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Research Article

Identifying Knowledge Gaps on Ecotoxicological Assessment of Micro/Nanoplastics with Aquatic Keystone Species

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ABSTRACT

The pollution of aquatic environments by micro/nanoplastics (M/NPs) has been a growing public concern in recent years. This situation has the potential to threaten and affect both aquatic and terrestrial ecosystems simultaneously. Therefore, extensive research has been conducted over the past few years to determine the toxicity of M/NPs. This article investigated the global scientific literature on M/NPs' toxicity on *Daphnia magna* by integrating social network analysis with bibliometrics. A total of 100 publications were downloaded and analyzed with the majority being academic journal articles. Network maps and graphics displayed the correlations between keywords, countries, and journals. Moreover, the type, size, and exposure time of M/NPs were also evaluated to expose current research trends. The results demonstrate that PS and PE are the primary polymer types employed in most toxicity studies. Researchers in China and Germany have provided a great contribution. The Journal of Environmental Pollution published the highest number of research articles. The top publishing countries and the top-cited publications and authors will likely pave the way for standardization in both microplastic and nanoplastic research.

Keywords: Daphnia magna, environmental pollution, ecotoxicology, bibliometric analysis, polymer.

Mikro/Nanoplastiklerin Ekotoksikolojik Değerlendirilmesindeki Bilgi Eksikliklerinin Sucul Ekosistem Anahtar Türleri ile Belirlenmesi

Öz

Son yıllarda sucul alanların mikro/nanoplastikler (M/NP) tarafından kirlenmesi, giderek artan bir endişe haline gelmiştir. Bu durum hem su hem de kara ekosistemlerini aynı anda tehdit etme ve etkileme potansiyeline sahiptir. Bu nedenle, M/NP'lerin toksisitesini belirlemek için son birkaç yıldır kapsamlı araştırmalar yürütülmüştür. Bu makale, sosyal ağ analizini bibliyometri ile entegre ederek *Daphnia magna* üzerindeki M/NP'lerin toksisitesi hakkındaki küresel bilimsel literatürü incelemiştir. Çoğunluğu akademik dergi makaleleri olmak üzere toplam 100 yayın indirilmiş ve analiz edilmiştir. Ağ haritaları ve grafikler, anahtar kelimeler, ülkeler ve dergiler arasındaki korelasyonları göstermiştir. Ayrıca,

mevcut araştırma eğilimlerini ortaya koymak için M/NP'lerin türü, boyutu ve maruz kalma süresi de değerlendirilmiştir. Sonuçlar, PS ve PE'nin çoğu toksisite çalışmasında kullanılan birincil polimer türleri olduğunu göstermektedir. Çin ve Almanya'daki araştırmacılar büyük katkı sağlamıştır. En fazla araştırma makalesi Çevre Kirliliği Dergisi (Journal of Environmental Pollution) tarafından yayınlanmıştır. En iyi yayın yapan ülkeler ve en çok atıf alan yayınlar ve yazarlar muhtemelen hem mikroplastik hem de nanoplastik araştırmalarında standardizasyonun önünü açacaktır.

Anahtar Kelimeler: Daphnia magna, çevre kirliliği, ekotoksikoloji, bibliyometrik analiz, polimer.

I. INTRODUCTION

Currently, the prevailing concern revolves around global environmental contamination, which necessitates daily attention. The surge in pollution and waste over recent years can be primarily attributed to the advancement of contemporary society, encompassing industrial and agricultural domains. Among the myriad of contaminants disseminated worldwide, the prominent escalation in plastic utilization stands out prominently. Approximately 8.3 billion metric tons of plastic have been manufactured to this day, of which 6.3 billion metric tons are either discarded in regular storage or come into contact with the outside environment via the receiving environment [1]. Consequently, 10% of the waste released into the receiving environment reaches water bodies [2-3]. Notwithstanding, plastic artifacts disseminated within the environment undergo fragmentation, resulting in minute plastic fragments known as microplastics (MP) and nanoplastics (NP), classified based on their dimensions [4]. Despite the small ratio, the amount of plastic in the oceans is expected to reach 250 million tons by 2025 [5]. As a result, it is anticipated that aquatic organisms will be exposed to more micro and nano-sized plastics (M/NPs) [6].

