nanotubes we found exceeded those environmental concentrations. Gottschalk et al. (2013) only provide a modest number of field estimates to compare to their models.

Values reported by Gottschalk et al. (2013) need more field verification as well as more detailed modeling. For example, spatially resolved models of Ag and ZnO nanoparticles in streams suggested that agricultural sources could dominate, but that a relatively small amount of either particle was retained in the streams. Another study found that ZnO left the watershed more quickly because of its ability to dissociate into ionic Zn^{+} (Dale et al., 2015). Further, EC_{50} or LC_{50} values indicate when half the population experiences negative effects or death; however, a 10% mortality rate could have large implications for ecological processes and would likely occur at far lower concentrations.

We did find higher concentrations for EC_{50} in terrestrial than aquatic experiments when papers reported concentrations per unit liquid volume, which generally occurred because experimenters reported the concentration they added as a liquid solution to sediments. We have no way of knowing what the actual exposures were in these experiments as the soil or sediments could have diluted the ENMs. Alternatively, the ENMs could have adhered to the sediments leading to higher effective doses or physically changing the toxicity.

4.3. Specifics of the most commonly used ENM types

The most commonly studied ENMs were Ag, Cu (Cu and CuO), TiO_2, and ZnO. Each of these has different uses and base properties. The most heavily studied of these is Ag, used for its microbicide properties in clothing, medical equipment, water treatment, and many other applications (Longano et al., 2012). Around 400 metric tons of Ag ENMs per year are produced and ultimately will enter the environment (Keller and Lazareva, 2014). The use of Ag ENMs has probably decreased transmission rates of disease and had many positive aspects. However, these particles can pass through the blood-brain barrier and impair mitochondrial function related to their penetration and accumulation in the mitochondrial membrane among other negative effects (Akter et al., 2018). Ag ENMs have cytotoxic effects on human cell lines (Tortella et al., 2020). Negative effects on an array of organisms are reviewed in detail by Tortella et al. (2020). The use of these particles is accelerating and, presumably, environmental concentrations will increase accordingly because the particles will not degrade. Laboratory experiments indicate effects on laboratory cell lines at concentrations ranging from 0.4–250 ng/L (0.0027–2.3 nM) including several modes of action (Akter et al., 2018). These concentrations are less than those we report for whole-organism experiments and overlap the ranges of environmentally relevant concentrations reported by Gottschalk et al. (2013). As discussed above, environmental concentrations can far exceed these in “hotspots” such as near Ag ENM-impregnated clothing discarded into water. The experiments we analyzed indicated Ag ENM bioaccumulation factors of 0.1–1, indicating some bio-dilution, but perhaps not enough to keep concentrations within cells low enough to avoid damage in all environmental applications.

Cu and CuO ENMs have biocidal properties and are used in personal care products and as an enabler for agricultural pesticides (Angeler et al., 2003) among other potential uses. Consistent with our analyses, others have noted biological accumulation and toxicity of copper ENMs (Angeler et al., 2003). Dissolved copper can be toxic to many organisms including aquatic organisms. Repeated addition of ionic copper sulfate to lakes to control cyanobacterial blooms is probably the most widely studied aspect of purposeful copper additions to the environment. Hanson and Stefan (1984) analyzed 58 years of treatment in five Minnesota lakes. They found additions caused shifts to undesirable cyanobacteria, fish kills, loss of desirable fish species, loss of macrophytes, and reduction of macroinvertebrates. Additionally, copper continued to build up in the lake sediments over time. This is instructive, as copper ENMs will build up in aquatic sediments at potentially greater rates than ionic forms of copper as they are less likely to wash out with hydrologic exchange. Given that copper ENMs are an ingredient of some pesticides and there is likely potential for repeated applications, the buildup of copper in terrestrial soils is also of concern.

While TiO_2 in its bulk form is considered non-toxic and is used in many products including human food, the ENM form has photocatalytic properties that can be useful in breaking down pollutants but also increase its toxicity to some organisms (Schaumann et al., 2015). Most of the microbicide properties relate to this phototoxicity. Many of the experiments we analyzed did not document the source or intensity of the light used and may have underestimated toxicity relative to that which would occur in surface waters. Additionally, increased acidity, as seen in surface waters as atmospheric CO_2 increases, magnifies the toxicity of TiO_2 ENMs (Xia et al., 2018). Again, we found few experiments that controlled for pH, so it is not clear what continued effects would be on marine ecosystems with ongoing acidification.

The largest use of ZnO nanoparticles is in sunscreen because they allow visible light to pass while absorbing ultraviolet light, but these particles also have anti-microbial properties and manufacturers incorporate them in food packaging. The ZnO nanoparticles have similar properties to TiO_2 as they are photocatalytic, producing reactive hydroxyl and peroxide compounds upon exposure to light (Sirelkhatim et al., 2015). Rajput et al. (2018) found negative effects of ZnO on terrestrial and aquatic animals, plants, and soil microbes in their review. In contrast, we found negative but weak effects of ZnO on most organisms. Long term experiments with ZnO on mice indicated negative health effects not evident in shorter experiments (Rajput et al., 2018)

4.4. Potential for accumulation in the environment

We have found considerably more data on a wider variety of organisms than did Hou et al. (2013), indicating research has progressed. Their review did find a relationship between environmental concentrations and body burdens of ENMs for aquatic organisms (fishes and microcrustaceans mostly) and soil (earthworms). However, these authors noted numerous experimental deficiencies in methodology. A more recent review (Kuehr et al., 2021b) investigated methods for testing bioaccumulation in invertebrate test organisms. They note considerable progress in experimental methodology over the last decade and provide a guide to best practices in such studies. Many of the studies we reviewed did not follow these suggested best practices.

We compare our results to some general principles derived from organic and metallic pollution research. A global analysis verified that organic chemicals have greater rates of trophic magnification if an organism metabolizes them at low rates and the compounds have high hydrophobicity (Walters et al., 2016). In contrast, functional relationships between concentrations and inputs or excretion alone control metal bioaccumulation (the metals cannot be metabolized). While organic forms of some metals (e.g. methylmercury) clearly biomagnify, the data for other forms of metals are more scant. Some metals apparently do biomagnify (Mann et al., 2011). Hou et al. (2013) noted that lipid content of animals correlated poorly to bioconcentration of ENMs, even the carbon-based ones. Sanchís et al. (2020) confirmed this observation with fullerenes (a non-polar compound expected to biomagnify), biofilms, and snails.

Most (although not all) compounds were not bioaccumulated or bioconcentrated in short experiments, and ENM experiments are prone to experimental errors that tend to overestimate these rates (Petersen et al., 2019). It is possible that short-term experiments underestimate bioaccumulation. While the average length of experiment we found was 11 days, Zeumer et al. (2020) found it took at least 14 days for rainbow trout to reach equilibrium concentrations of accumulated Ag ENMs.

We found a wide range of estimates for bioaccumulation and bioconcentration. An aquatic feeding experiment of the bivalve *Corbicula fluminea* found bioaccumulation factors for Ag ENMs of 31 after two days and values of 6,000 to 9,000 for TiO_2 ENMs (Kuehr et al., 2020). These values are much greater than those we found in our papers, but consistent with the observation of Ti bioaccumulating more than Ag. Kuehr et al. (2021a) also tested the amphipod *Hyallela azteca* and found biomagnification factors of 0.25–0.93, 0.02, and 0.02–0.03 for Ag, TiO_2, and Au ENMs respectively. These numbers are more consistent with those we found in earlier studies. A terrestrial mesocosm study found Ag concentrations decreased 100-fold with each trophic step (Unrine et al., 2012). However, a whole-lake dosing study found Ag biomagnified in Northern Pike livers (Martin et al., 2018). A five-fold biomagnification for Ce occurred in a terrestrial mesocosm study with plants and invertebrates (Majumdar et al., 2016), but there were decreases in La_2O_3 with increased trophic level in feeding studies with terrestrial insects (De la Torre Roche et al., 2015). Scant data exists for biomagnification of most types of ENMs and most experiments were too short to cover reproductive lifespans of larger organisms, which could lead to underestimates of long-term bioaccumulation.