According to ecotoxicity studies, after being digested by several living organisms, microplastics (MPs) reach the intestine, then the circulatory system, and finally various tissues such as the gills, liver, and kidneys [7]. It has been observed that nutrition, growth, and fertility may be affected as a result of the exposure of some types of zooplankton to MPs [8-11]. Moreover, because aquatic organisms are a source of food for humans, M/NP exposure of organisms also poses a threat to human safety and public health [12-14]. As one of the most critical zooplanktonic species in freshwater ecosystems, *Daphnia magna (D. magna)* serves as a link between primary producers and the higher trophic levels in various ecosystems [15-17]. It is utilized as a model test organism in ecotoxicology studies due to its ease of generation in the laboratory, low cost, and high sensitivity [18]. D. magna plays a critical role in determining the effect of pollutants on organisms, especially in aquatic areas [19-23].

Due to the fact that *D. magna* is a widely preferred test organism in ecotoxicology, it is also commonly used to investigate the toxicity of M/NPs [9, 23-29]. In the earliest studies, MPs were detected in the digestive tracts of *D. magna* exposed to primary and secondary microplastics in the size range of 2–5 μ m, and this was interpreted as evidence that even small-built creatures consume M/NPs [9]. Some studies show that MP fragments and additives have distinct toxicological mechanisms in terms of their intergenerational effect [30].

The focus of this research was to assess the ecotoxicological investigations of M/NPs conducted on *D. magna*, a keystone species among aquatic animals. In this respect, studies on M/NP toxicity with *D. magna* have been examined in the literature regardless of the year. The findings examined under the headings of M/NPs were physical and chemical properties, toxicity, duration of exposure, and toxicity analyses, and studies are categorized under the headings of the sociology of supporting experiments. Unlike previous studies, synthetic bibliometric methodologies and indicators were used to investigate the research trend and pressing issues of microplastic toxicity. In addition to paving the path for standardization in both of microplastic and nanoplastic research, the findings will be valuable for mapping the research landscape and technology forecasting.

II. MATERIAL AND METHODS

A. LITERATURE SEARCH CRITERIA

In the realm of scientific research, bibliometric analysis has garnered significant attention in recent times. This surge in interest can be ascribed to two primary factors: (1) the progress, availability, and ease of access to bibliometric software tools like Gephi, Leximancer, VOSviewer, alongside esteemed scientific databases such as Scopus and Web of Science, and (2) the cross-disciplinary dissemination of bibliometric methodologies from information science to the domain of business research [31]. In this study, it was preferred to use the Scopus scientific database. Because, the Scopus search engine, the bibliographic database of academic journal publications which covers more than 20,000 peer-reviewed journals published by more than 5,000 publishers, was used in this study [32-34]. An advanced search was performed using the following strings: "*Daphnia magna*", "microplastic", and "nanoplastic". Using restrictive keywords in the database, 2 distinct limited searches were conducted without restrictions on country or year. To improve the accuracy of the data, the first search included the keywords "*Daphnia magna*", and "Microplastics", and the second search included "*Daphnia magna*" and "Nanoplastics".

B. DATA PROCESSING

On June 21, 2021, 68 studies were discovered for the first search and 32 for the second search, each consisting of various types of documents. The documents were further screened based on their titles and abstracts; articles with research directions such as studies conducted with organisms other than *D. magna* were omitted. Accordingly, a total of 56 microplastic articles and 31 nanoplastic articles were evaluated in the first and second searches, respectively. Within the scope of this study, the results were separated into subcategories such as M/NP structure (morphological and chemical properties), the origin of M/NPs (purchase or obtained in a laboratory environment), M/NP types, and duration of exposure. The numerical data obtained from the study via bibliometric analyses were interpreted and visualized with social network analysis maps made via VOSViewer, and the corresponding figures were drawn using Origin 10.0. Figure 1 illustrates the research pattern and process flowchart employed in this study.



Figure 1. Research pattern and process flowchart

III. RESULTS AND DISCUSSION

A. PHYSICAL AND CHEMICAL PROPERTIES OF M/NPS

When the chemical polymer structures of the studied microplastics are examined, it is revealed that polystyrene (PS) (%35) and polyethylene (PE) (%28) are preferred for use in the vast majority of studies. PE, polypropylene (PP), PS, polyamide (nylon), and polyesters account for approximately %90 of the world's total plastic production [35-37]. PS is a widely utilized industrial plastic, particularly due to its strong stability, cost performance, and electrical properties [38-39]. This situation also drew the attention of researchers who have conducted toxicity studies. PS appears to be the dominant polymer type used in toxicity studies [40]. PE offers a broad range of applications. Its primary uses are in food packaging, chemical packaging, bottles, bags, water pipelines, and automotive parts, as well as its other practical daily applications. Additionally, since most plastics we use in our daily lives have a polymer structure, researchers have easy and inexpensive access to this type of polymer. Therefore, most researchers break down these substances to obtain secondary M/NPs. In addition, comparative studies of PE and other polymer types such as PVC, PET, PA, and PP have been carried out [41-43]. However, there is a limited number of studies on the toxicity of other polymers (PVC, PET, PA, PCB, PUR, PLA, and PMMA) [9, 41-48] (Figure 2).