Very few studies on trophic transfer used more than one trophic level, with carnivores very poorly represented in the published literature. In addition, most of the studies that report BMFs are in aquatic systems, possibly due to the ease and reliability of administering ENMs in an aqueous setting. More studies on biomagnification of ENMs in terrestrial systems, across greater numbers of trophic levels, and longer experiments will allow better characterization of ENM movements through food webs. A general lack of data is not surprising; in the United States and Europe, there are 75,000 to 140,000 different marketed chemicals of all types, but only approximately 11% of them have toxicity data and 1% are characterized with respect to bioconcentration (Johnson et al., 2020).

4.5. Conclusions

As we discussed in the introduction, there is a range of opinions on the potential danger of releases of ENMs to the environment. Our results indicate potential environmental risks of ENMs exist, but we found 74% of response ratios were negative. Ag ENMs generally had the most negative effects and relatively low values for EC_{50} but were also the most studied. Ag and Cu have antimicrobial properties, but can also negatively influence other groups, particularly animals. We found little information about interactions of different ENMs, though many environments probably receive multiple types.

There is a significant knowledge gap in our current understanding of their effects on differing organisms, while their use is accelerating, increasing the probability they will accumulate in the environment. The modest numbers of studies on ENMs were mostly conducted in controlled laboratory settings, were short experiments, and were not done under natural conditions. They often ignore multiple stressors and small-scale experiments can be difficult to extrapolate to the real world. We also subjected our data mining to constraints that would seem to allow any study that meets modern scientific standards to pass. However, we filtered many papers from our analyses because they did not report methods adequately to reproduce the study, did not use proper experimental design, or did not report basic results such as sample sizes and errors.

We recommend better experimental design and reporting and that reviewers insist that such practices be followed. We suggest focus on 1) longer experiments, 2) experiments under more realistic field conditions, 3) experiments that assess multiple stressors and/or combinations of different ENM particle types while accounting for individual effects as well, and 4) pursuit of methods that can better characterize environmentally relevant concentrations and effects at those concentrations. Novel ENMs continue to be synthesized and some of them are released into the environment either purposefully or indirectly. We urge researchers to pursue further investigation into field studies, life cycle analyses, multiple trophic level studies, long-term studies, and ultimately, the presence of potential pathways causing bioaccumulation of ENMs in humans. Our data suggest there may be substantial risks to unregulated releases of many different types of ENMs into the environment.

CRediT authorship contribution statement

All authors contributed approximately equally to project development, data collection, analysis, and manuscript preparation. All authors have given approval to the final version of the manuscript.

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.

Acknowledgment

We thank Rachel Keen, Crosby Hedden, Hannah Dea, and Benjamin Wiens for project development, data harvesting, and contributing to early drafts. The research was supported by the US National Science Foundation Award OIA-1656006. Contribution no. 22-007-J from the Kansas Agricultural Experiment Station.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.148843. The full dataset can be accessed at https://doi.org/10.6073/pasta/0720e5cbfd299b6617a91b543b112d04.

References

Akter, M., Sikder, M.T., Rahman, M.M., Ullah, A.K.M.A., Hossain, K.F.B., Banik, S., et al., 2018. A systematic review on silver nanoparticles-induced cytotoxicity: physicochemical properties and perspectives. J. Adv. Res. 9, 1–16.

Angeler, D.G., Chow-Fraser, P., Hanson, M.A., Sánchez-Carrillo, S., Zimmer, K.D., 2003. Biomanipulation: a useful tool for freshwater wetland mitigation? Freshw. Biol. 48, 2203–2213.

Bernhardt, E.S., Colman, B.P., Hochella, M.F., Cardinale, B.J., Nisbet, R.M., Richardson, C.J., et al., 2010. An ecological perspective on nanomaterial impacts in the environment. J. Environ. Qual. 39, 1954–1965.

Bour, A., Mouchet, F., Silvestre, J., Gauthier, L., Pinelli, E., 2015. Environmentally relevant approaches to assess nanoparticles ecotoxicity: a review. J. Hazard. Mater. 283, 764–777.