While determining the toxicity of nanoplastics, it seems that the vast majority of investigations have utilized PS (90%). With an annual production volume of 13 million, PS is the most commonly used type of polymer, especially in the packaging of food products [49]. In the conducted studies, data were obtained indicating that PS was found in nature as secondary M/NPs because of biodegradation, weather conditions and deterioration caused by UV photo-oxidation [50-51]. Therefore, the toxic effects of PS on *D. magna* have been greatly emphasized and well documented [52-53]. Nonetheless, there are a certain number of studies on determining nanoplastic toxicity with PE and PMMA [54-55].

To control the toxicity effect of the polymer types, MP studies using various non-polymer particles such as kaolin and glass beads have also been conducted [44, 47, 56]. Thus, they emphasized that the toxicity is not attributable to any particle that daphnids can ingest, but rather to the surface properties and chemical composition of MPs [57].



Figure 2. Distribution of preferred polymers for (a) microplastics and (b) nanoplastics

Based on the published studies, the most frequently studied MP size range is 1-10 μ m with a rate of 43%. *D. magna* is a freshwater *Cladocera* that feeds via active filtration and passive uptake, actively filtering particles as small as 200 nm [58] and as large as 80 μ m [59], depending on filtering apparatus size, as well as passively taking up smaller particles [60-61]. In current study, Jemec et al. (2016) demonstrated that *D. magna* readily ingests long synthetic fibers around 300 μ m in length, but also detected very long fibers around 1400 μ m in length inside the gut. Therefore, some researchers

investigated the toxicity caused by particles ranging in size from 100 to 1000 μ m. However, this is uncommon because the adult body size of *D. magna* ranges from 1.5 to 5.0 mm. Most studies conducted on NPs (40%) used a size range of 101-1000 nm (Figure 3-b). Considering the size of their filter combs, daphnids are thought to swallow microplastics more than nanoparticles. Consequently, as the particle size decreased, the number of studies were increased. However, some researchers claim that the nanoscale negative effects of plastics on the metabolism of daphnids are more significant [17].

Concerning the toxicity of MPs, their shape and size play an important role [9,45]. In general, it is not possible to compare the studies in the literature with one another in terms of microplastic type (bead, fragment, and fiber) due to the lack of widely accepted methodological standards in these investigations. Therefore, independent studies demonstrating the effects of polymer structures cannot be correlated with each other since they use polymers of different sizes [40]. In general, fragments and fibers are the most extensively studied microplastic types on D.magna, and it has been stated that they are more toxic than those in the form of spheres [62-63].



Figure 3. Distribution of preferred plastics by size ranges for (a) MPs and (b) NPs

In 73% of the studies, MPs were obtained from different companies, usually as commercial products. Cospheric LLC [64] and Sigma-Aldrich [65] are the most preferred companies. These are followed by companies such as BaseLine Chromtech Research Center (Tianjin, China) [66], Goodfellow GmbH [67], SonTek/Xylem Inc (San Diego, USA) [68] and Fluoresbrite® YG Microspheres, Polysciences, Inc. (GmbH, Germany) [69] (Fig. 4). Researchers who do not purchase them as commercial products obtained MPs by taking commercial polymers in the form of ready-made pellets [29] or by breaking down these products as mentioned previously [70].



Figure 4. Most preferred companies in M/NPs studies

The plastic fibers were cryogenically ground in liquid nitrogen using Freezer/Mill 6875 (SPEX® SamplePrep, US) to obtain MP fragments [62]. PS lids of disposable instant coffee cups were cut into small pieces, frozen in liquid nitrogen, and pulverized in a ball mill (Retsch MM400, Retsch GmbH, Germany) [70]. In a separate study, the original PET fabric was cut into 2 x 2 cm pieces and ground in a Retsch PM 100 planetary ball mill to produce microplastic fiber [71]. Milling of recycled LDPE was performed in two stages. First, the pellets were fragmented by a SM100 mill (Retsch GmbH, Haan, Germany) and then further cryo-milled using a homogenizer (MillMix 20, Domel, Železniki, Slovenia). Both LDPE were tested and analyzed as irregular fragments (microfragments) [72]. In another study by Na et al. [29], PE fibers were extruded three times to ensure the homogeneity of the mixture and then cryogenically ground in liquid nitrogen using Freezer/Mill 6875 (SPEX® SamplePrep, US) to obtain MP fragments.