Carley, L.N., Panchagavi, R., Song, X., Davenport, S., Bergemann, C.M., McCumber, A.W., et al., 2020. Long-term effects of copper nanopesticides on soil and sediment community diversity in two outdoor mesocosm experiments. Environ. Sci. Technol. 54, 8878–8889.

Cleveland, D., Long, S.E., Pennington, P.L., Cooper, E., Fulton, M.H., Scott, G.I., et al., 2012. Pilot estuarine mesocosm study on the environmental fate of silver nanomaterials leached from consumer products. Sci. Total Environ. 421-422, 267–272.

Dale, A.L., Lowry, G.V., Casman, E.A., 2015. Stream dynamics and chemical transformations control the environmental fate of silver and zinc oxide nanoparticles in a watershed-scale model. Environ. Sci. Technol. 49, 7285–7293.

De la Torre Roche, R., Servin, A., Hawthorne, J., Xing, B., Newman, L.A., Ma, X., et al., 2015. Terrestrial trophic transfer of bulk and nanoparticle La_2O_3 does not depend on particle size. Environ. Sci. Technol. 49, 11866–11874.

Dodds, W.K., 2019. Release of novel chemicals into the environment: responsibilities of authors, reviewers, and editors. Environ. Sci. Technol. 53, 14095–14096.

Forstner, C., Orton, T.G., Wang, P., Kopittke, P.M., Dennis, P.G., 2020. Wastewater treatment processing of silver nanoparticles strongly influences their effects on soil microbial diversity. Environ. Sci. Technol. 54 (21), 13538–13547.

Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-Bovy, G., Ellison, S., et al., 2012. Package 'Car'. R Foundation for Statistical Computing, Vienna.

Galhano, V., Hartmann, S., Monteiro, M.S., Zeumer, R., Mozhayeva, D., Steinhoff, B., et al., 2020. Impact of wastewater-borne nanoparticles of silver and titanium dioxide on the swimming behaviour and biochemical markers of Daphnia magna: an integrated approach. Aquat. Toxicol. 220, 105404.

Geitner, N.K., Ogilvie Hendren, C., Cornelis, G., Kaegi, R., Lead, J.R., Lowry, G.V., et al., 2020. Harmonizing across environmental nanomaterial testing media for increased comparability of nanomaterial datasets. Environ. Sci. Nano 7, 13–36.
Georgantzopoulou, A., Farkas, J., Ndungu, K., Coutris, C., Carvalho, P.A., Booth, A.M., et al., 2020. Wastewater-aged silver nanoparticles in single and combined exposures with titanium dioxide affect the early development of the marine copepod Tisbe battagliai. Environ. Sci. Technol. 54, 12316–12325.
Gottschalk, F., Sun, T., Nowack, B., 2013. Environmental concentrations of engineered nanomaterials: review of modeling and analytical studies. Environ. Pollut. 181, 287–300.
Handy, R.D., Owen, R., Valsami-Jones, E., 2008. The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs. Ecotoxicology 17, 315–325.
Hanson, M.J., Stefan, H.G., 1984. Side effects of 58 years of copper sulfate treatment of the fairmont lakes, MINNESOTA 1. JAWRA J. Am. Water Resour. Assoc. 20, 889–900.
Hedges, L.V., Gurevitch, J., Curtis, P.S., 1999. The meta-analysis of response ratios in experimental ecology. Ecology 80, 1150–1156.
Hochella, M.F., Mogk, D.W., Ranville, J., Allen, I.C., Luther, G.W., Marr, L.C., et al., 2019. Natural, incidental, and engineered nanomaterials and their impacts on the Earth system. Science 363, eaau8299.
Hou, W.-C., Westerhoff, P., Posner, J.D., 2013. Biological accumulation of engineered nanomaterials: a review of current knowledge. Environ Sci Process Impacts 15, 103–122.
Jeevanandam, J., Barhoum, A., Chan, Y.S., Dufresne, A., Danquah, M.K., 2018. Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. Beilstein J. Nanotechnol. 9, 1050–1074.
Johnson, A.C., Jin, X., Nakada, N., Sumpter, J.P., 2020. Learning from the past and considering the future of chemicals in the environment. Science 367, 384–387.
Keller, A.A., Lazareva, A., 2014. Predicted releases of engineered nanomaterials: from global to regional to local. Environ. Sci. Technol. Lett. 1, 65–70.
Kuehr, S., Meisterjahn, B., Schröder, N., Knopf, B., Völker, D., Schwirn, K., et al., 2020. Testing the bioaccumulation of manufactured nanomaterials in the freshwater bivalve Corbicula fluminea using a new test method. Environ. Sci. Nano 7, 535–553.
Kuehr, S., Kaegi, R., Maletzki, D., Schlechtriem, C., 2021a. Testing the bioaccumulation potential of manufactured nanomaterials in the freshwater amphipod Hyalella azteca. Chemosphere 263, 127961.
Kuehr, S., Kosfeld, V., Schlechtriem, C., 2021b. Bioaccumulation assessment of nanomaterials using freshwater invertebrate species. Environ. Sci. Eur. 33, 9.
Lajeunesse, M.J., 2015. Bias and correction for the log response ratio in ecological meta-analysis. Ecology 96, 2056–2063.
Lead, J.R., Batley, G.E., Alvarez, P.J.J., Croteau, M.-N., Handy, R.D., McLaughlin, M.J., et al., 2018. Nanomaterials in the environment: behavior, fate, bioavailability, and effects—an updated review. Environ. Toxicol. Chem. 37, 2029–2063.
Liu, W., Wu, Y., Wang, C., Li, H.C., Wang, T., Liao, C.Y., et al., 2010. Impact of silver nanoparticles on human cells: effect of particle size. Nanotoxicology 4, 319–330.
Longano, D., Ditaranto, N., Sabbatini, L., Torsi, L., Cioffi, N., 2012. Synthesis and antimicrobial activity of copper nanomaterials. Nano-antimicrobials. Springer, pp. 85–117.
Majumdar, S., Trujillo-Reyes, J., Hernandez-Viezcas, J.A., White, J.C., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2016. Cerium biomagnification in a terrestrial food chain: influence of particle size and growth stage. Environ. Sci. Technol. 50, 6782–6792.
Mann, R.M., Vijver, M.G., Peijnenburg, W., 2011. Metals and metalloids i n terrestrial systems: bioaccumulation, biomagnification and subsequent advers e effects. Ecological Impacts of Toxic Chemicals. Bentham Science Publishers, pp. 43–62.
Martin, J.D., Frost, P.C., Hintelmann, H., Newman, K., Paterson, M.J., Hayhurst, L., et al., 2018. Accumulation of silver in yellow perch (Perca flavescens) and northern pike (Esox lucius) from a lake dosed with nanosilver. Environ. Sci. Technol. 52, 11114–11122.
Mengersen, K., Schmidt, C., Jennions, M., Koricheva, J., Gurevitch, J., 2013. Statistical models and approaches to inference. In: Koricheva, J., Gurevitch, J., Mengersen, K. (Eds.), Handbook of Meta-analysis in Ecology and Evolution, pp. 89–107.