B. RESEARCH TENDENCY OF TOXICOLOGY OF M/NPS STUDIES

B. 1. Evaluation of Toxic Exposure

In the studies, tests for acute and chronic exposure were carried out at a rate of 64% and 36%, respectively. In immobilization studies with *D. magna*, the time of onset for acute exposure is usually studied for 24 or 48 hours. However, examining the research reveals that they had worked for 72 hours, 96 hours, and even up to 7 days. Accordingly, researchers working with more than one MP generally favored chronic exposure since MP species are examined at different durations. In a group study, the rate of chronic examination was increased in order to accurately examine all types of MP. However, unlike microplastics, nanoplastics have a shorter observed acute toxicity duration. Test times of 2 or 4 hours and 24 hours have been investigated in acute toxicity. The distribution of toxicity tests versus the number of articles is given in Fig. 5.



Figure 5. The distribution of toxicity tests vs number of articles for (a) MPs and (b) NPs

B. 2. Toxicity Endpoints

Toxicity test organisms are characterized as being "relatively easy to culture and maintain in the laboratory". Toxicity tests with *D. magna* are mostly based on the use of live test biota, which must be obtained from reliable sources or cultured and preserved in the laboratory in which the tests are performed. Numerous environmental factors must be considered in order to obtain an adequate quantity of healthy daphnia. Even in cultured laboratories, the availability of sufficient numbers of test organisms can often interfere with routine testing and monitoring of large numbers of samples [73]. For this reason, some researchers use commercially available *D. magna* biotests for their research. The effect concentrations of toxicity studies are expressed in units of mass per volume such as mg/L. The reported

toxicity endpoint of micro and nanoplastics is in the range of mg/L or μ g/L. It is occasionally expressed as particles/L. For example, *D. magna* immobilization test results of polystyrene (PS) beads are 66.97 mg/L or 0.11 µm. Uncertainties in the difference between particle/L and mg/L (endpoint unit in toxicity literature) were attempted to be eliminated with an equation developed by Leusch and Ziajahromi [74].

B. 3. Auxiliary Analysis Methods

In the majority of the studies, in addition to the toxicity tests performed, the stereomicroscope [14, 75], the fluorescent microscope [47], and the inverted microscope [76] were utilized to examine the exposure of daphnids to M/NPs. To determine the concentration of exposure, however, the number or mass of M/NPs in aqueous media must be known. Spectrofluorimetry [14] is used to measure the number of microplastics in experimental environments (aqueous solutions). In addition, the laser diffraction method [43, 72], and the Laser Particle Counter [77] are employed to determine the particle size and distribution to which daphnids are exposed. In addition to the concentration and particle size of the studied M/NPs, ATR-FTIR [72, 78-80] and Raman Spectrophotometry [39] are the favored techniques for determining chemical polymer structure. Micro FT-IR is used to determine the chemical polymer structure of smaller particles than ATR-FTIR. Advanced analysis methods such as SEM [39], SEM-EDS [43], FE-SEM [29, 81], and transmission electron microscopy (TEM) [45,82] were used for the microstructural calcification and elemental analysis of M/NPs.

Nanoplastic studies are more interested in the dynamic light scattering (DLS) method than microplastic investigations [55, 57, 83-84]. DLS is a technique in physics for determining the size distribution profile of small particles in suspension or polymers in solution. In nanoplastic research, TEM [22, 54, 85] and Zetasizer [86] are among the most frequently preferred analysis methods.

B. 4. General Trends Of Publications And Co-Occurrence Analysis

Bibliometric analysis frequently complements network visualization software, spanning from fully graphical user interface-based tools like VOSviewer [87] to command-based applications such as the Bibliometrix package in the R [88] programming language [31]. However, the most preferred VOSviewer is a scientific mapping application designed for bibliometric network visualization. It can perform a variety of bibliometric network analyses, such as keyword co-founding analysis and co-authorship analysis, in addition to citation network visualization. Fig. 6 displays the keywords that have appeared at least twice in 87 published articles. "Microplastics" is the most sought-after keyword in the search results, followed by "Daphnia magna". Depending on the relationship between keywords and article search content, this sequence is followed by the relationship between microplastic names, toxicity kinds, and aquatic creatures.

According to the keyword mapping for nanoplastics, bioaccumulation and uptake of "Daphnia magna" and nanoplastics are nearly equal in relation to toxicity. As a result, "uptake", "toxicity", "bioaccumulation" and "polystyrene" are frequently used in searches on aquatic organisms, and the frequency of their occurrence indicates the significance of terms to the subject. As shown in Fig. 7, the top three countries for microplastic research are China, Germany, and Slovenia, whereas the top three nations for nanoplastic research are China, Canada, and Denmark. In general, China is the leader in studies of M/NPs toxicity. China is indeed engaged in various international scientific organizations and has conducted many national kinds of research which were supported by official government funds [89].



Figure 6. Co-occurrence network of keywords generated by VOSviewer based on (a) "microplastic" OR "Daphnia magna" and (b) "nanoplastic" OR "Daphnia magna".



Figure 7. The country distribution of the total number of published articles

The publication sources that published the most articles on "microplastic" OR "*Daphnia magna*" and "nanoplastic" or "*Daphnia magna*" are given in Fig. 8. For studies found with the terms "microplastic" OR "*Daphnia magna*", Environmental Pollution had the most publications (17, 32%) with the second-highest IF2020 (IF=8.071). Science of The Total Environment ranked second (8, 15%) in the number of articles with an IF2020 of 7.963, and Journal of Hazardous Materials ranked third (4, 7.5%), although it had the highest IF2020 (IF= 10.588). The rest of the articles (45.5%) were published in 17 different journals. Moreover, for "nanoplastic" and "*Daphnia magna*" studies, Environmental Pollution published the highest number of articles (6, 20%) with the third-highest IF2020 (IF=8.071). Environmental Science and Technology ranked second (5, 16.7%) in the number of articles with the highest IF2020 (IF=9.028). Two journals, Environmental Toxicology and Chemistry and Environmental Science, published 3 articles each, which corresponds to 20% of the total publications. The rest of the articles (43.3%) were published by 11 different journals.



Figure 8. The journal distribution of the total number of published articles

IV. CONCLUSION

We know that small-sized plastics are easily dispersed and can have a direct impact on the toxicity in the aquatic environment. Therefore, it is essential to better understand the toxicity of M/NPs. Effects of nanoplastics on organisms may be related to particle toxicity, the toxicity of plastic-associated chemicals, or both; however, little is known about such effects and how particle shape, size, age, or polymer type influence them further. *Daphnia* shows considerable potential as an aquatic model for the risk assessment field due to its certain characteristics such as high sensitivity to environmental stressors, known genome, short generation time, and is therefore utilized in most studies.

In this study, 100 papers in the Scopus database were screened for bibliometric study using the keywords "*Daphnia magna*", "microplastic" and "nanoplastic". The results demonstrate that PS and PE are the primary polymer type employed in most toxicity studies.

Bibliometric analysis showed that the contributions of China and Germany to the evaluation of toxicity of M/NPs to *D. magna* are huge. Environmental Pollution was the journal that published the most research articles for M/NPs. According to our keyword analysis, more studies were conducted on microplastics (#56) compared to nanoplastics (#31). These initial results will be influential for future toxicology studies on M/NP effects on *D. magna*. While the ecotoxicological effects and health risks of microplastics are a current research trend, an increase in the number of publications focusing on the toxicity of M/NPs is anticipated in the near future.

Nevertheless, current studies on M/NPs are extremely controversial due to the inability to establish a standard method. Moreover, the long-term effects of chronic exposure on aquatic keystone species remain poorly understood, and studies on the effects on ecosystem dynamics are needed. In addition, the interactive effects of micro/nanoplastics on aquatic ecosystems need to be further investigated in conjunction with other stressors such as pollution and climate change. Understanding the trophic transfer of micro/nanoplastics from key species to other organisms can shed light on their broader impacts. Future perspectives should focus on emerging nanoplastics, use multi-omics approaches to reveal molecular responses, and investigate the impact of microplastic mixtures in natural environments. By addressing these gaps and considering future perspectives, ecotoxicological assessments can provide a comprehensive understanding of the ecological impacts of micro/nanoplastics on aquatic keystone species and contribute to effective mitigation measures.

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