Notter, D.A., Mitrano, D.M., Nowack, B., 2014. Are nanosized or dissolved metals more toxic in the environment? A meta-analysis. Environ. Toxicol. Chem. 33, 2733–2739.
Nowack, B., Ranville, J.F., Diamond, S., Gallego-Urrea, J.A., Metcalfe, C., Rose, J., et al., 2012. Potential scenarios for nanomaterial release and subsequent alteration in the environment. Environ. Toxicol. Chem. 31, 50–59.
Owen, R., Handy, R., 2007. Formulating the problems for environmental risk assessment of nanomaterials. Environ. Sci. Technol. 41, 5582–5588.
Petersen, E.J., Mortimer, M., Burgess, R.M., Handy, R., Hanna, S., Ho, K.T., et al., 2019. Strategies for robust and accurate experimental approaches to quantify nanomaterial bioaccumulation across a broad range of organisms. Environ. Sci. Nano 6, 1619–1656.
Rajput, V.D., Minkina, T.M., Behal, A., Sushkova, S.N., Mandzhieva, S., Singh, R., et al., 2018. Effects of zinc-oxide nanoparticles on soil, plants, animals and soil organisms: a review. Environ. Nanotechnol. Monit. Manag. 9, 76–84.
RCoreTeam. R, 2019. A Language and Environment for Statistical Computing (Version 3.0. 2) [Computer Software]. R Foundation for Statistical Computing, Vienna, Austria.
Rearick, D.C., Telgmann, L., Hintelmann, H., Frost, P.C., Xenopoulos, M.A., 2018. Spatial and temporal trends in the fate of silver nanoparticles in a whole-lake addition study. PLoS One 13.
Rohatgi, A., 2019. V4. 2. WebPlotDigitizer, San Francisco, CA.
Rubin, B.S., 2011. Bisphenol A: an endocrine disruptor with widespread exposure and multiple effects. J. Steroid Biochem. Mol. Biol. 127, 27–34.
Sanchís, J., Freixa, A., López-Doval, J.C., Santos, L.H.M.L.M., Sabater, S., Barceló, D., et al., 2020. Bioconcentration and bioaccumulation of C60 fullerene and C60 epoxide in biofilms and freshwater snails (Radix sp.). Environ. Res. 180, 108715.
Schaumann, G.E., Philippe, A., Bundschuh, M., Metreveli, G., Klitzke, S., Rakcheev, D., et al., 2015. Understanding the fate and biological effects of Ag- and TiO_2-nanoparticles in the environment: the quest for advanced analytics and interdisciplinary concepts. Sci. Total Environ. 535, 3–19.
Schindler, D.W., 1998. Replication versus realism: the need for ecosystem-scale experiments. Ecosystems 1, 323–334.
Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N.H.M., Ann, L.C., Bakhori, S.K.M., et al., 2015. Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. Nano-Micro Lett. 7, 219–242.
Sterne, J.A., Becker, B.J., Egger, M., 2005. The funnel plot. In: Rothenstein, H.R., Sutton, A.J., Borenstein, M. (Eds.), Publication Bias in Meta-analysis: Prevention, Assessment and Adjustments, pp. 75–98.
Tortella, G.R., Rubilar, O., Durán, N., Diez, M.C., Martínez, M., Parada, J., et al., 2020. Silver nanoparticles: toxicity in model organisms as an overview of its hazard for human health and the environment. J. Hazard. Mater. 390, 121974.
Unrine, J.M., Shoults-Wilson, W.A., Zhurbich, O., Bertsch, P.M., Tsyusko, O.V., 2012. Trophic transfer of Au nanoparticles from soil along a simulated terrestrial food chain. Environ. Sci. Technol. 46, 9753–9760.
Walters, D.M., Jardine, T.D., Cade, B.S., Kidd, K.A., Muir, D.C.G., Leipzig-Scott, P., 2016. Trophic magnification of organic chemicals: a global synthesis. Environ. Sci. Technol. 50, 4650–4658.
Westerhoff, P., 2020. From Nano-risk to Nano-solutions in Drinking Waters. 2020 NSF Nanoscale Science and Engineering Grantees Conference. http://www.nseresearch.org/2020/.
Whiles, M.R., Dodds, W.K., 2002. Relationships between stream size, suspended particles, and filter-feeding macroinvertebrates in a Great Plains drainage network. J. Environ. Qual. 31, 1589–1600.
Wu, S., Gaillard, J.-F., Gray, K.A., 2021. The impacts of metal-based engineered nanomaterial mixtures on microbial systems: a review. Sci. Total Environ. 780, 146496.
Xia, B., Sui, Q., Sun, X., Han, Q., Chen, B., Zhu, L., et al., 2018. Ocean acidification increases the toxic effects of TiO_2 nanoparticles on the marine microalga Chlorella vulgaris. J. Hazard. Mater. 346, 1–9.
Zeumer, R., Galhano, V., Monteiro, M.S., Kuehr, S., Knopf, B., Meisterjahn, B., et al., 2020. Chronic effects of wastewater-borne silver and titanium dioxide nanoparticles on the rainbow trout (Oncorhynchus mykiss). Sci. Total Environ. 723, 137974.